Milestones on Steroids and the Nervous System: 10 Years of Basic andTranslational Research

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Abstract

During the last ten years, the conference on "Steroids and Nervous System" held in Torino (Italy) was an important international point of discussion for scientists involved in this exciting and expanding research field. The present review aimed to recapitulate the main topics that were presented through the various editions of the meeting. Two broad areas were explored: the impact of gonadal hormones on brain circuits and behaviour, and the mechanism of action of neuroactive steroids. Relationships among steroids, brain and behaviour, the sexual differentiation of the brain and the impact of gonadal hormones, the interactions of exogenous steroidal molecules (endocrine disrupters) with neural circuits and behaviour, and how gonadal steroids modulate the behaviour of GnRH neurones were the topics of several lectures and symposia during this series of meetings. At the same time, many contributions were dedicated to the biosynthetic pathways, the physiopathological relevance of neurosteroids, and the demonstration of the cellular localization of different enzymes involved in neurosteroidogenesis, the mechanisms by which steroids may exert some of their effects, both classical and non-classical action of different steroids, the role of neuroactive steroids on neurodegeneration, neuroprotection and the response of the neural tissue to injury. In these 10 years, this field has significantly advanced and neuroactive steroids have emerged as new potential therapeutic tools to counteract neurodegenerative events.

Keywords: neurosteroids, brain, peripheral nerve, sex difference, neuroprotection, GnRH, kisspeptin, behaviour

The conference on "Steroids and the Nervous System" emerged as a "spin-off" from a conference specifically dedicated to the neuroendocrine controls of behaviour. The International Conference on Hormones, Brain and Behaviour" (ICHBB) met several times in various locations in Europe during the eighties and nineties (Bielfeld, Germany, 1982; Liege, BE, 1984 and 1989; Tours, FR, 1993; Torino, IT, 1996 and finally Madrid, SP, 2000). After ICHBB was merged with the activities of the Society for Behavioral Neuroendocrinology (SBN) that had been created in 1997, Gian Carlo Panzica (University of Torino) and Roberto C. Melcangi (University of Milan) decided that it would be important to keep a conference regularly meeting in Europe and dealing with steroid action in the brain. A cycle of conferences using essentially the same format as ICHBB was therefore initiated that has now met every two years for the past 10 years (2001, 2003, 2005, 2007, 2009 and 2011, fig.1) (see http://www.dafml.unito.it/anatomy/panzica/neurosteroids/ABSTRACTBOOKS.htm).

The scope of the conference has been expanded from the behavioural effects of steroids in the brain to cover all forms of steroid actions, the controls of steroid synthesis in the brain and in the peripheral nervous system, as well as the emerging translational models.

Steroids and behaviour at the Torino meeting

Glancing through the programs of these 6 conferences summarizing 10 years of research on steroids, one can identify a large number of symposia that were essentially or even exclusively dedicated to "Steroids, Brain and Behaviour". The topics that were covered in these symposia concern many aspects of the active research that took place in this field during the last decade. To list just a few, we had over the years the chance of attending symposia dedicated to behavioural effects of steroids as well as to the action of environmental oestrogens on behaviourally relevant neural circuits (2003) (1), on brain sexual differentiation (2005), on the importance of co-regulatory factors for steroid receptor action in the brain (2009) and on experimental murine models (2011).

Several round tables were also organised within the meeting during which we discussed the action of endocrine disrupter action on behaviour and neuroendocrine system (2005, 2011), and that of steroid hormones on sexually dimorphic brain circuits (2007). It must be mentioned that, as impressive as they are, all these symposia only provide a partial view of the time and talks that were devoted to behaviour during the meeting on Steroids and Nervous System. There were indeed many individual presentations on behaviour embedded in other symposia and these are far too numerous to be cited here. Starting from 2003, each meeting had additionally a few (usually 3) key-note speakers and many of the key-note lectures concerned, at least in part, the mechanisms of behaviour. During the 2003 meeting the attention was focused on the oestradiol modulation of astrocytes and the establishment of sex differences in the brain (2) and on the role of sex chromosomes in sexual differentiation of the brain (3). In the 2005 meeting, the speakers presented data on the rapid changes in the production and behavioural action of oestrogens (4) and on genetic models for the study of gonadal steroid dependent behaviours (5). In 2007 the attention was on the stress system in the human brain in depression and neurodegeneration (6). In 2009 meeting one of the key-note lectures was on the intracellular signal transduction cascades mediating behavioural effects of ovarian steroids (7). Finally, in 2011 we had lectures on comparative and functional implications of neurosteroidogenesis (8) and on oestrogen-induced plasticity and cognitive function (9). And that is without counting the large number of posters that were presented on themes related to the main talks and symposia and that were very often using behaviour as their dependent (or sometimes independent) variable.

