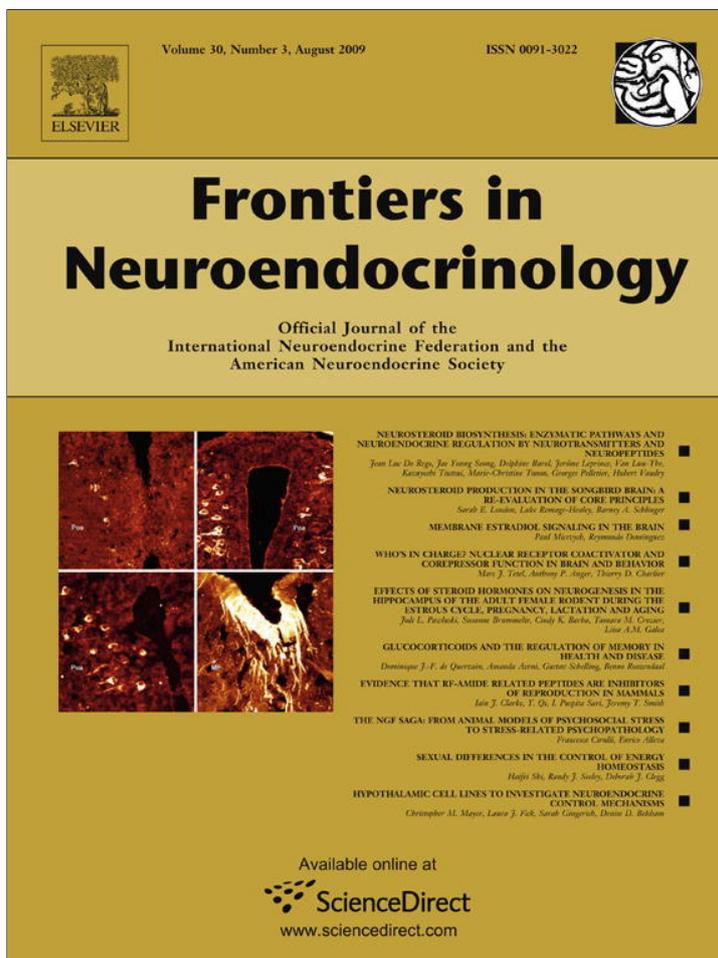


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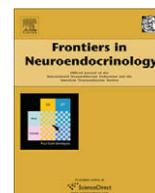
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## Review

## Who's in charge? Nuclear receptor coactivator and corepressor function in brain and behavior

Marc J. Tetel<sup>a,\*</sup>, Anthony P. Auger<sup>b</sup>, Thierry D. Charlier<sup>c</sup><sup>a</sup>Neuroscience Program, Wellesley College, 106 Central St., Wellesley, MA 02481, USA<sup>b</sup>Department of Psychology, University of Wisconsin, 1202 West Johnson St., Madison, WI 53706, USA<sup>c</sup>GIGA Neurosciences, University of Liege, 1 Avenue de l'Hôpital, B-4000 Liège, Belgium

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## ABSTRACT

Steroid hormones act in brain and throughout the body to regulate a variety of functions, including development, reproduction, stress and behavior. Many of these effects of steroid hormones are mediated by their respective receptors, which are members of the steroid/nuclear receptor superfamily of transcriptional activators. A variety of studies in cell lines reveal that nuclear receptor coregulators are critical in modulating steroid receptor-dependent transcription. Thus, in addition to the availability of the hormone and the expression of its receptor, nuclear receptor coregulators are essential for efficient steroid-dependent transactivation of genes. This review will highlight the importance of nuclear receptor coregulators in modulating steroid-dependent gene expression in brain and the regulation of behavior.

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## 1. Introduction

Steroid hormones have profound effects on homeostasis, development, reproduction and behavior. Many of the biological effects of steroid hormones are mediated through their respective receptors, which are members of the steroid/nuclear receptor

superfamily of transcriptional activators [111,205]. Receptors for the gonadal steroids, estrogens (ER), progesterone (PR) and androgens (AR), can act in a classic, genomic mechanism by interacting directly with DNA to regulate gene transcription [111,205]. Nuclear receptor coregulators enhance (coactivators) or repress (corepressors) the transcriptional activity of these steroid receptors, as well as other nuclear receptors [176,156]. In brain, while ER, PR and AR can also function independent of ligand on DNA or at the membrane to rapidly activate cytoplasmic signaling pathways [112,155,128,86,210,54], these receptors elicit many changes in behavior and physiology by acting through a classic, genomic mechanism of action. Approximately 300 coactivators and corepressors have been identified to date [157,101], indicating the growing complexity of these coregulators in receptor action. This review will focus on the function of some of these important nuclear receptor coregulators in genomic mechanisms of steroid action in brain and behavior.

## 2. Classic genomic mechanism of steroid action

Steroid receptors, including ER, PR and AR, have a modular domain structure consisting of an amino-terminal region (N-domain), a central DNA-binding domain (DBD) and a carboxy-terminal ligand-binding domain (LBD) [111,205]. In general, steroid

**Abbreviations:** AF, activation function domain; AR, androgen receptor; ARC, arcuate nucleus; AS, antisense oligodeoxynucleotides; CBP, CREB-binding protein; DNMT, DNA methyltransferase; E6-AP, E6-associated protein; EB, estradiol benzoate; ER, estrogen receptor; ERAP140, estrogen receptor associated protein 140; GnRH, gonadotropin-releasing hormone; GR, glucocorticoid receptor; GST, glutathione S-transferase tag; Hipp, hippocampus; HDAC, histone deacetylase; hsp, heat shock protein; Hyp, hypothalamus; IHC, immunohistochemistry; IP, immunoprecipitation; IR, immunoreactivity; MCG, midbrain central gray; MPOA, medial preoptic area; NCoR, nuclear receptor corepressor; ODN, oligodeoxynucleotides; p/CAF, p300/CBP-associated factor; PR, progesterone receptor; R5020, a progesterone receptor agonist; RAR $\alpha$ , retinoic acid receptor alpha; RPL7, ribosomal large subunit protein 7; RXR, retinoid X receptor; SDN, sexually dimorphic nucleus; SERM, selective estrogen receptor modulator (e.g. tamoxifen); SMRT, Silencing mediator of retinoid and thyroid hormone receptors; SPRM, selective progesterone receptor modulator (e.g. RU486); SRA, steroid receptor RNA activator; SRC-1/2/3, steroid receptor coactivator-1/2/3; SRE, steroid response element; TR, thyroid hormone receptor; TRAP220, thyroid hormone receptor associating protein 220; VMH, ventromedial hypothalamus; VMN, ventromedial nucleus of the VMH.

\* Corresponding author. Fax: +1 781 283 3642.

E-mail address: [mtetel@wellesley.edu](mailto:mtetel@wellesley.edu) (M.J. Tetel).

receptors have two transcriptional activation domains: one in the amino terminal (AF-1) and one in the carboxyl terminal LBD (AF-2) [202]. Intracellular ER exist in two forms,  $\alpha$  and  $\beta$ , which are transcribed from different genes [79,95]. These subtypes differ in their abilities to bind different ligands [96], distribution in brain [187,61,161,132], and functions in brain and behavior [151,142,152,18]. In addition, cell culture experiments indicate that ER $\alpha$  is a stronger transcriptional activator than ER $\beta$ , due to differences in the AF-1 region [41]. In most species, PR are expressed in two forms; the full-length PR-B and the truncated PR-A, which are encoded by the same gene but are regulated by different promoters [85]. Under certain conditions, *in vitro* studies indicate that human PR-B is a stronger transcriptional activator than PR-A [213,206,60], due to an additional AF domain in the N-terminus of PR-B [178]. These two PR isoforms appear to have distinct functions in reproductive behavior and physiology [113,141].

In the absence of hormone, steroid receptors are complexed with several chaperone molecules, including heat shock proteins (hsp). These interactions are requisite for proper protein folding and assembly of stable receptor–hsp heterocomplexes that are competent to bind ligand [169]. Upon binding hormone, steroid receptors undergo a conformational change that causes dissociation of these hsp and allow receptors to dimerize [44]. Activated receptors bind directly to specific steroid response elements (SREs) and SRE-like sequences in the promoter regions of target genes [111,205]. Binding of receptors to DNA increases or decreases gene transcription by altering the rate of recruitment of general transcription factors and influencing the recruitment of RNA polymerase II to the initiation site [89,88]. Thus, in brain it is thought that steroids can act via their respective receptors to alter neuronal gene transcription, resulting in profound changes in behavior and physiology [167,16].

