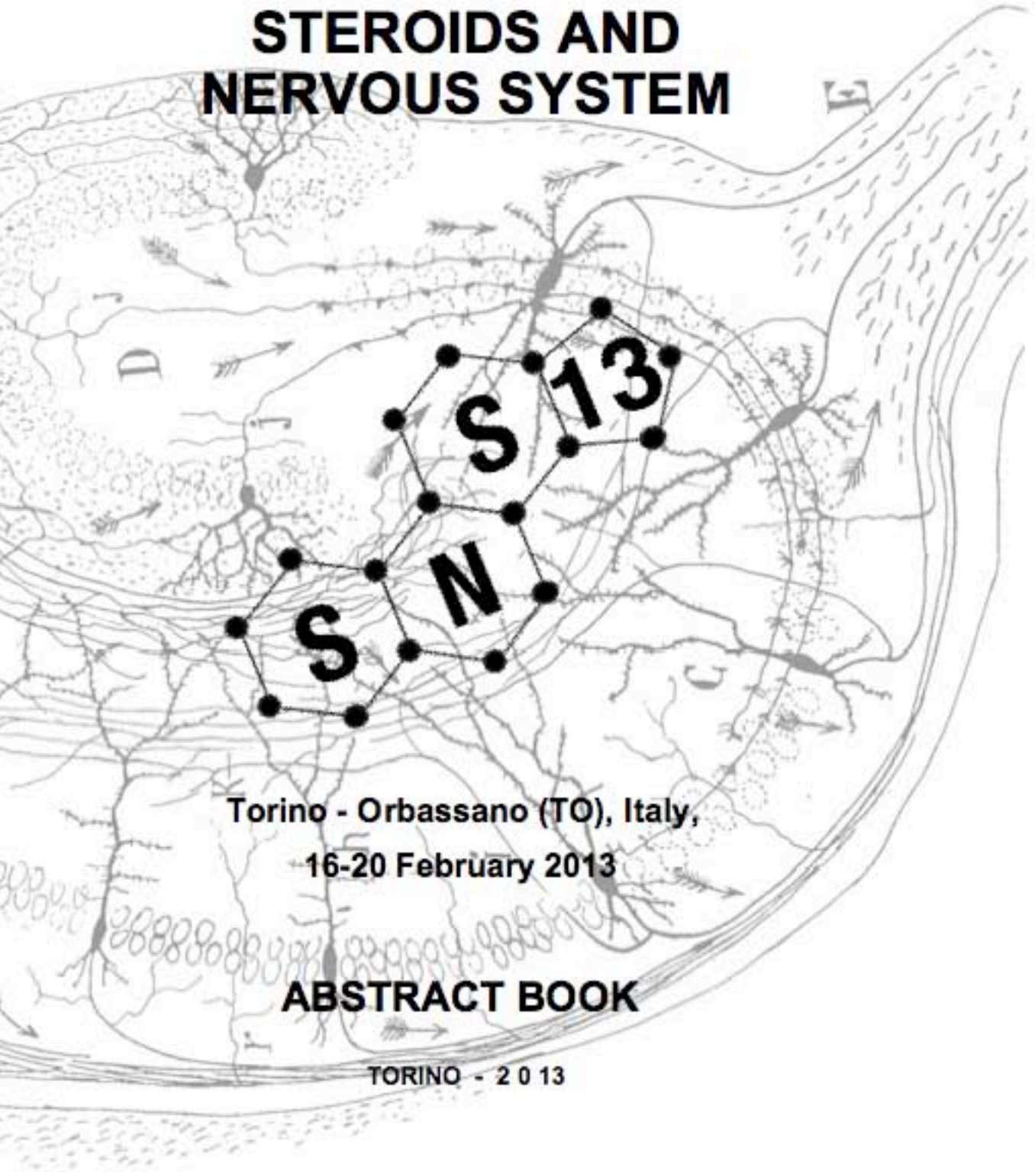
7th International Meeting

STEROIDS AND NERVOUS SYSTEM

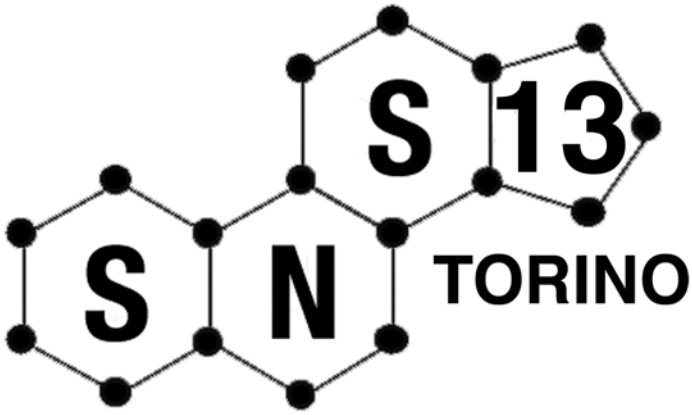


Torino - Orbassano (TO), Italy,

16-20 February 2013

ABSTRACT BOOK

TORINO - 2013



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STEROIDS AND NERVOUS SYSTEM

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February 16-20, 2013

ABSTRACTS OF INVITED LECTURES AND FREE CONTRIBUTIONS

G.C. Panzica and S. Gotti, editors

TORINO – 2013

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SATURDAY, 16th February 2013
09.30 – 16.30

Satellite Symposium
Allopregnanolone: state of art

(Organizers: Brinton R.D., Melcangi R.C., Panzica G.C.)

Satellite Symposium
Allopregnanolone: state of art

(Organizers: Brinton R.D., Melcangi R.C., Panzica G.C.)

- **Melcangi R.C.,** Giatti S., Calabrese D., Mitro N., Viviani B., Garcia-Segura L.M., Caruso D. **(Italy)** *Neuroactive steroid levels in physiological and pathological status: focus on progesterone metabolites*

- **Lambert J.J.,** Brown A.R., Gunn B.G., Belelli D. **(UK)** *Neurosteroids: local modulators of neuronal $GABA_A$ receptors*

- **Nothdurfter C.,** Schüle C., Rupprecht R. **(Germany)** *The role of allopregnanolone in depression and anxiety*

- Darbra S., Mòdol L., Llidó A., Casas K., Navarro X., Vallée M., **Pallarès M. (Spain)** *Neonatal allopregnanolone levels alteration: effects on behaviour and role of the hippocampus*

- **Brinton R.D. (USA)** *Allopregnanolone as a regenerative therapeutic for Alzheimer's disease: current status and future promise*

- Patte-Mensah C., Meyer L., Taleb O**., Mensah-Nyagan A.G. (France)** *Therapeutic potential of neurosteroid allopregnanolone for the treatment of neuropathic pain*

- **Hirst J.,** Bennett G.A., Kelleher M.A., Walker D.W., Palliser H.K. **(Australia)** *Neurosteroid disruption leads to adverse outcomes following compromised pregnancy and premature birth*

- **Brunton P.J.,** Donadio M.V., Yao S.T., GreenwoodM., Paris J.J., Frye C.A., Murphy D., Russell J.A. **(UK)** *Sex-dependent overwriting of prenatally programmed stress responses with neuroactive steroids*

- **Bäckström T.,** Nyberg S., Strömberg J., Ragagnin G., Timby E., vanWingen G., Ossewaarde L., Bixo M., Savic I. **(Swedeen)** *Allopregnanolone and mood disorders*

- **Schumacher M.,** Liu A., Liere P., Sitruk-Ware R., Ghoumari A., Guennoun R. **(France)** *Allopregnanolone and progesterone receptors: their respective significance in myelination and neuron viability*

NEUROACTIVE STEROID LEVELS IN PHYSIOLOGICAL AND PATHOLOGICAL STATUS: FOCUS ON PROGESTERONE METABOLITES

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Neuroactive steroid family includes steroid hormones produced in peripheral glands and steroids directly synthesized in the nervous system (i.e., neurosteroids). These molecules act as important physiological regulators of nervous function, affecting mood, behavior, reproduction and cognition, as well as acting like protective agents in models of injury, neurodegenerative diseases and psychiatric disorders [9-13,15]. As it will be reported, observations obtained by liquid chromatography-tandem mass spectrometry show that neuroactive steroid levels in central and peripheral nervous system are sex dimorphic and are influenced by hormonal environment [4]. Data obtained in experimental models of Alzheimer's disease, Parkinson's disease, multiple sclerosis, peripheral neuropathy, psychiatric disorders etc. have shown that pathological conditions also alter the levels of neuroactive steroids and in some cases these changes occur in a sex-dimorphic way [2,3,7,8,10]. In particular, changes on the levels of progesterone (PROG) and its metabolites are very interesting. In this context it is important to recall that PROG is converted into dihydroprogesterone (DHP) and subsequently into allopregnanolone, also known as tetrahydroprogesterone (THP) or into isopregnanolone [9]. PROG metabolism has a deep impact in the mechanism of action of this neuroactive steroid. Indeed, while DHP, like PROG, is still able to interact with the PROG receptor [9], THP is a potent ligand of GABA-A receptor [9]. Isopregnanolone does not bind directly to the GABA-A receptor [1] but it antagonizes the effect of THP on this neurotransmitter receptor [14]. PROG metabolites and particularly THP have been demonstrated in several experimental models to exert important neuroprotective effects [5,6,9-15]. Thus, PROG metabolites may have an important diagnostic and therapeutic perspective in the field of neurodegenerative and psychiatric disorders.

Reference list

[1] Bitran D, Hilvers RJ, Kellogg CK. Anxiolytic effects of 3 alpha-hydroxy-5 alpha[beta]-pregnan-20-one: endogenous metabolites of progesterone that are active at the GABAA receptor. Brain Res. 561:157-161, 1991.
[2] Caruso D, Barron AM, Brown MA, Abbiati F, Carrero P, Pike CJ, Garcia-Segura LM, Melcangi RC. Age-related changes in neuroactive steroid levels in 3xTg-AD mice. Neurobiol Aging. 2012. doi: 10.1016/j.neurobiolaging.2012.10.007.
[3] Caruso D, D'Intino G, Giatti S, Maschi O, Pesaresi M, Calabrese D, Garcia-Segura LM, Calza L, Melcangi RC. Sex-dimorphic changes in neuroactive steroid levels after chronic experimental autoimmune encephalomyelitis. J Neurochem. 114:921-932, 2010.
[4] Caruso D, Pesaresi M, Maschi O, Giatti S, Garcia-Segura LM, Melcangi RC. Effects of Short- and Long-Term Gonadectomy on Neuroactive Steroid Levels in the Central and Peripheral Nervous System of Male and Female Rats. J Neuroendocrinol. 22:1137-1147, 2010.
[5] Giatti S, Boraso M, Melcangi RC, Viviani B. Neuroactive steroids, their metabolites and neuroinflammation. J Mol Endocrinol. 49:R125-134, 2012.
[6] Giatti S., Caruso D., Boraso M., Abbiati F., Ballarini E., Calabrese D., Pesaresi M., Rigolio R., Santos-Galindo M., Viviani B., Cavaletti G., Garcia-Segura L.M., Melcangi R.C. Neuroprotective effects of progesterone in chronic experimental autoimmune encephalomyelitis. J. Neuroendocrinol. 24: 851-861, 2012,
[7] Giatti S, D'Intino G, Maschi O, Pesaresi M, Garcia-Segura LM, Calza L, Caruso D, Melcangi RC. Acute experimental autoimmune encephalomyelitis induces sex dimorphic changes in neuroactive steroid levels. Neurochem Int. 56:118-127, 2010.

[8] Melcangi RC, Caruso D, Levandis G, Abbiati F, Armentero MT, Blandini F. Modifications of Neuroactive Steroid Levels in an Experimental Model of Nigrostriatal Degeneration: Potential Relevance to the Pathophysiology of Parkinson's Disease. J Mol Neurosci. 46:177-183, 2012.
[9] Melcangi RC, Garcia-Segura LM, Mensah-Nyagan AG. Neuroactive steroids: state of the art and new perspectives. Cell Mol Life Sci. 65:777-797, 2008.
[10] Melcangi RC, Garcia-Segura LM. Sex-specific therapeutic strategies based on neuroactive steroids: In search for innovative tools for neuroprotection. Horm Behav. 57:2-11, 2010.
[11] Melcangi R.C., Giatti S., Pesaresi M., Calabrese D., Mitro N., Caruso D., Garcia-Segura L.M. Role of neuroactive steroids in the peripheral nervous system. Frontiers in Endocrinology, 2: 104, 2011.
[12] Panzica GC, Balthazart J, Frye CA, Garcia-Segura LM, Herbison AE, Mensah-Nyagan AG, McCarthy MM, Melcangi RC. Milestones on Steroids and the Nervous System: 10 years of basic and translational research. J Neuroendocrinol. 24:1-15, 2012.
[13] Schumacher M, Hussain R, Gago N, Oudinet JP, Mattern C, Ghoumari AM. Progesterone synthesis in the nervous system: implications for myelination and myelin repair. Front Neurosci. 6:10, 2012.
[14] Wang M, He Y, Eisenman LN, Fields C, Zeng CM, Mathews J, Benz A, Fu T, Zorumski E, Steinbach JH, Covey DF, Zorumski CF, Mennerick S3beta -hydroxypregnane steroids are pregnenolone sulfate-like GABA(A) receptor antagonists. J Neurosci. 22:3366-3375, 2002.
[15] Zorumski CF, Paul SM, Izumi Y, Covey DF, Mennerick S. Neurosteroids, stress and depression: Potential therapeutic opportunities. Neurosci Biobehav Rev. 37:109-122, 2013.

NEUROSTEROIDS: LOCAL MODULATORS OF NEURONAL $GABA_A$ RECEPTORS

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$GABA_A$ receptors mediate the majority of "fast" synaptic inhibition in the mammalian brain and are the target of clinically important drugs used in the treatment of a variety of medical conditions including: anxiety, sleep disorders and epilepsy. Furthermore, such drugs may be used as analgesics, to induce sedation and a state of general anaesthesia. The discovery, approximately a quarter of a century ago, that certain naturally occurring pregnane steroids such as allopregnanolone can potently and specifically enhance $GABA_A$ receptor function, raised the prospect that they might act as endogenous modulators to influence the activity of the brain's major inhibitory receptor. In common with other positive allosteric modulators of the $GABA_A$ receptor these steroids exhibit anxiolytic, analgesic, anticonvulsant, sedative and hypnotic properties. Such steroids were initially perceived to act as remote endocrine messengers. However, the subsequent discovery that the brain and spinal cord can synthesise such "neurosteroids" raised the prospect that they might additionally act nearby. Here evidence will be presented that certain neurons within the cortex, and thalamus can synthesise these GABA-active steroids and hence act as autocrine messengers to locally to "fine tune" neuronal inhibition. Neuronal neurosteroid synthesis appears to be particularly prevalent during early postnatal development, but also occurs in mature neurons. Future studies highlighting what triggers neurosteroid synthesis are required to better appreciate the physiological and pathophysiological role of these endogenous $GABA_A$ receptor modulators.

Referente list

Belelli and Lambert (2005). Neurosteroids: endogenous regulators of the $GABA_A$ receptor. *Nature Reviews Neuroscience* 6**,** 565-575.

THE ROLE OF ALLOPREGNANOLONE IN DEPRESSION AND ANXIETY

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The treatment of depression and anxiety disorders, which are very frequent and highly disabling, is still a challenge. Benzodiazepines have a rapid onset of action. However, they exert sedation and may induce tolerance, abuse liability and withdrawal symptoms. Antidepressants as long-term treatment show a more favorable side effect profile, but their onset of action is delayed. It is obvious that there is a need for novel pharmacological approaches. Considerable evidence has emerged that neuroactive steroids do not only affect the regulation of gene expression but may also alter neuronal excitability through interaction with specific neurotransmitter receptors. In particular, 3α-reduced neuroactive steroids such as allopregnanolone have been shown to act as positive allosteric modulators of the $GABA_A$ receptor, thereby playing an important role in the pathophysiology of depression and anxiety [5]. During depression, the concentration of allopregnanolone is decreased in plasma and cerebrospinal fluid. Furthermore, low levels of allopregnanolone are supposed to be associated with anxiety-like behavior. Antidepressant drugs such as selective serotonin reuptake inhibitors (SSRIs) have an impact on key enzymes of neurosteroid synthesis and have been shown to normalize the dysequilibrium of neuroactive steroids in depression [3]. Moreover, allopregnanolone itself has been shown to possess antidepressant- and anxiolytic-like effects. These effects appear to be brain region specific, in particular allopregnanolone levels in the hippocampus, the amygdala, the olfactory bulb and the medial prefrontal cortex seem to be involved in the regulation of anxiety and depression related behavioral dysfunction [2]. Furthermore, allopregnanolone is stress responsive and plays a major role in regulating hypothalamic-pituitary-adrenal (HPA) axis function. Reduced allopregnanolone levels following chronic stress have been shown to cause HPA hyperactivity [1]. As a target for the promotion of neurosteroidogenesis, the translacator protein (18 kDa) (TSPO) is a promising candidate for novel drugs in the treatment of depression and anxiety disorders [4]. TSPO ligands may promote the synthesis of neuroactive steroids via induction of cholesterol translocation to the inner mitochondrial membrane, thereby leading to an increased availability of neurosteroids in the brain.

Reference list

1. Evans J, Sun Y, McGregor A, Connor B. Allopregnanolone regulates neurogenesis and depressive/anxiety-like behaviour in a social isolation rodent model of chronic stress. Neuropharmacology. 2012; 63: 1315-1326.
2. Nelson M, Pinna G. S-norfluoxetine microinfused into the basolateral amygdala increases allopregnanolone levels and reduces aggression in socially isolated mice. Neuropharmacology. 2011; 60: 1154-1159.
3. Pinna G. In a mouse model relevant for post-traumatic stress disorder, selective brain steroidogenic stimulants (SBSS) improve behavioral deficits by normalizing allopregnanolone biosynthesis. Behav Pharmacol. 2010; 21: 438-450.
4. Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, Groyer G, Adams D, Schumacher M. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. Nat Rev Drug Discov. 2010; 9: 971-988.
5. 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.**

NEONATAL ALLOPREGNANOLONE LEVELS ALTERATION: EFFECTS ON BEHAVIOUR AND THE ROLE OF THE HIPPOCAMPUS

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Previous works have shown the importance of endogenous levels of the neurosteroid allopregnanolone (AlloP) during development for the adolescent and adult behaviour and for the nervous system maturation. Neonatal manipulation of AlloP levels, by means of systemic administration of AlloP or its synthesis inhibitor Finasteride (Fin), alters adolescent and adult behaviours. For instance, AlloP administration at postnatal day 5 (PN5) increases novelty-exploration in the open field and decreases the anxiolytic-like profile of the benzodiazepine lorazepam in the elevated plus-maze test (EPM) in adult male rats [1]. Also, AlloP administration from PN5 to PN9 deteriorates prepulse inhibition (PPI) [2], which indicate sensory gating alterations, and induces anxiolytic-like profile in the EPM in adult age [3]. Instead, neonatal Fin administration (from PN5 to PN9) increases emotional reactivity in situations of stress or conflict in the adolescent age, as reflected by the reduction in exploration of a novelty situation (decrease in novelty-directed activity and head-dips exploration) [2].

The alteration of the physiological levels of neurosteroids in early neonatal phases provokes alterations in the maturation of cerebral structures such as the hippocampus [4], the GABAergic thalamic-cortical system [5] or the meso-striatal dopaminergic systems [6]. AlloP has important modulatory effects in the hippocampus during the postnatal period where the adult pattern of inhibitory transmission is being established (PN9-PN12) [4]. Changes in AlloP biosynthesis during development and in early neonatal period could affect GABAA receptor subunit expression in the hippocampus. Thus, we hypothesized that neonatal AlloP levels would be involved on the maturation of inhibitory hippocampal system and related behavioural functions, i.e. exposure to a novel environment, emotional processing and sensorial gating. Consequently, the behavioural response to intrahippocampal neurosteroids administration would be different depending on neonatal AlloP levels.

Our results indicate that the anxiolytic-like profile of an intrahipocampal AlloP administration [7] is not present in those animals that were neonatally injected with AlloP of Fin [8]. The analysis of locomotor activity recorded in the open field (20min) also shows that the sedative effects of an intrahippocampal AlloP infusion, reflected as a decrease in locomotor activity, is not seen in those rats that were neonatally treated with AlloP [9]. Moreover, we have studied the passive avoidance learning, an aversive learning with important emotional component that is, in part, hippocampus-dependent [7]. In this paradigm, intrahippocampal infusions of pregnenolone sulphate (a GABAA positive modulator with promnesic profile) can produce an impairment of passive avoidance in situations of high environmental stress [10], but this effect is not present in the animals neonatally injected with Fin [11]. Regarding sensory gating, the facilitation of PPI induced by an intrahippocampal administration of AlloP in the adult age [12] is not present in the animals that were neonatally injected with AlloP or Fin [13]. Globally, these results indicate that behavioural responses to neurochemical modulation of the hippocampus are

altered depending on neonatal AlloP levels manipulation, suggesting that neonatal alteration of AlloP levels alters the normal function of the hippocampus.
At a molecular level, AlloP positively modulates GABAA receptors, being alpha4 and delta GABAA receptor subunits especially sensitive to the AlloP fluctuating levels [14]. Recent experiments from our lab indicate that neonatal Fin increases alpha4 and delta GABAA receptor subunits expression in the hippocampus during early development (from P6 to P15) by the alteration of AlloP and pregnenolone levels. Fin administration also anticipates the natural expression peaks (observed in no treated animals) from PN12 to PN10 in alpha4 and from PN12 to PN9 in delta [15]. Importantly, we have also shown that the increase in alpha4 and delta GABAA receptor subunits expression is maintained in the adult age in neonatal Fin-treated animals, and it could be related to some of the behavioural effects observed in previous experiments. Instead, neonatal AlloP administration decreases the neonatal expression of delta subunits [15], effect that seems related to a pharmacological down-regulation, as the increase of hippocampal AlloP levels after systemic AlloP administration is very high in relation to controls [8,13].
Taken together, and without excluding other brain structures, our results point out the important role of neonatal AlloP levels for the hippocampus maturation and for behavioural responses to environmental stress, emotional processing and sensorial gating. Our results also highlight the importance of neonatal AlloP levels and its impact on GABAA receptor expression that can lead to an adult altered system that responds different to environmental cues.

Reference list

[1] Darbra S, Pallarès M (2009) Neonatal allopregnanolone increases novelty-directed locomotion and disrups behavioural responses to GABAA receptor modulators in adulthood. Int.J.Devl.Neuroscience 27:617-625
[2] Darbra S, Pallarès M (2010) Alterations in neonatal neurosteroids affect exploration during adolescence and prepulse inhibition in adulthood. Psychoneuroendocrinology 35(4):525-535
[3] Darbra S, Pallarès M (2012) Effects of early postnatal allopregnanolone administration on elevated plus-maze anxiety scores in adult male Wistar rats. Neuropsychobiology 65(1):20-27
[4] Cooper EJ, Johnston GA, Edwards FA (1999) Effects of a naturally occurring neurosteroid on GABAA IPSCs during development in rat hippocampal or cerebellar slices. J.Physiol. 52:437-49
[5] Grobin AC, Heenan E J, Lieberman JA, Morrow AL (2003) Perinatal neurosteroid levels influence GABAergic interneuron localization in adult rat prefrontal cortex. J.Neurosci. 23:1832–1839
[6] Muneoka KT, Takigawa M (2002) A neuractive steroid, pregnenolone, alters the striatal dopaminergic tone before and after puberty. Neuroendocrinology 75:288-295
[7] Mòdol L, Darbra S, Pallarès M (2011) Neurosteroids infusion into the CA1 hippocampal region on exploration, anxiety-like behaviour and aversive learning. Behav. Brain Res. 222(1):223-229
[8] Mòdol L, Darbra S, Vallée M, Pallarès M (2013) Alteration of neonatal Allopregnanolone levels affects exploration, anxiety, aversive learning and adult behavioural response to intrahippocampal neurosteroids. Behav. Brain Res., in press
[9] Darbra S, Pallarès, M (2011) Interaction between early postnatal neurosteroid manipulations and adult infusion of neurosteroids into CA1 hippocampal region on the open field behavior. Behav. Brain Res. 216(2):705-711
[10] Martín-García E, Pallarès M (2008) A post-training intrahippocampal anxiogenic dose of the neurosteroid pregnenolone sulphate impairs passive avoidance retention. Exp. Brain Res. 191(2) :123-131
[11] Martin-Garcia E, Darbra S, Pallares M (2008) Neonatal finasteride induces anxiogenic-like profile and deteriorates passive avoidance in adulthood after intrahippocampal neurosteroid administration. Neuroscience 154:1497-1505
[12] Darbra S, Mòdol L, Pallarès M (2012) Allopregnanolone infused into the dorsal (CA1) hippocampus increases prepulse inhibition of startle response in Wistar rats. Psychoneuroendocrinology 37(4):581-585
[13] Darbra S, Mòdol L, Vallée M, Pallarès M (2013) Neonatal neurosteroid levels are determinant in shaping adult prepulse inhibition response to hippocampal allopregnanolone in rats. Psychoneuroendocrinology, in press
[14] Belelli D, Lambert JJ (2005) Neurosteroids: endogenous regulators of the GABA(A) receptor. Nat.Rev.Neurosci. 6(7):565-575
[15] Mòdol L, Casas C, Llidó A, Navarro X, Pallarès M, Darbra S (2012) Alteration of allopregnanolone levels affects alpha4 and delta GABAA receptor subunit expression and adult behavioural hippocampal response to neurosteroids. Poster presented at the 8th FENS forum of neuroscience

ALLOPREGNANOLONE AS A REGENERATIVE THERAPEUTIC: CURRENT STATUS AND FUTURE PROMISE

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Regenerative therapeutics hold the promise of self-renewal and repair. Aging and age-associated neurodegenerative diseases, while marked by a decline in these functions, retain capacity for regeneration. Molecular therapeutics promoting self-renewal in the nervous system face the challenge of activating a system of regenerative responses and a system of repair responses often in the context of a system undergoing degeneration. Neurosteroids regulate both regeneration and repair systems in brain and among this class of molecules, allopregnanolone, has been broadly investigated. In both the central and peripheral nervous systems, allopregnanolone promotes regeneration. In brain, generation and survival of new neurons was induced by allopregnanolone in both the aged and Alzheimer's mouse hippocampus and was accompanied by restoration of associative learning and memory function. In the Alzheimer's mouse brain, allopregnanolone increased liver-X-receptor (LXR) and pregnane-X-receptor (PXR) expression and reduced beta-amyloid and microglial activation. In parallel, allopregnanolone also increased myelin and white matter density. Therapeutic windows for efficacy were evident in both the normal aging and Alzheimer's mouse brain. Dosing and regenerative treatment regimens were determining factors regulating therapeutic efficacy. Allopregnanolone, serves as proof of concept for therapeutics that target endogenous regeneration, their windows of opportunity, and critical therapeutic development factors that will determine their efficacy.

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THERAPEUTIC POTENTIAL OF NEUROSTEROID ALLOPREGNANOLONE FOR THE TREATMENT OF NEUROPATHIC PAIN

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The International Association for the Study of Pain defines neuropathic pain as pain generated by a lesion or disease affecting the somatosensory system. Neuropathic pain may have multiple causes such as nerve injury, brain trauma, tumors, metabolic deficit, vascular infarction, surgical rhizotomy, thoracotomy, drug toxicity, infection etc….The symptoms characterizing neuropathic pain include a chronic discomfort with burning sensation, sharp, stabbing or shooting pain, allodynia, hyperalgesia and/or hyperpathia. Owing to its complex etiology and features, the treatment and management of neuropathic pain are extremely complicated. Consequently, neuropathic pain, which is refractory to several analgesic drugs, belongs to the category of stubborn pains that constitute major health concerns generating suffering in millions of patients and serious socio-economical problems. Development of novel strategies to treat effectively neuropathic pain is an urgent medical need and a crucial challenge for biomedical researchers. Progress has been made over the past two decades in the management and treatment of neuropathic pain but the substances developed induce several side effects including dependence, tolerance, nausea, reflex dysfunctions, treatment-emergent nervousness, anorexia or stomach burning. Therefore, characterization of novel effective analgesic and neuroprotective molecules exhibiting a safe toxicological profile may allow the development of innovative and efficient therapeutic strategies against neuropathic pain.
Allopregnanolone (AP) also called 3α,5α-tetrahydroprogesterone, is a neurosteroid produced from progesterone in the nervous system of all vertebrate species including rodents and humans. Several investigations revealed that AP is a natural molecule which exerts analgesic, neuroprotective, neurogenic, antidepressant, anaesthetic and anxiolytic effects. These important and multi-target neuroactive effects of AP are due to its ability to modulate various channels and receptors such as $GABA_A$, glycine, L- and T-type calcium channels. Most importantly, it has been shown that the administration of synthetic AP induces various beneficial actions in humans and animal models with no toxic side effects. In particular, a multi-parametric analysis revealed that AP efficiently prevented and suppressed neuropathic painful symptoms evoked in rats by chemotherapeutic drugs including vincristine and oxaliplatin. It has also been demonstrated that the modulation of the key AP-producing enzyme, 3α-hydroxysteroid oxido-reductase (3α-HSOR), in the spinal cord regulates thermal and mechanical pain thresholds of neuropathic rats subjected to peripheral nerve chronic constriction injury. Indeed, the painful symptoms were exacerbated in these neuropathic rats by intrathecal injections of provera, a pharmacological inhibitor of 3α-HSOR which decreased AP production in the spinal cord. By contrast, the enhancement of AP concentration in the intrathecal space induced analgesia and suppression of pathological symptoms in the peripheral nerve injured neuropathic rats. Moreover, in vivo knockdown of 3α-HSOR expression in healthy rat dorsal root ganglion using 6-carboxyfluorescein-3α-HSOR-siRNA increased thermal and mechanical pain perceptions whereas AP evoked a potent peripheral analgesia mediated by both T-type calcium and $GABA_A$ channels. In humans, blood levels of endogenous AP were inversely associated with low back pain and chest pain. Furthermore, oral administration of synthetic analogs of AP induced antinociceptive properties.
Altogether, these data indicate that AP, which possesses a high therapeutic potential and a good toxicological profile, offers an interesting opportunity to develop effective and safe strategies against chronic neuropathic pain.

NEUROSTEROID DISRUPTION LEADS TO ADVERSE OUTCOMES FOLLOWING COMPROPMISED PREGNANCY AND PREMATURE BIRTH

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Allopregnanolone concentrations are remarkably high in the fetal brain in long gestation species such as the sheep and guinea pigs. Placental progesterone production is key to maintaining allopregnanolone concentrations in the fetal brain, which decline after removal of the placenta at birth [1]. The placenta expresses 5α-reductases, and so provides not only progesterone, but also 5α-reduced precursors to the fetal brain, as well as maintaining elevated basal allopregnanolone in the fetal circulation. We have previously shown that these endogenous allopregnanolone concentrations suppress excitability and maintain an appropriate balance of fetal sleep-like behaviours in late gestation and, in turn, are essential of normal brain development. The resultant global suppression of excitability creates resistance to excitotoxcity and protects the fetal brain from injury that may otherwise result from hypoxia/ischemia insults. Hypoxia-induced brain injury is markedly increased if allopregnanolone concentrations are suppressed, and there is increased seizure activity [2]. We have further reported that acute hypoxia/ischemia increased the expression of 5α-reductases and allopregnanolone concentrations in the fetal brain. These allopregnanolone responses, thus, represent an endogenous mechanism protecting against acute hypoxic stress. In contrast to acute stress, chronic growth restriction that induces moderate hypoxia does not lead to increased allopregnanolone concentration; indeed, 5α-reductase-2 expression was reduced in the brain of growth-restricted fetuses [3]. In addition, we have shown that repeated exposure to synthetic glucocorticoids suppressed 5α-reductases in the placenta and lowered fetal allopregnanolone concentrations in the fetal circulation, however, this was only observed in male fetuses [4]. These finding suggest that the chronic stress producing fetal growth restriction does not lead to an adaptive neurosteroid response, perhaps contributing to the increased vulnerability of growth-retarded fetuses to perinatal brain injury. Furthermore, differences in the suppression of neurosteroids between male and female fetuses may explain the greater vulnerability of males to poor outcome following adverse events in pregnancy.

We have recently examined the effect of maternal psychosocial stress during pregnancy on neurosteroid synthesis pathways in the fetal brain. In these studies pregnant guinea pigs were subjected to stress induced by exposure to a strobe light at 50, 55, 60 and 65 days of gestation (term ~70 days) and which resulted in raised maternal and fetal cortisol concentrations after exposure. This resulted in significantly reduced expression of myelin basic protein and glial fibrillary acidic protein (GFAP) in brains of male fetuses, and reduced expression of microtubule associated protein-2 in both male and female fetal brains. These findings indicate effects of prenatal stress on fetal brain development are sexually dimorphic, in agreement with clinical observations in infants. Maternal administration of allopregnanolone resulted in increased fetal plasma concentrations in control pregnancies, but not in fetuses after maternal strobe light stress. Hence, maternal stress can block the effectiveness of exogenous allopregnanolone treatment for the fetsus, suggesting a stress–induced dysregulation of either neurosteroid transfer or metabolism.

Preterm birth leads to a premature loss of the placenta, and therefore of progesterone in circulation and brain, and this may be a major cause of some of adverse outcomes for preterm neonates. We have measured allopregnanolone concentrations following preterm

delivery in neonatal guinea pigs, and assessed the potential for postnatal progesterone replacement as a treatment to return brain and plasma concentrations to levels typical of late of gestation. In these studies guinea pig pups were delivered by ceasarean section at 62-63 days of gestation, or at term. Progesterone and allopregnanolone concentrations and brain 5α-reductase expression were measured at 24 hours after delivery. As expected, that brain allopregnanolone concentrations were significantly reduced 24 hours after delivery in both preterm and term. Preterm neonates were developmentally immature and had reduced myelination in the hippocampus. 5α-Reductase expression throughout the brain was also significantly reduced in neonates compared to both the gestation-equivalent fetal expression, and to expression in term neonates. Postnatal progesterone treatment (16mg/kg) in preterm neonates markedly increased allopregnanolone concentrations in both plasma and brain. We conclude stresses during pregnancy and exposure to exogenous glucocorticoids reduce allopregnanolone and 5α-reductase responses, and preterm birth leads to a marked decline in neurosteroid concentrations. We suggest that this increases the vulnerability of the brain to injury in late gestation. Postnatal progesterone therapy successfully re-establishes allopregnanolone levels in the brain of preterm neonates, and further studies should evaluate if this approach improves outcome in high-risk pregnancies.

Reference list

1. P.N. Nguyen, S.S. Billiards, D.W. Walker, J.J. Hirst, Changes in 5alpha-pregnane steroids and neurosteroidogenic enzyme expression in the perinatal sheep, Pediatr Res 53(6) (2003) 956-964.
2. T. Yawno, E.B. Yan, J.J. Hirst, D.W. Walker, Neuroactive steroids induce changes in fetal sheep behavior during normoxic and asphyxic states, Stress 14(1) (2011) 13-22.
3. M.A. Kelleher, H.K. Palliser, D.W. Walker, J.J. Hirst, Sex-dependent effect of a low neurosteroid environment and intrauterine growth restriction on foetal guinea pig brain development, Journal of Endocrinology 209 (2012) 301 - 309.
4. A.A. McKendry, H.K. Palliser, D.M. Yates, D.W. Walker, J.J. Hirst, The effect of betamethasone treatment on neuroactive steroid synthesis in a foetal Guinea pig model of growth restriction, Journal of Neuroendocrinology 22(3) (2010) 166-174.

SEX-DEPENDENT OVERWRITING OF PRENATALLY PROGRAMMED STRESS RESPONSES WITH NEUROACTIVE STEROIDS

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The perinatal period is a time of enhanced neuroplasticity and events such as stress can have detrimental effects on brain development. Maternal social stress exposure during pregnancy detrimentally 'programmes' the offspring's brain, resulting in profound alterations in physiology and behaviour in later life. The principal neuroendocrine stress response system, the hypothalamo-pituitary-adrenal (HPA) axis, is particularly sensitive to adverse fetal programming. Prenatally stressed (PNS) male and female adult offspring display enhanced HPA axis responses to both physical (systemic interleukin-1β, IL-1β) and psychological (restraint) stressors in adulthood, compared with controls [1]. Moreover, PNS males, but not females show greater anxiety-like behaviour on the elevated plus maze (EPM) compared with controls [1]. The central mechanisms underlying the hyper-responsive stress responses involve increased drive to the parvocellular corticotropin releasing hormone (CRH) neurones in the paraventricular nucleus (PVN)[1].

Neurosteroids such as allopregnanolone have anxiolytic actions and reduce HPA axis responses [2,3]. We have tested whether the 5α-reduced (5αR) metabolite of progesterone (allopregnanolone) reverses the 'programmed' hyperresponsive HPA phenotype in PNS rats. Short-term allopregnanolone treatment (3 and 1 mg/kg s.c. 20 and 2h before IL-1β challenge, respectively) normalised the ACTH response to IL-1β (500ng/kg i.v.) in PNS females, and reduced the response in control rats; however allopregnanolone had no effect in either the control or PNS males. The same enzymes that convert progesterone to allopregnanolone (5αR and 3α-hydroxysteroid dehydrogenase, 3αHSD) also convert testosterone into another neuroactive steroid, androstandiol (5α-androstane-3β,17β-diol) . Androstandiol (1mg/kg s.c. 20 and 2h before IL-1β challenge) had no effect on HPA axis responses to IL-1β in control males, but reversed the hyper-responses in PNS males, measured as significant reductions in ACTH and corticosterone secretion and in CRH mRNA expression in the PVN. The same androstandiol treatment regime also reduced anxiety-like behaviour on the EPM in PNS males.

To establish whether central levels of allopregnanolone are altered in adult PNS rats we measured brain allopregnanolone concentrations. Allopregnanolone levels were significantly lower in hypothalamic, midbrain and whole brain homogenates from male PNS rats compared with controls; this effect was not observed in PNS females. Strikingly, correlation analysis revealed a significant inverse relationship between the level of aggression the dam had experienced during the social stress in her pregnancy and whole brain allopregnanolone concentrations in her male, but not female PNS offspring.

We next tested whether there was reduced capacity for central neurosteroid production in PNS rats by quantifying 5αR mRNA (the rate-limiting enzyme) and 3αHSD expression by *in situ* hybridisation. In males, PNS was associated with reduced expression of 5αR mRNA in brain regions that provide excitatory drive to the HPA axis, such as the PVN and nucleus tractus solitarii (NTS). Conversely, 5αR mRNA expression was significantly increased in PNS males in limbic areas that exert an inhibitory influence over HPA axis activity, such as the medial prefrontal cortex and the dorsal part of the lateral septum. In PNS females 5αR mRNA expression was significantly reduced compared with control

females only in the NTS. There was no significant difference in 3α-HSD mRNA expression between control and PNS rats in the PVN or NTS in either males or females.
As 5αR mRNA was down-regulated in the NTS in both male and female PNS rats we hypothesised that up-regulation of 5αR expression here would reverse the hyperactive HPA axis responses to IL-1β in PNS rats. We used adenovirus (AdV)-mediated gene transfer to up-regulate expression of 5αR and 3αHSD in the NTS in PNS females. As expected, AdV-5αR-3αHSD treatment increased 5αR and 3αHSD mRNA in the NTS. Moreover, AdV-5αR-3αHSD treatment normalised HPA axis responses in the PNS rats. Hence, 5α-reduced steroids can sub-acutely over-write programming of hyperactive HPA axis responses to immune challenge, in PNS male and female rats. Evidently, down-regulation of neurosteroid production in the brain underlies, at least in part, HPA axis hyper-responsiveness in prenatally programmed offspring.

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Reference list

[1] Brunton PJ & Russell JA (2010) *J Neuroendocrinol* 22: 258-271.
[2] Patchev VK, Hassan AHS, Holsboer F & Almeida OFX (1996) *Neuropsychopharmacology* 15: 533-540.
[3] Frye CA, Koonce CJ, Edinger KL, Osborne DM, Walf AA (2008) Horm Behav 54: 726-734.

ALLOPREGNANOLONE AND MOOD DISORDERS

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Certain women experience negative mood symptoms during progesterone addition in sequential hormone therapy (HT). Women with Premenstrual Dysphoric Disorder (PMDD) increased negative mood symptoms related to allopregnanolone increase during the luteal phase of ovulatory menstrual cycles. In anovulatory cycles no symptom or sex steroid increase occurs. The symptoms are not mediated by the classical hormonal progesterone receptor. Another explanations has been put forward that symptoms are paradoxically provoked by allopregnanolone on the GABA-A receptor system. Sedative modulators of the GABA-A receptor include allopregnanolone, pregnanolone, benzodiazepines and barbiturates. Studies with GABA-A receptor modulators show that in certain individuals GABA-A receptor modulators, including allopregnanolone, have biphasic effects. In low concentrations they paradoxically give adverse, anxiogenic effects whereas in higher concentrations show sedative, calming properties. Positive GABA-A receptor modulators induce strong paradoxical effects e.g. negative mood in 3%–8% of those exposed, while up to 25% have moderate symptoms. Similar prevalence is found for PMDD, 3%–8% among women in fertile ages, and up to 25% have moderate symptoms of premenstrual syndrome (PMS).

In women taking progesterone in HT, the severity of these mood symptoms are related to the allopregnanolone serum concentrations in an inverted U-shaped curve. Negative mood symptoms occur when serum concentrations of allopregnanolone are similar to endogenous luteal phase levels, lower and higher concentrations have less effect on mood. Low concentrations of progesterone/allopregnanolone increase the activity in the amygdale (measured with functional Magnetic Resonance Imaging, fMRI) when aversive pictures are shown, similar to the changes seen during anxiety reactions. Higher allopregnanolone concentrations relate to decreased amygdale activity, thus the reactivity shows biphasic effect related to allopregnanolone concentration.

In women studied during stress, negative affect was highest during late luteal phase. At control conditions, fMRI data showed elevated amygdala and medial prefrontal cortex responses during luteal phase. Stress induced opposite unexpected lower fMRI response in late luteal phase. In addition, there was a negative correlation between the increase in allopregnanolone concentration across the menstrual cycle and a smaller the amygdala and medial prefrontal cortex response in late luteal phase.

Patients with PMDD, show increased GABA-A receptor sensitivity to allopregnanolone (5alpha-3alpha-OH-pregnanlo-20-one) compared to controls measures with saccadic eye velocity (SEV). However, to diazepam and pregnanolone PMDD patients show decreased sensitivity compared to controls. It is well known that diazepam does not act on receptors without gamma. Pregnanolone (5beta-3alpha-OH-pregnanol-20-one) has been shown to be a negative modulator on receptors containing alpha4,beta3,delta subunits while inert on alpha4beta3gamma2 receptors and a positive modulator on receptors with alpha1betaxgamma2 constellation. Alphaxolone (5alpha-3alpha-OH-pregnan-11,20-dione, a potent anesthetic steroid similar to allopregnanolone) is having the opposite effect being a positive modulator on alpha4beta3delta receptor. It is also known the alpha4 subunit prefer the delta subunit instead of gamma. These results indicates that patients with PMDD

have an up regulated alpha4betaxdelta GABA-A receptor. This agrees with findings in animals models of PMDD.
Another patient group, women with burn-out-syndrome, also show increased allopregnanolone sensitivity in SEV compared to controls suggesting up regulated delta subunit. In addition they show a positive modulating effect of flumazenil (a benzodiazepine antagonist) on SEV. In controls Flumazenil had no effect as expected. It is known from animal work that Flumazenil changes its effect in alpha4 receptors from being a benzodiazepine antagonist or inert compound to become a positive modulator like a benzodiazepine. As alpha4 prefer the delta subunit it is not unreasonable to suspect that burnout patients have an up regulation of the alpha4,delta containing receptors in the brain areas controlling SEV. Such receptor subtype up-regulation would explain the increased sensitivity to allopregnanolone as well as the agonistic effect of flumazenil,
Conclusion: These findings suggest that allopregnanolone induce negative mood symptoms in women with PMDD. Symptoms are caused by the paradoxical effect of allopregnanolone mediated via the GABA-A receptors in amygdale and emotional circuts. In addition two patient groups PMDD and burn-out-syndrome show pharmacological responses suggesting an up regulation of the alpha4,betax,delta subunit of the GABA-A receptor in SEV controlling circuits.

Reference list

Adkins CE, Pillai GV, Kerby J, Bonnert TP, Haldon C, McKernan RM, Gonzalez JE, Oades K, Whiting PJ, Simpson PB. Alpha4,beta3delta GABA(A) receptors characterized by fluorescence resonance energy transfer-derived measurements of membrane potential. J Biol Chem 2001;276:38934–38939.
Bäckström T, Bixo M, Nyberg S, Savic I. Increased neurosteroid sensitivity – an explanation to symptoms associated with chronic work related stress in women? Psychoneuroendocrinology 2012,In Press.
Bäckström T, Haage D, Löfgren M, Johansson IM, Strömberg J, Nyberg S, Andréen L, Ossewaarde L, Wingen GA, Turkmen S, Bengtsson SK. Paradoxical effects of GABA-A modulators may explain sex steroid induced negative mood symptoms in some persons. Neuroscience: 2011;191:46-54.
Ossewaarde L, Hermans E J, van Wingen G A, Kooijman S C, Johansson I M, Bäckström T, Fernández G, Neural mechanisms underlying changes in stress-sensitivity across the menstrual cycle, *Psychoneuroendocrinology* 2010;35:47-55.
Rahman M, Borra V B, Isaksson M, Johansson I M, Ragagnin G, Bäckström T, Wang M D, A comparison of the pharmacological properties of recombinant human and rat alpha(1)beta(2)gamma(2L) GABA(A) receptors in Xenopus oocytes, *Clin Exp Pharmacol Physiol* 2008;35:1002-1011.
van Wingen GA, Ossewaarde L, Bäckström T, Hermans EJ, Fernández G. Gonadal hormone regulation of the emotion circuitry in humans. Neuroscience. 2011;191:38-45.

ALLOPREGNANOLONE AND PROGESTERONE RECEPTORS: THEIR RESPECTIVE SIGNIFICANCE IN MYELINATION AND NEURON VIABILITY

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The brain is an important target of progesterone. Already in the early seventies, the uptake of tritiated progesterone by hypothalamic neurons has been demonstrated by autoradiography, pointing to direct actions of the hormone on neural cells. With the advent, a few years later, of a synthetic progestin with high affinity and selectivity for the progesterone receptors (PR), it became possible to clearly demonstrate their presence in the brain [4]. Although highest levels of PR were observed in brain regions involved in the control of reproductive functions such as the hypothalamus, binding studies showed consistent levels of receptors throughout the brain. A wide distribution of brain PR has subsequently been confirmed by mRNA analysis, immunohistochemistry and more recently by immunoelectron microscopy [7]. Moreover, expression studies have reported elevated levels of PR mRNA in cerebral cortex and subcortical regions, comparable to the ones observed in hypothalamic nuclei. Surprisingly, the significance of the wide distribution of PR in the brain has largely remained unexplored, and only hypothalamic PR have been extensively studied for their role in the regulation of female reproductive behavior.

This lack of information about the brain functions of PR is unexpected, as progesterone is known to exert multiple effects on neural cells. Moreover, the neuroprotective and regenerative effects of progesterone have recently received much attention. The little attention devoted to the brain functions of PR may relate to the widely accepted assumption that non-reproductive actions of progesterone may be mainly mediated by its metabolite allopregnanolone, which does not bind to PR, but to membrane gamma-aminobutyric acid type A ($GABA_A$) receptors, a major inhibitory neurotransmitter receptor [1]. It is indeed widely acknowledged that allopregnanolone has anxiolytic, anesthetic, antidepressant and anticonvulsant actions involving the modulation of $GABA_A$ receptors. Historically, since the forties, non-reproductive functions of progesterone have indeed always been linked to membrane actions. More recently, it has been proposed that allopregnanolone may also be the neuroactive metabolite mediating the neuroprotective effects of progesterone. However, these studies do not always take into account the fact that allopregnanolone can be converted back to 5alpha-dihydroprogesterone, a potent transcriptional activator of PR [5]. A major problem is that no selective inhibitors of allopregnanolone formation are available. As a consequence, the mechanisms by which progesterone exerts its neuroprotective actions are still not well understood.

Our recent experimental studies demonstrate a key role for PR in neuronal survival after stroke, induced by the transient occlusion of the middle cerebral artery (MCAO) in male mice [3]. We show that PR deficiency, and even haploinsufficiency, markedly increases the vulnerability of the mouse brain to ischemic injury, resulting in increased infarct volume and poor functional outcomes. Within a time window of about 24 hours, PR-dependent signaling of endogenous brain progesterone limits the extent of tissue damage and the impairment of motor functions. Levels of progesterone, analyzed by gas chromatography/mass spectrometry (GC/MS), were indeed strongly increased, more than 25-fold, in the brains of mice as early as 6h after MCAO. In contrast, brain levels of allopregnanolone did not change in response to ischemic stroke, suggesting that

endogenous allopregnanolone in the brain may not be involved in neuroprotective responses. However, the rise in endogenous progesterone is transient and not sufficient for a lasting improvement of neurological outcomes. To provide longer-term protection against focal cerebral ischemia, it is necessary to treat mice with progesterone. Notably, the protective effect of exogenous progesterone also requires the presence of PR and can be mimicked by administration of a low dose of the very potent and selective PR agonist Nestorone, a 19-norprogesterone derivative [6]. Consistent with previous reports, the administration of a large dose of allopregnanolone also protected neural cells against ischemic damage in our experimental model. But in contrast to progesterone, the neuroprotective effect of allopregnanolone was also observed in PR knockout mice, pointing to the existence an additional PR-independent neuroprotective pathway.
Our findings thus identify PR as a key therapeutic target for neuroprotective interventions after stroke, the most common cause of neurological disability. Importantly, PR haploinsufficiency observed in heterozygous PR knockout mice strongly suggests that the amount of PR may be a limiting factor in progesterone-dependent neuroprotection. This contrasts with reproduction functions of progesterone, for which mono-allelic expression of PR does not lead to a particular phenotype. Interestingly, PR haploisufficiency has also been demonstrated for another important non-reproductive function of progesterone: the stimulation of myelin formation [2]. Identification of PR as a neuroprotective and promyelinating drug target opens new therapeutic indications for selective synthetic progestins, already validated for contraception or hormone therapy. Although our data do not support a neuroprotective role of endogenous brain allopregnanolone, they nevertheless provide support for a therapeutic potential of allopregnanolone treatment in neuroprotective strategies.

Reference list

1. Belelli D, Lambert JJ (2005) Neurosteroids: endogenous regulators of the GABA(A) receptor. Nat Rev Neurosci 6:565-575.
2. Hussain R, el-Etr M, Gaci O, Rakotomamonjy J, Macklin WB, Kumar N, Sitruk-Ware R, Schumacher M, Ghoumari AM (2011) Progesterone and nestorone facilitate axon remyelination: a role for progesterone receptors. Endocrinology 152:3820-3831.
3. Liu A, Margaill I, Zhang S, Labombarda F, Coqueran B, Delespierre B, Liere P, Marchand-Leroux C, O'Malley BW, Lydon JP, De Nicola AF, Sitruk-Ware R, Mattern C, Plotkine M, Schumacher M, Guennoun R (2012) Progesterone receptors: a key for neuroprotection in experimental stroke. Endocrinology 153:3747-3757.
4. Parsons B, Rainbow TC, MacLusky NJ, McEwen BS (1982) Progestin receptor levels in rat hypothalamic and limbic nuclei. J Neurosci 2:1446-1452.
5. Rupprecht R, Reul JM, Trapp T, van S, Wetzel C, Damm K, Zieglgansberger W, Holsboer F (1993) Progesterone receptor-mediated effects of neuroactive steroids. Neuron 11:523-530.
6. Sitruk-Ware R, Small M, Kumar N, Tsong YY, Sundaram K, Jackanicz T (2003) Nestorone: clinical applications for contraception and HRT. Steroids 68:907-913.
7. Waters EM, Torres-Reveron A, McEwen BS, Milner TA (2008) Ultrastructural localization of extranuclear progestin receptors in the rat hippocampal formation. J Comp Neurol 511:34-46.

SUNDAY, 17th February 2013
09.00 - 12.00

Neuroactive steroids and psychiatric disorders

Neuroactive steroids and psychiatric disorders

(Chairs: Backstrom T., Riva M.)