Finally, in association with the "Torino meeting", as it has often been colloquially named, a satellite one-day symposium entirely dedicated to the endocrine control of behaviour was organised in 2009. It was named 7th ICHBB to celebrate the synchronised 60th birthday of the organisers of both the Torino Steroid meeting (Gian Carlo Panzica) and of the former ICHBB (Jacques Balthazart). At a more scientific level, this 7th ICHBB also coincided with the 50th anniversary of the publication of the seminal paper of Phoenix and collaborators (10) universally recognised as the founding paper for the research analyzing the endocrine controls of sexual differentiation of brain and behaviour.

With the exception of this satellite symposium, many of the talks and symposia mentioned above were not exclusively dedicated to the analysis of behaviour. They also concerned other topics such as the non-classical effects of steroids or the effects of steroids on the sexual differentiation of the brain. But in each case, they were behaviourally relevant in that either the changes in brain structure or function could contribute to explain behaviour or changes in behaviour were the driving force leading to changes in the brain or in steroid synthesis.

Ten years of progress in understanding sexual differentiation of the brain.

What we knew at the beginning of the 21st Century.

It has been a busy ten years for the field of behavioural neuroendocrinology and the topic of sexual differentiation of the brain in particular. As we entered this century we had a strong foundation of immutable facts about the physiological process of sexual differentiation of brain and behaviour; 1) hormones of gonadal origin are the preeminent determinant of sex differences in brain and behaviour, 2) sex differences in levels of gonadal hormones during a sensitive period of brain development will organise the brain into a sex-specific phenotype and 3) sex differences in levels of gonadal hormones in adulthood will activate the previously determined sex-specific brain phenotype in order to drive sex-specific physiology and behaviour. These are the basic facts but many aspects of the details vary by species, by physiological or behavioural endpoint and by brain region. In many cases the basic facts do not even apply. Nonetheless, the sturdy framework of the Organizational/Activational Hypothesis (10), which essentially codifies the three basic facts just enumerated, continues to provide a valuable backdrop against which to address all questions of the origins and significance of sex differences in the brain. Nothing is more valuable to scientific investigation than a dogma to be over thrown.

Dogma's overthrown.

There have been several major challenges to the dogma in the past 10 years, some have indeed created a paradigm shift in our thinking while others have offered refinements and qualifiers, notable exceptions or a more nuanced understanding. The biggest impact was the development of a mouse model that allowed for distinguishing between genetic, or chromosomal sex, and gonadal sex. The generation of animals with an XX genotype and a male phenotype (i.e. testes) or an XY genotype and a female phenotype (i.e. ovaries), allowed Art Arnold and his collaborators to ask for the first time whether all sex differences in the brain are determined by hormones (3, 11). The answer is, not surprisingly, mixed. Based on the current data to-date, it would appear that the sexual differentiation of endpoints that are directly relevant to reproduction, i.e. sexual behaviour and control of gonadotropin secretion and the brain areas that mediate them, are indeed subject to the classic hormonally mediated sexual differentiation of the brain. However, sex differences in endpoints that involve cognition, emotion or sensory integration are often influenced by chromosomal sex, sometimes markedly so. The next ten years will no doubt further advance our knowledge on this front by using genetic models such as the steroidogenic factor 1 (Nr5a1) knock-out mice which lack gonads (12) and by identifying specific X or Y genes and the associated mechanism of action.

Discoveries more in the realm of refinements to the theory are found in the characterization of genetically modified mice in which aromatase, androgen receptor or either isoform of the oestrogen receptor (ER) is either globally or locally and conditionally ablated. We have learned that in the rodent the long held dominance of oestradiol as the masculinizing hormone needs to make some room for androgens as important contributors to the natural process (13-18), and that ERα versus ERβ expression in a particular brain region mediates different responses (19-21). Our views of oestrogens effects have been further refined as well. First, steroid receptors are no longer mere transcription factors that mediate gene expression in a slow stately and direct manner, but instead can act rapidly at the membrane and integrate signal transduction pathways across a wide range of avenues (22, 23). Second, we now know oestradiol is more than just a gonadal hormone, it is also synthesised locally and rapidly and on demand, so much so that its resemblance to a neurotransmitters has been noted (24). Rapid membrane-mediated effects of oestradiol have been confirmed to contribute to the process of sexual differentiation of brain and behaviour (25), but what role local steroidogenesis plays in the process is not yet clear.

