

**Steroid Receptor Coactivator-1 from Brain Physically Interacts Differentially
with Steroid Receptor Subtypes**

Abbreviated title: SRC-1 from brain interacts with ER and PR

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ABSTRACT

In vitro studies reveal that nuclear receptor coactivators enhance the transcriptional activity of steroid receptors, including estrogen (ER) and progesterin receptors (PR), through ligand-dependent interactions. While work from our lab and others shows that steroid receptor coactivator-1 (SRC-1) is essential for efficient ER and PR action in brain, very little is known about receptor-coactivator interactions in brain. In the present studies, pull-down assays were used to test the hypotheses that SRC-1 from hypothalamic and hippocampal tissue physically associate with recombinant PR or ER in a ligand-dependent manner. SRC-1, from hypothalamus or hippocampus, interacted with PR-A and PR-B in the presence of an agonist, but not in the absence of ligand or in the presence of a selective PR modulator, RU486. Interestingly, SRC-1 from brain associated more with PR-B, the stronger transcriptional activator, than with PR-A. In addition, SRC-1 from brain, which was confirmed by mass spectrometry, interacted with ER α and ER β in the presence of agonist, but not when unliganded or in the presence of the selective ER modulator, tamoxifen. Furthermore, SRC-1 from hypothalamus, but not hippocampus, interacted more with ER α than with ER β , suggesting distinct expression patterns of other cofactors in these brain regions. These findings suggest that interactions of SRC-1 from brain with PR and ER are dependent on ligand, receptor subtype and brain region to manifest the pleiotropic functional consequences that underlie steroid-regulated behaviors. The present findings reveal distinct contrasts with previous cell culture studies and emphasize the importance of studying receptor-coactivator interactions using biologically-relevant tissue.

INTRODUCTION

The steroid hormones, estradiol and progesterone, exert many of their effects on reproductive behavior and physiology by binding to their respective intracellular receptors in specific brain regions (1-3). Intracellular estrogen receptors (ER) exist in two forms, α and β , which are transcribed from different genes (4-6). These subtypes differ in their functions (7), abilities to bind different ligands (8-11) and distribution in brain (12-16). In addition, cell culture experiments indicate that ER α is a stronger transcriptional activator than ER β , due to differences in the activation function-1 (AF-1) region of the amino-terminus (17). In most species, progesterin receptors (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 under the regulation of different promoters (18, 19). *In vitro* studies indicate that human PR-B is a stronger transcriptional activator than PR-A (20-24), due to an additional AF domain in the N-terminus of PR-B (25, 26). These two PR isoforms appear to have distinct functions in reproductive behavior and physiology (27-30).

Nuclear receptor coactivators dramatically enhance the transcriptional activity of steroid receptors *in vitro*, including ER and PR (31-33). In addition to early models of nuclear receptor coactivators functioning as a bridge between receptors and the general transcriptional machinery, nuclear receptor coactivators are thought to contribute to nuclear receptor transcription through a variety of processes, including phosphorylation, methylation, acetylation and chromatin remodeling (32, 34-36). The first steroid receptor coactivator to be cloned was steroid receptor coactivator-1 (SRC-1, also known as NcoA-1) (33), which was later found to be a member of a larger family of p160 proteins that includes SRC-2 (GRIP1, TIF2 and NCoA-2) (37) and SRC-3 (AIB1, TRAM-1, p/CIP, ACTR, RAC3) (38). Under most conditions, the p160 family and other coactivators physically interact with steroid receptors, including ER and PR, in the presence of an agonist, but not in the absence of ligand or in the presence of an antagonist or selective receptor modulators (33, 39-44) (but

c.f. (45, 46)). It is well established that selective estrogen receptor modulators (SERMs) regulate ER activity in a tissue specific manner (47). For example, tamoxifen can block ER action through competitive binding or can activate ER depending on the cellular environment, including the ratio of coactivators and corepressors (48). Using this same rationale, it has been suggested that RU486 is a selective PR modulator (SPRM) (49, 50).

A variety of studies have begun to investigate nuclear receptor coactivator function in hormone action in brain. SRC-1 mRNA and protein are expressed at high levels in the rodent hypothalamus, hippocampus, cerebellum, paraventricular nucleus, thalamus and amygdala (51-57) (and for review see (58)). Moreover, recent work reveals that hypothalamic neurons coexpress ovarian steroid receptors (ER and PR) and SRC-1 (59). In addition, we and others have found that SRC-1 is important for ER and PR action in brain, including regulation of ER transcriptional activity (55, 60) and hormone-dependent sexual differentiation of the brain (61) and sexual behavior (55, 60-64). Finally, the p160 coactivators appear to function in glucocorticoid receptor action in glial cells (65).

While cell culture studies indicate that receptor-coactivator interactions occur in a ligand-dependent manner, it is not known if coactivators from brain physically associate with receptors. Therefore, we tested the hypothesis that SRC-1, from brain regions rich in steroid receptors, physically associates with steroid receptors in a ligand-dependent manner. To test this hypothesis, we developed pull-down assays using recombinant PR and ER subtypes and SRC-1 from female rat hypothalamus and hippocampus. The present findings are in contrast with those of previous cell culture receptor-coactivator interaction studies and reveal the importance of investigating these interactions using biologically-relevant brain tissue. In addition, such studies may lead to the discovery of new cofactors that modulate steroid receptor action in brain.

MATERIALS AND METHODS

Experimental animals

Adult female (175-200 g) Sprague-Dawley rats from Charles River Breeding Laboratories, Inc. (Wilmington, MA) were housed singly in a 14:10 light-dark cycle, with lights off at 11 a.m. Animals were given food and water *ad libitum*. Female rats were anesthetized with Ketamine/Xylazine cocktail (100 mg Ketamine and 18 mg xylazine/0.75 ml/kg in saline) and ovariectomized. A one-week recovery period followed to allow clearing of endogenous hormones. All animals were overdosed with sodium pentobarbital (89 mg/kg) and chloral hydrate (425 mg/kg) and then decapitated. Hypothalamic and hippocampal (containing a small portion of the cortex dorsal to the Hipp) tissues were dissected out and flash frozen on dry ice. Tissue was then stored at -80°C. All animal procedures were approved by the Institutional Animal Care and Use Committees of Skidmore College and Wellesley College.