- **Biagini G.,** Rustichelli C., Curia G., Lucchi C., Pugnaghi M., Meletti S., Avoli M. **(Italy)** *Neurosteroids and epileptogenesis*

- **Bortolato M. (USA)** *Role of 5alpha-reductase in behavioral regulation: relevance to schizophrenia and other neuropsychiatric disorders*

- **Frye C.A. (USA)** *Pregnane xenobiotic receptors and membrane progestin receptors, sources and targets, for neurosteroid-mediated motivated behaviors*

- Iacobas D.A., Iacobas S., Chachua T., Sidyelyeva G., Goletiani C., Velíšková J., **Velíšek L. (USA)** *Modification of glutamatergic and GABAergic synapse gene fabric by prenatal corticosteroids*

- **Dalla C.,** Kokras N., Pastromas N., Kafetzopoulos V., Balthazart J., Cornil C.A., Papadopoulou-Daifoti *Z.* **(Greece)** *Blockade of neuroestrogens enhances depressive behavior of female, but not male rats in the forced swim test*

- Wong P., Chang C.C.R., Marx C.E., Caron M.G., Wetsel W.C., **Zhang X. (Singapore)** *Pregnenolone rescues schizophrenia-like behavior in dopamine transporter knockout mice*

NEUROSTEROIDS AND EPILEPTOGENESIS

Biagini G.[1], Rustichelli C.[2], Curia G.[1], Lucchi C.[1], Pugnaghi M. [1], Meletti S. [1], Avoli M.[3]

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Epileptogenesis is defined as the process by which a normal brain is reorganized to produce a recurrent epileptic activity [1]. This definition fully complies with the series of events observed in temporal lobe epilepsy (TLE) and in models mimicking TLE. Traditionally, in TLE models based on induction of *status epilepticus* (SE), epileptogenesis corresponds to the latent period, at the end of which a chronic phase characterized by spontaneous recurrent seizures is established. However, this view has been challenged by data suggesting a continuum in the progression of epilepsy, with seizures appearing after a very short latent period and, subsequently, increasing continuously in frequency for more than 3 months [2]. Interestingly, this new view of epileptogenesis takes into account the reported progressive nature of TLE, suggested by several studies on TLE patients resistant to antiepileptic drugs [1]. However, among the various mechanisms proposed to underlie epileptogenesis, only few of those manifested during the latent period are still active in the chronic phase, especially neuroinflammation [3]. Glial cells such as astrocytes and microglia play a major role in modulating neuroinflammation in the brain. These cells are able to synthesize a variety of trophic factors, regulators of inflammation as well as modulators of neurotransmission.

We have consistently found in the hippocampal formation that, after SE, glial cells present with increased expression of the cytochrome P450 cholesterol side-chain cleavage (P450scc) enzyme, which is critically involved in the synthesis of neurosteroids that reinforce inhibition by interacting with $GABA_A$ receptors [4]. In addition, we reported that the upregulation of P450scc was more pronounced in rats exposed to longer intervals of SE and that these animals presented with delayed onset of spontaneous recurrent seizures (Fig.1). Finally, this phenomenon was completely abolished by the 5alpha-reductase inhibitor finasteride, that precludes the synthesis of allopregnanolone and tetrahydrodeoxycorticosterone. A proconvulsive effect of finasteride has also been demonstrated in female rats previously exposed to pilocarpine-induced SE [reviewed in Ref. 4]. Interestingly, this effect was observed during the chronic phase, suggesting that neurosteroids exert an antiseizure effect in female rats. This observation is consistent with data demonstrating a role of neurosteroids in mediating the antiepileptic properties of progesterone in catamenial epilepsy. In line with these findings, we have recently observed a case of exacerbation of seizures in a women affected by TLE, treated with finasteride for hirsutism, who recovered sensitivity to antiepileptic drugs only by stopping finasteride. Indeed, as shown in Fig.2, finasteride is able to significantly abate allopregnanolone levels in the brain, even acutely. Overall, these findings demonstrate a major role of neurosteroids in the progression of epilepsy in TLE and its related models, thus suggesting a possible antiepileptogenic role of neurosteroids.

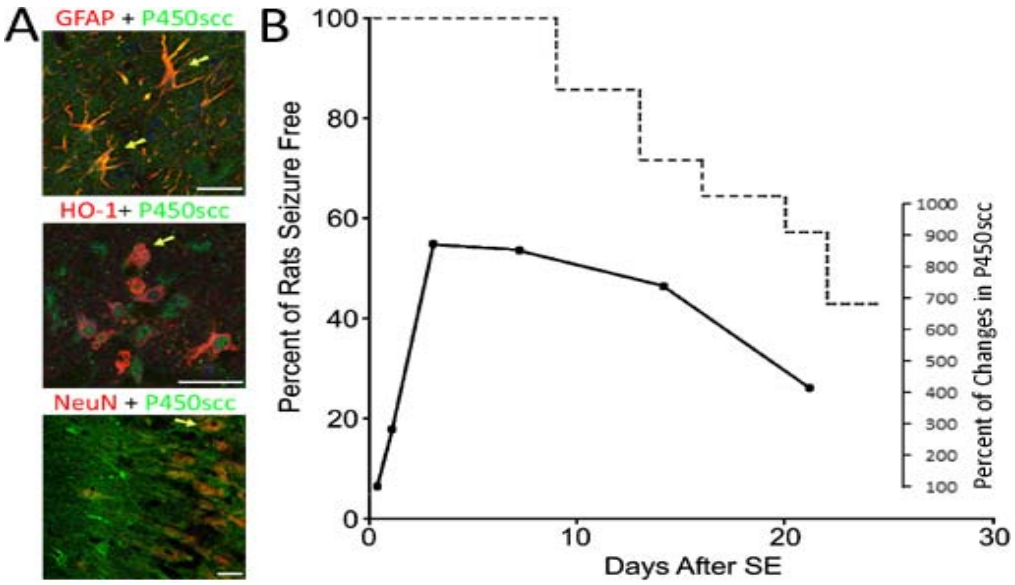

Fig.1. Induction of neurosteroid synthesis and epileptogenesis. In **A**: double localization experiments with markers of astrocytes (glial fibrillary acidic protein, GFAP), microglia (heme oxygenase-1, HO-1) and neurons (neuronal specific nuclear protein, NeuN) after pilocarpine-induced SE demonstrate that P450scc is mainly expressed in glial cells. Arrows point to double-labeled cells. Scale bars, 25 microns. In **B**, the decline of P450scc immunoreactivity (continuous line) is accompanied by the appearance of spontaneous recurrent seizures (dashed line) [modified from Ref. 4].

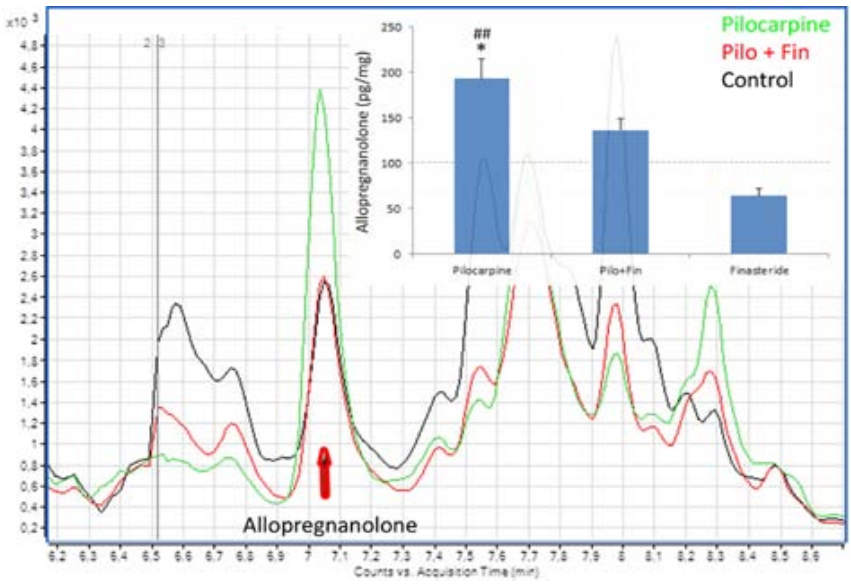

Fig.2. Chromatograms showing allopregnanolone levels in the hippocampus. Rats sacrificed approximately 30 min after pilocarpine injection present with a significant (*$p<0.05$, Fisher's LSD test) increase in allopregnanolone levels with respect to basal values (dotted line). Finasteride (100 mg/kg), given 10 minutes before pilocarpine injection, prevented the increase in allopregnanolone levels and, in control rats, even reduced allopregnanolone ($^{\#\#}p<0.01$ pilocarpine vs finasteride, Fisher's LSD test).

Reference list

1. Pitkanen A, Sutula TP. (2002) Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. Lancet Neurol. 1:173–181.
2. Williams PA, White AM, Clark S, Ferraro DJ, Swiercz W, Staley KJ, Dudek FE. (2009) Development of spontaneous recurrent seizures after kainate-induced status epilepticus. J. Neurosci. 29:2103-12.
3. Vezzani A, French J, Bartfai T, Baram TZ. (2011) The role of inflammation in epilepsy. Nat. Rev. Neurol. 7:31-40.
4. Biagini G, Panuccio G, Avoli M. (2010). Neurosteroids and epilepsy. Curr. Opin. Neurol. 23:170-6.

ROLE OF 5ALPHA-REDUCTASE IN BEHAVIORAL REGULATION: RELEVANCE TO SCHIZOPHRENIA AND OTHER NEUROPSYCHIATRIC DISORDERS

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The enzyme 5alpha-reductase (5AR) catalyzes the conversion of ketosteroid precursors - such as testosterone and progesterone - into their 5alpha-reduced metabolites. Over the last decade, converging lines of evidence have highlighted the role of 5alpha-reduced steroids and their precursors in brain neurotransmission and behavioral regulation. Capitalizing on these premises, we and other groups have investigated the role of 5AR in several animal models of neuropsychiatric disorders. Our preclinical and clinical findings suggest that 5AR inhibitors may elicit therapeutic effects in a number of disorders associated with dopaminergic hyperreactivity, including Tourette syndrome, schizophrenia and impulse control disorders. These effects appear to reflect antidopaminergic properties of finasteride, which are dissociated from extrapyramidal symptoms possibly through the selective modulation of D1 and D3 receptors in the nucleus accumbens, in coordination with other targets, such as sigma and beta-estrogen receptors.

PREGNANE XENOBIOTIC RECEPTORS AND MEMBRANE PROGESTIN RECEPTORS, SOURCES AND TARGETS, FOR NEUROSTEROID-MEDIATED MOTIVATED BEHAVIORS

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Background**:** Progesterone (P) has actions in the midbrain ventral tegmental area (VTA) to mediate reproductive behaviors, such as lordosis, among female rodents. In this region, some of P's effects occur through actions of 5alpha-pregnan-3alpha-ol-20-one (3alpha,5alpha-THP). Formation and actions of 3alpha,5alpha-THP in the VTA are necessary and sufficient to facilitate lordosis and these are site-specific to VTA (but not to nearby regions such as central grey, raphe nucleus, substantia nigra; Frye, 2009). Although 3alpha,5alpha-THP can be produced following metabolism of ovarian P, 3alpha,5alpha-THP is also a neurosteroid produced *de novo* in brain regions, such as the VTA. There can be dynamic changes in 3alpha,5alpha-THP production associated with behavioral experience. For example, biosynthesis of 3alpha,5alpha-THP occurs with reproductive experience. Pregnane Xenobiotic Receptor (PXR) may be involved in 3alpha,5alpha-THP's actions in the VTA. PXR. PXR is a nuclear receptor that regulates gene transcription for cytochrome P450 enzymes, which are involved in 3alpha,5alpha-THP biosynthesis [1]. We have identified PXR in the midbrain of female rats [2]. Thus, PXR may be a novel source for 3alpha,5alpha-THP in the VTA for lordosis.
Of interest are the mechanisms of progestogens in the VTA for lordosis. Rapid change in lordosis of rodents is one of the best-studied *in vivo* models for actions of progestogens independent of cognate, nuclear progestin receptors (PRs). Progestogens have rapid actions in the VTA, a midbrain area in which few PRs are expressed in adult rodents. The rapidity of the effects of progestogens and the low expression of nuclear PRs in this region suggest that progestogens have effects for lordosis of rodents independent of PRs. A question is the targets for these effects. 3alpha,5alpha-THP-mediated lordosis in the midbrain VTA involves actions through $GABA_A$, dopamine, and glutamate receptors and their downstream signaling targets [3]. Recent studies have focused on the role of membrane progestin receptors (mPRs). Rapid non-genomic signaling has been shown to be mediated by mPRs in various *in vitro* model systems, including fish oocytes [4]. We have analyzed expression of two of the common forms of these receptors (mPRalpha and mPRbeta) in female rats. Expression of mPR was examined by reverse-transcriptase polymerase chain reaction (RT-PCR) in central and peripheral tissues of proestrous Long-Evans rats. Expression of mPRalpha was observed in peripheral tissues and brain areas, including hypothalamus and midbrain. Expression of mPRbeta was only observed in brain tissues and was abundant in the midbrain and hypothalamus. To our knowledge, studies of these receptors in mammalian models have been limited to expression and regulation instead of function. A question that was addressed was the functional effects of progestogens via mPRalpha and mPRbeta in the midbrain of hormone-primed rats for lordosis.
Hypotheses**:** 1) If PXR is involved in biosynthesis of 3alpha,5alpha-THP, then knocking down PXR expression in the VTA will alter 3alpha,5alpha-THP synthesis in the midbrain and lordosis responding of female rats. 2) If mPRs are a target in the midbrain for

progestogen-mediated lordosis, then knocking down mPR expression in the VTA will reduce lordosis responding of female rats.

Methods & Results: In Experiment 1, the role of PXR was assessed. Rats had estrous cycle stage determined and were tested when in diestrus or proestrus. Diestrous and proestrous rats were infused to the VTA with control or anti-sense oligodeoxyribonucleotides (AS-ODNs) targeted against PXR. Rats were then tested for anxiety (open field, plus maze), social (social interaction) and sexual behavior (paced mating). Expression of PXR in the midbrain was verified with Western blotting. Levels of estradiol, P, dihydroprogesterone (DHP), and 3alpha,5alpha-THP in plasma and P, DHP, and 3alpha,5alpha-THP in midbrain were determined by radioimmunoassay. Results supported the hypothesis that formation of 3alpha,5alpha-THP requires PXR, and may be important for lordosis. PXR AS-ODN, compared to control, infusions to the VTA reduced PXR expression and 3alpha,5alpha-THP levels in the midbrain, and attenuated sexual receptivity of proestrous rats.

In Experiment 2, mPRs in the midbrain were assessed as targets for progestogens for lordosis. Ovariectomized rats were estradiol (E_2; 0.9 mg/kg, SC), P (4 mg/kg, SC) or vehicle (oil) primed and infused with AS-ODNs targeted against mPRalpha and/or mPRbeta intracerebroventricularly or to the VTA. Rats were assessed for anxiety (open field, elevated plus maze), social (social interaction) and sexual (lordosis) behavior. Progestogen-facilitated lordosis was significantly reduced with administration of AS-ODNs for mPRalpha, mPRbeta, or co-administration of mPRalpha and mPRbeta infused intracerebroventricularly, compared to vehicle. Administration to the VTA of mPRbeta AS-ODNs, or co-administration of mPRalpha and mPRbeta AS-ODNs, but not mPRalpha AS-ODNs alone, attenuated P-facilitated lordosis, compared to vehicle infusions. Our results suggest that mPRbeta in the VTA may be required for P-facilitated lordosis of rats.

Conclusions**:** Together, these studies demonstrate that PXR is involved in production of 3alpha,5alpha-THP in the midbrain VTA. Moreover, mPRs may be a target for progestogens' actions in the VTA for lordosis.

*Supported By**:** This research was funded by NIMH (MH0676980), NSF (IOS-0957148) and Alaska INBRE.*

Reference list

[1] Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterström RH, Perlmann T, Lehmann JM. (1998). An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. Cell. 92, 73-82.

[2] Frye CA, Paris JJ, Walf AA, Rusconi JC. (2011). Effects and Mechanisms of 3alpha,5alpha,-THP on Emotion, Motivation, and Reward Functions Involving Pregnane Xenobiotic Receptor. Front Neurosci. 5- Article 136, 1-18.

[3] Frye CA. (2009). Neurosteroids-From Basic Research to Clinical Perspectives. In: Hormones/ Behavior Relations of Clinical Importance. Eds: RT Rubin, DW Pfaff. 395-416. San Diego: AP.

[4] Zhu Y, Hanna RN, Schaaf MJ, Spaink HP, Thomas P. (2008). Candidates for membrane progestin receptors--past approaches and future challenges. Comp Biochem Physiol C Toxicol Pharmacol. 148, 381-9.

PRENATAL EXPOSURE TO CORTICOSTEROIDS: HYPOTHALAMIC CHANGES RELEVANT FOR POSTNATAL BEHAVIORAL IMPAIRMENTS

Iacobas D.A.[2], Iacobas S.[2], Chachua T.[1], Sidyelyeva G.[1], Goletiani C.[1], Velíšková J.[1,3,4], Velíšek L.[1,4,5]

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Arcuate nucleus (ARC) of the hypothalamus is a gate for peripheral signaling and a source of multiple intrahypothalamic connections [5,6], which in rats continue developing postnatally and mature between 3-4 postnatal weeks. In addition to its function in food intake, sexual behavior and energy metabolism control, ARC neurons strongly express c-fos during vagus nerve stimulation [4] suggesting that activation of ARC and its networks may constitute a part of endogenous seizure control system. ARC GABAergic neurons, which contain either neuropeptide Y/agouti-related peptide (NPY/AgRP) or pro-opiomelanocortin/cocaine-amphetamine-related transcript (POMC/CART)- [7] project both inside and outside of ARC. POMC/CART glutamatergic neurons connect mainly locally within the ARC. NPY/AgRP neurons from the ARC synapse on corticotropin releasing factor (CRF)-containing parvocellular neurons in the paraventricular hypothalamic nucleus (PVN) and regulate CRF release. CRF has significant proconvulsant effects via the CRF-R1 receptor system [1,2] and regulates glutamatergic transmission [2] depending on the structure, receptor system, and its localization. These findings suggest strong links between CRF and glutamate (and its KA/AMPA and NMDA receptors), which are abundantly present in the ARC. However, because the hypothalamus is an important control center for endocrine function, energy homeostasis, as well as inflammation, there is plethora of other (neuro) peptides and their receptor systems found in hypothalamic nuclei. All these peptides and their G-protein-based receptor systems interact within hypothalamic nuclei and especially within the main upstream control center, the ARC [6]. Finally, ARC contains a significant dopaminergic component as a part of ventral forebrain dopaminergic system.

Prenatal exposure to synthetic corticosteroids can significantly alter postnatal development through changes in neurotransmitters, peptides and their receptors, and thus having long-lasting behavioral effects. Some of these changes have been observed in animal experiments, others also in humans prenatally exposed to synthetic corticosteroids. Here, we focused on transcriptomic changes within the ARC of rats prenatally exposed to either betamethasone or saline. The expression of transcriptome has been assessed by novel computational tools to determine complex changes that may have life-long effects on phenotype, i.e., behavior. Concentration of proteins corresponding to some of the affected transcripts was also determined either by western blots in ARC samples or by immunohistochemistry on postnatal days 14-15 (P14-15). Behavioral effects were evaluated using novel object recognition, Barnes maze and Morris Water maze between P20-30.

Total of 18,094 unigenes were quantified in the hypothalamic ARC of P14 male and female rats prenatally exposed to betametasone used in this experiment. Out of these genes, Kyoto Encyclopedia for Genes and Genomes (http://www.genome.jp) selected 112 for the dopaminergic synapse, 75 for the GABAergic and 97 for the glutamatergic synapse. We further analyzed composition, topology and modulatory networks of the genomic fabric [3] of the dopaminergic, GABAergic, and glutamatergic synapse (the transcriptome

of the most interconnected and stably expressed gene network responsible for specific transmission). Finally we investigated the "transcriptomic landscape" of the GSF in the ARC of P14 males (M) and females (F) prenatally (G15) exposed to betamethasone (B) or saline (S). We combined in one measure (PWR = Pair-Wise Relevance) expression levels, controls and coordination of all pairs that can be formed by synapse genes with the other synapse genes, higher PWRs indicating larger influence of that gene pair to the fabric modulation.

We found that prenatal exposure to betamethasone caused sex-dependent changes in the dopaminergic/GABA/glutamatergic synapse genes:. In males, 10 dopaminergic (9%), 4 GABAergic (5%) and 5 glutamatergic synapse genes (5%) were down-regulated. While in females, 9 dopaminergic (8%), 3 GABAergic (4%) and 6 glutamatergic (6%) synapse genes were downregulated. The data indicate that in both sexes the dopaminergic synapse was the most affected. In contrast, in control animals, no significant differences between male and female were present in these synapse genes.

Since the most noticeable transcritpomic changes were found in the transcriptome of DA glutamatergic synapse, we investigated the expression of tyrosine-hydroxylase (TH) NMDA receptor subunits in the ARC. The western blot analyses and immunohistochemistry confirmed the sex-specific differences between prenatally betamethasone-exposed and saline-exposed P15 rats.

Accordingly to the changes in gene expression, prenatal exposure to synthetic corticosteroids was associated with postnatal changes in behavior and susceptibility to certain types of seizures. While we did not find any significant impairements in normal behavioral patterns (open field activity), there was a sex-specific change in the novel object recognition test. We found that behavioral lateralization in females is lost after prenatal betamethasone exposure and both male and female prenatally betamethasone exposed rats were avoiding novelty. This trait is similar to children with autism and suggests that certain elements of autistic behaviors can be present after prenatal exposure to synthetic corticosteroids. Additionally, there were changes in the search patterns in the Morris water maze as well as in the Barnes maze.

In conclusion, our work is consistent with findings of profound reprogramming changes in the brain after prenatal corticosteroid exposure associated with alterations cognitive functions and seizure susceptibility.

Reference list

1. Campbell, RE, Grove, KL, Smith, MS. Distribution of corticotropin releasing hormone receptor immunoreactivity in the rat hypothalamus: coexpression in neuropeptide Y and dopamine neurons in the arcuate nucleus. *Brain Res* 2003; 973:223-232.

2. Gallagher, JP, Orozco-Cabal, LF, Liu, J, Shinnick-Gallagher, P. Synaptic physiology of central CRH system. *Eur J Pharmacol* 2008; 583:215-225.

3. Iacobas, S, Iacobas, DA. Astrocyte proximity modulates the myelination gene fabric of oligodendrocytes. *Neuron Glia Biol* 2011; 6:157-169.

4. Naritoku, DK, Terry, WJ, Helfert, RH. Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. *Epilepsy Res* 1995; 22:53-62.

5. Schwartz, MW, Woods, SC, Porte, D, Jr., Seeley, RJ, Baskin, DG. Central nervous system control of food intake. *Nature* 2000; 404:661-671.

6. Simerly, RB. Hypothalamic substrates of metabolic imprinting. *Physiol Behav* 2008; 94:79-89.

7. Wynne, K, Stanley, S, McGowan, B, Bloom, S. Appetite control. *J Endocrinol* 2005; 184:291-318.

BLOCKADE OF NEUROESTROGENS ENHANCES DEPRESSIVE BEHAVIOR OF FEMALE, BUT NOT MALE RATS IN THE FORCED SWIM TEST

Dalla C.[1], Kokras N.[1,2], Pastromas N.[1], Kafetzopoulos V.[1], Balthazart J.[3], Cornil C.A.[3], Papadopoulou-Daifoti Z.[1]

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3. Behavioral Neuroendocrinology Research Group, GIGA Neurosciences, University of Liège, Belgium

Depression is an important psychiatric disorder, which affects women twice as much as men. Fluctuations in peripheral estrogen levels in women have been implicated in the etiology of this sex difference [1]. Recently, local *de novo* estrogen synthesis has also been discovered in the brain and emerging evidence suggests that these neuroestrogens are responsible for the fine-tuning of neuronal circuits in males and females. However, it has not been identified whether neuroestrogens have an effect in stress response and depression. In the present study, we investigated the role of gonadal hormones and neuroestrogens in the open field and the Forced Swim Test (FST), which measure activity, exploration and depressive-symtomatology.

Male and female adult Wistar rats were used. Animals received a sham-operation or gonadectomy and left undisturbed to recover for 3 weeks. Then, they were injected, i.p. for one week once a day, with either vehicle or the aromatase inhibitor letrozole (1 mg/kg). Aromatase is the rate-limiting enzyme that catalyses the conversion of androgens to estrogens and is located in both sexes in the gonads as well as in other tissues including the brain. Thus, by treating gonadectomized male and female rats with letrozole, we aimed to eliminate gonadal hormone secretion and to further investigate the role of neuroestrogens in behavior. After one week of letrozole treatment, all rats were subjected for 10 min to the open field test in automated chambers (Med Associates). One day later, all rats were subjected to a 15min FST pretest session and twenty-four hours later, they were subjected to a second 5min FST test session. FST videos (5 min test sessions) were manually scored with the in-house developed software *Observador*. Immobility duration was recorded as an index of passive behavior. Furthermore, swimming and climbing behaviors were recorded as indices of active serotonergic and noradrenenergic behavioral responses, respectively. During treatment and behavioral testing, the estrous phase of female rats was monitored.

Immediately after the second FST session, rats were killed by rapid decapitation and the hypothalamus, the hippocampus, the amygdala and the prefrontal cortex were isolated and stored in -80^{o}C. Aromatase activity in the hypothalamus was determined by the production of tritiated water associated with the conversion of [1β-^{3}H]-androstenedione into estrone, as before [3-4]. Analysis verified that letrozole inhibited aromatase in the brain, since no aromatase activity was detectable in letrozole-treated animals.

Results were analyzed using a two-way multivariate analysis of variance (MANOVA). Behavioral analysis revealed that females were overall more active and explorative than males in the open field test (sex effect: $p<0.05$), while gonadectomy eliminated this sex difference. This was mainly due to the fact that ovariectomy in females decreased ambulatory and vertical counts in the open field test (surgery effect: $p<0.05$). Inhibition of estrogen synthesis with letrozole had no effect in the open field test neither in sham nor in gonadectomized male and female rats ($p>0.05$). On the other hand, in the FST, letrozole

treatment enhanced immobility and decreased swimming duration (interaction of sex with treatment $p<0.05$) only in ovariectomized females; a finding indicative of enhanced "depressive-like" symptomatology. Notably, letrozole treatment had no effect on sham-operated or gonadectomized males.

These results indicate that estrogens originating from the gonads and the brain significantly affect the response of the animals depending on the behavioral test. Specifically, neuroestrogen's inhibition enhances depressive behavior only in female ovariectomized rats. Future studies will explore the involvement of the serotonergic, glutamatergic and dopaminergic changes in selected brain regions. Furthermore, the present data suggest a possible role of estrogen depletion in the development of affective disorders in post-menopausal women treated with aromatase inhibitors.

The research project is supported by the framework of the Action «Supporting Postdoctoral Researchers» of the Operational Program "Education and Lifelong Learning" (Action's Beneficiary: General Secretariat for Research and Technology), and is co-financed by the European Social Fund (ESF) and the Greek State.
Dr. C.A. Cornil is a F.R.S.-FNRS Research Associate.

Reference list

1. C. Dalla, K. Antoniou, Z. Papadopoulou-Daifoti, J. Balthazart and J. Bakker. "Oestrogen-deficient female aromatase knockout (ArKO) mice exhibit "depressive-like" symptomatology". *European Journal of Neuroscience 20, 1–12, 2004.*
2. C. Dalla, P. M. Pitychoutis, N. Kokras and Z. Papadopoulou-Daifoti "Sex differences in the neurobiological substrate of depression and in antidepressant response". *Basic and Clinical Pharmacology and Toxicology, 106, 226-33, 2010.*
3. Roselli, C.E., Horton L.E. and Resko J.A. "Distribution and regulation of aromatase activity in the rat hypothalamus and limbic system" *Endocrinology* 117: 2471-2477, 1985
4. De Bournonville C., Dickens M.J., Ball G.F., Balthazart J. and Cornil C.A. "Dynamic changes in brain aromatase activity following sexual interactions in males: Where, when and why?" *Psychoneuroendocrinology* In press, doi 10.1016/j.psyneuen.2012.09.001

PREGNENOLONE RESCUES SCHIZOPHRENIA-LIKE BEHAVIOR IN DOPAMINE TRANSPORTER KNOCKOUT MICE

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Pregnenolone belongs to a class of endogenous neurosteroids in the central nervous system (CNS), which has been suggested to enhance cognitive functions through GABAA receptor signaling by its metabolites. It has been shown that the level of pregnenolone is altered in certain brain areas of schizophrenic patients, and clozapine enhances pregnenolone in the CNS in rats, suggesting that pregnenolone could be used to treat certain symptoms of schizophrenia. In addition, early phase proof-of-concept clinical trials have indicated that pregnenolone is effective in reducing the negative symptoms and cognitive deficits of schizophrenia patients. Here, we evaluate the actions of pregnenolone on a mouse model for schizophrenia, the dopamine transporter knockout mouse (DAT KO). DAT KO mice mirror certain symptoms evident in patients with schizophrenia, such as the psychomotor agitation, stereotypy, deficits of prepulse inhibition and cognitive impairments.

Following acute treatment, pregnenolone was found to reduce the hyperlocomotion, stereotypic bouts and pre-pulse inhibition (PPI) deficits in DAT KO mice in a dose-dependent manner. At 60mg/kg of pregnenolone, there were no significant differences in locomotor activities and stereotypy between wild-type and DAT KO mice. Similarly, acute treatment of 60mg/kg of pregnenolone fully rescued PPI deficits of DAT KO mice. Following chronic treatment with pregnenolone at 60mg/kg, the cognitive deficits of DAT KO mice were rescued in the paradigms of novel object recognition test and social transmission of food preference test. Pregnenolone thus holds promise as a therapeutic candidate in schizophrenia.

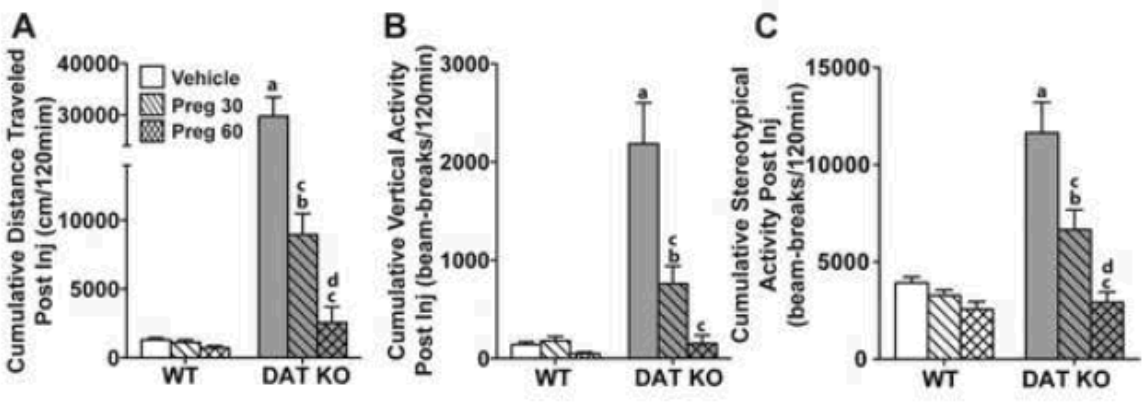

Fig 1. Dose-dependent effects of pregnenolone, haloperidol and clozapine on activities of WT and DAT KO mice in the open field. (A-C) Cumulative distance traveled (A), cumulative vertical activity (B), and cumulative stereotypical activities (C) were monitored over a 2h period following injection of vehicle, or 30 or 60 mg/kg Preg. N=10-15 mice/genotype/treatment condition; ap<0.05, WT-Veh versus KO-Veh; bp<0.05, WT-Preg30 versus KO-Preg30; cp<0.05, within groups versus Veh; dp<0.05, within groups Preg30 versus Preg60.

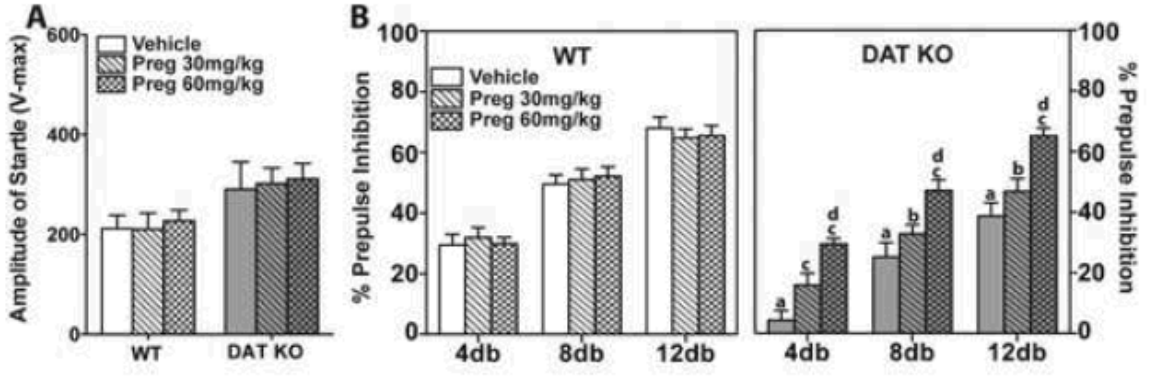

Fig 2. Pregnenolone rescues PPI in DAT KO mice. WT and DAT KO mice were injected (i.p.) with vehicle, or 30, or 60mg/kg Preg and were tested in PPI 5 min later. (A) Amplitude of the startle responses of WT and DAT KO mice. (B) PPI levels of WT and DAT KO mice. White bars represent WT and grey bars represent DAT KO performance. N=9-14 mice/genotype/treatment condition; ap<0.05, WT-Veh versus KO-Veh; bp<0.05, WT-Preg30 versus KO-Preg30; cp<0.05, within groups versus Veh; dp<0.05, within groups Preg30 versus Preg60.

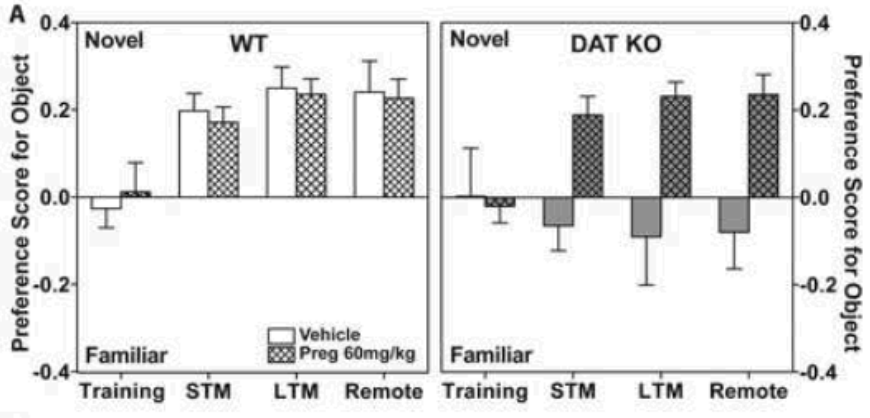

Fig 3. Pregnenolone normalizes the episodic memory deficits in DAT KO mice. WT and DAT KO mice were injected (s.c.) with vehicle or 60mg/kg Preg for 14 consecutive days and were tested in the novel object recognition for short-term (STM), long-term (LTM), and remote memory. White bars represent WT and grey bars represent DAT KO mice. (N=9-12)

SUNDAY, 17th February 2013
12.00 - 13.00

Plenary Lecture:

Belelli D. (UK)

NEUROSTEROIDS AND $GABA_A$ RECEPTOR INTERACTIONS: A FOCUS ON STRESS

Belelli D., Gunn B.G., Cunningham L., Lambert J.J.

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Stressful experiences engage a co-ordinated neuronal and hormonal response, orchestrated by the hypothalamic-pituitary-adrenocortical (HPA) axis *via* activation of CRF-releasing parvocellular neurons of the hypothalamic paraventricular nucleus (PVN). Dysfunction of this fundamental survival mechanism, for example as a consequence of adverse early life experiences, has been crucially implicated in various psychiatric disorders including depression. The mechanisms of stress dysfunction are complex but may involve $GABA_A$ receptors ($GABA_ARs$), as these receptors curtail stress-induced activation of the HPA axis [1]. 5α-pregnan-3α-ol-20-one (5α3α-THPROG) is a potent and selective endogenous modulator of $GABA_A$ receptors ($GABA_ARs$) and exhibits clear stress-protective actions [2]. The levels of 5α3α-THPROG rise rapidly during acute stress therefore, suggesting a possible role as regulator of the stress response. A compelling case can be made to implicate neurosteroids in stress-related disturbances. Here we will critically appraise how brain-derived neurosteroids may impact on the stress response to acute and chronic challenges. The pathological implications of such actions in the development of psychiatric disturbances will be discussed.

Reference list

[1] Cullinan et al, (2008). Functional role of GABAergic influences on the HPA axis. Brain Structure & Function 213(1-2) 63-72.

[2] Belelli and Lambert (2005). Neurosteroids: endogenous regulators of the $GABA_A$ receptor. Nature Reviews Neuroscience 6, 565-575.

SUNDAY, 17th February 2013
15.00 - 18.00

Neuroprotective effects

Neuroprotective effects

(Chairs: Garcia-Segura L.M., Viviani B.)

- **De Nicola A.F.,** Gonzalez-Deniselle M.C., Garay L., Meyer M., Gargiulo-Monachelli G., Guennoun R., Schumacher M., Carreras M.C., Poderoso J.J. **(Argentina*)*** *Progesterone protective effect in neurodegeneration and neuroinflammation*

- **Gravanis A. (Greece)** *Cross-talk of neurosteroids and neurotrophins in controlling neuronal survival*

- **Sohrabji F.,** Selvamani A., Miranda R.C. **(USA)** *Estrogen-IGF-microRNA interactions in stroke neuroprotection*

- **Saldanha C.J.,** Duncan K.A. **(USA)** *Cell specific provision of estrogens in the songbird brain*

- **Yadid G.,** Croitoru O., Sudai E., Ben-Tzion M., Gispan I. **(Israel)** *Dehydroepiandrosterone reverse cocaine-induced decrease in hippocampus neurogenesis and attenuate reinstatement*

- **Ghandour M.S.,** Hussain R., Ghoumari M.A., Schumacher M. **(France)** *Androgen receptor is a therapeutic target for myelin repair*

PROGESTERONE PROTECTIVE EFFECTS IN NEURODEGENERATION AND NEUROINFLAMMATION

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Far beyond its role in reproduction, progesterone exerts neuroprotective, promyelinating and antiinflammatory effects in the nervous system [1]. These effects are maximized under pathological conditions, implying that changes of the local environment sensitize the nervous tissues to steroid treatment [2]. In this presentation, we will discuss our results of progesterone neuroprotection in a motoneuron neurodegeneration model and a neuroinflammation model. In the Wobbler mouse, a mutation of the Vsp54 gene leads to motoneuron degeneration, astrogliosis and motor impairment. Motoneurons in the cervical spinal cord of Wobblers show cytoplasmic vacuolation, decreased immunoreactivity for choline-acetyltransferase (ChaT), decreased expression for Na,K-ATPase and brain-derived neurotrophic factor (BDNF) mRNAs, decreased axonal transport and increased activity of nitric oxide synthase (NOS), without the typical signs of apoptosis [3]. Additionally, Wobbler mitochondrial present membrane rupture, cristolysis, increased intramitochondrial NOS and decreased activity of respiratory chain complexes [3]. Clinically, Wobblers suffer several degrees of motor impairment. Treatment with progesterone (20 mg implant) from 3 weeks to 2 months markedly modifies spinal cord neuropathology. Thus, progesterone reverses the impaired expression of BDNF, ChAT and Na,K-ATPase, prevents oxidative damage of motoneurons and their vacuolar degeneration, attenuates mitochondrial morphological abnormalities, decreases the activity of NOS and enhances respiratory chain enzyme activities [3]. Long-term treatment with progesterone also increases muscle strength, biceps weigth and survival. Altogether, these data show that progesterone strongly protects Wobbler motoneurons from degeneration. To study progesterone effects in a neuroinflammation model resembling multiple sclerosis (MS), we induced experimental autoimmune encephalomyelitis (EAE) in C57Bl6 mice. In EAE mice we have analyzed if progesterone effects in the inflamed spinal cord involves the decreased transcription of local inflammatory mediators and the increased transcription of myelin proteins and myelin transcription factors. To this purpose, C57Bl/6 female mice divided into controls, EAE and EAE receive progesterone (100 mg implant), 7 days before EAE induction. This procedure produces pregnancy progesterone levels for the mouse. Tissues are collected on day 17 post-immunization [4]. Real time PCR technology has demonstrated that progesterone blocks the EAE-induced increase of the proinflammatory mediators tumor necrosis factor alpha (TNFα) and its receptor TNFR1, the microglial marker CD11b and toll-like receptor 4 (TLR4) mRNAs, and increases the mRNA expression of PLP and MBP, the myelin transcription factors NKx2.2 and Olig1 and enhances CC1+ oligodendrocyte density respect of untreated EAE mice. Immunocytochemistry has demonstrated decreased Iba1+ microglial cells. Using doble immunofluorescence labelling and confocal microcoscopy, we have shown that TNFα colocalized with glial-fibrillary acidic protein+ astrocytes and OX-42 + microglial cells. Progesterone treatment also attenuates the clinical signs of EAE. Therefore, the decreased inflammatory glial reactivity and increased myelination from EAE mice receiving

progesterone, supports that steroid neuroprotection involves the modulation of transcriptional events in the spinal cord of EAE mice. It is hoped that experimental data provided by animal models of neurodegeneration and neuroinflammation open the ground for testing the usefulness of neuroactive steroids for the prognosis and treatment of human neurological disorders [5].

Reference list

[1] Schumacher M, Sitruk-Ware R, De Nicola AF. Progesterone and progestins: neuroprotection and myelin repair. Curr Opin Pharmacol. 2008;8 :740-746.
[2] De Nicola AF, Labombarda F, Deniselle MC, Gonzalez SL, Garay L, Meyer M, Gargiulo G, Guennoun R, Schumacher M. Progesterone neuroprotection in traumatic CNS injury and motoneuron degeneration. Front Neuroendocrinol. 2009 ;30:173-187.
[3] Gonzalez Deniselle MC, Carreras MC, Garay L, Gargiulo-Monachelli G, Meyer M, Poderoso JJ, De Nicola AF. Progesterone prevents mitochondrial dysfunction in the spinal cord of wobbler mice. J Neurochem. 2012;122 :185-195.
[4] Garay LI, González Deniselle MC, Brocca ME, Lima A, Roig P, De Nicola AF. Progesterone down-regulates spinal cord inflammatory mediators and increases myelination in experimental autoimmune encephalomyelitis. Neuroscience 2012 (in press).
[5] Gargiulo Monachelli G, Meyer M, Rodríguez GE, Garay LI, Sica RE, De Nicola AF, González Deniselle MC. Endogenous progesterone is associated to amyotrophic lateral sclerosis prognostic factors. Acta Neurol Scand. 2011;123 :60-67.

CROSS-TALK OF NEUROSTEROIDES AND NEUROTROPHINS IN CONTROLLING NEURONAL SURVIVAL

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Neurotrophins control brain development and maintenance during adulthood and in aging. They act through prosurvival tyrosine kinase Trk and pan-neurotrophin p75NTR receptors, exerting potent neuroprotective and neurogenic effects in various neurodegenerative diseases. Unluckily, their polypeptidic nature limits their therapeutic potential. We have recently shown that neurosteroid dehydroepiandrosterone (DHEA) binds with high affinity to NGF receptors (*Lazaridis et al, PLoS Biol 2011*). DHEA exert potent neuroprotective effects, inducing the expression of anti-apoptotic Bcl-2 proteins (*Charalampopoulos et al, PNAS 2004*). It appears that DHEA exerts at least part of its anti-apoptotic effects by directly interacting with TrkA and p75NTR receptors (Kd: 5-10 nM), efficiently inducing TrkA phosphorylation, and NGF receptor-mediated prosurvival signaling, resulting in the induction of neuroprotective mir21 and anti-apoptotic Bcl-2 proteins. This sequence of events prevents the apoptotic loss of NGF receptor positive sensory and sympathetic neurons in ngf -/- mice. However, DHEA is metabolized to estrogens and androgens, affecting the endocrine system and increasing the risk for hormone-dependent tumors. We have synthesized 17-spiro analogs of DHEA with strong neuroprotective properties (EC50 at nanomolar levels), which are deprived of endocrine effects (*Calogeropoulou et al, J Med Chem 2009, Gravanis et al, Science Signaling 2012*). These findings render the development of neurotrophin-like small molecules (microneurotrophins) a realistic immediate target. Microneurotrophins interact with NGF receptors; activate phosphorylation of TrkA and dissociation of RhoGDI from p75NTR receptor. They also rescue NGF-dependent embryonic sensory and sympathetic neurons of ngf-/- mice. Microneurotrophins are exert potent in vivo and in vitro neurogenic effects: they increase the number of BrdU positive neurons in the hippocampus of adult mice and induces self-renewal of neural stem cells, isolated from E14 mouse embryos. These findings suggest that neurosteroidal microneurotrophins may serve as lead molecules to develop CNS bioavailable neurotrophin-like small molecules with potential neuroprotective properties and applications in the treatment of neurodegenerative diseases.

ESTROGEN-IGF-1-miRNA INTERACTIONS IN STROKE NEUROPROTECTION

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Stroke is a leading cause of mortality and disability, and both the risk and severity of stroke are elevated in older women. The relative stroke protection observed in younger, premenopausal women is usually attributed to ovarian hormones, however, estrogen therapy to older women, paradoxically, increases their risk for stroke. We propose that the impact of estrogen therapy cannot be considered in isolation, but should instead include consideration of other endocrine mediators that collaborate with estrogen to produce neuroprotective effects in younger, relatively healthy populations, but are reduced or dysregulated in aging and in conditions that predispose stroke. Due to its modulation of ischemic cell death, the post-stroke inflammatory response, neuronal survival and regeneration, and general somatic health, IGF-1 may be a crucial biochemical marker that determines the neuroprotective "window" of hormone therapy. This is supported by our data that acyclic middle-aged female rats (that have low IGF-1 levels) sustain a more severe infarction as compared to adult female (with higher IGF-1 levels). Estrogen exacerbates stroke severity in the former while it is neuroprotective in younger females. Post-stroke IGF-1 treatment reinstates estrogen's protective effects on middle-aged females, while post stroke treatment with an IGF-1 receptor inhibitor abolishes estrogen's neuroprotective effects in young females. These data support the hypothesis that IGF-1 bioavailability modulates estrogen-mediated neuroprotection. Failure of the insulin/IGF-1 axis is associated with metabolic disease, which increases stroke risk and severity, and may be targeted by other endocrine regulators, acting independently or cooperatively with estrogen. Our current studies focus on a class of small non-coding RNA (microRNA) that act as translation repressors. Using a bioinformatics approach, miRNA with consensus sequences in the IGF-1 gene were identified and two of these miRNA, miR1 and Let7f were selected for further study. Both miR-1 and Let7f are elevated in middle-aged females as compared to young females. Antagomirs to each miRNA injected into animals 4 h post stroke improved infarct volume in the cortex (for miR-1) and improved both cortical and striatal volume in the case of Let7f. Anti-Let7f antagomirs had no effect on infarct volume in males or ovariectomized females, indicating that the hormonal environment modulates the effects of miRNA. Together, these studies show that estrogen/IGF-1 interactions mediate stroke neuroprotection and that age-associated changes in these hormones can impair stroke outcomes. Further, regulatory factors targeting IGF-1 availability, such as miRNA, offer new options for stroke neuroprotection

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CELL-SPECIFIC PROVISION OF ESTROGENS IN THE SONGBIRD BRAIN

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Steroids like estrogens have profound effects on the vertebrate brain during development and adulthood. In many vertebrates, including passerine songbirds, estrogens are made available to neural circuits from peripheral sources, but importantly, also by neural sources. In the zebra finch (*Taeniopygia guttata*), neural provision of estrogens is particularly high due the abundant and widespread expression of aromatase (estrogen synthase). Importantly, the aromatase transcript and protein are only detected in neurons. However, both the aromatase transcript and protein are rapidly and dramatically induced in astroglia (astrocytes and radial glia) in the songbird brain following mechanical damage or neuro-inflammation. Thus, while aromatase is constitutive in neurons, its expression is inducible in astroglia.

Inhibition of astrocytic aromatization increases, and central estradiol (E_2) replacement decreases apoptosis around sites of damage, respectively. Central E_2 provision also increases injury-induced neurogenesis within proliferative zones of the finch brain. Since bone-morphogenetic proteins, (BMPs) have established roles in neurogenesis and proliferation during vertebrate neurodevelopment, we probed the association between glial aromatization and BMPs following brain damage in the adult songbird. BMPs 1,2,3 & 4 were cloned and partially sequenced from finch brain. BMP2 transcription in injured hemispheres was found to be up-regulated, and this up-regulation was mitigated by ipsilateral aromatase inhibition. At the protein level, BMP2 is constitutively expressed in neurons at multiple areas of the passerine brain, but induced in microglia around the site of damage. Interestingly, antagonism of BMP4 with noggin, another signaling molecule critical in the differentiation of progenitor cells into neurons, dramatically increased lesion size following mechanical damage to the songbird brain. Thus, brain injury, the control of secondary damage, and repair, in the songbird brain may recruit developmental mechanisms critical in brain development. Finally, the dramatic role of astrocytic aromatization is underscored by the observation that the wave of secondary damage characteristic of the mammalian brain following multiple forms of neural insult, including stroke, is only revealed in the songbird following inhibition of induced aromatase in reactive astrocytes. The songbird continues to provide an excellent model towards understanding the role of hormones in the regulation of natural and pathological neuroplasticity.

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DEHYDROEPIANDROSTERONE REVERSE COCAINE-INDUCED DECREASE IN HIPPOCAMPUS NEUROGENESIS AND ATTENUATE REINSTATEMENT

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Drug addiction is a chronic brain disorder, characterized by loss of control over drug consumption. The neurobiology of addiction is traditionally thought to involve the reward system of the brain, the VTA–NAC circuit, as a key detector of the reward stimulus; however, the hippocampus receives renewed interest for its potential role in addiction. Part of this attention is due to the fact that drugs of abuse are potent negative regulators of the neurogenesis in the adult hippocampus.
The neurosteroid dehydroepiandrosterone (DHEA) affects brain-cells morphology and differentiation as well as neurotransmission. Recently, DHEA was suggested as a potential treatment to attenuate cocaine-seeking behavior and relapse, but its precise mechanism is not yet known.
Our first aim was to examine whether DHEA can attenuate cocaine seeking behavior during extinction and relapse. Next we tested the possibility that DHEA protects against the decreased neurogenesis in the dentate gyrus of the hippocampus, caused by cocaine consumption. Rats were trained (FR-1 schedule) to self-administer cocaine (0.13 ml/infusion; 1.5 mg/kg/20s) and number of lever responses was recorded. When stable maintenance was attained, rats underwent cocaine extinction. Each day, 90 min prior to placement in the operant chambers rats were injected with DHEA (2 mg/kg) or saline. Following the extinction phase, one group of rats was injected with 5-bromodeoxyuridine (BrdU 50 mg/kg) and 24 h or 28 days later they were euthanized and their brains stained with antibodies to BrdU and NeuN. Another group received a priming injection of cocaine (10 mg/kg, i.p.), 28 after the extinction phase.
DHEA treated rats showed significantly lower number of active lever presses compared to saline treated rats in the extinction and relapse phases. Quantification of BrdU+ cells and of BrdU+ NeuN+ cells in the in the dentate gyrus of all groups revealed significantly less cells and neurons in cocaine treated rats versus saline treated rats. This decrease in hippocampal proliferation and neurogenesis was normalized by treatments with DHEA [1][2].
In conclusion, DHEA treatment decreased cocaine-seeking behavior during extinction, decreased reinstatement to cocaine-seeking behavior and increased hippocampal newly-formed cells and neurons. Our postulation is that DHEA attenuation of cocaine seeking behavior may be due to its effect on neurogenesis.

Reference list

1. Sudai E, Croitoru O, Shaldubina A, Abraham L, Gispan I, Flaumenhaft Y, Roth-Deri I, Kinor N, Aharoni S, Ben-Tzion M, Yadid G. High cocaine dosage decreases neurogenesis in the hippocampus and impairs working memory. Addict Biol. 16, 251-60, 2011.
2. Yadid G, Sudai E, Maayan R, Gispan I, Weizman A. The role of dehydroepiandrosterone (DHEA) in drug-seeking behavior. Neurosci Biobehav Rev. 35, 303-14, 2010.

ANDROGEN RECEPTOR IS A THERAPEUTIC TARGET FOR MYELIN REPAIR

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In the present study, we demonstrated that testosterone treatment efficiently restores the myelin deficit by the formation of new myelin in a mouse brain affected by severe demyelination resulted from long-term administration of cuprizone, which is toxic for oligodendrocytes. In addition to myelin repair, the number of activated astrocytes and microglial cells was reduced to normal level, indicating a decrease of neuroinflammatory responses. We identify that the neural androgen receptor (AR) represents a novel therapeutic target for myelin repair. Also, the remyelination induced by testosterone can be mimicked by 5α-dihydrotestosterone (DHT). Reversibly, this action was blocked by the AR antagonist flutamide. Testosterone treatment failed to promote remyelination in demyelinated mice carrying the testicular feminization mutation. Moreover, testosterone failed to stimulate the myelin repair after specific knockout of the AR in neural cells (neurons and macroglial cells). Thus, the neural brain AR is required for testosterone-induced remyelination. The synthetic testosterone analog 7α-methyl-19-nortestosterone, which does not stimulate prostate growth, also efficiently promotes remyelination.

MONDAY, 18th February 2013
09.00 - 12.00

Mechanisms of rapid regulation of behaviour by estrogens

Mechanisms of rapid regulation of behaviour by estrogens

(Chairs: Cornil C.A., Panzica G.C.)