Advances made.

The distinction between the active processes of masculinisation and defeminisation of the male brain has long puzzled behavioural neuroendocrinologists and the last decade has seen several advances along this front. Characterization of null mutant mice suggests that the beta isoform of the oestrogen receptor is central to defeminisation (26), but how this is so is not clear. During the 2011 meeting a symposium was dedicated to the role of ERβ in adult brain function (27). The surprising discovery that the final common pathway mediating masculinisation of sex behaviour in the rat is the prostaglandin PGE2, also included the observation that prostaglandin mediated masculinisation does not influence defeminisation, and provided a unique tool for parsing out these separate processes in the same animal (28, 29). Lastly, feminisation of brain development has always been the poor cousin to the more tractable process of masculinisation but recent findings (30, 31) has revealed a heretofore unappreciated second sensitive period in which elevated oestradiol feminises the brain. This period is about a week to 10 days later than masculinisation in the rodent and elucidating the origins, sites of action and mechanisms of action of oestradiol during this later period will be an important topic in the coming years.

Future directions

At this writing we are at the beginning stages of several important new developments in the study of sex differences in the brain, some mechanistic and others theoretical. On the mechanistic front, it is apparent that the enduring organizational effects of steroids on the brain likely involve some sort of epigenetic changes to the genome. These include changes to the chromatin (32, 33) and the DNA (34-36), but how these changes are integrated, maintained or perhaps modulated, remains to be determined. Epigenetic changes are certainly regionally specific, and may be an important component of the regional specificity of hormone action in general. This regional specificity compels us to reconsider the Organisational/Activational Hypothesis as many early hormonally mediated effects on the brain do not seem to follow the rules of this simple theory, suggesting new rules or guidelines are waiting for us to elucidate them. An important first step in that process comes from the novel view that we should also consider that the purpose of some sex differences in the brain is to make males and females more alike than different (37).

Brain and behaviour, targets for the endocrine disrupters.

The concept that exogenous substances may interfere with the normal development of brain and behaviour is not new, and it is at the basis of a large number of experimental studies. For instance, many studies on the sexual differentiation of rodent preoptic-hypothalamic circuits were conducted by using more powerful synthetic oestrogens like diethylstilbestrol [DES, (38)] or ethynylestradiol [EE_2, (39)]. However, during the years it appeared that these substances and many others that are able to bind oestrogen or androgen receptors are not limited to the laboratory use, but, due to their large-scale use in pharmaceutical or other industries, they are also widely present in the environment. In addition, some molecules of natural origin, like phytoestrogens produced by a large number of plants and normally present in the animal and human food, may also interact with gonadal hormone receptors.

These substances were collectively named *endocrine disrupters* or endocrine disrupting chemicals (EDCs), a term that was coined early in 90'. In early papers (40), EDCs were defined as molecules that may disrupt the development of the endocrine system. In addition, the effects of EDCs' exposure during development are often permanent. A large consensus on this idea came from the Endocrine Society that released a scientific statement outlining mechanisms and effects of EDCs (41). Even if neuroendocrinology was specifically mentioned, for many years the study of EDCs involved almost exclusively the toxicological aspects, whereas the neuroendocrine and behavioural implications of precocious exposure to EDCs were less investigated.

Just from the first Torino's meeting in 2001 the issue of neuroendocrine and behavioural effects of EDCs emerged as one of the main topics of the conference. In fact, in that occasion were presented data on the effects of phytoestrogens contained in the food on the expression and regulation of cerebral androgen and progesterone metabolizing enzymes (42), as well as on anxiety behaviour and visual-spatial memory (43, 44).

During the 2nd meeting in 2003, a satellite symposium was dedicated to the action of environmental oestrogens on behaviourally relevant neural circuits. This symposium was the follow up of a series of meetings centered on the actions of EDCs on behaviour and associated neural circuits, considered as more sensitive endpoints than other targets (45, 46).

The proceedings of this symposium (1) covered different experimental models including teleost fishes [somatostatin receptor (47)], birds [the vasotocin system (48, 49), the catecholaminergic system (50), and the male copulatory behaviour (48, 51) of the Japanese quail], and rodents [catecholaminergic system (52), socio-sexual behaviours (53-55), oestrogen receptors (56), and brain plasticity (57, 58)]. These contributions provided important information on the action of single EDCs, as well as insights into the neural mechanisms by which these EDCs exert their effects.