Recombinant GST- and Flag-tagged steroid receptors

Recombinant ER and PR fusion proteins were expressed in *Spodoptera frugiperda* (Sf9) insect cells by the Tissue Culture CORE Facility of the Univ. of Colorado Cancer Center and the Baculovirus/Monoclonal Antibody Facility of the Baylor College of Medicine as described previously (66, 67). Briefly, full-length human PR-A or PR-B was fused to a glutathione *S*-transferase (GST) tag. Insect cell cultures for PR-GST (viruses kindly provided by David Bain, Univ Colorado HSC) were incubated with 200 nM of the PR agonist R5020, 200 nM of the SPRM RU486, or in the absence of PR ligand. Full-length human ER α or ER β was fused to a flag tag (viruses kindly provided by Lee Kraus, Cornell) (67, 68). Sf9 cell cultures for ER-flag were incubated with 200 nM estradiol, 200 nM 4-OH-tamoxifen, or no ligand.

Tissue preparation

Brain tissue from female rats (n=54) was pooled in groups of three for each sample and homogenized in buffer (10 mM Tris, 10% glycerol, 400 mM NaCl, 1 mM DTT, 1mM EDTA, pH = 7.4) with protease inhibitors (1:10

dilution, P2714, Sigma, Saint Louis, MO). Samples were incubated on ice for 30 min., and then centrifuged for 30 min. at 4°C at 12,000 rpm and supernatants were aliquotted and frozen at -80°C.

PR GST pull-down assay procedure

All procedures were carried out at 4°C. Twenty-five μ l of Glutathione Sepharose 4B packed resins (Amersham Biosciences, Uppsala, Sweden) were added to siliconized centrifuged tubes and washed with TG buffer (20 mM Tris-HCl, pH 8.0, plus 10% glycerol) containing 100 mM NaCl (TG + NaCl). The resin was then pretreated with ovalbumin (1 mg/ml, Fisher Scientific, Hampton, NH) for 15 minutes on an end-over-end rotator. Following 3 rinses with TG + NaCl, equal amounts of recombinant human PR-GST in 100 mM salt was added to resins and incubated on a rotator for 1 hour. The resins were washed with TG + NaCl. Equal amounts of pooled hypothalamic or hippocampal whole cell extracts were added to the immobilized PR-GST, or GST alone as a control, and incubated on a rotator for 1 hour. Resins were washed with TG + NaCl to eliminate non-specific binding, then samples were eluted with 2% SDS sample buffer by boiling samples for 5 minutes and stored at -80°C until use.

Samples were analyzed by Western blot as described previously (62) for detection of SRC-1 interactions with PR. Briefly, SRC-1 from brain was probed for using a mouse monoclonal antibody generated against amino acids 477-947 of human SRC-1 (1135-H4, 0.5 μ g/ml, kindly provided by Dean Edwards, Bert O'Malley, Ming Tsai and Sergio Oñate, Baylor College of Medicine) (43) or a rabbit polyclonal antibody generated against aa 350-690 of mouse SRC-1 (M-341, 1:750, Santa Cruz Biotechnology). Membranes were incubated in a sheep anti-mouse secondary (1:6000, Amersham) or a donkey anti-rabbit secondary (1:10,000, Amersham) antibody. Immunoreactive bands were detected with an enhanced chemiluminescence kit (ECL; New England Biolabs) and membranes exposed to film (Blue Sensitive X-ray film, Laboratory Products Sales, Rochester, NY). Membranes were stripped for 3 hours at 70°C in stripping

buffer (2% sodium laurel sulfate, 62.5 mM Tris HCl, 100 mM 2-mercaptoethanol, H₂O, pH = 6.7), and then reprobed for PR using a mouse monoclonal antibody which recognizes N-terminal amino acids 165-534 of both PR-A and PR-B (PR 1294, 0.1 µg/ml, kindly provided by Dean Edwards). Films were placed on a light box (Fotodyne, New Berlin, WI) and photographed with an Olympus Camedia digital camera. Images were imported into the NIH Image analysis program (v 1.62, National Institutes of Health) on a Power Macintosh G3 computer and analyzed for immunoreactive band area as measured by number of pixels, which has been found to be consistent with optical density data (62).

ER Flag-tagged pull-down assay procedure

All steps were conducted at 4°C. Twenty-five µl of packed Anti-flag M2 affinity gel resin (Sigma) was added to each siliconized centrifuge tube and pre-washed 3 times with TBS and 2 times with 100 mM glycine HCl (100mM glycine, water, pH = 3.5). Resins were next washed 3 times with Wash Buffer + NaCl (50mM Tris-HCl, 100mM NaCl, 1% glycerol, 50mM Na Fluoride, water, pH = 7.4) + TX-100 (0.1% Triton X-100). Equal amounts of recombinant flag-tagged ER were added to the resin column and rotated on an end-over-end rotator for 1 hour. The resins with immobilized ER were washed 3 times with Wash Buffer + NaCl. Equal amounts of pooled hypothalamic, or hippocampal, whole cell extracts were added to the immobilized ER-flag and incubated on a rotator for 1 hour. The resins were washed 3 times with Wash Buffer + NaCl to eliminate non-specific binding, and then samples were eluted with 2% SDS sample buffer as described above and stored at -80°C.

Samples were analyzed by Western blot, as described above, for detection of SRC-1 interactions with ER. After probing for SRC-1, membranes were stripped and re-probed for flag-tagged ERα and ERβ using a mouse monoclonal antibody generated against the flag-tag (0.25 µg/ml, anti-Flag M2, Sigma) and a horseradish peroxidase linked sheep anti-mouse secondary antibody (1:80,000 dilution, Amersham Biosciences).