### 3. General mechanisms of molecular action of nuclear receptor coregulators

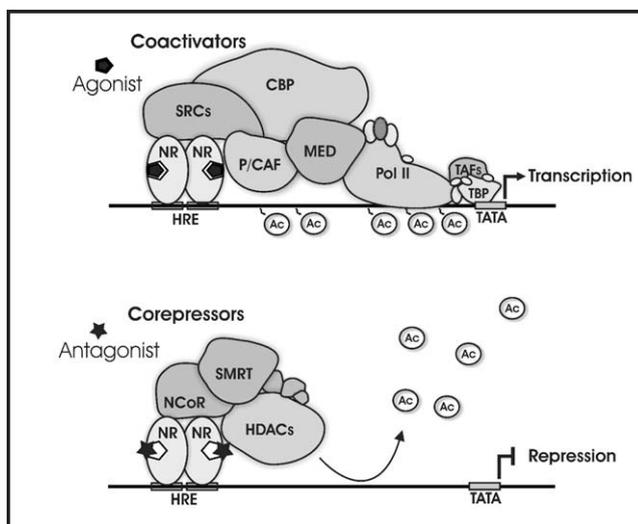
Nuclear receptor coregulators are required for efficient transcriptional regulation by nuclear receptors [176,156] (Fig. 1). Nuclear receptor coregulators dramatically enhance the transcriptional activity of nuclear receptors, including ER and PR [176,156]. *In vitro* studies using antibodies against nuclear receptor coregulators indicate that recruitment of coregulators is rate-limiting in steroid receptor-mediated gene transcription [176,203]. In further support for nuclear receptor coregulator-dependent facilitation of transcription *in vitro*, squelching, or the repression of the transcriptional activity of one steroid receptor by another, is reversed by the addition of coregulators [159]. Thus, a critical component of efficient steroid receptor transcription is the recruitment of nuclear receptor coregulators, which dramatically enhance transcriptional activity. Under most conditions, steroid receptors interact with coregulators in the presence of an agonist, but not in the absence of ligand or in the presence of an antagonist or a selective receptor modulator [159,120,196,186] but *c.f.* with findings revealing coregulator interactions with antagonist-bound receptors [160,221,50]. While nuclear receptor coregulators usually interact with the C-terminal AF-2 of the receptor [120,160,123,173,216,91], there are coregulators that associate with the N-terminus of the receptor [219]. Nuclear receptor coregulators influence receptor transcription through a variety of mechanisms, including acetylation, methylation, phosphorylation, chromatin remodeling and mRNA splicing [176,105]. The importance of these coregulators in a variety of human diseases, including cancer and some neurological disorders, is becoming more apparent [107].

### 4. Nuclear receptor coregulators of steroid receptors

#### 4.1. The p160 family

Steroid receptor coregulator-1 (SRC-1/NcoA-1) was one of the first coregulators found to interact with hormone-bound steroid receptors [159]. SRC-1 is a member of a larger family of p160 proteins that includes SRC-2 (also known as GRIP1, TIF2 and NCoA-2) [216,71] and SRC-3 (AIB1, TRAM-1, p/CIP, ACTR and RAC3) [6,194]. The SRC family of coregulators physically interacts with steroid receptors, including ER, PR and GR, in a ligand-dependent manner [176,156,159]. The SRCs physically associate with agonist-bound receptors through multiple LXXLL motifs (L, leucine; X, any amino acid) that make up nuclear receptor (NR) boxes [227]. *In vitro* experiments reveal that depletion of SRC-1 in cultured cells by micro-injection of antibodies to SRC-1 prevents receptor-dependent transcription, suggesting that SRC-1 is important for transcriptional activity of steroid receptors [203]. In cell culture, hormone induced transactivation of PR is reduced by coexpression of ER $\alpha$ , presumably due to squelching or sequestering of shared coregulators [159]. This squelching can be reversed by over-expression of SRC-1, suggesting that coregulators are a limiting factor necessary for full transcriptional activation of receptors [159]. In further support, over-expression of SRC-1 relieves thyroid hormone receptor inhibition of ER $\alpha$ -mediated transcription of the preproenkephalin gene in transient transfection assays in CV-1 cells [211].

The SRC family of coregulators appear to act as a platform for the recruitment of other coregulators (Fig. 1), including CREB-binding protein (CBP) and p300/CBP-associated factor (p/CAF), that possess histone acetyltransferase activity and aid in chromatin remodeling [122,83]. In addition, SRC-1 itself is known to possess endogenous histone acetyltransferase activity [192]. The p160 coregulators contain two activation domains, AD1 and AD2, in the C-terminal region. AD1 mediates interactions with CBP [31],



**Fig. 1.** Simplified models of coactivator and corepressor matrices. Top panel shows how agonist-bound nuclear receptors associate with coactivators and other proteins to regulate gene transcription. Coactivators can increase gene transcription via the acetylation of histones and through the recruitment and stabilization of the transcriptional complex. Bottom panel shows how antagonist-bound nuclear receptors can associate with corepressors and additional proteins to repress gene transcription. Corepressors can repress gene expression by causing the deacetylation of histones via the recruitment of HDACs to the genome. HRE, hormone response element; NR, nuclear receptor; SRCs, p160 steroid receptor coactivator family; CBP, CREB-binding protein; p/CAF, p300/CBP-associated factor; MED, mediator complex; HDACs, histone deacetylases; Ac, acetyl groups; Pol II, RNA Polymerase II; TBP, TATA-binding protein; TAFs, TBP-associated factors and TATA, TATA box.

while AD2 allows binding of other proteins, including the protein arginine methyltransferase CARM1 [33].

While much is known about the molecular mechanisms of nuclear receptor coactivators from a variety of *in vitro* studies [176,106], we are just beginning to understand their role in hormone action *in vivo*. SRC-1 knockout mice, while fertile, have decreased responsiveness in progesterone target tissues [229], partial resistance to thyroid hormone [222] and delayed development of cerebellar Purkinje cells [147]. However, it should be noted that SRC-2 is up-regulated in steroid-sensitive tissues, including brain and testes, suggesting that increased expression of this coactivator compensates for the loss of SRC-1 [229]. In addition, SRC-1 is critical in maintaining energy balance by regulating both energy intake and expenditure [218]. As discussed below, SRC-1 has profound effects in brain on hormone-dependent development, gene expression and behavior. It is interesting to note that SRC-1 exists in at least two isoforms, SRC-1a and the truncated SRC-1e [82,125], which appear to have different functions [82,209].

As is the case with SRC-1, SRC-2 enhances transcriptional activity of a variety of nuclear receptors, including ER, PR and AR [216,71]. The mid-region of the SRC-2 protein, which mediates interactions with steroid receptors, has relatively low homology with SRC-1, suggesting functional differences between these two proteins [216,71]. SRC-2 knock-out mice reveal that this coactivator is important in fertility and ductal branching in mammary gland [59,140,53]. Generation of mice in which SRC-2 is ablated specifically in cell types that express PR (PR<sup>Cre/+</sup>SRC-2<sup>flox/flox</sup>) have allowed the investigation of the function of this coactivator in progesterone action [53]. Disruption of SRC-2 expression in uterine PR-positive cells led to an early block in embryo implantation. Furthermore, removal of SRC-1 in PR<sup>Cre/+</sup>SRC-2<sup>flox/flox</sup> uteri caused a block in decidualization, suggesting that both SRC-1 and SRC-2 are required for complete PR-dependent decidualization. In addition, SRC-2 is important for PR action in mammary gland as demonstrated by the lack of significant branching and alveolar morphogenesis in the PR<sup>Cre/+</sup>SRC-2<sup>flox/flox</sup> mammary gland [53,139]. Microarray analysis of uteri from SRC-2 null mice reveal that SRC-2 is involved in the ability of progesterone to repress specific genes involved in a variety of functions, including cell cycle and immunity [80].

SRC-3/AIB1, which is amplified in human breast tumors [6], coactivates a variety of nuclear receptors, including ER and PR [203,6,102]. Phosphorylation of SRC-3 is thought to be important for its ability to coactivate specific steroid receptors and has been associated with its oncogenic properties [226]. Female SRC-3 null mice, while fertile, have delayed puberty, longer estrous cycles, ovulate fewer eggs and have impaired mammary gland development [230,63]. Recent studies in SRC-3 null mice reveal that this coactivator is critical for normal PR-dependent mammary gland development and function [63]. Using chromatin immunoprecipitation (ChIP) assays, gonadotropin-releasing hormone (GnRH) stimulated more efficient recruitment of SRC-3 by PR, on the progesterone response element of a luciferase reporter gene or the gonadotropin  $\alpha$  subunit gene promoter, than progesterone [5]. These findings suggest that phosphorylation of PR and its interaction with SRC-3 and binding to DNA may play an important role in the possible ligand-independent activation of PR by GnRHs [5].

