0013-7227/98/$03.00/0
Endocrinology

Vol. 139, No. 4
Printed in U.S.A.

Androgen Receptor in Mouse Brain: Sex Differences and Similarities in Autoregulation*

SHI-FANG LU, SUZANNE E. McKENNA†, ATHENA COLOGER-CLIFFORD‡, EUGENE A. NAU, AND NEAL G. SIMON

Department of Biological Sciences, Lehigh University, Bethlehem, Pennsylvania 18015

ABSTRACT

The androgen receptor (AR) is generally considered an autoregulated protein. However, studies in brain have produced mixed results regarding sex differences, which should be present given the higher endogenous levels of androgens in males, and the effects of gonadectomy, which presumably should lead to a loss of AR. Resolving these issues is a necessary step in developing a model of AR regulation in the central nervous system and, more broadly, in determining how regulation of this receptor may mediate neural target tissue responsiveness to androgen. To further investigate these issues, the distribution, density, and regulation of neural AR were compared among male and female mice that were intact, gonadectomized, or gonadectomized and given testosterone propionate (TP) through immunocytochemical and Western blot analyses. Four brain areas that have been linked to the regulation of male-typical behavior were evaluated: bed nucleus of the stria terminalis, posterior aspect, medial preoptic area, and dorsal and ventral aspects of the lateral septum. In the immunocytochemical study, integrated particle density, which reflects the average intensity of AR staining, was assessed among the six groups 24 h after surgery using PG-21, a peptide-based AR antiserum. Major findings included regional differences in the intensity of immunostaining; a robust sexual dimorphism in each region, with males exhibiting more intense staining than females; a loss of AR in both sexes after gonadectomy, with more dramatic changes evident in males; and significant up-regulation of AR in response to TP that was equivalent in both sexes. The Western blot analyses of AR in limbic system extracts prepared from the six groups showed a pattern of differences that mirrored the immunocytochemical results, indicating that PG-21 recognized both liganded and unliganded AR. In addition, a dose-response study, in which gonadectomized males and females were administered from 25–1000 μg TP, demonstrated a significant linear trend in up-regulation of AR in both males and females, with no sexual dimorphism in the response to hormone treatment. These results demonstrate that the regulation of AR in both male and female neural tissue is comparable and that the critical determinant of AR expression is the presence or absence of androgen. (*Endocrinology* **139:** 1594–1601, 1998)

THE FUNCTION and integrity of the androgen receptor (AR), which is considered an autoregulated protein, are generally thought to depend on adequate circulating levels of androgen (1–4). However, immunochemical studies conducted since 1990 that examined castration/androgen replacement effects in males and sex differences in AR have yielded mixed results regarding this concept. For example, in adult male rat, opossum, and mouse brain, AR levels were either very low or undetectable 1 or 4 days postcastration (the two time points sampled) in all regions that were evaluated, and androgen replacement up-regulated AR in anywhere from 15 min to 24 h after hormone treatment (5–8). In guinea pig, however, there was no effect on neural AR immunoreactivity 4 days after males were gonadectomized (9), and in hamsters, castration produced either a loss of nuclear AR (10) or no effect on immunoreactivity, except in lateral septum, where enhanced staining was noted (11). Lastly, the retrodorsal nucleus of the spinal cord required substantially longer exposure to testosterone (T) than either the dorsolateral nucleus or spinal nucleus of the bulbocavernosus for the induction of AR after androgen treatment (5).

Concerning sex differences, the intensity of AR immunostaining was greater in intact male *vs.* female rats in the posterior bed nucleus of the stria terminalis (BST_p) and periventricular nucleus, but not in the lateral septum (12), whereas in spinal motoneurons, qualitative immunostaining intensity did not differ between males and females (13). Using the number of positively labeled cells as an index, no significant sex difference was found between male and female cynomolgus monkeys in seven major limbic system areas, although possible differences in staining intensity were not reported (14). In mouse brain, a random sample of intact male and female mice (n = 3/group) was compared, and pronounced sex differences (males≫females) were found in the intensity of AR immunoreactivity in all receptor-positive regions using integrated particle density as a semiquantitative measure (15).

These mixed results have led to the suggestion of regional and/or species differences in neural AR regulation (5, 14, 16, 17). However, concerns about the properties of different AR antisera have limited efforts to resolve these issues. In particular, whether PG-21, the most widely used antiserum, recognizes unliganded AR remains a question (5, 6, 8, 18), which

Received September 15, 1997.
Address all correspondence and requests for reprints to: N. G. Simon, Ph.D., Department of Biological Sciences, 111 Research Drive, Lehigh University, Bethlehem, Pennsylvania 18015. E-mail: ngs0@lehigh.edu.
* This work was supported in part by grants from the NSF (IBN95–12015), the NIH (1-HD-31696), and the H. F. Guggenheim Foundation (to N.G.S.).
† Current address: National Institute of Environmental and Health Sciences, 111 TW Alexander Drive, P.O. Box 12233, MD E4–01, Research Triangle Park, North Carolina 27709.