Mass Spectrometry

Rat hypothalamic extracts (approximately 40 mg of tissue per condition) were exposed to immobilized ERα in the presence of 200 nM estradiol or no ligand. Eluted samples were resolved in adjacent lanes by SDS-PAGE and the region of the gel corresponding to SRC-1 was excised, digested with trypsin and desalted as described previously (69, 70). The peptide mixture was injected onto a C18 trap and then separated on a reversed phase nano-HPLC column (PicoFrrt™, 75 µm x 10 cm; tip ID 15 µm) with a linear gradient of 0-50 % mobile phase B (0.1 % formic acid-90 % acetonitrile) in mobile phase A (0.1 % formic acid) over 120 min at 200 nl/min. LC-MS/MS experiments were performed with a LTQ linear ion trap mass spectrometer (ThermoFinnigan, San Jose, CA) equipped with a nanospray source; the mass spectrometer was coupled on-line to a ProteomX® nano-HPLC system (ThermoFinnigan, San Jose, CA). The mass spectrometer was operated in the data-dependent mode using Xcalibur software. The most intense seven ions in each MS survey scan were automatically selected for MS/MS. This approach allows the detection of individual proteins in the nanogram range and has been used to identify proteins in complexes using immunoaffinity purification as well as low abundance transcription factors such as RelA/p65 NFκB (69, 70). The acquired MS/MS spectra were searched with SEQUEST algorithm from the SWISSPROT Protein Database on the Bioworks 3.2 platform (ThermoFinnigan, San Jose, CA).

Statistical Analysis

Films from Western blots were analyzed as described previously (62). Data were analyzed as a ratio of area of SRC-1 immunoreactive band to area of PR-A or PR-B band, or area of ERα or ERβ band. Unless stated otherwise, the area of immunoreactive bands was analyzed using a two-way ANOVA in StatView V. 5.0.1 (SAS Institute Inc., Cary, NC) to determine differences between receptor subtypes and ligand conditions. Differences were considered significant at a probability of less than 0.05.

RESULTS

PR interacts with neural SRC-1 in a ligand-dependent and subtype-specific manner

GST pull-down assays were used to investigate whether SRC-1 from brain physically associates with PR-A and PR-B, and whether these interactions depend on the ligand condition. SRC-1 from the hippocampus interacted with PR-A in the presence of the agonist R5020 (Fig. 1, lane 2), but not in the absence of ligand (Fig. 1, lane 3). SRC-1 did not interact with the GST tag bound to resin (Fig. 1, lane 4), or to the resin alone (lane 5), indicating that there was no non-specific binding of SRC-1 to the GST tag or resin alone.

SRC-1 from brain associated with both PR-A and PR-B when bound to agonist (Fig. 2, lanes 2 & 5, and Fig. 3). In dramatic contrast, little to no SRC-1 from the hippocampus (Fig. 2, lanes 4 & 7) or hypothalamus associated with PR-A and PR-B in the absence of ligand or in the presence of the SPRM RU486 ($F(5,32)=17.08$, $p<0.0001$; Fig. 3A and B). In confirmation of these results using the 1135-H4 monoclonal antibody to human SRC-1, similar findings were observed using the rabbit polyclonal antibody to mouse SRC-1 (data not shown). These findings indicate that SRC-1 from brain interacts with PR in a ligand-dependent manner. Figure 2 reveals lower molecular weight bands labeled with the SRC-1 monoclonal antibody that appear to interact with PR-A and PR-B in a manner that is not dependent on the ligand condition, because they are present in all three ligand conditions. However, these same immunoreactive bands were observed using the polyclonal SRC-1 antibody (data not shown), suggesting that these bands are fragments of SRC-1 from brain.

Initial findings suggested that SRC-1 associated more strongly with PR-B than with PR-A in the presence of agonist (Figure 2, lanes 2 & 5). Indeed, SRC-1 from hippocampus (Figure 3A) and hypothalamus (Figure 3B) interacted more with PR-B, than with PR-A, in the presence of agonist ($F(5,32)=11.75$, $p<0.0001$).

ER associates with SRC-1 from the hypothalamus in a receptor subtype-specific manner

Flag-tagged pull-down assays were used to investigate if ER α and ER β physically associate with SRC-1 from brain, and if these interactions occur in a ligand-dependent manner. Hypothalamic SRC-1 interacted with ER in a ligand-dependent manner (Figures 4 and 5B). Estradiol promoted the interactions of hypothalamic SRC-1 with ER α and ER β (Figures 4, lanes 2 & 5, and 5B). In contrast, in the absence of ligand or in the presence of the SERM, tamoxifen, ER α and ER β had little to no association with hypothalamic SRC-1 ($F(5,18)=28.86$, $p<0.0001$; Figure 4, lanes 3 & 6, 4 & 7, and Fig. 5B). SRC-1 from the hippocampus interacted strongly with both hippocampal ER α and ER β in the presence of estradiol (Figure 5A). In the absence of ligand or the presence of the SERM, tamoxifen ($F(5,24)=22.10$, $p<0.0001$), there was little interaction between hippocampal SRC-1 with either ER α or ER β (Figure 5A). Similar SRC-1 and ER interactions were observed using the polyclonal antibody to SRC-1 (data not shown).

Interestingly, SRC-1 from the hypothalamus physically associated more with ER α than with ER β in the presence of estradiol (Figure 4, lanes 2 & 5 and Fig. 5B). In contrast, we did not observe this differential interaction between SRC-1 from the hippocampus and ER α (0.42 ± 0.07) and ER β (0.32 ± 0.04 ; $p = 0.24$, two-tailed t -test) (Figure 5A). Taken together, these data suggest that ER subtypes interact with SRC-1 in a brain region specific manner.