During the 3rd meeting, data on the rapid influence of oestrogens on the excitability of adult rat hippocampal neurones were presented (59-61). These findings have led researchers to postulate the existence of so-called membrane or non-genomic oestrogen effects. EDCs able to bind oestrogen receptors (xenoestrogens) also act rapidly in the adult brain. For example, the oestradiol-induced enhancement of the long-term potentiation in CA1 upon tetanic stimulation was considerably suppressed by the co-perfusion with bisphenol A (BPA), although the perfusion of BPA alone did not alter the LTP-induction (62). On the other hand, DES enhanced the LTP by an almost identical magnitude to that obtained by oestradiol. EDCs can reach the brain via the blood circulation and by crossing the blood–brain barriers.

A symposium on the cerebral effects of xenoestrogens was again organised during the 4th meeting. This symposium included studies on the effects of BPA on the modulation of long-term depression and spinogenesis in the hippocampus (63), on the expression of oestrogen receptor (64), and on the development of the rodent (65) and avian brain (66).

During the 5th meeting, endocrine disruptors were considered among the wide family of steroid receptors coactivators (67), in particular modulating the expression of sexually dimorphic social and emotional behaviours (68). Finally, during the last meeting, whose proceedings are collected in this special issue, a round table on endocrine disrupter action on behaviour and neuroendocrine system has been organised (69).

In summary, during these ten years we observed an increasing interest in the field of EDCs, mainly related to the potentially adverse effects on the sexual differentiation of brain and behaviour. Some important facts emerged in this field:

- sexual behaviour and neural circuits related to its control are more sensitive endpoints than others currently used in toxicological studies (70, 71);
- neuropeptides and enzymes are major targets for the action of EDCs in the vertebrate brain (72);
- among different peptidergic systems kisspeptin in rodents (73-77), vasotocin in birds (48, 78, 79), as well as the enzyme aromatase in fishes (80-82), or the enzyme NO-synthase in rodents (83, 84) appear the most sensitive to low levels of EDCs during early development;
- alterations of these circuits may induce profound effects on sexual behaviour (85), puberty (74), reproductive physiology (86), and feeding behaviour (87);
- neural circuits can be altered also at synaptic levels, for example in the hippocampus (63, 88-90) and have profound effects on learning and memory (91);
- the putative mechanisms of action needs to be more thoroughly explored (69), but in addition to the EDCs binding to steroid or thyroid hormone receptors, they include the aryl hydrocarbon receptor, its interactions with ERβ, the activation of the P450 cytochromes, which are involved in the metabolism of most steroid

hormones, the PPARγ and retinoid receptors particularly important in adipose tissue.

Synthesis of neurosteroids

In the research area on steroids and nervous system, the 3 last decades were significantly marked by a major finding that revealed that neurones and glial cells have the ability to synthesise bioactive steroids, also called neurosteroids (92). This important discovery stemmed from a series of pioneer works showing the persistence of substantial amounts of pregnenolone, dehydroepiandrosterone and their sulfated derivatives in the rodent brain after adrenalectomy and/or gonadectomy (93, 94). However, the consolidation of the concept of neurosteroids required several investigations performed in different animal species (92, 95-97).

Since its creation, the International Meeting Steroids and Nervous System has steadily contributed through various symposia and plenary lectures to the elucidation of the biosynthetic pathways and mechanisms of action of neurosteroids. For instance, the first meeting (2001) has been launched with a symposium that provided key data on neurosteroid biosynthesis in mammalian and non-mammalian vertebrates (98, 99). The second meeting allowed fruitful discussion from talks on neurosteroid metabolism in the human brain (100) or neurosteroid production in the retina (101). During the 3rd meeting (2005), a satellite symposium made it possible to discuss the neuroprotective effects of steroids locally produced by the spinal cord and peripheral nervous system (102). In addition, a symposium of the main meeting discussed the role of steroidogenic acute regulatory protein and peripheral benzodiazepine receptors in neurosteroid biosynthesis (103, 104). Novel technological tools allowing high-sensitive dosage of neurosteroids were presented in a satellite symposium of the 4th meeting (105). To review and update the current knowledge on neurosteroid synthesis and functions, the opening lecture of the 6th meeting was dedicated to a comparative and functional analysis of neurosteroidogenesis (8), and a satellite symposium was focused to neuroactive steroids in the human brain (106).