## 4.2. Other coactivators of steroid receptors

### 4.2.1. CREB-binding protein (CBP)

While CBP was initially discovered to be a transcriptional activator of cAMP response element-binding protein (CREB) [36,98], it is also now known to function as an integrator of nuclear receptors with other cell signaling pathways, including CREB and AP-1 [83,98,232]. As is the case with the p160 family, CBP is important

in ligand-dependent transcriptional activity of nuclear receptors, including ER and PR [189]. While p300 is closely related to CBP, genetic knockout mice for CBP and p300 exhibit different phenotypes, suggesting a functional distinction of these coactivators [215]. Interestingly, mutation of the CBP gene causes Rubinstein-Taybi syndrome, which results in severe mental retardation and a variety of physiological deformities in humans [166]. In mice, mutations of CBP lead to similar physical deformities as well as impaired memory [154]. A variety of *in vitro* studies indicate that SRC-1 and CBP act synergistically to enhance ER and PR transcriptional activity and function [189,199,104,231]. In support of this concept, SRC-1 physically interacts with CBP and recruits it to the coactivator complex to form a ternary complex at target gene promoters [83,189]. For ligand-bound PR to induce transcription of target genes, SRC-1 must be recruited to the receptor dimer complex first, followed by CBP [104]. Deletion of either the CBP/p300-binding site, or the C-terminal region containing the PR-binding site, of SRC-1 dramatically reduces PR transactivation [104].

### 4.3.1. E6-associated protein (E6-AP)

E6-AP was originally identified as an E3 ubiquitin ligase [73,74] and is best known for its role in Angelman Syndrome. Nawaz et al. [144] demonstrated that E6-AP acts also as a coactivator for steroid receptors, including PR, ER, AR, GR, as well as RAR $\alpha$  and TR. The binding of E6-AP to nuclear receptors is hormone-dependent and the over-expression of this coactivator can reverse the squelching between ER and PR. E6-AP contains an intrinsic activation function independent of the ligase activity and only the loss of ligase activity, and not the coactivator function, is responsible for the symptoms of Angelman Syndrome. It should be noted that the ubiquitin ligase activity and the coactivator function are both necessary during the transcription process. Although the exact identity of the ubiquitin ligase(s) involved in the proteolysis of the steroid receptors is currently controversial, some results suggest that the stability of ER $\alpha$  and AR is directly linked to the expression levels of E6-AP [58,87]. Moreover, E6-AP is required for the degradation of the coactivator SRC-3 [114] and may also target itself [149]. E6-AP is expressed in most brain regions, including the hippocampus, cerebellum, cortex and thalamus [45]. It is also expressed in peripheral tissues and has been shown to be important in various aspects of reproduction, including the hormonal induction of breast and prostate growth, sperm production and ovulation [191]. Electron microscopic studies in various cell lines found that E6-AP is present in both cytoplasm and nucleus, in agreement with the ubiquitin ligase and nuclear receptor coactivator activities [207].

### 4.3.2. Estrogen receptor-associated protein 140 (ERAP140)

ERAP140 interacts in a ligand-dependent manner with, and enhances the transcriptional activity of, ER $\alpha$ , ER $\beta$ , TR, PPAR $\gamma$  and RAR $\alpha$ . Even in the absence of the classical LXXLL motifs common for most coactivators, ERAP140 interact with ER $\alpha$  through a binding site similar to that of other coactivators [184]. Contrary to the wide distribution of many nuclear receptor coregulators, high levels of expression of ERAP140 is mainly restricted to brain tissue and kidney, with very low levels in mammary gland, lungs and testis and undetected in liver or intestine. *In situ* hybridization studies suggest an exclusive neuronal localization, in areas such as cortex, hippocampus, thalamus, hypothalamus and cerebellum [184].

### 4.3.3. Steroid receptor RNA activator (SRA)

SRA is a distinctive coactivator in that it functions as an RNA transcript to enhance transcriptional activity of steroid receptors, including PR, ER, GR and AR [24,99]. While liganded ER reduced

PR transcriptional activation, addition of SRA reversed this quenching effect of ER [99]. Treatment of cells with SRC-1 and SRA antisense, greatly reduced activity of ER $\alpha$  or PR [24,99]. Antisense to either SRA or SRC-1 alone had a less dramatic effect on ER $\alpha$  activity, suggesting SRA association with SRC-1 [24]. In further support, SRA co-purified with SRC-1, indicating that SRA exists in a ribonucleoprotein complex containing SRC-1 [99]. Expression of SRA is tissue specific, with Northern blot analyses revealing that SRA mRNA expressed at high levels in liver, skeletal muscle and heart, and at lower levels in brain and placenta [99]. Finally, over-expression of SRA in transgenic mice reveals a role for SRA in estrogen-induced expression of PR in mammary gland [100].

#### 4.3.4. Ribosomal large subunit protein 7 (RPL7)

Similar to E6-AP, RPL7 (also known as L7/SPA) was not identified as a coregulatory protein at first, but as a cytoplasmic and nuclear protein involved in translational regulation [146]. It was only later that a dual role of RPL7 in both transcription and translation was described [127,66,217]. RPL7 increases the transcriptional activity of ER and PR only when bound to the selective ER modulator (SERM), tamoxifen, or the selective PR modulator (SPRM), RU486, respectively [78]. In contrast, estradiol, or the full estrogen receptor antagonist ICI 164,384, does not elicit binding of RPL7 to ER [78]. RPL7 is expressed in estrogen-sensitive regions of reproductive organs, including the uterus [145,69]. In zebra finch brain, RPL7 is expressed at high levels in the song system of the adult male [47]. Furthermore, RPL7 mRNA concentration is higher in male telencephalon the day of hatch (P1) and in adult compared to female [47]. Western blot analyses suggest that the expression of three different functional peptides of RPL7, the full length peptide (44 kDa), the ribosomal functional unit (27 kDa) and the coactivator functioning peptide (31 kDa), is sex and age dependent in zebra finches [47]. While the role of RPL7 in the modulation of ER in brain remains unknown, it is possible that the sexually differentiated expression of RPL7 in brain is involved in modulating retinoic acid or vitamin D action, both of which are important in brain development [109,119]. Although this has not been directly studied in brain, another mechanism by which RPL7 functions to modulate vitamin D receptor is by interfering with the binding of the receptor to its response element [15]. When vitamin D receptor binds hormone, it can form a heterodimer with retinoid X receptor (RXR). This dimer can then recognize a specific vitamin D response element and enhance transcription of target genes. In the presence of RPL7, the heterodimer is no longer able to enhance transcription due to repression of dimer-binding to the response element by RPL7. Depletion of RPL7 by antibodies restored binding of the heterodimer on the response element [15]. Thus, RPL7 can coactivate antagonist-bound steroid receptors and repress ligand-bound VDR in the presence of RXR. Therefore, because the actions of RPL7 are context-dependent, the term “selective coactivator” has been used to describe this coregulator and others [158]. In future studies it will be important to determine the biological function of RPL7 in brain.

Finally, there are a variety of other coactivators, including TRAP220 [77], PGC-1 [197], chromatin high mobility group proteins 1 and 2 [19] and TIP60 [22], that are known to interact with ER and PR. Thus, there is much more to be learned about the function of coactivators in steroid receptor action. In addition to the study of individual nuclear receptor coactivators, it should be noted that coregulators are usually present in preformed complexes, or “coregulatorsomes”, ready to be recruited when needed [122]. Therefore, it is important to determine the different coregulators involved in specific physiological responses and define whether these coactivators act together, in parallel, or independently from each other, in the modulation of nuclear receptor action.

#### 4.4. Nuclear receptor corepressors

##### 4.4.1. Overview of nuclear receptor corepressors in gene silencing

While much is being discovered about the functional roles of steroid receptor coactivators in modulating steroid receptor function at the genome, less is known about the role of corepressors, especially in brain. However, the recent expansion in investigating the epigenetic suppression of gene transcription will allow for a better understanding of how these repressor molecules also regulate gene transcription. Whether or not the diversity of directional coding exists with corepressors, as it does with coactivators, is yet to be determined. That is, it is becoming clear that coactivator molecules can be modified by phosphorylation, methylation, ubiquitination, sumoylation, and acetylation, which can determine the functional role of the transcriptional complex [176,106,188]. While the major function of nuclear receptor coactivators is to increase gene transcription, corepressors generally suppress or silence gene transcription (Fig. 1). The basic model of gene silencing occurs following methylation of DNA at CpG sites by a particular DNA methyltransferase (DNMT) [171,90]. While methylation alone can interfere with protein-binding, it is the binding of methyl-CpG-binding proteins (MBDs), which subsequently recruit corepressor and histone deacetylase (HDAC) repressor complexes, that mainly result in gene repression. Some notable repressor complexes that form on DNA to repress gene transcription are the Sin3, NuRD, CoREST, and the NCoR/SMRT repressor complexes [40]. These multi-protein complexes are associated with relatively unique combinations of HDACs and transcriptional regulating factors.