Mass spectrometry confirms hypothalamic SRC-1 interacts with ER α

In order to independently confirm the western blot data for estradiol-dependent binding of ER to SRC-1 from rat brain, we employed an unbiased mass spectrometry approach. Rat hypothalamic extracts were exposed to immobilized ER α in the presence of estradiol or no ligand and eluted samples were resolved by SDS-PAGE. Gel slices corresponding to the putative SRC-1 region of the 2 lanes were digested with trypsin, and peptides analyzed by LC-MS/MS. Database searching identified an abundant, doubly

charged peptide with MH⁺ of 1336.65907. While no matches were found in the rat SwissProt database, a search of the far more completely annotated human database matched the amino acid sequence SDISSSSQGVIEK with highly significant scores of XCorr = 3.62 and DeltaCn = 0.45. Furthermore, 18 of 24 of the observed fragment ions matched the predicted fragment ions. This peptide corresponds to amino acids 97-109 of the human nuclear receptor coactivator 1 (EC 2.3.1.48) with gene name of NCoA-1 (SRC-1) and SwissProt accession number Q15788. It is important to note a match in the rat database was not found because, in spite of 100% identity of this human peptide with mouse, chicken, pig and many other species, the rat NCoA-1 sequence is not currently in the SwissProt database. Interestingly, this peptide was found in the slice from the lane eluted from estradiol-bound ER α , and not in the slice eluted from unliganded ER α , confirming our findings from the Western blot analysis.

DISCUSSION

To test the hypotheses that SRC-1 from brain physically associates with PR and ER subtypes in a ligand-dependent manner, we developed pull-down assays with brain tissue from female rats. We found that SRC-1 from hypothalamic or hippocampal extracts interacted with both GST-tagged PR-A and PR-B when bound to the agonist R5020. In contrast, very little to no SRC-1 from brain associated with PR-A or PR-B in the absence of ligand or in the presence of the SPRM, RU486. These findings are consistent with previous studies using recombinant SRC-1 and the concept that SRC-1 and PR interactions are agonist-dependent (33, 71). The present findings support our previous work indicating a role for SRC-1 action in the hypothalamus in PR-dependent female sexual behavior (62) and suggest that SRC-1 may contribute to the effects of progestins on memory in the hippocampus (72).

Interestingly, we found that SRC-1 from hypothalamus or hippocampus interacts more with PR-B, than with PR-A, in the presence of agonist (Figure 3). The present results are in contrast to other pull-down assays using

recombinant SRC-1. In one study, full-length recombinant SRC-1 interacted equally with PR-A and PR-B when bound to agonist (43). In another pull-down study, an SRC-1 fragment interacted with PR-B, but not PR-A (71). Taken together, the present findings suggest the importance of using biologically-relevant tissue, in contrast to the use of cell lines alone, in these pull-down assays. It may be that other cofactors and proteins, that are present in brain, are important for appropriate SRC-1 and PR interactions.

In vitro studies indicate that human PR-B is a stronger transcriptional activator than PR-A (20, 22-24), due to the additional AF-3 region of PR-B (25, 26). It is likely that this additional AF domain in PR-B allows for enhanced recruitment of coactivators, thus augmenting the transcriptional activity of PR-B (24, 25, 73). Interestingly, a recent study indicates that both PR isoforms are required for the complete expression of female sexual behavior in mice (27). While it is not known if PR-B is a stronger transcriptional activator than PR-A in brain, our findings suggest that PR-B is a stronger activator of SRC-1 dependent progesterone signaling pathways in brain than PR-A.

SRC-1 from hypothalamus or hippocampus interacted with ER α and ER β when bound to estradiol (Figures 4 and 5). In contrast, very little to no association of SRC-1 from brain was detected with ER α or ER β in the absence of ligand or in the presence of the SERM, tamoxifen (Figures 4 and 5). Our findings are consistent with a variety of studies using cell lines demonstrating that estradiol facilitates the association of SRC-1 with ER, while antagonists prevent this association (40, 48, 74, 75). In contrast to the present findings, under certain phosphorylation conditions, cell culture studies suggest that both ER α and ER β can recruit coactivators to AF-1 in the absence of ligand (45, 46). While we detected little to no interactions between receptor and SRC-1 from brain in the absence of ligand, it will be important to investigate whether physiologically-relevant events that modulate ligand-independent activation impact on receptor-coactivator interactions in brain. Furthermore, under the present experimental conditions, it appears that the selective receptor

modulators, tamoxifen and RU486, function as antagonists to prevent receptor-coactivator interactions.

In the present studies, SRC-1 from the hippocampus appears to interact equally with ER α and ER β (Figure 5A). Association of SRC-1 with ligand-bound ER α and ER β in the hippocampus may be an integral component of estrogen's effects on cognition and memory (76, 77). Interestingly, in contrast to the hippocampus, SRC-1 obtained from hypothalamic extracts interacted more with ER α than with ER β (Figures 4 and 5B). ER α , and to a lesser extent ER β , are expressed in the hypothalamus (12-16). In the hypothalamus, ER α is necessary for the full expression of rodent female sexual behavior (78-82), while ER β in this region appears to influence anxiety and the stress response (77, 83). These different functions of the ER subtypes in brain may be explained in part by the different transcriptional abilities of these receptors. The amino-terminus is shorter in ER β than ER α , which may account for the lower transcriptional activity of ER β observed in particular cell lines (17). These differences in transcriptional abilities between ER α and ER β may be attributed to differential recruitment of coactivators, or differences in the ability of the same coactivator to facilitate transcription of the ER subtypes (84). While some studies using recombinant SRC-1 are consistent with our findings that SRC-1 interacts more with ER α than with ER β (84), other findings suggest that SRC-1 associates equally with each ER subtype (74, 85). While these later findings are consistent with our results using SRC-1 from hippocampus, we observed that SRC-1 from hypothalamus interacted more with ER α than with ER β . These data suggest that ER α is a more efficient transcriptional activator of SRC-1 dependent signaling pathways in the hypothalamus than ER β . In support, previous findings from our lab indicate that SRC-1 function in the hypothalamus is important for maximal expression of ER-mediated female sexual behavior (62), which appears to be ER α -dependent (78, 79). 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 cofactors

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 cofactors are present in the receptor-coactivator complex and/or if SRC-1 undergoes differential phosphorylation.