- **Srivastava D.P. (UK)** *Molecular mechanisms underlying rapid estrogenic-modulation of cortical connectivity*

- **Remage-Healey L. (USA)** *Rapid synthesis and action of estrogens within forebrain circuits in the context of behavior*

- **Cornil C.A. (Belgium)** *Rapid control of reproductive behavior by locally synthesized estrogens - focus on aromatase*

- Phan A., Ervin K., Gabor C.S., **Choleris E. (Canada)** *Rapid estrogenic regulation of social and non-social learning*

- Filová B., Malinová M., Bábíčková J., Tóthová Ľ., Ostatníková D., Celec P., **Hodosy J. (Slovakia)** *Non-genomic effect of steroid hormones on behavior*

- **Seredynski A.L.,** Balthazart J., Ball G.F., Cornil C.A. **(Belgium)** *Acute effects of putative membrane estrogen receptors agonists and antagonists on male sexual motivation in Japanese Quail*

MOLECULAR MECHANISMS UNDERLYING RAPID ESTROGENIC-MODULATION OF CORTICAL CONNECTIVITY

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There is increasing evidence that the regulation of structure and function of neuronal circuits is an essential component of normal cognitive function and behaviour. Indeed, multiple studies have demonstrated concurrent changes in connectivity between neurons during and following the acquisition of learned behaviours. Estrogens have repeatedly been shown to have powerful influences over cognitive function and behaviour, which is believed to be, in part, driven by estrogenic-regulation of neuronal connectivity. It is now becoming clear that estrogens have two modes of actions. In addition to the classic mode of action, which takes hours to days to manifest and relies on gene transcription, it is emerging that estrogens can act in a rapid manner, with effects occurring within minutes to hours. These rapid effects can result in the initiation of signalling pathways, leading to a number of cellular events, many of which are independent of gene transcription. Moreover, it is becoming clear that estrogens can also rapidly regulate specific behaviours. However, the molecular and cellular mechanisms that underlie this rapid regulation of behaviour have yet to be fully elucidated.

As the remodelling of cortical connectivity is believed to be an essential component of cognitive function, we have focused on understanding how estrogens can regulate dendritic spines, the site for the majority of excitatory synapses, on cortical neurons. We are currently investigating the signalling pathways that are initiated by acute estrogenic treatment, and are interested in how these pathways drive estrogen-dependent spine formation. In addition, we are interested in understanding which estrogen receptor(s) mediate the rapid (acute) actions of estrogens to the remodelling of dendritic spines on cortical neurons. By further elucidating the molecular underpinnings of rapid estrogenic-modulation of cortical connectivity, we hope to add to the growing understanding of how estrogens rapidly regulate behaviour.

RAPID SYNTHESIS AND ACTION OF ESTROGENS WITHIN FOREBRAIN CIRCUITS IN THE CONTEXT OF BEHAVIOR

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It is now clear that estrogens are not just circulating reproductive hormones, but that they also have neurotransmitter-like properties in a wide variety of brain circuits. The view of estrogens as intrinsic neurocircuit modulators that shape behavior has been bolstered by a series of recent developments from multiple vertebrate model systems. Here, I will describe several recent findings from studies of songbirds showing how the identified neural circuits that govern auditory processing, sensorimotor integration and motor output (song) are modulated by the local and acute production of estrogens. First, studies using in vivo microdialysis demonstrate that estrogens fluctuate in auditory cortex (30-min time bin resolution) when songbirds are hearing song and interacting with conspecifics. Second, estrogens rapidly boost the auditory-evoked activity of neurons in the same auditory cortical region, enhancing auditory processing. Third, local pharmacological blockade of estrogen signaling in this region impairs auditory neuronal responsiveness as well as behavioral song preferences. Lastly, the rapid estrogen actions that occur within the auditory cortex can propagate upstream (transsynaptically) to sensorimotor and premotor circuits to enhance the neural representation of song. Together, these and other emergent studies provide support for the critical roles for rapid neuro-estrogen signaling in sensorimotor integration, learning, perception and cognition. These findings are observed in both males and females, and the general implications and possible compensatory mechanisms will be discussed.

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RAPID CONTROL OF REPRODUCTIVE BEHAVIOR BY LOCALLY SYNTHESIZED ESTROGENS – FOCUS ON AROMATASE

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Estrogens are generally considered as slow acting messengers that exert a long-term control over physiology and behavior through the transcriptional activity of their liganded nuclear receptors [5]. Along with these genomic effects, estrogens also activate a large variety of cellular signaling pathways through membrane-initiated events that are too rapid to result from de novo protein synthesis [6]. Evidence had recently accumulated indicating that these cellular effects translate into acute changes at the organismal level [1]. Interestingly, however, the origin of the estrogens able to trigger these non-genomic actions is rarely examined. Moreover, genomic and non-genomic actions of estrogens are often studied independently such that we do not exactly know how they cooperate to control a same physiological or behavioral response. Recent data addressed these issues in the context of the study of male sexual behavior in Japanese quail.

A first series of experiments showed that the central administration of estradiol rapidly facilitates sexual motivation in male Japanese quail whose sexual behavior had been inhibited by a chronic deprivation of estrogens (chronic treatment with an aromatase inhibitor) [4]. This acute effect of estradiol was mimicked by an injection of the membrane-impermeant estradiol analog, estradiol-BSA. However, no such effect of estradiol was observed on copulatory behavior, all subjects remaining sexually inactive. Conversely, the acute blockade of estradiol's action (by estrogen receptor antagonists) or synthesis (by aromatase inhibitors) rapidly impaired sexual motivation but not performance [4]. This effect on sexual motivation was prevented by the co-administration of estradiol or the membrane-impermeant estradiol analog, estradiol-biotin, along with the aromatase inhibitor suggesting it is initiated at the cell membrane. These results indicate that estrogens have evolved complementary mechanisms (non-genomic vs genomic) acting in different time frames (short- vs long-term) to control different components (motivation vs performance) of the same behavioral response and improve reproductive fitness.

In parallel, we showed that the enzymatic activity of aromatase, the limiting enzyme for estrogen synthesis, rapidly changes in a brain-, sex- and stimulus-dependent manner following changes in the social or environmental context [2,3]. Specifically, copulation results in a rapid and reversible inhibition of aromatase activity. Interestingly, this response appears to depend on the female's presence suggesting that it may reflect changes in the motivational state of the males. These data thus demonstrate that brain aromatase activity is rapidly modulated in vivo providing a mechanism of rapid control of local estrogen provision with a spatial and time resolution compatible with the rapid effects observed on behavior.

Together, these data support the idea that brain derived estrogens should be considered as neuromodulators. Moreover, given the critical role played by aromatase in the control of social behavior, it is likely that this general observation extends to other behavioral systems.

Reference list

1. Cornil CA, Ball GF, Balthazart J. Rapid control of male typical behaviors by brain-derived estrogens. *Front Neuroendocrinol*. 2012; 33(4): 425-46.

2. de Bournonville C, Dickens MJ, Ball GF, Balthazart J, Cornil CA. Dynamic changes in brain aromatase activity following sexual interactions in males: where, when and why? *Psychoneuroendocrinology*. In press. doi : 10.1016/J.Psyneuen.2012.09.001.
3. Dickens MJ., Cornil CA., Balthazart J. Acute stress differentially affects aromatase activity in specific brain nuclei of adult male and female quail. Endocrinology. 2011 ; 152(11) : 4242-51
4. Seredynski AL, Balthazart J, Christophe VJ, Ball GF, Cornil, CA. Neuroestrogens rapidly regulate sexual motivation but not performance. *J Neurosci*. In press. doi: 10.1523/Jneurosci.2557-12.2012.
5. Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 1994; 63:451– 486.
6. Vasudevan N, Pfaff DW. Non-genomic actions of estrogens and their interaction with genomic actions in the brain. *Front Neuroendocrinol*. 2008; 29(2): 238-57

RAPID ESTROGENIC REGULATION OF SOCIAL AND NON-SOCIAL LEARNING

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Steroid hormones have been traditionally considered transcription regulators of gene expression, this action taking hours to days to show behavioral effects. In more recent years rapid actions, taking place within minutes of administration, have also been demonstrated. We have been investigating the rapid effects of estrogen and its receptors ERalpha, ERbeta and the G-protein coupled estrogen receptor (GPER) in various learning paradigms in female mice. These paradigms measure different types of learning and include social recognition, object recognition, object location and social learning paradigms. Social and object recognition assess recognition memory of a social or a non-social stimulus respectively. In social recognition when mice are given a choice between investigating a familiar or an unfamiliar conspecific, they will typically investigate the novel social stimulus longer than the familiar stimulus, indicating they recognize the familiar mouse. In a similar choice test, mice that recognize previously encountered objects will preferentially investigate novel over familiar objects. The object location test assesses spatial learning and memory; when mice are given a choice between two familiar objects one of which has been moved to a novel location, they will preferentially investigate the displaced object over one that has not been moved. We also investigated social learning, where an individual acquires novel adaptive information from a conspecific, using the social transmission of food preferences paradigm, where an "observer" mouse acquires information about the diet through a social interaction with a recently fed "demonstrator" conspecific. When given a choice between two novel diets the observer will preferentially consume the diet it previously smelled on the breath of its demonstrator. For each of these paradigms we have developed versions that enable the assessment of rapid hormonal effects, with the mice learning and testing occurring within 40 min or less from the time of hormone or drug administration.

We have found that systemic administration of 17-beta estradiol to ovariectomized mice rapidly enhances performance in each paradigm [3]. However, this effect appears to be differently mediated by ERalpha, ERbeta and GPER in the various learning tasks. Systemic treatment with the selective ERalpha agonist propyl pyrazole triol (PPT) rapidly enhanced performance in the social recognition, object recognition, and object location test [4], but impaired the social transmission of food preferences [1]. Similarly, systemic GPER activation with the selective agonist G1 rapidly enhanced social recognition, object recognition and object placement learning [2]. However, G1 either impaired or enhanced social learning in the social transmission of food preferences paradigm, depending upon the duration of the social exposure to the food and post-treatment timing [1, 2]]. Conversely, systemic administration of the ERbeta selective agonist diarylpropionitrile (DPN) rapidly impaired social recognition at higher doses and had no effect on object recognition and social learning [1, 4].

Systemic treatment with estradiol also rapidly induced the formation of new dendritic spines in the CA1 region of the hippocampus, as measured with Golgi staining [3]. Similarly, systemic treatment with ERalpha agonist PPT and GPER agonist G1 resulted in increases of dendritic spine density in the CA1 hippocampus [2, 4]. Conversely, systemic administration of ERbeta agonist DPN decreased dendritic spine density [4]. These results support the notion that estrogens can rapidly affect brain plasticity via the ERalpha and

GPER receptors and point at the hippocampus as one possible site of rapid estrogenic action on learning and memory.
In subsequent investigations using brain area-specific administrations via implanted guide cannulae we showed that intrahippocampal administration of 17-beta estradiol improved performance in the social recognition, object recognition and object location learning paradigms within 40min of infusion [5]. In view of the known involvement of the hippocampus in spatial learning and memory we subsequently investigated whether spatial and contextual cues are necessary for the hippocampus to mediate estradiol's rapid facilitation of social and object recognition. Using a Y-apparatus, which minimizes the spatial and contextual information available to mice, we found intrahippocampal 17β-estradiol still enhanced object recognition performance, but not social recognition [5]. Therefore, in the hippocampus estradiol appears to be able to directly improve object recognition and placement learning, but it may only indirectly facilitate social recognition *via* enhanced processing of spatial contextual information. Other brain areas are likely to be responsible for estradiol's direct rapid enhancing effects on social recognition. On going investigations are assessing the effects of intrahippocampal estradiol in the social transmission of food preferences and of the estrogen receptor alpha, beta and GPER specific agonists on social recognition, object recognition, object location and social learning paradigms.
Taken together, the results of these investigations show a clear facilitatory role for the rapid actions of estrogens on learning and memory. They also show that these effects are receptor- and task-dependent. Overall, it appears that social recognition, object recognition and object location recognition are similarly affected by treatments with selective estrogen receptor agonists, while social learning is often affected in opposite or different manners, suggesting the interplay of multiple factors in the regulation of learning when it requires a social context. These investigations also suggest that rapid ERbeta involvement in learning is often different than that of the other estrogen receptors, ERalpha and GPER. Generally, we find that while ERalpha and GPER1 seem to have strong rapid effects on learning, the involvement of ERbeta is less profound, possibly modulatory, rather than regulatory.

References list

1. Ervin K, Friesen J, Gallagher N, Roussel V, Zicherman J, Clipperton Allen A, Phan A, Choleris E (2012). Estrogens rapidly modulate performance on a social learning task in mice. Society for Neuroscience Abstracts, 92.14.
2. Gabor CS, Lymer J, Systerova U, Phan A, Choleris E (2011). Rapid effects of estrogen receptor GPER/GPR30 agonist G-1 on learning and memory in female mice. Society for Neuroscience Abstracts, 728.03.
3. Phan A, Gabor CS, Favaro KJ, Kaschak SL, Armstrong JN, MacLusky NJ, Choleris E (2012). Low doses of 17beta-estradiol rapidly improve learning and increase hippocampal dendritic spines. Neuropsychopharmacology 37: 2299–2309.
4. Phan A, Lancaster KE, Armstrong JN, MacLusky N, Choleris E (2011). Rapid effects of estrogen receptor alpha and beta selective agonists on learning and dendritic spines in female mice. Endocrinology 152(4): 1492–1502.
5. Phan A, Suschkov S, Pecchioli N, Seguin L, Winters BD, Choleris E (2012). Estrogen's rapid effects on learning are mediated by the CA1 hippocampus. Society for Neuroscience Abstracts, 92.12.

NON-GENOMIC EFFECT OF STEROID HORMONES ON BEHAVIOR

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Introduction. The effects of sex hormones are largely mediated by intracellular receptors. These genomic effects of sex hormones are relatively slow and their influence manifests itself after one hour to several days. In contrast to the slow genomic effects of steroids, their quick reactions that are too fast for the activation of DNA transcription and translation (within few seconds to minutes) were also described. These effects are referred to as non-genomic effects. Non-genomic activity of steroids causes rapid introduction of conventional second messenger signal transduction cascades, including the rapid increase in intracellular calcium concentration and activation of protein kinase A (PKA), protein kinase C (PKC) or MAPK.
Effects of testosterone and its metabolites 5α-dihydrotestosterone and estradiol on behaviour are not well known. So far regarding the sex hormone research on behaviour, controversies exist, and these might be due to different approach to the behavioural testing. Although doubts about the existence of non-genomic effects of steroids have disappeared, their role in behaviour change is still not understood. The aim of our study was to examine the rapid effects of testosterone and estradiol on anxiety and depressive behaviour in male rats.
Materials and Methods. This study was performed on 30 male Wistar rats (AnLab Prague, Czech Republic). Rats were housed in groups of 6 animals on a 12 hrs light: 12 hrs dark cycle (lights on at 07:00 h), at a temperature of 25 ± 2°C and 55 ± 10% humidity. Food and water were available *ad libitum* in their home cages. Rats were divided into 4 groups: control sham males (n =8), castrated males (n = 8), castrated males treated with testosterone (n = 7) and castrated males treated with estradiol (n = 7). At the age of 15 weeks, animals were castrated or sham operated. One week after the surgery sham castrated animals were treated intramuscularly with olive oil (Oleum Olivae, VULM SK s.r.o, Slovakia, 100 µl; n = 8) and castrated groups were treated with either olive oil (n = 8), testosterone (5 mg/kg; Testosterone propionate T1875, SigmaAldrich LLC, USA; n = 7) or estradiol (0.5 mg/kg; 17-β estradiol propionate, 46556 Fluka, SigmaAldrich LLC, USA; n = 7). Five minutes after the application of olive oil, testosterone or estradiol, rats underwent a series of behavioural test battery in subsequent order: Open field test (5 min), Novel object recognition test (5 min), Light/Dark box (5 min) and Forced swim test (3 min). All tests were performed within 30 minutes and after the last testing, blood samples from the tail vein were collected. To determine the concentration of testosterone and estradiol in plasma, we have used commercial ELISA kits (DRG Diagnostics, Marburg, Germany).
Results. We have found significantly lower testosterone levels in castrated group and E group, when compared to the control group ($p<0.01$). On contrary, not castrated but T group have significantly higher testosterone levels in plasma ($p<0.001$) Significantly higher level of estradiol was observed in E group in comparison with control ($p < 0.001$), castrated groups and T group). In open field test, group treated with estradiol spent more time in the central zone in comparison with castrated and testosterone group (Fig. 4,

$p<0.05$). No differences with control group were observed. In the test of preference of light and darkness (light/dark box) we confirmed that testosterone and estradiol showed anxiogenic anxiety effect. T group ($p<0.05$) and the E group ($p<0.05$) spent less time in the light zone compared to the control group and castrated group. We have found no significant differences in simple novelty object recognition (Fig. 5) and in forced swim test (Fig. 6) between groups. However, there is sign of depressive behaviour in the testosterone group, as evidenced by immobility.

Conclusion. In conclusion, we observed anxiolytic behaviour and a sign of depressive-like behaviour in castrated male rats, immediately after testosterone application. The influence of rapid effect of estradiol on anxiety has differed in depending of the used test. Further studies using a larger number of animals per group should help with reproduction of results, reducing the interindividual variability and description non-genomic effect of steroids in females.

ACUTE EFFECTS OF PUTATIVE MEMBRANE ESTROGEN RECEPTORS AGONISTS AND ANTAGONISTS ON MALE SEXUAL MOTIVATION IN JAPANESE QUAIL

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Genomic effects of estradiol (E_2) are mediated by transcriptional actions of nuclear estrogen receptors (ERs) bound to a ligand that occur within hours to days (1). In addition, E_2 modulates cell functions within minutes through non-genomic actions involving membrane-associated receptors (mERs) (2). These cellular effects can result in the modulation of behavioral processes including male sexual behavior (3,4). Male sexual behavior is divided into an appetitive component that is indicative of the motivational aspect and a consummatory component, which represents the actual performance of the male and its ability to copulate with a female. We previously showed that blocking the action of estrogens or their synthesis by a single intracereboventricular injection of estrogen receptor antagonists or aromatase inhibitors respectively decreases sexual motivation (appetitive behavior) within minutes without affecting performance (consummatory behavior) (5). However, the specific receptors mediating these behavioral responses are largely unknown. Several putative membrane estrogen receptors have been proposed (6). First, classical ERs (ERα and ERβ) can mediate rapid E_2 actions. Second, the G protein-coupled receptor 30 (GPR30) has also been shown to activate estrogen-dependent signaling pathways in breast cancer cells that lack known ERs and in neuronal cells. Third, two mERs with a yet undefined molecular structure have been described: ER-X which activates estrogen-dependent signaling pathways in αERKO and Gq-mER which activates estrogen-dependent signaling pathways in αβERKO mice and in GPR30 knockout mice.

The aim of this study was to determine which putative mERs are involved in the rapid modulation of male sexual motivation by estrogens. We tested whether acute intracerebroventricular injections of various mERs agonists (the ERα-specific agonist PPT, the ERβ-specific agonist DPN, the GPR30-specific agonist G1, the ER-X-specific agonist 17α-estradiol and the Gq-mER-specific agonist STX) would facilitate sexual motivation after its transient inhibition by an acute injection of the aromatase inhibitor Vorozole™ in castrated male Japanese quail chronically treated with a testosterone implant (CX+T). Additionally, we tested whether acute intracerebroventricular injections of mERs antagonists (the ERα-specific antagonist MPP and the GPR30-specific antagonist G15) would alter sexual motivation in castrated male Japanese quail similarly treated with exogenous testosterone (CX+T). Sexual motivation was assessed by the frequency of rhythmic cloacal sphincter movements (RCSM) (5). All copulatory tests were performed in a large arena so that deficits in sexual motivation would additionally be reflected in a decrease of sexual performance as reported previously (5). Birds were assigned to different sub-groups (2 or 3 sub-groups) and pre-tested after a vehicle injection to insure that they were in the appropriate conditions and behaviorally similar. These sub-groups were then repeatedly tested in a counterbalanced order on different days (tests every three days) with different drugs. To make sure that the treatments did not exert long lasting effects on the behavior due to activation of genomic actions, all birds were post-tested after a vehicle injection. In each experiment, results obtained in pre- and post-tests were compared with

paired-samples t-tests. The effects of experimental treatments were analyzed by two-way ANOVAs with treatments as the repeated factor and their order (2 or 3 sub-groups) as the independent factor in order to exclude the possibility of long-term effects of treatments that would interfere with the short-term effects under investigation. When significant, these ANOVAs were followed by post-hoc Fisher LSD, Newman-Keuls' tests or HSD Tukey's tests (as appropriate depending on the number of comparisons) comparing all conditions.
As previously shown (5), acute treatment with vorozole inhibited both appetitive and consummatory aspects of male sexual behavior. This inhibition was prevented by the administration of DPN, the ERβ-specific agonist (see Table 1). The activation of ERβ

Membrane receptors	Agonist (+)/ antagonists (-)	Appetitive behavior	Consummatory behavior
ERα	PPT (+)	–	–
	MPP (-)	–	–
ERβ	DPN (+)	↑	↑
GPR30	G1 (+)	–	–
	G15 (-)	–	–
Gq-mER	STX (+)	–	–
ER-X	17α-estradiol (+)	↑	↑

Table 1: Effects of membrane estrogen receptors agonists and antagonists on appetitive and consummatory sexual behavior in male quail.

almost doubled the frequency of appetitive and consummatory sexual behavior within 15 min. No effects were observed when we tested the ERα-specific agonist and antagonist on both aspects of male sexual behavior. Similar negative results were obtained for GPR30 and the Gq-mER. 17α-estradiol, possibly acting through ER-X, increased significantly the frequency of appetitive and consummatory sexual behavior. Together, these results confirm that brain-derived estrogens rapidly modulate male sexual motivation. This effect seems to be mediated through membrane-associated ERβ and perhaps also ER-X. This receptor specificity thus appears to be different from the specificity reported for the genomic effects of estrogens on male sexual behavior. Further work on this and other experimental models will be needed to determine whether this difference between genomic and membrane-initiated effects of estrogens has or not a general character.

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References list

1. Tsai M-J, O'Malley BW (1994) Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annual Reviews of Biochemistry 63: 451-486.
2. Micevych P, Dominguez R (2009) Membrane estradiol signaling in the brain. Frontiers in Neuroendocrinology 30:315-327.
3. Cross E, Rosseli CE (1999) 17β-estradiol rapidly facilitates chemioinvestigation and mounting in castrated male rats. American Journal of Physiology 276:R1346-R1350.
4. Cornil CA, Taziaux M, Baillien M, Ball GF, Balthazart J (2006) Rapid effects of aromatase inhibition on male reproductive behaviors in Japanese quail. Hormones and Behavior 49:45-67.
5. Seredynski AL, Balthazart J, Christophe V, Ball GF, Cornil CA (in press) Neuro-estrogens rapidly regulate sexual motivation but not performance. Journal of Neuroscience.
6. Cornil CA, Ball GF, Balthazart J (2012) Rapid control of male typical behaviors by brain-derived estrogens. Frontiers in Neuroendocrinology (4): 425-46.

MONDAY, 18th February 2013
12.00 - 13.00

Plenary Lecture:

Galea L.A.M. (Canada)

WHY SEX AND SEX HORMONES MATTER FOR BRAIN HEALTH: STEROID HORMONE MODULATION OF COGNITION, EMOTION AND HIPPOCAMPAL NEUROPLASTICITY

Galea L.A.M.

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Sex differences exist in neuroplasticity and the prevalence and symptoms of some neuropsychiatric and neurodegenerative disorders, such as depression and Alzheimer's disease. Anytime a sex difference is observed this suggests that gonadal hormones are involved. As anyone who has gone through adolescence, pregnancy or menopause can attest hormones can exert powerful effects on brain and behaviour. My laboratory has focussed primarily on three main areas of research: how motherhood, stress and sex hormones affect neuroplasticity, cognition and emotional behaviours. I will talk about how steroid hormones affect hippocampal neurogenesis differently in males and females, how motherhood affects neurogenesis and the relationship between hormones and neurogenesis, the effects of steroid hormones on hippocampus-dependent cognition and in animal models of depression. For example ttestosterone and DHT upregulate neurogenesis in the adult male, but not female, rat (Spritzer & Galea, 2007) while estradiol upregulates cell proliferation in the female, but not male, rats (Barker & Galea, 2008). Males, but not females, show an acute stress-induced suppression of cell proliferation (Falconer & Galea, 2003) and a greater response to corticosterone-induced suppression in neurogenesis, although this effect varies across the lifespan (Barha et al., 2011; Brummelte & Galea, 2010). Neurogenesis in the hippocampus may be related to hippocampus dependent learning and memory and we have found that estradiol has a dissociable effect on differential memory processes, with higher levels impairing reference and working memory, and lower levels facilitating working memory in hippocampus-dependent tasks (Galea et al., 2001; Holmes et al., 2002). Furthermore evidence suggests that estradiol modulates effort-based decision making (Uban et al, 2012) and context fear conditioning (Barha et al., 2010) in female rodents. Reproductive experience (pregnancy and mothering) affects both hippocampus-dependent learning and memory and neurogenesis long after weaning (Barha and Galea, 2011; Pawluski et al., 2009). Recently we found that reproductive experience modulates the ability of the hippocampus to respond to ovarian hormones in middle age (Barha and Galea, 2011). Motherhood itself is associated with increased risk to develop anxiety and depression. Indeed, the time of greatest risk to develop depression in a women's lifetime is during the postpartum. Thus we created two animal models of postpartum depression (PPD). In the ovarian hormone withdrawal model, withdrawal from a hormone simulated pregnancy increased depressive-like behavior (Galea et al., 2001). In our corticosterone (CORT)-induced model, high chronic levels of CORT during the postpartum increased depressive-like behavior in the dams and increased anxiety-like behavior in the offspring (Brummelte et al., 2006). Importantly, the hippocampus is vulnerable to the effects of stress and is implicated in depression; depressed patients have a smaller hippocampus which is related to the duration of disease. Animal models of depression, including our own, show reduced neurogenesis and synaptic plasticity in the hippocampus. Together these studies show that 1) sexes differ in neuroplasticity and behaviour in response to steroid hormones; and that 2) females have a unique physiology that renders them more vulnerable to stress-related disorders such as depression and perhaps Alzheimer's disease.

Reference list

Barha CK, Brummelte S, Lieblich SE, Galea LAM. (2011). Chronic restraint stress in adolescence differentially influences HPA axis function and adult hippocampal neurogenesis in male and female rats. Hippocampus 21:1216-1227

Barha CK, Dalton GL, Galea LAM. (2010). Low doses of 17α-estradiol and 17β-estradiol facilitate, while higher doses of estrone and 17α- and 17β-estradiol impair, contextual fear conditioning in adult female rats. Neuropsychopharmacology, 35, 547-559.

Barha CK, Galea LAM. (2011) Motherhood alters the cellular response to estrogens in the hippocampus later in life. Neurobiology of Aging, 32:2091-2095.

Barker JM, Galea LAM. (2008). Repeated estradiol administration increases cell proliferation and decreases cell death in female, but not male, rats. Neuroscience, 152, 888-902.

Brummelte S, Pawluski JL, Galea LAM. (2006).High postpartum levels of corticosterone given to dams influence postnatal neurogenesis and behaviour of offspring: A model of post-partum stress and possible depression. Hormones and Behavior, 50, 370-382.

Falconer EM, Galea LAM. (2003). Sex differences in defensive behaviors, cell proliferation and survival in the dentate gyrus in response to acute predator odor stress. Brain Research, 975, 22-36.

Galea LAM, Wide J, Barr A. (2001). Estradiol alleviates depressive-like symptoms in a novel animal model of post-partum depression. Behavioral Brain Research, 122, 1-9

Galea, L.A.M., Wide, J.K., Paine, T.A., Holmes, M.M., Ormerod, B.K. & Floresco, S.B.(2001). High levels of estradiol disrupt conditioned place preference learning, stimulus response learning and reference memory but have no effects on working memory. Behavioral Brain Research, 126, 115-126.

Holmes, M.M., Wide, J.K. & Galea, L.A.M. (2002). Low levels of estradiol facilitate while high levels of estradiol impair working memory performance in the working/reference memory version of the radial arm maze. Behavioral Neuroscience, 116, 928-934.

Pawluski, J.L., Brummelte, S., Barha, C.K., Crozier,T.M., Galea L.A.M. (2009). Effects of steroid hormones on neurogenesis in the hippocampus of the adult female rodent during the estrous cycle, pregnancy, lactation and aging. Frontiers in Neuroendocrinology, 30, 343-357.

Spritzer MD, Galea LAM. (2007). Testosterone and Dihydrotestosterone, but not Estradiol, Enhance Hippocampal Neurogenesis in Adult Male Rats. Developmental Neurobiology, 67, 1321-1333.

Uban KA, Rummel J, Floresco SB, Galea LAM. (2012). Estradiol Modulates Effort-Based Decision Making in Female Rats. Neuropsychopharmacology, 37: 390-401.

MONDAY, 18th February 2013
16.00 - 18.00

Aging

Aging

(Chairs: Garcia-Segura L.M., Schumacher M.)

- **Urbanski H.F. (USA)** *Regulation of neurosteroidogenesis in the nonhuman primate*
- **Gibson G. (UK)** *Progesterone and ischemia in ageing mice*
- **Hogervorst E. (UK)** *Effects of gonadal hormones on cognitive behavior in aged men and women*
- **Morrison J.H.,** Hao J., Hara Y., Dumitriu D., Wang A.C.J., Bailey M.E., Bloss E.B., Ohm D.T., Janssen W.G.M., Yuk F., Puri R., Baxter M.G., Rapp P.R. **(USA)**, *Synaptic correlates of cognitive performance: Implications for cognitive aging and menopause*

NEUROSTEROIDOGENESIS IN THE AGING NONHUMAN PRIMATE

Urbanski H.F.

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Like humans, rhesus macaques (*Macaca mulatta*) are large, long-lived diurnal primates, and show similar age-related changes in the secretion of many steroid hormones, including estradiol, testosterone, cortsiol and dehydroepiandrosterone (DHEA). Consequently, they represent a pragmatic animal model in which to examine the mechanisms by which these steroidal changes contribute to perturbed sleep-wake cycles and cognitive decline in the elderly. Using remote serial blood sampling we have found the circulating levels of DHEA, as well as estradiol and testosterone, decline markedly in old monkeys [1,2,5,6]. Furthermore, using real-time PCR, we have shown that genes associated with the conversion of DHEA to estradiol and testosterone (e.g., *3BHSD*, *17BHSD*, and aromatase) are highly expressed in brain areas associated with cognition and behavior, including the hippocampus, prefrontal cortex and amygdala [5,6]. Taken together, these findings suggest that administration of supplementary DHEA in the elderly may have therapeutic potential for cognitive and behavioral disorders, but with fewer negative side effects outside of the central nervous system. To test this we have developed a novel steroid supplementation paradigm for use in old animals; this involves oral administration of DHEA and testosterone at physiologically-relevant times of the day to mimicking the circadian hormone patterns observed in young adults [4]. We are currently evaluating the efficacy of this steroid supplementation paradigm at reversing age-associated disorders, including perturbed sleep-wake cycles, cognitive decline as well as impaired immune response.

Reference list

[1] Downs JL, Urbanski HF (2006). Neuroendocrine changes in the aging reproductive axis of female rhesus macaques (macacaca mulatta). Biology of Reproduction, 75:539-546.
[2] Downs JL, Mattison JA, Ingram DK, Urbanski HF (2008). Effect of age and caloric restriction on circadian adrenal steroid rhythms in rhesus macaques. Neurobiology of Aging, 29:1412-1422.
[3] Sorwell K, Urbanski HF (2010). Dehydroepiandrosterone and age-related cognitive decline. Age (Dordr), 32:61-67.
[4] Sorwell KG, Garten J, Renner L, Weiss A, Garyfallou VT, Kohama SG, Neuringer M, Urbanski HF (2012). Hormone supplementation during aging: how much and when? Rejuvenation Research, 15:128-131.
[5] Sorwell KG, Kohama SG, Urbanski HF (2012). Perimenopausal regulation of steroidogenesis in the nonhuman primate. Neurobiology of Aging, 33:1487.E1-1487.E13.
[6] Urbanski HF, Sorwell K (2012). Age-related changes in neuroendocrine rhythmic function in the rhesus macaque. Age (Dodr), 34:1111-1121.

PROGESTERONE AND ISCHEMIA IN AGING MICE

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Cerebral stroke continues to kill 5.5 million people worldwide per annum and is the leading cause of long-term disability in countries such as the UK. The total incidence of stroke is projected to rise substantially over the next 20 years due to the rising elderly population. Although age is one of the most significant prognostic markers for poor outcome following stroke very few studies are conducted in aged animals. Importantly, gender differences in both vulnerability to stroke and outcome following cerebral ischemia have frequently been reported and attributed to the action of steroid hormones. Progesterone is a candidate neuroprotective factor for stroke yet the majority of pre-clinical studies have focused on using young, adult animals. In terms of cerebral stroke, postmenopausal females, along with males, represent the group at highest risk of cerebral stroke and can be modelled using either aged or ovariectomized female animals. Data will be presented from experiments where the neuroprotective effect of progesterone administration following cerebral ischemia in aged and ovariectomized mice was examined. In addition, we will focus on reviewing the studies have that have been conducted to date examining the neuroprotective potential of progesterone in aged animals. In our experiments, female (aged or ovariectomized) mice underwent 60 minutes of middle cerebral artery occlusion (MCAO) and were administered progesterone (or vehicle) at 1h, 6h and 24h post-MCAO. At 48h post-MCAO, progesterone significantly reduced lesion volume but had no effect on neurological score in aged female mice. However, in ovariectomized mice, at 48h post-MCAO, progesterone had no effect on lesion volume but did improve neurological function. In a further study of ovariectomized mice, allowed to survive for 7 days post-MCAO, progesterone treatment significantly improved motor outcome as assessed using both the grid and rotarod test. In fact, by 7 days post-MCAO, progesterone-treated ovariectomized mice did not differ significantly in performance with shams, whereas vehicle-treated ovariectomized mice displayed a significant functional impairment following ischemia. Our data demonstrated that progesterone has different neuroprotective effects whether it is administered to aged or ovariectomized female mice thus suggesting that ovariectomized mice cannot always be used as a substitute for aged animals. In addition, when pre-clinical stroke research is considering the efficacy of potential neuroprotective therapies it should take into account both age and gender in order to confirm a neuroprotective benefit as efficacy may vary across groups.

EFFECTS OF GONADAL HORMONES ON COGNITIVE BEHAVIOR IN AGED MEN AND WOMEN

Hogervorst E.

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Over the last four decades, animal and cell culture studies have shown that sex steroids can have manifest protective effects on the aging brain. Estrogens and testosterone can affect most of the markers of brain pathology, such as those associated with vascular and Alzheimer's disease either directly (e.g. by acting on amyloid toxicity) or indirectly. Both steroids have been associated with most of the mechanisms thought to be responsible for cognitive decline and dementia which risks increase with age.

In sharp contrast to these positive findings, our reviews show that estrogen treatment can exert negative effects on cognitive function in women over 65 years of age, when treatment duration exceeds one year. While the 'Window of opportunity theory' suggests that this may be because these women are too far beyond the age at menopause, some short term positive effects (2-4 months) were also found in women with dementia, who on average would be at least 15 years beyond the average age at menopause. Overall, we mainly found time limited positive treatment effects, with the largest cognitive effects in women who had undergone surgical menopause. These data reflect those of animal studies and 3 human observational studies (US, China, Denmark) showing that women who undergo surgical menopause without treatment have an increased risk for cognitive decline and dementia. However, this may be related to their earlier age at menopause. In Indonesia we found that (independent of genetic polymorphisms associated in other studies with early menopause) if natural menopause occurred age 47 or before, women had an increased risk for memory dysfunction. In the Oxford Project To Investigate Memory and Ageing, we also found that associations of estrogen levels with a higher risk for dementia in older women was independent of genetic polymorphisms associated with estrogen synthesis and metabolism.

Small treatment trials focusing on cognitive improvement using testosterone were usually done in combination with estrogens in relatively recent menopausal women and were only short term. These limited trials suggested added value of testosterone for improving executive functions. It is unclear whether long term exposure to higher testosterone levels at an older age might confer risk for dementia and accelerated cognitive decline in older women, as is thought to be the case for estrogens. For instance, in MRC Foresight Challenge in Oxfordshire, in older women high testosterone levels also had a negative association with memory and speed of information processing.

Older men in general did not show positive associations of high estrogen levels with cognition and in several studies also showed increased risk for cognitive dysfunction and dementia with higher estrogen levels. For instance, in the MRC Foresight Challenge cohort we reported negative associations for older men between high estrogen levels and verbal memory. Meta-analyses suggested that hypogonadal older men were also not found to benefit from testosterone treatment. On the other hand, this may be different for eugonadal men where some limited positive effects of testosterone treatment have been found. In addition, our observational data showed that optimal testosterone levels at baseline were

associated with better global cognitive function in healthy elderly from Nottingham (MRC CFAS substudy) and protected against a sharp drop in cognitive function after a 2 year follow-up. In older women, however, again the reverse was found with higher testosterone levels at baseline conferring risk for cognitive decline. Professor Brinton Diaz showed in her 'healthy cell bias' theory how estrogens confer protective effects to healthy neurons but that cells undergoing pathological change show acceleration in their demise when exposed to estrogens. Most of testosterone is converted to estrogens in the brain. Age, health status of cells, duration of treatment and gender may thus modify effects of sex steroid treatment on brain function. In this talk we will discuss associations of levels of sex steroids with brain function in elderly men and women.

Reference list

Hogervorst, E. (in press) Estrogen and the brain: does estrogen treatment improve cognitive function? Invited review for special issue Menopause International

Hogervorst, E. (2012). Prevention of dementia with sex hormones: can androgens be an alternative to improve cognition in postmenopausal women. Minerva Medica(invited paper for special issue)

Holland, J., Bandelow, S., Hogervorst, E., (2011). Testosterone levels and cognition in elderly men: a review Maturitas. 2011 Aug;69(4):322-37. Epub 2011 Jun 2 (IF 2.03) invited

Thornton V, Warden D, Talbot C, S S Mastana, Bandelow S, Hogervorst E. (2011) Modification of estrogen's association with Alzheimer's disease risk by genetic polymorphisms. Brain Res. Mar 16;1379:213-23.

Hogervorst. E. & Bandelow, S. Sex steroids to maintain cognitive function in women after the menopause: A meta-analyses of treatment trials. Maturitas, 2010 May; 66(1) 56-71 Epub 2010 Mar 4 (IF 2.03) invited

Hogervorst, E., Matthews, F.M., Brayne, C. (2010). Are optimal levels of testosterone associated with better cognitive function in healthy elderly women and men ? Biochim Biophys Acta. 2010 Oct;1800(10):1145-52. Epub 2010 Jan 7. (IF 2.713): invited for special issue

Hogervorst E, Bandelow S. Brain and cognition. Is there any case for improving cognitive function in menopausal women using estrogen treatment? Minerva Ginecol. 2009 Dec;61(6):499-515.invited

Helen T Butler, Donald R Warden, Eva Hogervorst, Jiannis Ragoussis, A David Smith, Donald J Lehmann
(2009) Association of the aromatase gene with AD in women Neurosci Let14;468(3): 202-6 (IF 2.2)

Hogervorst, E., Yaffe, K., Richards, M., Huppert, F.A. (2009) Hormone replacement therapy to maintain cognitive function in women with dementia-update. Cochrane-Database-Syst-Rev.(2): CD003799. 100

(Reference can be requested via e.hogervorst@lboro.ac.uk)

SYNAPTIC CORRELATES OF COGNITIVE PERFORMANCE: IMPLICATIONS FOR COGNITIVE AGING AND MENOPAUSE

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We have been addressing the synaptic basis of age-related cognitive decline as well as potential mechanisms that preserve a healthy synaptic profile and result in improved cognitive performance in aged individuals. Although rodent studies have highlighted the vulnerability of the hippocampus to aging, studies of macaque monkeys suggest that the prefrontal cortex (PFC) is perhaps even more susceptible to both synaptic alterations and cognitive impairment with age. In a nonhuman primate (NHP) model, we have found age-related synaptic alterations in PFC that correlate with cognitive decline, and which differ both qualitatively and quantitatively from the age-related alterations that occur in hippocampus. These studies have examined normal aging in gonadally-intact monkeys as well as the interactions between estrogen and aging in ovariectomized (OVX) monkeys. Monkeys in these studies have had extensive behavioral assessment, and accordingly, a key goal in all cases has been to correlate specific synaptic indices with cognitive performance [1].

Cognitive decline is associated with the loss of a select class of highly plastic glutamatergic synapses in the aged monkey PFC, whereas other classes of excitatory synapses are resistant to age-related changes. More specifically, thin spines on pyramidal cell dendrites in PFC suffer a dramatic loss with age that is highly correlated with cognitive decline, whereas mushroom and stubby spines appear unaffected by age [2]. The thin spines are thought to be particularly plastic, and NMDA-receptor dominated, whereas the large mushroom spines are highly stable and AMPA receptor-dominated [3]. The link between loss of thin spines and cognitive decline suggests that the unique cognitive capacities supported by the PFC require an enriched representation of thin spines, conferring enhanced potential for synapse turnover and plasticity. Interestingly, the same class of highly plastic spines in PFC that is vulnerable to aging is protected by cyclical estradiol treatment in OVX subjects, i.e., a treatment that also ameliorates age-related decline in cognitive function supported by PFC [4, 5]. This suggests that while endocrine senescence may be linked to synaptic and cognitive aging, protection against age-related decline is feasible. However, our recent studies suggest that while cyclical estradiol treatment rescues thin spines and cognitive performance, chronic treatments similar to common clinical practice fail to provide benefit [6-8]. Our current thinking is that this dependence on the temporal pattern of hormone delivery may be linked to the dynamic regulation of estrogen receptors.

Concurrent analyses of the hippocampus in the same gonadally-intact monkeys used for the PFC analyses [2] have shown that the pattern of synaptic aging and the synaptic correlates of cognitive ability in hippocampus are quite different from PFC [9]. Our hippocampal studies have concentrated on the perforant path terminal zone in the dentate gyrus (DG), a circuit known to be highly vulnerable to aging [10]. In this region, there is minimal synapse loss if all axospinous synapses are considered. However, the complex multi-synaptic boutons suffer an age-related decline and the large and strong perforated synapses are lost with menopause [11, 12]. Interestingly, it is the frequency of multi-

synaptic boutons making perforated synapses that correlate with memory scores [11]. In addition, there appears to be an age-related failure of AMPA receptor insertion in the large, stable synapses that correlates with cognitive decline [13]. Thus, the DG and PFC analyses from the same monkeys suggest that these two structures differ in their "synaptic strategy" for cognition as well as their vulnerability to aging.

In humans, protection of the vulnerable classes of spines/synapses described above may be a reasonable target for early intervention, prior to the degenerative cascade that results in extensive neuron death and the disastrous cognitive decline that occurs in Alzheimer's Disease.

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Reference list

1. Hara, Y., P.R. Rapp, and J.H. Morrison, *Neuronal and morphological bases of cognitive decline in aged rhesus monkeys.* Age (Dordr), 2012. **34**(5): p. 1051-73.
2. Dumitriu, D., et al., *Selective changes in thin spine density and morphology in monkey prefrontal cortex correlate with aging-related cognitive impairment.* J Neurosci, 2010. **30**(22): p. 7507-15.
3. Kasai, H., et al., *Structural dynamics of dendritic spines in memory and cognition.* Trends Neurosci, 2010. **33**(3): p. 121-9.
4. Hao, J., et al., *Interactive effects of age and estrogen on cognition and pyramidal neurons in monkey prefrontal cortex.* Proc Natl Acad Sci U S A, 2007. **104**(27): p. 11465-70.
5. Rapp, P.R., J.H. Morrison, and J.A. Roberts, *Cyclic estrogen replacement improves cognitive function in aged ovariectomized rhesus monkeys.* J Neurosci, 2003. **23**(13): p. 5708-14.
6. Ohm, D.T., et al., *Clinically relevant hormone treatments fail to induce spinogenesis in prefrontal cortex of aged female rhesus monkeys.* J Neurosci, 2012. **32**(34): p. 11700-5.
7. Baxter, M.G., et al., *Multiple clinically-relevant hormone therapy regimens fail to improve cognitive function in aged ovariectomized rhesus monkeys.* . Neurobiology of Aging, 2012 In Press.
8. Bailey, M.E., et al., *Continuously delivered ovarian steroids are less effective than cyclic treatments at increasing morphological metrics of plasticity in macaque dorsolateral prefrontal cortical neurons.* Submitted.
9. Morrison, J.H. and M.G. Baxter, *The ageing cortical synapse: hallmarks and implications for cognitive decline.* Nature reviews. Neuroscience, 2012. **13**(4): p. 240-50.
10. Morrison, J.H. and P.R. Hof, *Life and death of neurons in the aging brain.* Science, 1997. **278**(5337): p. 412-9.
11. Hara, Y., et al., *Synaptic characteristics of dentate gyrus axonal boutons and their relationships with aging, menopause, and memory in female rhesus monkeys.* J Neurosci, 2011. **31**(21): p. 7737-44.
12. Hara, Y., et al., *Synaptic correlates of memory and menopause in the hippocampal dentate gyrus in rhesus monkeys.* Neurobiol Aging, 2012. **33**(2): p. 421 e17-28.
13. Hara, Y., et al., *Synaptic distributions of GluA2 and PKMzeta in the monkey dentate gyrus and their relationships with aging and memory.* J Neurosci, 2012. **32**(21): p. 7336-44.

TUESDAY, 19th February 2013
09.00 - 11.00

Epigenetics

Epigenetics

(Chairs: McCarthy M.M., Mitro N.)

- **Frick K.M. (USA)** *Epigenetic regulation of estrogen-induced memory enhancement*

- **Bale T.L. (USA)** *Prenatal stress reprogramming of offspring stress dysregulation: miRNA and placental contributions*

- **Auger A.P. (USA)** *Epigenetic organization of the juvenile social brain and sex-biased vulnerability to mental health disorders*

- **McCarthy M.M.,** Nugent B.M. **(USA)** *Epigenetics of sex differentiation*

EPIGENETIC REGULATION OF ESTROGEN-INDUCED MEMORY ENHANCEMENT

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Epigenetic alterations of histone proteins and DNA have recently been implicated in synaptic plasticity and cognitive function, as well as the etiology of psychiatric disorders and neurodegenerative diseases. Histone alterations and DNA methylation are critical for the formation of hippocampal-dependent memory, and increasing evidence suggests that regulation of these epigenetic processes by modulatory factors such as environmental enrichment and stress substantially influences memory function. We recently demonstrated that histone H3 acetylation and DNA methylation in the dorsal hippocampus are necessary for the sex-steroid hormone 17β-estradiol (E_2) to enhance object recognition memory consolidation in young adult female mice [1, 2]. Aged female rodents and humans experience a loss of cognitive responsiveness to estrogens that could result from age-related deterioration of epigenetic processes. Our preliminary studies in middle-aged female mice show that E_2 in middle-aged females can increase histone H3 acetylation and decrease protein levels of histone deacetylase 2 as in young females. However, the E_2-induced increase in DNA methyltransferase 3A protein is delayed in middle-aged females, suggesting that DNA methylation may be compromised in older females. Collectively, these findings have important implications for understanding how hormones influence cognition in adulthood and aging. This talk will provide a brief overview of the literature on epigenetics and memory, and will describe in detail our findings demonstrating that epigenetic alterations regulate E_2-induced memory enhancement in female mice.

Reference list

1. Zhao Z, Fan L, Fortress AM, Boulware MI, Frick KM (2012) Hippocampal histone acetylation regulates object recognition and the estradiol-induced enhancement of object recognition. Journal of Neuroscience, 32:2344-51.
2. Zhao Z, Fan L, Frick KM (2010) Epigenetic alterations regulate the estradiol-induced enhancement of memory consolidation. Proceedings of the National Academcy of Sciences USA, 107:5605-10.

PRENATAL STRESS REPROGRAMMING OF OFFSPRING STRESS DYSREGULATION: miRNA AND PLACENTAL CONTRIBUTIONS

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Neurodevelopmental disorders including autism and schizophrenia show strong sex biases in presentation, onset and treatment. Such disorders have been associated with fetal antecedents including maternal stress. The programming mechanisms through which stress contributes to disease development are not well understood; though likely involve a complex interaction between the maternal environment and effects on the placenta. We have previously identified a sensitive period of early gestation where maternal stress produces sex-dependent epigenetic programming effects on offspring stress pathway neurodevelopment. We have examined the developing brain for changes in the miRNA environment following prenatal stress by ABI Taqman array. To determine the mechanisms by which epigenetic programming changes the miRNA landscape, comparisons in miRNA expression patterns were examined in neonates administered an aromatase inhibitor (formestane) or a HDAC inhibitor (VPA) at birth. We found dramatic changes in the neonatal brain miRNA environment in response to either formestane or VPA. Large-scale bioinformatics analyses specifically identified the miR-200 family in the developing hypothalamus. Gene targets of this family are being identified using HITS-CLIP bioinformatics assessment in the developing hypothalamus for deep sequencing of miRNA:mRNA associations in the RISC complex. We have also focused on examining the upstream signaling mechanisms that associate changes in the maternal hormonal milieu with reprogramming of the fetal brain. To identify potential biomarkers, we conducted analyses by Affymetrix Array in male and female placentas across pregnancy. One candidate gene, O-glycosyltransferase, was identified and further examined for its role in altering neurodevelopment by biochemical, proteomic and genomic analyses. ChIP analyses further identified a reduced association with the transcriptional activational mark, H3K4me3, with this gene, decreasing its expression in stressed male placentas. This X-linked gene was similarly regulated in human placental tissue supporting its translational potential. These results may provide critical insight into the mechanisms contributing to sex-biased disease vulnerability to maternal stress during early pregnancy impacting the developing brain via effects at the placenta that are transmitted to reprogram critical neuroendocrine systems.

EPIGENETIC ORGANIZATION OF THE JUVENILE SOCIAL BRAIN AND SEX-BIASED VULNERABILITY TO MENTAL HEALTH DISORDERS

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While multiple factors can influence ones vulnerability to mental health risk, early-life experiences appear to be a contributing factor in shaping ones later risk for developing a mental health disorder. The mechanisms by which gene x environmental interactions program brain development are still being elucidated; however, emerging data indicate that early alterations of the epigenome within the developing amygdala appear to have lasting consequences on juvenile social interactions. Also intriguing, is the role of one's gender in influencing risk for atypical social development. As there appears to be sex differences in the epigenome, it is plausible that sexual differentiation of the epigenome may partly underlie sex-biased mental health disorders. Indeed, there appears to be sex differences in DNA methylation patterns, methyl-binding proteins, and co-regulatory proteins levels during brain development. While natural variations in these epigenetic mechanisms appear to underlie sex differences in brain physiology and behavior, they may also influence the way neurons respond to endogenous and exogenous cues perturbations. These differential responses may be beneficial or may place one at greater mental health risk. We will discuss how sex differences in the epigenome may occur within the developing amygdala and how these differences impact social development. Therefore, we suggest that sexual differentiation of the epigenome produces a sex-biased vulnerability to mental health disorders.

THE EPIGENETICS OF SEXUAL DIFFERENTIATION OF THE BRAIN

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The hormonally mediated sexual differentiation of the brain is characterized by an early developmental organizational phase and a second, post-pubertal activational phase. Effective execution of the activational phase requires that the neuronal restructuring that occurred during the organizational phase both matches the hormonal milieu of the adult phase and that a memory of the organizational changes endures until the adult phase. Steroid hormones act via nuclear receptors which interact both directly with the DNA and with components of the transcriptional complex that includes proteins capable of exerting epigenetic changes to the DNA. The combination of these facts has led to an increased interest in the potential for an epigenetic underpinning to sexual differentiation (McCarthy et al., 2009).

In the laboratory rodent the brain masculinizing hormone is estradiol derived via aromatization from testicular androgens. Estrogens bind to two receptor isoforms, ER-alpha and ER-beta. Both receptors appears to play a role in sexual differentiation of the brain but that role varies by endpoint and region.

Epigenetic changes consist of alterations to the DNA or surrounding chromatin that impact on gene expression and can be transduced to the next generation, but do not involve changes to the sequence of nucleotides. The dominant forms of epigenetic changes are those to the chromatin, which involves alterations of the histone cores of nucleosomes, and methylation marks added directly to the DNA at CpG cites, meaning cytosines located next to guanines. Whether the changes to the DNA and surrounding chromatin repress or enhance gene transcription is variable and depends upon the precise locations, nature or and timing of the changes.

We have explored both chromatin and DNA methylation changes in the context of sex differences in the brain and the sexual differentiation of neuronal morphology and reproductive behavior. Both parameters are subject to change in response to the hormones modulating sexual differentiation in the Preoptic Area, a key brain region controlling male sexual behavior (Schwarz, Nugent & McCarthy, 2010; Matsuda et al., 2011). Manipulation of the enzymes controlling epigenetic changes to the chromatin and DNA alters the process of sexual differentiation in predictable ways, thereby implicating the centrality of these changes to the enduring effects of hormone acting during the perinatal sensitive period. However, aspects of this process were not predictable and suggest that instead of a direct interaction of the estrogen receptor, with the DNA to either transcribe or repress gene expression, the enzymes regulating DNA methylation and possibly chromatin changes is well, is the target of hormonal regulation. This profoundly alters our view of how steroids differentiates the brain and opens new vistas of exploration in to the mechanism of organization actions of gonadal steroids.

References list

McCarthy MM, Auger AP, Bale TL, De Vries GJ, Dunn GA, Forger NG, Murray EK, Nugent BM, Schwarz JM, Wilson ME. (2009) The epigenetics of sex differences in the brain. J Neurosci. 29:12815-12823.

Schwarz JM, Nugent BM, McCarthy MM. (2010) Developmental and hormone-induced epigenetic changes to estrogen and progesterone receptor genes in brain are dynamic across the life span. Endocrinology. 151:4871-81.

Matsuda KI, Mori H, Nugent BM, Pfaff DW, McCarthy MM, Kawata M. (2011) Histone deacetylation during brain development is essential for permanent masculinization of sexual behavior. Endocrinology. 152:2760-7.