‡ Current address: Laboratoire de Ethologie et Neurobiologie, Université Louis Pasteur, URA CNRS 1295, 7 rue de la Université, 67000 Strasbourg, France.

makes the interpretation of immunochemical findings regarding the effects of castration problematic. As a consequence, it is difficult to conceptualize how neural AR regulation may be, for example, linked to behavioral sensitivity to androgen.

In the present study, the effects of castration with or without T replacement on AR expression were assessed in male and female mouse brain in four regions that have been implicated in the regulation of male-typical behaviors: BST_p, medial preoptic area (MPO), and dorsal and ventral aspects of the lateral septum (LS_D, and LS_V, respectively) (see reviews in Refs. 7 and 19). Including females provided an immunocytochemical assessment of potential sexual dimorphisms in AR regulation that has not previously been reported and may be important for understanding a cellular mechanism linked to androgen sensitivity. The male-female comparisons are a useful model for assessing the latter, because there is a robust sex difference in behavioral sensitivity to androgen (reviewed in Ref. 7). Western blot analyses were conducted with brain extracts prepared from comparably treated males and females to provide an additional test of quantitative differences in AR. The findings demonstrated that although there is a sexual dimorphism in AR density, regulatory responses to both gonadectomy and T treatment are essentially identical in both sexes.

Materials and Methods

Animals and treatments

Adult CF-1 mice (8 weeks old) were purchased from the Charles River Laboratories (Wilmington, MA). They were housed in groups of three or four per cage at 23 ± 2 C under a 12-h light, 12-h dark cycle, with food and water provided *ad libitum*. All maintenance procedures fully complied with federal guidelines for animal care. Six groups were included in the immunocytochemistry (ICC) and Western blot studies: females and males that were intact (INT-F and INT-M), gonadectomized (GDX-F and GDX-M), or gonadectomized and treated with T propionate (TP; GDX-F+T and GDX-M+T). In the ICC study, a single supraphysiological dose of TP (1000 μg) was used. In the subsequent Western analyses, from 25–1000 μg TP were administered to test for dose-response effects and whether changes in AR immunoreactivity were consistent across a range of doses. The hormone was injected sc in 0.1 ml oil vehicle immediately upon completion of surgery. Three independent determinations were made for each dose in each sex.

Reagents

Rabbit AR antiserum (PG-21) was a generous gift from Dr. Gail Prins. The ABC staining kit was obtained from Vector Laboratories (Burlingame, CA). Precast minigels, nonfat milk, and biotin-labeled mol wt standards were purchased from Bio-Rad (Hercules, CA). Horseradish peroxidase-conjugated antirabbit antiserum, nitrocellulose membranes, enhanced chemiluminescence (ECL) detection reagents, and Hyperfilm were obtained from Amersham (Arlington Heights, IL). TP was purchased from Steraloids (Wilton, NH). Hemo De and Permount medium were obtained from Fisher Scientific (Fairlawn, NJ). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

ICC

Twenty-four hours after the injection of TP, mice were anesthetized and perfused with PBS, followed by 4% formaldehyde in PBS. The brains were postfixed for 24 h in the same fixative, and 40-μm frozen sections were cut on a rotary microtome. Sections were treated with 0.15% Triton X-100/PBS for 40 min, then with 10% FBS/PBS for 30 min. Free floating sections were incubated for 48 h at 4 C in 1% BSA/PBS containing a peptide-based polyclonal AR antiserum against N-terminal amino acids 1–21 (1 μg/ml). The sections were washed and placed in biotinylated goat antirabbit IgG solution (1:400) for 2 h, washed in PBS, and then incubated in avidin-biotin-peroxidase complex solution (prepared according to the manufacturer's instructions) for 2 h. Nickel (0.16%)-intensified diaminobenzadine (1 mg/ml) was used as the chromogen. After ICC staining, sections were mounted on gelatin-treated glass slides, air-dried, dehydrated through a graded ethanol series, cleaned in Hemo De, and coverslipped with Permount medium. Although PG-21 is a fully characterized AR antiserum (20), these were the initial studies with mouse brain, and control procedures that included excess unlabeled peptide and exclusion of primary antiserum were run. Staining was absent under these conditions in all sections (not shown).