These pull-down assays allow us to directly address the differential interactions of SRC-1 with the PR and ER subtypes. In addition, this approach allows the efficient detection of protein-protein interactions and the application of mass spectrometry. However, one must be careful in interpreting the results from these assays given that non-specific interactions can occur. In the present studies, little to no interactions were detected between SRC-1 from brain and the fusion protein tags alone (GST or Flag tags) or the resins only (Figure 1), suggesting there were no significant non-specific interactions between SRC-1 and fusion tags or resins. Moreover, Western blot analysis and mass spectrometry revealed that SRC-1 interacted with receptor when bound to agonist, but not when bound to antagonist or unliganded, suggesting these coactivator-receptor interactions were specific. It should be noted that human ER and PR proteins were used to investigate interactions with SRC-1 protein from rat brain. It is possible that SRC-1 from rat brain may interact differently with human ER and PR compared to rat receptor. However, the human PR ligand binding domain (LBD), the receptor region most critical for SRC-1 interactions (32, 86), has a high degree of protein sequence homology (92%) with the rat PR LBD (BLAST) (18, 87). Furthermore, the LBDs of human ER α and ER β are 89% and 90% identical in protein sequences to the LBDs of rat ER α and ER β , respectively (88). However, given that discrete differences in protein structure can lead to differences in protein interactions, it will be important to investigate endogenous interactions between SRC-1 and steroid receptors in brain using co-immunoprecipitation assays in future studies. Nevertheless, the high degree of homology between the rat and human LBDs of PR and ER,

taken together with the ligand-dependent nature of the interactions in the present studies, suggest that our findings provide important insights into the physical associations of SRC-1 from brain and these receptors.

In conclusion, the present data indicate that SRC-1 from hypothalamus and hippocampus physically associate with ER and PR in a ligand-dependent manner. These findings extend our previous studies showing that SRC-1 is expressed in ER and PR containing cells in brain regions important for reproductive behavior (59). In addition, these protein-protein interaction studies provide further support for work from our lab and others that reveal an important role for SRC-1 in ER and PR action in brain (55, 60-63). Moreover, the present studies reveal that SRC-1 from brain interacts differentially with ER and PR subtypes

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

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REFERENCES

1. **Mani SK, O'Malley BW** 2002 Mechanism of progesterone receptor action in the brain. In: Arnold AP, Etgen AM, Fahrbach SE, Rubin RT, Pfaff DW eds. *Hormones, Brain and Behavior*. San Diego: Academic Press; 643-682
2. **Blaustein JD, Erskine MS** 2002 Feminine sexual behavior: cellular integration of hormonal and afferent information in the rodent forebrain. In: Pfaff DW ed. *Hormones, Brain and Behavior*. New York: Academic Press; 139-214
3. **Pfaff DW** 1980 *Estrogens and Brain Function*. New York: Springer-Verlag
4. **Jensen EV, Suzuki T, Kawasima T, Stumpf WE, Jungblut PW, de Sombre ER** 1968 A two-step mechanism for the interaction of estradiol with rat uterus. *Proceedings of the National Academy of Sciences USA* 59:632-638
5. **Shyamala G, Gorski J** 1969 Estrogen receptors in the rat uterus. Studies on the interaction of cytosol and nuclear binding sites. *J Biol Chem* 244:1097-1103
6. **Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson J** 1996 Cloning of a novel estrogen receptor expressed in rat prostate and ovary. *Proceedings of the National Academy of Sciences USA* 93:5925-5930
7. **Carpenter KD, Korach KS** 2006 Potential biological functions emerging from the different estrogen receptors. *Ann N Y Acad Sci* 1092:361-373
8. **Kuiper GGJM, Carlsson B, Grandien K, Enmark E, Häggblad J, Nilsson S, Gustafsson J** 1997 Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* 138:863-870
9. **Hall JM, McDonnell DP** 1999 The estrogen receptor beta-isoform (ERbeta) of the human estrogen receptor modulates ERalpha transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. *Endocrinology* 140:5566-5578
10. **Jones PS, Parrott E, White IN** 1999 Activation of transcription by estrogen receptor alpha and beta is cell type- and promoter-dependent. *J Biol Chem* 274:32008-32014
11. **Damdimopoulos AE, Spyrou G, Gustafsson JA** 2008 Ligands Differentially Modify the Nuclear Mobility of Estrogen Receptors {alpha} and. *Endocrinology* 149:339-345
12. **Shughrue PJ, Lane MV, Merchenthaler I** 1997 Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. *J Comp Neurol* 388:507-525
13. **Greco B, Allegretto EA, Tetel MJ, Blaustein JD** 2001 Coexpression of ER beta with ER alpha and progestin receptor proteins in the female rat forebrain: Effects of estradiol treatment. *Endocrinology* 142 5172 -5181
14. **Kuiper GG, Shughrue PJ, Merchenthaler I, Gustafsson JA** 1998 The estrogen receptor beta subtype: a novel mediator of estrogen action in neuroendocrine systems. *Front Neuroendocrinol* 19:253-286
15. **Osterlund M, Kuiper GG, Gustafsson JA, Hurd YL** 1998 Differential distribution and regulation of estrogen receptor-alpha and -beta mRNA within the female rat brain. *Brain Res Mol Brain Res* 54:175-180
16. **Mitra SW, Hoskin E, Yudkovitz J, Pear L, Wilkinson HA, Hayashi S, Pfaff DW, Ogawa S, Rohrer SP, Schaeffer JM, McEwen BS, Alves SE** 2003 Immunolocalization of estrogen receptor beta in the mouse brain: comparison with estrogen receptor alpha. *Endocrinology* 144:2055-2067

