Endogenous neurosteroids influence synaptic GABA$_A$ receptors during post-natal development

Belelli, D.; Brown, A. R.; Mitchell, S. J.; Gunn, B. G.; Herd, M. B.; Phillips, G. D.

Published in:
Journal of Neuroendocrinology

DOI:
10.1111/jne.12537

Publication date:
2018

Citation for published version (APA):
Belelli, D., Brown, A. R., Mitchell, S. J., Gunn, B. G., Herd, M. B., Phillips, G. D., Seifi, M., Swinny, J. D., & Lambert, J. J. (2018). Endogenous neurosteroids influence synaptic GABA$_A$ receptors during post-natal development. *Journal of Neuroendocrinology*, *30*(2), 11-15. Article e12537. https://doi.org/10.1111/jne.12537

DR JEREMY LAMBERT (Orcid ID : 0000-0002-4204-3602)

Article type : Review Article

Corresponding Author Email Id : j.j.lambert@dundee.ac.uk

Endogenous neurosteroids influence synaptic $\mathrm{GABA_A}$ receptors during post-natal development.

Delia Belelli, Adam R. Brown, Scott J. Mitchell, Ben G. Gunn, Murray B. Herd,
Grant Phillips, Mohsen Seifi, Jerome D. Swinny, Jeremy J. Lambert.

A review article to be considered for the "Steroids & the Nervous System" Special Issue.

Abstract

GABA plays a key role in both embryonic and neonatal brain development. For example, during early neonatal nervous system maturation, synaptic transmission, mediated by $\mathrm{GABA_A}$ receptors ($\mathrm{GABA_A}$Rs), undergoes a temporally specific form of synaptic plasticity, to accommodate the changing requirements of maturing neural networks. Specifically, the duration of miniature inhibitory postsynaptic currents (mIPSCs), resulting from vesicular GABA activating synaptic $\mathrm{GABA_A}$Rs, is reduced, permitting neurons to appropriately influence the window for postsynaptic excitation. Conventionally, programmed expression changes to the subtype of synaptic $\mathrm{GABA_A}$R are primarily implicated in this plasticity. However, it is now evident that in developing thalamic and cortical principal- and inter-neurons an endogenous neurosteroid tone *e.g.* allopregnanolone, enhances synaptic $\mathrm{GABA_A}$R function. Furthermore, a cessation of steroidogenesis, due to a lack of

This is the peer reviewed version of the following article: 'Endogenous neurosteroids influence synaptic GABAA receptors during post-natal development', *Journal of Neuroendocrinology*, which has been published in final form at http://dx.doi.org/10.1111/jne.12537. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

substrate, or a co-factor, appears primarily responsible for early neonatal changes to GABA-ergic synaptic transmission, followed by further refinement, which results from subsequent alterations of the $GABA_AR$ subtype. The timing of this cessation of neurosteroid influence is neuron specific, occurring by postnatal day 10 (P10) in thalamus, but about a week later in cortex. Neurosteroid levels are not static, but change dynamically in a variety of physiological and pathophysiological scenarios. Given that GABA plays an important role in brain development, abnormal perturbations of neonatal $GABA_AR$-active neurosteroids, may have a considerable immediate, but also longer term impact, upon neural network activity. Here we review recent evidence that changes in neurosteroidogenesis substantially influence neonatal GABA-ergic synaptic transmission. We discuss the physiological relevance of these findings and how interference of neurosteroid-$GABA_AR$ interaction early in life may contribute to psychiatric conditions later in life.

Introduction

From an early embryonic age, GABA-ergic signalling plays a fundamental role in neuronal development, influencing neuronal proliferation, migration and differentiation and is important in the establishment of neuronal networks[1–4]. These effects of GABA are mediated by ionotropic $GABA_A$ receptors ($GABA_ARs$), or by G-protein coupled $GABA_B$ receptors. Certain steroids are potent, endogenous, positive allosteric modulators (PAMs) of the $GABA_AR$[5–8] (neuroactive steroids). Furthermore, such steroids may be synthesised locally in the brain, or spinal cord and are classed as neurosteroids. Here we consider the role of neurosteroids and GABA in the developing neonatal central nervous system (CNS).

Neurosteroids: endogenous modulators of the $GABA_AR$.

The finding in 1966 by Craig and colleagues that the anticonvulsant properties of certain endogenously occurring steroids could be distinguished from classical hormonal actions[9], paved the way nearly two decades later to the discovery that the synthetic general anaesthetic steroid alphaxalone[10] and subsequently certain endogenous metabolites of progesterone (5α-pregnan-3α-ol-20-one; allopregnanolone) and deoxycorticosterone (5α-pregnan-3α,21 diol-20-one; 5α-THDOC) act as potent and selective positive allosteric modulators (PAMs) of the major inhibitory receptor in the mammalian brain, the $GABA_AR$[5,10–14] (Figure 1).

$GABA_AR$s belong to the transmitter-gated ion channel superfamily and are composed of five subunits arranged around a central pore to form an anion-conducting ion channel[15,16]. Low nM aqueous concentrations of these neuroactive steroids enhance the actions of GABA by promoting the anion-conducting state of the associated channel[11,14,17,18]. Their activity at such low concentrations suggests the presence of a high affinity steroid binding site on the $GABA_AR$. However, these steroids are highly lipophilic, resulting in relatively high membrane concentrations in close proximity to the proposed transmembrane binding site on the $GABA_AR$[19,20] . Therefore, the steroid may have a relatively low affinity for the receptor, but be optimally concentrated, locally in the membrane, leading to an increased probability that the steroid will occupy the receptor binding site[19,20]. The $GABA_AR$ is the target for clinically important drugs, *e.g.* benzodiazepines and certain general anaesthetics, which enhance the $GABA_AR$ function [5]. In common with such drugs, administration of neuroactive steroids produce anxiolytic, analgesic, anticonvulsant and sedative effects, with higher doses capable of inducing a general anaesthetic state[5,8].

Initially, endocrine glands such as the adrenals and ovaries were considered the exclusive source of steroid, necessitating it to cross the blood brain barrier to influence neural activity. Subsequently, compelling evidence emerged for *de novo* brain synthesis *i.e.* neurosteroids[21]. Hence, these endogenous $GABA_AR$-modulators may potentially act in an endocrine, paracrine, or autocrine manner, to influence neuronal signalling in various physiological and pathological situations. Indeed, neurosteroid levels are dynamically changed in a variety of physiological (*e.g.* development, puberty, pregnancy, stress, ovarian cycle) and pathophysiological (*e.g.* major depression, postpartum depression, premenstrual tension, panic attacks and schizophrenia) scenarios [5].

In the brain and spinal cord the $GABA_AR$-active steroids such as allopregnanolone and 5α-THDOC may *a)* originate from peripheral sources *e.g.* placenta, ovaries and adrenals necessitating them crossing the blood brain barrier[22]; *b)* be derived from their peripheral hormonal precursors, progesterone and deoxycorticosterone, or *c)* be synthesised *de novo* from cholesterol *via* a series of multi-enzymatic steps[23]. Diverse approaches including *in situ* hybridization, immunohistochemistry, gas chromatography mass spectrometry, electrophysiology and behaviour, purport that neurosteroids, synthesised by neurons and/or glia may achieve levels sufficient to influence in a paracrine and/or autocrine fashion $GABA_AR$ signalling from the onset of embryonic GABAergic transmission into maturity [5,24–29]. The suite of enzymes that synthesise neurosteroids exhibit regional and cellular-selective expression patterns, which change within discrete temporal windows and are susceptible to external challenges, *e.g.* stress[23,30–33]. In the CNS the production of $GABA_AR$-active neurosteroids from cholesterol (Figure 1) first requires the translocation of the steroid across the mitochondrial membrane by translocator protein 18 kDa (TSPO)[34,35]. Cholesterol is then metabolised to pregnenolone by the mitochondrial P450 side-chain cleavage enzyme CYP11A1, this metabolite is then exported across the mitochondrial membrane, where it may be converted to $GABA_AR$-active neurosteroids such as allopregnanolone following three sequential enzyme reactions catalysed by 3β-hydroxysteroid dehydrogenase (3β-HSD) to form progesterone, 5α-reductase (5α-R) to produce 5α-dihydroprogesterone (5α-DHP) and 3α-hydroxysteroid dehydrogenase (3α-HSD) to form allopregnanolone (Figure 1) [7]. Note both in the CNS and the periphery the enzymes 5α-R and 3α-HSD may sequentially participate in the conversion of deoxycorticosterone derived primarily from the adrenals into the $GABA_AR$-active 5α-THDOC [22]. We have recently reviewed the expression of 5α-R and 3α-HSD in mammalian brain[24], (see also [30-33]). As GABA plays a crucial role in neurodevelopment, during this critical time of neonatal development it is conceivable that the changing levels of neurosteroids, from CNS-located paracrine, or autocrine sources, may influence the establishment of mature neuronal circuits and communication. Additionally, neonatal endocrine glands such as the adrenals may provide the brain and spinal cord with either the $GABA_AR$-active steroids *per se*, or their precursors [22,36,37].

$GABA_A$Rs.

The $GABA_A$R subunits are drawn from a repertoire of 19 gene products belonging to distinct families including: α1-6; β1-3; γ1-3, δ, ε, θ, π and ρ1-3[15,16]. In the adult mammalian brain this diversity underpins the expression of 20-30 $GABA_A$R subtypes, that are uniquely distributed and consequently influence particular behaviours[38–40]. Although neuroactive steroids are highly selective for $GABA_A$Rs, they exhibit limited $GABA_A$R subtype selectivity[5]. However, the $GABA_A$R subunit composition does influence *a)* neuronal subcellular location *e.g.* synaptic *vs* extra/peri-synaptic expression [41] *b)* the impact of enzymes (*e.g.* kinases) upon receptor function/location/expression[42–44], *c)* the pharmacology of the receptor *e.g.* benzodiazepines and certain general anaesthetics[15,40] and *d)* the physiological properties of the receptor, including the kinetics of receptor activity influenced by rates of deactivation, desensitization, and GABA affinity[42].