TUESDAY, 19th February 2013
11.30 - 12.30

Young investigators symposium

Young investigators symposium

(Chairs: Frye C.A., Mensah-Nyagan A.G.)

- **Kudova E.**, Chodounska H., Slavikova B., Kapras V., Vales K., Rambousek L., Borovska J., Vyklicky V., Krausova B., Vyklicky L.jr. **(Czech Republic)** *Interaction of neurosteroids with NMDA receptors: current insight into structure-activity relationships and their neuroprotective effect*

- **Jayaraman A.**, Lent D., Christensen A., Pike C.J. **(USA)** *The effects of testosterone and high-fat diet on neuroinflammation*

- **López Rodríguez A.B.**, Mateos Vicente B., Romero-Zerbo S.Y., Rodriguez-Rodriguez N., Bellini M.J., Rodriguez de Fonseca F., Bermudez-Silva F.J. Azcoitia I., Garcia-Segura L.M., Viveros M.P. **(Spain)** *Estradiol effects on cortical reactive astrogliosis after brain injury involve cannabinoid receptors CB1 and CB2*

- **Ruiz-Palmero I.**, Hernando M., Garcia-Segura L.M., Arevalo M.A. **(Spain)** *G protein-coupled estrogen receptor is involved in the neuritogenic mechanism of 17beta-estradiol in developing hippocampal neurons*

- **Boraso M.**, Valero M., Gardoni F., Marco E.M., Corsini E., Galli C.L., Di Luca M., Marinovich M., López-Gallardo M., Viveros M.P., Viviani B. **(Italy)** *Early maternal deprivation immunologically primes hippocampal synapses by redistributing interleukin-1 receptor type I in a sex dependent manner*

- **De Felice A.**, Scattoni M.L., Tait S., Ricceri L., Calamandrei G. **(Italy)** *Sex-dependent vulnerability to a neuroendocrine disruptor in a mouse model of autism spectrum disorders*

INTERACTION OF NEUROSTEROIDS WITH NMDA RECEPTORS: CURRENT INSIGHT INTO STRUCTURE-ACTIVITY RELATIONSHIPS AND THEIR NEUROPROTECTIVE EFFECT

Kudova E.*, Chodounska H.*, Slavikova B.*, Kapras V.*, Vales K., Rambousek L.**, Borovska J.***, Vyklicky V.***, Krausova B.***, Vyklicky L.Jr.*****

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Neurosteroids exert their effects through modulation of the synaptic and/or extrasynaptic neurotransmitter–receptors, and are utilized by the brain for fine-tuning the function of the neural network. Often, excitatory transmissions are mediated by L-glutamic acid, namely through the N-methyl-D-aspartate receptor (NMDA) receptor. This receptor forms a Ca^{2+} permeable ion channel, and under physiological conditions the activation of this receptor is essential for synaptic plasticity, learning, and memory. However, overexcitation of the NMDA receptor can also induce cell death. This excitotoxicity is often seen in conjunction with ischemia injuries in the brain, and is also thought to contribute to the neurodegeneration associated with various forms of dementia. Of our interest is the fact that progesterone and its reduced metabolites also behave as endogenous neuroprotectives. In particular, 3alpha,5beta-pregnanolone sulfate has been shown to be an effective, use-dependent antagonist of the NMDA receptor with a high neuroprotective potential. We have synthetised and examined vast family of neurosteroids (currently almost 200 compounds) while searching for new and even more effective ligands for NMDA receptors. Our database covers series of compounds bearing substituents at C-3 with negatively and positively charged substituents, as well as zwitterions. These C-3 substituents differ by a type and lenght and new structure-activity relationships have been established. Also, we have introduced various substituents to different position of steroid skeleton (e.g. C-7, C-11, C-17, C-20). Subsequently, the patch-clamp and imagining recordings from HEK293 cells expressing NR1/NR2B receptors and cultured rat hippocampal neurons were used to establish the NMDAR inhibition and IC_{50} values. Moreover, our *in vivo* experiments show that these neurosteroid ligands are able to cross the blood brain barrier and do not induce psychotomimetic symptoms (such as hyperlocomotion and sensorimotor gating deficit). These findings provide a possible new therapeutic approach for the treatment of diseases induced by NMDA receptor overactivation.
The structure-activity relationship, detailed synthesis, and relationship of structure vs. IC_{50} values will be discussed.

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THE EFFECTS OF TESTOSTERONE AND HIGH-FAT DIET ON NEUROINFLAMMATION

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Inflammatory pathways contribute to the pathogenesis of several diseases, including type 2 diabetes mellitus (T2D). Inflammation is regulated by many factors. For example, testosterone can attenuate inflammation in part by decreasing the expression of pro-inflammatory cytokines such as TNFalpha and IL-1beta. On the other hand, high-fat diet is associated with activation of pro-inflammatory pathways. High-fat diet also induces obesity, promotes T2D, and is associated with decreased testosterone levels. We hypothesize that interactions between low testosterone and high-fat diet-induced metabolic changes both independently and cooperatively regulate inflammatory pathways and accelerate the development of T2D related pathology. In this study, we investigate the effects of experimental manipulation of testosterone levels combined with high-fat diet on neuroinflammation and development of T2D. In particular, we determine the effects of low testosterone levels in the presence and absence of a high-fat diet on (i) expression of pro-inflammatory pathways (ii) metabolic indices of T2D, and (iii) levels of reactive astrocytes and activated microglia. Our preliminary results suggest that low testosterone levels and high-fat diet significantly elevate blood glucose levels, reduce insulin sensitivity, and increase expression levels of TNFalpha and IL-1beta. In addition, we show that neurons exhibit reduced survival and poorer neurite outgrowth when co-cultured with glial cultures generated from high-fat fed animals in comparison to glial cultures from animals maintained on a normal diet. These results demonstrate neuroinflammatory effects of high-fat diet, a relationship that is affected by testosterone levels. Together, our findings suggest that low testosterone and obesity are interactive regulators of neuroinflammation that may increase risk of downstream disorders such as T2D and Alzheimer's disease.

ESTRADIOL EFFECTS ON CORTICAL REACTIVE ASTROGLIOSIS AFTER BRAIN INJURY INVOLVE CANNABINOID RECEPTORS CB1 AND CB2

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Brain injuries activate inflammatory responses and trigger the release of potential harmful molecules such as cytokines or reactive oxygen species leading to a worsening in secondary damage [7]. One of the most studied aspects is the astrocitic reactivity and the glial scar formation after lesion. Previous studies of the group show that the neuroactive steroid estradiol reduces reactive astroglia after brain injury [1,2,4,5] by mechanisms similar to those involved in the regulation of reactive gliosis by endocannabinoids, for example inhibiting NFKB-induced transcription of proinflammatory chemokines and cytokines [3,6]. The similitude in the anti-inflammatory actions of cannabinoids and estrogens on astrocytes suggests a possible interaction of these two families of molecules in the regulation of reactive astroglia. To test this hypothesis, in this study, we have assessed the effect of estradiol, the cannabinoid CB1 antagonist/inverse agonist AM251, and the cannabinoid CB2 antagonist/inverse agonist AM630 on reactive astroglia in the cerebral cortex of male rats after a stab wound brain injury. Our results showed that estradiol reduced the number of vimentin immunoreactive astrocytes and the number of glial fibrillary acidic protein (GFAP) immunoreactive astrocytes in the proximity of the wound. The protective effect of estradiol was significantly inhibited by the administration of either CB1 or CB2 receptor antagonists. The effect of estradiol may be in part mediated by alterations in endocannabinoid signaling because the hormone also altered the levels of mRNA for CB2 receptor in the injured cerebral cortex as well as the levels of monoacylglycerol lipase (MAGL) and N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD), two enzymes involved in the synthesis and metabolism of endocannabinoids. These findings suggest that estradiol may decrease reactive astroglia in the injured brain by regulating the activity of the endocannabinoid system .

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Reference list

[1] Barreto G, Veiga S, Azcoitia I, Garcia-Segura LM, Garcia-Ovejero D. Testosterone decreases reactive astroglia and reactive microglia after brain injury in male rats: role of its metabolites, oestradiol and dihydrotestosterone. Eur J Neurosci. 25:3039-3046 (2007).

[2] Barreto G, Santos-Galindo M, Diz-Chaves Y, Pernía O, Carrero P, Azcoitia I, Garcia-Segura LM. Selective estrogen receptor modulators decrease reactive astrogliosis in the injured brain: effects of aging and prolonged depletion of ovarian hormones. Endocrinology 150, 5010-5015 (2009).

[3] Cerciat M, Unkila M, Garcia-Segura LM, Arevalo MA. 2010. Selective estrogen receptor modulators decrease the production of interleukin- 6 and interferon-gamma-inducible protein-10 by astrocytes exposed to inflammatory challenge in vitro. Glia. 58:93-102 (2010).

[4] Garcia-Estrada,J., Del Rio,J.A., Luquin,S., Soriano,E., & Garcia-Segura,L.M. Gonadal hormones down-regulate reactive gliosis and astrocyte proliferation after a penetrating brain injury. Brain Res. 628, 271-278 (1993).
[5] Garcia-Estrada J, Luquin S, Fernandez A, Garcia-Segura LM. Dehydroepiandrosterone, pregnenolone and sex steroids downregulate reactive astroglia in the male rat brain after a penetrating brain injury. Int J Dev Neurosci. 17:145-151 (1999).
[6] Kozela E, Pietr M, Juknat A, Rimmerman N, Levy R, Vogel Z. Cannabinoids D9-tetrahydrocannabinol and cannabidiol differentially inhibit the lipopolysaccharide-activated NF-jB and interferonb/ STAT proinflammatory pathways in BV-2 microglial cells. J Biol Chem. 285:1616-1626 (2010).
[7] Sofroniew,M.V. & Vinters,H.V. Astrocytes: biology and pathology. Acta Neuropathol. 119, 7-35 (2010).

G PROTEIN-COUPLED ESTROGEN RECEPTOR IS INVOLVED IN THE NEURITOGENIC MECHANISM OF 17β-ESTRADIOL IN DEVELOPING HIPPOCAMPAL NEURONS

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Estradiol promotes neuritogenesis in developing hippocampal neurons by a mechanism involving the upregulation of neurogenin 3, a Notch-regulated transcription factor (1, 2). In this study we have explored whether G-protein coupled estrogen receptor 1 (GPER) participates in this hormonal action. GPER agonists (17β-estradiol, G1, ICI 182,780) increased neurogenin 3 expression and neuritogenesis in mouse primary hippocampal neurons and this effect was blocked by the GPER antagonist G15 and by a siRNA for GPER. In addition, GPER agonists increased Akt phosphorylation in ser473, which is indicative of the activation of phosphoinositide-3-kinase (PI3K). However, GPER agonists did not significantly affect the phosphorylation of Akt in the presence of G15. Furthermore, the PI3K inhibitor wortmannin prevented the effect of G1 and estradiol on neurogenin 3 expression and the effect of estradiol on neuritogenesis. These findings suggest that GPER participates in the control of hippocampal neuritogenesis by a mechanism involving the activation of PI3K signaling.

Reference list

1 Arevalo, M.A., Ruiz-Palmero, I., Scerbo, M.J., Acaz-Fonseca, E., Cambiasso, M.J., Garcia-Segura, L.M., 2012. Molecular mechanisms involved in the regulation of neuritogenesis by estradiol: Recent advances. J. Steroid. Biochem. Mol. Biol. 131, 52-56.

2 Ruiz-Palmero, I., Simon-Areces, J., Garcia-Segura, L.M., Arevalo, M.A., 2011. Notch / neurogenin 3 signalling is involved in the neuritogenic actions of oestradiol in developing hippocampal neurones. J. Neuroendocrinol. 23, 355-364.

EARLY MATERNAL DEPRIVATION IMMUNOLOGICALLY PRIMES HIPPOCAMPAL SYNAPSES BY REDISTRIBUTING INTERLEUKIN-1 RECEPTOR TYPE I IN A SEX DEPENDENT MANNER

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Adverse early-life events represent triggers, which cause an enduring phenotypic shift of immune cells towards a sensitized state. A recent hypothesis suggests that an enhanced immune response later in life might contribute to promote an increased susceptibility to neurodegenerative and psychiatric disorders. Peripheral and central inflammation adversely affect neuronal functions through cytokines like IL-1. We studied whether a single prolonged episode of maternal deprivation (MD) during the neonatal period at PND9 affects rat neuron sensitivity to inflammatory events later in life. To this aim we evaluated the synapse distribution of interleukin receptor type I (IL-1RI) and its interaction with GluN2B subunit of NMDA receptors. These endpoints were investigated in association with the distribution of both the NMDA and AMPA receptors subunits (respectively GluN2A, GluN2B and GluA1, GluA2). The analysis have been performed in the hippocampus and in prefrontal cortex of male and females rats at PND45.
MD at significantly increases the levels of IL-1RI and its interaction with GluN2B, at the synapse of male hippocampal neurons, without affecting the total amount of IL-1RI and NMDAR subunits. Furthermore, GluN2B and GluN2A ratio was significantly decreased in the hippocampus of male MD rats, together with the levels of GluA1 and GluA2 subunits. None of the observed alterations occurred in females and in prefrontal cortex of both sexes. Thus MD induces a long-lasting and sex-dependent alteration of the receptors setting at the hippocampal post-synapses. We suggest that MD-induced enrichment of IL-1RI contributes to "immunologically" prime hippocampal synapses to the action of IL-1beta, thus prompting a novel molecular basis to the critical role for the immune response in early-life programming of later-life brain functions and behaviour.

SEX-DEPENDENT VULNERABILITY TO A NEUROENDOCRINE DISRUPTOR IN A MOUSE MODEL OF AUTISM SPECTRUM DISORDERS

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Autism is a neurodevelopmental disorder defined by core symptoms that include impaired social and communicative skills and repetitive behaviours, and it is strongly biased towards males, with a male-female ratio of 4:1. The etiological bases of autism are still largely unexplained, but there is general agreement that autism results from complex interaction between multiple genes conferring vulnerability and diverse environmental factors, including early prenatal exposure to environmental contaminants. A role for steroid hormones has been also hypothesized in autism etiology, as fetal exposure to higher levels of testosterone could contribute to the hypermasculinization of sex dimorphic brain areas reported in some autistic individuals (the Extreme Male Brain theory of autism, see [1]). Notably perinatal exposure to widespread environmental pollutants with known endocrine disrupting activity (i.e. heavy metals, organophosphate pesticides) appears to increase risk of developing autism spectrum disorders [3].

BTBR T+tf/J (BTBR) is an inbred strain of mice that displays several behavioural traits relevant to autism, including social deficits and repetitive behaviours in adults, as well as an unusual pattern of ultrasonic vocalizations in pups, resembling the atypical crying seen in some autistic infants. Furthermore, BTBR pups show faster acquisition of some developmental milestones [5]. Alterations in brain levels of serotonin and oxytocin have been reported in this strain of mice, but surprisingly few information is available on steroid hormones status of BTBR.

Chlorpyrifos (CPF) is a non-persistent organophosphate (OP) largely used as insecticide in both agricultural and urban communities. It acts as a developmental neurotoxicant by targeting immature central nervous system at doses below the threshold that elicits overt cholinergic toxicity. A recent study [4] indicates that environmentally relevant prenatal exposure to CPF in children can alter the morphology of some brain areas involved in cognitive and behavioural processes, inducing loss or reversal of expected sex differences. Numerous animal studies have confirmed long-term effects of developmental exposure to CPF both on behaviour and neural systems in absence of significant brain acetylcholinesterase (AChE) inhibition. In particular, several evidences indicate that CPF acts as a neuroendocrine disrupter: developmental exposure to this OP in rodent models induces changes in social and anxiety responses and influences neuroendocrine markers (oxytocin, vasopressin, estrogen receptors) in hypothalamic and amygdaloidal regions [2,6].

In the present study we analyzed the effects of developmental exposure to CPF in BTBR mice. We aimed at evaluating in this mouse model of autism whether prenatal exposure to low CPF doses i) interfered with early neurobehavioural development of the offspring, ii) modulated the characteristic autistic-like behavioural profile of these mice at adulthood, iii) affected the expression of oxytocin, vasopressin and their related receptors, and estrogen receptor ERα and β in brain areas involved in the control of social responses.

Pregnant BTBR mice were administered from gestational day 14 to gestational day 17 with either vehicle or CPF at a daily dose of 6 mg/kg by oral gavages. Offspring of both sexes underwent assessment of sensorimotor milestones and ultrasound emission on postnatal

days 4, 6, 8 and 12. At adulthood, the social responses of females were assessed in a free social interaction test with a same-sex companion, whereas the courtship behaviour of adult males (including ultrasonic calls) was analyzed upon presentation of a sexually receptive untreated female.
Our findings indicated that CPF did not grossly alter the neurodevelopmental profile of BTBR mice, but significantly reduced spontaneous motor activity and head movements while increasing immature motor patterns such as pivoting. CPF exposure also increased both frequency and duration of ultrasonic calls emitted by pups in isolation. Of note all these effects were more marked in the male sex.
At adulthood, CPF associated alterations were found predominantly in males, while in social interactions between females we did not found any significant deficit in social investigation. Specifically, CPF-exposed males presented a particular shift in social investigation when presented with a receptive female, as they increased sniffing of the head and body areas and reduced sniffing of the anogenital area of their female partners. Such abnormalities in social investigation were associated with marked increase in the rate of ultrasonic vocalizations emitted during the courtship.
Still in progress analyses of mRNA expression as for several neuroendocrine markers in hypothalamus and amygdala evidenced in males a significant CPF-induced decrease of vasopressin receptor 1A in the hypothalamus and ERα in the amygdala, and a general trend towards a diminished expression of ERβ and oxytocin precursor in the hypothalamus. Such findings, though still preliminary, suggest that in BTBR male mice prenatally exposed to CPF long-term changes in limbic/hypothalamic circuits might contribute to the unusual courtship profile.
Altogether, we showed here that BTBR male mice are specifically vulnerable to a widely-diffused neurotoxicant acting as a neuroendocrine disruptor in both rodents and humans. These findings open the way for future experimental studies on the interaction among vulnerable gene backgrounds, hormonal homeostasis and environmental neurotoxicants in the etiology of sex-biased neurodevelopmental disorders.

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Reference list

[1] Baron-Cohen S, Lombardo V, Auyeung B, Ashwin E, Chakrabarti B, Knickmeyer R: Why are spectrum conditions more prevalent in males? *PLOS Biology* 2011, 9(6):e1001081.
[2] Frye CA, Bo E, Calamandrei G, Calzà L, Dessì-Fulgheri F, Fernàndez M, Fusani L, Kah O, Kajta M, Le Page Y, Patisaul HB, Venerosi A, Wojtowicz AK, Panzica GC: Endocrine disrupters: a review of some sources, effects, and mechanism of actions on behaviour and neuroendocrine systems. J Neuroendocrinol. 2012, 24(1):144-59.
[3] Landrigan PJ: What causes autism? Exploring the environmental contribution. Curr. Opin. 2010, 22(2):219-25.
[4] Rauh VA, Perera FP, Horton MK, Whyatt RM, Bansal R, Hao X, Liu J, Barr DB, Slotkin TA, Peterson BS: Brain anomalies in children exposure prenatally to a common organophosphate pesticide. *Proc Natl Acad Sci* U S A 2012, 109(20):7871-6.
[5] Scattoni ML, Gandhy SU, Ricceri L, Crawley JN: Unusual repertoire of vocalizations in the BTBR T+tf/J mouse model of autism. *PLoS One* 2008, 3(8):e3067.
[6] Tait S, Ricceri L, Venerosi A, Maranghi F, Mantovani A, Calamandrei G: Long-term effects on hypothalamic neuropeptides after developmental exposure to chlorpyrifos in mice. *Environ Health Perspect* 2009, 117:112-6.

TUESDAY, 19th February 2013
14.00 - 16.00

Round table:

Effects of Finasteride treatment on the nervous system

Effects of Finasteride treatment on the nervous system

(Chairs: Melcangi R.C., Panzica G.C.)

- **Worthington A. (USA)** *Introducing the post-Finasteride syndrome foundation*
- **Traish A.M. (USA)** *5α-reductase inhibitors therapy is associated with risk of sexual dysfunction*
- **Melcangi R.C.,** Caruso D., Abbiati F., Giatti S., Calabrese D., Cavaletti G. **(Italia)** *Neuroactive steroid levels in post-Finasteride patients showing persistent sexual side effects and anxious/depressive symptomatology*
- **Bortolato M.,** Frau R., Soggiu A., Roncada P., Bini V., Devoto P. **(USA)** *Towards a characterization of the molecular bases of the behavioral effects of Finasteride*
- **Walf A.A., Frye C.A. (USA)** *Finasteride increases depression behavior, whereas administration of 3alpha-androstanediol reduces depression behavior and increases brain-derived neurotrophic factor, without increasing prostate growth, of male mice*

INTRODUCING THE POST-FINASTERIDE SYNDROME FOUNDATION

Worthington A.*

*Research Coordinator, Post-Finasteride Syndrome Foundation, New Jersey, USA

The Post-Finasteride Syndrome (PFS) is characterized by persistent sexual, neurological, hormonal and mental side effects in men who have taken the prescription drug finasteride to treat AGA (androgenic alopecia) or BPH (benign prostatic hyperplasia). The syndrome can persist indefinitely, has no known cure and few, if any, effective treatments.

Reported persistent symptoms include loss of libido, erectile dysfunction, Peyronie's Disease, penile atrophy, gynecomastia, muscle atrophy and weakness, cognitive impairment, depression, sleep disorders, severe anxiety, severely dry skin, skeletal and joint pain, metabolic and digestive disorders. The condition often has a life-altering impact on the relationships and careers of affected patients, and a number of suicides have been reported.

The New Jersey based Post-Finasteride Syndrome Foundation is dedicated to supporting and funding basic science research aimed at discovering the underlying molecular mechanisms of PFS. Our ultimate goal is to use this knowledge to develop more effective therapies for PFS patients. Past research has laid the ground work on which more targeted, multi-disciplinary research projects can now operate. In order to achieve optimal effectivity, a certain level of information exchange and coordination between future PFS research projects will be necessary. The Post-Finasteride Syndrome Foundation is here to help facilitate this coordination and provide a platform for scientific information exchange.

Our current research interests include: genomic and non-genomic actions of the androgen receptor in both the peripheral and nervous system context, integrated genomic and proteomic analysis related to androgen responsive genes, gene regulatory networks and epigenetics, and the effect of PFS on the nervous systems and on neurosteroids.

Research and scientific collaboration enquiries can be directed to research@pfsfoundation.org.

5α-REDUCTASE INHIBITORS THERAPY IS ASSOCIATED WITH RISK OF SEXUAL DYSFUNCTION

Traish A.M.

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Erectile function is a complex neurovascular process, regulated by multiple biochemical and physiological signaling pathways [1-10]. Penile erection is dependent on health of the penile vascular bed, and the perineal and ischiocavernous muscles that support the proximal penis. Erection depends on adequate arterial blood inflow and trapping within the cavernosal bodies (venoocclusion) to maintain increasing pressure and volume to achieve penile rigidity [1-10]. The venoocclusive mechanism depends on the integrity of neural, vascular, and endocrine systems as well as the fibroelastic properties of the corporal cavernosal tissue [1-10].

Androgens are critical for penile-tissue development, growth, and maintenance of function and androgen deficiency is thought to be is a risk factor for erectile dysfunction [1-10]. In erectile tissue, androgens are thought to regulate the expression and activity of neural and endothelial nitric oxide synthase and maintain the health of the enthodelium and the trabeculae. Androgens also regulate the balance between the trabeculae and connective tissue in erectile tissue [10]. In the animal model, androgen deprivation produced significant structural and functional alterations concomitant with reduced erectile function as assessed by changes in intracavernosal pressure in response to pelvic nerve stimulation [10]. The cavernosal and dorsal nerves undergo structural alterations in response to androgen deprivation [11,12]. Androgen deprivation produced marked reduction in neural and nitric oxide synthase activities [13-16]. Thus, perturbations in the endocrine milieu, and more specifically androgens may contribute to erectile dysfunction.

5α-reductases are a family of isozymes expressed in a wide host of tissues, including the central nervous system (CNS), and play a pivotal role in sexual differentiation, development and physiology [17]. 5α-reductases transform not only testosterone5α-dihydrotestosterone but also progesterone, deoxycorticosterone, aldosterone and corticosterone into their respective 5α-dihydro-derivatives [17, 18]. The latter steroids serve as substrates for 3α-hydroxysteroid dehydrogenases. These enzymes transform these 5α-reduced metabolites into a sub-class of neuroactive steroid hormones with distinct physiological functions. Steroid hormones modulate a multitude of functions in human physiology encompassing regulation of sexual differentiation, sexual function, neuroprotection, memory enhancement, anxiety, sleep and stress, among others [17, 18].

The family of 5α-reductases was targeted for drug development to treat pathophysiological conditions, such as benign prostatic hyperplasia and androgenetic alopecia [19-23]. While the clinical use of 5α-reductase inhibitors, such as Finasteride and Dutasteride was well established in management of benign prostatic hyperplasia [19-22], their use in young men with androgenetic alopecia may be associated with adverse events such as depression and erectile dysfunction [17, 18, 24, 25, 26]. The scope and magnitude of the adverse side effects of such drugs on cardiovascular function, sexual function and the CNS, remains poorly understood. The physiological responses elicited by steroid hormones, as a result of the activity of this family of enzymes, warrants deeper investigation.

Investigations in animals and humans have revealed that Finasteride and Dutasteride may be associated with decreased libido, erectile function, orgasm [27-29]. In the animal model, these agents produce marked structural and functional alterations in penile tissue which contribute to erectile dysfunction [28, 29]. In humans, these agents may also be associated with depression and discontinuation of these drugs may be associated with persistent adverse events in a subset of patients [17, 18, 24-26]. There is an urgent need to better understand the function of 5α-reductases and the role of neuroactive steroids in the physiology of sexual function in order to minimize the potential adverse side effects of inhibitors, such as Finasteride and Dutasteride, in targeting 5α-reductases in treatment of benign prostatic hyperplasia and androgenic alopecia. In Summary, physicians should make every effort to educate their patients on the potential risks associated with use of 5alpha reductase inhibitors of BPH or hair restoration, especially depression and persistent sexual dysfunction.

Reference list

1. Traish AM, Goldstein I, Kim NN. Testosterone and erectile function: from basic research to a new clinical paradigm for managing men with androgen insufficiency and erectile dysfunction. Eur Urol. 2007 Jul;52(1):54-70.
2. Traish AM, Guay AT. Are androgens critical for penile erections in humans? Examining the clinical and preclinical evidence. J Sex Med. 2006 May;3(3):382-404.
3. Traish A, Kim N. The physiological role of androgens in penile erection: regulation of corpus cavernosum structure and function. J Sex Med. 2005 Nov;2(6):759-70.
4. Traish AM. Androgens play a pivotal role in maintaining penile tissue architecture and erection:. J Androl. 2009 Jul-Aug;30(4):363-9.
5. Traish AM, Abu-Zahra H, Guay AT. The brain, the penis and steroid hormones: clinical correlates with endothelial dysfunction. Curr Pharm Des. 2008;14(35):3723-36.
6. Kurbatov D, Kuznetsky J, Traish A. Testosterone improves erectile function in hypogonadal patients with venous leakage. J Androl. 2008 Nov-Dec;29(6):630-7
7. Yassin AA, Saad F, Traish A. Testosterone undecanoate restores erectile function in a subset of patients with venous leakage: a series of case reports. J Sex Med. 2006 Jul;3(4):727-35.
8. Armagan A, Kim NN, Goldstein I, Traish AM. Dose-response relationship between testosterone and erectile function: evidence for the existence of a critical threshold. J Androl. 2006 Jul-Aug;27(4):517-26.
9. Traish AM, Toselli P, Jeong SJ, Kim NN. Adipocyte accumulation in penile corpus cavernosum of the orchiectomized rabbit: a potential mechanism for veno-occlusive dysfunction in androgen deficiency. J Androl. 2005 Mar-Apr;26(2):242-8.
10. Traish AM, Park K, Dhir V, Kim NN, Moreland RB, Goldstein I. Effects of castration and androgen replacement on erectile function in a rabbit model. Endocrinology. 1999 Apr;140(4):1861-8.
11. Armagan A, Hatsushi K, Toselli P. The effects of testosterone deficiency on the structural integrity of the penile dorsal nerve in the rat. Int J Impot Res. 2008 Jan-Feb;20(1):73-8.
12. Rogers RS, Graziottin TM, Lin CS, Kan YW, Lue TF. Intracavernosal vascular endothelial growth factor (VEGF) injection and adeno-associated virus-mediated VEGF gene therapy prevent and reverse venogenic erectile dysfunction in rats. Int J Impot Res. 2003 Feb;15(1):26-37.
13. Lugg JA, Rajfer J, González-Cadavid NF.Dihydrotestosterone is the active androgen in the maintenance of nitric oxide-mediated penile erection in the rat. Endocrinology. 1995 Apr;136(4):1495-501.
14. Lugg J, Ng C, Rajfer J, González-Cadavid N. Cavernosal nerve stimulation in the rat reverses castration-induced decrease in penile NOS activity. Am J Physiol. 1996 Aug;271(2 Pt 1):E354-61.
15. Penson DF, Ng C, Cai L, Rajfer J, González-Cadavid NF. Androgen and pituitary control of penile nitric oxide synthase and erectile function in the rat. Biol Reprod. 1996 Sep;55(3):567-74.
16. Restoration of normal adult penile erectile response in aged rats by long-term treatment with Garban H, Marquez D, Cai L, Rajfer J, Gonzalez-Cadavid NF. androgens. Biol Reprod. 1995 Dec;53(6):1365-72.
17. Traish AM. 5α-reductases in human physiology: an unfolding story. Endocr Pract. 2012 Nov 1;18(6):965-75.
18. Traish AM, Hassani J, Guay AT, Zitzmann M, Hansen ML. Adverse side effects of 5α-reductase inhibitors therapy: persistent diminished libido and erectile dysfunction and depression in a subset of patients. J Sex Med. 2011 Mar;8(3):872-84.
19. Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, Lieber MM, Cespedes RD, Atkins JN, Lippman SM, Carlin SM, Ryan A, Szczepanek CM, Crowley JJ, Coltman CA Jr. The influence of finasteride on the development of prostate cancer. N Engl J Med. 2003 Jul 17;349(3):215-24. Epub 2003 Jun 24.
20. Gormley GJ, Stoner E, Bruskewitz RC, Imperato-McGinley J, Walsh PC, McConnell JD, Andriole GL, Geller J, Bracken BR, Tenover JS, et al. The effect of finasteride in men with benign prostatic hyperplasia. The Finasteride Study Group. N Engl J Med. 1992 Oct 22;327(17):1185-91.
21. Theoret MR, Ning YM, Zhang JJ, Justice R, Keegan P, Pazdur R. The risks and benefits of 5α-reductase inhibitors for prostate-cancer prevention. N Engl J Med. 2011 Jul 14;365(2):97-9.
22. Andriole GL, Bostwick DG, Brawley OW, Gomella LG, Marberger M, Montorsi F, Pettaway CA, Tammela TL, Teloken C, Tindall DJ, Somerville MC, Wilson TH, Fowler IL, Rittmaster RS; REDUCE Study Group. Effect of dutasteride on the risk of prostate cancer. N Engl J Med. 2010 Apr 1;362(13):1192-202.
23. Olsen EA, Whiting DA, Savin R, Rodgers A, Johnson-Levonas AO, Round E, Rotonda J, Kaufman KD; Male Pattern Hair Loss Study Group. Global photographic assessment of men aged 18 to 60 years with male pattern hair loss receiving finasteride 1 mg or placebo. J Am Acad Dermatol. 2012 Sep;67(3):379-86
24. Irwig MS. Depressive symptoms and suicidal thoughts among former users of finasteride with persistent sexual side effects. J Clin Psychiatry. 2012 Sep;73(9):1220-3.
25. Irwig MS. Persistent sexual side effects of finasteride: could they be permanent? J Sex Med. 2012 Nov;9(11):2927-32
26. Irwig MS, Kolukula S. Persistent sexual side effects of finasteride for male pattern hair loss. J Sex Med. 2011 Jun;8(6):1747-53.
27. Gur S, Kadowitz PJ, Hellstrom WJ. Effects of 5-alpha reductase inhibitors on erectile function, sexual desire and ejaculation. Expert Opin Drug Saf. 2013 Jan;12(1):81-90.
28. Oztekin CV, Gur S, Abdulkadir NA, Lokman U, Akdemir AÖ, Cetinkaya M, Hellstrom WJ. Incomplete recovery of erectile function in rat after discontinuation of dual 5-alpha reductase inhibitor therapy. J Sex Med. 2012 Jul;9(7):1773-81
29. Pinsky MR, Gur S, Tracey AJ, Harbin A, Hellstrom WJ. The effects of chronic 5-alpha-reductase inhibitor (dutasteride) treatment on rat erectile function. J Sex Med. 2011 Nov;8(11):3066-74.

NEUROACTIVE STEROID LEVELS IN POST-FINASTERIDE PATIENTS SHOWING PERSISTENT SEXUAL SIDE EFFECTS AND ANXIOUS/DEPRESSIVE SYMPTOMATOLOGY

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The enzyme 5α-reductase converts neuroactive steroids, such as testosterone (T) and progesterone (PROG) into their metabolites. Thus, T is converted into dihydrotestosterone (DHT) and subsequently into 5α-androstane-3α,17β-diol (3α-diol) or 5α-androstane-3β,17β-diol (3β-diol), PROG into dihydroprogesterone (DHP) and then into tetrahydroprogesterone (THP) or isopregnanolone [6]. An inhibitor of this enzyme, the finasteride, is used for androgenic alopecia (male pattern hair loss). Observations performed in a subset of man taking finasteride for male pattern hair loss seem to indicate that sexual dysfunction as well as anxious/depressive symptomatology may occur [1,2,7,8]. Very important, persistent sexual side effects as well as depression persist despite the discontinuation of the treatment [3-5]. A possible hypothesis to explain depression symptoms after finasteride treatment might be impairment in the levels of neuroactive steroids [9]. To this aim, neuroactive steroids levels were evaluated in paired plasma and CSF samples obtained from 3 male patients who received finasteride for the treatment of androgenic alopecia and that after drug discontinuation still show long-term sexual side effects as well as anxious/depressive symptomatology. Data obtained by liquid chromatography-tandem mass spectrometry show a general decrease of neuroactive steroid levels, and particularly of 5α-reduced metabolites of PROG and T in CSF and plasma of post-finasteride patients. The present results confirm that an impairment of neuroactive steroid levels, associated to depression symptoms, is present in androgenic alopecia patients despite the discontinuation of the finasteride treatment.

Reference list

[1] Altomare G, Capella GL (Depression circumstantially related to the administration of finasteride for androgenetic alopecia. J Dermatol 29:665-669.2002).
[2] Kaufman KD, Olsen EA, Whiting D, Savin R, DeVillez R, Bergfeld W, Price VH, Van Neste D, Roberts JL, Hordinsky M, Shapiro J, Binkowitz B, Gormley GJ (Finasteride in the treatment of men with androgenetic alopecia. Finasteride Male Pattern Hair Loss Study Group. J Am Acad Dermatol 39:578-589.1998).

TOWARDS A CHARACTERIZATION OF THE MOLECULAR BASES OF THE BEHAVIORAL EFFECTS OF FINASTERIDE

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Converging lines of clinical evidence indicate that finasteride, the selective 5alpha-reductase inhibitor currently approved for benign prostatic hyperplasia and male-pattern alopecia, exerts psychoactive effects. In particular, a subset of male patients treated with finasteride has been shown to display severe depressive symptoms often resistant to standard mood-enhancing therapies. On the other hand, preliminary clinical data collected by our group suggest that finasteride may have therapeutic potential for a number of disorders characterized by dopaminergic hyperactivity and behavioral dyscontrol, such as Tourette syndrome, pathological gambling and hypersexuality. The neurobiological underpinnings of these behavioral effects remain elusive. In the quest to shed light on the mechanisms of finasteride, we have studied the proteomic effects of acute and prolonged administration of this drug in the brain of male rats. The results of these studies point to changes in a number of key proteins implicated in steroidogenic metabolism, synaptic plasticity and GABAergic neurotransmission. Based on these and other results, we are currently testing a number of novel therapeutic approaches to counter finasteride-induced depressive effects and explain its antidopaminergic actions.

FINASTERIDE INCREASES DEPRESSION BEHAVIOR, WHEREAS ADMINISTRATION OF 3ALPHA-ANDROSTANEDIOL REDUCES DEPRESSION BEHAVIOR AND INCREASES BRAIN-DERIVED NEUROTROPHIC FACTOR, WITHOUT INCREASING PROSTATE GROWTH, OF MALE MICE

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Background: Androgens regulate aspects of the reproductive, central nervous system, skeletal, and cardiovascular systems throughout the lifespan. Testosterone (T), the primary androgen secreted from the testes, can be aromatized into estradiol and 5apha-reduced to dihydrotestosterone (DHT). DHT is metabolized to 5alpha-androstane,3alpha,17beta-diol (3alpha-diol) and 5alpha-androstane,3beta,17beta-diol (3beta-diol). Men experience a decade-by-decade decline in androgens, with marked androgen deficiency typically observed in the 6th-8th decade of life [1]. This decline can be associated with diminished libido, fatigue, decreased muscle mass, osteoporosis, depression, and/or cognitive dysfunctions among some men. However, men are often reported to use androgen replacement therapies even in their 4th decade, suggesting that some men may be particularly sensitive to androgen decline. There is also use of androgens among young men for effects on physical growth (e.g. anabolic steroids). A serious concern is that such treatments have potential to increase risk for prostate pathologies.

Androgen therapy is intended to alleviate the physical and psychological detriments that age-related T decline has on men but it can increase risk of prostate cancer by progressing the disease [2]. T and DHT bind to androgen receptors (ARs), and some of the proliferative effects of androgens on the prostate may be through this mechanism. However, there are non-AR mechanisms of other T metabolites to consider. Estradiol readily binds to estrogen receptors (ER) alpha and beta, and can enhance benign prostatic hyperplasia and adenocarcinoma, likely via actions at ERalpha, not ERbeta [3]. 3alpha-diol, which is sufficient to produce androgens' affective and cognitive-enhancing effects, has actions via ERbeta and GABA receptors [4-6].

Some men use treatments, such as finasteride, a 5alpha-reductase inhibitor, to treat growth related effects of androgens (e.g. alopecia, benign prostatic hyperplasia) and to reduce side effects of anabolic steroids. An example of this is "OPERATION WHICH DOCTOR," an ongoing legal case involving illicit steroids and drugs, like finasteride, being obtained via internet orders and non-licensed prescribers and pharmacists. This resulted in legislation to change internet drug and steroid trafficking. Moreover, there can be long-term effects of such treatment. Finasteride use has been linked to sexual dysfunctions and mental side effects, including depression, anhedonia and dysphoria. Self-reports, obtained from a case study and internet blogs) demonstrate the long-term and robust nature of the side-effects from these treatments in young men. A question is whether the beneficial trophic effects of androgens may be via 5alpha-reduction.

Methods & Results: In Experiment 1, the behavioral effects of finasteride in a mouse model of depression were assessed. Using the forced swim test, a well-characterized animal assay of depression, we tested the behavioral effects of finasteride (75 mg/kg, SC) administered once (acute) or 5 times (chronic) in homozygous 5alpha-reductase knockout mice (5a-RKO) and their wildtype counterparts. We hypothesized that gonadally-intact male mice administered finasteride would have increased immobility in the forced swim test. Finasteride significantly increased time spent immobile in the forced swim test.

In experiment 2, a question was whether mice that have a reduced capacity to form 5alpha-reduced metabolites can respond to 3alpha-diol and 3beta-diol. Our hypothesis was that 5alpha-reductase mice would have greater anxiety-like (mirror maze) and depressive-like (forced swim test) behavior than wildtype mice, and this would be abrogated by administration of 3alpha-diol or 3beta-diol. Gonadally-intact male mice were administered vehicle, 3alpha-diol (1 mg/kg), or 3beta-diol (1 mg/kg) 1 hour before behavioral testing in the mirror maze and forced swim task on separate testing occasions. After the forced swim test, mice had the testes and prostate gland collected. Results indicated that wildtype mice spent more time in the mirrored section of the mirror maze than did 5a-RKO mice, an indication of anti-anxiety-like type behavior. 3alpha-diol and 3beta-diol increased time spent in the mirrored section of the mirror maze compared to vehicle administration. A similar pattern was observed for the forced swim test with wildtype mice spending less time immobile than 5a-RKO mice. 3alpha-diol reduced time spent immobile compared to vehicle. The typical peripheral growth-enhancing effects of androgens were not observed with administration of 3alpha-diol and 3beta-diol. Thus, mice lacking 5alpha-reductase can respond to administration of 3alpha-diol and 3beta-diol.
In experiment 3, a focus was on further understanding the role of ERbeta as a target of androgens' trophic effects. A previous study has shown that 3alpha-diol or 3beta-diol to gonadectomized male ERbeta knockout (BERKO) mice was ineffective in enhancing cognitive performance or producing anti-anxiety effects (Frye et al., 2008). A question is the trophic effects of such manipulations in the body versus the brain. Studies in BERKO mice demonstrate prostate hyperplasia in aging, suggesting a potential inhibitory role of ERbeta for prostate growth [7]. We hypothesized that 3alpha-diol would have anti-anxiety and anti-depressant-like effects and enhance a central indication of growth (levels of brain-derived neurotrophic factor- BDNF) among wildtype, but not BERKO, mice. Gonadally-intact male mice were administered 3alpha-diol (1 mg/kg) or vehicle 1 hour before testing in the mirror maze and the forced swim task. We found that 3alpha-diol administration compared to vehicle produced greater effects in wildtype compared to BERKO mice. There was no indication that 3alpha-diol increased prostate weight. BDNF was increased by 3alpha-diol compared to vehicle in the hippocampus and prefrontal cortex of wildtype > BERKO mice. Thus, 3alpha-diol may act via ERbeta to enhance affective processing and central BDNF, without producing prostate growth.
Conclusions: The key to developing androgen therapies that do not increase risk for prostate pathologies, or producing other negative effects on androgen-sensitive tissues (e.g. reproductive tract, brain), relies on discovering how androgens' exert their beneficial effects. These studies using mouse models suggest that 5alpha-reduced metabolites acting via ERbeta may have trophic effects in brain that can be parsed from growth in the body.

This research was supported in part by Karo Bio and UAF INBRE.

Reference list

[1] Morley JE, Kaiser FE, Perry HM 3rd, et al. (1997). Longitudinal changes in testosterone, luteinizing hormone, and follicle-stimulating hormone in healthy older men. Metabolism. 46, 410-3.
[2] Marks LS, Mazer NA, Mostaghel E, et al. (2006). Effect of testosterone replacement therapy on prostate tissue in men with late-onset hypogonadism: a randomized controlled trial. JAMA. 296, 2351-61.
[3] Bonkhoff H, Berges R. (2009). The evolving role of oestrogens and their receptors in the development and progression of prostate cancer. Eur Urol. 55, 533-42.
[4] Frye CA, Koonce CJ, Edinger KL, et al. (2008). Androgens with activity at estrogen receptor beta have anxiolytic and cognitive-enhancing effects in male rats and mice. Horm Behav. 54, 726-34.
[5] Gee KW. (1988). Steroid modulation of the GABA/benzodiazepine receptor-linked chloride ionophore. Mol. Neurobiol. 2, 291–317.
[6] Pak TR, Chung WC, Lund TD, et al. (2005). The androgen metabolite, 5alpha-androstane-3beta, 17beta-diol, is a potent modulator of estrogen receptor-gene transcription in neuronal cells. Endo. 146 , 147–155.
[7] Jarred RA, McPherson SJ, Bianco JJ, et al. (2002). Prostate phenotypes in estrogen-modulated transgenic mice. Trends Endocrinol Metab. 13, 163-8.

WEDNESDAY, 20th February 2013
09.00 - 11.30

Estrogen-regulated synaptic connectivity: new approaches of signaling

Estrogen-regulated synaptic connectivity: new approaches of signaling

(Chairs: Balthazart J., Rune G.M.)

- **Kramár E.A. (USA)** *Estrogens rapid influence on synaptic plasticity: interactions with integrins and actin cytoskeleton*

- **Micevych P.E. (USA)** *Membrane estrogen actions: mGluRs, spines and sexual receptivity*

- **Rune G.M. (Germany)** *Phosphorylation of aromatase by calcium-induced calcium release: implications for synaptic connectivity*

- **Abraham I.M. (New Zealand)** *Single molecule analysis of non-classical estradiol action in live neurons*

- **Zhang L.,** Hernández V.S. **(Mexico)** *Ovariectomy down-regulates the hypothalamic vasopressin PVN and SON volume, AVP/Aromatase co-expressing cell numbers and AVP fibres In amydgala and hippocampus: Its impact on anxiety and spatial learning*

ESTROGENS RAPID INFLUENCE ON SYNAPTIC PLASTICITY: INTERACTIONS WITH INTEGRINS AND ACTIN CYTOSKELETON

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Estrogen has been described as a potent modifier of spine anatomy and synaptic function by rapidly enhancing fast excitatory postsynaptic potentials, facilitating long-term potentiation, and increasing spine numbers. All of these effects are likely to contribute to the steroids significant influence on cognition and memory. Beside their importance for our understanding of spine dynamics, and the behavioral effects associated with the estrus cycle, estrogen's influence in the adult brain is poorly understood. The first part of this presentation will describe previous work showing that estrogen's acute effects on synaptic responses and LTP in adult hippocampal tissue involve signaling pathways leading to actin polymerization within dendritic spines. The second half of the presentation will present recent findings showing that estrogen produces two synaptic effects essential for the EPSP enhancement found with LTP: a transient shift of beta1-integrins into their activated conformation and prolonged phosphorylation of CaMKII. Neutralizing antisera against the integrins eliminated estrogen's effects on transmission. Evidence for a time dependent change in the subunit balance of AMPA receptors was also obtained, a process previously linked to CaMKII activation. Discussion of the general hypothesis regarding estrogen as a synaptic modifier regulating acute actions in synaptic signaling and transmission, and spine morphology will conclude the presentation.

MEMBRANE ESTROGEN ACTIONS: METABOTROPIC GLUTAMATE RECEPTORS, IMMEDIATE EARLY GENES, SPINES & SEXUAL RECEPTIVITY

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Our understanding of estradiol actions in the brain has undergone a major shift in the past decade. It is now well accepted that estradiol has both direct nuclear actions and membrane-initiated cell signaling. One of the most studied of estradiol actions in the induction of sexual receptivity. Even prior to the past ten years, the neurochemistry, neural circuits and steroid dependence had been worked out to a great extent. And while it is clear that estradiol induces sexual receptivity, the mechanism is complex. Our laboratory has uncovered a part of the lordosis regulating circuitry of the hypothalamus that mediates the initial, rapid response of estradiol controlling sexual receptivity. This arcuate nucleus (ARH) to medial preoptic nucleus (MPN) to ventromedial nucleus (VMH) pathway underlies an estradiol-induced inhibition of lordosis. This transient inhibition is a necessary component of sexual receptivity. Interestingly, this initial action of estradiol is mediated through membrane estrogen receptor-α (mERα) that is trafficked to the membrane in a complex with metabotropic glutamate receptor-1a (mGluR1a). Membrane-initiated estradiol signaling is temporally constrained through an estradiol autoregulation that controls trafficking of the mERα-mGluR1a complex to the cell membrane and the internalization of the complex from the cell surface. mERα-mGluR1a signaling underlies the activation of a novel protein kinase, PKCθ, and an ARH microcircuit leading to the release of β-endorphin in the MPN and μ-opioid receptor (MOR) activation. Estradiol membrane signaling also activates LIM kinase which phosphorylates an active depolymerizing agent, cofilin. Phospho-cofilin is deactivated to allow the formation of dendritic spines in the ARH. Initially these spines had an immature, filapodial shape. Although the spine density did not change for 48 hours after estradiol treatment, spines morphology became more mature with the appearance of mushroom-shaped spines at the time lordosis behavior began to appear. This suggested that spines were important for sexual receptivity. Indeed these newly formed spines were shown to be critical for the estradiol induction of sexual receptivity. Blocking spinogenesis with cytochalasin D, a drug that inhibits actin polymerization, prevented spinogenesis and estradiol induced lordosis behavior.

Although the sex steroids, and in particular estradiol, are critical for lordosis behavior, tactile stimulation from the flanks and perineum is also important. A marker for such salient sensory input to lordosis regulating ARH and VMH can be visualized by measuring the induction of the immediate early gene, activity-regulated cytoskeleton-associated protein/activity-regulated gene 3.1 (Arc/Arg3.1). Arc/Arg3.1 is induced in the female ARH by sexual interaction with a male, but not by estradiol, olfactory stimuli or more general social interaction. Interestingly, progesterone both prevented the induction of Arc/Arg3.1 and its downstream effects. In females where estradiol alone was used to induce lordosis, Arc/Arg3.1 induction attenuated sexual receptivity when rats were retested for lordosis behavior. Knockdown of Arc/Arg3.1 induction prevented the loss of sexual receptivity in repeatedly tested females.

In the ARH, steroid stimulation and sensory input are integrated to signal sexual receptivity. Estradiol mediates cell signaling and rapidly triggers plastic changes in the

neuronal morphology that activate a multisynaptic circuit involving NPY, β-endorphin and MOR whose activity is modulated by sensory input that induces Arc/Arg3.1.

Supported by NIH grant DA013185

PHOSPHORYLATION OF AROMATASE: IMPLICATIONS FOR SYNAPTIC CONNECTIVITY IN THE HIPPOCAMPUS

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Spine synapse density in females, specifically in the hippocampus, widely depends on aromatase activity in hippocampal neurons, in other words depends on hippocampus-derived estradiol (Kretz et al., 2004). Inhibition of aromatase activity in hippocampal slice cultures originating from females and systemic treatment of females mice with aromatase inhibitors reduced significantly the number of spines and spine synapses (Kretz et al. 2004, Zhou et al., 2010), the expression of synaptic proteins (Prange-Kiel et al., 2006), and LTP could not be induced in the presence of aromatase inhibitors (Vierk et al., 2012). Impairment of LTP and as a consequence spine and spine synapse loss was specifically seen in females but not in males.
Aromatase activity in hippocampal neurons is regulated by substrate availability, such as cholesterol and testosterone (Fester et al., 2009), neuronal activity (Hojo et al., 2004), and gonadotropins, such as GnRH (Prange-Kiel et al., 2008). On the cellular level by using cell lines, it was furthermore shown that Ca^{2+} release from intracellular stores downregulates aromatase activity via phosphorylation of the enzyme by Ca^{2+}-dependent kinases and in contrast to other enzymes aromatase becomes inactivated by this process (Balthazart et al., 2005; 2006). In hippocampal neurons, we found consistently immunoreactivity of aromatase upregulated and estradiol synthesis enhanced when Ca^{2+} release was prevented. Opposite effects were seen when Ca^{2+} release was experimentally induced. Along this line, short term treatment with NMDA, inducing Calcium-induced calcium release, inhibits estradiol synthesis, while long-term treatment with NMDA resulted in an increase in estradiol content of the supernatant, presumably due to depletion of internal calcium stores. Most importantly, application of estradiol to hippocampal neurons also phosphorylates aromatase and thereby inhibits its activity, as shown by immunoprecipitation. Estradiol treatment is paralleled by calcium influx and as a consequence by inducing calcium release from internal stores. Inhibition of aromatase activity by estradiol likely underlies the failure of estradiol to increase spine synapses in hippocampal slice cultures of female animals (Kretz et al., 2004) but not of male animals.

SINGLE MOLECULE ANALYSIS OF NON-CLASSICAL ESTRADIOL IN LIVE NEURONS

Ábrahám I.

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Besides estrogen-receptor mediated slow classical genomic mechanisms estradiol (E2) also exerts faster, non-classical effects via membrane receptors and the intracellular signal-transduction system. We have demonstrated that E2 exerts direct actions upon intracellular signaling pathways via extracellular signal-regulated kinase 1/2 (ERK1/2) activation in the brain. One of the key mechanisms in ERK1/2 activation is the transient immobility of surface movement of neurotrophin membrane receptors such as the TrkA receptor (TrkAR). In this study we investigated the rapid non-classical effect of E2 on TrkAR. Total internal reflection fluorescence microscopy (TIRFM) in combination with immunohistochemistry and Western blotting was used for super-resolution real-time live cell imaging of quantum dot (Qdot) labelled-TrkAR plasmamembrane trafficking and detection of ERK activation. Experiments were performed on PC12 cells and isolated basal forebrain neurons from cholinergic-GFP mouse brain. Immunohistochemical studies validated membrane associated TrkAR labelling in PC12 cells and Western blot study showed that nerve growth factor (NGF, 100 nM) stimulation of Qdot-labelled TrkA receptors induced ERK phosphorylation. TIRFM investigations demonstrated that Qdot-labelled TrkAR followed confined diffusion in control conditions in both cell types. The TrkAR had two distinct motility states on the membrane surface, characterized as mobile and immobile phases. NGF (100 nM) increased the frequency and time interval of the immobile periods in both PC12 cells and adult neurons. Low concentrations of E2 (100 pM) also rapidly increased the frequency of immobile periods of TrkAR trafficking in PC12 cells and adult neurons. These results demonstrate for the first time the confined diffusion of TrkAR with transient immobile periods in the receptor trajectory in the neuronal membrane. Furthermore single molecule analysis showed a rapid NGF-like membrane effect of E2 on TrkAR mobility, suggesting the presence of membrane-initiated E2-induced TrkAR signalling in adult cholinergic neurons and PC12 cells.