17. **Delaunay F, Pettersson K, Tujague M, Gustafsson JA** 2000 Functional differences between the amino-terminal domains of estrogen receptors alpha and beta. *Mol Pharmacol* 58:584-590
18. **Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H, Chambon P** 1990 Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO Journal* 9:1603-1614
19. **Conneely OM, Maxwell BL, Toft DO, Schrader WT, O'Malley BW** 1987 The A and B forms of the chicken progesterone receptor arise by alternate initiation of translation of a unique mRNA. *Biochemical and Biophysical Research Communications* 2:493-501
20. **Vegeto E, Shahbaz MM, Wen DX, Goldman ME, O'Malley BW, McDonnell DP** 1993 Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. *Molecular Endocrinology* 7:1244-1255
21. **McDonnell DP, Goldman ME** 1994 RU486 exerts antiestrogenic activities through a novel progesterone receptor A form-mediated mechanism. *Journal of Biological Chemistry* 269:11945-11949
22. **Tung L, Kamel Mohamed M, Hoeffler JP, Takimoto GS, Horwitz KB** 1993 Antagonist-occupied human progesterone B-receptors activate transcription without binding to progesterone response elements and are dominantly inhibited by A-receptors. *Molecular Endocrinology* 7:1256-1265
23. **Wen DX, Xu YF, Mais DE, Goldman ME, McDonnell DP** 1994 The A and B isoforms of the human progesterone receptor operate through distinct signaling pathways within target cells. *Molecular and Cellular Biology* 14:8356-8364
24. **Giangrande PH, Pollio G, McDonnell DP** 1997 Mapping and characterization of the functional domains responsible for the differential activity of the A and B isoforms of the human progesterone receptor. *Journal of Biological Chemistry* 272:32889-32900
25. **Sartorius CA, Melville MY, Hovland AR, Tung L, Takimoto GS, Horwitz KB** 1994 A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform. *Molecular Endocrinology* 8:1347-1360
26. **Tung L, Abdel-Hafiz H, Shen T, Harvell DM, Nitao LK, Richer JK, Sartorius CA, Takimoto GS, Horwitz KB** 2006 Progesterone receptors (PR)-B and -A regulate transcription by different mechanisms: AF-3 exerts regulatory control over coactivator binding to PR-B. *Mol Endocrinol* 20:2656-2670
27. **Mani SK, Reyna AM, Chen JZ, Mulac-Jericevic B, Conneely OM** 2006 Differential response of progesterone receptor isoforms in hormone-dependent and -independent facilitation of female sexual receptivity. *Molecular Endocrinology* 20:1322-1332
28. **Mulac-Jericevic B, Mullinax RA, Demayo FJ, Lydon JP, Conneely OM** 2000 Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. *Science* 289:1751-1754
29. **Mulac-Jericevic B, Conneely OM** 2004 Reproductive tissue selective actions of progesterone receptors. *Reproduction* 128:139-146
30. **Mulac-Jericevic B, Lydon JP, DeMayo FJ, Conneely OM** 2003 Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. *Proc Natl Acad Sci U S A* 100:9744-9749
31. **O'Malley BW** 2006 Molecular biology. Little molecules with big goals. *Science* 313:1749-1750

32. **Rosenfeld MG, Lunyak VV, Glass CK** 2006 Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. *Genes Dev* 20:1405-1428
33. **Oñate SA, Tsai SY, Tsai MJ, O'Malley BW** 1995 Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* 270:1354-1357
34. **Lonard DM, O'Malley BW** 2006 The expanding cosmos of nuclear receptor coactivators. *Cell* 125:411-414
35. **Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen CA, McKenna NJ, Oñate SA, Tsai SY, Tsai MJ, O'Malley BW** 1997 Steroid receptor coactivator-1 is a histone acetyltransferase. *Nature* 389:194-197
36. **Bannister AJ, Kouzarides T** 1996 The CBP co-activator is a histone acetyltransferase. *Nature* 384:641-643
37. **Voegel JJ, Heine MJS, Zechel C, Chambon P, Gronemeyer H** 1996 TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *EMBO Journal* 15:3667-3675
38. **Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM, Meltzer PS** 1997 AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science* 277:965-968
39. **Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL** 1998 The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* 95:927-937
40. **Margeat E, Poujol N, Boulahtouf A, Chen Y, Muller JD, Gratton E, Cavailles V, Royer CA** 2001 The human estrogen receptor alpha dimer binds a single SRC-1 coactivator molecule with an affinity dictated by agonist structure. *Journal of Molecular Biology* 306:433-442
41. **Tanenbaum DM, Wang Y, Williams SP, Sigler PB** 1998 Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains. *Proc Natl Acad Sci U S A* 95:5998-6003
42. **Williams SP, Sigler PB** 1998 Atomic structure of progesterone complexed with its receptor. *Nature* 393:392-396
43. **Oñate SA, Boonyaratankornkit V, Spencer TE, Tsai SY, Tsai MJ, Edwards DP, O'Malley BW** 1998 The steroid receptor coactivator-1 contains multiple receptor interacting and activation domains that cooperatively enhance the activation function 1 (AF1) and AF2 domains of steroid receptors. *Journal of Biological Chemistry* 273:12101-12108
44. **McInerney EM, Tsai MJ, O'Malley BW, Katzenellenbogen BS** 1996 Analysis of estrogen receptor transcriptional enhancement by a nuclear hormone receptor coactivator. *Proceedings of the National Academy of Sciences USA* 93:10069-10073
45. **Dutertre M, Smith CL** 2003 Ligand-independent interactions of p160/steroid receptor coactivators and CREB-binding protein (CBP) with estrogen receptor-alpha: regulation by phosphorylation sites in the A/B region depends on other receptor domains. *MolEndocrinol* 17:1296-1314
46. **Tremblay A, Tremblay GB, Labrie F, Giguere V** 1999 Ligand-independent recruitment of SRC-1 to estrogen receptor beta through phosphorylation of activation function AF-1. *MolCell* 3:513-519