While the components of corepressor complexes are still being identified, there are some shared and some unique molecules that make up these different corepressor complexes [40]. For example, the Sin3 and NuRD complexes both contain HDAC1, HDAC2, RbAp46 and RbAp48; however, the Sin3 complex also contains Sin3a, SAP18 and SAP30; whereas, the NuRD complex contains MBD3, MTA-2 and Mi-2. The CoREST complex contains HDAC1, HDAC2, CoREST, SHARP and Sin3. Lastly, the SMRT/NCoR complex contains HDAC3, SMRT and NCoR. It is likely that these different combinations contribute to different functional consequences on the genome. While NCoR and SMRT can function within the same complex, they can act independent of each other to regulate steroid receptor function and gene transcription. The recruitment of corepressor complexes to DNA can occur via interacting with steroid receptors directly or by interacting with the MBDs-bound to methylated CpG sites. Thus, there exists the potential for specification of function by which complexes are formed on DNA. Further specification is obtained by MBDs exhibiting distinct associations with particular corepressors complexes. For example, MeCP2 associates with the Sin3 corepressor complex, whereas, MBD2 is associated with the NuRD complex. The general mechanism by which corepressor complexes suppress gene expression is by deacetylating the histones via the HDACs in the complex [40]. The removal of acetyl groups on the histones restores a positive charge on the histone tails, allowing them to become tightly associated with DNA, condensing DNA, and ultimately suppressing gene transcription. Thus, corepressors oppose the biological properties of coactivators, which lead to the acetylation of histones to increase gene transcription. The first corepressors of nuclear receptors to be identified were NCoR (nuclear receptor corepressor) [72] and SMRT (silencing mediator of retinoid and thyroid hormone receptors) [30].

##### 4.5. Nuclear receptor corepressor (NCoR)

NCoR was one of the first corepressors of nuclear receptors to be discovered and was identified via its interaction with thyroid receptors [72]. Unliganded thyroid hormone receptors bind DNA

and suppress gene transcription. It was hypothesized that additional proteins are bound to unliganded thyroid receptors on DNA that are responsible for gene repression. This led to the discovery of a 270 kDa protein bound to unliganded thyroid receptors which was termed NCoR [72]. While the functional role of NCoR is still being elucidated, its presence appears to be critical for survival as global deletion of NCoR is embryonic lethal [81]. Interestingly, this suggests that the loss of NCoR function cannot be compensated by another corepressor, in contrast to the loss of SRC-1 which can be partially compensated by increased SRC-2 [229]. While NCoR is known to repress the activity of thyroid hormone [9], it is believed that NCoR may be a non-selective repressor of nuclear receptor function. However, the mechanism by which NCoR suppresses gene transcription seems to have some selectivity with which HDAC it associates with on DNA. In particular, NCoR appears to assemble with HDAC3 and SMRT [84]. NCoR has also been associated with the Sin3 corepressor complex via its interaction with Sap30. The transcriptional repression by NCoR appears to occur mainly via its associations with Sin3 and HDACs [2,65,143]. Interestingly, while NCoR and SMRT co-immunoprecipitate with many of the same molecules, the methyl-CpG-binding protein, Kaiso, appears to associate only with NCoR [235]. Due to NCoR's association with particular methyl-binding proteins, such as MeCP2 and Kaiso, it has been suggested that NCoR plays a larger role in methyl-directed gene repression. As NCoR forms distinct corepressor complexes, it is likely to be involved in the regulation of a small subset of genes.

Differences in the sub-cellular localization of NCoR have been observed and appear critical for normal cell development and function. For example, activation and cytoplasmic localization of NCoR allows neural stem cells to differentiate into astrocytes [67]. This would suggest that NCoR functions during development as a key factor inhibiting neural differentiation into glia. It also supports the concept that sub-cellular localization of coregulator proteins can dictate not only gene transcription, but also cellular differentiation. The differential sub-cellular localization of NCoR can also be associated with abnormal cell function, such as the increased cytoplasmic localization following abnormal IKK $\alpha$  activation in colorectal cancer cells [143]. Interestingly, the presence of NCoR within a cell can also dictate whether the SERM tamoxifen can be agonistic or antagonistic on ER function [183]. If NCoR is present, then tamoxifen blocks ER function. However, in the absence of NCoR, SRC-1 is recruited to the complex resulting in a tamoxifen-induced increase in ER activity [183]. Therefore the presence and sub-cellular localization of NCoR, as well as the availability of coactivators, can be critical for normal cell function.

#### 4.6. Silencing mediator of retinoid and thyroid hormone receptors (SMRT)

While SMRT represses thyroid hormone receptor function and interacts with NCoR [30], it is also known to modulate ER activity in a ligand-dependent and cell-type specific manner. Some studies in MCF-7 cells suggest that SMRT may be involved in the suppressive actions of raloxifene on ER activity. For example, while estradiol binding to ER causes recruitment of coactivators and increased expression of IGF-1 and c-Myc, raloxifene binding to ER causes the recruitment of NCoR, SMRT, and HDAC2 [179]. Interestingly, while depletion of NCoR in MCF-7 cells can increase estradiol-induced ER activity; in the same study, depletion of SMRT led to a reduction of estradiol-induced ER activity [165]. These findings suggest that while SMRT is involved in gene silencing of thyroid hormone receptors, its presence appears to be important for full ER activity within a cell. It is likely that this enhancement of ER activity is occurring through a direct interaction with ER or coactivators, rather than through the formation of the SMRT/NCoR

repressor complex. These data indicate that the biological role of SMRT is functionally different than NCoR, even though they can appear within the same corepressor complex. It is likely that the functional role of SMRT and NCoR in either enhancing, or suppressing, gene transcription is dependent upon the sub-cellular localization and expression of coactivators and corepressors within a cell. Therefore, it seems to be important to determine the cellular diversity of coactivators and corepressors in order to predict a biological outcome on DNA.

## 5. Nuclear receptor coregulator function in brain and behavior

### 5.1. Nuclear receptor coactivators

#### 5.1.1. Neuroanatomical expression and regulation of coactivators

While much is known about the molecular mechanisms of nuclear receptor coactivators from a variety of cell culture studies [176,156], we are just beginning to understand their role in hormone action in brain. SRC-1 mRNA and protein are expressed at high levels in the cortex, hypothalamus, hippocampus and cerebellum of rodents [147,129,185,116,124,11,133,153] and birds [25,26]. SRC-2 is also expressed at high levels in the hypothalamus and hippocampus [147,118,7], while SRC-3 is mainly expressed in the hippocampus [147,7]. Male and female guinea pig fetuses have high expression of SRC-1 mRNA and protein in the limbic system, which decrease in the hippocampus with advancing gestation [182]. In contrast, SRC-1 mRNA levels increase in the guinea pig dentate gyrus near birth [182]. In addition to the p160s, other nuclear receptor coactivators, including CBP, TRAP220 and ERAP140, are expressed at high levels in steroid-sensitive brain regions [184,133,193,12,57]

In order for coactivators to function with steroid receptors, they must be expressed in the same cells. Indeed, SRC-1 is expressed in the majority of estradiol-induced PR cells in reproductively-relevant brain regions, including the VMN, medial preoptic area and arcuate nucleus [201]. In addition, the majority of estradiol-induced PR cells in these same brain regions coexpress CBP [201]. Given that virtually all estradiol-induced PR cells in the hypothalamus contain ER $\alpha$  [17,220], these findings suggest that these specialized cells represent functional sites of interaction between ovarian steroid receptors and coactivators (SRC-1 and CBP) in brain [201]. These findings provide neuroanatomical evidence that nuclear receptor coactivators are integral in mediating steroid hormone action in reproductively-relevant brain regions. It is important to note that not all ER and PR cells express SRC-1 [153,201], suggesting that the receptors in these cell populations use other coactivators. Thus, different populations of ER cells, as defined by the coactivators they express, could have different responses (*i.e.* activation of different target genes) to ligand.