The neuronal location of the receptor influences the GABA inhibitory signalling repertoire of the neuron. Receptors clustered in the post-synaptic domain primarily serve to mediate a fast, phasic form of inhibition in response to relatively high local concentrations of neurotransmitter, which occur in the synapse following the vesicular release of GABA[41]. By contrast, receptors in peri-synaptic and extra-synaptic locations are activated by ambient concentrations of GABA to mediate a sustained, or tonic form of neuronal inhibition[41]. However, in some neurons during physiological bursts of high frequency presynaptic stimulation the spill-over of GABA from the synapse is sufficient to additionally engage these extrasynaptic receptors to produce a greatly prolonged form of slow phasic inhibition[45–47]. The majority of $GABA_A$Rs contain two α and two β subunits together with a single copy of the γ2 subunit. Synaptic receptors often contain the γ2 subunit, although receptors incorporating this subunit may be located out with the synapse. Receptors incorporating the δ subunit, in place of the γ2 subunit are expressed extra- or peri-synaptically[41,42,48,49].

GABA$_A$R subunit expression and function during embryonic and post-natal development.

In the adult brain, activation of GABA$_A$Rs usually causes an inhibitory effect on neuronal excitability, conferred both by a net inward directed flow of Cl$^-$ ions and the shunting action of GABA[50]. However, early in development (embryonic and first postnatal week), GABA$_A$R activation results in neuronal depolarization, due to a relatively high intracellular Cl$^-$ concentration, resulting from limited expression of KCC2, the principal neuronal transporter for Cl$^-$ ion extrusion[51]. Although the validity of this developmental ionic perturbation has been challenged, under certain experimental conditions[52–54], the depolarizing nature of these early GABA-ergic signals may be sufficient to activate voltage-dependent calcium, or sodium channels and appear pivotal to the neurotrophic actions of the neurotransmitter[55–57]. Furthermore, during embryogenesis, such depolarizations are primarily mediated by activation of extra-synaptic GABA$_A$Rs, which are expressed prior to and indeed facilitate the establishment of functionally relevant synaptic connections after birth[2,3].

During early development the subunit composition of GABA$_A$Rs is highly plastic, consequently impacting upon the physiological and pharmacological properties of the receptors and influencing their subcellular localization[15,42]. Preceding the generation, migration, and differentiation of most telencephalic and mesencephalic neurons[58,59], the first detection of GABA$_A$R subunits (including α1-5, β1-3 and γ1-3), coincides with the appearance of GABA-positive neurons at approximately E13-14 in the marginal zone, sub-plate, and sub-ventricular zone[60,61]. For rodent brain, the end of the first postnatal week generally signals a change to a hyperpolarizing GABA$_A$R response[1]. Depending on the specific brain region, postnatal weeks two to three mark the establishment of a near-mature synaptic network, which reaches an adult stage by about two months of age following sexual maturation. From approximately the end of the first postnatal week synaptic GABA$_A$R signals, resulting from the vesicular release of GABA, begin to play an important role in shaping the development of neuronal circuits[62].

As the postnatal brain develops there is an increased requirement for temporal precision within neuronal networks and accordingly, in many neurons the duration of phasic events *e.g*. miniature inhibitory postsynaptic currents (mIPSCs) mediated by the activation of synaptic GABA$_A$Rs by GABA released from a single vesicle, is progressively reduced[42,63–69]. This critical development of GABA-ergic signalling is generally considered to result from a genetically programmed change of the subtype of synaptic GABA$_A$R. In this regard the α-subunit isoform exerts a profound role on the duration of GABA$_A$R-mediated phasic events. For example, electrophysiological studies with expressed recombinant GABA$_A$Rs demonstrates the duration of GABA-evoked currents mediated by receptors incorporating either α2-, or α3-subunits are prolonged compared to equivalent receptors containing the α1 subunit[70], although the β and γ subunit isoforms may also influence the mIPSC decay[71,72]. Of physiological relevance, in different neurons (*e.g*. principal glutamatergic neurons and GABAergic interneurons) expression of different synaptic GABA$_A$R isoforms results in kinetically distinct synaptic events, underpinning the integration of neuronal signals and network activity and allowing the establishment of behaviourally relevant neuronal rhythms[73,74].

Many neurons express multiple GABA$_A$R isoforms, complicating evaluation of their role in neonatal development. To study this in detail we have used the ventrobasal (VB) neurons of the mouse thalamus. These neurons provide an ideal model as they exhibit a clear developmental transition from synaptic α2-GABA$_A$Rs from postnatal weeks 1-2, to α1-GABA$_A$Rs in weeks 2-3[75] (Figure 2).They are innervated by a band of GABA-ergic neurons, the nucleus reticularis (nRT neurons), which release GABA onto the VB neurons. Our studies, described below, reveal an important role for GABA-modulatory neurosteroids in determining the duration of mIPSCs during neonatal development.

During postnatal development of thalamic VB neurons neurosteroids, in concert with changes to GABA$_A$R isoforms, influence phasic and tonic inhibition.

We reported that the duration of thalamocortical VB neuron mIPSCs is greatly reduced over a critical, short period of 1-2 days between postnatal days (P) 9-11[75]. Synaptic receptors incorporating the α1-subunit are associated with brief mIPSCs[63,75]. Implicating changes to synaptic GABA$_A$Rs, our immunohistochemical and electrophysiological studies with an α1 "knock-out" mouse and benzodiazepine-insensitive knock-in (α1H101R; α2H101R) mice revealed P20 neurons to exclusively express synaptic $\alpha1\beta\gamma2$ GABA$_A$Rs, whilst younger mice expressed the $\alpha2\beta\gamma2$ isoform[75] (Figure 2). However, the major change to the mIPSC duration, occurred prior (P9-11) to the synaptic α2- to α1-GABA$_A$R transition [75] and were replicated in an $\alpha1^{-/-}$ mouse[75]. Clearly, this initial P9-11 synaptic plasticity does not result from expression of α1-GABA$_A$Rs[75]. During postnatal development the levels of GABA$_A$R-modulatory neurosteroids change[76]. Do neurosteroids influence neural inhibition during postnatal development (P7-24)?

Preventing neurosteroid synthesis in P7 thalamus with the 5α-reductase inhibitor finasteride significantly reduced mIPSC duration[77]. Intracellular delivery of the neurosteroid scavenger γ-cyclodextrin [γ-CD][78–80], produced the same effect[77], but by P10 was inert (Figure 2,3). Exogenous 5α3α was equi-effective in prolonging mIPSCs of P7 and P20-24 neurons. Therefore, the developmental change in the mIPSCs is not a consequence of synaptic GABA$_A$Rs steroid-insensitivity, but results from a loss of the neurosteroid[77]. Note that the duration of both control and γ-CD-treated P20-24 mIPSCs are further reduced *c.f.* P10 mIPSCs, a change which corresponds temporally with the exchange of synaptic α2-GABA$_A$Rs by α1-GABA$_A$Rs[77] (Figure 2). Hence, prior to P10 the mIPSC duration is reduced, caused by decreased neurosteroid levels, with subsequent further kinetic refinement resulting from a change in the synaptic GABA$_A$R subtype (Figure 2). A recent study suggested that hippocampal expression of the GABA$_A$R α2-subunit was increased by GABA$_A$R-active neurosteroids[81]. Therefore, it is conceivable that a waning neurosteroid tone heralds the programmed subsequent loss of thalamic synaptic α2-GABA$_A$Rs *post* P10.

The source of the neurosteroid influencing P7-8 neurons is not known. Studies using an antibody against the neurosteroid suggest that the VB neurons contain $5\alpha3\alpha$[82]. Inclusion in the recording pipette of the membrane-impermeant γ-CD (0.5 mM), caused a time-dependent (> 6 min) decrease in the mIPSC duration of P7 VB neurons, mirroring the effect of pre-incubated γ-CD and of finasteride pre-treatment[77]. These studies demonstrate that VB neurons contain $GABA_A$R-active neurosteroids, but do not elucidate whether their source is autocrine, paracrine, or indeed a consequence of prior endocrine release[5].

We suggest that by P10 the local neurosteroid levels are insufficient to influence GABA-ergic neurotransmission. Can the more mature thalamus synthesise neuroactive steroids? Incubation of thalamic slices with 5α-DHP, the immediate precursor of allopregnanolone (Figure 1), caused a substantial prolongation of mIPSCs of both P20-24 and P7 neurons[77], which was prevented by indomethacin, an inhibitor of 3α-hydroxysteroid dehydrogenase (3α-HSD), the enzyme that converts 5α-DHP to the $GABA_A$R-active allopregnanolone (Figure 1). Although indomethacin is not a selective inhibitor of the 3α-HSD enzyme, this effect of 5α-DHP to prolong the mIPSCs was reversed by γ-CD treatment. Collectively, these results demonstrate that the more mature (P20-24) thalamic slice retains the ability to synthesise $GABA_A$R-active neurosteroids and that the change in mIPSC duration at P9-10 results from a loss of steroid substrate, or a co-factor *e.g.* NADPH, see[83].

P20-24 VB neurons express synaptic $\alpha1\beta2\gamma2$ and extrasynaptic $\alpha4\beta2\delta$ $GABA_A$Rs that mediate phasic and tonic inhibition respectively[75,84]. Although the P20-24 mIPSCs were insensitive to γ-CD treatment, suggesting that local neurosteroid levels are insufficient to impact upon synaptic $GABA_A$Rs, neurosteroids are particularly efficacious upon recombinant receptors incorporating the δ-subunit[85,86]. However, the magnitude of the thalamic tonic current mediated by $\alpha4\beta2\delta$-$GABA_A$Rs was not influenced by γ-CD pre-incubation, suggesting that in P20-24 VB neurons the neurosteroid levels are not sufficient to influence either synaptic, or extrasynaptic $GABA_A$Rs[77]. Nevertheless, incubation with the precursor 5α-DHP, resulted in a large increase of the tonic current, illustrating that neurosteroid production can be reinitiated sufficiently to influence both phasic and tonic inhibition[77].