47. **Lewis-Wambi JS, Jordan VC** 2005 Treatment of Postmenopausal Breast Cancer with Selective Estrogen Receptor Modulators (SERMs). *Breast Dis* 24:93-105.:93-105
48. **Smith CL, Nawaz Z, O'Malley BW** 1997 Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. *Molecular Endocrinology* 11:657-666
49. **Han SJ, Tsai SY, Tsai MJ, O'Malley BW** 2007 Distinct Temporal and Spatial Activities of RU486 on PR Function in Reproductive Organs of Ovariectomized Mice. *Endocrinology*.
50. **Wardell SE, Edwards DP** 2005 Mechanisms controlling agonist and antagonist potential of selective progesterone receptor modulators (SPRMs). *SeminReprodMed* 23:9-21
51. **Martinez de Arrieta C, Koibuchi N, Chin WW** 2000 Coactivator and corepressor gene expression in rat cerebellum during postnatal development and the effect of altered thyroid status. *Endocrinology* 141:1693-1698
52. **Meijer OC, Steenbergen PJ, de Kloet ER** 2000 Differential expression and regional distribution of steroid receptor coactivators SRC-1 and SRC-2 in brain and pituitary. *Endocrinology* 141:2192-2199
53. **Misiti S, Schomburg L, Yen PM, Chin WW** 1998 Expression and hormonal regulation of coactivator and corepressor genes. *Endocrinology* 139:2493-2500
54. **Ogawa H, Nishi M, Kawata M** 2001 Localization of nuclear coactivators p300 and steroid receptor coactivator 1 in the rat hippocampus. *Brain Research* 890:197-202
55. **Molenda HA, Griffin AL, Auger AP, McCarthy MM, Tetel MJ** 2002 Nuclear receptor coactivators modulate hormone-dependent gene expression in brain and female reproductive behavior in rats. *Endocrinology* 143:436-444
56. **Yousefi B, Jingu H, Ohta M, Umezu M, Koibuchi N** 2005 Postnatal changes of steroid receptor coactivator-1 immunoreactivity in rat cerebellar cortex. *Thyroid* 15:314-319
57. **Charlier TD, Lakaye B, Ball GF, Balthazart J** 2002 Steroid receptor coactivator SRC-1 exhibits high expression in steroid-sensitive brain areas regulating reproductive behaviors in the quail brain. *Neuroendocrinology* 76:297-315
58. **Molenda HA, Kilts C, Allen RL, Tetel MJ** 2003 Nuclear receptor coactivator function in reproductive physiology and behavior. *Biology of Reproduction* 69:1449-1457
59. **Tetel MJ, Siegal NK, Murphy SD** 2007 Cells in behaviourally relevant brain regions coexpress nuclear receptor coactivators and ovarian steroid receptors. *J Neuroendocrinol* 19:262-271
60. **Apostolakis EM, Ramamurphy M, Zhou D, Onate S, O'Malley BW** 2002 Acute disruption of select steroid receptor coactivators prevents reproductive behavior in rats and unmasks genetic adaptation in knockout mice. *Mol Endocrinol* 16:1511-1523
61. **Auger AP, Tetel MJ, McCarthy MM** 2000 Steroid receptor coactivator-1 mediates the development of sex specific brain morphology and behavior. *Proceedings of the National Academy of Sciences USA* 97:7551-7555
62. **Molenda-Figueira HA, Williams CA, Griffin AL, Rutledge EM, Blaustein JD, Tetel MJ** 2006 Nuclear receptor coactivators function in estrogen receptor- and progestin receptor-dependent aspects of sexual behavior in female rats. *Hormones and Behavior* 50:383-392

63. **Charlier TD, Ball GF, Balthazart J** 2005 Inhibition of steroid receptor coactivator-1 blocks estrogen and androgen action on male sex behavior and associated brain plasticity. *Journal of Neuroscience* 25:906-913
64. **Charlier TD, Harada N, Ball GF, Balthazart J** 2006 Targeting steroid receptor coactivator-1 expression with locked nucleic acids antisense reveals different thresholds for the hormonal regulation of male sexual behavior in relation to aromatase activity and protein expression. *Behav Brain Res* 172:333-343
65. **Trousson A, Grenier J, Fonte C, Massaad-Massade L, Schumacher M, Massaad C** 2007 Recruitment of the p160 coactivators by the glucocorticoid receptor: dependence on the promoter context and cell type but not hypoxic conditions. *J Steroid Biochem Mol Biol* 104:305-311
66. **Tetel MJ, Giangrande PH, Leonhardt SA, McDonnell DP, Edwards DP** 1999 Hormone-dependent interaction between the amino- and carboxyl-terminal domains of progesterone receptor in vitro and in vivo *Molecular Endocrinology* 13:910-924
67. **Melvin VS, Harrell C, Adelman JS, Kraus WL, Churchill M, Edwards DP** 2004 The role of the C-terminal extension (CTE) of the estrogen receptor α and β DNA binding domain in DNA binding and interaction with HMGB. *The Journal of Biological Chemistry* 279:14763-14771
68. **Kraus WL, Kadonaga JT** 1998 p300 and estrogen receptor cooperatively activate transcription via differential enhancement of initiation and reinitiation. *Genes Dev* 12:331-342
69. **Tilton RG, Haidacher SJ, Lejeune WS, Zhang X, Zhao Y, Kurosky A, Brasier AR, Denner L** 2007 Diabetes-induced changes in the renal cortical proteome assessed with two-dimensional gel electrophoresis and mass spectrometry. *Proteomics* 7:1729-1742
70. **Zhao Y, Zhang W, White MA, Zhao Y** 2003 Capillary high-performance liquid chromatography/mass spectrometric analysis of proteins from affinity-purified plasma membrane. *Anal Chem* 75:3751-3757
71. **Giangrande PH, Kimbrel A, Edwards DP, McDonnell DP** 2000 The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding. *MolCell Biol* 20:3102-3115
72. **Sandstrom NJ, Williams CL** 2001 Memory retention is modulated by acute estradiol and progesterone replacement. *Behav Neurosci* 115:384-393
73. **Hovland AR, Powell RL, Takimoto GS, Tung L, Horwitz KB** 1998 An N-terminal inhibitory function, IF, suppresses transcription by the A-isoform but not the B-isoform of human progesterone receptors. *Journal of Biological Chemistry* 273:5455-5460
74. **Cowley SM, Parker MG** 1999 A comparison of transcriptional activation by ER α and ER β . *J Steroid Biochem Mol Biol* 69:165-175
75. **Yi P, Driscoll MD, Huang J, Bhagat S, Hilf R, Bambara RA, Muyan M** 2002 The effects of estrogen-responsive element- and ligand-induced structural changes on the recruitment of cofactors and transcriptional responses by ER α and ER β . *Molecular Endocrinology* 16:674-693
76. **Fugger HN, Foster TC, Gustafsson J, Rissman EF** 2000 Novel effects of estradiol and estrogen receptor α and β on cognitive function. *Brain Res* 883:258-264
77. **Bodo C, Rissman EF** 2006 New roles for estrogen receptor β in behavior and neuroendocrinology. *Front Neuroendocrinol* 27:217-232