Because coactivators play a major role in the transcriptional activity of nuclear receptors, it has been suggested that different nuclear receptors present in the same cell could compete for the same coregulator protein [126]. The decrease of one coactivator would increase the competition between different hormonal systems and thus reduce the response of one or both of the hormone systems. For example, it seems that the thyroid-mediated decrease of estrogen-induced sexual behavior is controlled at the transcriptional level though the competition by TR and ER for SRC-1. The coactivator would thus be a limiting factor in hormone-mediated gene expression [43,42,212]. Therefore, it is important to evaluate the potential regulation of coactivator concentrations by hormones and other factors.

A limited number of studies have focused on the potential regulation of coactivator expression in brain. Misiti and colleagues [129] were the first to suggest a sex difference in coactivator

expression, with the concentration of SRC-1 mRNA higher in the pituitary gland in adult male rats compared to females. This difference was later confirmed at the protein level [20]. Sex differences in SRC-1 expression were also observed in the song control nuclei HVC and Area X in canaries [26] and in the preoptic area/hypothalamus region in Japanese quail [25,28]. While a sex difference in SRC-1 expression was not detected in the hypothalamus of adult rats [20], likely candidates to explain these sex differences in adult birds are 17 $\beta$ -estradiol and testosterone. 17 $\beta$ -estradiol treatment reduces the levels of SRC-1 in rat pituitary and *in vitro* [129], while treatment with this same hormone reverses the down-regulation of SRC-1 expression in the rat ventromedial hypothalamus caused by ovariectomy [131]. In the hypothalamus of cycling female rats, SRC-1 levels were lowest during diestrus, and highest at proestrus and estrus [23]. Interestingly, the endocrine disruptor 4-methylbenzylidene camphor (4-MBC), which has estrogenic activity and also interferes with the thyroid axis, alters SRC-1 expression in brain [110]. Pre- and post-natal exposure to 4-MBC increased SRC-1 mRNA in the VMH and MPOA of female rats [110], which could further accentuate the estrogenic effect of this disruptor and alter other nuclear receptor signaling pathways.

In castrated male Japanese quail, testosterone treatment increases the level of SRC-1 mRNA and protein in the preoptic area region/hypothalamus [28]. However, testosterone had no effect on SRC-1 expression in the male hamster medial preoptic region, BNST, arcuate nucleus and amygdala [200], in the male rat hypothalamus [118] or in the telencephalon of male zebra finches [48]. While anabolic androgenic steroids did not alter SRC-1 expression in the hypothalamus of male rats, testosterone decreased SRC-2 protein in this brain region [118]. Finally, thyroid hormone decreases expression of SRC-1 in neonatal mouse cerebellum [170] and rat cortex and dentate gyrus [75].

In addition to gonadal steroids, there is evidence that glucocorticoids regulate SRC-1 [97]. Stress and the associated increase in glucocorticoids were shown to reduce SRC-1 mRNA in the brain, as well as in the stomach, heart, liver and kidney [97]. More specifically, a reduction of SRC-1 expression in the hypothalamus of both male and female rats is found after acute restraint stress [20]. Interestingly, acute stress also decreased SRC-1 in the frontal cortex in male rats, but increased its concentration in the male pituitary and the female hippocampus [20]. While chronic exposure of adrenalectomized male rats to relatively high levels of corticosterone did not affect SRC-1 mRNA levels in the hippocampus, it did lead to a down-regulation of the SRC-1 splice variant, SRC-1e, in the anterior pituitary [125]. In addition, stress blocks the testosterone-induced increase in SRC-1 expression in brains of castrated male quail [28]. Taken together, these studies suggest that coactivators are important in modulating the glucocorticoid-mediated stress response.

In addition to steroids and stress, it appears there is photoperiodic regulation of SRC-1 expression in brain. SRC-1 concentration fluctuates through the day in Japanese quail in the hippocampus, hindbrain and optic lobes, independently of sex, testosterone treatment and stress [28]. In male Siberian hamsters, short days reduced SRC-1 expression in the posteromedial bed nucleus of the stria terminalis and posterodorsal medial amygdala [200]. This photoperiodic regulation of SRC-1 may modulate androgen regulation of seasonal reproduction. Both the Japanese quail and Siberian hamster show an important seasonal cycle, with high levels of gonadal steroids during reproduction in the spring and low to undetectable levels in the winter when reproduction is suppressed [164,175]. Superimposed on these seasonal changes are the fluctuations of steroid release during the day with high levels early in the morning and low levels during the night [93]. It may be that the central nervous system responds to changes in circulating levels of steroids by altering the concentration of available coactivators.

While coactivators are regulated at the transcriptional level, numerous studies indicate that post-translational modifications of coactivators play a major role in modulating the properties of these important proteins. The total concentration of the coactivator, as measured at the mRNA or protein level, is sometimes not sufficient to determine whether the coactivator will function with a specific nuclear receptor. Numerous studies have shown that coactivators such as the SRCs, CBP and peroxisomal proliferator-activated receptor  $\gamma$  coactivator-1alpha (PGC-1alpha) are modified by phosphorylation, methylation, acetylation and sumoylation [35,29,32,92,177]. For example, phosphorylation of SRCs is essential for optimal activity of the coactivator, notably through the regulation of protein–protein interactions. Phosphorylation of SRC-1 by cAMP enhances both ligand-dependent and -independent activity of PR [177]. In addition, phosphorylation is important for the physical and functional interaction of SRC-1 [177] and SRC-3 [226] with CBP. This phosphorylation of the SRCs is induced by multiple kinases, including MAPK and I $\kappa$ B kinases, which are involved in a variety of cellular signaling pathways [226,225]. Post-translational modifications also seem to be involved in defining the specificity of coregulators for a given nuclear receptor. Because nuclear receptor coactivators interact with a broad range of transcription factors, it has been suggested that coactivators could distinguish one downstream class of transcription factors from another, so as to prevent global gene activation [227]. Post-translational modifications of the coactivators, such as phosphorylation, seem to be involved in the specificity of these interactions with various transcription factors. Androgens or estrogens induce phosphorylation at six specific amino acids of SRC-3, all of which are required for the transactivation of target genes by ER [226]. But the phosphorylation of only five specific sites on SRC-3 is induced by TNF $\alpha$ -stimulated signaling pathway and is required for transactivation of NF- $\kappa$ B [226]. In addition to influencing protein–protein interactions between the coactivator and a specific nuclear receptor or another coactivator, post-translational modifications are involved in sub-cellular distribution [3,34,70], stabilizing the protein [70,228] and regulation of enzymatic activity [68,234]. Finally, studies suggest that degradation of coactivators is an important aspect of their regulation. Treatment with a 26S proteasome inhibitor (MG132) increases SRC-1 expression in the hypothalamus, preoptic area and hippocampus [214]. In addition, this same treatment up-regulated PR and ER $\beta$  in the same brain regions, while ER $\alpha$  was increased in the POA only [214]. These findings suggest that degradation of important components of steroid action, the receptors and coactivators, are regulated in a brain region-specific manner.

The study of coregulator expression and post-translational modifications has most often focused on one or a few specific coregulators. However, it is important to note that coactivators are not assembled into static and stable complexes, but rather exist in dynamic complexes that are rapidly dissociating and recruiting other coregulatory proteins. In addition, these coregulators are constantly undergoing a variety of post-translational modifications that may further alter their function [121] and also see [www.nur-sa.org/10.1621/datasets.01001](http://www.nur-sa.org/10.1621/datasets.01001) for datasets. Relatively little is known about the control of the expression of the proteins in these complexes in a brain region of interest or in defined cell types. All together, cellular differences in coactivator ratio, as well as their unique post-translational modifications, are likely to be involved in cell-specific responses to hormone in a promoter and ligand-dependent manner [236,10].

