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

G. C. Panzica*, J. Balthazart‡, C. A. Frye§, L. M. Garcia-Segura*, A. E. Herbison**, A. G. Mensah-Nyagan†, M. M. McCarthy†† and R. C. Melcangi§§

*Laboratory of Neuroendocrinology, Department of Anatomy, Pharmacology and Forensic Medicine, Neuroscience Institute of Turin (NIT), University of Turin, Turin, Italy.
†Neuroscience Institute Cavalieri-Ottolenghi (NICO), Orbassano (Torino), Italy.
‡University of Liège, GIGA Neuroscience, Research Group in Behavioral Neuroendocrinology, Liège, Belgium.
§Department Psychology, University at Albany, Albany, NY, USA.
¶Instituto Cajal, CSIC, Madrid, Spain.
**Centre for Neuroendocrinology, Department of Physiology, University of Otago, Dunedin, New Zealand.
††Equipe Stéroides, Neuromodulateurs et Neuropathologies, EA-4438 Université de Strasbourg, Strasbourg, France.
‡‡Departments of Physiology, School of Medicine, University of Maryland, Baltimore, MD, USA.
§§Department of Endocrinology, Pathophysiology and Applied Biology – Center of Excellence on Neurodegenerative Diseases, University of Milano, Milano, Italy.

During the last 10 years, the conference on ‘Steroids and Nervous System’ held in Torino (Italy) has been an important international point of discussion for scientists involved in this exciting and expanding research field. The present review aims to recapitulate the main topics that have been presented through the various meetings. Two broad areas have been explored: the impact of gonadal hormones on brain circuits and behaviour, as well as 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 gonadotrophin-releasing hormone neurones, have been the topics of several lectures and symposia during this series of meetings. At the same time, many contributions have been dedicated to the biosynthetic pathways, the physiopathological relevance of neurosteroids, the demonstration of the cellular localisation of different enzymes involved in neurosteroidogenesis, the mechanisms by which steroids may exert some of their effects, both the classical and nonclassical actions 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.

Key words: neurosteroids, brain, peripheral nerve, sex difference, neuroprotection, GnRH, kisspeptin, behaviour.

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for the past 10 years (2001, 2003, 2005, 2007, 2009 and 2011) (Fig. 1) (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 actions of steroids, the controls of steroid synthesis in the brain and in the peripheral nervous system (PNS), as well as the emerging translational models.

Steroids and behaviour at the Torino meeting

Glancing through the programmes of these six conferences summarising 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 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 meetings, during which we discussed the action of endocrine disrupter action on behaviour and neuroendocrine system (2005, 2011), as well as that of steroid hormones on sexually dimorphic brain circuits (2007). It must be noted 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 the Nervous System. There were indeed many individual presentations on behaviour embedded in other symposia and these are far too numerous to be cited in the present review. Starting from 2003, each meeting had additionally a few (usually three) key-note speakers, and many of the key-note lectures concerned, at least in part, the mechanisms of behaviour. During the 2003 meeting, attention was focused on the modulation of astrocytes by oestradiol and the establishment of sex differences in the brain (2), as well as on the role of sex chromosomes in sexual differentiation of the brain (3). In 2005, speakers presented data on the rapid changes in the production and behavioural action of oestrogens (4), as well as on genetic models for the study of gonadal steroid dependent behaviours (5). In 2007, attention focused on the stress system in the human brain in depression and neurodegeneration (6). In 2009, one of the key-note lectures was on the intracellular signal transduction cascades mediating the behavioural effects of ovarian steroids (7).

Finally, in 2011, we had lectures on comparative and functional implications of neurosteroidogenesis (8), as well as on oestrogen-induced plasticity and cognitive function (9). All of the above was augmented by the large number of posters that were presented on themes related to the main talks and symposia, which very often were 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 1-day symposium entirely dedicated to the endocrine control of behaviour was organised in 2009. It was named the 7th ICHBB to celebrate the synchronised 60th birthday of one 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 et al. (10) universally recognised as the founding paper for the research analysing the endocrine controls of sexual differentiation of brain and behaviour.

With the exception of this satellite symposium, many of the talks and symposia referred to above were not exclusively dedicated to the analysis of behaviour. They also concerned other topics, such as the nonclassical effects of steroids or the effects of steroids on the sexual differentiation of the brain. However, 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 10 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: (i) hormones of gonadal origin are the pre-eminent determinants of sex differences in brain and behaviour; (ii) sex differences in levels of gonadal hormones during a sensitive period of brain development will organise the brain into a sex-specific phenotype; and (iii) sex differences in levels of gonadal hormones in adulthood will activate the previously determined sex-specific brain phenotype to drive sex-specific physiology and behaviour. These are the basic facts, although many details vary by species, by physiological or behavioural end-point, 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 it is possible to address all questions concerning the origins and significance of sex differences in the brain. Nothing is more valuable to scientific investigation than a dogma to be overthrown.