Image analysis

Sections were matched across the six groups according to the method of Slotnick and Leonard (21). For each region, sections were prepared from at least three animals. Images were taken with a CCD-72 video camera connected to a Macintosh computer equipped with an AG-5 Scion frame grabber at a magnification of 20 × 3.3 using an Olympus BH-2 microscope. Measurements of staining intensity were taken in a matched 725 × 725-μm field in each area after the images were thresholded by density slicing to the same value. The mean background density for each image was determined after density slicing the background area and was used as a correction factor. Data generated by this approach include the total area of stained particles and mean particle density, which were used for calculating integrated particle density (IPD), defined by the following formula: IPD = total area of stained particles × (mean particle density − mean background density). The IPD measure provided a semiquantitative index of average staining intensity within a given region and was used as the unit of analysis. A similar approach for comparing AR immunostaining across brain regions was employed by Menard and Harlan (17).

Western blot and ECL detection

Twenty-four hours after gonadectomy and hormone administration, the animals were killed by cervical dislocation, and blocks containing the regions included in the ICC analysis plus hypothalamus (which also exhibited strong AR staining in the presence of T; data not shown) were isolated from the rest of the brain and minced on an iced stage. Tissues were then placed in 10 mM Tris (pH 7.4), 0.12 M sucrose, 2.5 mM $MgCl_2$, 5% SDS, 1 mM phenylmethylsulfonylfluoride, and 0.02% NaN_3 and sonicated. Samples were then boiled for 7 min to denature proteins and reduce proteinase activity and were centrifuged at 7000 rpm for 2 min to pellet any undissolved debris. Supernatants were saved, and protein concentrations were quantitated using the Bio-Rad protein assay method. Each sample was adjusted to 60 μg total protein before loading. Proteins were separated on Bio-Rad precast 10% polyacrylamide mini gels. The buffers and electrophoresis procedure have been described previously (22). Proteins were transferred to nitrocellulose membranes by electroblotting. For immunoblot detection the Amersham ECL detection system was used. In brief, blots were blocked with 5% dry milk in TBS buffer (20 mM Tris, pH 7.6; 137 mM NaCl; and 0.1% Tween-20) for 1 h, transferred into TBS buffer containing 1% dry milk and 0.1 μg/ml PG-21 primary antiserum, and incubated for 1 h followed by washes with TBS. Blots were then treated with horseradish peroxidase-conjugated antirabbit IgG diluted in TBS buffer containing 1% dry milk for 1 h and again washed. Finally, blots were reacted with ECL detection reagents, and exposed to Hyperfilm for 1 h. Films were processed with Kodak GBX developer and fixative (Eastman Kodak, Rochester, NY). The area and intensity of each band were quantitated using NIH Image (version 1.59) installed in an Apple Macintosh IIvx computer connected to a Fotodyne photographic system (Fotodyne Inc., Hartland, WI). Three independent replicates were run in each sex at each dose. Preliminary studies showed that the presence of excess peptide completely blocked the immunoreactive bands (not shown).

Data analysis

A two-way ANOVA was used to assess general trends in the ICC results. A series of one-way ANOVAs was conducted to provide more detailed information about effects within each group and each region. The Western blot results were evaluated using ANOVA and trend analysis. In all cases, *post-hoc* comparisons used Duncan's new multiple range test with $\alpha = 0.05$.

Results

ICC

In general, positive AR immunostaining was restricted almost exclusively to cell nuclei in all groups and regions. Cytoplasmic staining was not seen in intact or TP-treated animals. In gonadectomized males and females, there was a significant reduction in the intensity of nuclear staining, and a concomitant increase in cytoplasmic staining was not observed. When detected, the levels of reaction product were very low in this compartment.

The results are shown graphically in Fig. 1, and a representative series of sections is presented in Fig. 2. This pattern of group differences was consistent within each of the areas analyzed. A two-way ANOVA showed that there were significant differences among the six groups in the density of AR staining independent of region [$F(5,124) = 252$; $P < 0.001$]. Duncan's multiple range test demonstrated the INT-M, GDX-F+T, and GDX-M+T groups did not differ from each other, but exhibited significantly greater staining intensity than the INT-F, GDX-F, and GDX-M animals ($P < 0.05$; the latter groups also did not differ from each other). There also were significant overall differences in AR immunostaining among the four regions