Taken together, all of the data provided by renowned experts in symposia and proceedings of the International Meeting Steroids and Nervous have significantly contributed to clarify the biosynthetic pathways and physiopathological relevance of neurosteroids. Nowadays, a consensual definition of neurosteroids considers these molecules as endogenous steroidal compounds synthesised in neurones or glial cells of the CNS and PNS. To be qualified as a neurosteroid, the candidate steroidal molecule must persist in substantial amounts in the nervous system after removal of the peripheral or traditional steroidogenic glands such as the adrenals and gonads. The demonstration of neurosteroid biosynthesis requires the localization in nerve cells of the translocator protein 18 kDa, the steroidogenic acute regulatory protein and active steroidogenic key enzymes such as cytochrome P450 side chain cleavage, 3β-hydroxysteroid dehydrogenase, cytochrome P450c17, 5α-reductase, 3α-hydroxysteroid oxido-reductase, 17β-hydroxysteroid dehydrogenase and aromatase (92, 95-97, 107, 108).

Finally, it should also be noticed that endogenous neurosteroids act as paracrine or autocrine factors, regulating the activity of classical nuclear steroid receptors or membrane receptors including G protein-coupled receptors (109, 110), $GABA_A$ and T-type calcium channels (111-114) or NMDA (115, 116), P2X (117) and sigma receptors (118, 119).

Neuroendocrine control of reproduction by steroids

Another area of research that has featured strongly at the Torino meetings over the last ten years has been that of how gonadal steroids modulate the gonadotropin-releasing hormone (GnRH) neurones that control fertility. Since 2001 much has changed in this field and this has been reflected in the Torino presentations. Firstly, the techniques used by GnRH neurone investigators have changed considerably. This has been driven primarily by the use of genetic manipulations in mice that have greatly facilitated investigation of the GnRH neurone and its network. As reflected in the 2001 meeting, the mainstay approaches of the field at that time were *in situ* hybridization for GnRH mRNA, one of the few direct indices of GnRH neurones at the turn of the century (120), and use of the immortalised embryonic GT1 cell lines that synthesise GnRH (121). By 2011, a range of sophisticated transgenic and cell- or receptor-specific gene mutation approaches were being used to establish the electrical properties, gene expression profiles and *in vivo* significance of GnRH neurone-selective receptor manipulations. The second major change in this field has been the discovery of kisspeptin. Initially discovered in humans in 2003 (122, 123), GnRH neurone investigators rapidly took up the challenge of deciphering how kisspeptin regulates fertility and this topic has been present at meetings since 2007 (124-126).

The key gonadal steroid-GnRH neurone milestones at Torino meetings over the last 10 years have been summarised in the following sub-chapters.

Understanding rapid gonadal steroid actions on GnRH neurones.

The meeting has witnessed the gradual unfolding of how oestrogens, androgens and progesterone derivatives exert rapid, sometimes direct, actions upon GnRH neurones. At the 2001 meeting, the role of allopregnanolone on $GABA_A$-mediated effects on GnRH neurones in GT1 cells (127) and native adult GnRH neurones (128) was discussed. This was followed at the next meeting in 2003 by descriptions of how oestradiol rapidly activates specific intracellular signaling cascades in GnRH neurones, including calcium dynamics. These actions were mediated directly by ERβ expressed by GnRH neurones as well as indirectly through $GABA_A$ receptors (129, 130). This line of work was brought up to date at the most recent meeting in 2011 where studies detailing the complex, dose-dependent direct- and indirect- effects of oestradiol (131, 132) and androgen metabolites (133, 134), on GnRH neurone electrical activity were presented. Although the issue of the physiological relevance of rapid steroid actions remains unknown (135), it is clear that progesterone and androgen derivatives, as well as oestradiol itself, can exert rapid actions on mammalian GnRH neurones both directly, and indirectly through GABA and glutamatergic inputs to these cells.

Examining the role of glial cells and growth factors in the steroid regulation of GnRH neurones.

The importance of astrocytic growth factors such as TGFβ and βFGF on the functioning of GT1 cells (121) was elucidated during the 2001 meeting. This was expanded in 2003 to document the role that oestradiol played in regulating glial production of these growth factors (136). At the same meeting, the key roles for IGF-1 interactions with oestradiol in modulating adrenergic tone within the GnRH neuronal network *in vivo* were illustrated (137). This was to be expanded further in 2007 meeting by showing that oestradiol acts on membrane ERs on glial cells to promote progesterone synthesis that, in turn, impacts on the ability of GnRH neurones to exhibit the preovulatory surge (138). Alongside many other talks at the Torino meeting on steroid hormone-growth factor interactions, these studies have provided the impetus for considering the potentially important impact of glial cells on GnRH neurone functioning. The lack of good tools to dissect the roles of specific groups or regional locations of glia *in vivo* seems to remain a significant problem for understanding the roles of these cells beyond their normal "neuronal support roles".