The mIPSC frequency of P10-24 VB neurons was much greater than that of P7 neurons. Unexpectedly, incubation with finasteride greatly increased the mIPSC frequency of P7, but not of P10 VB neurons. Furthermore, 5α-DHP greatly reduced the mIPSC frequency of P20-24 VB neurons, an effect probably mediated by allopregnanolone, as indomethacin prevented this suppression of vesicular release by 5α-DHP. Hence, vesicular GABA release from P7 nRT neurons is governed by a neurosteroid tone which, as it wanes, results in an increased mIPSC frequency for P10-24 neurons[77]. These effects of the steroid metabolite appear unlikely to be mediated by presynaptic nRT GABA$_A$Rs as other enhancers of GABA$_A$R function (pentobarbital, etomidate and zolpidem) had no effect on quantal GABA release[77] and therefore the steroid may act *via* alternative targets *e.g* voltage-gated T-type calcium channels[87].

A neurosteroid influence on GABA-ergic transmission of the developing mouse cortex.

Given the sudden loss of neurosteroid influence for P10 VB neurons we investigated whether GABA-ergic transmission in another brain region was similarly influenced and if so determine whether the temporal neurosteroid profile was similar to thalamus. These thalamic relay VB neurons send projections to cortical layer 4 (L4) neurons during P4-8 and subsequently (P10-14) L4 cortical neurons project to L2/3 neurons[88,89]. We therefore investigated in L2/3 pyramidal neurons and GAD-67$^+$ cortical interneurons the temporal profile of phasic inhibition and the influence of neurosteroid tone.

In L2/3 cortical pyramidal neurons the mIPSC profile changed considerably from neonatal (P7-15), to juvenile stages (P20-24)[90]. Similar to VB neurons, the mIPSC frequency increased and their duration decreased between P7-8 and P20-24. In common with VB neurons, the mIPSC kinetic change did not result from neurosteroid insensitivity of synaptic GABA$_A$R, but resulted from a loss of neurosteroid production as the mIPSC decay time of P7, but not of P20-24 cortical neurons was substantially reduced by pre-incubation with γ-CD, or by finasteride pre-treatment[90]. In contrast to the thalamic VB neurons, γ-CD influenced the mIPSC decay time at P10 and at P15, but as described above was inert by P20-24 (Figure 3). Hence, the neurosteroid profile is temporally distinct from that of the thalamocortical relay neurons. Mirroring the VB neurons, incubation of the cortical slice with 5α-DHP greatly prolonged mIPSCs of P20-24 pyramidal neurons. In common with thalamic VB neurons, this effect of 5α-DHP was prevented by indomethacin and reversed by γ-CD treatment. Collectively, these results demonstrate that when supplied with the immediate steroid precursor, the cortical slice can metabolise 5α-DHP into a $GABA_A$R-active neuroactive steroid[90]. Indeed, phasic inhibition in hippocampal dentate gyrus granule cells and the medium spiny neurons of the nucleus accumbens is similarly influenced by 5α-DHP (unpublished), suggesting this capacity to synthesise $GABA_A$R-active neurosteroids may be a common feature across different brain regions.

Even in the presence of γ-CD, the mIPSC decay time decreased from P7-8 to P20-24 suggesting synaptic $GABA_A$Rs to be influenced by additional factor(s) during neonatal development. As described above, the $GABA_A$R subunit composition may influence the mIPSC duration, with particularly the α1 subunit associated with brief events. We found the mIPSCs recorded from $\alpha1^{-/-}$ cortical L2/3 pyramidal neurons to be prolonged *c.f.* their wild type counterparts at all ages studied (P7-8, P10, P15 and P20-24)[90]. In common with WT neurons, treatment with γ-CD, or finasteride, reduced the mIPSC decay time of P7-15 $\alpha1^{-/-}$ neurons, but with no effect on P20-24 neurons (Figure 3). Therefore, during cortical neuron development from P7, to at least P15, the duration of phasic inhibitory events is simultaneously influenced by a neurosteroid tone and by the subunit composition of the synaptic $GABA_A$Rs, with a waning influence of the neurosteroid occurring between P15 and P20. By contrast, for thalamocortical neurons the neurosteroid influence dissipates by P10, but the mIPSC duration continues to decrease up to P20-24 due to the exchange of synaptic α2-$GABA_A$Rs by α1-$GABA_A$Rs (Figure 2,3). Finally, the frequency of mIPSCs increased during development in both cortical and thalamic neurons. However, whereas in thalamus the increased mIPSC frequency was due to a waning neurosteroid presynaptic influence, the frequency of cortical mIPSCs was not influenced by γ-CD pre-treatment[90] and therefore may reflect increased GABA-ergic innervation.

Immunohistochemical and *in situ* hybridisation studies reveal cortical principal neurons to express both the 5α-reductase and the 3α-HSD enzymes required for allopregnanolone synthesis, whereas interneurons do not[30]. We therefore investigated whether phasic inhibition of cortical GABA-ergic interneurons is similarly influenced by neurosteroids during neonatal development.

Recordings were made from GAD67 GFP$^+$ mice, engineered to co-express green fluorescent protein (GFP) with the GABA-synthesising γ-amino decarboxylase (GAD67) enzyme, which identifies, but does not distinguish between the three major interneuron classes in mouse cortex (*i.e.* somatostatin-positive (SS$^+$), parvalbumin-positive (PV$^+$), 5-HT$_3$R-positive (5-HT$_3$R$^+$)] interneurons[89,91–93]. Recordings from GFP$^+$ neurons revealed P7-8 mIPSCs to be greatly prolonged *c.f.* equivalent recordings from P20-24 neurons. Intracellular γ-CD reduced the P7-8 mIPSC duration, with no effect on P20-24 neurons. Therefore, in common with cortical pyramidal neurons, interneuron phasic inhibition is influenced by neurosteroids early in postnatal development, an effect that waned by P20-24. As these interneurons do not appear to express the enzymes to synthesize allopregnanolone, these observations suggest such steroid may originate from paracrine, or endocrine sources and not emanate directly from autocrine synthesis[30].

Neurosteroid modulation of GABA$_A$R function during postnatal development: Physiological relevance.

The findings presented above provide a compelling case for endogenous pregnane steroids enhancing GABA$_A$R-mediated phasic inhibition in both cortex and thalamus during the 2nd and 3rd post-natal weeks. During early postnatal maturation a programmed cessation of neurosteroidogenesis and changes to the expression of synaptic GABA$_A$R subtypes, act in concert to influence phasic inhibition. This interplay of neurosteroidogenesis with GABA$_A$R expression follows a neuron-specific temporal pattern. In somatosensory thalamus *i.e*. VB neurons, the neurosteroid tone dissipates by P10, prior to the subsequent exchange of synaptic α2-GABA$_A$Rs, by α1-GABA$_A$Rs, which further refines phasic inhibition. By contrast, in cortical L2/3 pyramidal neurons a neurosteroid influence on phasic inhibition persists for at least another week (Figure 3). The fading neurosteroid influence results from a loss of steroid substrate, or an essential co-factor, as incubation of P20-24 brain slices with 5α-DHP prolongs both cortical and thalamic mIPSCs.

Neurosteroids enhance the function of both synaptic and extrasynaptic $GABA_ARs$[5,48], thereby potentially influencing both depolarising (embryonic and early post-natal) and hyperpolarising (later post-natal) actions of GABA. The mechanism(s) that dynamically regulate neonatal neurosteroid levels are not known, although NMDA receptor activation reportedly triggers their synthesis in CA1 neurons, providing an intriguing link between neural excitation and enhancement of neural inhibition[94,95].

What is the physiological significance of these neonatal changes to GABA-ergic transmission? As discussed, generally GABA-ergic signals mediate a depolarizing response up to the first post-natal week (but see below). Such an effect can cause activation of specific voltage-gated conductances, *e.g.* Ca^{2+}, which act to initiate intracellular processes crucial to neuronal migration and maturation *e.g.* neurite growth and synapse formation, thus permitting the subsequent establishment and synchronization of neuronal networks[2,96,97]. Neurosteroid levels are elevated during these early phases of neurodevelopment, *i.e.* during late gestation and early neonatal life, but have decreased by the third postnatal week, *e.g.* in the cortex[76] (See also Figure. 3 below).

The neurosteroid decline coincides temporally with the switch to a hyperpolarizing GABA signal[1]. These events may be associated, as inhibition of neurosteroid synthesis, by finasteride, influences KCC2 expression in postnatal hippocampus, implying aberrant neuronal inhibition[98,99]. Is the prolongation of the phasic GABA depolarisation by the neurosteroid action required for increased KCC2 expression? The differential neurosteroid profile of neonatal thalamic and cortical neurons (Figure 3), clearly warrants a comparison of the temporal expression of KCC2 in these neurons and the timing of their depolarising/hyperpolarising switch. Furthermore, certain cortical interneurons are of interest as, even for mature interneurons, $GABA_AR$ activation may cause their depolarisation[100,101], an action important in the emergence of brain rhythms in the fast γ- frequency domain[102–104] (Figure 3).

Enhancement by neurosteroids of the GABA-evoked depolarisation may influence the temporal window of crucial processes such as neuronal migration, morphological maturation and synapse formation[3,57]. In support, allopregnanolone promotes cell proliferation both in human and rodent brain and regulates cell-cycle and gene expression[105,106]. Although some of these actions appear to be mediated by GABA$_A$Rs, a putative involvement of G-protein-coupled membrane progesterone receptors requires consideration[107].

Our recordings from neonatal cortical L2/3 pyramidal and GABA-ergic interneurons reveal a role for neurosteroids in the normal maturation of the cortical network. The formation of functional GABAergic synapses on interneurons precedes the development of glutamatergic synapses in principal neurons[97,108,109], although there are exceptions[110–112]. Whether neurosteroids influence the GABA$_A$R-mediated regulation of both GABAergic and glutamatergic transmission in these distinct neuronal types remains to be determined. We have not compared in detail the temporal pattern of neurosteroid regulation of GABAergic transmission of cortical interneurons, with that now established for pyramidal neurons. Furthermore, our recordings were from GAD-67$^+$ neurons, therefore the neurosteroid influence may differ across distinct GABAergic , SS$^+$, PV$^+$, 5-HT$_3$R$^+$ interneurons[89,93,113]. The 5-HT$_3$R$^+$ interneurons dominate in layers II-III of mature cortex[93]. However, PV$^+$ interneurons may be of particular interest as they exhibit a distinctive, extended developmental expression pattern *c.f.* other interneurons[89,114] and a window of functional maturation exquisitely susceptible to extrinsic modulation during the second to third postnatal week[89]. In particular, the overall homogenous distribution of PV$^+$ interneurons across all cortical layers except for layer 1[93,114] appears crucial for normal development of cortical connectivity. Importantly, in schizophrenics (see below) and in animal models of this condition, there is a layer selective reduction of the cortical PV and GAD67 mRNA signal[113].