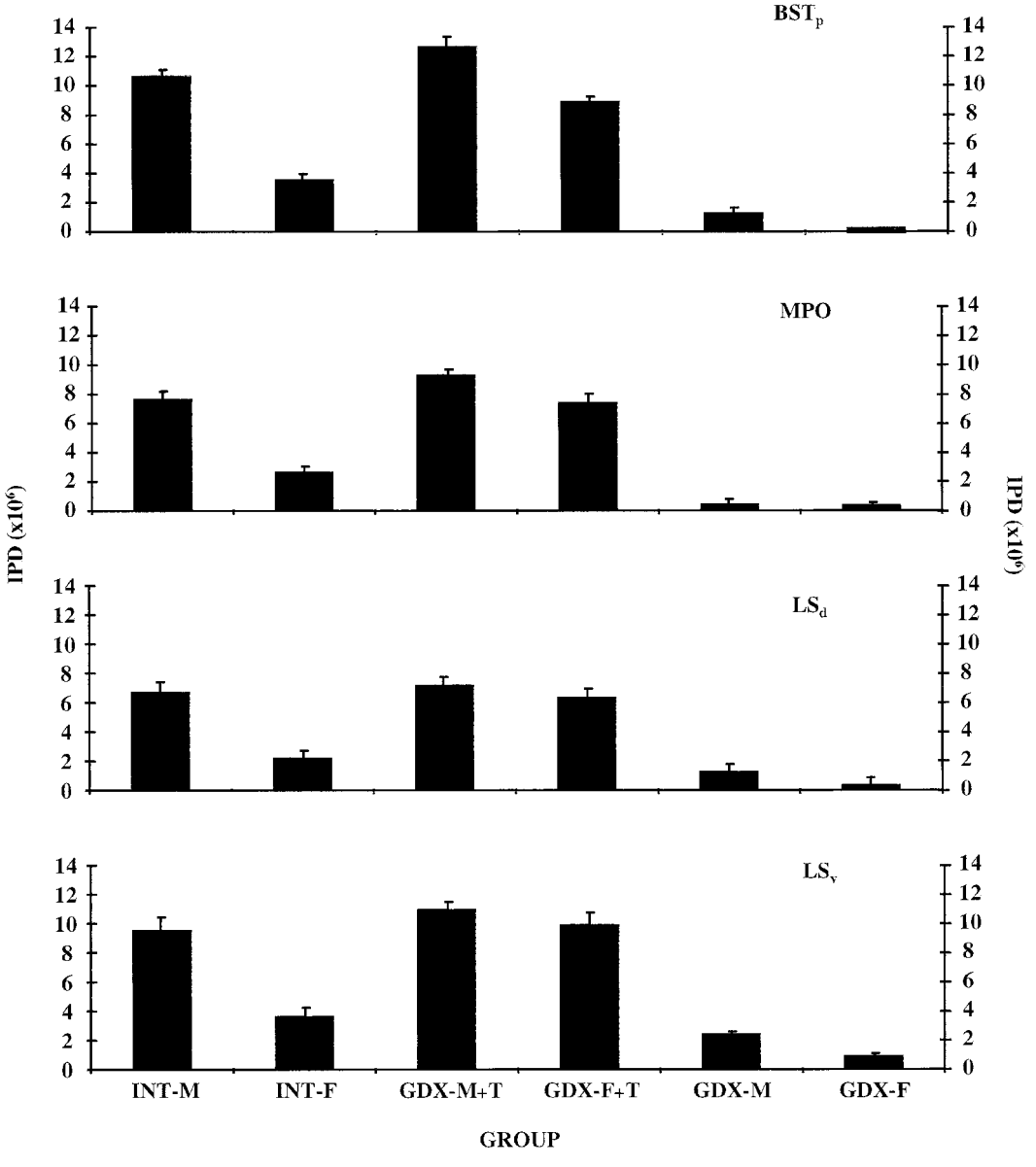

FIG. 1. A summary of AR immunoreactivity in each of the six groups by brain region. Data shown are integrated particle densities (mean ± SEM). See text for details of the statistical analyses.