OVARIECTOMY DOWN-REGULATES THE HYPOTHALAMIC VASOPRESSIN PVN AND SON VOLUME, AVP/AROMATASE CO-EXPRESSING CELL NUMBERS AND AVP FIBRES IN AMYDGALA AND HIPPOCAMPUS: ITS IMPACT ON ANXIETY AND SPATIAL LEARNING

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We have previously shown that vasopressinergic hypothalamic magnocellular neurons project to amygdala (Hernández and Zhang, 2012) and hippocampus (Zhang and Hernández, 2012) influencing anxiety behaviours (Zhang, et al, 2010) and spatial learning (Hernández et al, 2012). The vasopressin gene expression is up-regulated by a promoter region for the oestrogen receptor. Hence, it is interesting to evaluate the effects of ovariectomy on hypothalamic vasopressin system rat and its behavioural consequences. Ovariectomy was performed to 2 month-old Wistar rats. After 1 month of the surgery, elevated plus maze (EPM) and Vogel conflict test (VCT) were performed. Rats were perfused 90 min after the VCT procedure. Immunohistochemistry against aromatase (Ar+), arginine vasopressin (AVP) and Fos were performed to evaluate: 1) The volume occupied by neurons expressing vasopressin in the paraventricular (PVN) and supraoptic (SON) nuclei; 2) The co-localization ratio of AVP+&Ar+/ AVP+ neurons in the PVN and SON; 3) The neuronal activation in hypothalamus and amygdala (basolateral (BLA), central (CeA) and medial (MeA)). Our results showed that ovariectomy down-regulated the volumes occupied by vasopressin expressing neurons in hypothalamus (82% in PVN, $p<0.05$; and SON 83%, $p<0.001$; ctrl = 100%), as well as the ratio of the co-expression AVP/Ar cell number *vs* the total of the vasopressin neurons numbers per region: in the PVN (27.7%, $p<0.001$) and SON (50.5%, $p<0.001$). Regarding the presence of AVP immunopositive fibres, there were marked reductions in both ventral and dorsal hippocampus (around 80%) and the medial postero-dorsal amygdala (around 60%). Behavioural tests showed impaired spatial learning performance revealed by Morris Water Maze (MWM), an increased unconditioned anxiety measured by EPM ($p<0.01$) and a decrease in conditioned anxiety measured by the VCT ($p<0.01$), which is in accordance with our previous study suggested that this water-deprivation-conditioned anxiety behaviour (VCT) is strongly modulated by hypothalamic vasopressinergic system (Zhang et al, 2010). Fos analysis showed a diminished neuronal activation in CeA ($p<0.05$), MeA ($p<0.05$), BLA ($p<0.01$) and PVN ($p<0.01$) 90 min after VCT. Our data indicate that the ovarian hormones are important regulator(s) for the hypothalamic nonapeptide AVP system functions.

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Reference list

Hernandez, V. S., S. Ruiz-Velazco, et al. (2012). "Differential effects of osmotic and SSR149415 challenges in maternally separated and control rats: The role of vasopressin on spatial learning." Neurosci Lett 528(2): 143-7.

Hernandez, V.S., Zhang, L (2012) "Vasopressin containing fibers distribution and synaptic innervation in the medial and central amygdala: an immunohistochemical study using light and electron microscopy" Society For Neuroscience Meeting, New Orleans.

Zhang, L. and V. S. Hernandez (2013). "Synaptic innervation to rat hippocampus by vasopressin-immuno-positive fibres from the hypothalamic supraoptic and paraventricular nuclei." Neuroscience 228:139-162.

Zhang, L., V. S. Hernandez, et al. (2012). "Hypothalamic vasopressin system regulation by maternal separation: its impact on anxiety in rats." Neuroscience 215: 135-48.

Zhang, L., M. P. Medina, et al. (2010). "Vasopressinergic network abnormalities potentiate conditioned anxious state of rats subjected to maternal hyperthyroidism." Neuroscience 168(2): 416-428.

WEDNESDAY, 20th February 2013
11.45 - 13.15

Plenary Lecture

Bourguignon J.-P. (Belgium)

NEUROENDOCRINE DISRUPTION ACROSS REPRODUCTION AND ENERGY BALANCE

Bourguignon J.-P., Franssen D., Gérard A., Lebrethon M.-C., Rasier G., Parent A.-S.

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During the past decades, epidemiological studies have shown increasing prevalence of disorders such as obesity, metabolic syndrome and early onset of sexual maturation. While direct effects of environmental factors on end organs have been largely incriminated, the possible role of neuroendocrine mechanisms has emerged recently. The hypothalamus is a unique place where homeostasis of essential functions like reproduction and energy balance are jointly controlled. Both functions share involvement of same neuropeptides locally (e.g. NeuroPeptide Y, Agouti-Related Peptide -AGRP, Corticotrophin Releasing Hormone, kisspeptin) as well as regulation by same hormones of peripheral origin (e.g. leptin, ghrelin, sex steroids). Another common feature of reproduction and energy balance is that the neuroendocrine control is organized during the critical prenatal and early postnatal periods of life, in a manner that will determine those functions for the entire life cycle.

The physiological concepts summarized above could apply to the consequences of exposure to endocrine disrupting chemicals (EDCs). Early evidence has been obtained that exposure to EDCs such as diethylstilbestrol (DES) and bisphenol A (BPA) could be associated with impairment of both the reproductive axis and the control of energy balance. Moreover, the tragic story of female reproductive tract cancer in the offspring of women treated with DES during pregnancy and rodent data after fetal and neonatal exposure to BPA illustrate the lifelong impact of exposure during early critical windows of life, consistent with Barker's concept of early life origins of health and diseases.

The rodent paradigm we use for evaluation of neuroendocrine function is the pulsatile release of Gonadotrophin releasing hormone (GnRH) from hypothalamic explants *in vitro*. More precisely, we study the developmental increase in GnRH pulse frequency that precedes the onset of sexual maturation. This process can be facilitated by *in vitro* and *ex vivo* exposure to estradiol or leptin whereas it is inhibited by AGRP and Ghrelin.

Our initial studies were performed using the insecticide DDT after clinical observation that girls exposed to DDT in early life and moving to Belgium for international adoption showed markedly increased risk of sexual precocity. Using the above female rodent model, we confirmed that neonatal exposure to DDT could account for early acceleration of GnRH pulse frequency through complex mechanisms involving the estrogen receptor, the AMPA subtype of glutamate receptor and the arylhydrocarbon dioxin receptor. More recently, we have exposed rodents neonatally for 5 days to either DES or BPA. In such conditions, the EDC dose appears to be critical since pubertal timing is advanced after exposure to the highest dose but delayed after using the lowest dose. The changes in GnRH pulse frequency are consistent with the changes in pubertal timing after BPA but not after DES. The mRNA expression of Kiss is reduced by BPA, confirming estrogen like effects reported by others. DES accounts for a reduction of leptin stimulatory effects on pulsatile GnRH secretion. This effect is more obvious when neonatal exposure to DES was preceded by moderate food restriction during fetal life. Taken together, those data indicate that, during the critical period of fetal and neonatal life, nutritional insults and EDCs could interact with the neuroendocrine crosstalk between control of reproduction and energy balance.

Supported by the European Commission (EDEN), the Belgian Fonds de la Recherche Scientifique Médicale, the University of Liège and the Belgian Study Group for Pediatric Endocrinology

WEDNESDAY, 20th February 2013
15.00 - 17.30

Genomic and non-genomic effects of neuroactive steroids: mechanisms of action

Genomic and non-genomic effects of neuroactive steroids: mechanisms of action

(Chairs: Lambert J.J., Melcangi R.C.)

- **Marin R.,** Casañas V., Pérez J.A., Fabelo N., Fernandez C.E., Diaz M. **(Spain)** *Membrane estrogen receptor as part of signalosomes in neurons. The concept of "lipid raft aging"*

- **Tetel M.J. (USA)** *Nuclear receptor coactivators in steroid action in brain*

- **Petersen S.,** Intlekofer K.A., Moura-Conlon P.J., Lopez J.A., del Pino J. **(USA)** *Non- classical progesterone signaling molecules in the nervous system*

- **Levine J. (USA)** *Consequences of non-classical ER alpha signaling in brain*

- **Schütz G.,** Berger S. **(Germany)** *Dissection of glucocorticoid action in the brain using inducible gene inactivation*

- **Kajta M.,** Rzemieniec J., Litwa E., Wnuk A., Lason W., Nalepa I., Zelek-Molik A., Wojtowicz A. **(Poland)** *Impact of G-protein-coupled receptor 30 on the effects of daidzein and dichlorodiphenyltrichloroethane in mouse embryonic neuronal cells*

ESTROGENS AS MODULATORS OF NEURONAL SIGNALOSOMES AND BRAIN LIPID HOMEOSTASIS

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Estrogens have been identified as neuroprotectors against degeneration by means of a variety of genomic and non-genomic mechanisms [1]. In particular at the plasma membrane, several pathways triggered by estrogens through its interaction with different targets, including membrane estrogen receptors (mERs), have been shown to exert beneficial actions against neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). Part of these actions takes place in lipid rafts, which are membrane domains with a particular lipid composition. These microdomains also represent a preferential site for signaling protein complexes, or signalosomes, relevant for neuronal functionality. Furthermore, recent findings indicate a potential role of estrogens in the preservation of neuronal membrane physiology related to lipid homeostasis.

We have found that ERs are localized in neuronal lipid rafts, taking part of a macromolecular complex together with insulin growth factor 1-receptor (IGF-1R), a voltage-dependent anion channel (VDAC), caveolin-1 as a pivotal anchoring protein, and probably other still uncharacterized molecules [2]. Estradiol binding to its receptor at this level enhances neuroprotection against amyloid-β degeneration through the activation of different signal transduction pathways. Part of this strategy occurs through modulation of VDAC gating by favouring the phosphorylation of the channel, and thereby reducing Aβ-induced neurotoxicity and extrinsic apoptosis [3]. This mechanism may be estrogen-dependent since selective estrogen receptor modulators, such as tamoxifen, do not enhance VDAC phosphorylation and neuroprotection against Aβ. Therefore, a plausible hypothesis is that the dynamic interaction of signalosomes with different extracellular ligands may be at the basis of neuronal maintenance against different neuropathologies.

Moreover, part of the stability and functionality of signaling platforms lays on the distribution of lipid hallmarks in these microstructures, which modulate membrane physicochemical properties, thus favoring protein interactions and functionality. In this sense, neuronal raft interactions of ERs have been shown altered in AD brains, a phenomenon that may contribute to neuronal impairment [4]. In agreement with this, our recent work has demonstrated alterations in the lipid composition of these microdomains in human AD, PD and incidental PD, as well as in AD transgenic mice [5,6]. Furthermore, lipid rafts show age-dependent changes in the brain, a phenomenon that we have coined as "Lipid-raft aging", which is considerably intensified as a consequence of AD [7]. This lipid derangement is particularly relevant in the case of polyunsaturated fatty acids (PUFA), such as docosahexanoic acid (DHA), which is highly abundant in the brain and crucial for the establishment and maintenance of lipid rafts. DHA has been shown significantly altered in lipid rafts from AD and PD brains, thus contributing to membrane architecture impairment.

A recent finding is that, in nerve tissues, physiological doses of estradiol regulate cholesterol and DHA levels, important components of lipid rafts, through different mechanisms. Especially, estradiol activates DHA mobilization from the liver to the brain [8]. In addition, we have observed that PUFA can be locally produced in the brain, and this activity is under hormonal and dietary influences. Indeed, estrogens and DHA may act synergistically to stabilize brain lipid structure by regulating neuronal synthesis. These data suggest that part of the neuroprotective effects elicited by estrogens occur through brain homeostatic preservation of PUFA (and DHA) amounts.

In general, estrogen mechanisms of neuroprotection may occur not only by its interaction with neuronal protein targets through non-genomic and genomic mechanisms, but also through its participation in membrane architecture stabilization via "lipidomic" mechanisms.

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Reference list

[1] Marin R., Guerra B., Alonso R., Ramírez C.M. and Díaz M. (2005) Estrogen captivates classical and alternative mechanisms to orchestrate neuroprotection. Curr. Neurovasc. Res. 2(4): 287-301.
[2] Marin R., Diaz M., Alonso R., Sanz A., Arevalo M.A., Garcia-Segura LM. (2009) Role of estrogen receptor alpha in membrane-initiated signaling in neural cells: interaction with IGF-1 receptor. J. Steroid Biochem. Mol. Biol. 114, 2-7.
[3] Marin R., Ramirez CM., Morales A., Gonzalez M., Alonso R., Diaz M. (2008) Modulation of A -induced neurotoxicity by estrogen receptor alpha and other associated proteins in lipid rafts. Steroids 73, 992-996.
[4] Marin R. (2011) Signalosomes in the brain: relevance in the development of certain neuropathologies such as Alzheimer's disease. Front. Physiol. 2 (23) 1-4.
[5] Martin V., Fabelo N., Santpere G., Puig B., Marin R., Ferrer I., Diaz M. (2010) Lipid alterations in lipid rafts from Alzheimer's disease human brain cortex. J. Alzheimer Dis. 19, 489-502.
[6] Fabelo N., Martin V., Santpere G., Marin R., Torrent L., Ferrer I., Diaz M. (2011) Severe alterations in lipid composition of frontal cortex lipid rafts from Parkinson's disease and incidental Parkinson's disease. Mol Med. 17, 1107-1118.
[7] Fabelo N., Martin V., Marin R., Santpere G., Aso E., Ferrer I., Diaz M. (2012) Evidence for premature lipid raft aging in APP/PS1 double-transgenic mice, a model of familial premature lipid raft aging in APP/PS1 double-transgenic mice, a model of familial Alzheimer disease. J. Neuropathol. Exp. Neurol. 71, 868-881.
[8] Fabelo N., Martin V., Gonzalez C., Alonso A., Diaz M. (2011) Effects of oestradiol on brain lipid class and fatty acid composition: comparison between pregnant and ovariectomised oestradiol-treated rats. J. Neuroendocrinol. 24, 292-309.

NUCLEAR RECEPTOR COACTIVATORS IN STEROID ACTION IN BRAIN

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The steroid hormones, estradiol and progesterone, exert many of their effects on reproductive behavior and physiology by binding to their respective intracellular receptors in specific brain regions. Studies done in cell culture indicate that nuclear receptor coactivators profoundly influence transcriptional activity of steroid receptors through a variety of mechanisms, including acetylation of histones, methylation, phosphorylation and chromatin remodeling. Moreover, the critical role of these coactivators in a variety of human diseases, including cancer and neurological disorders, is becoming more evident [1, 2]. While we know much about coactivator mechanisms *in vitro*, we are beginning to understand the function of these important regulatory molecules in steroid action in brain and behavior. Our lab has been exploring the function of three of the more important coactivators, steroid receptor coactivator-1 (SRC-1), SRC-2 and CREB binding protein (CBP), in estrogen receptor (ER) and progestin receptor (PR) action in brain and behavior in rodents. Our work reveals that nuclear receptor coactivators are coexpressed in estradiol-induced PR cells in reproductively-relevant brain regions in female rats and mice, suggesting that these specialized cells represent functional sites of interaction between steroid receptors and coactivators in brain [3, 4]. Moreover, antisense studies from our lab and others reveal that these coactivators influence ER-mediated transactivation of the behaviorally-relevant PR gene in the ventromedial nucleus of the hypothalamus (VMN) [5, 6], an important region in the regulation of hormone-dependent female reproductive behavior. Using this same antisense approach, we found that nuclear receptor coactivator action in the VMN is essential for both ER- and PR-dependent aspects of female sexual behavior in rats [5, 7]. Recently, our lab has taken a proteomics-based approach to studying steroid hormone action in brain. Using protein-protein interaction assays, we found that SRC-1 and SRC-2 from the hypothalamus or hippocampus interact with ER and PR in a ligand-dependent manner, which was confirmed by mass spectrometry [8, 9]. Interestingly, these coactivators from brain interact with ER and PR in a receptor subtype-specific, and brain region-specific, manner. These findings reveal distinct contrasts with previous cell culture studies and emphasize the importance of studying receptor-coactivator interactions using biologically-relevant tissue. It is our goal to use this proteomics-based approach to identify potentially novel proteins involved in hormone action in brain. Understanding the recruitment of different coactivator complexes to the promoter, which is likely to be cell and tissue specific, will be critical to understanding steroid action in brain and the regulation of complex behaviors.

Reference list

[1] D.M. Lonard, R. Kumar, B.W. O'Malley, Minireview: the SRC family of coactivators: an entree to understanding a subset of polygenic diseases?, Molecular Endocrinology (Baltimore, Md, 24 (2010) 279-285.
[2] O.C. Meijer, E.R. De Kloet, Coregulators in CNS function and disease, in: R. Kumar, W. O'Malley B (Eds.) Nuclear Receptor Coregulators and human disease, World Scientific Books, 2008.
[3] M.J. Tetel, N.K. Siegal, S.D. Murphy, Cells in behaviourally relevant brain regions coexpress nuclear receptor coactivators and ovarian steroid receptors, J Neuroendocrinol, 19 (2007) 262-271.
[4] C.M. Tognoni, J.G. Chadwick, Jr., C.A. Ackeifi, M.J. Tetel, Nuclear receptor coactivators are coexpressed with steroid receptors and regulated by estradiol in mouse brain, Neuroendocrinology, 94 (2011) 49-57.
[5] H.A. Molenda, A.L. Griffin, A.P. Auger, M.M. McCarthy, M.J. Tetel, Nuclear receptor coactivators modulate hormone-dependent gene expression in brain and female reproductive behavior in rats, Endocrinology, 143 (2002) 436-444.

[6] E.M. Apostolakis, M. Ramamurphy, D. Zhou, S. Onate, B. O'Malley, Acute disruption of select steroid receptor coactivators prevents reproductive behavior in rats and unmasks genetic adaptation in knockout mice, Molecular Endocrinology, 16 (2002) 1511-1523.
[7] H.A. Molenda-Figueira, C.A. Williams, A.L. Griffin, E.M. Rutledge, J.D. Blaustein, M.J. Tetel, Nuclear receptor coactivators function in estrogen receptor- and progestin receptor-dependent aspects of sexual behavior in female rats, Hormones and Behavior, 50 (2006) 383-392.
[8] H.A. Molenda-Figueira, S.D. Murphy, K.L. Shea, N.K. Siegal, Y. Zhao, J.G. Chadwick, L.A. Denner, M.J. Tetel, Steroid receptor coactivator-1 from brain physically interacts differentially with steroid receptor subtypes, Endocrinology, 149 (2008) 5272-5279.
[9] M.A. Yore, D. Im, L.K. Webb, Y. Zhao, J.G. Chadwick, H.A. Molenda-Figueira, S.J. Haidacher, L. Denner, M.J. Tetel, Steroid receptor coactivator-2 expression in brain and physical associations with steriod receptors, Neuroscience, 169 (2010) 1017-1028.

NON-CLASSICAL PROGESTERONE SIGNALING MOLECULES IN NEURAL CELLS

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Since the 1980s, studies investigating neural effects of progesterone (P_4) have focused mainly on genomic and non-genomic actions of the classical progestin receptor (Pgr). More recently two groups of non-classical P_4 signaling molecules have been identified: 1) Class II progestin and adipoQ receptor (Paqr) family that includes Paqr 5,6,7,8 and 9, also called membrane progestin receptor α (mPRα; Paqr7), mPRβ (Paqr8), mPRγ (Paqr5), mPRδ (Paqr6) and mPRε (Paqr9) [1-6]; and 2) Progesterone receptor membrane component (Pgrmc) family that includes Pgrmc1[1-3], Pgrmc2 [4] and neudesin [5, 6]. The Class II Paqr family members bind to P_4, have seven transmembrane domains and activate either inhibitory [7-9] or stimulatory [10, 11] G proteins. Pgrmc family members are cytochrome b_5-like heme/steroid-binding proteins [12-14] that act through multiple pathways including mitogen-activated protein kinase (MAPK) [12], phosphatidylinositol 3-kinase (PI-3K) [15], and protein kinase G (PKG) [16-19]. To determine which of the non-classical signaling molecules are most likely to mediate neuroendocrine effects of P_4, we first mapped the distribution of the mRNAs encoding Paqr 7 and 8 and Pgrmc1 and 2 throughout the forebrain of the female rat brain [20]. These molecules have been studied more intensively than other candidates. We also mapped the expression of the mRNA encoding serpine mRNA binding protein 1 (Serbp1; also called plasminogen activator inhibitor 1; Pairbp1) [21-23], a binding partner of Pgrmc1 that is critical for some Pgrmc1 functions [23]. We next used quantitative polymerase chain reaction (QPCR) to measure levels of each of these molecules in tissue microdissected from the anteroventral periventricular nucleus (AVPV) of the preoptic area (POA) and the ventromedial nucleus of the hypothalamus (VMN). We focused on these nuclei because the AVPV controls female-typical gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) surge release [12-15] and the VMN regulates female reproductive behaviors [24]. Both of these functions are modulated through rapid actions of P_4.

Results of our studies showed that Paqr7 mRNA levels are low with no distinct localization pattern in POA or hypothalamic nuclei [20]. Moreover, *paqr7* expression is not altered by hormone treatment in the AVPV or the VMN. Paqr8 mRNA levels are also low and homogeneous in the female POA and hypothalamus, but more abundant in the hippocampus and thalamic nuclei [20]. Interestingly, E_2 and P_4 induce changes in Paqr8 mRNA levels that parallel changes in Pgr in the AVPV; E_2 alone increases levels, P_4 alone decreases levels, and combined E_2 and P_4 has the same effect as E_2 alone. No steroid treatment affects *paqr8* expression in the VMN. Our findings suggest that Paqr7 is probably not a key player in P_4-mediate effects on neuroendocrine functions, but Paqr8 may mediate P_4 regulation of GnRH and LH release. It is notable that Paqr6 and Paqr9 expression patterns have not yet been mapped in the brain, but are detected in human hypothalamus and other brain regions using quantitative polymerase chain reaction (QPCR) [10]. Thus, these molecules may also be involved in P_4 regulation of neuroendocrine functions in females. In contrast to the Paqr molecules, several preoptic and hypothalamic nuclei contain relatively high levels of mRNAs encoding Pgrmc family members, particularly Pgrmc1 and Serbp1 [20], as well as neudesin (Lopez, J.A. and Petersen, S.L.). The distribution patterns of these molecules were similar to that of Pgr mRNA, except that *pgrmc1* expression is more widespread in regions outside the POA and hypothalamus. Unlike any of the other nonclassical P_4 receptors examined, the distribution of neudesin mRNA overlaps that of Pgr mRNA completely with no expression in regions lacking Pgr (Lopez, J.A. and Petersen, S.L., unpublished). These findings suggest that Pgrmc family members regulate some of the same functions as the classical Pgr. Results of our hormonal regulation studies of Pgrmc family members support this idea. P_4 alone or combined E_2 and P_4 increases Serbp1 and Pgrmc1 mRNA levels in the VMH [25]. Moreover, although Pgrmc1 mRNA levels are not altered by ovarian steroids in the AVPV, combined E_2 and P_4 significantly upregulate Serbp1levels in the region. These data are interesting in view of evidence that Serbp1 binds Pgrmc1 and dramatically increases cellular responsiveness to P_4 by 10-fold in granulosa cells that lack Pgr [21, 23]. Interestingly, neudesin is the only Pgrmc family member we found to be down-regulated by E_2. Overall our findings indicate that Pgrmc1, Pgrmc2 and Serbp1, as well as neudesin, are the most likely candidates for mediating non-classical effects of P_4 on neuroendocrine functions. Pgrmc1 is a particularly interesting candidate because it was recently identified as the elusive sigma type 2 receptor [26] and ligands of this receptor regulate dopamine (DA) transporter activity through a Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) transduction system [27]. It is

intriguing that P_4 facilitation of female mating behavior involves rapid changes in CaMKII kinase activity [28] through a mechanism upstream from DA receptor activation [29]. Pgrmc1 is also interesting in the context of sexual differentiation of brain nuclei, particularly of POA and hypothalamic nuclei that develop through sex-specific and E_2-regulated apoptosis. Sexual differentiation of the AVPV occurs during the perinatal period when the developing testes, but not ovaries, are active. In the male AVPV, testosterone is aromatized to E_2 and this hormone triggers apoptosis [30-32] and defeminization of LH release mechanisms. Importantly, Pgrmc1 prevents apoptosis in non-neural tissue [33] and we recently found that *pgrmc1* expression is lower in the neonatal AVPV of males than females (del Pino, J. and Petersen, S.L.). Moreover, Pgrmc1 was originally identified as a target of the arylhydrocarbon receptor (AhR) ligand [34] and developmental exposure to AhR ligands block defeminization of LH release patterns [35].
Our recent findings and those of others suggest that neudesin may also play a role in sexual differentiation of the AVPV. Neudesin is a neurotrophic factor [5, 6, 36] and we showed that *neudesin* expression is lower in the developing male than in the female AVPV (Lopez, J.A. and Petersen, S.L.), consistent with a higher rate of apoptosis is the male. Moreover, administration of E_2 to neonatal females increases apoptosis [30] and down-regulates neudesin in the AVPV (Lopez, J.A. and Petersen, S.L.). We are actively investigating the roles of Pgrmc1, Pgrmc2 and neudesin in the development and adult functioning of sex-specific neuroendocrine functions using conditional knockout mice and *in vitro* models. These studies will increase our understanding of how non-classical P_4 signaling molecules regulate neuroendocrine signaling.

Reference list

1. Losel, R.M., et al. Steroids, 2008. 73(9-10): p. 929-34.
2. Peluso, J.J. Biol Reprod, 2006. 75(1): p. 2-8.
3. Peluso, J.J. Steroids, 2011. 76(9): p. 903-9.
4. Gerdes, D., et al. Biological chemistry, 1998. 379(7): p. 907-11.
5. Kimura, I., et al. Journal of Neuroscience Research, 2006. 83(8): p. 1415-24.
6. Kimura, I., et al. Journal of Neuroscience Research, 2005. 79(3): p. 287-94.
7. Karteris, E., et al. Molecular endocrinology, 2006. 20(7): p. 1519-34.
8. Thomas, P., et al. Endocrinology, 2007. 148(2): p. 705-18.
9. Zhu, Y., et al. Proceedings of the National Academy of Sciences, 2003. 100(5): p. 2231-2236.
10. Pang, Y., et al. Endocrinology, 2013. 154(1): p. 283-95.
11. Tubbs, C. and P. Thomas. Endocrinology, 2009. 150(1): p. 473-84.
12. Kimura, I., et al. Biochemical and biophysical research communications, 2009. 381(1): p. 75-80.
13. Mifsud, W. and A. Bateman. Genome biology, 2002. 3(12): p. RESEARCH0068.
14. Min, L., et al. The FEBS journal, 2005. 272(22): p. 5832-43.
15. Kimura, I., et al. Journal of Neuroscience Research, 2006. 83(8): p. 1415-1424.
16. Bashour, N.M. and S. Wray. Endocrinology, 2012. 153(9): p. 4457-4469.
17. Peluso, J.J., et al. Endocrinology, 2007. 148(5): p. 2037-44.
18. Peluso, J.J. and A. Pappalardo. Biology of reproduction, 2004. 71(6): p. 1870-8.
19. Peluso, J.J., et al. Endocrinology, 2008. 149(2): p. 534-43.
20. Intlekofer, K.A. and S.L. Petersen. Neuroscience, 2011. 172: p. 55-65.
21. Peluso, J.J., et al. Endocrinology, 2004. 145(6): p. 3014-22.
22. Peluso, J.J., et al. Biology of reproduction, 2005. 73(2): p. 261-70.
23. Peluso, J.J., et al. Biology of reproduction, 2012.
24. Mani, S.K. and J.D. Blaustein. Neuroendocrinology, 2012. 96(2): p. 152-161.
25. Intlekofer, K.A. and S.L. Petersen. Neuroscience, 2011. 176: p. 86-92.
26. Xu, M., et al. Endocrinology, 2000. 141(9): p. 3319-27.
27. Weatherspoon, J.K. and L.L. Werling. J. of Pharmacology and Experimental Therapeutics, 1999. 289(1): p. 278-284.
28. Balasubramanian, B., et al. Endocrinology, 2008. 149: p. 5518-5526.
29. Balasubramanian, B. and S.K. Mani. Journal of Neuroendocrinology, 2009. 21(11): p. 954-960.
30. Arai, Y., et al. Neurosci Res, 1996. 25(4): p. 403-7.
31. Forger, N.G. Journal of Neuroendocrinology, 2009. 21(4): p. 393-399.
32. Tsukahara, S. Journal of Neuroendocrinology, 2009. 21(4): p. 370-376.
33. Peluso, J.J., et al. J Clin Endocrinol Metab, 2009. 94(7): p. 2644-9.
34. Selmin, O., et al. Carcinogenesis, 1996. 17(12): p. 2609-2615.
35. Mably, T.A., et al. Toxicol Appl Pharmacol, 1992. 114(1): p. 108-117.
36. Kimura, I., et al. The Journal of biological chemistry, 2008. 283(7): p. 4323-31.

CONSEQUENCES OF NON-CLASSICAL ERα SIGNALING IN BRAIN

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Ovarian estrogens exert critically important actions in hypothalamic neurons to regulate ovulatory cyclicity, reproductive behaviors, and energy homeostasis. Estrogen receptor alpha (ERα) appears to mediate most of these effects, as disruption of ER signaling leads to infertility and metabolic syndrome. ER signaling mechanisms may include "classical genotropic"effects mediated by direct binding of receptor dimers to DNA, "non-classical genotropic" effects involving tethering of ERs to other transcription factors, and "non-classical non-genotropic" actions mediated by cytoplasmic ERs coupled to membrane-initiated signal transduction pathways. Our studies make use of novel ER mutant mouse models to ascertain the cellular mechanisms by which ERα mediates E_2effects on these physiological and behavioral processes. We have utilized a novel mutant ER knock-in mouse model, which confers non-classical genotropic and non-genotropic signaling in the absence of classical signaling, to determine that non-classical ER signaling can convey E_2 effects integral to homeostatic feedback control of reproductive hormone secretions, as well as E_2 actions governing paracopulatory behavior and body weight regulation.

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DISSECTION OF GLUCOCORTICOID ACTION IN THE BRAIN USING INDUCIBLE GENE INACTIVATION

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Glucocorticoid action in the brain is mediated by two ligand-activated transcription factors, the mineralocorticoid (MR) and the glucocorticoid receptor (GR). To investigate the specific role of MR and GR in processes that are affected by changes in circulating glucocorticoid levels, e.g. the activity of the hypothalamic-pituitary-adrenal (HPA-) axis and hippocampal neurogenesis, we have generated mouse mutants that lack MR or GR in specific regions of the brain using the Cre/loxP recombination system. Since early-onset ablation of MR or GR expression affects brain development, we have established a transgenic mouse line that allows inactivating conditional (flox) alleles at desired time points after brain development is completed. In order to achieve inducible gene inactivation we used the CreERT2 fusion protein that translocates into the nucleus only after the application of the synthetic ligand tamoxifen and we restricted the gene inactivation to neurons in forebrain areas that show co-expression of the receptors by using the regulatory elements of the CaMKIIalpha gene to drive CreERT2 expression. We addressed possible compensatory actions in mutants with ablation of either MR or GR by analyzing mutants with simultaneous ablation of MR and GR. The analysis of the plasma corticosterone levels at circadian trough and peak revealed that MR in contrast to GR only participates in the control of HPA-axis activity at circadian peak. The investigation of hippocampal neurogenesis showed that only MR is crucial for sustained progenitor cell proliferation under basal conditions. Furthermore, we could show that the basal mRNA expression of neurotrophins and growth factors is neither dependent on MR nor on GR. Currently, we perform expression profiling and chromatin immunoprecipitation to identify MR and GR target genes and the corresponding binding sites in neurons of the adult hippocampus.

IMPACT OF G-PROTEIN-COUPLED RECEPTOR 30 ON THE EFFECTS OF DAIDZEIN AND DICHLORODIPHENYLTRICHLOROETHANE IN MOUSE EMBRYONIC NEURONAL CELLS

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Membrane estrogen receptors have received considerable attention because they provide an array of beneficial effects, including neuroprotection. Recent studies have shown that G-protein-coupled receptor 30 (GPR30), also known as G-protein-coupled ER 1, mediates non-genomic estradiol signaling in several cell lines as well as in brain and peripheral tissues [1, 2]. Although GPR30 is expressed in many regions of the brain [3], little is known about its impact on responses of developing nervous system to neuroprotective action of phytoestrogens and neurotoxic action of endocrine disrupting chemicals (EDCs) such as pesticides. To better understand the molecular and functional link between the membrane estrogen receptor GPR30 and apoptotic signaling, we investigated the effects of phytoestrogen daidzein and EDC representative dichlorodiphenyltrichloroethane (DDT) on mouse embryonic neuronal cells in primary cultures. Our study demonstrated that a selective GPR30 antagonist G15 reversed the daidzein-mediated inhibition of glutamate-induced loss of membrane mitochondrial potential, caspase-3 activity, and LDH release, but a selective GPR30 agonist G1 intensified the effects of daidzein. Neuroprotective action of daidzein was not retained in siRNA GPR30-transfected cells. Furthermore, mRNA GPR30 silencing augmented the vulnerability of neuronal cells to DDT-induced apoptosis. Interestingly, DDT-induced activation of caspase-3 and LDH release were accompanied by an increase in expression of GPR30 mRNA. Here, we demonstrated that novel extranuclear GPR30 may be targeted by phytoestrogens and pesticides in embryonic neuronal cells. These data uncover a key role of GPR30 intracellular signaling in mediating the anti-apoptotic action of daidzein and may provide insight into new strategies to treat or prevent neural degeneration caused by an exposure to EDCs.

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Reference list

1. Albanito L, Madeo A, Lappano R, Vivacqua A, Rago V, Carpino A, Oprea TI, Prossnitz ER, Musti AM, Ando S, Maggiolini M (2007); *Cancer Res* 67:1859-1866
2. Prossnitz ER, Barton M (2011); *Nat Rev Endocrinol* 7:715-726
3. Brailiou E, Dun SL, Brailou GC, Mizou K, Sklar LA, Oprea T, Prossnitz ER, Dun NJ (2007); *J Endocrinol* 193:311-321

Posters' Exhibition: abstracts

SEX DIFFERENCES AND EFFECTS OF ESTROGENIC COMPOUNDS ON THE INFLAMMATORY RESPONSE OF ASTROCYTES EXPOSED TO THE INSECTICIDE DIMETHOATE

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Dimethoate (DMT) is an organophosphorous (OP) insecticide extensively used in horticulture for pest treatment and as an acaricide for treating gardens, vineyards, and field crops. A low dose of the DMT results in high oxidation of lipids and proteins and in impairment of mitochondrial function in the brain of male rats, together with a reduction of gonadal hormones in plasma. Here we have assessed whether DMT affected the inflammatory response, the production of reactive oxygen species (ROS) and the expression of steroidogenic proteins and estrogen receptors (ERs) in cortical astrocyte-enriched cultures obtained separately from male and female CD1 mice pups. Furthermore, we have analyzed whether estradiol may counteract the effects of DMT. A dose of DMT (2μg/mL) that did not affect cell viability increased the mRNA levels of interleukin (IL) 6, interferon-γ-inducible protein 10 (IP10), tumor necrosis factor (TNF) α, IL1β, ERα, ERβ, steroidogenic acute regulatory protein (StAR) and aromatase in male but not in female cultures. Estradiol decreased the mRNA levels of IL6, IP10, TNFα, and IL1β in male but not in female cultures treated with DMT. The effect of estradiol was prevented by the ER antagonist ICI 182,780, fully imitated by an ERβ agonist and partially imitated by an ERα agonist. Furthermore, DMT increased the production of ROS in male astrocytes while estradiol reduced ROS production to control levels. These findings indicate that a sublethal dose of DMT alters astrocyte function. The antiinflammatory action of estradiol on male astrocytes and the sexually dimorphic action of DMT suggest that the pesticide may have different neurological outcomes in males and females.

IMPORTANCE OF ENDOGENOUS ANDROGEN LEVEL ON A1-ADRENERGIC RESPONSIVENESS OF RAT SEMINAL VESICLE

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Resistance exercise and anabolic androgenic steroid (AAS) are often elicited associated, however there are still several side effects especially to men that were not reported. This study aimed to investigate possible changes during two protocols of resistance exercise and nandrolone decanoate in the expression and pharmacological characteristics of α1-adrenoceptor in adult rat seminal vesicle. The animals received 5 mg/Kg of nandrolone decanoate or vehicle, twice a week for 8 weeks and/or were exposed to traditional resistance exercise or pyramid training. The training protocol consisted of 8 weeks of training, with progressive workload and at the end of the experimental period, testosterone level was measured. The characteristics of α1-adrenoceptor in the seminal vesicle were determined using an isolated organ bath, radioligand receptor binding and real-time reverse transcription-polymerase chain reaction techniques. Functional studies showed a lower sensitivity in the seminal vesicle contractile response from animals treated with nandrolone associated or not to resistance training, and the maximum contractile response was also altered by them. Binding results of these adrenoceptors supported the functional studies findings. Both variables (resistance exercise/AAS) reduced endogenous testosterone level (with the exception of one pyramid/vehicle group) and also the density of α1-adrenoceptor in these experimental groups, although mRNA expression of all 3 α1-adrenoceptor subtypes was not altered among them. Taken together, the results suggest that the effects of nadrolone decanoate with or without the association of resistance exercise affect contractile response of the seminal vesicle, and probably indicate an ejaculatory dysfunction in individuals who make use of them. Nevertheless, pyramid training did not show the same pattern of response from the traditional resistance exercise to norepinephrine when combined with nandrolone, indicating a protective aspect of the protocol, maintaining the sensitivity to the agonist, probably because of the unaltered level of endogenous testosterone. These results corroborate with previous data from the same protocol, which reported altered seminal parameters due to steroid and/or resistance exercise effect, compromising the fertility of these animals [1] and shows the important role that endogenous androgen has for the homeostasis of the reproductive system.
Keywords: Nandrolone decanoate; resistance exercise; male reproduction; functional studies; seminal vesicle.

We declare no conflict of interest.

Reference list

1- AGATI, Leandro Barile, GARCIA, P. C., PEREIRA, Oduvaldo Camara Maques. Fertility parameters of nandrolone decanoate on male rats submitted to two different protocols of physical exercise In: The 6th International meeting on Steroids and Nervous System, 2011, Turin. Trabajos del Instituto Cajal. Madrid: Consejo Superior de Investigaciones Científicas, 2011. v.83. p.126 – 126

EFFECTS OF PROGESTERONE AND ALLOPREGNANOLONE ON THE TEMPORAL COURSE OF REACTIVE GLIOSIS AND THE FUNCTIONAL INTEGRITY OF THE HIPPOCAMPUS AFTER GLOBAL CEREBRAL ISCHEMIA IN RATS

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The steroid hormones progesterone (P4) and 3-alpha,5-alpha-allopregnanolone (ALLO), are known to exert neuroprotective effects in several models of damage. They promote neuroprotective and neurorestorative phenomena after global cerebral ischemia (GCI), leading to a reduction of both brain damage and functional deficits, and attenuate the astroglial and microglial reaction in other models of damage. No evidence exists of the possible effects of P4 and ALLO on the glial reaction triggered by GCI, and in particular, of its correlation at various periods after ischemia, with performance in hippocampus-dependent tasks. This study assessed the effects of P4 and ALLO on the astroglial and microglial reaction in the hippocampal Ammon's horn of rats after GCI, and on spatial learning and memory in the Morris Water Maze (MWM). Male adult *Sprague-Dawley* rats were subjected to 15 min of GCI by the four-vessel occlusion model and treated i.v. at 15 min, 2, 6, 24, 48 and 72 h after ischemia with: P4, (8 mg/kg); ALLO, (4 mg/kg); or Vehicle (2-hydroxypropyl-beta-cyclodextrin, 20% in sterile water). Rats subjected to Sham procedures were included as controls. Rats were sacrificed at 7 days post-ischemia, or at 14, 28, or 97 days, after being tested for the seven previous days in the MWM. Astrocytic and microglial reaction were analyzed by immunohistochemistry for GFAP and OX-42, respectively. Pyramidal neurons were counted in CA1, CA3 and the *hilus*. Global ischemia resulted in a severe neuronal loss and an intense glial reaction with hypertrophy of astrocytes and microglial cells along most of the Ammon´s horn, at 14, 28, and 97 days after ischemia. P4 treatment reduced the magnitude and extension of the glial reaction, as expressed by the number and/or location of hypertrophic cells, at 14, 28, and 97 days after GCI, and preserved spatial learning and memory in spite of a severe neuronal loss, with few (about 15%) pyramidal neurons remaining in CA1. ALLO exerted similar effects as those of P4, but with a slightly better preservation of pyramidal neurons in CA1. These results suggest a neurorestorative effect of both P4 and ALLO, by reducing the glial reaction possibly contributing to preserve the functional integrity of the hippocampus after GCI, in spite of a low sparing of pyramidal neurons.

EFFECT OF PROGESTERONE ON INTRACELLULAR SIGNALING PATHWAYS IN THE CNS OF FEMALE RATS SUBMITTED TO THE FORCED SWIMMING TEST

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Depression is a mood disorder with a high prevalence in the population, which is higher in women than in men [1]. Previous studies showed that the chronic administration of low doses of progesterone in diestrus female rats has an antidepressive effect in the forced swimming test (FST) [2]. Depression is associated with the neurodegeneration and the cell death of some brain circuits [3] and progesterone is a neuroprotective steroid that could at least partially prevent this neurodegeneration [4]. The aim of this study was to verify the effect of progesterone in the protein expression and activation of Akt and caspase-3 in the hypothalamus and in the olfactory bulb of diestrus female rats submitted to the forced swimming test (FST).

It was used 2-3 months-old female Wistar rats in diestrus. In the first day, the animals were trained in the FST. After, they received vehicle (C) or progesterone (P) (0.4 mg/kg) (i.p.) daily during two estrous cycles (8-10 days). After the last injection, the animals were submitted to the FST that was recorded for behaviorally analysis. Thirty minutes after the test, they were decapitated and the hypothalamus and the olfactory bulb were collected and frozen for molecular analysis. Protein expression of p-AKT, AKT and caspase-3 was evaluated by Western Blot. The protein expression of these enzymes was analyzed by Student's T-test in the hypothalamus and by two-way ANOVA/SNK in the olfactory bulb and are presented as mean ± SEM.

The treatment increased the ratio p-AKT/AKT (C= 0.96 ± 0.09; P= 1.50 ± 0.07) (t= 4.446, $P<0.001$). However, we did not find an increase in the activation of this pathway, since that the increase in the expression of the ratio p-AKT/AKT occurred through a reduction in the protein expression of AKT (C= 0.62 ± 0.06; P= 0.44 ± 0.03) (t= 2.795, $P=0.016$). Progesterone also decreased the expression of procaspase-3 in the hypothalamus (C= 0.31 ± 0.01; P= 0.27 ± 0.02) (t= 2.502, $P=0.046$). Progesterone did not change the activation and the expression of ERK in the hypothalamus. The treatment did not change the activation and the expression of these proteins in the olfactory bulb.

In summary, our findings indicate that progesterone decrease the expression of procaspase-3 in the hypothalamus of diestrus female rats. This neuroprotective effect could partially explain the antidepressive effect of progesterone in these animals on the FST. The pathways involved in the regulation of the expression of this enzyme remain to be clarified.

Reference list

[1] K.A. Yonkers, O. Brawman-Mintzer, The pharmacologic treatment of depression: is gender a critical factor? J Clin Psychiatry 63 (2002) 610-615..
[2] S. Andrade, S.L. Silveira, B.D. Arbo, B.A. Batista, R. Gomez, H.M. Barros, M.F. Ribeiro, Sex-dependent antidepressant effects of lower doses of progesterone in rats, Physiol. Behav. 99 (2010) 687-690.
[3] S. Campbell, G. Macqueen, An update on regional brain volume differences associated with mood disorders, Curr. Opin. Psychiatry 19 (2006) 25-33.
[4] D.N. Bryant, L.C. Sheldahl, L.K. Marriott, R.A. Shapiro, D.M. Dorsa, Multiple pathways transmit neuroprotective effects of gonadal steroids, Endocrine 29 (2006) 199-207.

CUCURBTACIN E, A PHYTOSTEROL EXERTING NEUROPROTECTIVE AND PRO-AUTOPHAGIC PROPERTIES IN A CELLULAR MODEL OF PARKINSON'S DISEASE

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As neurodegenerative disorders spread at an alarming rate across populations, the need for preventive and/or complementary therapies arises in the aging population. Currently, the management of such conditions is purely symptomatic. Estrogens have been widely studied for their neuroprotective potential, but their numerous side-effects hinder their direct involvement in the treatment of neurodegenerative diseases. On the other hand, more research is conducted on several phytoestrogens with interesting neuroprotective properties without or less estrogenic responses. [1] Among these compounds, cucurbitacins, tetracyclic triterpenoid structurally similar to estrogens, mostly found in the Cucurbitaceae plant family, have been demonstrated to have antioxidant [2], anticancer [3] and anti-inflammatory [4, 5] activities. Recently, it has been proposed that cucurbitacins might have a modulating effect on autophagy [6]. In particular, Cucurbitacin E (CuE), extracted from the Cucurbitaceae *Ecballium elaterium*, a Mediterranean species of squirting cucumber, possesses important antiproliferative effects on cancer cells [7], linked directly to its actin-binding abilities [8]. The autophagic process, involving bulk degradation of cytoplasmic content via the lysosomal compartment, is the subject of much recent research on neurodegenerative disorders. The well-known actions of CuE on the polymerisation of the cellular cytoskeleton have brought us to explore its involvement in the autophagic pathway as a possible pathway for neuroprotection. We evaluated the neuroprotective potential of Cucurbitacin E using a cellular model of Parkinson's disease (PD): PC12 rat pheochromocytoma cells differentiated to dopaminergic motor neurons by administration of nerve growth factor (NGF). Neuronal damages were induced by the administration of 1-methyl-4-phenylpyridinium (MPP+), a parkinsonian neurotoxin that causes the impairment of the mitochondrial respiratory chain function, thus promoting oxidative stress and subsequent apoptosis. In this cellular paradigm, very low doses (0.1 nM) of Cucurbitacin E have been administered before and during MPP+ treatment. Then, specific tests for cytotoxicity (LDH colorimetric assays) and apoptosis (ssDNA fragmentation) were performed to assess the overall neuroprotection. To determinate the cellular mechanism underlying the pro-survival effects of Cucurbitacin E on dopaminergic neurons, we proceed to study the cellular antioxidant potential by measuring intracellular reactive oxygen species (ROS) and superoxide anion production. We demonstrated the presence of autophagosomes, the autophagic organelles, in our neuronal cells using specific fluorescent dyes. Our results show that the overall number of mature autophagosomes is higher after Cucurbitacin E treatment. The use of immunofluorescence techniques also showed colocalization of LC3, a specific autophagosome marker, and HDAC6, a microtubule deacetylase protein known to target and traffic aggregated proteins for degradation. The expression of HDAC6, as assessed by Western blotting, was also upregulated after CuE treatment, which suggests a higher turnover rate of autophagic cargo. In conclusion, our results show that Cucurbitacin E is a potent neuroprotective molecule, capable of inhibiting the apoptotic process caused by MPP+ exposure on neuronal PC12 cells. The

neuroprotective activities of CuE have not proven to be related to an amplification of the cellular antioxidative potential or ROS scavenging abilities in our neuronal cellular system. However, we have observed that CuE enhances autophagic degradation, which could lead to a more efficient turnover of damaged mitochondria as well as oxidized and aggregated proteins. Our results also demonstrate that a modulatory effect of the autophagic process might be the mechanism used by Cucurbitacin E to exert its neuroprotective properties. Our findings, combined with its known anticancer and anti-inflammatory effects, qualify CuE as a potential compound for preventive and complementary therapies in neurodegenerative disease as well as in a wide range of health disorders.

Reference list

[1] Gélinas, S., Martinoli, M-G. Neuroprotective effect of estradiol and phytoestrogens on MPP+-induced cytotoxicity in neuronal PC12 cells. Journal of Neuroscience Research 2002, 70: 90-96.
[2] Tannin-Spitz, et al. Cucurbitacin glucosides: Antioxidant and free-radical scavenging activities. Biochemical and Biophysical Research Communications 2007, 364: 181-186.
[3] Sun, J., et al. Cucurbitacin Q: a selective STAT3 activation inhibitor with potent antitumor activity. Oncogene 2005, 24: 3236-3245.
[4] Abdelwahab, SI, et al. Anti-inflammatory activities of cucurbitacin E isolated from Citrullus lanatus var. citroides: Role of reactive nitrogen species and cyclooxygenase enzyme inhibition. Fitoterapia 2011, 81: 1190-1197.
[5] Attard, E. et al. Immunomodulatory activity of cucurbitacin E isolated from Ecballium elaterium. Fitoterapia. 2005 Jul;76(5):439-41
[6] Huang, WW, et al. (2012) Cucurbitacin E induces G_2/M phase arrest through STAT3/p53/p21 signaling and provokes apoptosis via Fas/CD95 and mitochondria-dependent pathways in human bladder cancer T24 cells. Evidence-based Complementary and Alternative Medicine, 2012.
[7] Sörensen, PM, et al. The natural product Cucurbitacin E inhibits depolymerisation of actin filaments. ACS Chemical Biology 2012, 7: 1502-1508.
[5] Zhang, T., et al. Cucurbitacin induces autophagy through mitochondrial ROS production which counteracts to limit caspase-dependent apoptosis. Autophagy 2012, 8:4, 559-576.

SYNTHESIS OF NEW ESTROGEN ANALOGUES WITH ANTIOXIDANT ACTIVITY

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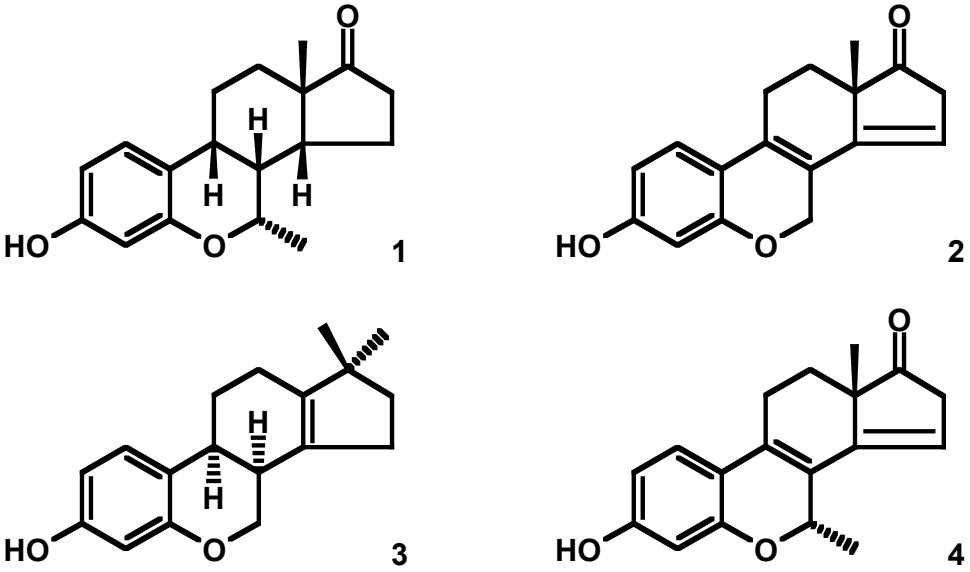
Among the benefits of steroidal estrogens containing phenolic A-ring and having antioxidant and antiradical activity is their potential for Alzheimer's disease, Parkinson's disease and other neurodegenerative diseases treatment [1]. This neuroprotective action is provided both by antioxidant properties and other effects mediated by estrogen receptors. One can look for modified estrogens with combination of neuroprotective action and reduced estrogenic activity [2,3], because the antioxidant neuroprotective action of estrogens does not depend on their estrogenic properties [4]. The properties of 6-oxa-analogues of steroid estrogens can differ strongly from these of corresponding 6-carba-analogues since the oxygen atom affects the distribution of electric charge in the A ring; the antiradical activity of these compounds is scarcely investigated [5]. We have synthesized below listed compounds and tested their antiradical activity.



The compound 3 is of special interest, because its antiradical activity is 6 times higher than that of ionol whereas its uterotropic activity is much lower than that of 17α-ethinylestradiol.

Reference list

[1] Simpkins J.A., Yang S-H., Liu R., Perez E., Cai Zu Y., Covey D.F. & Green P.S. Stroke. 2004, 1, 2648-2651.
[2] Prokai L., Simpkins J.W. Pharmacol. Therapeut. 2007, 114, 1-12.
[3] Römer W., Oettel M., Menzenbach B., Droescher P. & Schwarz S. Steroids, 1997, 62, 689-694.
[4] Moosmann B., Behl C. Proc. Natl. Acad. Sci. USA. 1999, 8867-8872.
[5] Shavva A., Morozkina S., Galkina O. Approaches for Searching of Modified Steroid Estrogen Analogues with Improved Biological Properties. In: Steroids – Basic Science, In Tech, Rijeka, Croatia, 2011. pp. 171-220.