Suggesting a putative role for neurosteroids in the development of cortical connectivity, neonatal administration of allopregnanolone at (P5), a time which precedes the functional maturation of PV$^+$ interneurons and when neurosteroids levels are relatively high (Figure 3), alters their distribution between superficial (II/III) and deep (V/VI) layers of the adult prefrontal cortex. Additionally, allopregnanolone reduces their abundance in the medial dorsal thalamus[115–117]. These neurosteroid effects appear to be GABA$_A$R-mediated as they are mimicked by benzodiazepines[118]. Future genetic and pharmacological approaches to manipulate embryonic and postnatal neurosteroids may elucidate and differentiate their role in the three main classes of cortical interneurons[89]. Similar studies are required across different cortical layers and areas *e.g.* visual *vs* somatosensory cortex, as translaminar GABAergic inhibition is sub-served by different interneuron classes[119].

A similar complexity may apply to somatosensory thalamic neurons (*e.g.* VB and nRT), where the neurosteroid influence wanes, approximately a week prior to that of cortical pyramidal neurons (Figure 2,3). The significance of this temporally distinct neurosteroidogenic profile is not known. However, unilateral lesion of neonatal thalamic lateral geniculate nucleus, or the VB, substantially alters the developmental expression of α1- and α5-GABA$_A$R subunits, across the layers of the corresponding cortical territories *i.e.* visual, V1 and somatosensory, S1, emphasising the profound influence of thalamic inputs on the development of cortical GABAergic circuits[120]. Therefore, it is conceivable that thalamic neurosteroidogenesis may influence thalamo-cortical connectivity.

Neurosteroids, early life adversity and psychiatric disorders.

Given the proposed involvement of neurosteroids in brain development, experiences that perturb their levels during the establishment of neural circuitry, may subsequently influence juvenile and adult behaviour. In support, there are significant associations between abnormal levels of GABA$_A$R-active neurosteroids *e.g.* allopregnanolone and a variety of neuropsychiatric disorders[33] (see below). Moreover, strategies to restore neurosteroid levels in such conditions in humans, or equivalent animal models, have proved, beneficial[33,121–125]. Note the majority of psychiatric disorders, ~2/3, are diagnosed by 24 years old, or earlier, consistent with a neurodevelopmental component[126,127].

Environmental events, grouped under the umbrella of early-life adversity or early life stress (ELS), are now recognised as a major preventable cause of future psychiatric disorders including anxiety, depression and substance abuse[128–130]. Indeed, prior ELS may additionally be a risk factor for the future manifestation of some neurological disorders, *e.g.* certain forms of epilepsy [131]. Clinically, these terms describe a variety of negative experiences early in life, ranging from poverty, malnutrition, to physical and emotional trauma, or abuse[132].

Patchev and colleagues (1997) first suggested that abnormal regulation of neurosteroid action as a consequence of negative experiences early in life could affect neurodevelopmental trajectories to contribute to adult psychopathology[133]. In a rodent model of maternal separation the GABA$_A$R-active neurosteroid (5α-THDOC), administered during early experience of reduced maternal care, prevented in adulthood, the development of a variety of neuroendocrine dysfunctions and behavioural abnormalities *i.e.* anxiety, associated with prior exposure to early-life adversity[133]. Whether prior ELS subsequently influences adult levels of neurosteroids and/or the dynamic regulation of their levels under a variety of physiological conditions *e.g.* pregnancy is not known. However, such experiences greatly influence the functional effects of GABA$_A$R-active neurosteroids. Thus, using a naturalistic model of fragmented maternal care[134,135], we demonstrated that prior ELS produces a profound dysregulation of the neuronal circuits orchestrating the stress response in the paraventricular nucleus (PVN) of the hypothalamus[136]. Specifically, prior ELS greatly increased the glutamatergic excitatory drive to the CRF-releasing PVN neurons, sufficient to prevent the normal suppression of PVN firing by physiological levels of allopregnanolone[136]. Note that increased circulating allopregnanolone is purported to act as a part of a feedback circuit during acute stress, thereby limiting the duration of CRF release and consequently of glucocorticoids[21,24].

The nucleus accumbens is part of the reward pathway and is implicated in both depression and drug abuse[137–139]. In common with thalamus and cortex, it can synthesise GABA$_A$R-active neurosteroids (unpublished observations). Furthermore, we find that adult mice previously exposed to ELS, exhibit altered accumbal GABA$_A$R neurotransmission and an abnormal response to cocaine, *i.e.* an altered locomotor sensitization, a behaviour that recapitulates in rodents aspects of drug addiction[140].

Schizophrenia is negatively influenced by early-life adversity[141–143]. The findings by Grobin and colleagues[115,116], discussed above, suggest a possible link between early neurosteroid dysregulation and an abnormal developmental pattern for prefrontal cortex, a region implicated in schizophrenia[113]. In support, a recent investigation revealed abnormal neurosteroid levels in a population of schizophrenic patients[144]. Furthermore, Bortolato and co-workers have implicated altered neurosteroidogenesis in the accumbal-mediated expression of behavioural deficits *i.e.* altered pre-pulse inhibition, which is typically altered in animal models of schizophrenia[145]. Thus, the impact of abnormal neurosteroid levels upon neurodevelopment may extend beyond the cortex.

In conclusion, it is now evident that endogenous neurosteroids play a vital role in fine-tuning GABA-ergic transmission during neonatal development in a neuron specific manner. GABA is crucial to establishing and developing appropriate neural connections in the developing brain. Therefore perturbations of neurosteroid levels *e.g.* by stress, during this critical time of neonatal development may have a long term impact upon neuronal circuitry and plasticity. Further investigation of the influence of $GABA_A$R-active neurosteroids during development is now warranted. Such studies may allow a better understanding of the underlying neurobiology that results in the psychiatric disorders associated with early life adversity.

References

1. Ben-Ari Y. The yin and yen of GABA in brain development and operation in health and disease. *Front Cell Neurosci*. 2012;6:45

2. Cellot G, Cherubini E. Functional role of ambient GABA in refining neuronal circuits early in postnatal development. *Front Neural Circuits*. 2013;7:1-9.

3. Kilb W, Kirischuk S, Luhmann HJ. Role of tonic GABAergic currents during pre- and early post-natal rodent development. *Front Neural Circuits*. 2013;7:1-13.

4. Le Magueresse C, Monyer H. GABAergic interneurons shape the functional maturation of the cortex. *Neuron*. 2013;77(3):388-405.

5. Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the $GABA_A$ receptor. *Nat Rev Neurosci*. 2005;6(7):565-575.

6. Herd MB, Belelli D, Lambert JJ. Neurosteroid modulation of synaptic and extrasynaptic $GABA_A$ receptors. *Pharmacol Ther*. 2007;116(1):20-34..

7. Gunn BG, Brown AR, Lambert JJ, Belelli D. Neurosteroids and $GABA_A$ receptor interactions: a focus on stress. *Front Neurosci*. 2011;5:131.

8. Reddy DS, Estes WA. Clinical potential of neurosteroids for CNS disorders. *Trends Pharmacol Sci*. 2016;37(7):543-561.

9. Craig CR. Anticonvulsant activity of steroids: separability of anticonvusant from hormonal effects. *J Pharmacol Exp Ther*. 1966;153(2):337-343.

10. Harrison NL, Simmonds MA. Modulation of the GABA receptor complex by a steroid anesthetic. *Brain Res*. 1984;323:287-292.

11. Callachan H, Cottrell GA, Hather NY, Lambert JJ, Nooney JM, Peters JA. Modulation of the $GABA_A$ receptor by progesterone metabolites. *Proc Roy Sci Lond*. 1987;231:359-369.

12. Gee KW, Chang WC, Brinton RE, McEwen BS. GABA-dependent modulation of the Cl- ionophore by steroids in rat brain. *Eur J Pharmacol*. 1987;136(3):419-423.

13. Gee WK, Bolger B, Brinton R, Coirini H, McEwen B. Steroid modulation of the cholride ionophore in rat brain: structure-activity requirements, regional dependence and mechanism of action. *J Pharmacol Exp Ther*. 1988;246(2).

14. Peters JA, Kirkness EF, Callachan H, Lambert JJ, Turner AJ. Modulation of the $GABA_A$ receptor by depressant barbiturates and pregnane steroids. *Br J Pharmacol*. 1988;94(4):1257-1269.

15. Olsen RW, Sieghart W. $GABA_A$ receptors: Subtypes provide diversity of function and pharmacology. *Neuropharmacology*. 2009;56(1):141-148.

16. Olsen RW, Sieghart W. International union of pharmacology. LXX Subtypes of γ-Aminobutyric Acid$_A$ receptors: Classification on the basis of subunit composition pharmacology, and function. Update. *Pharmacol Rev*. 2008;60(3):243-260.

17. Zhu WJ, Vicini S. Neurosteroid prolongs GABA$_A$ channel deactivation by altering kinetics of desensitized states. *J Neurosci*. 1997;17(11):4022-4031.

18. Akk G, Covey DF, Evers AS, Steinbach JH, Zorumski CF, Mennerick S. Mechanisms of neurosteroid interactions with GABA$_A$ receptors. *Pharmacol Ther*. 2007;116(1):35-57.

19. Hosie AM, Wilkins ME, da Silva HMA, Smart TG. Endogenous neurosteroids regulate GABA$_A$ receptors through two discrete transmembrane sites. *Nature*. 2006;444(7118):486-489.

20. Chisari M, Eisenman LN, Covey DF, Mennerick S, Zorumski CF. The sticky issue of neurosteroids and GABA$_A$ receptors. *Trends Neurosci*. 2010;33(7):299-306.

21. Purdy RH, Morrow AL, Moore PH, Paul SM. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc Natl Acad Sci U S A*. 1991;88(10):4553-4557.