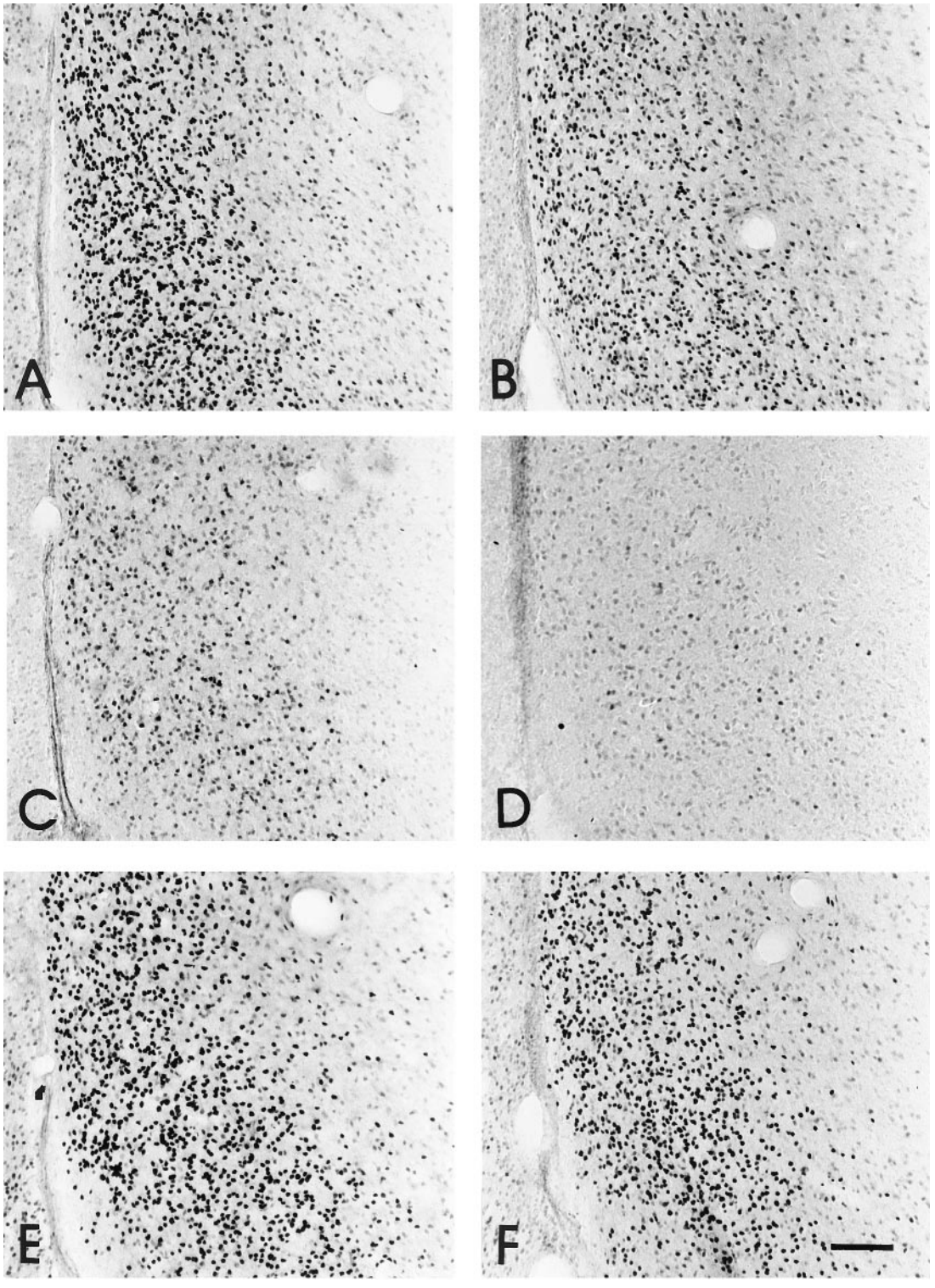

FIG. 2. Representative ICC sections from LS_V in each of the six groups. A, INT-M; B, INT-F; C, GDX-M; D, GDX-F; E, GDX-M+TP; F, GDX-F+TP. This pattern of group differences was seen in four regions. *Bar* = 100 μm.

[$F(3,124) = 29.86$; $P < 0.001$] independent of hormonal status. *Post-hoc* comparisons showed that BST_p and LS_V, although not different from each other, exhibited more intense staining than MPO and LS_D ($P < 0.05$). The latter two regions did not differ significantly. The group × region interaction also was statistically significant [$F(15,124) = 3.51$; $P < 0.01$]. This appeared to be primarily due to a very strong induction of AR in GDX-F+T in LS_V. The one-way ANOVA on results from this region provides more detailed information regarding this point.

Regional effects

LS_V. ANOVA of results within this area revealed a significant overall difference in the integrated density of AR immunostaining among the six groups [$F(5,36) = 81.62$; $P < 0.01$]. Duncan's test showed that the INT-M, GDX-M+T, and GDX-F+T groups did not differ from each other and had significantly higher IPD values than the INT-F, GDX-M, and GDX-F groups. Among the latter groups, INT-F and GDX-M differed significantly from GDX-F (see Fig. 2).

LS_D. The ANOVA showed that the groups differed significantly in the intensity of AR staining [$F(5,36) = 37.98$; $P < 0.01$]. *Post-hoc* comparisons revealed that GDX-M+T, INT-M, and GDX-F+T did not differ from each other and had significantly denser staining than INT-F, GDX-M, or GDX-F. Differences between INT-F and GDX-M were not significant, nor did GDX-M and GDX-F differ from each other. However, AR immunoreactivity in INT-F was significantly greater than that in GDX-F.