NEUROPATHIC PAIN AND DIABETES: A ROLE FOR TESTOSTERONE METABOLITES

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Neuropathic pain exerts a substantial impact on quality of life, particularly by causing considerable interference in sleep, daily activities, and enjoyment of life. Chronic neuropathic pain is present in 26% of diabetic patients, in 8.7% of individuals with impaired glucose tolerance, in 4.2% of individuals with impaired fasting glucose and in 1.2% of individuals with normal glucose tolerance [11]. The diabetes causes injury at all levels of the nervous system from the peripheral nerves (i.e., diabetic neuropathy) to the brain but it is unclear how these damages are induced. Painful diabetic neuropathy (PDN) is characterized by spontaneous pain and increased sensitivity to mechanical and chemical stimuli [2]. It has been proposed that hyperexcitability and spontaneous hyperactivity of primary afferents [3] and spinal dorsal horn neurons [8] detected in streptozotocin (STZ)-induced diabetes, might account for this symptomatology. The neurobiological mechanisms associated with the spinal hyperactivity and with the pain evoked remain, however, unclear. Glutamate seems to be involved since an increase in binding affinity and in density of NMDA and AMPA receptors were detected in the spinal cord of diabetic rats [9]. A higher glutamatergic excitation is also supported by pharmacological studies showing that hyperalgesia and allodynia in diabetic animals is reversed by NMDA and non-NMDA receptors antagonists [4]. Recent studies also indicate that a communication exists between the immune system and the nervous system [7]. Although multiple conditions may generate neuropathic pain, a common underlying mechanism is the presence of inflammation at the site of the damaged or affected nerve(s). The resultant neuroinflammatory environment can cause activation of microglia and astrocytes which appear to play a prominent role in nociception [6]. In order to understand and characterize the impact of diabetes on neuropathic pain we have analyzed the expression of neurotransmission and neuroinflammation markers both in the dorsal horn of spinal cord and in the dorsal root ganglia of male Sprague Dawley rats raised diabetic by STZ injection. We have observed that 1 month of diabetes is sufficient to induce mechanical hyperalgesia and allodynia. Thus, diabetic rats show a reduction of pain threshold evaluated by Randall-Selitto and Von Frey test. Moreover, the data obtained on neurotransmission revealed: *i*) an increase of the Na,K-ATPase activity; *ii*) an increased presynaptic activity confirmed by the enhance of the levels of proteins such as synapsin 1 and syntaxin 1; *iii*) an increased postsynaptic activity evaluated through the expression of NMDA receptor subunits NR2A, NR2B and the activating phosphorylation of NR2B (NR2Bp). The increase in the excitatory neurotransmission is further reinforced by the high levels of substance P observed in the dorsal root ganglia of the STZ-rats. In addition to the evaluation of these neurotransmission parameters, we have analyzed also neuroinflammatory markers. An increase of astroglial reactivity, confirmed also by the production of pro-inflammatory citokines (i.e., TNFalpha and IL-1beta) in the dorsal horn of spinal cord of diabetic rats, was observed. Neuroactive steroids are important

modulators of a variety of physiological and pathological functions [5]. They can alter synaptic transmission by interacting with ionotropic neurotransmitter receptors and/or voltage-dependent Ca2+ or K+ channels as well as by influencing second-messenger pathways [1]; in particular 5alpha-reduced neuroactive steroids are very effective in alleviating peripheral nociception in both acute and chronic pain conditions in various animal models of pain [10]. On the basis of these observations we have tested two metabolites of testosterone, the 5alpha-Androstan-17beta-ol-3-one (dihydrotestosterone, DHT) and the 5alpha-Androstane-3alpha,17beta-diol (3alpha-diol) in order to analyze their possible analgesic effects on the PDN. Preliminary results obtained from behavioral tests have revealed that these neuroactive steroids show analgesic effects by decreasing the pain threshold in diabetic rats.

Reference list

[1] Belelli, D., Lambert, J.J., 2005. Neurosteroids: endogenous regulators of the GABAA receptor. Nat. Rev. Neurosci. 6, 565—575.
[2] Chen S.R., Pan H.L. Hypersensitivity of spinothalamic tract neurons associated with diabetic neuropathic pain in rats, J. Neurophysiol. 87 (2002) 2726–2733.
[3] Khan G.M., S.R. Chen, Pan H.L. Role of primary afferent nerves in allodynia caused by diabetic neuropathy in rats, Neuroscience 114 (2002)291–299.
[4] Malcangio M., Tomlinson D.R. A pharmacologic analysis of mechanical hyperalgesia in streptozotocin/diabetic rats, Pain 76 (1998) 151–157.
[5] Melcangi, R.C., Garcia-Segura, L.M., Mensah-Nyagan, A.G., 2008. Neuroactive steroids: state of the art and new perspectives. Cell Mol. Life Sci. 65, 777—797.
[6] Ricardo Vallejo, Dana M. Tilley, MS, Laura Vogel, Ramsin Benyamin. The Role of Glia and the Immune System in the Development and Maintenance of Neuropathic Pain. Pain Practice, Volume 10, Issue 3, (2010) 167–184 .
[7] Safieh-Garabedian B, Poole S, Haddad JJ, Massaad CA, Jabbur SJ, Saade NE. The role of the sympathetic efferents in endotoxin-induced localized inflammatory hyperalgesia and cytokine upregulation. Neuropharmacology. 2002;42:864– 872.
[8] Sima A.A. New insights into the metabolic and molecular basis for diabeticneuropathy. Cell. Mol. Life Sci. 60 (2003) 2445–2464.
[9] Tomiyama M., Furusawa K., Kamijo M., Kimura T., Matsunaga M., Baba M. Upregulation of mRNAs coding for AMPA and NMDA receptor subunits and metabotropic glutamate receptors in the dorsal horn of the spinal cord in a rat model of diabetes mellitus. Brain Res. Mol. Brain Res. 136 (2005) 275–281
[10] Vesna Jevtovic-Todorovic, Douglas F. Covey , Slobodan M. Todorovic. Are neuroactive steroids promising therapeutic agents in the management of acute and chronic pain? Psychoneuroendocrinology (2009) 34S, S178—S185
[11] Ziegler D, Rathmann W, Dickhaus T, Meisinger C, Mielck A; KORA Study Group. Neuropathic pain in diabetes, prediabetes and normal glucose tolerance. The MONICA/KORA Augsburg Surveys S2 and S3. Pain Med 2009;10:393–400

GLUCOCORTICOID RECEPTOR AND FKBP5 EXPRESSION IS ALTEREDFOLLOWING EXPOSURE TO CHRONIC STRESS: MODULATION BY ANTIDEPRESSANT TREATMENT

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Major depression is thought to originate from the interaction between susceptibility genes and adverse environmental events, in particular stress. The HPA axis is the major system involved in stress response and its dys-regulation is an important element in the pathogenesis of depression. The stress response is therefore finely tuned through a series of mechanisms that control the trafficking of glucocorticoid receptors (GR) to the nucleus, including binding to the chaperone protein FKBP5 and receptor phosphorylation, suggesting that these elements may also be affected under pathologic conditions.
On these bases, we investigated FKBP51 and GR expression and phosphorylation in the hippocampus (ventral and dorsal) and in the prefrontal cortex of rats exposed to chronic mild stress (CMS) and we analyzed the effect of a concomitant antidepressant treatment. We found that animals exposed to CMS show increased expression of FKBP5 as well as enhanced cytoplasmic levels of GR, primarily in ventral hippocampus and prefrontal cortex. Chronic treatment with the antidepressant duloxetine is able to normalize such alterations, primarily in the prefrontal cortex. Moreover we demonstrate that altered trafficking of GR to the nucleus may also be sustained by changes in receptor phosphorylation, which is also modulated by pharmacological intervention.
In summary, while GR-related changes after CMS might be relevant for the depressive phenotype, the ability of antidepressant treatment to normalize some of these alterations may contribute to the normalization of HPA axis activity observed in depressed patients.

PROGESTERONE RECEPTOR ISOFORMS EXPRESSION IS REGULATED BY DNA METHYLATION BUT NOT BY HISTONE ACETYLATION IN HUMAN ASTROCYTOMA CELL LINES

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Progesterone (P) exerts diverse actions in the central nervous system, including the regulation of brain tumors growth. P increases the number of cells in lines derived from human astrocytomas, the most frequent and aggressive brain tumors. Many P effects are mediated through its intracellular receptor (PR), which is expressed as two main isoforms (PR-A, 94 kDa and PR-B, 116 kDa) with different regulation and function. PR isoforms are differentially expressed in several cancer cells, and this difference in PR regulation has been associated to a PR isoform-specific promoter methylation. We have reported a differential expression of PR isoforms both in biopsies and cell lines from human astrocytomas. PR expression increases according to the tumor evolution grade, being PR-B the predominant isoform expressed in human astrocytomas grades III and IV. However, the epigenetic mechanisms involved in the regulation of PR isoforms expression in these tumors are unknown. Therefore, the role of DNA methylation and histone acetylation in the regulation of PR expression was studied. We evaluated the effect of the demethylating agent 5-aza-2'-deoxycytidine (5AzadC, 5 and 10 μM) and the histone deacetylase inhibitor trichostatin A (TSA, 0.25, 0.50, 1.0 μM) on PR isoforms expression in human astrocytoma cell lines U373 and D54, derived from tumors grades III and IV, respectively, by RT-PCR and Western blot. 5AzadC treatments increased PR-B mRNA and protein expression in U373 cells but not in D54 cells. PR-A protein content increased with 10 μM 5AzadC in U373 cells. TSA did not modify PR-B expression in U373 cells. The treatment with 5AzadC in combination with TSA induced PR-B expression to the same extent as 5AzadC alone in U373 cells. We next studied whether the increase in PR expression induced by 5AzadC (5 μM) exerted an effect on the P induced proliferation in U373 cells. P treatment (10 nM) increased the number of U373 cells, while 5AzadC and the co-treatment with P reduced the number of cells. Our data suggest that epigenetic processes such as DNA methylation participate in the regulation of PR-B expression in U373 cells and that this regulation is lost in the higher tumor grade derived cell line D54. Besides, PR gene demethylation reduces cell number in the astrocytoma line U373.

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NEONATAL CORTICOSTERONE AND ENVIRONMENTAL ENRICHMENT DURING DEVELOPMENT MODULATE BRAIN METABOLISM AND INDIVIDUAL RESPONSES TO CANNABINOID AGONIST IN MICE

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Clinical and experimental studies indicate that adverse environmental conditions may favour the onset of emotional disturbances and increase the vulnerability towards the effects of the consumption of psychoactive drugs. Conversely, stimulating environmental conditions may exert a protective or compensatory role. Neurobiological systems such as the hypothalamic-pituitary-adrenal (HPA) and the endocannabinoid system (ECS) interact to modulate the expression of emotions. Here we analysed how exposure to stimulating environments during development (childhood and adolescence) modulates individual response to the administration of high doses (0.3 mg/kg) of an ECS agonist (JWH-018) during adolescence. We exposed newborn CD1 mice to a moderate dose of corticosterone (33.3 mg/l) supplemented in the maternal drinking water; an independent group of adolescent mice were housed in environmental enrichment (EE) conditions (physical and social stimuli were provided). Exposure to JWH-018 during adolescence is associated, in the short-term, with hypomotility, analgesia and reduced body temperature; in the long-term, adolescent exposure to JWH-018 is associated with anhedonia, anxiety, increased rearing, and major alterations in brain concentrations of the following metabolites (measured through ^{1}H imaging-guided magnetic resonance spectroscopy): glutamate, glutamine, phospho-choline plus glicero-phospho-choline, creatine plus phospho-creatine, inositol, N-acetyl-aspartate. Beside exerting independent behavioural and neurochemical effects, exposure to stimulating environments contrasts some of the consequences of JWH-018 administration on behaviour (hypolocomotion during adolescence and increased anxiety in adulthood) and brain metabolic profiles. These data support the hypothesis that moderate neonatal stress, environmental enrichment and pharmacological stimulation of the ECS contribute to the expression of emotions throughout the entire course of development.

NEUROACTIVE STEROIDS UPREGULATE THE EXPRESSION OF GENES INVOLVED IN FATTY ACID BIOSYNTHESIS IN PRIMARY SCHWANN CELLS

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The myelin sheath is a multilayered membrane produced in the peripheral nervous system by differentiation of the plasmatic membrane of Schwann cells [4]. The main role of this membrane is to allow efficient transmission of nerve impulses along the axons, which it surrounds [6]. Diminished or abnormal fatty acid could affect myelin fluidity and function, ultimately contributing to the pathogenesis of diabetic peripheral neuropathy (DPN) [5; 7]. Studies in animal models have revealed a protective role for administration of fatty acids on DPN [3]. However, the specific changes induced by diabetes on myelin lipid and protein composition, as well as the molecular mechanism responsible for these defects, remains to be identified. Recently we have demonstrated that DPN is characterized by myelin abnormalities, which are associated with lipid synthesis alteration. The activation of LXR, a nuclear receptor regulating cholesterol and fatty acid homeostasis, causes the restoring of neuroactive steroids levels and myelin lipid composition in the sciatic nerve of diabetic rats [1], as well as prevents the structural alterations observed in myelin of this tissue [2]. Now we would define whether neuroactive steroids exert their protective effects in DPN by directly regulating fatty acid metabolism. To this aim, we have performed experiments in primary Schwann cells treated with progestagens and androgens to assess their ability to modulate fatty acid biosynthesis. Our data indicate that some of the tested steroids, such as the 3alpha-diol (5α-androstane-3α17b-diol), is able to induce fatty acid biosynthetic gene expression. These data highlight a new role for some neuroactive steroids in regulating lipid biosynthesis.

Reference list

1. Cermenati G et al., J Neurosci, 30:11896-11901, 2010.
2. Cermenati G et al., J Lipid Res, 53(2):300-10, 2012.
3. Coste TC et al., Cell Mol Biol, 50:845-853, 2004.
4. Garbay B et al., Prog Neurobiol, 61:267-304, 2000.
5. Verheijen MH et al., Pnas, 106:21383-21388, 2009.
6. Waxman SG and Ritchie Jm, Ann Neurol, 33:121-136, 1993.
7. Zuvic-Butorac M et al., Biochim Biophys Acta, 1537:110-116, 2001.

DEUTERODERIVATIVES OF NEUROSTEROID ANALOGUES. SYNTHESIS AND USE

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The naturally occurring neurosteroids exert their effects through various neuronal receptors. The important neuroprotective activity is a use-dependent inhibition of NMDA receptors by 3α5β-pregnanolone sulfate (3α5βP-S) [1]. Thus, its analogues could be promising molecules in the therapy of central nervous system diseases. We have designed and evaluated electrophysiological activity of series of such analogues. Some of the molecules proved neuroprotective activity in animal experiments. The example is 3α5β-pregnanolone glutamate [2].

In order to examine its pharmacologically relevant properties, it was necessary to synthesize isotopicaly labeled derivative. The incorporation of deuterium labels is generally exploited. Substitution of three or four hydrogen atoms by the deuterium is the method of choice. While deuterated *L*-glutamic acid is commercially available, its deuterium labels are adjacent to a carbonyl group, and are susceptible to deuterium/proton exchange. Moreover, the isotopic labels are unsuitably placed on a hydrolysable substituent. However, deuterium labels that are incorporated directly into the steroid core will remain intact during chemical processes, analytical procedures, or metabolic degradation.

We will describe first efficient synthesis of conveniently labeled 5beta steroids; a trideuterated [9,12,12-$^2H_3$]- [3], [18,18,18-$^2H_3$]-, and [19,19,19-$^2H_3$]-3alpha5beta-pregnanolone derivatives from the commercially available precursors. Despite of relatively long synthetic pathways the overall yields are slightly under 10%.

Moreover, all deuterium labels are located in specific positions such that both the chemical and metabolic stability is guaranteed. The three synthesized isotopicaly labeled compounds make possible bioavailability and pharmacokinetic studies and additionally, more complicated metabolic experiments.

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Reference list

[1] Kapras V, Slavíčková A, Šťastná E, Vyklický L. Jr, Valeš K, Chodounská H. (2012)
Synthesis of deuterium labeled NMDA receptor inhibitor - 20-Oxo-5beta-[9,12,12(2)H(3)]pregnan-3alpha-yl-L-glutamyl 1-ester. Steroids 77(3): 282-287.
[2] Petrovič M, Sedláček M, Horák M, Chodounská H. VyklickýL. (2005)
20-Oxo-5beta-pregnan-3alpha-yl sulfate is a use-dependent NMDA receptor inhibitor. J. Neurosci. 25(37): 8439-50.
[3] Valeš K, Rambousek L, Holubová K, Svoboda J, Bubeníková-Valešová V. Chodounská H, Vyklický L, Stuchlík A. (2012) 3alpha5beta-pregnanolone glutamate, a use-dependent NMDA antagonist, reversed spatial learning deficit in an animal model of schizophrenia. Behavioral Brain Research. 235: 82-88.

CHRONIC TREATMENT WITH THE PHOSPHODIESTERASE TYPE 5 INHIBITOR, SILDENAFIL, DISPLAYS ANTIDEPRESSIVE EFFECTS AND TESTOSTERONE SECRETION INCREASE VIA STIMULATION OF LEYDIG CELLS IN CD1 MALE MICE

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Family-selective PDE inhibitors have been widely studied as therapeutic agents for treatment of various human diseases, including cardiotonics, vasodilators, smooth muscle relaxants, antidepressants, antithrombotics, antiasthmatics, and agents for improving learning and memory. Some years ago, though, in the US FDA Adverse Event Reporting System, Sildenafil has been implicated in adverse emotional and aggressive behaviors. Previous studies from our lab showed that chronic Sildenafil increases aggressive behaviors in several mice strain, restoring agonistic and normal sexual behavior in subordinated male mice in a chronic psychosocial stress model. The present study aimed to observe the Sildenafil effect to the neuroendocrine responses to stress and vulnerability to stress-induced changes. Subordinated male CD1 mice were injected with Sildenafil (10mg/kg) for 4 weeks. Competitive aggression, anxiety, explorative behavior, social and sexual behavior, leydig cell morphology and serum testosterone were examined. The results confirmed that Sildenafil increased competitive aggression, environmental and social exploration, and reduced anxiety as compared to controls. In addition, in treated mice, areas of endocrine tissue containing Leydig cells were larger, with a greater number of cells involved in testosterone secretion, and total serum testosterone was higher compared to controls. Under present conditions, behavioral effects observed were due to an elevation in Testosterone levels caused by inhibition of phosphodiesterase 5, which in turns stimulates Leydig cell steroidogenesis. The results of the present study confirmed that the accumulation of cyclic guanosine monophosphate by PDE5 inhibition is involved in the androgen biosynthesis stimulation. Overall these data support the hypothesis of Sildenafil being a promising molecule for treatment of stress-related human pathologies.

LOCAL ESTRADIOL SYNTHESIS IN THE BRAIN AND ITS IMPLICATION IN MALE AND FEMALE SEXUAL MOTIVATION OF JAPANESE QUAIL

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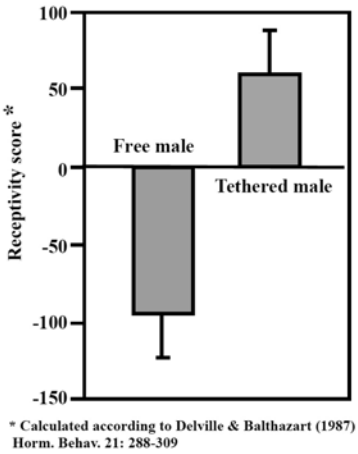
Introduction. Estrogens act through two mechanisms that affect a variety of physiological and behavioral processes in two distinct time frames: genomic effects develop with relatively long latencies and persist for relatively long durations while non-genomic actions occur rapidly and are usually transient [5, 1]. It was recently demonstrated that, in quail, estrogens acutely control measures of male sexual motivation but not performance [4]. This suggested the intriguing hypothesis that estrogens could genomically regulate in the long term the ability of males to perform the highly stereotyped copulatory sequence, while the short term control of the motivation to engage in sexual behavior would depend on their non-genomic mode of action.
In addition, we recently showed that, in quail, local estrogen synthesis as assessed by changes in brain aromatase activity could also vary rapidly in response to sexual interactions [2]. Interestingly, simply seeing the female induces similar changes in aromatase activity suggesting that estrogen synthesis could also be related to sexual motivation. However, direct evidence of such causal relationship is still lacking.
Interestingly, the female brain also contains aromatase in the brain regions known to control their sexual behavior although its concentration tends to be lower than in males. Yet, we know almost nothing concerning the role of brain-derived estrogens on their behavior, even less their sexual motivation.
Aims. The first objective of this research was to identify behavioral tests that would allow us to manipulate sexual motivation in order to assess the effect of changes in sexual motivation on brain aromatase activity. Secondly, we intended to design tests of sexual motivation and performance for females in order to test the effects of estrogens on these responses.
Methodology.
(1) Manipulation of sexual motivation in males: These procedures were based on the induction of sexual satiety which is characterized by a fall of sexual motivation and on the Coolidge effect that is the re-activation of sexual motivation in satiated males by the presentation of a novel female. The frequency of the rhythmic contractions of the cloacal sphincter muscles (RCSM) was used as a measure of sexual motivation and the cloacal contact movements (CCM), the final step of the copulatory sequence, were used as a measure of sexual performance (consummatory sexual behavior).
(2) Development of tests for sexual behavior in females: The social proximity test involved the measure of the time spent near a male located behind a glass partition at the narrow end of a long rectangular arena as compared to the time spent in the same area when no stimulus male is present. A variant of this test involved the analysis of the behavior toward a tethered male placed at the narrow end of the test chamber. During this test, females were able to display proceptive and receptive behavior that was quantified as previously described [3]. We also used a choice test in which the time spent sand bathing at one end of a long runway and time spent with a tethered male simultaneously available at the other end of the runway (competition between sexual motivation and another strongly desirable stimulus, the sand bath) were measured.

Results. As expected, males housed with a female for 24 hours (that were supposed to have copulated to satiation) displayed significantly reduced frequencies of RCSM and CCM. This data provides additional evidence that the RCSM constitutes a good measure of sexual motivation. However, the presentation of a novel female after a 10 min copulatory opportunity did not restore high CCM frequency suggesting that the Coolidge effect cannot be observed at the level of copulatory performance in quail. Yet, the presentation of a novel female after visual exposure to a female for 10 min resulted in an increased RCSM frequency suggesting that the introduction of a novel sexual stimulus can override the reduction of sexual motivation observed after a prolonged exposure to the view of the female.



In females, the results of one-trial social proximity tests showed that females spend more time on the male side when the male is present behind the glass partition. Moreover, when a tethered male is placed at the narrow end of the chamber and females can pace their interaction with the male, they regularly approach and squat in front of him (high receptivity scores) thus soliciting mounts and copulation. This is in sharp contrast with what happens when males are free to move and copulate with the female (low receptivity scores; see Figure). Females thus display appetitive behavior as well as sexual receptivity. Finally, when given the choice between sand bathing and a tethered male, females spend more time in the compartment containing the male compared to the time spent in this compartment in the control trial (without any male).

Discussion. The results show that sexual motivation can be easily manipulated behaviorally in male quail. Moreover, we developed new tests, similar to those used in rodents, to quantify sexual motivation and receptivity in females. With these new behavioral tools, we are now able to investigate the causal relationship between estrogens and sexual motivation in both sexes. Future experiments will use male subjects in known motivational states (as induced by the manipulations described above) and assay their aromatase activity to determine whether estrogen synthesis is acutely modulated by changes in sexual motivation. In addition, we will examine whether pharmacological treatments known to interfere with estrogen (genomic and non-genomic) signaling affect these new measures of sexual motivation and receptivity.

Reference list

1. Cornil CA, Ball GF, Balthazart J. Rapid control of male typical behaviors by brain-derived estrogens. Front Neuroendocrinol. 2012; 33(4): 425-46.
2. De Bournonville C, Dickens MJ, Ball GF, Balthazart J, Cornil CA. Dynamic changes in brain aromatase activity following sexual interactions in males: where, when and why? Psychoneuroendocrinology. In press. doi : 10.1016/J.Psyneuen.2012.09.001.
3. Delville Y, Balthazart J. Hormonal control of female sexual behavior in the japanese quail. Horm Behav. 1987; 21(3): 288-309.
4. Seredynski AL, Balthazart J, Christophe VJ, Ball GF, Cornil, CA. Neuroestrogens rapidly regulate sexual motivation but not performance. J Neurosci. In press. doi : 10.1523/Jneurosci.2557-12.2012.
5. Vasudevan N, Pfaff DW. Non-genomic actions of estrogens and their interaction with genomic actions in the brain. Front Neuroendocrinol. 2008; 29(2): 238-57.

PRENATAL STRESS INCREASES THE EXPRESSION OF PROINFLAMMATORY CYTOKINES AND EXACERBATES THE INFLAMMATORY RESPONSE TO LIPOPOLYSACCHARIDE (LPS) IN THE HIPPOCAMPAL FORMATION OF ADULT MALE MICE

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It is known that development and plasticity of the neuroendocrine system can be affected by many factors, and that adverse events during the prenatal period can result in long-lasting changes in adulthood [1-3]. Indeed, prenatal stress results in an enhancement of certain aspects of immune function, including elevated levels of inflammatory cytokines in the periphery and in the brain [4-6]. This proinflammatory status of prenatally stressed animals may affect normal brain function, since cytokines such as IL6, IL-1β and TNF-α have different physiological roles, including regulation of neuronal development, ionic homeostasis, neuropeptide release and synaptic plasticity [7-10]. In addition, cytokines could take part in the pathogenesis and progression of neurodegenerative diseases [11-12].
In this study we have assessed whether prenatal stress affects the inflammatory response of the hippocampal formation of male mice to an inflammatory challenge during adulthood.

Methods: Animals used in our experiments were derived from four different reproductions performed at separated seasonal periods throughout the year. Adult virgin C57BL/6 female mice (2 months of age) from the Complutense University animal colony were group-housed (6 per cage) to coordinate their estrous cycle. Females in estrous were individually housed for 24h in the presence of a sexually experienced male. Females were then examined to detect for the possible presence of a vaginal plug, which was used to confirm mating. Pregnant mice were randomly assigned to stress (n=10) or nonstress (n=10) groups. Animals of the stress group were placed in plastic transparent cylinders and exposed to bright light for 3 sessions of 45 min every day from gestational day 12 to parturition. Nonstressed pregnant mice were left undisturbed. At four months of age, non stressed and prenatally stressed male offspring were killed, 24h after the systemic administration of lipopolysaccharide (LPS) or vehicle.
Plasma corticosterone was measured by radioimmuneassay. Interleukin 1β (IL1β) and tumor necrosis factor-α (TNF-α) levels were assessed in the hippocampus by quantitative real-time polymerase chain reaction. Immunohistochemistry analysis for Iba1 (marker of microglia) and GFAP (marker of astroglia) and TNF alpha were performed. Also the morphology of microglia was assessed.

Results: Under basal conditions, prenatally stressed animals showed increased expression of interleukin 1β and tumor necrosis factor-α (TNF-α) in the hippocampus and an increased percentage of microglia cells with reactive morphology in CA1 compared to non-stressed males. Furthermore, prenatally stressed mice showed increased TNF-α immunoreactivity in CA1 and increased number of Iba-1 immunoreactive microglia and GFAP-immunoreactive astrocytes in the dentate gyrus after LPS administration. In contrast, LPS did not induce such changes in non-stressed animals.

Conclusion: These findings indicate that prenatal stress induces a basal proinflammatory status in the hippocampal formation during adulthood that results in an enhanced activation of microglia and astrocytes in response to a proinflammatory insult.

Reference list

[1] Darnaudéry, M., Maccari, S., 2008. Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. Brain Research Reviews 57, 571-85

[2] de Kloet, E.R., Joëls, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nat Rev Neurosci. 6, 463-75.

[3] Weinstock, 2005. Weinstock, M., 2005. The potential influence of maternal stress hormones on evelopment and mental health of the offspring. Brain, Behavior, and Immunity 19, 296-308.

[4] Laviola, G., Rea, M., Morley-Fletcher, S., Di Carlo, S., Bacosi, A., De Simone, R.,Bertini, M., Pacifici, R., 2004. Beneficial effects of enriched environment on adolescent rats from stressed pregnancies. European Journal of Neuroscience 20,1655-64.

[5] Vanbesien-Mailliot, C.C., Wolowczuk, I., Mairesse. J., Viltart, O., Delacre, M., Khalife, J., Chartier-Harlin, M.C., Maccari, S., 2007. Prenatal stress has proinflammatory consequences on the immune system in adult rats. Psychoneuroendocrinology 32,114-24.

[6] Diz-Chaves, Y., Pernía, O., Carrero, P., Garcia-Segura, L.M., 2012. Prenatal stress causes alterations in the morphology of microglia and the inflammatory response of the hippocampus of adult female mice. Journal of Neuroinflammation 9, 71.

[7] Raber, J., Sorg, O., Horn, T.F., Yu, N., Koob, G.F., Campbell, I.L., Bloom, F.E., 1998. Inflammatory cytokines: putative regulators of neuronal and neuro-endocrine function. Brain Research Brain Research Reviews 26,320-326.

[8] Albensi, B.C., Mattson, M.P., 2000. Evidence for the involvement of TNF and NF-κB in hippocampal synaptic plasticity. Synapse 35,151-159.

[9] Skoff, A.M., Zhao, C., Adler, J.E., 2009. Interleukin-1alpha regulates substance P expression and release in adult sensory neurons. Experimental Neurology 217, 395-400.

[10] Park, K,M., Bowers, W.J., 2010. Tumor necrosis factor-alpha mediated signaling in neuronal homeostasis and dysfunction. Cellular Signalling 22, 977-983.

[11] Griffin, W.S., 2006. Inflammation and neurodegenerative diseases. The American Journal of Clinical Nutrition 83, 470S-4S.

[12 Perry, V.H., Cunningham, C., Holmes, C., 2007. Systemic infections and inflammation affect chronic neurodegeneration. Nat. Rev. Immunol. 7, 161-7.

PHYSIOLOGICAL ROLES OF ENDOGENOUS NEUROSTEROIDS AT ALPHA-2 SUBUNIT-CONTAINING GABA(A) RECEPTORS

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Neurosteroids are important endogenous modulators of the major inhibitory neurotransmitter receptor in the brain, the gamma-aminobutyric acid type A (GABA(A)) receptor. They are involved in numerous physiological processes, and are also linked to several central nervous system disorders, including depression and anxiety. The neurosteroids allopregnanolone and allo-tetrahydro-deoxycorticosterone (THDOC) have multiple effects in animal models, including: anxiolysis, analgesia, sedation, anticonvulsion, antidepressive actions, suggesting they could be useful therapeutic agents, for example, in anxiety, stress and mood disorders.

Neurosteroids potentiate GABA-activated currents by binding to a conserved site within alpha subunits. Potentiation can be eliminated by hydrophobic substitution of a conserved glutamine residue in the first transmembrane domain of alpha subunits. Previous studies suggest that alpha-2 subunits are key components in neural circuits affecting anxiety and depression, and that neurosteroids could be endogenous anxiolytics. It is therefore possible that this anxiolysis occurs via potentiation at alpha-2 subunit-containing receptors. To examine this hypothesis, alpha-2(Q241M) knock-in mice were generated, and used to define the roles of alpha-2 subunits in mediating the effects of endogenous and exogenous neurosteroids.

Biochemical and imaging analyses indicated that relative expression levels and localization of GABA(A) receptor alpha-1—alpha-5 subunits were unaffected, suggesting the knock-in had not caused any compensatory effects. Electrophysiological characterization of cells in acute hippocampal and nucleus accumbens brain slices revealed faster-decaying inhibitory synaptic transmission in alpha-2(Q241M) mice. Furthermore, the response to applied THDOC was markedly reduced compared to wild-type cells. Alpha-2 subunits therefore constitute a major component of synaptic $GABA_A$ receptors in these areas. Tonic currents recorded from dentate gyrus granule cells of alpha-2(Q241M) mice lost sensitivity to applied THDOC, implicating a role for alpha-2-type GABA(A) receptors in tonic currents.

With regard to behavioural phenotype, the alpha-2(Q241M) knock-in mice showed greater anxiety levels in two classical rodent anxiety paradigms (light-dark box and elevated plus maze). This is consistent with endogenous neurosteroids mediating anxiolysis via alpha-2-type GABA(A) receptors. In addition, the anxiolytic response to injected THDOC is impaired by the alpha-2(Q241M) mutation, which would identify the alpha-2 isoform as an appropriate target for generating receptor subtype-selective neurosteroid therapeutics for anxiety disorders.

PROGESTERONE REDUCES CASPASE-3 ACTIVATION AND DNA FRAGMENTATION IN THE HIPPOCAMPUS FOLLOWING GLOBAL CEREBRAL ISCHEMIA

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Global cerebral ischemia (GCI) causes neuronal death of selectively vulnerable cells as the CA1 pyramidal neurons of the hippocampus. In response to ischemia, some proteases, such as caspases, which are constitutively expressed in the brain, are specifically activated. Particularly, an increase in caspase-3 mRNA [4,8,15], as well as in protein expression [4,24], and activation [4,8,16,18,20,22,24,25], suggest that caspase-3 may be involved in neuronal death after GCI. In turn, the translocation to the nucleus of a DNAase activated by caspase-3 [2,24] seems to contribute to DNA fragmentation induced by ischemia [4,8,10,11,19-22,26]. Progesterone (P4) is neuroprotective in several models of brain injury [3,23]. P4 has several cellular and molecular mechanisms of action and exerts a variety of effects on the brain, capable to counteract some of the pathophysiological phenomena triggered by ischemia and reperfusion. In particular, P4 reduces caspase-3 expression and activation, and DNA fragmentation resulting from traumatic brain injury [5-7,17], middle cerebral artery occlusion [9], and oxygen-glucose deprivation [1]. In the present study, we assessed the effect of P4 on the expression and activation of caspase-3 and DNA fragmentation in the hippocampus following GCI. Male, adult *Sprague-Dawley* rats were subjected to GCI for 13 minutes by the four-vessel occlusion model. P4 (8 mg/kg, i.v.) or its vehicle (2-hydroxypropyl-beta-cyclodextrin 20% in sterile water), were administered at 15 min, 2, 6, 24, 48, and 70 h of reperfusion. This dose has been reported to be neuroprotective in improving anatomical and behavioral outcome after GCI [12-14] in rats. Rats subjected to sham procedures were used as controls. Rats were sacrificed either at 72 h or 7 days after ischemia. Remaining pyramidal neurons were evidenced by the Nissl staining technique. Caspase-3 expression and activation were evaluated by immunohistochemistry and a specific activity assay, respectively. The intensity of the caspase-3 immunolabeling and activation were measured by optical density (OD). DNA fragmentation was assessed as the number of TUNEL positive neurons. GCI induced a severe neuronal loss in CA1, an increase in the intensity of caspase-3 immunolabeling and its activation, as well as an increase in DNA fragmentation. Post-ischemic administration of P4 significantly reduced both the intensity of caspase-3 immunolabeling and activation at 72 h, as well as DNA fragmentation at 7 days after GCI, but only partially prevented CA1 neuronal loss. Present results suggest the reduction of caspase-3 expression and activation, and DNA fragmentation, as a part of the neuroprotective effects of progesterone.

Reference list

1. Ardeshiri et al. Eur. J. Neurosci. 24 (2006) 2567-2574.
2. Cao et al. J. Neurosci. 21 (2001) 4678-4690.
3. Cervantes et al. Advances in the Preclinical Study of Ischemic Stroke. In Tech (2012) 305-46.
4. Chen et al. J. Neurosci. 18 (1998) 4914-4928.
5. Cutler et al. Exp. Neurol. 195 (2005) 423-429.
6. Djebaili et al. Neuroscience 123 (2004) 349-359.
7. Djebaili et al. J. Neurotrauma 22 (2005) 106-118.

8. Gillardon et al. Brain. Res. Mol. Brain. Res. 50 (1997) 16-22.
9. Ishrat et al. Neuroscience. 210 (2012) 442-450.
10. Iwai et al. Brain Res. 671 (1995) 305-308.
11. MacManus et al. Neurosci. Lett. 164 (1993) 89-92.
12. Morali et al. Neurosci. Lett. 382 (2005) 286-290.
13. Morali et al. Restor. Neurol. Neurosci. 29 (2011) 1-15.
14. Morali et al. Restor. Neurol. Neurosci. 30 (2012) 1-8.
15. Ni et al. J. Cereb. Blood Flow Metab. 18 (1998) 248-256.
16. Niwa et al. Neurosci. Lett. 300 (2001) 103-106.
17. O´Connor et al. Exp. Neurol. 205 (2007) 145-153.
18. Ouyang et al. J. Cereb. Blood Flow Metab. 19 (1999) 1126-1135.
19. Petito et al. J. Cereb. Blood Flow Metab. 17 (1997) 967-976.
20. Rami et al. Neurochem. Int. 43 (2003) 211-223.
21. Sei et al. Neurosci. Lett. 171 (1994) 179-182.
22. Shimizu et al. Biol. Pharm. Bull. 30 (2007) 1950-1953.
23. Stein. Horm. Behav. (2012). In press. http://dx.doi.org/10.1016/j.yhbeh.2012.05.004
24. Tanaka et al. J. Neurosci. 24 (2004) 2750-2759.
25. Teschendorf et al. Neurosci. Lett. 448 (2008) 194-199.
26. Volpe et al. Neurosci. Lett. 186 (1995) 157-160.

THE FIRST ANTIOXIDANT ESTROGEN ANALOGUE WITHOUT A RING PHENOLIC HYDROXYL GROUP

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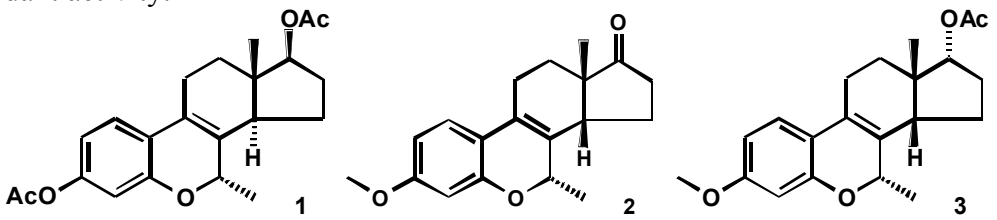
Estrogens are known to be powerful antioxidants independently of their binding to the estrogen receptors and can be used for neurodegenerative diseases (for example, Alzheimer and Parkinson disorders) treatment [1]. But the pro- and antioxidant properties of estrogens are subject of debate. Strong estrogens activity has been associated with an increase of risk to develop breast cancer [2]. Thus, search for new antioxidants without hormonal activity is doubtless of strong interest [3]. 6-oxa-Analogues of steroid estrogens possess a broad spectrum of biological activities and can differ strongly from these of corresponding 6-carba-analogues [4]. We have synthesized below listed compounds and tested their antioxidant activity.



It is known that estrogen analogues without A ring phenolic hydroxyl group do not possess antioxidant activity [5]. The antioxidant activity of compound 2 was confirmed in experiment on rats. It is significant to note that compound 2 has a methoxyl group at position 3 and is the first antioxidant estrogen analogue without A ring phenolic hydroxyl group.

	Shiff bases, units/mg of total lipides	Diene conjugates, nmol/mg of total lipides	Trien conjugates, units/mg of total lipides	Clein Coefficient	Malonic dialdehyde, nmol/mg of protein
Brain					
Control	31,6 ± 3,4	5,4 ± 0,2	0,104±0,011	0,396±0,021	1,64 ± 0,12
Com-pound 2	12,6 ± 3,2 P < 0,02	4,8 ± 0,4 P > 0,05	0,120±0,014 P > 0,05	0,342±0,022 P < 0,05	1,53 ± 0,10 P > 0,05

Reference list

[1] Simpkins J.A., Yang S-H., Liu R., Perez E., Cai Zu Y., Covey D.F. & Green P.S. Stroke. 2004, 1, 2648-2651.
[2] Key T., Appleby P., Barnes I. Reeves G. J. Natl. Cancer Inst., 2002, 94, 606—616.
[3] Non-estrogenic estradiol derivative compounds with anti-oxidative activity. US Pat. 6436917.
[4] Shavva A., Morozkina S., Galkina O. Approaches for Searching of Modified Steroid Estrogen Analogues with Improved Biological Properties. In: Steroids – Basic Science, In Tech, Rijeka, Croatia, 2011. pp. 171-220.
[5] Badeau, M.; Adlercreutz, H.; Kaihovaara, P.; Tikkanen, M. J. Estrogen A-ring structure and antioxidative effect on lipoproteins. Steroid Biochem. Mol. Biol., 2005, 96, 271-278.

BEHAVIORAL EFFECTS OF UNPREDICTABLE CHRONIC MILD STRESS (UCMS) IN A MURINE MODEL OF DEPRESSION

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Many social factors and various aversive situations may induce affective disorders, like depression and anxiety-related disorders, suggesting complex relationships among stressful situations and the onset of clinical depression.
The unpredictable chronic mild stress (UCMS) protocol is a valid, reliable and sensitive model for studying depressive disorders in rodents: it involves a period of mild socio-environmental stressors and this procedure replicates several depression-related behavioral and physiological impairments [1].
The aim of our study was to investigate the impact of a six weeks UCMS paradigm (random pattern of mild stressors twice a day) on the development of a depressive and anxiogenic phenotype in CD1 mice.
Overall, at the end of the experiment, the measured parameters indicated a sexually dimorphic effect.
For assessing the anxiety-like behavior, we used the Elevated Plus Maze (EPM) and Open Field (OF): the analysis of mice activity on EPM revealed an anxiolitic effect of UCMS exposure in female (increased number of entries into the open arms) and an anxiogenic effect (decrease in the number of entries) in male. For OF, Two-way ANOVA indicated a significant interaction gender-treatment [$p<0,05$] about the time spent in centre of the arena and a significant effect of the gender [$p<0,001$] about the total distance travelled in the central zone.
To evaluate the depressive profile we used the Forced Swimming Test: the analysis of the time wich animal spent in immobile posture (floating) during a five min trial revealed no differences of both gender and treatment.
For the hedonic behavior, we performed the sucrose preference test (SPT) calculating the consumption of sucrose solution and water. The results show that UCMS treatment causes a reduction in the fluids intake (sucrose, water and total), in particular UCMS male, suggesting a less anhedonic state. No effect of UCMS were observed about the sucrose preference.
Present results indicate that UCMS treatment may represent a good model to test the sexually dimorphic onset of affective disorders.
Were are currently investigating the vasopressin systems in sexually dimorphic circuits and in future studies we will analyze other circuits involved on anxiety behaviour (i.e. nNOS system) and on regulation of stress response (i.e serotonin system).

This research was supported by Compagnia di San Paolo (Progetto Neuroscienze) - Turin

Reference list

[1] Isingrini E, Surget A., Belzung C., Freslon J-L., Frisbee J., O'Donnell J., Camus V., d'Audiffret A., Altered aortic vascular reactivity in the unpredictable chronic mild stress model of depression in mice UCMS causes relaxation impairment to Ach; Physiology & Behavior 2011 103:540–4

PRELIMINARY OBSERVATIONS OF NEUROSTEROIDS IN HIBERNATION RELFECT STRESS AND NEUROPLASTICITY ASSOCIATED WITH INTERBOUT AROUSAL IN THE ARTIC GROUND SQUIRREL

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Background: To deal with stressors in their environment, animals have adapted neuroendocrine, behavioral and physiological responses. Hibernation is a suite of adaptations which is characterized in mammals by a state of profoundly reduced requirements for food and water in times when resources are limited, such as in the Arctic during winter [1]. In hibernating arctic ground squirrels (AGS; *Urocitellus parryii*), metabolic rate can drop to 1-2% and body temperature can be at or below 0°C [2]. During hibernation, prolonged bouts of torpor are interrupted by regular, brief periods of arousal (interbout arousal; IBA), where body temperature and metabolic rate return to typical levels of summer active (euthermic) animals. Arousal is energetically costly, physiologically stressful, and associated with synaptogenesis [3-6].

Neurosteroids, such as allopregnanolone, increase in response to acute stressors and may reinstate parasympathetic tone. Footshock, restraint, and social challenges increase allopregnanolone [7-12]. How stress is associated with IBA to change hormones is of interest.

Neurosteroids and hibernation can be neuroprotective. Progesterone and allopregnanolone have beneficial effects in animal models of aging and neuronal compromise (i.e., seizure, cortical contusion, ischemia, diabetic neuropathy) [9]. Protection from neural insults (seizures and traumatic brain injury) is observed with neurosteroids and hibernation, respectively [9, 13]. AGS resists cerebral ischemia/reperfusion injury even when not hibernating [14]. Neuroplasticity may underlie some of these neuroprotective effects. One marker of neuroplasticity, Brain-derived Neurotrophic Factor (BDNF), in addition to steroids and glucose were determined in AGS in different states (hibernating, IBA, and summer active).

Hypotheses: Because hibernation is an adaptation for energy conservation that minimizes stress, we hypothesize that the hormonal profile of AGS will reflect lower levels of stress associated with hibernation. In contrast, due to the energetic challenge of IBA, we expect hormone profiles consistent with physiological and cellular stress associated with transition to euthermy [4, 15].

Methods: In this preliminary study, we examined hormonal factors in plasma and brain tissue collected from captive AGS. During the active season, AGS were housed at 20ºC, 12:12, L:D. During the hibernation period, AGS were housed in environmental chambers at 2ºC, 4:20 L:D and hibernation/arousal state monitored by behavioral observations and respiratory rate. Samples were obtained during hibernation in winter (October, December; n = 2-3), IBA (October, December; n =3), and during the summer active season (June-August; n = 3). Enzyme-linked immunosorbent assay kits were used for cortisol, allopregnanolone, and BDNF and colormetric readers for glucose analyses. Radioimmunoassay was used to determine progesterone, dihydroprogesterone (DHP), and allopregnanolone levels in AGS brain or plasma.

Results: Hibernating AGS had lower levels of plasma cortisol and progesterone, but not allopregnanolone, compared to IBA or summer active squirrels. Unexpectedly, no change was observed in plasma glucose levels as a function of hibernation, IBA, or summer active

period. Among hibernating AGS, progesterone levels were lower in the hippocampus, thalamus, hypothalamus, midbrain and hindbrain compared to AGS in IBA or active summer. Progesterone levels in the cortex were similarly high among AGS in hibernation, IBA, and active summer compared to other species [16]. BDNF levels were highest during IBA compared to hibernating and summer active animals. Among hibernating AGS, allopregnanolone levels were lower in the striatum, thalamus and hypothalamus compared to AGS in IBA or active summer. However, in the cortex and hippocampus, allopregnanolone levels were greatest during hibernation. Levels of DHP showed a less consistent pattern.

Conclusions: Among AGS, hibernation compared to IBA or active summer is associated with lower levels of stress hormones, plasma and subcortical progesterone and striatal, thalamic, hypothalamic, and hindbrain allopregnanolone. Low level of plasma cortisol during hibernation is consistent with an absence of cellular and physiological stress during this state of energy conservation. The surge in cortisol during IBA reflects energetic stress associated with arousal as well as the increase in gluconeogensis characteristic of AGS during arousal [17]. High BNDF coupled with the surge in cortisol during arousal could be related to pronounced synaptogenesis and cognitive enhancement following arousal [5, 18].

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Reference list

[1] Carey, H.V., M.T. Andrews, and S.L. Martin, Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. Physiol Rev, 2003. 83(4): p. 1153-81.
[2] Barnes, B.M., Freeze avoidance in a mammal: body temperatures below 0 degree C in an Arctic hibernator. Science, 1989. 244(4912): p. 1593-5.
[3] Karpovich, S.A., et al., Energetics of arousal episodes in hibernating arctic ground squirrels. J Comp Physiol B, 2009. 179(6): p. 691-700.
[4] Ma, Y.L., et al., Absence of cellular stress in brain after hypoxia induced by arousal from hibernation in Arctic ground squirrels. Am J Physiol Regul Integr Comp Physiol, 2005. 289(5): p. R1297-306.
[5] von der Ohe, C.G., et al., Ubiquitous and temperature-dependent neural plasticity in hibernators. J Neurosci, 2006. 26(41): p. 10590-8.
[6] Magarinos, A.M., et al., Rapid and reversible changes in intrahippocampal connectivity during the course of hibernation in European hamsters. Proc Natl Acad Sci U S A, 2006. 103(49): p. 18775-80.
[7] Barbaccia, M.L., et al., The effects of inhibitors of GABAergic transmission and stress on brain and plasma allopregnanolone concentrations. Br J Pharmacol, 1997. 120(8): p. 1582-8.
[8] Frye, C.A., Progestins influence motivation, reward, conditioning, stress, and/or response to drugs of abuse. Pharmacol Biochem Behav, 2007. 86(2): p. 209-19.
[9] Frye, C.A., Neurosteroids-From Basic Research to Clinical Perspectives, in Hormones/ Behavior Relations of Clinical Importance, R. Rubin and D. Pfaff, Editors. 2009, AP: San Diego. p. 395-416.
[10] Patchev, V.K., et al., The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. Neuroscience, 1994. 62(1): p. 265-71.
[11] Patchev, V.K. and O.F. Almeida, Gonadal steroids exert facilitating and "buffering" effects on glucocorticoid-mediated transcriptional regulation of corticotropin-releasing hormone and corticosteroid receptor genes in rat brain. J Neurosci, 1996. 16(21): p. 7077-84.
[12] Purdy, R.H., et al., 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): p. 4553-7.
[13] Zhou, F., et al., Hibernation, a model of neuroprotection. Am J Pathol, 2001. 158(6): p. 2145-51.
[14] Drew, K.L., et al., Hibernation: a natural model of tolerance to cerebral ischemia/reperfusion, in Innate Neuroprotection for Stroke, J. Gidday and M. Perez-Pinzon, Editors. 2012, Springer: New York. p. 37-50.
[15] Zhu, X., et al., MAPKs are differentially modulated in arctic ground squirrels during hibernation. J Neurosci Res, 2005. 80(6): p. 862-8.
[16] Walf, A.A., et al., Levels of 5alpha-reduced progesterone metabolite in the midbrain account for variability in reproductive behavior of middle-aged female rats. Brain Res, 2011. 1379: p. 137-48.
[17] Galster, W. and P.R. Morrison, Gluconeogenesis in arctic ground squirrels between periods of hibernation. Am J Physiol, 1975. 228(1): p. 325-30.
[18] Weltzin, M.M., et al., Arousal from hibernation alters contextual learning and memory. Behav Brain Res, 2006. 167(1): p. 128-33.

THE STEROID 5-ALPHA REDUCTASES MEDIATE THE ABNORMALITIES IN INFORMATION PROCESSING AND ATTENTION MECHANISMS INDUCED BY REM SLEEP DEPRIVATION

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Sleep is essential for basic survival and prolonged sleep loss has long been associated to a variety of behavioral aberrations, such as extreme irritability, as well as manic-like and psychotic manifestations. Accordingly, rodents subjected to protracted sleep deprivation exhibit characteristic behavioral phenotypes reminiscent of schizophrenia symptoms, including hyperactivity, aggression, stereotyped behavior, impaired perceptual and emotional processing.

We previously reported that rats exposed to 72h of REM sleep deprivation (SD), displayed robust sensorimotor gating deficits related to hyperdopaminergic states, which were reversed by dopamine receptor antagonists. However, the neural underpinnings of these and other SD-induced behavioral perturbances remain poorly understood.

Recent findings highlighted that steroid 5-alpha reductases (5ARs) 1 and 2, the enzymes catalyzing the main rate-limiting step in neurosteroidogenesis, are key modulators of stress responsiveness, sensorimotor gating and affective behaviors. Furthermore, we showed that in rodents, the prototypical 5AR inhibitor finasteride (FIN) elicits antipsychotic-like effects without producing extrapyramidal manifestations.

Building on these premises, we hypothesized a role for 5ARs in the regulation of the neurobiological mechanisms underlying the phenotypic alterations induced by REM SD.

Adult male Sprague-Dawley rats were selectively deprived of REM sleep for 72h using the single platform method performed in the water-tank apparatus. Rats maintained in their home cages were used as controls. Startle and prepulse inhibition, two operational indices of emotional reactivity and sensorimotor gating integrity, were measured immediately after the SD period. Furthermore, at the end of the behavioral sessions, animals were euthanized and their brain regions were harvested to assess the expression of 5ARs as well as neurosteroid levels.

In accord with previous results, SD-subjected rats exhibited profound PPI deficits and changes in startle parameters, which were accompanied by significant enhancements of 5AR1 and 5AR2 expression and activity (as measured by the progesterone/allopregnanolone ratio) in the prefrontal cortex (PFC) and ventral striatum. Notably, PPI deficits were correlated with the increase in 5AR levels. In a different set of experiments, FIN (25-100mg/kg, IP) dose-dependently reduced the PPI deficits and normalized the neurosteroid imbalances induced by REM SD. Finally, progesterone (25mg/kg, IP) and its 5-alpha reduced metabolite allopregnanolone (10mg/kg, IP) respectively ameliorated and worsened SD-induced PPI disruption.

These results collectively suggest that 5ARs may mediate some of the emotional and cognitive changes ensuing REM SD, through alterations of neurosteroid homeostasis in critical regions of the dopaminergic system, such as PFC and ventral striatum. Furthermore, the present results strengthen our previous evidence on the role of FIN and other 5AR inhibitors in the therapy of neuropsychiatric disorders featuring dopaminergic imbalances and gating disturbances, such as schizophrenia and Tourette syndrome.

EFFECTS OF PROGESTERONE ON MYELIN AND INFLAMMATION IN ACUTE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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A common model to study Multiple Sclerosis (MS) is experimental autoimmune encephalomyelitis (EAE) in rodents. Both share histopathological characteristics such as demyelination, cell infiltration and neurodegeneration [1].