78. **Rissman EF, Early AH, Taylor JA, Korach KS, Lubahn DB** 1997 Estrogen receptors are essential for female sexual receptivity. *Endocrinology* 138:507-510
79. **Ogawa S, Eng V, Taylor J, Lubahn DB, Korach KS, Pfaff DW** 1998 Roles of estrogen receptor-alpha gene expression in reproduction-related behaviors in female mice. *Endocrinology* 139:5070-5081
80. **Kudwa AE, Rissman EF** 2003 Double oestrogen receptor alpha and beta knockout mice reveal differences in neural oestrogen-mediated progestin receptor induction and female sexual behaviour. *J Neuroendocrinol* 15:978-983
81. **Musatov S, Chen W, Pfaff DW, Kaplitt MG, Ogawa S** 2006 RNAi-mediated silencing of estrogen receptor {alpha} in the ventromedial nucleus of hypothalamus abolishes female sexual behaviors. *ProcNatlAcadSciUSA* 103:10456-10460
82. **Ogawa S, Chan J, Chester AE, Gustafsson JA, Korach KS, Pfaff DW** 1999 Survival of reproductive behaviors in estrogen receptor beta gene- deficient (betaERKO) male and female mice. *ProcNatlAcadSciUSA* 96:12887-12892
83. **Isgor C, Cecchi M, Kabbaj M, Akil H, Watson SJ** 2003 Estrogen receptor beta in the paraventricular nucleus of hypothalamus regulates the neuroendocrine response to stress and is regulated by corticosterone. *Neuroscience* 121:837-845
84. **Wong C, Komm B, Cheskis BJ** 2001 Structure-Function evaluation of ER alpha and beta interplay with SRC family coactivators. *ER selective ligands. Biochemistry* 40:6756-6765
85. **Monroe DG, Johnsen SA, Subramaniam M, Getz BJ, Khosla S, Riggs BL, Spelsberg TC** 2003 Mutual antagonism of estrogen receptors alpha and beta and their preferred interactions with steroid receptor coactivators in human osteoblastic cell lines. *JEndocrinol* 176:349-357
86. **Feng W, Ribeiro RCJ, Wagner RL, Nguyen H, Apriletti JW, Fletterick RL, Baxter JD, Kushner PJ, West BL** 1998 Hormone-dependent coactivator binding to a hydrophobic cleft on nuclear receptors. *Science* 280:1747-1750
87. **Kato J, Hirata S, Nozawa A, Mouri N** 1993 The ontogeny of gene expression of progestin receptors in the female rat brain. *J Steroid BiochemMolBiol* 47:173-182
88. **Harris HA, Bapat AR, Gonder DS, Frail DE** 2002 The ligand binding profiles of estrogen receptors alpha and beta are species dependent. *Steroids* 67:379-384

FIGURE LEGENDS

Figure 1. SRC-1 from Hippocampal Whole Cell Extracts Associates with PR-A in a Ligand-Dependent Manner. SRC-1 from hippocampal whole cell extracts (WCE) associates with PR-A in the presence of the agonist R5020 (lane 2), but not in the absence of ligand (lane 3). SRC-1 does not interact with GST (glutathione *S*-transferase, lane 4) tag alone or with the glutathione resin (lane 5). Input (1% of total) of SRC-1 from hippocampal extract is shown in Lane 1.

Figure 2. SRC-1 from Hippocampal Whole Cell Extracts Associates with PR-A and PR-B in a Ligand-Dependent Manner. SRC-1 from the hippocampus associates with PR-A and PR-B in the presence of the agonist R5020 (lanes 2 and 5), but not in the absence of ligand (lanes 3 and 6), or in the presence of the SPRM, RU486 (lanes 4 and 7). Input (1% of total) of SRC-1 from hippocampal extract is shown in Lane 1.

Figure 3. SRC-1 from the Hippocampus and Hypothalamus Associates with PR in a Ligand-Dependent and Receptor Subtype-Specific Manner.

A) SRC-1 from hippocampal extracts interacted with both PR-A and PR-B in the presence of R5020, but not in the absence of ligand or the presence of RU486. * $p < 0.0001$, significantly different from PR-A + R5020. # $p < 0.01$, significantly different from PR-B + R5020. SRC-1 from hippocampus interacted more with PR-B, than with PR-A, when bound to R5020. ** $p < 0.05$, *t*-test.

B) Hypothalamic SRC-1 interacts with PR-A and PR-B when bound to R5020, but little to no interactions were detected in the absence of ligand or when receptors were bound to RU486, * $p < 0.01$, significantly different from PR-A + R5020. # $p < 0.001$, significantly different from PR-B + R5020. SRC-1 from the hypothalamus interacted more with PR-B, than with PR-A, when bound to R5020. ** $p < 0.05$, *t*-test, $n = 5-7$ per treatment group.

Figure 4. SRC-1 from Hypothalamic Whole Cell Extracts Associates with ER α and ER β in a Ligand-Dependent Manner. SRC-1 from the hypothalamus associates with ER α and ER β 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.

Figure 5. ER Recruits SRC-1 from the Hypothalamus in a Receptor Subtype-Specific Manner.

A) In the presence of estradiol, both ER α and ER β interacted with hippocampal SRC-1, but little to no interactions were detected in the absence of ligand or when receptors were bound to Tamoxifen. * $p < 0.0001$, significantly different from ER α + estradiol. # $p < 0.0001$, significantly different from ER β + estradiol.

B) Hypothalamic SRC-1 interacted more strongly with both ER α and ER β in the presence of estradiol, than in the absence of ligand or when receptors were bound to Tamoxifen. * $p < 0.0001$, significantly different from ER α + estradiol. # $p < 0.01$, significantly different from ER β + estradiol. SRC-1 interacted more with ER α , than ER β , when bound to estradiol. ** $p < 0.05$, *t*-test, $n = 4-5$ per treatment group.