### 5.2.1. Hormone-dependent brain development

Steroid hormones act within the developing brain to have lasting organizational effects on adult sexually dimorphic brain physiology and behavior. On the day of birth, testosterone

secretion from the testis results in masculinization (increased adult male sexual behavior) and defeminization (decreased adult female sexual behavior) of adult reproductive behavior. In the brain, testosterone is converted into dihydrotestosterone and estradiol, which then bind to AR and ER, respectively. To determine if nuclear receptor coactivators are necessary for the organizing actions of steroid hormones in developing brain, antisense oligonucleotides were targeted at SRC-1 mRNA within the hypothalamus during the first few days of life and their impact on steroid-mediated differentiation of the sexually dimorphic nucleus (SDN) of the preoptic area and adult sexual behavior was assessed [11]. It is well known that testosterone exposure, via its aromatization and binding to ERs, increases the size of the female SDN [46,117]. It was found that testosterone-treated females receiving hypothalamic SRC-1 antisense infusions during development had a smaller SDN size compared to testosterone-treated control females, suggesting that interfering with SRC-1 disrupts differentiation of the SDN [11]. To test if SRC-1 was important in mediating the long-term steroid-mediated differentiation of adult sexual behavior, males, testosterone-treated females, and control females were infused with either SRC-1 antisense, scrambled controls or vehicle into the hypothalamus during the first few days of neonatal life and sexual behavior was examined in adulthood. As neonatal testosterone exposure defeminizes adult sexual behavior, it was confirmed that males and testosterone-treated females receiving control infusions exhibited lower levels of adult female sexual behavior contrasted to control females [11]. However, males and testosterone-treated females infused with SRC-1 antisense during the neonatal period exhibited high levels of adult female sexual behavior that were comparable to control females, suggesting that SRC-1 is critical in mediating the defeminizing actions of testosterone on adult female sexual behavior. Interestingly, there were no differences between rats treated with SRC-1 antisense or controls in the number of mounts directed toward an estrous female or in the latency to mount an estrous female. These findings suggest that reducing SRC-1 had no long-term effect on the organization of male sexual behavior. Therefore, SRC-1 appears crucial for testosterone-mediated defeminization of female sexual behavior, but not for testosterone-mediated masculinization of male sexual behavior. In addition, SRC-1 appears to have a role in development of other brain regions. SRC-1 null mice have delayed development of cerebellar Purkinje cell precursors at an early embryonic stage, delayed maturation of Purkinje cells after birth, and impaired motor function in adulthood [147]. These findings suggest that SRC-1 is important in Purkinje cell development and normal motor function [147].

The divergent outcome of SRC-1 in the development of sexual behavior appears to be the same for the role of CBP in steroid-mediated sexual differentiation of adult sexual behavior. In a similar follow-up study, the role of CBP in sexual differentiation of adult sexual behavior was examined. It was hypothesized that reducing postnatal CBP levels within the hypothalamus would block the defeminizing actions of testosterone on adult female sexual behavior in female rats [13]. As predicted, testosterone-treated female rats infused with CBP antisense neonatally exhibited higher levels of female sexual behavior in adulthood contrasted to scrambled controls. This indicates that reducing CBP expression interfered with testosterone's ability to defeminize or reduce female sexual behavior. Comparable to SRC-1 antisense infusions, reducing CBP expression did not interfere with the masculinization of male sexual behavior. Although these data do not rule out a possible role for CBP and SRC-1 in modulating the development of some components of male sexual behavior, they do suggest that some coactivators, such as CBP and SRC-1, may be more important in processes mediating defeminization than those mediating masculinization of sexual behavior. It is possible that both CBP and SRC-1 need to

be reduced in order to influence masculinization. Indeed, as discussed below, simultaneous reduction of both CBP and SRC-1 was required to interfere with steroid-induced sexual behavior in adult female rats [133].

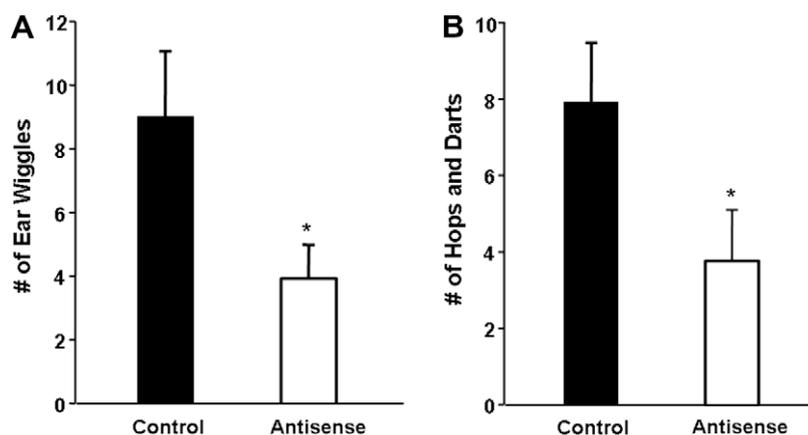
#### 5.2.2. Hormone-dependent gene expression in brain

The function of nuclear receptor coactivators in hormone-dependent gene expression in brain and behavior in adult rodents has also been investigated [133,7]. Estradiol-induction of PR gene expression in the VMN is important for hormone-dependent female sexual behavior [168]. Therefore, studies have addressed if SRC-1 and CBP are critical in modulating ER-mediated transactivation of the PR gene in the VMN. Infusions of antisense oligodeoxynucleotides to SRC-1 and CBP mRNA into one side of the VMN of adult female rats reduced the expression of ER-mediated activation of PR gene expression compared to the contralateral control ODN-treated VMN [133]. These findings are supported by previous *in vitro* studies indicating that SRC-1 and CBP function together to modulate ER activity [189]. In further support of SRC-1 and CBP/p300 functioning together in brain, neurons in the rat hippocampus and dentate gyrus coexpress SRC-1 and p300 [153]. Another study in brain supports these findings and extend them to include a role of SRC-2 in ER-mediated induction of PR in the VMN [7]. This same study found that SRC-3 is not involved in estradiol induction of PR. Finally, the p160 coactivators function in glucocorticoid receptor (GR) action in glial cells [62] and in GR-mediated repression of the corticotropin-releasing hormone gene [209]. Taken together, these findings indicate that nuclear receptor coactivator action in brain is essential for full steroid receptor transcriptional activity.

#### 5.2.3. Steroid-dependent behaviors

Given that nuclear receptor coactivators are critical for hormone-dependent gene expression in brain, it was hypothesized that coactivators act in brain to modulate the expression of hormone-dependent behaviors [133]. Female rats treated with antisense to both SRC-1 and CBP mRNA into the VMN displayed reduced levels of hormone-dependent female sexual receptivity compared to scrambled treated controls [133]. Another study supported these findings with SRC-1 and extended them to include a role for SRC-2 in hormone-dependent lordosis [7]. Moreover, the effects of these nuclear receptor coactivators on specific ER- and PR-dependent aspects of female sexual behavior have been investigated [134]. There are two modes of hormone regulated female reproductive behavior in rats: estrogen-mediated (elicited by estradiol alone) and progesterone-facilitated (requires estradiol priming followed by progesterone) [16]. To test the hypothesis that nuclear receptor coactivators function in brain to modulate ER-mediated aspects of female reproductive behavior, animals were injected with estradiol only [134]. Antisense to SRC-1 and CBP infused into the VMN of animals treated with estradiol alone decreased lordosis intensity, suggesting that these coactivators modulate ER-mediated aspects of female sexual behavior. Proceptive behaviors by the female, which serve to solicit interaction by the male, are PR-dependent and include ear-wiggling and hopping and darting [64,223,198,51,52,150]. Infusion of antisense to SRC-1 and CBP mRNA into the VMN around the time of progesterone administration reduced PR-dependent ear-wiggling and hopping and darting (Fig. 2) [134]. Thus, it appears that nuclear receptor coactivators function in brain to modulate PR and ER action in brain and influence specific aspects of hormone-dependent sexual behaviors in rodents.

The function of coactivators has also been studied in hormone action in bird brain. To test whether SRC-1 was involved in steroid-dependent male sexual behavior in Japanese quail, locked nucleic acid-derived antisense oligodeoxynucleotides were used to



**Fig. 2.** Nuclear receptor coactivators modulate PR action in brain. Female rats were ovariectomized, primed with estradiol and progesterone and exposed to male rats. Infusions of antisense to both SRC-1 and CBP mRNA in the ventromedial nucleus of the hypothalamus of female rats, decreased PR-dependent (A) ear-wiggling and (B) hops and darts compared to scrambled control-treated animals [134]. \* $p < 0.05$ ; one-tailed  $t$  test.