Previous work from our laboratory demonstrated that pretreatment with progesterone improves clinical signs in EAE mice and decreases the loss of myelin basic protein (MBP) and proteolipid protein (PLP) measured in the acute phase by immunohistochemistry and *in situ* hybridization [2]. The aim of the present study was to analyze if progesterone protective effects in the spinal cord of C57Bl/6 female EAE mice during the acute phase of the disease, involved the decreased transcription of local inflammatory mediators and the increased transcription of myelin proteins and myelin transcription factors. Real time PCR technology demonstrated that progesterone blocked the EAE-induced increase of the proinflammatory mediator tumor necrosis factor alpha (TNFα), which plays a detrimental role in MS and EAE, and its receptor TNFR1. Progesterone treatment also attenuated the microglial/macrophage marker CD11b and toll-like receptor 4 (TLR4) mRNAs. Conversely, progesterone increased the mRNA expression of PLP and MBP, the myelin transcription factors NKx2.2 and Olig1 and enhanced CC1+ oligodendrocyte density respect of untreated EAE mice. Additionally, immunocytochemistry demonstrated decreased microgliosis, demonstrated by the significantly decreased number of Iba1+ cells in gray and white matter of animals receiving the steroid. Double fluorescence immunostaining with specific markers demonstrated the colocalization of TNFα with glial-fibrillary acidic protein+ astrocytes and OX-42 + microglial cells. Therefore, progesterone treatment improved the clinical signs of EAE, decreased inflammatory reactivity of microglia and astrocytes and increased myelination. These data suggest that progesterone neuroprotection involves the modulation of several transcriptional events in the spinal cord of mice with acute EAE. This work contributes to strengthen the potential therapeutic use of neuroactive steroids in MS.

Reference list

[1] McCarthy DP, Richards MH, Miller SD.Mouse Models of Multiple Sclerosis: Experimental Autoimmune Encephalomyelitis and Theiler's Virus-Induced Demyelinating Disease Methods Mol Biol. 2012; 900:381-401.
[2] Garay L, Deniselle MC, Lima A, Roig P, De Nicola AF. Effects of progesterone in the spinal cord of a mouse model of multiple sclerosis. J Steroid Biochem Mol Biol. 2007; 107(3-5):228-37.

ANTI-INFLAMMATORY PROPERTIES FOR A TESTOSTERONE METABOLITE IN ACUTE EXPERIMENTAL MODEL OF MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a spontaneous, autoimmune and demyelinating pathology of unknown etiology. Neuroinflammation is one of the most important feature of MS, being probably one of the first events that occurs in patients. It has been reported that MS is influenced by sex hormones; indeed, pregnancy and menopause can alter disease course [4] and clinical observations revealed different incidence between males and females. To date, clinical therapies are aimed to contrast inflammation, but neuroprotective agents, able to reduce the natural worsening of MS, are not yet available. Neuroactive steroids, cholesterol-derived molecules that exert their actions on the nervous system [10], have been proposed for MS treatment [7]. A pilot clinical trial involving male MS patients demonstrated improvement of cognitive performance, slowing of brain atrophy and reduction for the delayed type hypersensitivity skin recall responses after one-year treatment with testosterone [12]. Neuroprotective effects of testosterone, and of its metabolite dihydrotestosterone (DHT), have also been observed in experimental autoimmune encephalomyelitis (EAE) rodents [6,11], the best characterized and widely used animal models for MS. Moreover, the protection exerted by androgens on EAE is further suggested by observations on castrated male mice, where pathology course was worse compared to sham animals [1]. Beside this, *in vitro* evidences pointed out anti-inflammatory effects of testosterone, such as the reduction of inflammatory cytokines produced by human macrophages [5] and monocytes [8,9]. Finally, testosterone seems to modulate naïve T cell exposed to central nervous system antigens towards a Th2 response, increasing the production of interleukin (IL)-5 and IL-10 [2]. To explain these results, it has been proposed that some of the neuroprotective effects observed after testosterone treatment could be due to its aromatization to estradiol, a well-known anti-inflammatory and protective steroid [3,13]. On the other hand, some effects are mediated by the direct action on androgen receptor (AR), as demonstrated after administration of the non-aromatizable metabolite of testosterone, i.e. DHT[11]. Moreover, DHT could be subsequently (and reversibly) converted into 3 alpha-diol, a GABAA receptor agonist, increasing the possibility for neuroprotection, due to the different mechanism of action exerted by this metabolite. In light of these evidences, and considering the lack of observations for the neuroprotective action of androgens in rat models of EAE, we induced the pathology in male Lewis rats, in order to develop an acute form of EAE, mainly characterized by a neuroinflammatory profile. EAE animals were subcutaneously treated with 3 alpha-diol or vehicle (sesame oil) every other day, whereas control rats (i.e., rats not induced with EAE) received in the same days only vehicle. Animals were monitored through the experiment, assessing the neurological deficits associated to the pathology,

and, at the end of the experiment (i.e., at 15 days post induction), spinal cords were dissected. 3 alpha-diol treatment did not modify the clinical course of EAE animals, compared to vehicle-only EAE rats, nor the loss of weight associated to the pathology. From a molecular point of view, inflammatory parameters, like the glial fibrillary acidic protein (GFAP) and the major histocompatibility complex class II (MHC-II), were assessed in the dorsal funiculus of lumbar spinal cord. Immunostaining analysis revealed that the increase of GFAP+ and MHC-II+ cells, associated to EAE, was reduced by 3 alpha-diol treatment. In agreement with this observation, spinal cord pro-inflammatory cytokine expression (i.e., tumor necrosis factor alpha) was high in vehicle EAE animals and decreased after 3 alpha-diol treatment. Instead, the expression of the anti-inflammatory cytokine transforming growth factor beta 1 was not different between vehicle and 3 alpha-diol treated EAE rats, suggesting a specific modulation on the pro-inflammatory components after 3 alpha-diol administration. Liquid chromatography tandem mass spectrometry analysis were subsequently performed to assess neuroactive steroid levels in spinal cord of EAE and controls animals. A significant increase of 3 alpha-diol levels was observed in steroid-treated EAE animals; interestingly, also levels of DHT were raised significantly after treatment, indicating the retroconversion of 3 alpha-diol. These data may suggest that the reduction of inflammatory parameters in spinal cord could be mediated by both the action of DHT on AR and/or of 3 alpha-diol on GABAA receptor. These observations pointed out, for the first time, the effects of 3 alpha-diol on inflammatory parameters in EAE Lewis rats. Future studies will be aimed to elucidate the involvement of androgen and/or GABAA receptors on the observed effects.

Reference list

[1] Bebo BF Jr, Zelinka-Vincent E, Adamus G, Amundson D, Vandenbark AA, Offner H. Gonadal hormones influence the immune response to PLP 139-151 and the clinical course of relapsing experimental autoimmune encephalomyelitis J Neuroimmunol. 1998 Apr 15;84(2):122-30.

[2] Bebo BF Jr, Schuster JC, Vandenbark AA, Offner H. Androgens alter the cytokine profile and reduce encephalitogenicity of myelin-reactive T cells. J Immunol. 1999 Jan 1;162(1):35-40.

[3] Brinton RD. Estrogen-induced plasticity from cells to circuits: predictions for cognitive function. Trends Pharmacol Sci. 2009 Apr;30(4):212-22. Review.

[4] Confavreux C, Hutchinson M, Hours MM, Cortinovis-Tourniaire P, Moreau T. Rate of pregnancy-related relapse in multiple sclerosis. Pregnancy in Multiple Sclerosis Group. New Engl J Med 1998;339:285–291.

[5] D'Agostino P, Milano S, Barbera C, Di Bella G, La Rosa M, Ferlazzo V, Farruggio R, Miceli DM, Miele M, Castagnetta L, Cillari E. Sex hormones modulate inflammatory mediators produced by macrophages. Ann N Y Acad Sci 1999;876:426–429.

[6] Dalal M, Kim S, Voskuhl RR. Testosterone therapy ameliorates experimental autoimmune encephalomyelitis and induces a T helper 2 bias in the autoantigen-specific T lymphocyte response. Journal of Immunology 1997 159 3–6.

[7] Gold SM, Voskuhl RR. Estrogen and testosterone therapies in multiple sclerosis. Prog Brain Res. 2009;175:239-51. Review.

[8] Li ZG, Danis VA, Brooks PM. Effect of gonadal steroids on the production of IL-1 and IL-6 by blood mononuclear cells in vitro. Clin Exp Rheumatol 1993;11:157–162.

[9] Liva SM, Voskuhl RR. Testosterone acts directly on CD4+ T lymphocytes to increase IL-10 production. J Immunol. 2001 Aug 15;167(4):2060-7.

[10] Melcangi RC, Garcia-Segura LM, Mensah-Nyagan AG Neuroactive steroids: state of the art and new perspectives. Cellular and Molecular Life Sciences 2008 65 777–797.

[11] Palaszynski KM, Loo KK, Ashouri JF, Liu HB, Voskuhl RR. Androgens are protective in experimental autoimmune encephalomyelitis: implications for multiple sclerosis. J Neuroimmunol.2004;146(1-2):144-52.

[12] Sicotte NL, Giesser BS, Tandon V, Klutch R, Steiner B, Drain AE, Shattuck DW, Hull L, Wang HJ, Elashoff RM, Swerdloff RS, Voskuhl RR. Testosterone treatment in multiple sclerosis: a pilot study. Arch Neurol. 2007 May;64(5):683-8.

[13] Spence RD, Voskuhl RR. Neuroprotective effects of estrogens and androgens in CNS inflammation and neurodegeneration. Front Neuroendocrinol. 2012 Jan;33(1):105-15. Review.

ESTRADIOL AND TESTOSTERONE REGULATE ARGININE-VASOPRESSIN EXPRESSION IN SH-SY5Y NEUROBLASTOMA CELLS THROUGH ESTROGEN RECEPTORS ALPHA AND BETA

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The expression of arginine-vasopressin (AVP) is regulated by estradiol and testosterone in different neuronal populations by mechanisms that are not yet fully understood. Estrogen receptors (ERs) have been shown to participate in the regulation of AVP neurons by estradiol. In addition, there is evidence of the participation of ER beta in the regulation of AVP expression exerted by testosterone via its metabolite 5alpha-dihydrotestosterone (5alpha-DHT) and its further conversion in the androgen metabolite and ER beta ligand 3beta-diol. In this study we have explored the role of ERs and androgen receptor in the regulation exerted by estradiol and testosterone on AVP expression, using the human neuroblastoma cell line SH-SY5Y.
Estradiol treatment increased AVP mRNA levels in SH-SY5Y cells in comparison to cells treated with vehicle. The stimulatory effect of estradiol on AVP expression was imitated by the ER alpha agonist-PPT and blocked by the ER antagonist, ICI 182,780 and the ER alpha antagonist-MPP. In contrast, the ER beta agonist-DPN reduced AVP expression, while the ER beta antagonist-PHTPP enhanced the action of estradiol on AVP expression. Testosterone increased AVP expression in SH-SY5Y cells by a mechanism that was not affected by the androgen receptor antagonist-flutamide, was not imitated by the testosterone metabolite 5alpha-DHT and was blocked by the ER alpha antagonist-MPP. In contrast, 5alpha-DHT had a similar effect than the ER beta agonist-DPN, reducing AVP expression.
These findings suggest that estradiol and testosterone regulate AVP expression in SH-SY5Y cells through ERs, exerting a stimulatory action via ER alpha and an inhibitory action via ER beta.

ENHANCED GLUTAMATERGIC TRANSMISSION IN A MOUSE MODEL OF EARLY LIFE STRESS: RELEVANCE TO THE STRESS PROTECTIVE ACTIONS OF NEUROSTEROIDS

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Adverse early-life experiences, in the form of poor maternal care, are recognized to program an abnormal stress response. The mechanisms of stress dysfunction are complex but may involve $GABA_A$ receptors ($GABA_ARs$), as these receptors curtail stress-induced activation of the HPA axis [1]. 5α-pregnan-3α-ol-20-one (5α3α-THPROG) is a potent and selective endogenous modulator of $GABA_A$ receptors ($GABA_ARs$) and exhibits clear stress-protective actions [2]. The levels of 5α3α-THPROG rise rapidly during acute stress, therefore suggesting a possible role as regulator of the stress response. Here, we utilize a paradigm of early-life stress (ELS) to investigate the relevance of 5α3α-THPROG actions at $GABA_ARs$ in CRF-releasing neurones of the neonatal mouse hypothalamus [3]. In recordings from CRF-releasing dorsal-medial parvocellular (mpd) neurons 5α3α-THPROG potently inhibited neuronal discharge of control neurons whereas this action was greatly compromised for the ELS group. 5α3α-THPROG was less effective in potentiating $GABA_AR$ function in ELS *cf* control mice. Furthermore for ELS neurons the excitatory drive was greatly enhanced *cf* control mpd neurons. Neurosteroid suppression of mpd firing was also blunted for mice lacking δ-subunit-containing $GABA_ARs$ ($\delta^{0/0}$). Intriguingly, $\delta^{0/0}$ offspring show hallmarks of abnormal maternal care similar to ELS pups. Notably, $\delta^{0/0}$ mpd neurons also exhibit enhanced glutamatergic drive but not reduced neurosteroid potentiation of $GABA_A$ receptor function. Collectively, these findings identify increased glutamatergic transmission as a common maladaptation to early-life stressors and draw attention to neurosteroids as possible important early regulators of the stress response.

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Reference list

[1] Cullinan *et al*, (2008). Functional role of GABAergic influences on the HPA axis. *Brain Structure & Function* **213**(1-2) 63-72.

[2] Belelli and Lambert (2005). Neurosteroids: endogenous regulators of the $GABA_A$ receptor. *Nature Reviews Neuroscience* **6,** 565-575.

[3] Rice *et al.* (2008). A novel mouse model for acute and long-lasting consequences of early life stress. Endocrinology **149**(10), 4892-900.

VARIATIONS OF THE GLUTAMATE LEVELS IN INFERIOR FRONTAL GYRUS ACROSS SEX AND THE MENSTRUAL CYCLE

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Introduction

Estrogen has been suggested to influence performance on tasks dependent upon prefrontal cortex (PFC) functioning. In humans, the physiological evidence of this influence is limited. One of the conducted studies, however, demonstrates higher glutamate levels within the anterior cingulate cortex (ACC) in the follicular compared to the luteal phase. Similarly, the current study aims to explore whether glutamate levels within the inferior frontal gyrus (IFG) change across the menstrual cycle, expecting a peak in the follicular phase. IFG is a region related to functions like working memory and cognitive control. For the current sample, cognitive control has already been shown to increase in the follicular phase.

Methods

Fifteen male and fourteen female subjects were tested three times within a month period, whereby the women were tested in their menstrual, follicular, and luteal phase (verified by saliva samples). Men were tested in corresponding time intervals. Magnetic resonance spectroscopy (MRS) data acquisition was done using a 3T MR scanner. In vivo short echo ^{1}H-spectra from the left (Brocas area) and right inferior frontal gyrus were obtained by using a single-voxel point-resolved spectroscopy (PRESS) sequence (voxel size 20 × 20 × 20 mm^3, time repetition/time echo [TE] = 1500/35 ms, 128 averages). Here, we report the ratio of the combined signal of glutamate and glutamine to creatine (Glx/Cre)

Results

Hormone levels for the three phases were: Menstrual phase E 2.8(±1.3), P 55.1(±20.5); Follicular phase E 3,7(±1,5), P 59,4(±31,8), L 4.7(±1.7), P 195.2(±97.2). For group analysis an ANOVA with sex, phase, and side was conducted and revealed a main effect of side ($F(1,27)=5.05$;$p=0.03$; $\eta^2=0.16$), but no further main effects or interaction effects were detected. However, to explore the data further, ANOVAs with sex and phase were conducted for each side separately. While there was no significant effect for the left side, ANOVA for the right side resulted in a sex × phase interaction ($F(2, 54)=3.41$;$p=0.04$;$\eta^2=0.11$). Post hoc testing with Fishers LSD revealed a difference in the follicular phase compared to menstrual ($p=0.01$, $d= 0.58$), luteal phase ($p=0.054$, $d=0.48$), and men ($p=0.01$, $d=0.96$)

Conclusion

The results show overall higher levels of glutamate in the right IFG compared to the left. Furthermore, the Glx/Cre levels within the right IFG is stable for men, however for women they increase from menstrual to follicular phase and decreases again in the luteal phase. This is consistent with previous findings in the ACC. It can be speculated that the effect observed on the right side is due to reduced inhibition of the left hemisphere onto the right through corpus callosum fibers. It can also be speculated whether the peak in Glx/Cre level is related to the peak in cognitive control observed in the follicular phase.

EFFECTS OF ALLOPREGNANOLONE ON FEEDING BEHAVIOR IN MALE WISTAR RATS

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Background: The action of many endogenous factors that contribute to excess eating is still not well understood. GABAergic transmission in the hypothalamus is needed for normal feeding regulation [1, 2]. The stress-induced progesterone metabolite allopregnanolone is one highly potent endogenous positive $GABA_A$-receptor modulating steroid (GAMS) [3] and in the light of this, we were encouraged to investigate the effect of allopregnanolone in different feeding situations. We hypothesize that the effect of allopregnanolone depends on the activity state of AgRP-neurons, which have in rats a higher activity in the active (dark) than in the inactive (light) period of the day. In rats, an increased meal size, but not number of meals, has been linked to diet-induced obesity [4, 5]. To further elucidate the role of allopregnanolone in this aspect of feeding, we also observed feeding behavior and investigated parameters such as meal latency, frequency and duration. **Methods**: Chow intake was measured after acute subcutaneous injections of allopregnanolone in both the active and inactive period of the day. Eating sessions were recorded by a digital film camera, and analyzed to detect meal patterns. **Results:** Acute injections of allopregnanolone dose-dependently increased intake of standard chow by up to four times in the male Wistar rat, with a larger effect in the active period ($Z = -4.29$; $p \leq 0.001$). In the inactive period only a few vehicle treated rats ate, but administration of allopregnanolone increased this fraction significantly. In contrast, in the active period, 80% of the vehicle treated rats ate and after administration of allopregnanolone the amount of ingested food was further increased. Allopregnanolone treated rats also started their meal earlier compared to vehicle (chi-sq(df=1)=8.02; $p \leq 0.05$) and ate their first meal during a significant longer period of time ($Z = -3.06$; $p \leq 0.01$). **Conclusion**: Allopregnanolone seems to act at several levels of feeding, i.e. both initiating food intake, increasing the amount of food ingested as well as the duration of a meal. The disparity in the effect of allopregnanolone between the active and the inactive period may be the diurnal differences in activity of GABAergic feeding regulating neurons.

Reference list

1. Tong, Q., Ye, C. P., Jones, J. E., Elmquist, J. K., & Lowell, B. B. (2008). Synaptic release of GABA by AgRP neurons is required for normal regulation of energy balance. *Nat Neurosci, 11*(9), 998-1000.
2. Wu, Q., Boyle, M. P., & Palmiter, R. D. (2009). Loss of GABAergic signaling by AgRP neurons to the parabrachial nucleus leads to starvation. *Cell, 137*(7), 1225-1234.
3. Majewska, M. D., Harrison, N. L., Schwartz, R. D., Barker, J. L., & Paul, S. M. (1986). Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science, 232*(4753), 1004-1007.
4. Farley, C., Cook, J. A., Spar, B. D., Austin, T. M., & Kowalski, T. J. (2003). Meal pattern analysis of diet-induced obesity in susceptible and resistant rats. *Obes Res, 11*(7), 845-851.
5. Furnes, M. W., Zhao, C. M., & Chen, D. (2009). Development of obesity is associated with increased calories per meal rather than per day. A study of high-fat diet-induced obesity in young rats. *Obes Surg, 19*(10), 1430-1438.

IMMUNOHISTOCHEMICAL ANALYSIS OF INTERACTIONS BETWEEN KISSPEPTIN NEURONS AND HYPOTHALAMIC TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS IN OLD FEMALE RATS

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BACKGROUND: Kisspeptin is a pivotal regulator of the reproductive functions. Kisspeptin neurons and their fibers are distributed abundantly throughout the arcuate nucleus (ARC) of the rat hypothalamus. We clarified the possibility that the kisspeptin neurons contact with tuberoinfundibular dopaminergic (TIDA) neurons which regulate the prolactin (PRL) secretion, and regulate TIDA neurons through synapses. The aim of this study was to investigate interactions between kisspeptin neuron and TIDA neuron in female rats with aging. Therefore, we observed the contact between kisspeptin neuron and TIDA neuron in 24-month-old female rats.

METHODS: 24-month-old femal rats and 8-week-old femal rats were perfused with 4% paraformaldehyde. After removing brains, coronal cryosections were cut and stained with anti-kisspeptin antibody and anti-tyrosine hydoroxylase (TH) antibody which is a marker of dopaminergic neuron. The numbers of TH cells contacting with kisspeptin-immunoreactive (ir) fibers were counted.

RESULTS: Apparent TH-ir cell bodies and kisspeptin-ir fibers were found in the dorsal ARC of 24-month-old female and 8-week old female rats. The number of TH-ir cells in the dorsal ARC did not differ significantly between groups. Moreover, there were no significant differences in the number of TH-ir cells contacting with kisspeptin-ir fibers between 24-month rats and 8-week rats. On the other hand, the number of ARC kisspeptin-ir cell bodies in 24-month rats was significantly fewer than that in 8-week rats. Plasma levels of PRL in 24-month rats were higher than that in 8-week rats.

CONCLUSION: This study revealed that the contacts between TIDA neurons and kisspeptin neurons are maintained after menopause. Because kisspeptin-ir cell bodies were decreased in 24-month rats compared to 8-week rats, PRL release might be regulated by differences in the expressions or releases of kisspeptin through TIDA neurons in female after menopause.

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EFFECT OF CHRONIC EXPOSURE TO ANDROGEN ON KISSPEPTIN EXPRESSION AND LUTEINIZING HORMONE RELEASE IN FEMALE RATS

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Polycystic ovary syndrome (PCOS) is a women endocrine disorder, and is characterized by anovulation, menstrual irregularity and hyperandrogenism. The etiology of PCOS remains unclear. Kisspeptin is implicated in ovulation and follicular development by stimulation of GnRH/luteinizing hormone (LH) secretion. The purpose of this study was to determine whether chronic exposure to androgen affects expression of kisspeptin and LH release in female rats, because women with PCOS show hyperandrogenism. Female rats were weaned, and were implanted subcutaneously with 90-d continuous-release pellets of 5α-dihydrotestosterone (DHT) [1]. After 10 wk of age (7 wk after pellet implantation), animals were used for experiments. *Kiss1* mRNA-expressing cells in both the anteroventral periventricular nucleus (AVPV) and the arcuate nucleus (ARC) were significantly decreased in DHT-implanted female rats compared to normal female rats. AVPV kisspeptin neurons are known to be involved in LH surge generation and are positively regulated by estrogen. We tested whether AVPV kisspeptin neurons respond to high level of estrogen. Normal female rats showed estrogen-induced LH surge in the afternoon, whereas estrogen-induced LH surge could not be detected in DHT-implanted rats even if animals were administrated high concentration of estrogen. On the other hand, there were no significant differences in numbers of AVPV *Kiss1* mRNA-expressing cells, kisspeptin-immunoreactive (ir) cells and PoA GnRH-ir cells between DHT-female rats and normal female rats. ARC kisspeptin neurons are considered to participate in pulsatile LH release and are a target of estrogen-negative feedback action. We tested whether DHT implantation affects ARC kisspeptin expression and pulsatile LH release in ovariectomized (OVX) female rats. ARC *Kiss1* mRNA-expressing cells were significantly decreased in OVX DHT-female rats compared to OVX female rats. Neurokinin B (NKB) is known to coexpress kisspeptin in the ARC. NKB-ir cells in OVX DHT-female rats were also significantly fewer than that in OVX female rats. Pulsatile LH secretion was suppressed in OVX DHT-female rats. These results suggest that chronic exposure to androgen suppresses both ovulation and follicular development. Hyperandrogenism may suppress pulsatile LH secretion through inhibition of ARC kisspeptin neurons. On the other hand, AVPV kisspeptin neurons might not be involved in suppression of LH surge by androgen. Thus, hyperandrogenism in PCOS patients may be associated with anovulation and menstrual irregularity through partial inhibition of kisspeptin neurons.

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Reference list

[1] Manneras L, Cajander S, Holmang A, Seleskovic Z, Lystig T, Lonn M, Stener-Victorin E. *Endocrinology* 2007; 148: 3781-3791.

TESTOSTERONE, CORTICOSTERONE AND UNACYLATED GHRELIN CONCENTRATIONS IN SERUM OF THE CHRONICALLY ALCOHOLIZED RATS

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Background. A challenging question that continues unanswered in the field of addiction is why some individuals are more vulnerable to substance use disorders than others. Numerous risk factors for alcohol and other drugs of abuse, including exposure to various forms of stress, have been identified in clinical studies. However, the neurobiological mechanisms that underlie this relationship remain unclear [1, 2]. During last decade, the focus of scientific researches of the brain reinforcing mechanisms was shifted from typical neurotransmitter approach to participation of steroid hormonal systems and peptide hormones like corticoliberin (CRF), ghrelin and orexins in emotional supply of dependence phenomena, in alcohol addiction in particular [3, 4]. But special information concerning this subject is rather pure up to now. That is why the aim of this work consisted in simultaneous quantitative determination of steroid hormones and ghrelin level in the serum of chronically alcoholized rats.

Material and methods. The 50 Wistar male rats were alcoholized with 15% ethanol as a one source of liquid for 6 months, 40 rats consumed water for this period (control rats, group 1). After that 13 alcoholized and 10 water consuming rats were decapitated and both the blood and the brain tissue were taken for hormonal immunoassay. 2 h (equal immediately after last intake of alcohol, 14 rats, group 2), 24 h (13 rats, group 3) and 7 days (13 rats, group 4) after withdrawal of ethanol the same investigation was processed as well. The immunoassays were carried out by the EIA test kits of IBL Int. (Germany), DBC Inc. (Canada) and Bertin Pharma (France).

Data analysis. The row data were treated by means of parametric and nonparametric tests. Data were analyzed using the SPSS and Systat after the Kolmogorov–Smirnov test for normality. All animal groups were compared one to another by the ANOVA test and correspondent “post hoc” paired tests of Newman–Kruskall–Wallis test and Dunn’s test.

Results. The experimental investigation included the quantitative determination of testosterone, corticosterone and unacylated ghrelin in the rat serum after prolonged alcoholization followed by alcohol withdrawal. The mean concentrations of testosterone were 1.62±0,35; 2.83±0,93; 3.37±0,51 and 2.38±0,50 ng/ml respectively in groups 1 to 4. Such variability in experimental data can be explained by different individual reactions of rats exposed to ethanol. The testosterone concentrations in serum slightly increased in the course of experimental alcoholization and immediately after withdrawal of ethanol. There were statistically significant differences ($p<0.001$) between compared 2nd (alcoholization) and 3rd (withdrawal) groups. The corticosterone concentrations were 138.2±3,14; 139.0±1,73; 138.1±1,45 and 137.7±1,80 ng/ml in the groups studied. These values reflected the high levels of normal concentrations of corticosterone. But there were no statistically significant differences between all groups. The unacylated ghrelin concentrations were 0.986±0,11; 0.476±0,07; 0.497±0.0,07 and 0.669±0,08 ng/ml in groups 1-4. These changes can be qualified as more informative compared to testosterone and corticosterone. In general we registered 2-fold decrease ($p<0.001$) in chronically alcoholised rats (2nd group) and immediately after withdrawal of ethanol (3rd group). 7 day

after withdrawal of ethanol (4th group) we revealed tendency to normalisation in the unacylated ghrelin concentration ($p<0.05$). In all cases the use of Kolmogorov – Smirnov test showed the distribution of Gaussian curve type. There were no correlation between testosterone and corticosterone profiles and corticosterone and unacylated ghrelin ones.

Discussion. The findings concerning testosterone during alcoholization are rather contradictory [1, 3]. As a rule, the chronic alcoholization led to decrease of testosterone concentrations both in clinic and in experimental conditions. Some investigations showed that the testosterone profiles could be connected with degree of alcohol addiction. On the contrary, the acute administration of alcohol increases hormone concentration in the serum. The same data were received with corticosterone which concentrations during chronic alcoholization were high (the high level of normal range variation). That indicated that chronic alcoholization was not heavy stressful factor. Moreover, the corticosterone levels were stable high without significant variations. Also there was no data suggesting general depression of the hypothalamus-pituitary-adrenal axis. The unacylated ghrelin represents the most stable version of ghrelin family polypeptides and constitutes about 90% of the blood circulating ghrelins. We found significant (2-fold) decrease of the unacylated ghrelin after chronic alcoholization. The withdrawal of ethanol recovered the unacylated ghrelin level but only partially. It seems to us the ghrelin system has an important role in adaptation of the organism to alcoholization compared to other hormones (testosterone and corticosterone, for example).

Conclusions. If the steroid hormonal systems are rather stable during alcoholization, the unacylated ghrelin can initiate the chain from stomach (place of synthesis) to the CNS and participate in the process of reward from food and other positive reinforcing signals. There is a negative feedback between positive reinforcing reply on alcohol intake and synthesis of ghrelin in the gastral mucosa.

Reference list

1. Boyd, K.N., Kumar, S., O'Buckley, T.K., Porcu, P., Morrow, A.L. (2010) Ethanol induction of steroidogenesis in rat adrenal and brain is dependent upon pituitary ACTH release and de novo adrenal StAR synthesis. Neurochemistry, 112(3): 784–796.
2. Landgren, S., Engel, J.A., Hyytia, P., Zetterberg, H., Blennow, K., Jerlhag, E. (2011) Expression of the gene encoding the ghrelin receptor in rats selected for differential alcohol preference. Behav. Brain Res., 221 : 182–188.
3. Rose, A.K., Shaw, S.G., Prendergast, M.A., Little, H.J. (2010) The Importance of Glucocorticoids in Alcohol Dependence and Neurotoxicity. Alcohol Clin. Exp. Res. 34(12): 2011–2018.
4. Shabanov, P.D., Lebedev, A.A., Bychkov, E.R. (2012) The effect of ethanol exposure in pregnancy on maturation of monoaminergic systems in the developing rat brain. Ross. Fiziol. Zh. Im. I M Sechenova. 98(2): 202-211.

NEUROPROTECTIVE EFFECT OF ESTRADIOL IN MICE WITH SELECTIVE CHOLINERGIC LESIONS IN NUCLEUS BASALIS MAGNOCELLUALARIS: MORPHOLOGICAL AND BEHAVIOURAL STUDIES

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Basal forebrain cholinergic neurons play a critical role in learning and memory and are highly affected in neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Previous neurodegenerative lesion studies have demonstrated a neuroprotective effect of 17β-estradiol (E2) treatment on cholinergic neurons. However, these lesion models lacked selective damage to cholinergic neurons, limiting the interpretation of behavioural deficits observed after such lesion and the correlation with the neuroprotective potential of E2. In this study, we have examined the effect of E2 treatment on cholinergic neurons and associated learning behaviours using an *in vivo* mouse model with selective cholinergic lesion in the nucleus basalis magnocellularis (NBM). We applied a novel, highly selective mouse cholinotoxin, mu p75-Saporin (mp75SAP), by means of microinjection into the NBM of ovariectomised adult female mice. Unilateral injection of mp75-SAP resulted in cholinergic cell loss in the NBM and ipsilateral cholinergic fibre loss in the somatosensory cortex. The 0.9μg/μl mp75-SAP and 12 days survival time were selected as being optimal for the model which exhibited ~70% of cholinergic cell loss in the NBM and ~75% of cholinergic fibre loss in the cortex. A single injection of E2 1h after mp75Sap lesion increased the ipsilateral cholinergic fibre density in the cortex but it did not have an effect on cholinergic cell loss in the NBM ($p < 0.005$; $n = 6$). The functional consequences of E2 treatment on selective cholinergic lesion in the NBM was tested by performing skilled motor reaching task and novel object recognition test. Mice given bilateral injection of mp75-SAP into the NBM demonstrated impaired motor learning skills. Effect of E2 on learning behaviours is currently ongoing investigation and the data will be discussed on the poster. These findings demonstrate that a single E2 treatment is able to restore the basal forebrain fiber loss in a cholinotoxic *in vivo* model. Therefore, E2-induced neuroprotective actions may have therapeutic relevance in neurodegenerative diseases associated with cholinergic cell death.

PSYCHOACTIVE PROPERTIES OF BNN27-A NOVEL NEUROSTEROID DERIVATIVE

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Introduction

Dehydroepiandrosterone (DHEA) is one of the most potent neuroactive neurosteroids. A novel synthetic analogue of DHEA, named BNN27 ((20R)-3β,21-dihydroxy-17R,20-epoxy-5-pregnene), has been developed. In contrast to DHEA, this compound is deprived of estrogenic or androgenic effects[1]. Although, BNN27's neurotrophic/neuroprotective actions have been thoroughly studied, its potential psychoactive properties have not been investigated to date. Hence, the purpose of the present study was to screen BNN27 for potential anxiolytic and antidepressant actions.

Method

Male and female adult Wistar rats (N=48) were treated acutely with BNN27 (10, 30, 90 mg/kg, i.p.) or vehicle (DMSO 100%) and 30mins later they were placed to an open field arena for 1hr. Horizontal and vertical activity, as well as time spent in the center of the arena were measured using an automated computer program (Med Associates). The following day rats were again injected with the same dose of BNN27 or vehicle and 30min later they were subjected to the light/dark test for 10min. Total number of transitions and per cent of time spent into the illuminated compartment were calculated using an in-house developed computer program (*Observador*). Six days later all rats were subjected to the modified forced swim test, as before[2]. Specifically, rats were subjected to a 15min pretest session, thereafter all rats received three injections of BNN27 (10, 30, 90 mg/kg, i.p.) or vehicle at 1, 19, 23 hrs after the pretest session. 24 hrs after the pretest session, all rats were subjected to a 5min forced swim test. Immobility, swimming and climbing behavior were scored using the same in-house developed computer program. Three weeks later, all rats had again one single injection of either BNN27 (10, 30, 90 mg/kg, i.p.) or vehicle, killed with rapid decapitation and brain tissue samples from hippocampus and prefrontal cortex were collected for biogenic monoamine assays using HPLC-ED, as before[3].

Results

Preliminary results clearly show that 30min post-injection, BNN27 has a marked impact on spontaneous motor activity of male and female rats. Specifically, both male and female rats displayed lower horizontal and vertical activity when injected with progressively higher doses of BNN27. The highest BNN dose (90mg/kg) resulted in a clear suppression of motor activity in the open field, whereas the lower doses of BNN27 (10 and 30mg/kg) were only slightly different from control rats. Interestingly, male rats injected with 10 mg/kg BNN27 displayed increased time spent in the center of the arena, suggesting an anxiolytic effect. In the light/dark box, no differences were found in total transitions and per cent of time spent in the illuminated compartment for those rats injected with 10 and 90mg/kg BNN27, but the 30mg/kg BNN27 dose resulted in an increased time spent into the illuminated compartment, suggesting a possible anxiolytic action.

Discussion

Our preliminary results with regards to the acute administration of single doses of BNN27 to male and female rats clearly show that BNN27 has psychotropic properties. Whereas higher doses of BNN27 seemingly cause motor depression, the lower BNN27 doses appear to have anxiolytic properties. Differences however in the acute effects of BNN27, between male and female rats, warrant further investigation.

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Reference list

1. Calogeropoulou, T., N. Avlonitis, V. Minas, X. Alexi, A. Pantzou, I. Charalampopoulos, M. Zervou, V. Vergou, E.S. Katsanou, I. Lazaridis, M.N. Alexis, and A. Gravanis, *Novel dehydroepiandrosterone derivatives with antiapoptotic, neuroprotective activity.* J Med Chem, 2009. 52 6569-87.
2. Kokras, N., C. Dalla, A.C. Sideris, A. Dendi, H.G. Mikail, K. Antoniou, and Z. Papadopoulou-Daifoti, *Behavioral sexual dimorphism in models of anxiety and depression due to changes in HPA axis activity.* Neuropharmacology, 2012. 62 436-45.
3. Kokras, N., K. Antoniou, A. Polissidis, and Z. Papadopoulou-Daifoti, *Antidepressants induce regionally discrete, sex-dependent changes in brain's glutamate content.* Neurosci Lett, 2009. 464 98-102.

CIRCADIAN FACTORS INFLUENCE 3ALPHA-DIOL LEVELS AND DEPRESSIVE LIKE BEHAVIORS AMONG C57 MICE

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Background: Although there are strain differences among mice, mice exert a high neurosteroid capacity with circadian differences in pregnane neurosteriod, which has been demonstrated among females with considerable diurnal variation, that rivals estrous changes (Corpechot, 1997). Androgenic neurosteriods, such as 3alpha-diol, also play an important role in physiology and behavior. Some studies show that moderate to high levels of androgens are associated with anti-anxiety behaviors (Frye et al 2008). However, not all studies demonstrate this effect, some show lower levels of androgens associated with better performance. Important factors, such as age of individual, species, strain and/or time of day may influence the role of androgens on depressive-like behaviors.
Methods: Of interest are the behavioral effects of circadian patterns in a mouse model of depression. C57 mice, bred at the University at Albany, were assessed for heart rate via CODA monitor and tested in the Force Swim Task (FST), a well-characterized animal assay of depression. Tissues were collected immediately after testing so that plasma and brain 3alpha-diol levels could be determined. Regression analyses were performed with time of day as the independent variable and immobility, heart rate, and 3alpha-diol levels as the dependent variable. Further, the relationship between tissue specific 3alpha-diol levels and the time spent immobile, a measure of depressive like behavior in the FST, was examined through simple regression analyzes. We hypothesized that there will be variations of 3alpha-diol as a function of time of day and this may influence the expression of depressive like behaviors.
Results: Mice spent more time immobile when tested in morning then did mice tested during midday or evening periods. There was an inverse relationship between time of day and duration of time spent immobile. 3alpha-diol levels in plasma, prefrontal cortex and hypothalamus also significantly declined throughout the day. Interestingly, heart rate levels were lower in morning and increased during midday and evening periods. Additionally, similar patterns were seen in 3alpha-diol levels in the hippocampus. When considering the relationship between 3alpha-diol levels and immobility, we found lower levels of 3alpha-diol in plasma, hippocampus and prefrontal cortex were associated with greater immobility. Whereas in the hypothalamus, increased time spent immobile was associated with higher levels of 3alpha-diol.
Conclusions: Circadian factors and differences in 3alpha-diol levels between brain regions may influence the expression of depressive phenotypes in the FST among C57 mice

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Reference list

C. Corpéchot, B.E. Collins, M.P. Carey, A. Tsouros, P. Robel, J.P. Fry. Brain neurosteroids during the mouse oestrous cycle. Brain Res, 766 (1997), pp. 276–280

Frye CA, Koonce CJ, Edinger KL, et al. (2008). Androgens with activity at estrogen receptor beta have anxiolytic and cognitive-enhancing effects in male rats and mice. Horm Behav. 54, 726-34.

METHYLATION OF MIGRAINE-RELATED GENES IN DIFFERENT TISSUES

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Migraine is a paroxysmal neurovascular disorder which is known to be – at least partly - genetic in nature. However, genes have only clearly been identified for a rare type of migraine familial hemiplegic migraine. GWAS studies have identified genes that may be relevant for the more common forms of migraine, but an additional influence of environmental factors is likely. Interestingly, the prophylactic effectivity of valproate, a DNA methylation inhibitor, may point to the involvement of epigenetic mechanisms in migraine. Migraine is much more common in women of the fertile age than in men, pointing towards a role for 17β-estradiol, which is known to be involved in epigenetic mechanisms. Recently, it was demonstrated that CGRP, a neuropeptide and potent vasodilator that most likely plays a key role in the pathophysiology of migraine, can be regulated via epigenetic mechanisms. Methylation is often examined in blood, which can easily be obtained in clinical studies, but it is not sure whether these results can be compared to other tissues. The aim of our study was to investigate the methylation of genes that may be involved in the pathogenesis of migraine in different tissues relevant to the pathophysiology of migraine and examine the role of 17β-estradiol in the methylation. In addition to the 'migraine tissues', we investigated methylation in blood and aorta as a peripheral control. Female SD rats (n=11-12 per group) were ovariectomized or sham-operated and treated with 17β-estradiol or placebo. DNA was isolated from blood, aorta, dura mater, trigeminal caudal nucleus and trigeminal ganglion. DNA methylation of 10 migraine-related genes (MTHFR, eNOS, ESR1, GPER, CGRP, USF1, USF2, RAMP1, CRCP and CRLR) was assessed through bisulfite treatment and sequenom mass spectrometry. In none of the genes, we observed a significant effect of estradiol treatment. Tissue-specific methylation was seen in the CRCP, CRLR, ESR and NOS3 genes. The methylation in blood was not correlated to that in the rest of the tissues. Interestingly, the variation in methylation in some genes in some of the tissues was very high (e.g., mean: 41%, 5%-95% percentile: 18 to 67% methylation for the CRCP gene in caudal nucleus), while in other genes or tissues this variation of very small (e.g., mean 4%, 5%-95% percentile: 2 to 6% methylation for the ESR1 gene in blood). These different variations were specific for the combination of gene and tissue.
From our results we conclude that (i) DNA methylation is tissue specific and that methylation of DNA from blood cannot automatically be extrapolated to methylation of DNA from other tissues; (ii) certain genes in some tissues are prone to epigenetic regulation, while in other genes or tissues the methylation is conserved, being less influenced by environmental factors; and (iii) the variation in methylation that is present in some genes is likely to have reduced the power of our study when studying effects of estradiol treatment. Thus, we cannot categorically exclude that the methylation of the genes that we have studied may be influenced by estradiol.

GENE LOCI CODING FOR BRAIN SPECIFIC STEROID SYNTHESIZING ENZYMES OVERLAP WITH MULTIPLE SCLEROSIS SUSCEPTIBILITY LOCI

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Multiple Sclerosis (MS) is a demyelinating inflammatory disease of unknown origin that affects the central nervous system (CNS).
Epidemiological findings, such as hormone-associated effects of pregnancy on MS susceptibility and clinical course imply a causal involvement of steroid hormones in the pathogenesis of MS.[1] Progesterone derivatives promote myelin formation and stimulate the proliferation of oligodendrocyte precursors and differentiation of these cells into myelinating oligodendrocytes.
To approach the relation between genetic variation and the underlying pathophysiology of MS, we compared the chromosomal location of genes coding for steroidogenic enzymes with known susceptibility loci for MS. We obtained the physical position of MS susceptibility markers in the vicinity of steroid-synthesizing enzymes (SSE) from the website of the National Center for Biotechnology Information
(www.ncbi.nlm.nih.gov/mapview).

Chromosome	**MS susceptibility marker**	**Proximate loci of steroid hormone-synthesizing enzymes**
1q43	*RGS7*[2]	*RGS7*
5p15.3	D5S1953[3] D5S635[4] D5S676[5] D5S406[6]	5alpha-reductase 1: *SRD5A1*
6p21.3	D6S273[7] D6S2444[8]	21-Ohdehydrogenase *CYP21A2*
10p15	D10S591[9] D10S189[9]	Isoforms of 3alpha-hydroxysteroid dehydrogenase *AKR1C1-AKR1C4*
19q13	D19S879[10] D19S246[11] D19S585[8]	DHEA-sulfotransferase *SULT2A1, SULT2B1*
22q13.1	D22S417[11]	P450 2D6 oxidase *CYP2D6, CYP2D7*

Table 1: Genomic location of relevant genes related to steroid metabolism and proximate MS susceptibility loci

The most significant genetic association with MS in Northern Europeans known to date lies in the Major Histocompatibility Complex (MHC) located on chromosome 6p21.3. The MHC contains more than 200 genes, many of which are related to the immune system.
The MHC class III region contains the tandemly arranged genes coding for 21-hydroxylase, namely *CYP21A2*, tenascin *TNX*, complement *C4A/B*, the serine/threonine nuclear protein kinase *RP*, and related pseudogenes. These genes are duplicated together as a genetic unit known as the RCCX module. Population-specific copy number variations (CNVs) for this genomic region occur frequently; up to 13% of a population may have three copies of the functional gene *CYP21A2*.[12]
CYP21A2 codes for steroid 21-hydroxylase which converts progesterone to 11-deoxycorticosterone, a substrate required for the formation of cortisol. The presence of two or more of functional *CYP21A2* genes would imply a shift of steroid hormone metabolism away from the

synthesis of promyelinating progestins Allopregnanolone (ALLOPREG) and 5α-Dihydroprogesterone (DHP) towards the glucocorticoid pathway.
CYP2D6 on chromosome 22q13.1 encodes a member of the cytochrome P450 enzyme family. *CYP2D6* catalyzes 21-hydroxylation of progesterone in the human brain. The *CYP2D6* region exhibits CNV and contains two pseudogenes, i.e. *CYP2D7* and *CYP2D8*. A common *CYP2D7* variant exhibits tissue-specific activity in the human brain.[13]
It is worthy of note that both known genetic regions coding for enzymes that hydroxylate steroids at the 21-position exhibit CNV and contain highly homologous pseudogenes of potential functional importance. This may result in tissue-specific variations of the steroid hormones progesterone and cortisol.
Analysis of MS susceptibility markers of American individuals of mixed African-European descent who are affected by MS revealed a region of genomic European ancestry around chromosome 1 centromere that is associated with multiple sclerosis. This genomic region extends from 114.9 to 144.7 Mb in build 35 of the human genome assembly and may determine the difference in risk seen between the African and the European population.[14] Among 68 known genes, this region contains *HSD3B1* and *HSD3B2*, coding for 3 beta-hydroxysteroid dehydrogenase type 1 and 2, which are key enzymes involved in the biosynthesis of all classes of steroid hormones.
Signs of hyperandrogenism including hirsutism occur frequently in female patients affected by MS.[15] One of the enzyme defects causing hirsutism is a deficiency of 3α hydroxysteroid dehydrogenase type III.[16] This enzyme is encoded by the gene *AKR1C2* which is expressed in the skin and also in the human brain, where it catalyzes the step from DHP to ALLOPREG. Its expression is reduced in the CNS of patients with MS as compared to healthy persons.[17]
A putative susceptibility marker for MS is located on chromosome 1q43, corresponding to the gene *RGS7*, coding for one of the Regulator of G-protein signalling (RGS) proteins.[2] In the HERITAGE studies, this gene locus has also been identified as a population-specific marker that influences progesterone production in White, but not Black Americans.[18]
Quite different genetic constellations, modified by environmental factors, may result in a brain-specific deficiency of promyelinating progesterone derivatives. The relevant genes coding for SSE may, in various combinations, lead to a similar condition of lower-than-normal progestin levels in the CNS. A deficit of ALLOPREG and relevant enzymes has been demonstrated in the CNS of MS victims.[17]
The comparison of gene loci coding for SSE and known susceptibility markers suggests that genes coding for steroid hormone synthesizing enzymes might be useful in further candidate gene approaches to complete our current understanding of MS genetics.

Reference list

1. Leitner H. Med Hypotheses 2010; 75: 229–34
2. McCauley JL, Zuvich RL, Bradford Y, et al.. Genes Immun 2009; 10(7):624-30.
3. Goertsches R, Villoslada P, Comabella M, et al. J Neuroimmunol 2003; 143:124-28.
4. Dyment DA, Willer CJ, Scott B, et al. Neurogenetics 2001; 3: 145–51.
5. Eraksoy M, Hensiek A, Kurtuncu M, et al. J Neuroimmunol 2003;143:129–32.
6. Ebers GC, Kukay K, Bulman DE, Sadovnick AD, et al. Nat Genet 1996; 13: 472-76.
7. Harbo HF, Datta P, Oturai A, et al. J Neuroimmunol 2003; 143:101-106.
8. Yeo TW, Roxburgh R, Maranian M, Singlehurst S, Gray J, Hensiek A, Setakis E, Compston A, Sawcer S. J Neuroimmunol 2003; 143: 53–59.
9. Akesson E, Coraddu F, Marrosu MG, et al. J Neuroimmunol 2003; 143:31–38.
10. Haines JL, Ter-Minassian M, Bazyk A, et al. Nat Genet 1996; 13: 469–71.
11. Jonasdottir A, Thorlacius T, Fossdal R, et al. J Neuroimmunol 2003;143: 88–92.
12. Saxena K, Kitzmiller KJ, Wu YL, et al. Mol Immunol 2009; 46: 1289 303
13. Pai HV, Kommaddi RP, Chinta SJ, Mori T, Boyd MR, Ravindranath V. J Biol Chem 2004; 279: 27383–89.
14. Reich D, Patterson N, De Jager PL, et al. Nat Genet 2005; 37: 1113–18.
15. Falaschi P, Martocchia A, Proietti A, D'Urso R, Antonini G. Neuro Endocrinol Lett 2001; 22: 248–50.
16. Steiner AZ, Chang L, Ji Q, et al. J Clin Endocrinol Metab 2008; 93:1298–303.
17. Noorbakhsh F, Ellestad KK, Maingat F, et al. Brain 2011; 134: 2703–21
18. Ukkola O, Rankinen T, Gagnon J, Leon AS, et al. J Clin Endocrinol Metab 2002; 87:3708–20.

PRO-APOPTOTIC AND TOXIC EFFECTS OF 4-PARA NONYLPHENOL IN MOUSE EMBRYONIC NEURONAL CELLS: A ROLE OF ESTROGEN AND RETINOID RECEPTORS

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Endocrine disrupting chemicals (EDCs) are present in the environment and cause deleterious effects on human health. These substances are known to mimic, antagonize or modify the endogenous hormonal activity [2]. An exposure to EDCs during pregnancy or perinataly may cause strong and persistent developmental impairments. There is a growing body of evidence that children exposed to EDCs in those critical periods may later display neuropsychiatric disorders such as learning disabilities, attention deficit and hyperactivity disorder (ADHD) or even autism [1]. Little is known, however, about mechanisms of action of EDCs on developing brain. EDC representative 4-para-nonylphenol (NP) is alkylophenol present in plastic wrappings and bottles and may get an easy access to living organisms. In this study we investigated pro-apoptotic and toxic effects of NP on mouse embryonic neuronal cells in primary cultures in vitro. We demonstrated that NP induced loss of mitochondrial membrane potential and activated caspase-3 in hipocampal neurons, which was followed by substantial LDH release at 24 h of exposure. Estrogen receptor (ER) antagonists, MPP and PHTPP, intensified the NP-induced loss of mitochondrial membrane potential and LDH release, whereas ER agonists, PPT and DPN, partially reversed these effects. Retinoid X receptor (RXR) antagonist HX531 normalized the level of mitochondrial membrane potential and diminished NP-induced LDH release. Our study demonstrated that pro-apoptotic and neurotoxic actions of NP are mediated by an impairment of ER alpha and ER beta signaling, which is accompanied by an activation of RXR. These data may have implication for development of new protective strategies against neurotoxicity attributed to EDCs.

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Reference list

1. de Cock M, Maas YG, van de Bor M. (2012) *Acta Paediatr.*;101(8):811-8. Review.
2. Frye CA, Bo E, Calamandrei G, Calzà L, Dessì-Fulgheri F, Fernández M, Fusani L, Kah O, Kajta M, Le Page Y, Patisaul HB, Venerosi A, Wojtowicz AK, Panzica GC. (2012) *J Neuroendocrinol.*;24(1):144-59.

DEVELOPMENTAL CHANGES AGAINST THE HPA-AXIS FOLLOWING PRENATAL EXPOSURE TO STRESS

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Perinatal life is a period of high plasticity and vulnerability to stress [1]. Several studies, in various species, have reported that stressful events occurring during the perinatal period may impinge on various aspects of the neuroendocrine programming, subsequently amending the offspring's growth, metabolism, sexual maturation, stress responses, and immune system. Doubtless, the common feature of any gestational stress is the modification of the HPA axis reactivity of the offspring. Depending on the timing at which gestational stress occurs, PS-evoked maternal hormones, such as glucocorticoids, might diffuse through the feto-placental barrier and thus impair the fetal development.
To understand the mechanisms that underlie the effects of prenatal stress exposure on neuroendocrine alterations during development, we exposed pregnant Sprague-Dawley rats during the last week of gestation to repetitive immobilization three times a day for 45 minutes [2] and we investigated molecular alterations at different postnatal ages.
First, we found a significant reduction of the placental weight in PS animals, which may be associated with intrauterine growth retardation, which will eventually contribute to postnatal morbidity. Moreover, within the maternal part of the placenta, we found a significant reduction of 11β-Hydroxysteroid Dehydrogenase-2 mRNA expression, the enzyme that converts the maternal glucocorticoids into their inactive metabolites, thus protecting the fetus from high exposure to glucocorticoids. We next focused on offspring's alterations at different ages: immediately after birth (PND1), during infant life (PND7), at weaning (PND21), during adolescence (PND40) and in the adult life (PND62), in order to create a time-profile of the changes under investigation. We found that, at PND62, the expression of glucocorticoid receptors was significantly reduced in the hippocampus and prefrontal cortex of rats that were exposed to prenatal stress. Interestingly, particularly within the hippocampus, the effect become manifest around periadolescence, suggesting that such alteration may also represent the long-term consequence of developmental disturbances associated with PS exposure.
Our results provide further support to the notion that in-utero exposure to stress produces a long-lasting mark in the progeny. These events lead to a deflection in the normal trajectory of fetal development although the consequences of such experiences may remain silent until adolescence or early adulthood. This suggests that early intervention may hold the promise for preventing the manifestation of pathologic phenotypes associate with the exposure to early life adversities.