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, whereas 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 the distinction 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 question for the first time whether all sex differences in the brain are determined by hormones (3, 11). Not surprisingly, the answer is mixed. Based on the current data available to date, it would appear that the sexual differentiation of endpoints that are directly relevant to reproduction (i.e. sexual behaviour and the control of gonadotrophin 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, and sometimes markedly so. The next 10 years will no doubt further advance our knowledge on this front by using genetic models such as the steroidogenic factor 1 (Nr5a1) knockout mice that 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 characterisation 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 masculinising 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 the effects of oestrogens 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 neurotransmitter 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), although 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. Characterisation of null mutant mice suggests that the β isof orm of the oestrogen receptor is central to defeminisation (26), although 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 involves 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, although recent findings (30, 31) have revealed a previously unappreciated second sensitive period in which elevated oestradiol feminises the brain. This period is approximately 1 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

Currently, 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 organisational 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 DNA (34–36), although how these changes are integrated, maintained or perhaps
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 example, many studies on the sexual differentiation of rodent preoptic-hypothalamic circuits were conducted by using more powerful synthetic oestrogens such as diethylstilbesterol (38) or ethynylestradiol (39). However, it is apparent that these substances, as well as many others that are able to bind oestrogen or androgen receptors, are not limited to the laboratory use but, as a result of their large-scale use in pharmaceutical or other industries, they are also widely present in the environment. In addition, some molecules of natural origin, such as 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 the 1990s. In early studies (40), EDCs were defined as molecules that may disrupt the development of the endocrine system. In addition, the effects of exposure to EDCs during development are often permanent. A large consensus on this idea came from the Endocrine Society, which 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.

From the first Torino meeting in 2001, the issue of the neuroendocrine and behavioural effects of EDCs emerged as one of the main topics of the conference. Indeed, on that occasion, data were presented with respect to the effects of phytoestrogens contained in foods that may disrupt the development of the endocrine system. In addition, the effects of exposure to EDCs during development are often permanent. A large consensus on this idea came from the Endocrine Society, which 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.

During the third meeting, data on the rapid influence of oestrogens on the excitability of adult rat hippocampal neurons were presented (59–61). These findings have led researchers to postulate the existence of so-called membrane or nongenomic 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, although the perfusion of bisphenol A alone did not alter the long-term potentiation-induction (62). On the other hand, diethylstilbestrol enhanced the long-term potentiation 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 fourth meeting. This symposium included studies on the effects of bisphenol A 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 fifth meeting, endocrine disruptors were considered among the wide family of steroid receptors coactivators (67), in particular those modulating the expression of sexually dimorphic social and emotional behaviours (68). Finally, during the last meeting, whose proceedings are collected in this special issue of the Journal of Neuroendocrinology, a round table on endocrine disrupter action on behaviour and neuroendocrine system was organised (69).

In summary, during these 10 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 to be 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 EDCs binding to ste-
roid or thyroid hormone receptors, they include the aryl hydrocarbon receptor, its interactions with ERα, the activation of the P450 cytochromes, which are involved in the metabolism of most steroid hormones, the peroxisome proliferator-activated receptor γ and retinoid receptors important in adipose tissue.

Synthesis of neurosteroids

In the research area on steroids and nervous system, the three 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 or gonadectomy (93, 94). However, consolidation of the concept of neurosteroids required several investigations performed in different animal species (92, 95–97).

Subsequent to its creation, the International Meeting on Steroids and the Nervous System has steadily contributed through various symposia and plenary lectures to the elucidation of biosynthetic pathways and mechanisms of action of neurosteroids. For example, the first meeting (2001) was 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 third meeting (2005), a satellite symposium made it possible to discuss the neuroprotective effects of steroids locally produced by the spinal cord and PNS (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 fourth meeting (105). To review and update the current knowledge on neurosteroid synthesis and functions, the opening lecture of the sixth meeting was dedicated to a comparative and functional analysis of neurosteroidogenesis (8), and a satellite symposium focused on 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 on Steroids and the Nervous System have significantly contributed to clarify the biosynthetic pathways and physiopathological relevance of neurosteroids. Currently, a consensual definition of neurosteroids considers these molecules as endogenous steroidal compounds synthesised in neurones or glial cells of the CNS and PNS. To qualify 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 localisation 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 be noted 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 10 years has been that of how gonadal steroids modulate the gonadotrophin-releasing hormone (GnRH) neurones that control fertility. Since 2001, much has changed in this field and this has been reflected in the Torino presentations. First, 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 the 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 hybridisation for GnRH mRNA, one of the few direct indices of GnRH neurones at the turn of the century (120), and the 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 are summarised below.