22. Reddy DS. Physiological role of adrenal deoxycorticosterone-derived neuroactive steroids in stress-sensitive conditions. *Neuroscience*. 2006;138(3):911-920.

23. Do Rego JL, Seong JY, Burel D, Leprince J, Luu-The V, Tsutsui K, Tonon MC, Pelletier G, Vaudry H. Neurosteroid biosynthesis: Enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides. *Front Neuroendocrinol*. 2009;30(3):259-301.

24. Gunn BG, Cunningham L, Mitchell SJ, Swinny JD, Lambert JJ, Belelli D. $GABA_A$ receptor-acting neurosteroids: A role in the development and regulation of the stress response. *Front Neuroendocrinol*. 2015;36:28-48.

25. Melcangi RC, Mensah-Nyagan AG. Neurosteroids: measurement and pathophysiologic relevance. *Neurochem Int*. 2008;52(4-5):503-505.

26. Panzica GC, Melcangi RC. The endocrine nervous system: source and target for neuroactive steroids. *Brain Res Rev*. 2008;57(2):271-276.

27. Porcu P, Barron AM, Frye CA, Walf AA, Yang SY, He XY, Morrow AL, Panzica GC, Melcangi RC. Neurosteroidogenesis today: novel targets for neuroactive steroid synthesis and action and their relevance for translational research. *J Neuroendocrinol*. 2016;28(2):12351.

28. Vallée M. Structure-activity relationship studies on neuroactive steroids in memory, alcohol and stress-related functions: a crucial benefit from endogenous level analysis. *Psychopharmacology*. 2014;231:3243-3255.

29. Vallée M, Rivera JD, Koob GF, Purdy RH, Fitzgerald RL. Quantification of neurosteroids in rat plasma and brain following swim stress and allopregnanolone administration using negative chemical ionization gas chromatography/mass spectrometry. *Anal Biochem*. 2000;287(1):153-166.

30. Agis-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E, Guidotti A. Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. *Proc Natl Acad Sci*. 2006;103:14602-14607.

31. Agis-Balboa RC, Pinna G, Pibiri F, Kadriu B, Costa E, Guidotti A. Down-regulation of neurosteroid biosynthesis in corticolimbic circuits mediates social isolation-induced behavior in mice. *Proc Natl Acad Sci*. 2007;104(47):18736-18741.

32. Do Rego JL, Vaudry H. Comparative aspects of neurosteroidogenesis: from fish to mammals. *Gen Comp Endocrinol*. 2016;227:120-129.

33. Locci A, Pinna G. Neurosteroid biosynthesis downregulation and changes in GABA$_A$ receptor subunit composition: A biomarker axis in stress-induced cognitive and emotional impairment. *Br J Pharmacol*. 2017. DOI 10.1111/bph.13843.

34. Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacepere JJ, Lindemann P, Norenberg MD, Nutt D, Weizman A, Zhang MR, Gavish M. Translocator protein (18 kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol Sci*. 2006;27(8):402-409.

35. Papadopoulos V, Fan J, Zirkin B. Translocator protein (18 kDa): an update on its function in steroidogenesis. *J Neuroendocrinol*. July 2017. DOI 10.1111/jne.12500

36. Korosi A, Baram TZ. The pathways from mother's love to baby's future. *Front Behav Neurosci Neurosci*. 2009;3:1-8.

37. Schmidt MV. Molecular mechanisms of early life stress-Lessons from mouse models. *Neurosci Biobehav Rev*. 2010;34(6):845-852.

38. Atack JR. GABA$_A$ receptor subtype-selective modulators. I. α2/α3-selective agonists as non-sedating anxiolytics. *Curr Top Med Chem*. 2011;11(9):1176-1202.

39. Atack JR. GABA$_A$ receptor subtype-selective modulators. II. α5-selective inverse agonists for cognition enhancement. *Curr Top Med Chem*. 2011;11(9):1203-1214.

40. Crestani F, Rudolph U. *Behavioral Functions of GABA$_A$ Receptor Subtypes - The Zurich Experience*. Vol 72. 1st ed. Elsevier Inc.; 2015.

41. Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA$_A$ receptors. *Nat Rev Neurosci*. 2005;6(3):215-229.

42. Fritschy J-M, Panzanelli P. GABA$_A$ receptors and plasticity of inhibitory neurotransmission in the central nervous system. *Eur J Neurosci*. 2014;39(11):1845-1865.

43. Nakamura Y, Darnieder LM, Deeb TZ, Moss SJ. Regulation of GABA$_A$Rs by phosphorylation. *Adv Pharmacol*. 2015;72:97-146.

44. Jacob TC, Moss SJ, Jurd R. GABA$_A$ receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat Rev Neurosci*. 2008;9(5):331-343.

45. Herd MB, Brown AR, Lambert JJ, Belelli D. Extrasynaptic GABA$_A$ receptors couple presynaptic activity to postsynaptic inhibition in the somatosensory thalamus. *J Neurosci*. 2013;33(37):14850-14868.

46. Rovo Z, Matyas F, Bartho P, Slezia A, Lecci S, Pellegrini C, Astori S, David C, Hangya B, Luthi A, Acsady L. Phasic, nonsynaptic GABA$_A$ receptor-mediated inhibition entrains thalamocortical oscillations. *J Neurosci*. 2014;34(21):7137-7147.

47. Mesbah-Oskui L, Horner RL. Enhanced thalamic spillover inhibition during non-rapid-eye-movement sleep triggers an electrocortical signature of anesthetic hypnosis. *Anesthesiology*. 2016;125(5):964-978.

48. Belelli D, Harrison NL, Maguire J, Macdonald RL, Walker MC, Cope DW. Extrasynaptic GABA$_A$ receptors: Form, pharmacology, and function. *J Neurosci*. 2009;29(41):12757-12763.

49. Hausrat TJ, Muhia M, Gerrow K, Thomas P, Hirdes W, Tsukita S, Heisler FF, Herich L, Dubroqua S, Breiden P, Feldon J, Schwarz JR, Yee BK, Smart TG, Triller A, Kneussel M. Radixin regulates synaptic GABA$_A$ receptor density and is essential for reversal learning and short-term memory. *Nat*

Commun. 2015;6:6872.

50. Mody I, De Koninck Y, Otis TS, Soltesz I. Bridging the cleft at GABA synapses in the brain. *Trends Neurosci*. 1994;17(12):517-525.

51. Blaesse P, Schmidt T. K-Cl cotransporter KCC2—a moonlighting protein in excitatory and inhibitory synapse development and function. *Eur J Physiol*. 2015;467(4):615-624.

52. Ben-Ari Y. Is birth a critical period in the pathogenesis of autism spectrum disorders? *Nat Rev Neurosci*. 2015;16(8):498-505.

53. Zilberter Y. Commentary: GABA depolarizes immature neurons and inhibits network activity in the neonatal neocortex *in vivo*. *Front Pharmacol*. 2015;6(294):1-3.

54. Kirmse K, Kummer M, Kovalchuk Y, Witte OW, Garaschuk O, Holthoff K. GABA depolarizes immature neurons and inhibits network activity in the neonatal neocortex *in vivo*. *Nat Commun*. 2015;6:7750.

55. Sernagor E, Chabrol F, Bony G, Cancedda L. GABAergic conrol of neurite outgrowth and remodeling during development and adult neurogenesis: general rules and differences in diverse systems. *Front Cell Neurosci* 2010 14;4:11.

56. Ben-Ari Y. The GABA excitatory/inhibitory developmental sequence: A personal journey. *Neuroscience*. 2014;279:187-219.

57. Oh WC, Lutzu S, Castillo PE, Kwon H-B. *De novo* synaptogenesis induced by GABA in the developing mouse cortex. *Science*. 2016;353(6303):1037-1040.

58. Jacobson M. *Developmental Neurobiology*. 2nd ed. New York: Plenum Press; 1978.

59. Jones EG. *The Thalamus*. New York: Plenum Press; 1985.

60. Del Rio JA, Soriano E, Ferrer I. Development of GABA-immunoreactivity in the neocortex of the mouse. *J Comp Neurol*. 1992;326(4):501-526.

61. Laurie DJ, Wisden W, Seeburg PH. The distribution of thirteen $GABA_A$ receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. *J Neurosci*. 1992;12(11):4151-4172.

62. Luhmann HJ, Kirischuk S, Sinning A, Kilb W. Early GABAergic circuitry in the cerebral cortex. *Curr Opin Neurobiol*. 2014;26:72-78.

63. Okada M, Onodera K, Van Renterghem C, Sieghart W, Takahashi T. Functional correlation of $GABA_A$ receptor α subunits expression with the properties of IPSCs in the developing thalamus. *J Neurosci*. 2000;20(6):2202-2208.

64. Vicini S, Ferguson C, Prybylowski K, Kralic J, Morrow AL, Homanics GE. $GABA_A$ receptor α1 subunit deletion prevents developmental changes of inhibitory synaptic currents in cerebellar neurons. *J Neurosci*. 2001;21(9):3009-3016.

65. Jüttner R, Meier J, Grantyn R. Slow IPSC kinetics, low levels of α1 subunit expression and paired-pulse depression are distinct properties of neonatal inhibitory GABAergic synaptic connections in the mouse superior colliculus. *Eur J Neurosci*. 2001;13(11):2088-2098.

66. Goldstein PA. Prolongation of hippocampal miniature inhibitory postsynaptic currents in mice lacking the $GABA_A$ receptor α1 subunit. *J Neurophysiol*. 2002;88(6):3208-3217.

67. Bosman LW, Heinen K, Spijker S, Bussaard AB. Mice lacking the major adult $GABA_A$ receptor subtype have normal number of synapses, but retain juvenile IPSC kinetics until adulthood. *J Neurophysiol*. 2005;94(1):338-346.

68. Takahashi T. Postsynaptic receptor mechanisms underlying developmental speeding of synaptic transmission. *Neurosci Res*. 2005;53(3):229-240.

69. Deidda G, Bozarth IF, Cancedda L. Modulation of GABAergic transmission in development and neurodevelopmental disorders: investigating physiology and pathology to gain therapeutic perspectives. *Front Cell Neurosci*. 2014;8:1-23.