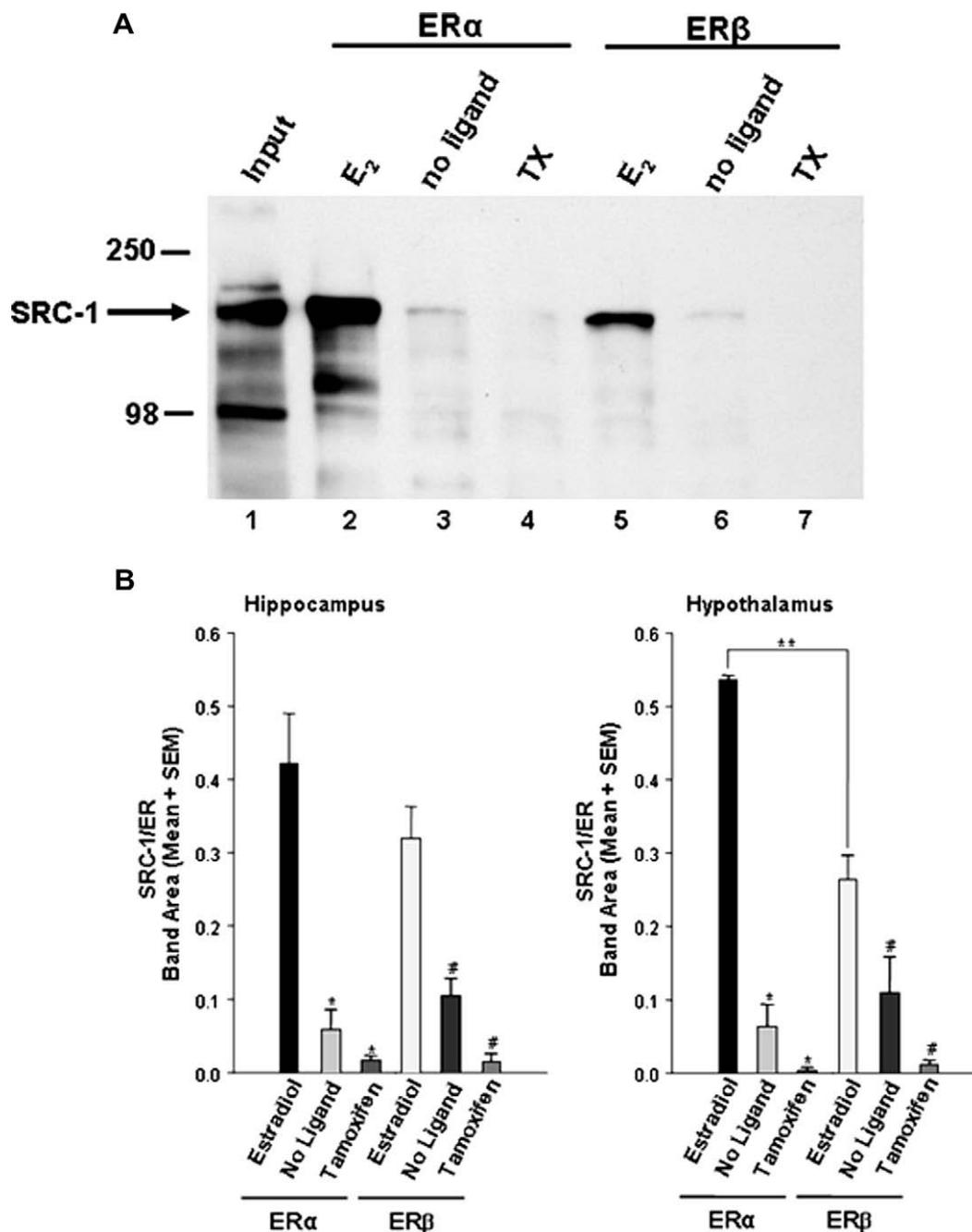
down-regulate the expression of SRC-1 in the medial preoptic nucleus, a key center in the control of male sexual behavior [14]. Reducing the expression of SRC-1 in this region decreased the testosterone-induced mounts and cloacal contact movement (similar to intromission and ejaculation in rat) and the number of struts, a pre- and post-ejaculatory display (Fig. 4A and B). It should be noted that the expression of the copulatory behavior is ER-dependent, while struts are known to be mediated by AR [1,181]. The behavioral inhibition induced by the blockade of SRC-1 expression was paralleled in the medial preoptic nucleus by major neuroanatomical and neurochemical modifications [27]. The volume of the nucleus defined by Nissl staining and by aromatase-immunoreactive material was reduced in SRC-1 depleted males compared to controls (Fig. 4C). The number of vasotocin fibers, another marker of testosterone action [163], was also reduced [27]. The confirmation of a significant role of SRC-1 for steroid-dependent behavior and physiology was obtained from birds in which SRC-1 was up-regulated. This up-regulation of SRC-1 protein in the medial preoptic region was observed 48 h after the termination of the antisense treatment, probably resulting from a rebound of SRC-1 expression (see [27] for further explanation). This up-regulation of SRC-1 expression in the medial preoptic area was associated with a pronounced increase in copulatory behavior and a parallel increase in the volume of the median preoptic nucleus [27]. Taken together, these data strongly support that idea that SRC-1 is an important modulator of estrogen and androgen-dependent gene transcription and behavior in quail brain. Surprisingly, further investigations of the role of SRC-1 on aromatase expression and activity suggested a dissociation between the effect of testosterone on male sexual behavior and the neurochemical plasticity of the median preoptic nucleus. Indeed, while sexual behavior was significantly reduced after inhibition of SRC-1 expression, no change in the preoptic area volume or aromatase activity was observed [28]. The behavioral inhibition is likely to have resulted from an effect of the SRC-1 depletion on other neurochemical systems, such as the peptidergic system, involved in the control of male sexual behavior [162,8]. The extent to which SRC-1 protein expression was down-regulated was smaller in these last studies [28] compared to the first experiment [27]. These studies reveal important information about the relative threshold of effects of steroids (e.g. testosterone) on related traits, such as male sexual behavior and aromatase expression.

#### 5.2.4. Receptor-coactivator interactions

Recently, a proteomics-based approach has been used to study the interactions of steroid receptors with coactivators from rat

brain. To test the hypotheses that SRC-1 from brain physically associates with ER and PR subtypes in a ligand-dependent manner, pull-down assays with brain tissue from female rats were developed [135]. SRC-1 from hypothalamus or hippocampus interacted with ER $\alpha$  and ER $\beta$  when bound to estradiol (Fig. 3A), which was confirmed by mass spectrometry [135]. SRC-1 may function with ER $\alpha$  in the hypothalamus to mediate expression of female sexual behavior [151,142,152,94], and with both ER subtypes in the hippocampus to differentially modulate estrogen's effects on cognition [18,56] and stress [18,76]. Very little to no association of SRC-1 from brain was detected with ER $\alpha$  or ER $\beta$  in the absence of ligand or in the presence of the selective ER modulator (SERM) tamoxifen (Fig. 3A). These findings suggest tamoxifen is functioning as an antagonist to prevent receptor-coactivator interactions, and are consistent with a variety of studies using cell lines demonstrating that estradiol facilitates, while antagonists prevent, SRC-1 association with ER [115,190,233]. In contrast to these findings using brain tissue, cell culture studies suggest that both ER $\alpha$  and ER $\beta$  can recruit coactivators to AF-1 in the absence of ligand under certain phosphorylation conditions [49,204]. In the absence of ligand, little to no interactions between receptor and SRC-1 from brain were detected, and therefore it will be important to investigate whether physiologically-relevant events that modulate ligand-independent activation impact on receptor-coactivator interactions in brain.

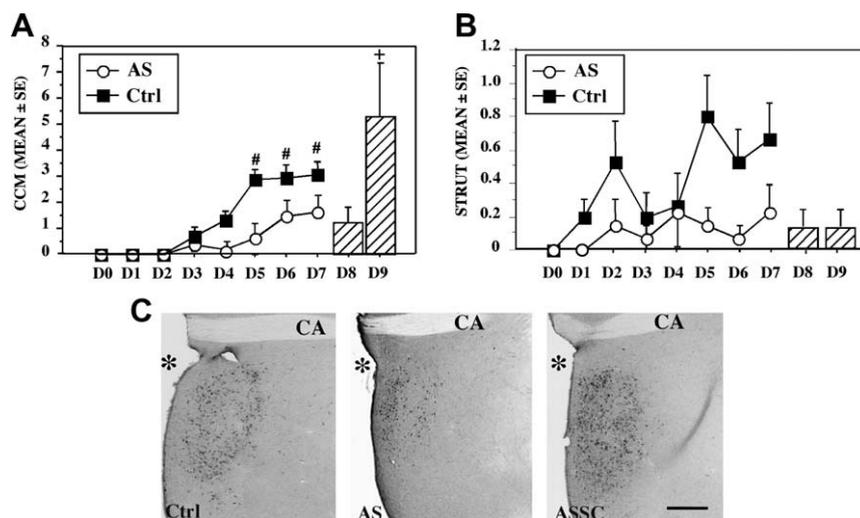
In the same studies discussed above [135], SRC-1 from the hippocampus appears to interact equally with ER $\alpha$  and ER $\beta$  (Fig. 3B). In contrast, SRC-1 obtained from hypothalamic extracts interacted more with ER $\alpha$  than with ER $\beta$  (Fig. 3B). The different functions of the ER subtypes in brain (discussed above) may be explained in part by the lower transcriptional activity of ER $\beta$  observed in particular cell lines [41]. These differences in transcriptional abilities between ER $\alpha$  and ER $\beta$  may be attributed to differential recruitment of coactivators, or differences in the ability of the same coactivator to facilitate transcription of the ER subtypes [224]. While some studies using recombinant SRC-1 [224] are consistent with the findings that SRC-1 from brain interacts more with ER $\alpha$  than with ER $\beta$ , other studies suggest that SRC-1 associates equally with each ER subtype [137,38]. While these later findings are consistent with the results using SRC-1 from hippocampus, it was found that SRC-1 from hypothalamus interacted more with ER $\alpha$  than with ER $\beta$ . These data suggest that ER $\alpha$  is a more efficient transcriptional activator of SRC-1 dependent signaling pathways in the hypothalamus than ER $\beta$  [135]. In support, previous findings indicate that SRC-1 function in the hypothalamus is important for maximal expression of