Reference list

[1] Cirulli, F., Francia, N., Berry, A., Aloe, L., Alleva, E., Suomi, S.J., 2009 Early life stress as a risk factor for mental health: role of neurotrophins from rodents to non-human primates. Neurosci Biobehav Rev 33, 573-585.
[2] Maccari, S., Morley-Fletcher, S., 2007 Effects of prenatal restraint stress on the hypothalamus-pituitary-adrenal axis and related behavioural and neurobiological alterations. Psychoneuroendocrinology 32 Suppl 1:S10-15.

INFLAMMATORY RESPONSE IN THE BRAIN OF RATS EXPOSED TO CHRONIC MILD STRESS

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Major depressive disorder (MDD) is a common disorder that represents a leading cause of disability in the world. It is thought to originate from the interaction between susceptibility genes and environmental events, such as stress, to which an individual can be exposed in different moments of life [1]. One of the major problems of depression is the relevant percentage of patients who do not show an adequate response to antidepressant therapy, as well as the high rate of relapse. Growing evidence suggests that the activation of the inflammatory/immune system contributes to the pathogenesis of depression. In particular, depression shows elevated comorbidity with cancer, arthritis rheumatoid, cardiovascular and neurodegenerative diseases, characterized by inflammatory alterations [2]. In addition, elevated blood levels of the pro-inflammatory cytokines including interleukin (IL)-1, interleukin (IL)-6 and tumor necrosis factor (TNF)-α are commonly found in depressed patients [3]. A role for inflammation in depression is also supported by the findings that cytokine administration induces depressive symptoms, as occurs in the 30% of hepatitis C patients who are treated with the immune activator interferon-alpha.
On these bases, it is important to characterize the changes of immune/inflammatory response in animal models of depression in order to establish their relationship with the depressive phenotype as well as the involvement in antidepressant response.
In order to do this we investigated the inflammatory response of rats exposed to a chronic mild stress (CMS) paradigm, which represents a well-establishes animal model of depression, for 8 weeks. Moreover, a group of animals (sham or CMS) were chronically treated with the antidepressant imipramine (10 mg/kg/day starting from week 2), in order to evaluate the ability of the antidepressant treatment to interfere with inflammatory alterations.
As expected, chronic mild stress caused a gradual decrease in the consumption of 1% sucrose solution over the 8-week period. When investigating the molecular changes of inflammatory markers, we found that the gene expression of several cytokines is modulated by stress. Indeed, IL-1, IL-6, and TNF-α were significantly increased in the hippocampus of stressed animals. Also the mRNA levels for CD11b, a marker of microglia activation, were increased after CMS. Moreover, we found that chronic imipramine treatment was able to normalize the depressive phenotype caused by CMS paradigm, although it did not alter the changes of the inflammatory response.
In summary, these data provide support for a link between inflammation and depression, suggesting that a depressive state may be associated with significant alterations of the inflammatory response in selected brain regions. Interestingly, the failure of antidepressant treatment to normalize the inflammatory changes, while correcting the 'anhedonic' phenotype, suggests that classical pharmacological intervention may not work on the full spectrum of changes that characterize the depressive phenotype. This concept, when translated to humans, may be relevant for the presence of residual symptoms that are associated with enhanced the risk of relapse.

Reference list

1. Caspi, A., Sugden, K., Moffitt, TE., Taylor, A., Craig, IW., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A., Poulton, R., 2003. Influence of life stress on depression:moderation by a polymorphin in the 5-HTT gene. Science. 301(5631):386-9.
2. Anisman, H., Merali, Z., Hayley, S. 2008. Neurotransmitter, peptide and cytokine processes in relation to depressive disorder: comorbidity between depression and neurodegenerative disorders. Prog Neurobiol. 85(1):1-74. Epub 2008 Feb 13. Review.
3. Connor, TJ., Leonard, BE. 1998. Depression, stress and immunological activatiom: the role of cytokines in depressive disorders. Life Sci. 1998;62(7):583-606.

ESTRADIOL AFFECTS THE EXPRESSION AND DISTRIBUTION OF THE EXOCYTOTIC PROTEINS IN NEONATAL RAT DEEP CEREBELLAR NUCLEI

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Estradiol (E2) is a potent steroid that affects several neurobiological functions. E2 has both gonadal and neuronal origin. Evidence for steroidogenesis in the deep cerebellar nuclei (DCN), a structure that controls the temporal and spatial features of the cerebellar output [5], have been recently provided [3]. E2 may affect brain development by means of the secretion of signalling molecules such as prostaglandins, GABA, glutamate, granulin and focal adhesion kinase, among others [2]. To date, no studies have been carried on the possible role of E2 in exocytotic process in non-classical neuroendocrine cells.
In this work we studied the effect of E2 on the exocytotic proteins SNAP-25 [1], VAMP1 and VAMP2 [4] in presynaptic terminals of the neonatal rat DCN. Double immunofluorescence procedures, quantitative analysis of colocalization, spectral confocal microscopy and electron microscopy analysis showed that E2 affects the exocytotic process modulating the expression and distribution of its regulating-proteins. Alterations at post-synaptic level were also studied in relationship with the modifications of the exocytotic patterns.
Taken together, our results provide new data for a better understanding of the underlying mechanisms by which E2 affects neonatal development of the cerebellum.

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Reference list

1. Blasi J, Chapman ER, Link E, Binz T, Yamasaki S, De Camilli P, Südhof TC, Niemann H, Jahn R. Botulinum neurotoxin A selectively cleaves the synaptic protein SNAP-25. Nature. 1993; 365(6442):160-3;
2. McCarthy MM., Estradiol and the developing brain. Physiol Rev. 2008; 88(1):91-124;
3. Manca P, Caria MA, Blasi J, Martín-Satué M, Mameli O. Cytochrome P450 17α-hydroxylase/C(17,20)-lyase immunoreactivity and molecular expression in the cerebellar nuclei of adult male rats. J Chem Neuroanat. 2012; 45(1-2):18-25;
4. Raptis A, Torrejón-Escribano B, Gómez de Aranda I, Blasi J. Distribution of synaptobrevin/VAMP 1 and 2 in rat brain. J Chem Neuroanat. 2005; 30(4):201-11;
5. Uusisaari M, De Schutter E. The mysterious microcircuitry of the cerebellar nuclei. J Physiol. 2011; 589(Pt 14):3441-57.

LXR-MEDIATED LIPOGENESIS PROTECTS PERIPHERAL NERVES FROM DIABETES-INDUCED MYELIN ALTERATIONS

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Diabetic peripheral neuropathy (DPN) represents a considerable medical problem and the molecular mechanisms of this disease are still obscure. DPN is associated with deleterious changes in peripheral nerves, such as myelin damage and decrease in nerve conduction velocity [2, 4, 5, 6, 7, 8]. Myelin is a biological membrane characterized by high lipid content providing the electrically insulating property required for the saltatory propagation of the nervous influx [3]. We previously showed that Liver X Receptors (LXRs) activation, by promoting cholesterol utilization, increased neuroactive steroid levels in peripheral nerves and exerted neuroprotective effects on DPN [1]. Besides regulating cholesterol homeostasis, LXR directly activates the expression of the lipogenic transcription factor Sterol Regulatory Element Binding Protein-1c (SREBP-1c), a gene involved in fatty acid synthesis. Recently, it has been highlighted the role of SREBP-1c in the regulation of lipid metabolism during peripheral nerve myelination.
In streptozotocin (STZ)-treated rats, an experimental model of DPN, we performed lipidomic analyses of purified myelin from peripheral nerves. We observed that diabetes alter the myelin lipid composition leading to morphological changes of the sheath. LXR activation counteracts alterations caused by diabetes by improving myelin lipid content and restoring the expression levels of all major enzymes involved in fatty acid synthesis. We also found that diabetes reduced the performance of STZ-treated rats in functional tests; parameters restored at the level of non-diabetic animals by LXR activation.
These results suggest that LXR activation protects peripheral nerves from neuropathy by modulating cholesterol and lipid metabolism in peripheral nerves of diabetic rats.

Refence list

1. Cermenati, G., S. Giatti, G. Cavaletti, et al. 2010. J Neurosci 30: 11896-11901.
2. Eckersley, L. 2002. Int Rev Neurobiol 50: 293-321.
3. Garbay, B., A. M. Heape, F. Sargueil, and C. Cassagne. 2000. Prog Neurobiol 61: 267-304.
4. Malik, R. A., S. Tesfaye, P. G. Newrick, et al. 2005. Diabetologia 48: 578-585.
5. Sima, A. A., and T. Brismar. 1985. Ann Neurol 18: 21-29.
6. Sugimoto, K., Y. Murakawa, and A. A. Sima. 2000. Diabetes Metab Res Rev 16: 408-433.
7. Valls-canals, J., M. Povedano, J. Montero, AND J. Pradas. 2002. Electromyogr Clin Neurophysiol 42: 3-6.
8. Vinik, A. I., T. S. Park, K. B. Stansberry, and G. L. Pittenger. 2000. Diabetologia 43: 957-973.

SEX DIFFERENCES IN AXON DIAMETER AND G-RATIO IN THE SPLENIUM OF THE RAT CORPUS CALLOSUM

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Previous work in our laboratory [2] has found that, in humans, white-matter volume increases more steeply during male adolescence than during female adolescence. At the same time, magnetization-to-transfer ratio (MTR), an indirect measure of myelination, decreases in males but not females. A possible hypothesis to explain these findings is an increase in axon diameter relative to its myelin sheath and, as such, a change in g-ratio (axon diameter/fiber diameter) in male adolescents. Both changes might be related to increases in testosterone (T) levels, especially in males with an "efficient" version of the androgen receptor gene [2,3]. Together, these observations point to the possible role of T in the regulation of axon caliber and, by extension, g-ratio. A subtle shift in the g-ratio could change the proportion of WM tissue occupied by axons and myelin, influencing, in turn, the properties of WM.

In order to determine whether sex differences exist in the diameter and g-ratio of myelinated axons in young rats (post-natal day [PND] 70), we evaluated the structural properties of axons in the splenium of the corpus callosum (CC) using electron microscopy (EM). We examined male and female rats under natural conditions (not castrated) as well as pre-pubertal male rats (PND 21) after real or sham castration.

Using EM, we carried out two experiments in Wistar rats. In Experiment 1, we examined the effect of sex on axon diameter and g-ratio in the CC splenium (6 males and 6 females; PND 70). In Experiment 2, we studied the effect of pre-pubertal orchidectomy, and the associated removal of endogenous testosterone, on axon diameter in the CC splenium (5 gonadectomized [gx] males, 5 intact males, 5 intact females; all PND 70). To remove the rats brains, animals were weighed, deeply anasthetized, and perfused. The brains were cut in the mid-sagittal plane and a slice (200 μm thick) of the medial section of the right hemisphere was taken, exposing the CC to plain view. For each slice, a digital image was taken to measure the length of the CC. The splenium (posterior 1/5 of the total length) was removed and embedded in resin. Silver/gold, ultrathin sections (70 nm) of the splenium were cut and stained with uranil acetate and lead citrate, and digital images were collected. For each rat, eight micrographs were taken at 10,000x magnification along the entire dorsal-to-ventral extent of the splenium. In both experiments, we measured the length of the CC and axon diameter using the software, ImageJ. Axon diameter was calculated using automatic analyses of the cross-sectional area of about 1,000 myelinated axons in each rat. In Experiment 1, we also calculated the g-ratio. This was done manually for approximately 400 myelinated axons in each rat. The g ratio was calculated as the ratio between d and D, where d is the axon diameter (excluding the myelin sheath) and D is the fiber diameter (axon diameter + myelin thickness); D was calculated by measuring the minimum and maximum diameters of each axon. Median values of axon diameter and g-ratio obtained for each rat were used in all group comparisons reported below.

As expected, body weight was significantly higher in males than females for both experiments; orchiectomized males had significantly less weight gain than intact males. The total anterior to posterior length of the CC was greater in females than in intact males

($p<0.001$). Orchidectomized males had a longer CC than intact males ($p< 0.01$), but showed no difference compared with intact females. In Experiment 1, mean axon diameter of myelinated axons was greater in males compared with females (Mean±SEM: males, 529.2 ±12.9 μm; females, 456.2±7.9 μm; $p<0.001$). We also found a predicted sex difference in g-ratio; males had a higher g-ratio than females (Mean±SEM: males, 0.767±0.004; females, 0.741±0.007; $p<0.05$). In Experiment 2, we confirmed these sex differences in axon diameter in intact rats (Mean±SEM: males, 536.2 ±7.5; females, 449.0±6.3; $p<0.001$). Removal of endogenous testosterone by orchiectomy was associated with a smaller axon diameter, as compared with intact males (Mean±SEM: males gx, 490.0±5.3; males, 536.2 ±7.5; $p<0.001$). Nonetheless, the mean axon diameter of orchidectomized males remained larger than that of females ($p<0.01$).

The present study revealed sex differences in both axon diameter and g-ratio in the splenium of the rat corpus callosum during early adulthood. This is the first demonstration of a sex difference in g-ratio; it supports our original hypothesis that, in male adolescents, the observed age-related increases in WM volume, occurring in the context of decreases in MTR [2], could be due to an increase in axon diameter relative to its myelin sheath (i.e., an increase in g-ratio). Interestingly, our finding that males have shorter corpus callosums compared with females confirms that the larger axons found in males were not due to an overall larger corpus callosum. The same pattern was observed for gonadectomized males. These results suggest that T can be, in part, responsible for determining the size of axon caliber. In fact, the removal of endogenous testosterone by orchidectomy resulted in a significant decrease in axon diameter, although the degree of this reduction did not reach female values. The possible involvement of estrogen in modulating axon caliber is unlikely given previous work by Yate and Jurasca (2008) [4] who showed that pre-pubertal ovariectomy of females rats does not affect the diameter of either myelinated or unmyelinated axons in the splenium. Our findings and those of Yate and Jurasca lead us to speculate that there may be additional (non-hormonal factors) playing a role in determining sex differences in axon caliber.

Further work will be necessary to understand the complex pattern of effects through which T modulates axonal size, likely via the cytoskeleton, whether through direct action of T on neurons or by way of interactions with glia.

Reference list

1. Paus T., Nawaz-Khan I., Leonard G., Perron M., Pike GB., Pitiot A., Richer L., Susman E., Veillette S., Pausova Z. Sexual dimorphism in the adolescent brain: Role of testosterone and androgen receptor in global and local volumes of grey and white matter. Hormones and Behavior 2010; 57: 63-75.
2. Perrin J. S., Leonard G., Perron M., Pike G. B., Pitiot A., Richer L., Veillette S., Pausova Z., and Paus T. Sex differences in the growth of white matter during adolescence. Neuroimage 2009; 45:1055–1066.
3. Perrin J.S., Hervé PY., Leonard G., Perron M., Pike G.B., Pitiot A., Richer L., Veillette S., Pausova Z., Paus T. Growth of white matter in the adolescent brain: role of testosterone and androgen receptor. Journal of Neuroscience. 2008; 28: 9519-9524.
4. Yates M.A., Juraska J.M. Pubertal ovarian hormone exposure reduces the number of myelinated axons in the splenium of the rat corpus callosum. Exp Neurol. 2008; 1: 284-7.

EFFECTS OF 17α-ESTRADIOL TREATMENT ON HIPPOCAMPAL ABNORMALITIES OF SPONTANEUSLY HYPERTENSIVE RAT (SHR)

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Hippocampal neuropathology is a recognized feature of the spontaneously hypertensive rat (SHR). In previous studies we have found that SHR present abnormalities in the hippocampus consisting of decreased cell proliferation in the dentate gyrus (DG), astroglial reactivity and decreased neuronal density in the hilus of the DG. These abnormalities are reversed by 17β-estradiol, an active neuroprotective agent. 17α-estradiol, an optical isomer of 17β-estradiol, has lower affinity for ER classical receptors and therefore much less feminizing and carcinogenic actions. However, neuroprotective actions of this molecule have been reported. These actions could be mediated by membrane receptors such as GABAa, GPR-30 or ERX.

In this work, we attempted to investigate possible neuroprotective actions of 17α-estradiol on the hippocampal neuropathology of SHR. We treated 16 week old male SHR (mean arterial pressure: 180 Hgmm) and normotensive WKY rats with 17α-estradiol or cholesterol pellets during two weeks. Animals were sacrificed and brains removed. We studied cellular proliferation in the dentate gyrus by the expression of Ki67 and astroglial reactivity by GFAP expression in stratum radiatum of the hippocampus. 17α-estradiol treatment was able to increase cellular proliferation in the DG of SHR rats ($p<0.05$ SHR vs WKY y SHRαE_2). SHR presented increased astrogliosis in all hippocampal areas. Treatment with α-estradiol was able to partially reverse this reactivity in CA1 ($p<0.01$) and DG (non significative tendency).

These results show that α-estradiol was capable of increasing cellular proliferation and decrease astrocitosis present during hypertension.

The use of this, and other non-feminizing compounds like SERMs, could be of great interest to the safe management of the cerebral complications of hypertension without the undesirable side effects of 17β-estradiol

ROLE OF TESTOSTERONE ON NEUROGENESIS IN THE VENTRICULAR-SUBVENTRICULAR ZONE OF ADULT RATS

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Neurogenesis continues along the walls of the lateral ventricle in the adult rodent brain. The primary progenitors, or stem cells, correspond to a subpopulation of local astrocytes (B1 cells) [1], [2], 3], which retain an apical contact with the ventricle. Therefore, this adult germinal layer includes not only a subventricular component, but also a ventricular zone; i.e. ventricular-subventricular zone (V-SVZ) [4]. B1 cells give rise to intermediate progenitors, or transit amplifying cells (C cells), which divide to generate neuroblasts (A cells) that continue to proliferate [5], [6] and migrate tangentially along the rostral migratory stream (RMS) to the olfactory bulb, a process of continuous neuronal replacement that occurs throughout life [7], [8], [9].
Endocrine system is tightly correlated with central nervous system (CNS). Beside signaling, hormones have a role in shaping the CNS both during critical ages in development or in adult neurogenesis [10-12]. Several studies demonstrated that estrogen receptor α (ERα) is critical for reproductive function, while ERβ may be involved in regulating non–reproductive behaviors and brain development [13]. Previous results indicated that adult neurogenesis is partly regulated by gonadal hormones [14]. Here we analyzed the effect of sexual hormones on neurogenesis in the adult rat ventricular-subventricular zone.
Adult male Wistar rats (21 days old) were either bilaterally castrated or sham operated. Two weeks after surgery animals received one intraperitoneal injection of either testosterone propionate (CX+T group n=5; 1.00 mg/0,1 mL sesame oil; Sigma-Aldrich) or the vehicle alone (CN and CX, n=5 each). Two days after treatment animals were sacrificed. A decreased cell proliferation rate was observed in the CX group in comparison to the other two groups This effect is mimicked by estradiol and not by dyhydrotestosterone suggesting that it is likely to be mediated by ERα (Farinetti et al., in preparation). I studied the distribution and expression of ERs mainly focusing on the V-SVZ in intact, castrated and castrate plus T male rats. The number of ERα+ cells was high in the hypothalamus (arcuate nucleus and periventricular nucleus) and did not change among experimental groups. Interestingly rare cells were ERα+ in the V-SVZ, while there were many in the septal region (bed nucleus stria terminalis and lateral ventral septal nucleus) where the number of ERα+ cells was higher in castrated mice then in either sham operated or castrated plus T treated rats.
The analysis of double labeling with NeuN, a marker of postmitotic neurons suggested that the majority of ERα+ cells were neurons. This percentage was not significantly different among groups indicating that the higher expression of ERα was not confined to a single cell population. Moreover we quantified the expression of ERα genes in different brain regions. Interestingly the occurrence of mRNA was much higher then the actual protein.
In order to confirm our immunohistochemical data we dissected hypothalamus, V-SVZ (medial, lateral and dorsal corner), hippocampus and striatum and analyzed the expression of mRNA for ERα in male and female adult rats. Since we observed a sexual dimorphism on the expression of β -actin, we used as internal control the expression of the housekeeping gene cyclophilin. The mRNA was detected in all the regions, but the expression was lower in the striatum. Interestingly the expression in the V-SVZ and in the

hippocampus was comparable to the expression in the hypothalamus.

Reference list

1. Doetsch, F., et al., *Subventricular zone astrocytes are neural stem cells in the adult mammalian brain.* Cell, 1999. 97(6): p. 703-16.
2. Lim, D.A., et al., *Noggin antagonizes BMP signaling to create a niche for adult neurogenesis.* Neuron, 2000. 28(3): p. 713-26.
3. Mirzadeh, Z., et al., *Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain.* Cell Stem Cell, 2008. 3(3): p. 265-78.
4. Fuentealba, L.C., K. Obernier, and A. Alvarez-Buylla, *Adult neural stem cells bridge their niche.* Cell Stem Cell. 10(6): p. 698-708.
5. Luskin, M.B., et al., *Neuronal progenitor cells derived from the anterior subventricular zone of the neonatal rat forebrain continue to proliferate in vitro and express a neuronal phenotype.* Mol Cell Neurosci, 1997. 8(5): p. 351-66.
6. Lois, C., J.M. García-Verdugo, and A. Alvarez-Buylla, *Chain migration of neuronal precursors.* Science, 1996. 271(5251): p. 978-81.
7. Petreanu, L. and A. Alvarez-Buylla, *Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction.* J Neurosci, 2002. 22(14): p. 6106-13.
8. Imayoshi, I., et al., *Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain.* Nat Neurosci, 2008. 11(10): p. 1153-61.
9. Lazarini, F. and P.M. Lledo, *Is adult neurogenesis essential for olfaction?* Trends Neurosci, 2011. 34(1): p. 20-30.
10. Azcoitia, I., et al., *Role of astroglia in the neuroplastic and neuroprotective actions of estradiol.* Eur J Neurosci, 2010. 32(12): p. 1995-2002.
11. Oboti, L., et al., *Integration and sensory experience-dependent survival of newly-generated neurons in the accessory olfactory bulb of female mice.* Eur J Neurosci, 2009. 29(4): p. 679-92.
12. Brock, O., et al., *Short term treatment with estradiol decreases the rate of newly generated cells in the subventricular zone and main olfactory bulb of adult female mice.* Neuroscience, 2010. 166(2): p. 368-76.
13. Lund, T.D., et al., *Novel actions of estrogen receptor-beta on anxiety-related behaviors.* Endocrinology, 2005. 146(2): p. 797-807.
14. Brock, O., et al., *Short term treatment with estradiol decreases the rate of newly generated cells in the subventricular zone and main olfactory bulb of adult female mice.* Neuroscience. 166(2): p. 368-76.

EMERGENCE FROM HIBERNATION IN BLACK BEARS IS ASSOCIATED WITH DIFFERENCES IN PLASMA CHOLESTEROL, STRESS, SEX AND NEURO STEROIDS AND BRAIN-DERIVED NEUROTROPHIC FACTOR

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Background: To deal with stressors in their environment, animals have adapted neuroendocrine, behavioral and physiological responses. Hibernation is a suite of adaptations, which is characterized in mammals by a state of profoundly reduced requirements for energy and water in times when resources are limited, such as in the Arctic during winter [1]. During hibernation, black bears (*Ursus americanus)* suppress metabolic rate to 25% of basal rates during summer while core body temperature decreases to no lower than 30°C [2]. Black bears enter a continuous state of hibernation for 5-7 months in contrast to smaller hibernators where torpor is interrupted by periodic arousals [2]. Few studies have investigated endocrine status in hibernating bears, although changes in serum sex steroids have been described as being independent of Hibernation [3]. Serum stress steroids, however, have been described as being elevated during Hibernation [4]. In this preliminary study, we examined hormonal factors in captive black bears sampled during hibernation in winter (March, n = 7), at spring emergence (end-March to end-April, n = 7), and during summer in the active season (May-July, n = 13). Bears were housed in individual outdoor enclosures with dens and materials for nesting and sampled for blood after immobilization with Telazol.

In non-hibernators, such as rats, stressors such as footshock and social challenges, including mating, are associated with elevated neurosteroid levels (e.g. allopregnanolone) [5-11]. This increase may be adaptive, because administering allopregnanlone to female rats reduces their stress responses to acute physical challenge (e.g. restraint) [7]. Administration of estradiol, progesterone, and allopregnanolone can also have beneficial effects in whole animal models of aging and neuronal compromise (i.e., seizure, cortical contusion, ischemia, and diabetic neuropathy models; reviewed in 7 and others). It is of interest what the hormonal profile of hibernating mammals is during different states (hibernation, emergence/transition from hibernation, and summer active).

Neurosteroids may exert their protective effects through enhancing neuroplasticity. Neuroplasticity is affected by differences in hyper-phosphorylated tau protein levels (one hallmark of Alzheimer's Disease) as well as synaptic changes, both of which are features of hibernation and may be associated with protection from insults, such as stroke. One marker of neuroplasticity, Brain-derived Neurotrophic Factor (BDNF), as well as steroids and glucose were determined in black bears in different states (hibernating, emergence from hibernation, and summer active).

Hypotheses: If emergence from hibernation is associated with changes in endocrine factors such as metabolism, stress, sex and neurosteroids, as well as plasticity, we expect to see differences in these parameters associated with these states.

Methods: Plasma samples were collected from captive male and female black bears active in the summer, during hibernation, and shortly after emergence from hibernation in spring by the Bear Hibernation Project at University of Alaska Fairbanks. Bears were immobilized by Telazol injection 15-30 minutes prior to blood sampling. Commercially available enzyme-linked immunosorbent assay kits (ELISA) were used for cortisol,

estradiol, allopregnanolone, and BDNF and colormetric readers for glucose and cholesterol analyses. Radioimmunoassay was used to measure serum levels of progesterone and 5α-Androstane-3α, 17β-diol (3α-diol).

Results: There was mixed evidence for differences in metabolic factors between male and female black bears particularly during emergence. There was an interaction between sex and state for these effects with levels of cholesterol in plasma being higher in females during emergence and lower in males, compared to during summer and hibernating states. Glucose levels were higher in feeding bears during summer in comparison with hibernating or newly emerged male and female bears.

There were sex differences in cortisol levels in black bears, except during emergence. Females had higher levels of circulating cortisol than did males except during emergence when their levels were low and male-typical. There was a significant interaction for sex and state with estradiol levels being lower among hibernating and emerging females, and higher among hibernating and emerging males, compared to summer levels. There was a tendency for state influencing progesterone, allopregnanolone, and 3α-diol levels, and an interaction between these variables and sex. Females, but not males, had lower levels of progesterone during hibernation compared to emergence and summer levels as well as lower levels of allopregnanolone during hibernation and emergence compared to summer levels. Males had higher levels of 3α-diol than females during emergence compared to during hibernation and summer.

There was an apparent interaction between sex and state for BDNF levels in plasma of black bears. Among females, BDNF levels were low during emergence from hibernation compared to hibernation. Among males, BDNF levels were highest during emergence compared to summer or hibernating state.

Conclusions: These pilot data suggest that the emergence from hibernation may be a particularly dynamic time related to endocrine regulation and neural plasticity.

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Reference list

[1] Barnes, B.M., 1989. Freeze avoidance in a mammal: body temperatures below 0 degree C in an Arctic hibernator. Science. 244, 1593-5.

[2] Tøien, Ø., Blake, J., Edgar, D.M., Grahn, D.A., Heller, H.C., Barnes, B.M., 2011. Hibernation in black bears: independence of metabolic suppression from body temperature. Science. 331, 906-9.

[3] Palmer. S.S., Nelson, R.A., Ramsay, M.A., Stirling, I., Bahr, J.M., 1988. Annual changes in serum sex steroids in male and female black (Ursus americanus) and polar (Ursus maritimus) bears. Biol Reprod. 38, 1044-50.

[4] Donahue, S. W., Vaughan, M. R., Demers, L. M., Donahue, H. J., 2003. Serum markers of bone metabolism show bone loss in hibernating bears. Clin. Orthop. Relat. 408, 295-301.

[5] Barbaccia, M.L., Roscetti, G., Trabucchi, M., Purdy, R.H., Mostallino, M.C., Concas, A., Biggio, G., 1997. The effects of inhibitors of GABAergic transmission and stress on brain and plasma allopregnanolone concentrations. Br J Pharmacol. 120, 1582-8.

[6] Frye, C.A., 2007. Progestins influence motivation, reward, conditioning, stress, and/or response to drugs of abuse. Pharmacol Biochem Behav. 86, 209-19.

[7] Frye, C.A., 2009. Neurosteroids-From Basic Research to Clinical Perspectives. In: Hormones/ Behavior Relations of Clinical Importance. Eds: RT Rubin, DW Pfaff. 395-416. San Diego: AP.

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

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

[10] Patchev, V.K., Almeida, O.F., 1996. Gonadal steroids exert facilitating and "buffering" effects on glucocorticoid-mediated transcriptional regulation of corticotropin-releasing hormone and corticosteroid receptor genes in rat brain. J Neurosci. 16, 7077-84.

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

NEUROSTEROIDS MODULATION OF CORTICAL NETWORK EXCITABILITY

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Neurosteroids (NSs) such as allopregnanolone (ALLO) and tetrahydrodeoxy-corticosterone (THDOC) are well known modulators of $GABA_A$ receptor function. While a large amount of data are available at the single cell level, their "global modulation" of network firing activity was not investigated in detail. NSs levels vary in the diverse brain regions and may change under different physiological and pathological conditions [1-3]. The NSs synthesizing enzymes, 5α-reductase type I and 3α-hydroxysteroid dehydrogenase have also different expression profiles, i.e. in the neocortex they co-localize in glutamatergic but not in GABAergic neurons [4]. Therefore these endogenous substances in excitatory neurons that co-express $GABA_A$ receptors and NSs synthetic enzymes can act in an autocrine manner [5] while in inhibitory cells their modulation is different.
The goal of our study was to investigate the effects of physiological NSs concentrations on the activity of networks formed by acutely dissociated mouse neocortical neurons. By using the multi-electrode arrays (MEA) technique the spontaneous reverberating activity of excitatory and inhibitory neurons was simultaneously recorded and analyzed in control and after perfusion with NSs. Their effects were analyzed, by using statistical methods [6], on physiological variables such as excitability (spikes-per neuron) and number of neurons engaged in bursts.
THDOC, at physiological concentrations, selectively decreased inhibitory interneuron activity, whereas at concentrations higher than 100 nM it inhibited both excitatory and inhibitory clusters. The analysis of the network activity after long time wash-out (8 hrs) highlight that the NS effect on inhibitory clusters persisted for hours producing a sort of long-term depression (LTD) in their excitability.
To further clarify this point we applied THDOC twice (after a 2h wash-out). The first application induced, as expected, a persistent depression of inhibitory neurons but after the second administration of THDOC no further LTD was observed. This experiment suggests that the first application of the NS produces some stable modifications, a sort of "memory" in the network that could be relevant also in vivo.
THDOC and ALLO, at low concentrations, are allosteric modulators of GABAergic neurotransmission but in the μM concentration range they also act as $GABA_A$ receptor agonists [7]. In agreement with these properties, our analysis of the global excitability of the network showed a sharp increase in NSs inhibitory effects at concentrations between 100 and 1000 nM, probably due to a direct agonistic activity at $GABA_A$ receptors.
Many single-cell studies of the effects of ALLO and THDOC have been performed, but no differences were reported between their effects. We show here that THDOC and ALLO, although consistently producing network inhibition at high concentrations, at low concentrations (10-100 nM) have different effects on excitatory and inhibitory clusters; moreover, the network recovered more easily from ALLO than from THDOC effect.
Previous studies suggested that tonic GABAergic currents are highly sensitive to NSs [8]. To investigate whether this happens also in our experimental mode we applied Gabazine (GBZ), a $GABA_A$ receptor antagonist, at concentrations that only block the phasic GABAergic current. GBZ 100 nM increased the activity of inhibitory neurons, leaving almost unaffected the excitatory neuron excitability suggesting that interneurons are controlling each other mainly through a phasic inhibition. In these conditions the

excitability of the network was reduced but also the sensitivity to THDOC was unexpectedly decreased by approximately one order of magnitude compared to control.
A detailed analysis [6, 9] of the burst properties showed that in the presence of NSs, the neuronal activity changed and became heterogeneous because of the appearance of different states with occupancy probabilities strongly dependent on the drug concentration. In particular, we observed the random appearance of novel up-states, characterized by excitability features and engaged neurons different from those observed in control.
While the analysis of the "global network effect" of NSs provide information about the average changes in excitability of inhibitory and excitatory clusters, the "states analysis " highlighted changes in the network connectivity: in the presence of neuromodulators different connectivity modes appear. What is the physiological significance of this effect?
Specific firing patterns recorded in selected neuronal populations encode information during physiological or pathological conditions [10] and changes in the connectivity induced by endogenous compounds are able to modify the response of the network.
Taken together, our results provide a new description of the mode of action of NSs and give some new insight in understanding the complexity of the network response to these endogenous modulators.

Reference list

[1] Reddy,D.S.,and Rogawski,M.A. (2002).Stress-induced deoxycorticosterone - derived neurosteroids modulate GABAA receptor function and seizure susceptibility. J. Neurosci. 22, 3795–3805.
[2] Serra,M.,Pisu,M.G., Littera,M., Papi, G., Sanna,E.,Tuveri,F.,Usala,L., Purdy,R.H.,and Biggio,G.(2000).Social isolation-induced decreases in both the abundance ofn euroactive Steroids and GABAAr eceptor function in rat brain. J. Neurochem. 75,732–740.
[3] Maguire J., & Mody I. (2012) Front. Cell. Neurosci.6:1-6
[4] Agis-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E & Guidotti A (2006). Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. Proc Natl Acad Sci U S A 103, 14602–14607.
[5] Gunn B.G,. Brown A.R, Lambert J.J.and Delia Belelli D. (2011) Neurosteroids and GABAA receptor interactions: a focus on stress. Front Neurosci. 5:131-151.
[6] Gullo F, Maffezzoli A, Dossi E, Lecchi M, Wanke E. (2012) Classifing heterogeneity of spontaneous up-states: A method for revealinf variations in firing probability, engaged neurons and Fano factor. J Neurosci Methods 203:407-417.
[7] Puia G., Santi M.R., Vicini S., Pritchett D.B., Purdy R.H., Paul S.M., Seeburg P.H., and Costa E. (1990) Neurosteroids act on recombinant human GABAA receptors. Neuron 4:759-765.
[8] Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. (2003) Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by γ sununit-containing GABAA receptors. Proc. Natl. Acad. Sci. 100: 14439-14444.
[9]Gullo F, Mazzetti S, Maffezzoli A, Dossi E, Lecchi M, Amadeo A, et al. Orchestration of presto and largo synchrony in up-down activity of cortical networks. Front Neural Circuits 2010;4(11):1–16.
[10] Luczak A, Bartho P, Harris KD. (2009) Spontaneous events outline the realm of possible sensory responses in neocortical populations. Neuron. 62:413–425.

AN IMAGING META-ANALYSIS OF THE NEUROBIOLOGY OF SEXUAL DIMORPHISM AND ITS RELATIONSHIP TO FETAL TESTOSTERONE

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Aims: To report the first systematic review of voxel-based morphometry (VBM) studies on sexual dimorphism in human brain volume. **Background:** Sexual dimorphism in the brain substantially reflects how both physiological processes and experience affect sexual differentiation [1]. Previous whole-brain and region-of-interest (ROI) studies into sex differences in brain volume in humans have led to contradictory results regarding where and how sex has an effect. This is likely due to insufficient sample size of individual studies. **Method:** We applied Gaussian-process regression (GPR) coordinate-based meta-analytic (CBMA) technique to measure sexual dimorphism in brain volume reported by 16 VBM studies. The total sample comprised of 2186 individuals (1076 females, 49%) with ages ranging from 7-80 years. Participants were mostly white Caucasian or Asian. The strength of GPR-CBMA is that it not only incorporates location information but also all significant and non-significant/trend-level effect-size information, to reduce the bias of just including significant results for the meta-analysis. **Results:** Males on average have larger volume than females in the right and left amygdalae, left pre-central gyrus and the left anterior cingulate gyrus. Females on average have larger volume than males in the right inferior and superior frontal gyri, left thalamus and right planum temporale. **Discussion:** This meta-analysis identifies human brain structures showing volumetric sexual dimorphism across previous VBM studies. Some of these regions, such as the amygdalae and planum temporale, have been shown to be influenced by fetal testosterone (FT), a sex-differentiating developmental factor, in boys [2]. FT has not yet been studies in girls in relation to regional brain volume. It is known that the amygdalae are manipulated by steroid hormones in animals [3]. Recently the amygdala has also been found to correlate with bioactive plasma testosterone in adults [4] and with sex chromosome dosage in humans [4, 5]. These results suggest that future studies investigating these brain structures in typically developing and atypical populations should be stratified by sex. In addition, the results suggest candidate regions for investigating physiological mechanisms, (such as sex steroid and/or sex chromosomal influences) which contribute to sexual dimorphism in the human brain.

Reference list

1. McCarthy, M. M. & Arnold, A. P. Reframing sexual differentiation of the brain. *Nat Neurosci* **14**(6), 677-683 (2011).
2. Lombardo, M. V., Ashwin, E., Auyeung, B., Chakrabarti, B., Taylor, K., Hackett, G., Bullmore, E. T., & Baron-Cohen, S. Fetal testosterone influences sexually dimorphic gray matter in the human brain. *J Neurosci* **32**(2), 674-680 (2012).
3. Cooke, B. M., Tabibnia, G., & Breedlove, S. M. A brain sexual dimorphism controlled by adult circulating androgens. *Proc Natl Acad Sci U S A* **96**(13), 7538-7540 (1999).
4. Lentini, E., Kasahara, M., Arver, S., & Savic, I. Sex differences in the human brain and the impact of sex chromosomes and sex hormones. *Cereb Cortex* (Epub ahead of print). doi:10.1093/cercor/bhs222
5. Good, C. D., Lawrence, K., Thomas, N. S., Price, C. J., Ashburner, J., Friston, K. J., Frackowiak, R. S., Oreland, L., & Skuse, D. H. Dosage-sensitive X-linked locus influences the development of amygdala and orbitofrontal cortex, and fear recognition in humans. *Brain* **126**(Pt 11), 2431-2446 (2003).

RALOXIFENE PROTECTS NEURONS AGAINST HYPOXIA IN ERβ-AND G-PROTEIN-COUPLED RECEPTOR 30 - INDEPENDENT PATHWAYS

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Diminished oxygen supply to the brain may result in complex cerebral dysfunctions like cerebral palsy and severe disabilities. Natural neuroprotection against hypoxia-induced injury in females is considered to be due to the effects of circulating estrogens and lost after reproductive senescence. Although anti-hypoxic effects of estrogen have been documented, its clinical use has serious limitations related to endocrine actions on peripheral tissues and risk of cancerogenesis. Selective estrogen receptor modulators (SERMs) represent an alternative to estrogen devoid of its side-effects and acting as estrogen receptor (ER) agonists or antagonists in a tissue-specific manner. SERM representative raloxifene is used nowadays in clinical practice to activate ERα in bone tissue, and to antagonize this receptor in breast and uterine cancers. Little is known, however, about mechanisms of action of raloxifene on neuronal cells exposed to hypoxia. Therefore, the aim of the present study was to investigate anti-hypoxic potential of raloxifene in mouse hippocampal cells with special concern on interactions with classical nuclear ERs (ERα, ER) and newly identified membrane ER G-protein-coupled receptor 30 (GPR30).
Our study demonstrated that eighteen h exposure of mouse hippocampal cells in primary cultures to hypoxia induced loss of membrane mitochondrial potential, which was followed by activation of caspase-3 and subsequent increase in lactate dehydrogenase (LDH) release. Moreover, hypoxic conditions inhibited expressions of classical ERs, but stimulated the expression of membrane GPR30. In this study, raloxifene partially reversed hypoxia-induced loss of membrane mitochondrial potential and LDH release, but it did not affect hypoxia-induced caspase-3 activity. Raloxifene treatment caused tremendous increase in the expression of ERα mRNA which was accompanied by decrease in ERβ and GPR30 mRNAs. In neuronal cells transfected with ERβ or GPR30 siRNAs, neuroprotective effects of raloxifene were still retained as they were observed in wild cells. However, in neuronal cells with silenced ERα, raloxifene lost its anti-hypoxic potential. Our data point to strong neuroprotective actions of raloxifene in hippocampal cells exposed to hypoxia. They also provide evidence for ERα-mediated, but ERβ- and GPR30-independent action of raloxifene, which may have implications for treatment or prevention of hypoxic brain injury.

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PLASTICITY OF OLIGODENDROCYTES IS DEFINED WITHIN A CRITICAL TIME-WINDOW NECESSARY FOR EFFICIENT MYELIN REPAIR IN POSTNATAL BRAIN

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Genetic or acquired myelin defects, during brain development, give rise to various severe and mild abnormalities of the central nervous system (CNS). In the present study we described the potential of oligodendrocyte plasticity required for myelin repair during postnatal brain development. We used a transgenic mouse, Oligo-TTK, which carries a truncated form of the thymidine kinase of herpes simplex1 (TK-HSV1) gene, under the control of MBP promoter. The transgene can induce time specific ablation of oligodendrocyte in postnatal brain. Furthermore, a hybrid mice carrying both transgenes, TK-HSV1 and *Plp*-EGFP (enhanced green fluorescent protein) was used to facilitate oligodendrocyte visualization. Two models of hypomyelination were generated for reversible and irreversible myelin repair. The reversible myelin abnormalities were achieved with Ganciclovir (GCV) treatment for two weeks (GCV1-14) while the irreversible hypomyelination model was obtained by three weeks GCV injection (GCV1-21). GCV1-14 model showed 78-85% reduction in OLs number at 2 weeks (W2) followed by a gradual recovery in OLs population and myelin until W6. Oppositely, in GCV1-21 model the severe loss of OLs at W3 was compensated by merely 40-50% recovery of OLs with persistent myelin deficiency. GCV1-21 model showed a cell density of olig2$^+$ cells restricted to 1.78 ± 0.11/ 100 μm^2 at W3, while in GCV1-14 mouse was 2.68 ± 0.14/100 μm^2 and then increased 3 folds during W4. Despite that only 25-30% of OLs are formed during W3 of normal brain development, a critical number of Olig2$^+$, *Plp*-EGFP or CA II$^+$ cells must be reached at the end of W3 to carry out normal myelination. The formation and regeneration of oligodendrocytes within a defined time frame is required to achieve normal myelination in postnatal brain.

EFFECTS OF MEDROXYPROGESTERONE ACETATE AND RELATED COMPOUNDS ON GABA-A RECEPTORS

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Medroxyprogesterone acetate (MPA) is a progestin widely used as a contraceptive, in hormone replacement therapy as well as in many other therapeutic indications.
Many metabolites of progestines have GABA-A receptor modulatory effects. GABA-A receptor modulators are known to affect memory and learning including the progesterone metabolite allopregnanolone. In humans chronic treatment with MPA during 5 years doubles the frequency of dementia and evidences have been found in rats about cognitive-impairing effects of MPA related to the GABAergic system.
We have synthesized and investigated the effect of MPA and four of its A-ring reduced derivatives. Patch-clamp methods were used in recombinant HEK-cells expressing human alpha5beta3gamma2L, human alpha2beta3gamma2S and human alpha1beta2gamma2L GABA-A receptors. Neither MPA nor any of its A-ring reduced metabolites had an agonist activity on the alpha1beta2gamma2L receptor. However, MPA itself had a strong positive allosteric effect on the alpha5beta3gamma2L and alpha2beta3gamma2S GABA-A receptor. MPA had also a direct effect on baseline shift and open the chloride ion channel by itself on both alpha5 and alpha2. The 3alpha-OH, 5alpha/beta-MPA or 3beta-OH, 5alpha/beta-MPA were not active as agonists on the alpha5beta3gamma2L GABA-A receptor.
These results show that MPA itself has direct influence on memory and cognitive processes via the alpha5 GABA-A receptor.

THE EFFECT OF DIBUTYL PHTHALATE (DBP) ON CASPASE-3 ACTIVATION AND LDH RELEASE IN MOUSE HIPPOCAMPAL CELLS

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Objectives: Phthalates are salts and esters of phthalic acid used to manufacture varnish, alkyd paints, adhesives, solvents, and laminates. Due to their properties phthalates are widely used as an additive to plastics containing polyvinyl chloride (PVC), because they increase their flexibility and softness. Dibuthyl phthalate (DBP), a diester of benzene-1,2-dicarboxylic(phthalic) acid, is used extensively in many industrial products such a plastic wrap, paper coatings, safety glass, nail polishes, dental materials and some pharmaceuticals. DBP is not covalently bound to the polymer and it can migrate from these products into the environment. Human are exposed to DBP through different ways, such a food, air and medical treatment. DBP was reported to affect reproductive and developmental processes. Several studies suggest that DBP interfered with gonadal hormones and had an estrogenic or antiandrogenic properties. For this reason DBP was classified as an endocrine disruptor. It was shown that DBP is capable of binding estrogen receptors-alpha (ER-alpha), inducing ER-alpha-mediated gene expression and enhancing the proliferation of MCF-7 human breast cancer cells. On the other hand, in the testis of DBP-treated rats the levels of androgen receptor (AR), ER-alpha, and retinoid X receptor-gamma (RXR-r) expression decreased significantly in a time- or dose-dependent manner. Moreover, DBP significantly increased the peroxisome proliferator-activated receptor-gamma (PPAR-gamma) and phosphorylated extracellular-signal-regulated kinase (p-ERK1/2) levels in the testis. However, until now, there are no studies have been conducted to explain how the DBP impacts the nerve cells. Recent in vivo studies demonstrated that exposure to the most widely used phthalate, di(2-ethylhexyl) phthalate (DEHP) reduced a number of mature neurons and their precursors in the hippocampus of young rats [3]. It was also proven that DEHP interferes with cholinergic transmission in neurons in *Drosophila melanogaster* [2], and inhibits the proliferation and differentiation into neurons in rat adrenal pheochromocytoma cell line PC12 [1]. Also data concerning the epidemiological impact of DEHP on the development of psychiatric disorders in children such as Attention Deficit Hyperactivity Disorder, (ADHD) and autism [4] has been published. Nevertheless, the molecular mechanism of phthalates action on nervous cells has not been clarified so far.

The aim: In the present study we investigated the effect of DBP on viability and apoptosis of cultured hippocampal mouse neurons. Additionally, the role of estrogen receptors (ERs) and PPAR-gamma in DBP-induced action of hippocampus neurons was studied.

Materials and methods: The cultures of hippocampal neurons were prepared from Swiss mouse embryos on 17/18 days of gestation. The cells were cultured in phenol red-free Neurobasal medium supplemented with glutamine and B27 onto poly-ornithine-coated plates. For experiment cells were exposed to DBP in a following concentrations: 10, 50, 100 nM, and 1, 10, 25, 50 and 100 μM. The involvement of ERs in DBP action was investigated using the estrogen receptor antagonist ICI 182,780. To study the involvement of PPAR-gamma in mechanism of DBP action, the specific agonist GW1929 and antagonist GW9662, were used. Cell cultures were exposed to all chemicals for 6 or 24 hours and after this time media were collected for measurement of LDH activity. In harvested cells the caspase-3 activity was measured. Additionally in the separate

experiments the number of apoptotic bodies in hippocampal cells cultures was determined by Hoechst 33342 staining.

Results: Our preliminary data demonstrated that the high concentration of DBP stimulated both the LDH activity as well as the caspase-3 activity in the cultured mouse hippocampal cells. The proapoptotic action of DBP was confirmed by the increase in the number of apoptotic bodies. Additionally, the cytotoxic as well as proapoptotic effect of DBP was enhances in the presence of ERs agonist, PPAR-gamma antagonist and agonist.

In summary, the study demonstrated that exposure to DBP can cause neuronal cell death. However, this neurotoxic effect may not be directly mediated through an ERs and PPAR gamma. Further research is required to delineate the signaling pathways responsible for ERs and PPAR gamma agonist- and antagonist-induced neurons apoptosis.

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Reference list

1. Chen T., Yang W., Li Y., Chen X., Xu S., Toxicol Lett. 2011 25;20:34-41.
2. Ran D., Cai S., Wu H., Gu H., Food Chem Toxicol. 2012 50:3291-7
3. Smith C.S., Macdonald A., Holahan M., R., Neuroscience. 2011 13;193:100-8.
4. Testa C., Nuti F., Hayek J., De Felice C., Chelli M., Rovero P., Latini G., Papini A.M., ASN Neuro. 2012, 30;4:223-9.

PROGESTERONE RECEPTOR ISOFORMS REGULATION BY ESTRADIOL IN HYPOTHALAMIC CULTURE CELL LINE AND ITS POSSIBLE IMPLICATION IN RODENT SEXUAL BEHAVIOR

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Progesterone regulates multiple brain functions related to reproduction, primarily by the interaction with its nuclear receptor (PR). PR has two main isoforms (PR-A and PR-B) which are regulated by estrogen through different mechanisms depending on the anatomical region. Particularly, there is a specific variation in the proportion of PR-B/PR-A in the hypothalamus of rodents during the estrous cycle and with estradiol and progesterone treatments, which has been related with sexual behavior. In this study we characterized a mouse hypothalamic cell model to study the molecular mechanisms involved in the regulation of the PR isoforms. Before treatments, mHypoE-N1cells were maintained in red phenol free media supplemented with 10% charcoal-stripped FBS for 72 hours. Protein levels were determined by western blot using a rabbit polyclonal antibody that recognizes both PR isoforms (sc-539). First, we evaluated the induction of the PR isoforms at different concentrations of estradiol (1-100 nM) by 24 hours. At the highest response concentration (20 nM) we performed time course experiments (1-48 hours), which maximal response for PR-B isoform was found at 24 hours. However, we did not found differential response with progesterone after estradiol priming. Interestingly, estradiol response was absolutely lost when cells were maintained in red phenol free media supplemented with 10% charcoal-stripped FBS for prolonged periods. In conclusion, this data demonstrates the importance of the mHypoE-N1 cell model to study the molecular mechanisms involved in the regulation of the PR isoforms.

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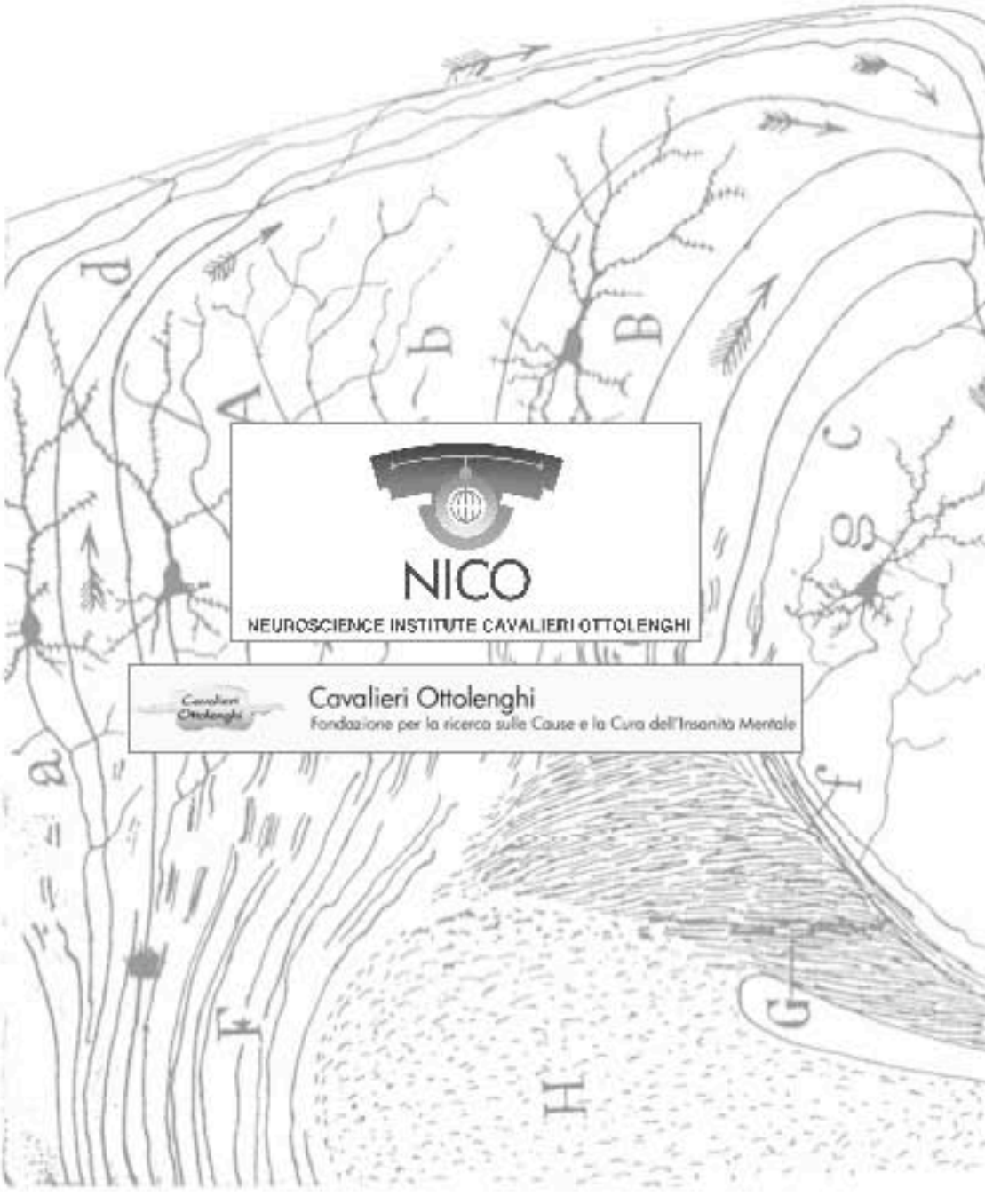
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