Defining the mechanisms of oestrogen positive and negative feedback.

Talks presented in 2001 meeting focused upon the roles of gonadal steroids in regulating GnRH gene transcription using *in situ* hybrization (120) and GnRH transgenics (139), respectively. This topic moved a considerable step forward with the data presented at the 2003 meeting detailing the effects of ovariectomy and oestrogen replacement upon GnRH neurone firing rates and the potential ion channels underlying these actions (140). It would not, however, be until the 2011 meeting that the data on single cell RT-PCR allowed to define the precise ion channel subunits modulated by oestradiol in GnRH neurones (141, 142). The GnRH neurone firing studies in 2003 were complemented by studies showing the effects of different steroid regimens upon pulsatile GnRH secretion from hypothalamic explants (143). Although from different species, this highlighted the continuing puzzle as to why the effects of ovariectomy and oestradiol replacement on GnRH neurone firing rates and GnRH secretion are so dissimilar. The 2007 meeting was presented with a series of genetic and ER-specific ligand studies (144, 145) that defined the mechanism and types of ERs involved in the positive feedback mechanisms in mice and rats. These studies concluded that oestradiol acted on ERα-expressing neurones in the rostral hypothalamus to activate GnRH neurones to evoke the GnRH surge (124). Other studies presented at that meeting highlighted the oestrogen-sensitivity of kisspeptin neurones (125). By the time of the 2011 meeting the promise of the oestradiol-sensitive kisspeptin neurones within the GnRH neuronal network had been fulfilled with three papers (126, 146, 147) detailing their now established key importance in different oestrogen feedback mechanisms.

Over the last 10 years, the Torino meeting has provided one focus meeting for promoting the understanding of how gonadal steroids modulate the behaviour of GnRH neurones. This is a large subject with too many active investigators to accommodate at the Torino meeting at one time. Nevertheless, those outside the field have been treated to a consistently high-quality overview of progress in the subject while GnRH neurones aficionados have had the luxury of discussing science in the delightful mid-winter setting of Torino.

Interactions with classical and non classical steroid receptors

Through the years at the International Conference on Steroids and the Nervous System, there has been much work presented on the mechanisms by which steroids may exert some of their effects. Nuclear steroid receptors (nSRs) were discovered over 50 years ago for oestrogen and were followed by discovery of specific nSRs for progestins and androgens (148). These classic nSRs are intracellular, are activated by the binding of steroids, and serve as transcription factors. Our discussions of oestrogen action in the brain via nSRs has included actions via the originally discovered ERα and its traditional role in reproduction, but also how these actions have effects in other brain regions such as the hippocampus, to influence processes relevant for aging and related functions (149). Various effects, from form to function, of the more recently discovered ERβ have been discussed (27, 150), with an emphasis on integrated actions via ERα and ERβ (5). The role of progestin receptors in reproduction, and their effects as neural integrators of hormonal and environment actions, have been proposed (151, 152). How actions at progestin receptors may occur through steroid activation or involve other ligands, such as dopamine, is intriguing (153). At this venue, we have also discussed the role of androgens receptors in sexual differentiation, and other processes, along with how there may be actions of androgens via other nSRs, including ERbeta, as well as actions apart from nSRs (15, 16, 154-158).

More recently, it has been demonstrated that steroids bound to nSR complexes, bind hormone response elements, and have actions through co-activators, to result in changes in their rates of transcription and translation. The importance of co-regulatory factors to influence nSRs action has been discussed at our venue (159). How steroids' actions in the brain via sNRs can also involve coactivators, which modulate hormone-dependent gene expression in brain and reproductive behaviour in rodents (67) and galliforms (159), and co-repressors, such as chromatin binding factors mediation of epigenetic organization of sex differences in the brain (160), has been the topic of recent symposia. Thus, as evidence has emerged regarding steroids actions via nSRs, these topics have been of ongoing interest and discussion.