BST_p. ANOVA revealed significant differences among the groups in AR immunoreactivity [$F(5,22) = 105.2$; $P < 0.01$]. Duncan's test showed that IPD was greater in GDX-M+T than in all other groups. Males and GDX-F+T, although not differing significantly from each other, had more intense staining than INT-F, GDX-M, and GDX-F. Within these three conditions, AR immunoreactivity was significantly greater in INT-F than in GDX-M or GDX-F.

MPO. ANOVA demonstrated significant differences in the IPD of AR immunoreactivity [$F(5,30) = 55.88$; $P < 0.01$]. *Post-hoc* comparisons showed that the GDX-M+T group exhibited significantly higher immunoreactivity than all other groups. GDX-F+T and GDX-M+T did not differ from each other, but were significantly higher than INT-F, GDX-M, and GDX-F. Significantly greater immunoreactivity was seen in INT-F than in GDX-M and GDX-F, and these latter two groups did not differ from each other.

Intragroup effects

INT-M. ANOVA of the results summarized in Fig. 1 showed that there were significant differences in AR immunoreactivity among the four regions [$F(3,20) = 4.09$; $P < 0.05$]. The intensity of AR-positive staining was highest in BST_P and was significantly greater than that seen in LS_D and MPO, but not in LS_V. The AR-positive staining intensity was significantly higher in the latter region than in LS_D. Values in the MPO and LS_D did not differ from each other.

INT-F. There were no significant differences in the density of AR staining across the four regions [$F(3,20) = 3.05$; P = NS].

GDX-M. The ANOVA showed significant overall differences among the four regions [$F(3,20) = 7.97$; $P < 0.01$]. *Post-hoc* comparisons revealed that IPD was significantly greater in LS_v than in the other regions. Values in BST_p, LS_D, and MPO did not differ significantly from each other.

GDX-F. ANOVA of the results summarized in Fig. 1 showed that there were no significant differences in the density of AR-positive staining among the four regions [$F(3,20) = 1.33$; P = NS].

GDX-M+T. There were significant regional differences in AR immunoreactivity [see Fig. 1; $F(3,20) = 18.7$; $P < 0.01$]. *Post-hoc* comparisons revealed that values in all regions differed significantly from each other, with the rank order being $BST_p > LS_v > MPO > LS_D$.

GDX-F+T. ANOVA revealed significant differences in AR density among the regions [$F(3,20) = 7.35$; $P < 0.01$]. *Post-hoc* comparisons showed that AR immunoreactivity in LS_V and BST_p did not differ, whereas staining intensity was significantly greater than in LS_D. The LS_V also was significantly higher in IPD than MPO.

Western blot

The results are shown in Fig. 3. A dominant 97-kDa band, which was regulated by androgen, was detected in all groups. The values reflect the integrated density of this band, which was calculated by multiplying intensity × area. A two-way ANOVA showed that there was a significant sex difference in the optical density of the AR band [$F(1,32) = 8.9$; $P < 0.01$]. As shown in the histogram, this effect was due primarily to the difference in normal and residual AR band densities in males and females when intact and after castration, respectively. The induction of AR in both sexes 24 h after exogenous TP administration was essentially identical. There also was a significant difference among the dosages independent of sex [$F(6,32) = 22.9$; $P < 0.01$], whereas the sex × treatment interaction was not significant [$F(6,32) = 1.18$; P = NS]. Trend analyses were employed to assess whether there was a linear response to the increasing doses of TP (in this analysis, data from the intact animals were excluded because of the sex difference in endogenous circulating androgen). In both males and females, a significant linear trend was evident through the 1000-μg dose [males: $F(1,12) = 33.62$; $P < 0.001$; females: $F(1,12) = 49.81$; $P < 0.001$], demonstrating that AR can be induced beyond levels seen in intact males in neural tissue. A second band at approximately 55 kDa also was detected (not shown). The identity of this band is unclear, because integrated density did not differ significantly among the groups, which would have been expected if the band represented a proteolytic fragment of the receptor.

Discussion

Four findings in this study extend previous investigations of neural AR regulation. One was the sexual dimorphism in AR immunoreactivity, which was observed in all four regions. This result is basically consistent with those reported in rats, with the exception of the lateral septum, where no difference was found between males and females (12). The discrepancy can be explained by differences in analytical methods, specifically the use of positively labeled cells *vs.* the integrated density of AR immunostaining. Perhaps more important was the observation of comparable regulation of AR in males and females after androgen administration. Although other studies also have shown up-regulation of AR by androgen in male brain in 24 h or less (5, 6, 8, 10), the present results are the first to demonstrate androgen-depen-