**Fig. 3.** SRC-1 from rat brain associates with ER $\alpha$  and ER $\beta$  in a ligand-dependent and receptor isoform-specific manner. (A) SRC-1 from the hypothalamus associates with ER $\alpha$  and ER $\beta$  in the presence of estradiol (lanes 2 and 5), but not in the absence of ligand (lanes 3 and 6), or in the presence of the SERM, tamoxifen (lanes 4 and 7). Input (1% of total) of SRC-1 from hypothalamic extract is shown in Lane 1. (B) In the presence of estradiol, both ER $\alpha$  and ER $\beta$  interacted with hippocampal SRC-1, but little to no interactions were detected in the absence of ligand or when receptors were bound to Tamoxifen. Hypothalamic SRC-1 interacted more strongly with ER $\alpha$  than ER $\beta$  in the presence of estradiol \* $p < 0.0001$ , significantly different from ER $\alpha$  + estradiol. # $p < 0.01$ , significantly different from ER $\beta$  + estradiol. \*\* $p < 0.05$ ,  $t$ -test,  $n = 4-5$  per treatment group. From [135], Copyright 2008, The Endocrine Society.

ER-mediated female sexual behavior [134], which appears to be ER $\alpha$ -dependent [151,172]. In addition, SRC-1 from brain interacts more with PR-B than with PR-A [135]. These differential interactions of SRC-1 from hypothalamus or hippocampus with the ER and PR subtypes suggest that these brain regions have distinct expression patterns of coregulators involved in these important protein-protein interactions. In addition, it is possible that SRC-1 undergoes differential phosphorylation in these two brain regions, leading to distinct patterns of interaction with receptors. Future experiments will need to apply mass spectrometry analysis to determine if, in a

brain region-specific manner, different coregulators are present in the receptor-coactivator complex and/or if SRC-1 undergoes differential phosphorylation. Finally, these findings suggest the importance of using biologically-relevant tissue, in contrast to the use of cell lines alone, in investigating receptor-coactivator interactions. It may be that other coregulators and proteins that are present in tissue (e.g. brain) are important for appropriate SRC-1 interactions with receptor. Understanding how nuclear receptor coactivators function with various steroid receptors, and their subtypes, is critical to understanding how hormones act in different brain regions to



**Fig. 4.** Down-regulation of SRC-1 affects testosterone-dependent male sexual behavior and medial preoptic area morphology in Japanese quail. Inhibitory effects of SRC-1 down-regulation on the frequency of two testosterone-dependent male sexual behaviors, (A) cloacal contact movements (CCM, estrogen-dependent) and (B) struts, a pre- and post-copulatory display (androgen-dependent). Frequency of behavior was recorded each day from D0 to D7. Average ( $\pm$ SE) are shown for controls (Ctrl) and antisense-injected (AS) subjects. Mixed-two way ANOVA revealed a significant main effect of antisense injections on the frequencies of both cloacal contact movements and struts. *Post hoc* Tukey's tests: # $p < 0.01$  versus control on the corresponding day. Bar graphs on the right illustrates the behavioral recovery after cessation of the antisense treatment in AS treated birds for which antisense injections were replaced on days 8 and 9 by control injections and behavioral testing was continued for two additional days (D8–D9). This group is referred as ASSC. + $p < 0.05$  versus AS at D7. It should be noted that only male copulatory behavior *per se* was restored and even increased compared to control group, while the frequency of struts remained low. C. Effects of manipulations of SRC-1 expression on the testosterone-dependent increase of the preoptic median nucleus (POM) volume identified in sections stained for aromatase. The figure illustrates the POM in a control (left), AS (middle) and ASSC (right) subject. CA, Anterior commissure; \*, third ventricle; scale bar, 200  $\mu$ m. [27]

profoundly influence physiology and behavior. Ultimately, investigation of these receptor-coactivator interactions using brain tissue may allow the identification of novel coregulators involved in the steroid receptor complex in brain.

## 6. Nuclear receptor corepressor function in brain and behavior

### 6.1. Corepressor expression and regulation in brain

Few studies have examined the expression and regulation of corepressor expression in brain. One particular study found that both NCoR and SMRT were expressed ubiquitously throughout the brain with some small anatomical differences in expression, such as higher levels of SMRT in the paraventricular nucleus and the substantia nigra [208]. The regulation of corepressor expression appears to be complex. Some early studies reported no influence of estradiol or thyroid hormone on NCoR and SMRT mRNA expression in male or female hypothalami [130]. Furthermore, another study examined the developmental profile of NCoR and SMRT and the potential impact of thyroid hormone on their expression within the cerebellum. It was found that NCoR, but not SMRT, expression increased over neonatal development within this area and this increase appeared to be independent of thyroid hormone [116]. A later study found that NCoR, as well as SRC-1, mRNA was decreased in the cortex and dentate gyrus, but not the CA3 region, in hypothyroid-induced rats [75]. Therefore, it appears that thyroid hormone may regulate corepressor expression in a brain region-specific manner. Interestingly, some of the same processes (*i.e.* hormones and 26S proteasome) that regulate ER and PR expression appear to regulate corepressors, such as SMRT, in adult female rat brain [214]. Indeed, recently it was reported that estradiol leads to down-regulation of NCoR, but not SMRT, expression in MCF-7 cells [55]. This down-regulation of NCoR by estradiol is likely to influence the expression of numerous downstream targets and provides a mechanism by which estradiol can have a broad genomic impact. It was also found recently that the levels

of SMRT were lowest at metestrus within the preoptic area of adult female rat brain (NCoR expression was not examined in this study) [23]. These findings suggest that some other hormone, besides estradiol, could regulate SMRT expression in adult female rat brain.

### 6.2. Corepressors and brain development

While the functional roles of corepressors in brain are currently unclear, mounting evidence indicates that NCoR, as well as other corepressors, play a major role in cellular differentiation during early brain development [148]. As mentioned above, NCoR appears to be critical for the suppression of glia differentiation and essential in maintaining neural stem cells in an undifferentiated multipotent state in developing brain [67]. In support, mice containing a disruption of NCoR have a 70% increase in cells expressing glia fibrillary acidic protein (GFAP), a marker of astroglia differentiation. As sex differences in glia differentiation exist in developing brain, it will be interesting to determine if sex differences in NCoR expression underlie these sex differences [136]. The sub-cellular localization of NCoR within the brain has also been correlated with abnormal brain function, such as the increased cytoplasmic localization found in individuals with Huntington's Disease [21]. In *Drosophila*, NCoR physically interacts with MeCP2 [39], the apparent cause of Rett syndrome [4]. NCoR also appears to associate with DISC1 (disrupted-in-schizophrenia) [180], a potential key factor in schizophrenia [108,138]. NCoR also interacts directly with CBP, suggesting that it can modify coactivator function [37]. Therefore, while NCoR is of key interest in investigating neurodevelopmental and behavioral disorders that are also sexually dimorphic, its function in modulating steroid receptor activity in developing or adult brain remains relatively unexplored.

## 7. Summary and future directions

The mechanisms by which steroids act in a region-specific, and cell type-specific, manner is a fundamental issue in steroid

hormone action in brain. Recent investigations indicate that, in addition to the bioavailability of hormone and receptor levels, nuclear receptor coregulators are critical molecules in modulating steroid receptor-mediated transcription. Studies from cell lines have revealed much about the molecular mechanisms of action of these coactivators and corepressors. Furthermore, work in brain, as well as other steroid-sensitive tissues, indicates that nuclear receptor coregulators are critical in the fine-tuning of steroid-responsiveness within individual cells. In order to understand how hormones function in the brain to regulate complex behaviors, it will be critical to understand the recruitment of different coactivator and corepressor complexes to the promoter, which is likely to be cell- and tissue-specific. Finally the distinction between coactivator and corepressor is becoming less clear. For example, some *in vitro* studies now suggest that SRC-1 can act as a repressor of ligand-activated TR [195] and GR [209,103,174]. It is increasingly apparent that the cellular environment and the complexity of the coregulator complex are important parameters in the modulation of steroid action in the brain and behavior.

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