This classical "genomic" mechanism of steroid action, involving the transcription of DNA and synthesis of proteins, can elicit a biological response within 10 minutes, hours or days. In addition to classical actions via nSRs, there has been an ongoing dialogue about non-traditional actions of steroids. Non-classical actions of steroids can occur much more rapidly (<10 minutes, and even in seconds) than actions at nSRs, in the absence of nSRs, and in the presence of inhibitors of transcription and/or translation. Non-classical, rapid steroid actions, often referred to as "non-genomic" actions of steroids, have been extensively studied over the past few decades, demonstrated for all the major classes of steroids, and are now well-recognised. Rapid, non-classical actions of oestrogens, progestogens, and androgens and their role in various hormone-sensitive functions, have been ongoing topics of discourse at this meeting (4, 69, 89, 161, 162).

An important question is which receptors mediate non-genomic actions? Several physiologically relevant membrane-associated proteins have been identified on plasma membranes suggesting the existence of specific membrane steroids receptors (22, 23, 163-165). However, identities of some of these membrane targets remain controversial. Neurotransmitter receptors have been foci of non-genomic signaling activity of steroids. The most widely studied (and discussed) neurotransmitter targets for steroid actions have been through GABA receptors (166-173). However, actions of steroids through glutamate (120, 174), dopamine (175), adrenergic (137, 176, 177), opiate (178), and sigma (179) receptors have been investigated and discussed at this meeting.

Some non-traditional effects of steroids may be downstream of actions at membrane targets. The intracellular signal transduction cascades, which mediate some behavioural effects of ovarian steroids have been discussed (137, 176). Some effects of steroids, such as progestagens, may be mediated in part through adenyl cyclase, G-proteins, PKA, PLC, and/or PKC pathways (180, 181). Other effects of oestrogen may be mediated through MAPK signaling, mitochondrial processes, or other intracellular pathways. (182). Extensive discussions of traditional and novel effects and mechanisms of steroids have taken place during the meetings organised in Torino. There have also been perspectives of how actions through classic nSR signaling may integrate with rapid, membrane action of steroids, and their downstream effectors (183, 184). The discourse to date about classic and non-traditional steroid action have been productive and will likely continue to expand the field in a substantive manner to elucidate new perspective regarding modulatory effects of steroid signalling.

Neuroactive steroids as neuroprotective agents: translational research

The role of neuroactive steroids on neurodegeneration, neuroprotection and the response of the neural tissue to injury has been a fundamental topic in the International Meeting on Steroids and Nervous System since its first edition in 2001. Since then, this field has significantly advanced and neuroactive steroids have emerged as new potential therapeutic tools to counteract neurodegenerative events.

Oestradiol and neuroprotection

By the time of the first Torino meeting extensive experimental evidence indicated that oestradiol is neuroprotective (126). However, a turning point was the publication of the results of the Women's Health Initiative (WHI) clinical trial on the effects of hormonal therapy in women (185, 186). The results of this study showed an increased risk of dementia and stroke in women over 65 years of age who received conjugated equine oestrogens plus medroxyprogesterone acetate (MPA) compared to women who received placebo. This finding was in contradiction with the evidence obtained in animal models of neurodegenerative diseases. Therefore, new studies have addressed in recent years the possible causes of this discrepancy. In particular, age at which hormones were administered relative to the perimenopausal transition has emerged as a critical issue. Observational studies and randomised clinical studies suggest that early initiation of hormone therapy may provide cognitive benefits, particularly to verbal memory and other hippocampus-mediated functions (187). In addition, new basic studies have shown that the neuroprotective activity of oestradiol depends on the duration of ovarian hormone deprivation (188) and is affected by age-associated modifications in the levels of other molecules, such as insulin-like growth factor-I (189).

Progesterone and other neurosteroids

Another neuroactive steroid whose neuroprotective activity has been frequently discussed in Torino meetings is progesterone. The neuroprotective activity of progesterone and its metabolites dihydroprogesterone and tetrahydroprogesterone has been characterised in the last decade (190-192). Progesterone and its metabolites promote remyelination in the CNS (193, 194) and the PNS (195-197). Furthermore, progesterone attenuates clinical severity, demyelination, neuronal dysfunction and axonal damage in experimental autoimmune encephalomyelitis, a well-established experimental model of multiple sclerosis (198-201) and in diabetic neuropathy (202). Progesterone is also protective after traumatic brain injury in animals (192). In addition, clinical trials have indicated a reduction in the mortality and an improvement of functional outcomes after traumatic brain injury in patients treated with progesterone (203).