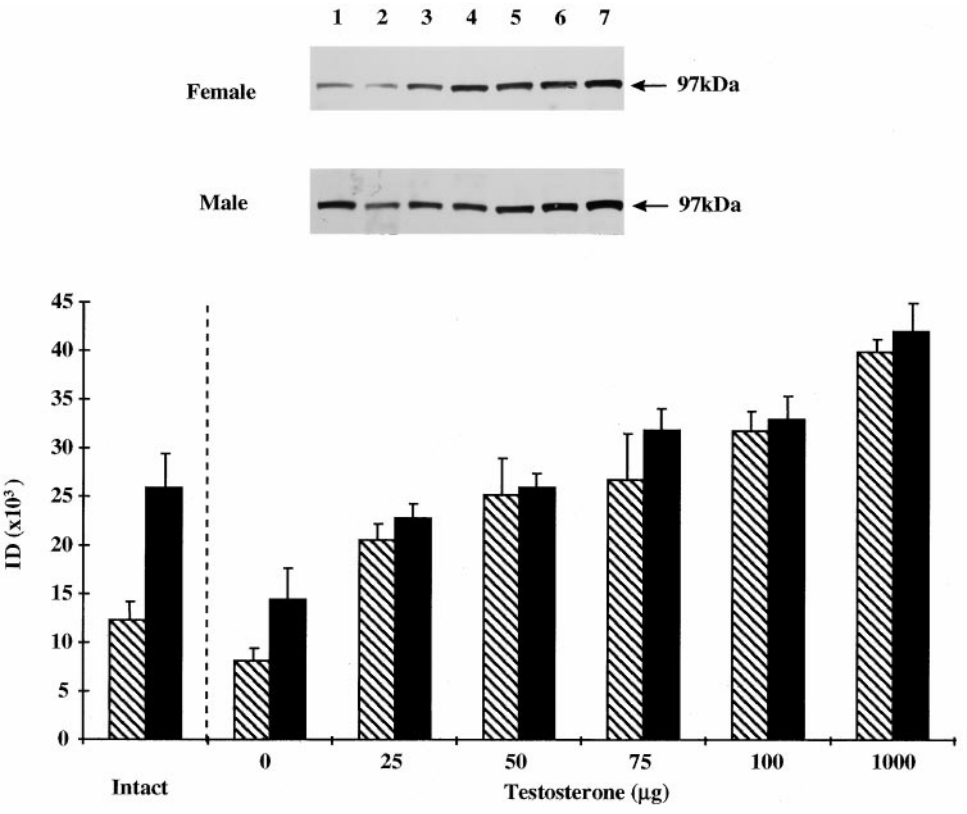

FIG. 3. A summary of the Western blot results and representative gels showing the regulated 97-kDa AR band in females and males that were intact, gonadectomized, or gonadectomized and treated with 0–1000 μg TP. Data shown are integrated densities of each band (mean ± SEM) of three independent replications in each sex for each condition. Lanes 1–7 on both gels: intact, gonadectomized (0), and 25, 50, 75, 100, and 1000 μg TP, respectively. ▨, Females; ■, males. The presence of excess peptide completely blocked the immunoreactive bands in other runs (not shown). See text for details of the statistical analyses.

dent regulation of AR in female brain in a manner essentially identical to that seen in males. This finding has implications for models of sex differences in neural target tissue sensitivity to androgen. Third, the consistency of castration and T-induced effects in both the ICC and Western blot analyses is noteworthy. Fourth, there was a linear increase in AR in response to TP in both males and females at doses up through 1000 μg. Androgen receptor band densities in both females and males exceeded those seen in intact males at all doses above 50 μg. This significant induction of AR was specifically dependent on androgen, because flutamide, an antiandrogen, did not increase AR density beyond that seen in castrated males at comparable doses (Lu, S., and N. G. Simon, unpublished data). A similar enhancement of the AR protein level was noted in several brain regions in intact male rats after 14 days of exposure to anabolic androgens (7), in spinal nuclei in castrated males 8 h after a 2-mg TP injection (5), and in castrated European male ferrets after 10 days of T treatment (16). These observations bear on a number of issues raised by recent immunochemical and regulatory studies of neural AR, including whether PG-21 recognizes unliganded receptor, the suggestion of region-specific differences in AR regulation, and, more broadly, whether changes in AR density contribute directly to neural sensitivity to androgen.