70. Lavoie AM, Tingey JJ, Harrison NL, Pritchett DB, Twyman RE. Activation and deactivation rates of recombinant $GABA_A$ receptor channels are dependent on alpha-subunit isoform. *Biophys J*. 1997;73(5):2518-2526.

71. Huntsman MM, Huguenard JR. Fast IPSCs in rat thalamic reticular nucleus require the $GABA_A$ receptor β1 subunit. *J Physiol*. 2006;572: 459-475

72. Ye Z, Yu X, Houston CM, Aboukhalil Z, Franks NP, Wisden W, Brickley SG. Fast and slow inhibition in the visual thalamus is influenced by allocating $GABA_A$ receptors with different ɣ subunits. *Front Cell Neurosci* 2017; 11:95.

73. Cannon J, McCarthy MM, Lee S, Lee J, Borgers C, Whittington MA, Kopell N. Neurosystems: Brain rhythms and cognitive processing. *Eur J Neurosci*. 2014;39(5):705-719.

74. Maris E, Fries P, van Ede F. Diverse phase relations among neuronal rhythms and their potential function. *Trends Neurosci*. 2016;39(2):86-99.

75. Peden DR, Petitjean CM, Herd MB, Durakoglugil MS, Rosahl TW, Wafford K, Homanics GE, Belelli D, Fritschy JM, Lambert JJ. Developmental maturation of synaptic and extrasynaptic $GABA_A$ receptors in mouse thalamic ventrobasal neurones. *J Physiol*. 2008;586(4):965-987.

76. Grobin AC, Morrow AL. 3α-hydroxy-5α-pregnan-20-one levels and $GABA_A$ receptor-mediated 36Cl(-) flux across development in rat cerebral cortex. *Brain Res Dev Brain Res*. 2001;131:31-39.

77. Brown AR, Herd MB, Belelli D, Lambert JJ. Developmentally regulated neurosteroid synthesis enhances GABAergic neurotransmission in mouse thalamocortical neurones. *J Physiol*. 2015;593(1):267-284.

78. Shu HJ, Eisenman LN, Jinadasa D, Covery DF, Zorumski C, Mennerick S. Slow actions of neurosteroids at $GABA_A$ receptors. *J Neurosci*. 2004;24(30):6667-6675.

79. Shu H-J, Zeng C-M, Wang C, Covey DF, Zorumski CF, Mennerick S. Cyclodextrins sequester neuroactive steroids and differentiate mechanisms that rate limit steroid actions. *Br J Pharmacol*. 2007;150(2):164-175.

80. Akk G, Shu H, Wang C, Steinbach JH, Zorumski CF, Covey DF, Mennerick S. Neurosteroid access to the $GABA_A$ receptor. *J Neurosci*. 2005;25(50):11605-11613.

81. Reddy DS, Gangisetty O, Wu X. PR-independent neurosteroid regulation of α2-$GABA_A$ receptors in the hippocampus subfields. *Brain Res*. 2017;1659:142-147.

82. Saalmann YB, Kirkcaldie MTK, Waldron S, Calford MB. Cellular distribution of the $GABA_A$ receptor-modulating 3α-hydroxy, 5α-reduced pregnane steroids in the adult rat brain. *J Neuroendocrinol*. 2007;19(4):272-284.

83. Forte N, Medrihan L, Cappetti B, Baldelli P, Benfenati F. 2-Deoxy-d-glucose enhances tonic inhibition through the neurosteroid-mediated activation of extrasynaptic $GABA_A$ receptors. *Epilepsia*. 2016;57(12):1987-2000.

84. Belelli D, Peden DR, Rosahl TW, Wafford KA, Lambert JJ. Extrasynaptic $GABA_A$ receptors of thalamocortical neurons: a molecular target for hypnotics. *J Neurosci*. 2005;25(50):11513-11520.

85. Belelli D, Casula A, Ling A, Lambert JJ. The influence of subunit composition on the interaction of neurosteroids with $GABA_A$ receptors. *Neuropharmacology*. 2002;43:651-661.

86. Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA. Pharmacological characterization of a novel cell line expressing human α4β3δ $GABA_A$ receptors. *Br J Pharmacol*. 2002;136(7):965-974.

87. Jevtovic-Todorovic V, Covey DF, Todorovic SM. Are neuroactive steroids promising therapeutic agents in the management of acute and chronic pain? *Psychoneuroendocrinology*. 2009;34:S178-S185.

88. van der Bourg A, Yang J-W, Reyes-Puerta V, Laurenczy B, Wieckhorst M, Stuttgen MC, Luhmann HJ, Helmchen F. Layer-Specific Refinement of Sensory Coding in Developing Mouse Barrel Cortex. *Cereb Cortex*. 2016:1-16.

89. Butt SJ, Stacey JA, Teramoto Y, Vagnoni C. A role for GABAergic interneuron diversity in circuit development and plasticity of the neonatal cerebral cortex. *Curr Opin Neurobiol*. 2017;43:149-155.

90. Brown AR, Mitchell SJ, Peden DR, Herd MB, Seifi M, Swinny JD, Belelli D, Lambert JJ. During postnatal development endogenous neurosteroids influence GABA-ergic neurotransmission of mouse cortical neurons. *Neuropharmacology*. 2016;103:163-173.

91. Tamamaki N, Yanagawa Y, Tomioka R, Miyazaki J-I, Obata K, Kaneko T. Green fluorescent protein expression and colocalization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse. *J Comp Neurol*. 2003;467(1):60-79.

92. Lee S, Hjerling-Leffler J, Zagha E, Fishell G, Rudy B. The largest group of superficial neocortical gabaergic interneurons expresses ionotropic serotonin receptors. *J Neurosci*. 2010;30(50):16796-16808.

93. Rudy B, Fishell G, Lee S, Hjerling-Leffler J. Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Dev Neurobiol*. 2011;71(1):45-61.

94. Tokuda K, Izumi Y, Zorumski CF. Ethanol enhances neurosteroidogenesis in hippocampal pyramidal neurons by paradoxical nmda receptor activation. *J Neurosci*. 2011;31(27):9905-9909.

95. Zorumski CF, Paul SM, Izumi Y, Covey DF, Mennerick S. Neurosteroids, stress and depression: potential therapeutic opportunities. *Neurosci Biobehav Rev*. 2013;37(1):109-122.

96. Owens DF, Kriegstein AR. Is there more to GABA than synaptic inhibition? *Nat Rev Neurosci*. 2002;3(9):715-727.

97. Cossart R. The maturation of cortical interneuron diversity: How multiple developmental journeys shape the emergence of proper network function. *Curr Opin Neurobiol*. 2011;21(1):160-168.

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

99. Mòdol L, Casas C, Llidó A, Navarro X, Pallarès M, Darbra S. Neonatal allopregnanolone or finasteride administration modifies hippocampal K+ Cl- co-transporter expression during early development in male rats. *J Steroid Biochem Mol Biol*. 2014;143:343-347.

100. Banke TG, McBain CJ. GABAergic input onto CA3 hippocampal interneurons remains shunting throughout development. *J Neurosci*. 2006;26(45):11720-11725.

101. Glickfeld LL, Roberts JD, Somogyi P, Scanziani M. Interneurons hyperpolarize pyramidal cells along their entire somatodendritic axis. *Nat Neurosci*. 2009;12(1):21-23.

102. Xiao-Jing Wang and György Buzsáki. Gamma oscillation by synaptic inhibition in a hippocampal interneuronal network model. *J Neurosci*. 1996;16(20):6402-6413.

103. Vida I, Bartos M, Jonas P. Shunting inhibition improves robustness of gamma oscillations in hippocampal interneuron networks by homogenizing firing rates. *Neuron*. 2006;49(1):107-117.

104. Doischer D, Hosp JA, Yanagawa Y, Obata K, Jonas P, Vida I, Bartos M. Postnatal differentiation of basket cells from slow to fast signaling devices. *J Neurosci*. 2008;28(48):12956-12968.

105. Wang JM, Johnston PN, Ball BG, Brinton RD. The neurosteroid allopregnanolone promotes proliferation of rodent and human neural progenitor cells and regulates cell-cycle gene and protein expression. *J Neurosci*. 2005;25(19):4706-4718.

106. Brinton RD. Neurosteroids as regenerative agents in the brain: therapeutic implications. *Nat Rev Endocrinol*. 2013;9(4):241-250.

107. Thomas P, Pang Y. Protective actions of progesterone in the cardiovascular system: potential role of membrane progesterone receptors (mPRs) in mediating rapid effects. *Steroids*. 2013;78(6):583-588.

108. Ben-Ari Y. Excitatory actions of GABA during development: the nature of the nurture. *Nat Rev Neurosci*. 2002;3(9):728-739.

109. Dehorter N, Vinay L, Hammond C, Ben-Ari Y. Timing of developmental sequences in different brain structures: physiological and pathological implications. *Eur J Neurosci*. 2012;35(12):1846-1856.

110. Luhmann HJ, Prince DA. Postnatal maturation of the GABAergic system in rat neocortex. *J Neurophysiol*. 1991;65(2):247-263.

111. Agmon A, Dowd DK. NMDA receptor-mediated currents are prominent in the thalamocortical synaptic response before maturation of inhibition. *J Neurophysiol*. 1992;68(1):345-349.

112. Sauer J-F, Bartos M. Recruitment of early postnatal parvalbumin-positive hippocampal interneurons by GABAergic excitation. *J Neurosci*. 2010;30(1):110-115.

113. Hoftman GD, Datta D, Lewis DA. Layer 3 excitatory and inhibitory circuitry in the prefrontal cortex: developmental trajectories and alterations in schizophrenia. *Biol Psychiatry*. 2017;81(10):862-873.

114. Tremblay R, Lee S, Rudy B. GABAergic interneurons in the neocortex: from cellular properties to circuits. *Neuron*. 2016;91(2):260-292.

115. Grobin AC, Heenan EJ, Lieberman JA, Morrow AL. Perinatal neurosteroid levels influence gabaergic interneuron localization in adult rat prefrontal cortex. *J Neurosci*. 2003;23(5):1832-1839.

116. Grobin AC, Gizerian S, Lieberman JA, Morrow AL. Perinatal allopregnanolone influences prefrontal cortex structure, connectivity and behavior in adult rats. *Neuroscience*. 2006;138(3):809-819.