The neuroprotective action of other neuroactive steroids has also been assessed during the last decade. Among these is allopregnanolone, whose cerebral levels are decreased in an experimental model of Niemann-Pick type C disease. The neonatal administration of allopregnanolone results in a delay of the onset of neurological symptoms, and a doubling the lifespan of the animals (204). Other studies have demonstrated the efficacy of treatment with dehydroepiandrosterone after spinal cord injury (205) and in diabetic neuropathy (206). Neuroactive steroids are also important endogenous modulators of mood and have therapeutic potential for the treatment of depression and anxiety disorders. Novel therapeutic strategies might either be based on synthetic derivates of endogenous 3alpha-reduced neuroactive steroids or on the modulation of neurosteroidogenic activity (207). Pregnenolone and dehydroepiandrosterone are also promising candidates for the treatment of schizophrenia (208, 209). Better performance on executive tasks is associated with increased plasma levels of dehydroepiandrosterone in schizophrenic patients (209) and clinical trials have demonstrated that pregnenolone is able to decrease negative symptoms and extrapyramidal side effects and to improve verbal memory, attention and working memory performance in these patients (208).

Alternatives to treatment with neuroactive steroids have been also explored in recent years. These include synthetic receptor modulators, like for instance selective oestrogen modulators (SERMs). Some SERMs have been shown to be neuroprotective and anti-inflammatory agents in experimental animal models of central neurodegeneration (210). Another alternative therapeutic strategy might be the use of pharmacological agents that increase the synthesis of endogenous neuroactive steroids within the nervous system (211). With this perspective, ligands of translocator protein (TSPO, previously known as peripheral benzodiazepine receptor (104)) may represent an interesting option (212-214). TSPO is mainly present in the mitochondrial outer membrane, where it promotes**,** in cooperation with steroidogenic acute regulatory protein (StAR), the translocation of cholesterol to the inner mitochondrial membrane. The mitochondrial translocation of cholesterol is a limiting step in steroidogenesis, since it allows the transformation of cholesterol into pregnenolone. Observations have shown that treatment with ligands of TSPO, like for instance Ro5-4864, exerts neuroprotective effects in aged peripheral nervous system

(215), in peripheral nerve during diabetes (216) and in CNS after neuronal injury (217). A similar approach has been obtained with a ligand of liver X receptors. Indeed, treatment of diabetic animals with a synthetic ligand of these receptors (i.e., GW3965) results in an increase of neuroactive steroidoigenesis in the sciatic nerve which is associated with neuroprotective effects (218).

Perspectives for the future

During the last decade several studies have shown that pathological events have an important impact on neuroactive steroid levels in nervous tissues. Changes in neurosteroid biosynthesis or in neurosteroid levels in the brain, spinal cord or peripheral nerves have been detected under different pathological conditions, including experimental models of diabetes (219-221), hereditary peripheral neuropathy (219), peripheral nerve injury (222), spinal cord injury (223, 224), multiple sclerosis (225, 226), autism (227), and Parkinson's disease (228, 229). Neuroactive steroid levels are also modified in the human brain under pathological conditions, including Alzheimer's disease, Parkinson's disease, multiple sclerosis and hepatic encephalopathy (97, 230-235). To develop adequate therapeutic tools based on neuroactive steroids (212-214) it would be necessary to increase our knowledge on the specific regional and temporal changes that occur in neurosteroid levels in the human brain at different phases of neurodegenerative diseases and during affective disorders. In addition, it would be also necessary to determine the implications of such changes for the manifestation and outcome of the pathological condition.

Another important issue is that different pathologies of the central and peripheral nervous system show sex differences in their incidence, symptomatology and/or neurodegenerative outcome (236). Interestingly, the levels of neuroactive steroids in the CNS and PNS under pathological conditions also show sex differences (219, 221, 224-226, 237, 238). In addition, the nervous system of males and females show different responses to neuroactive steroids. Therefore, it would be important to explore with detail the interaction of sex with neurosteroid levels and neurosteroid actions to develop adequate sex-specific neuroprotective strategies.

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Information about this series of conferences is available at a dedicated website http://www.dafml.unito.it/anatomy/panzica/neurosteroids/index.html, where the extended abstracts of all the editions of the meeting are available for downloading.

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Legend to the figure

Fig. 1 – Participants at the 6th International Meeting on Steroids and Nervous System, Torino, February 2011.

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Fig. 1 – Participants at the 6th International Meeting on Steroids and Nervous System, Torino, February 2011.
1483x880mm (72 x 72 DPI)