Understanding rapid gonadal actions of steroid 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 GABAA receptors has been discussed. This was followed at the next meeting in 2003 by descriptions of how oestradiol rapidly activates specific intracellular signalling cascades in GnRH neurones, including calcium dynamics. These actions were mediated directly by ERα expressed by GnRH neurones, as well as indirectly through GABAA 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 actions of steroids 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 transforming growth factor-β and basic fibroblast growth factor 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 the 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 the 2007 meeting by showing that oestradiol acts on membrane ERs on glial cells to promote progesterone synthesis, which, 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 appears 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 hybridisation (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 reverse transcriptase-polymerase chain reaction allowed a definition of 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, evoking 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 studies (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, whereas GnRH neurones aficionados have had the luxury of discussing science in the delightful mid-winter setting of Torino.

Interactions with classical and nonclassical steroid receptors

Over the years at the International Meeting 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, as well as how these actions have effects in other brain regions (e.g. the hippocampus) to influence processes relevant for ageing 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). A role for progesterin receptors in reproduction, as well as their effects as neural integrators of hormonal and environment actions, has been proposed (151, 152). How actions at progestin receptors may occur through steroid activation or involve other ligands, such as dopamine, is intriguing (153). We have also discussed the role of androgen receptors in sexual differentiation, and other processes, along with how there may be actions of androgens via other nSRs, including ERβ, 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, resulting in changes in their rates of transcription and translation. The importance of co-regulatory factors to influence nSRs action has been discussed (159). How the actions of steroids in the brain via sNRs can also involve coactivators, which modulate hormone-dependent gene expression in the brain and reproductive behaviour in rodents (67) and galliforms (159), and co-repressors, such as chromatin-binding factor mediation of the epigenetic organisation of sex differences in the brain (160), has been the topic of recent symposia. Thus, as evidence has emerged regarding the actions of steroids via sNRs, these topics have been of ongoing interest and discussion.

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

An important question is which receptors mediate nongenomic actions? Several physiologically relevant membrane-associated proteins have been identified on plasma membranes, suggesting the existence of specific membrane steroid receptors (22, 23, 163–165). However, identities of some of these membrane targets remain controversial. Neurotransmitter receptors have been focal of the nongenomic signalling activity of steroids. The most widely studied (and discussed) neurotransmitter targets for the actions of steroids 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 nontraditional 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, protein kinase A, phospholipase C and/or protein kinase C pathways (180, 181). Other effects of oestrogen may be mediated through mitogen-activated protein kinase signalling, 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 signalling may integrate with the rapid membrane actions of steroids, as well as their downstream effectors (183, 184). The discourse to date about classic and nontraditional actions of steroids has 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 meeting in 2001. Subsequently, this field has significantly advanced and neuroactive steroids have emerged as new potential therapeutic tools to counteract neurodegenerative events.

Oestriadiol 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 clinical trial on the effects of hormonal therapy in women (185, 186). The results of their studies showed an increased risk of dementia and stroke in women over 65 years of age who received conjugated equine oestrogens plus medroxyprogesterone acetate compared to women who received placebo. This finding was in contradiction with the evidence obtained in animal models of neurodegenerative diseases. Therefore, new studies conducted in recent years have addressed the possible causes of this discrepancy. In particular, the 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 dihydropregesterone and tetrahydroprogesterone has been characterised in the last decade (190–192). Progesterone and its metabolites promote remyelination in the central nervous system (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 derivatives of endogenous 3α-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, such as selective oestrogen modulators. Some selective oestrogen modulators 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, the translocation of cholesterol to the inner mitochondrial membrane. The mitochondrial translocation of cholesterol is a limiting step in steroidogenesis because it allows the transformation of cholesterol into pregnenolone. Observations have shown that treatment with ligands of TSPO (e.g. Ro5-4864) exerts neuroprotective effects in the aged PNS (215), in the peripheral nerve during diabetes (216) and in the 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 steroidogenesis 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 steroid 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 nervous tissues. Changes in neurosteroid biosynthesis or in neurosteroid levels 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 CNS and PNS 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 in detail the interaction of sex with neurosteroid levels and actions of neurosteroids to develop adequate sex-specific neuroprotective strategies.

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