117. Gizerian SS, Morrow AL, Lieberman JA, Grobin AC. Neonatal neurosteroid administration alters parvalbumin expression and neuron number in medial dorsal thalamus of adult rats. *Brain Res*. 2004;1012(1-2):66-74.

118. Grobin AC, Lieberman JA, Morrow AL. Perinatal flunitrazepam exposure causes persistent alteration of parvalbumin-immunoreactive interneuron

localization in rat prefrontal cortex. *Neurosci Lett*. 2004;359(1-2):9-1.

119. Marques-Smith A, Lyngholm D, Kaufmann AK, Stacey JA, Hoerder-Suabedissen A, Becker EB, Wilson MC, Molnar Z, Butt SJ. A transient translaminar GABAergic interneuron circuit connects thalamocortical recipient layers in neonatal somatosensory cortex. *Neuron*. 2016;89(3):536-549.

120. Paysan J, Kossel A, Bolz J, Fritschy JM. Area-specific regulation of gamma-aminobutyric acid type A receptor subtypes by thalamic afferents in developing rat neocortex. *PNAS*. 1997;94:6995-7000.

121. Pinna G. Targeting neurosteroidogenesis as therapy for PTSD. *Front Pharmacol*. 2014;4(166):1-5.

122. Pinna G, Rasmusson AM. Ganaxolone improves behavioral deficits in a mouse model of post-traumatic stress disorder. *Front Cell Neurosci*. 2014;8(256):1-11.

123. Schüle C, Eser D, Baghai TC, Nothdurfter C, Kessler JS, Rupprecht R. Neuroactive steroids in affective disorders: target for novel antidepressant or anxiolytic drugs? *Neuroscience*. 2011;191:55-77.

124. Scioli-Salter ER, Forman DE, Otis JD, Gregor K, Valovski I, Rasmusson AM. The shared neuroanatomy and neurobiology of comorbid chronic pain and PTSD. *Clin J Pain*. 2015;31(4):363-374.

125. Kanes SJ, Colquhoun H, Doherty J, Raines S, Hoffmann E, Rubinow DR, Meltzer-Brody S. Open-label, proof-of-concept study of brexanolone in the treatment of severe postpartum depression. *Hum Psychopharmacol*. 2017;32(2):2-7.

126. Kessler RC, Amminger GP, Aguilar-Gaxiola S, Alonso J, Lee S, Ustun TB. Age of onset of mental disorders: a review of recent literature. *Curr Opin Psychiatry*. 2007;20(4):359-364.

127. de Girolamo G, Dagani J, Purcell R, Cocchi A, McGorry PD. Age of onset of mental disorders and use of mental health services: needs, opportunities and obstacles. *Epidemiol Psychiatr Sci*. 2012;21(1):47-57.

128. Baram TZ, Solodkin A, Davis EP, Stern H, Obenaus A, Sandman CA, Small SL. Fragmentation and unpredictability of early-life experience in mental disorders. *Am J Psychiatry*. 2012;169(9):907-915.

129. Franklin TB, Saab BJ, Mansuy IM. Neural mechanisms of stress resilience and vulnerability. *Neuron*. 2012;75(5):747-761.

130. Teicher MH, Samson JA, Anderson CM, Ohashi K. The effects of childhood maltreatment on brain structure, function and connectivity. *Nat Rev Neurosci*. 2016;17(10):652-666.

131. Jones NC, O'Brien TJ, Carmant L. Interaction between sex and early-life stress: Influence on epileptogenesis and epilepsy comorbidities. *Neurobiol Dis*. 2014;72:233-241.

132. Heim C, Binder EB. Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions, and epigenetics. *Exp Neurol*. 2012;233(1):102-111.

133. Patchev VK, Montkowski A, Rouskova D, Koranyi L, Holsboer F, Almeida OFX. Neonatal treatment of rats with the neuroactive steroid tetrahydrodeoxycorticosterone (THDOC) abolishes the behavorial and neuroendocrine consequences of adverse early life events. *J Clin Invest*. 1997;99(5):962-966.

134. Ivy AS, Brunson KL, Sandman C, Baram TZ. Dysfunctional nurturing behavior in rat dams with limited access to nesting material: a clinically relevant model for early-life stress. *Neuroscience*. 2008;154(3):1132-1142.

135. Rice CJ, Sandman CA, Lenjavi MR, Baram TZ. A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology*. 2008;149(10):4892-4900.

136. Gunn BG, Cunningham L, Cooper MA, Corteen NL, Seifi M, Swinny JD, Lambert JJ, Belelli D. Dysfunctional astrocytic and synaptic regulation of hypothalamic glutamatergic transmission in a mouse model of early-life adversity: relevance to neurosteroids and programming of the stress response. *J Neurosci*. 2013;33(50):19534-19554.

137. Francis TC, Lobo MK. Emerging role for nucleus accumbens medium spiny neuron subtypes in depression. *Biol Psychiatry*. 2017;81(8):645-653.

138. Volkow ND, Morales M. The brain on drugs: from reward to addiction. *Cell*. 2015;162(4):712-725.

139. Lobo MK, Nestler EJ. The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons. *Front Neuroanat*. 2011;5(41):1-11.

140. Stephens DN, King SL, Lambert JJ, Belelli D, Duka T. $GABA_A$ receptor subtype involvement in addictive behaviour. *Genes, Brain Behav*. 2017;16(1):149-184.

141. Neuchterlein KH, Dawson ME, Ventura J, Miklowitz D, Konishi G. Information-processing anomalies in the early course of schizophrenia and bipolar disorder. *Schizophr Res*. 1991;5(3):195-196.

142. Walker E, Mittal V, Tessner K. Stress and the hypothalamic pituitary adrenal axis in the developmental course of schizophrenia. *Annu Rev Clin Psychol*. 2008;4:189-216.

143. Bahari-Javan S, Varbanov H, Halder R, Benito E, Kaurani L, Burkhardt S, Anderson-Schmidt H, Anghelescu I, Budde M, Stilling RM, Costa J, Medina

J, Dietrich DE, Figge C, Folkerts H, Gade K, Heilbronner U, Stockel J, Thiel A, Hagen MV, Zimmermann J, Zitzelsberger A, Schulz S, Schmitt A, Delalle I, Falkai P, Schulze TG, Dityatev A, Sananbenesi F, Fischer A. HDAC1 links early life stress to schizophrenia-like phenotypes. *Proc Natl Acad Sci*. 2017:E4686-E4694.

144. Bicikova M, Hill M, Ripova D, Mohr P, Hampl R. Determination of steroid metabolome as a possible tool for laboratory diagnosis of schizophrenia. *J Steroid Biochem Mol Biol*. 2013;133(1):77-83.

145. Frau R, Mosher LJ, Bini V, Pillolla G, Pes R, Saba P, Fanni S, Devoto P, Bortolato M. The neurosteroidogenic enzyme 5α-reductase modulates the role of D1 dopamine receptors in rat sensorimotor gating. *Psychoneuroendocrinology*. 2016;63:59-67.

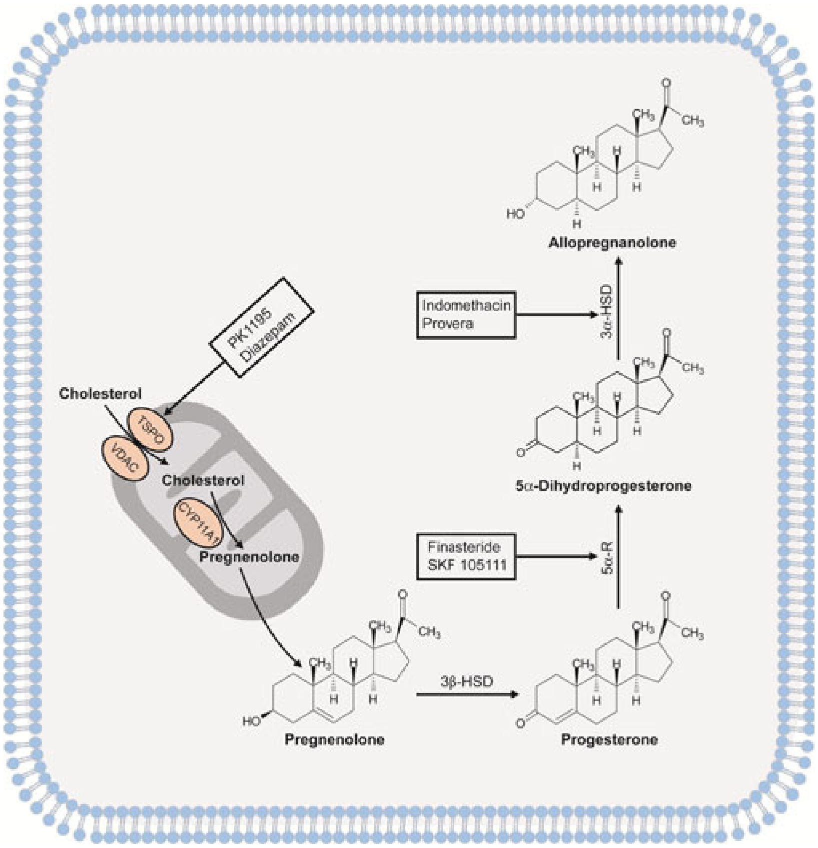
Figure Legends:

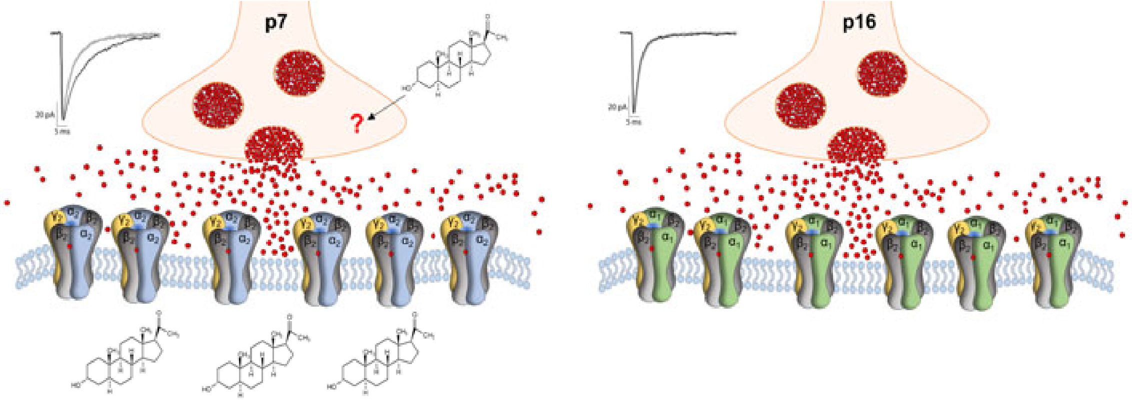
Figure 1: The schematic illustrates the d*e novo* neurosteroid synthesis. Initially cholesterol is translocated across the mitochondrial membrane by TSPO and accessory proteins (*e.g.* VDAC)[34,35]. The mitochondrial P450scc (CYP11A1) converts cholesterol to pregnenolone, which diffuses into the cytosol. Cytosolic pregnenolone is subsequently converted to progesterone by 3β-HSD. Progesterone, either newly synthesised locally, or of peripheral origin, is reduced to 5α-dihydroprogesterone (5α-DHP) and then to allopregnanolone (5α-pregnan-3α-ol-20-one, 5α3α) by the enzymes 5α-reductase (5α-R) and 3α-hydroxysteroid-dehydrogenase (3α-HSD) respectively. Pharmacological agents such as finasteride and indomethacin inhibit the enzymes 5α-R and 3α-HSD respectively. The agent γ-CD can be employed to sequester the active neurosteroid. Although agents such as ethanol and certain antidepressants such as fluoxetine are known to enhance the levels of $GABA_A$R-active neurosteroids, their specific site(s) of action are not known. TSPO; translocator protein, VDAC; the 32-kDa voltage-dependent anion channel (required for benzodiazepine binding); 5α-R; 5α-reductase, 3α-HSD; 3α-hydroxysteroid dehydrogenase.

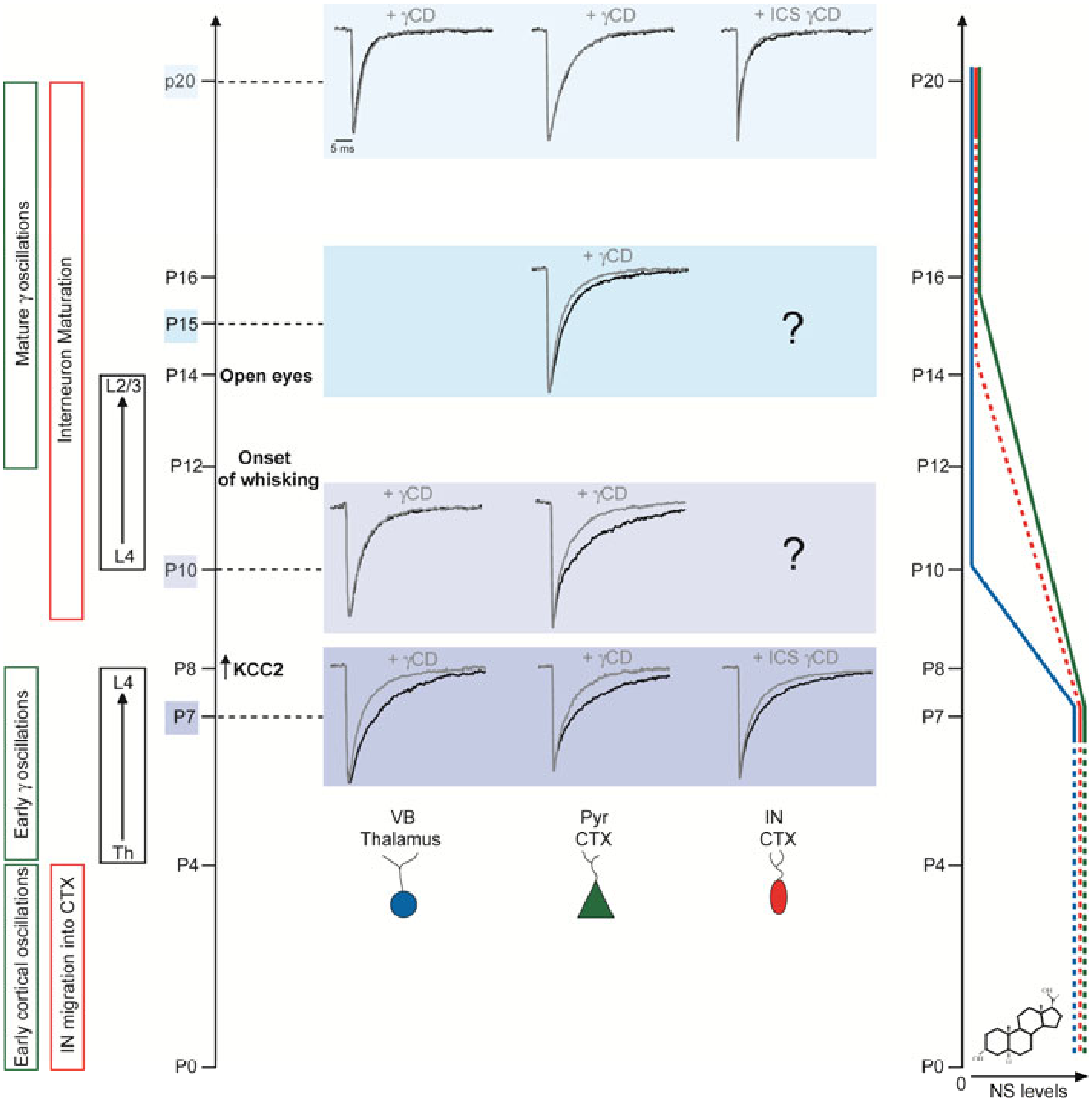
Figure 2: Illustrated is a diagrammatic representation of the changing temporal influence of an endogenous neurosteroid tone and the $GABA_A$R subunit composition upon the decay of mIPSCs recorded from VB neuron during development. During early developmental stages [postnatal day (P) 5-P14], the mIPSCs are exclusively mediated by α2-$GABA_A$R (blue). By P16, the α2 subunit is fully replaced by the α1 subunit (green). During this developmental window, mIPSCs recorded from these neurons transition from slow to fast decaying phasic events, a trait often associated with a change in the receptor subunit composition. However, the developing α2-$GABA_A$R containing VB synapses exhibit a significant endogenous neurosteroid tone that profoundly influences GABAergic neurotransmission, as indicated by the ability of γ-CD (grey lines) to shorten the mIPSC decay time course *c.f.* control (black line) up to P10. Importantly, this kinetic change precedes the switch from α2-$GABA_A$Rs to α1-$GABA_A$Rs. Thus, dissipation of an endogenous neurosteroid tone represents an alternative physiological mechanism to impart a rapid kinetic profile to GABAergic inhibitory signals. The steroid icon indicates 1) a postsynaptic action to enhance the actions of GABA interacting with $GABA_A$Rs and 2) a presynaptic effect of the neurosteroid to suppress the quantal release of GABA mediated

by an unknown target. Note that mature α1-$GABA_AR$ containing synapses lack an endogenous neurosteroid tone.

Figure 3: A schematic representation illustrating the relationship between the development of cortical (layers 2 & 3) and the somatosensory thalamic (VB complex) connectivity and endogenous neurosteroid levels between P0 and P24. Left Y axis: A diagrammatic representation of the temporal profile of the development of cortical circuits between postnatal day (P) 0 – P24 describing: the oscillatory capacity of the network (green box), interneuron maturation (red box) and maturation of cortical neuronal projections for layers (L) 2,3 and 4 (black box). The Centre Panel illustrates the influence of endogenous neurosteroid tone upon $GABA_AR$-mediated phasic inhibition between P0 and P20-24, for thalamic VB neurons (blue), L2/3 cortical pyramidal neurons (green) and interneurons (red) suggesting a physiologically relevant role for endogenous neurosteroids in the processes of developmental maturation: representative normalised mIPSCs recorded from VB neurons (blue), L2/3 cortical pyramidal neurons (green) and interneurons (red) in the presence and absence of γ-CD (grey lines) during discrete temporal windows, namely P7, P10, P15 and P20-24. The presence of an endogenous neurosteroid tone is revealed by the effect of γ-CD (grey lines) on the decay time course (*i.e*. shortening) of the mIPSC (control black lines). This effect of γ-CD is evident at ≤ P9 for VB neurons and ≤ P15 for cortical pyramidal neurons. An endogenous neurosteroid tone is additionaly revealed by γ-CD in cortical interneurons at P7, but it dissipates by P20-24. The endogenous neurosteroid tone profile between P7 and P20-24 for these interneurons remains to be determined. The Right Y axis illustrates developmental changes to neurosteroid levels (arbitrary units and deduced by their impact on $GABA_AR$ phasic transmission) for VB neurons (blue lines), cortical neurons (green lines) and cortical interneurons (red lines). Dotted lines indicate that we do not have equivalent data on neurosteroid levels within these temporal windows. Note an arbitrary neurosteroid level of 0 here is used to infer a level of neurosteroid that is insufficient to influence phasic inhibition.


Allopregnanolone
5α-Dihydroprogesterone
Indomethacin
Provera
3α-HSD
PK1195
Diazepam
Cholesterol
TSPO
VDAC
Cholesterol
CYP11A1
Pregnenolone
Finasteride
SKF 105111
5α-R
3β-HSD
Pregnenolone
Progesterone
CH3
HO
O
H


p7
20 pA
5 ms
?
p16
20 pA
5 ms
γ2
α2
β2
α1


Mature γ oscillations
Interneuron Maturation
Early γ oscillations
Early cortical oscillations
IN migration into CTX
L2/3
L4
L4
Th
p20
P16
P15
P14
Open eyes
P12
Onset of whisking
P10
P8
↑KCC2
P7
P4
P0
+ γCD
+ γCD
+ ICS γCD
5 ms
+ γCD
?
+ γCD
+ γCD
?
+ γCD
+ γCD
+ ICS γCD
VB Thalamus
Pyr CTX
IN CTX
P20
P16
P14
P12
P10
P8
P7
P4
P0
0
NS levels