Previous efforts to understand neural AR regulation in males had led to the suggestion that PG-21 did not recognize unliganded AR (5, 6, 8, 18). This position was derived from observations of AR after androgen withdrawal or treatment in, for example, BST and several androgen-responsive spinal nuclei and conflicting reports on whether castration did or did not cause a loss of AR immunoreactivity in neural target cells (c.f. Ref. 10 *vs.* Ref. 11). The present study, by combining both ICC and Western blot analyses, provides some insight into this question. The former consistently showed a loss of AR immunoreactivity 24 h after castration in both males and females in the four regions reported and has been observed throughout the mouse brain (Lu, S., and N. G. Simon, unpublished observations). Because the Western analyses yielded ratios of AR band densities that mirrored the ICC data, it seems reasonable to conclude that the loss of immunoreactivity in gonadectomized animals in the ICC study reflected a steep decline in AR protein levels rather than an inability of PG-21 antiserum to detect unoccupied AR. More specifically, if unoccupied receptor conformation *in situ* had made the targeted epitope unavailable for antibody recognition and thus caused the decreased staining in the ICC experiment, then the Western blot analyses, where proteins were denatured, would have shown more intense AR bands in the gonadectomized male and female conditions. Combined, these observations suggest that there is a rapid loss of AR in neural target tissue as circulating androgens decrease, which also has been seen in peripheral tissues such as ventral prostate (23, 24), and that androgen serves to stabilize the receptor. Support for the latter is seen in biochemical studies that showed an effect of ligand on AR half-life (1, 25, 26). One implication of this perspective about AR loss in the absence of androgen is that earlier equilibrium binding studies that used an interval of 24 h or more after castration before conducting the assays may have underestimated the normal endogenous AR population (27–30). Exchange assays thus appear to be necessary for accurate quantitation of AR (31, 32).

Males and females exhibited comparable responses to go-

nadectomy and TP replacement, which bears on the question of whether AR regulation by androgen is a factor in the sexual dimorphism in neural and behavioral sensitivity to androgen. The effects were similar across the four regions, particularly in response to TP treatment. Dose-response effects did not differ between males and females in the Western analyses; there was a linear response to the increasing level of androgen. These observations demonstrate that there is a common regulatory mechanism for AR in male and female neural tissue in mice and that the major factor is the presence or absence of androgen, findings that are consistent with autoregulation of AR by androgen. The progressive increase in AR band density that accompanied higher TP doses demonstrates that augmentation of AR levels is possible and is in keeping with recent immunochemical results. In one of these studies, AR expression also was increased beyond that seen in normal males 24 h after treatment (5), whereas in the other, enhanced AR levels were found 14 days after exposure to an anabolic androgen cocktail (17). Because androgen binding slows receptor degradation and extends the AR complex half-life (1, 26), it is likely that stabilization of AR is a contributing factor in the up-regulation of receptor levels. At the same time, *de novo* AR synthesis is required for a constant supply of receptor protein (23). Given that AR functions as a transcription factor, it is likely that alterations in its cellular level have direct effects on target gene expression.

The augmentation of AR levels and the significant up-regulation of this protein within 24 h of androgen administration have implications for efforts to understand behavioral changes associated with anabolic steroid abuse and mechanisms underlying neural target tissue sensitivity to androgen. Extended exposure to anabolic steroids has been linked to a set of personality changes, including "roid rage" (33–35). The elevated levels of AR seen in response to higher doses of androgen may represent part of the cellular events that underlie these changes. In this context, chronic, high level anabolic androgen treatment significantly increased the aggressiveness of pubertal male hamsters (36) and the optical density of AR in rat brain (17). At the same time, the comparable increases in AR immunoreactivity in females and males suggest that changes in the level of this protein are not sufficient to produce parallel changes in behavioral sensitivity to androgen. A prominent example of this dissociation is the induction of male-typical aggression in adult female mice. The activation of this response in females is a direct, androgen-dependent effect (37–39). Sixteen to 21 days of androgen exposure were required before aggression toward a stimulus male was seen, yet the present results showed that AR levels were dramatically increased in females within 24 h. This suggests that changes in AR content alone are not sufficient for the induction of male-typical behaviors. Rather, increased cellular AR content probably triggers progressively enhanced (or suppressed) transcription of other androgen-regulated genes (40), which, in combination, lead to the expression of behaviors such as aggression. A comparable view regarding the lack of a simple relationship between AR immunoreactivity and responsiveness to the masculine sexual behavior-promoting effect of T recently was expressed based on work in male hamsters (41), although it was noted that aromatization of T to estradiol was a cautionary factor in their conclusions.

Three additional issues require attention. One is the observed molecular weight of AR (97 kDa), the second is the absence of any apparent regional differences in AR regulation, and the third is the apparent lack of cytoplasmic immunostaining. Regarding the mol wt, 97 kDa is consistent with that expected based on the derived amino acid sequence for AR (4, 42). In addition, Puy *et al.* (43) isolated human neural AR from temporal lobe, and Western analysis demonstrated that it was a 98-kDa protein. However, other investigators have reported AR as an approximately 110-kDa molecule (44–46). Although explanations for this modest discrepancy probably include differences in extract preparation and electrophoresis conditions, the most important points are that the 97-kDa protein observed in the Western-ECL blots was immunoreactive with PG-21, a fully characterized anti-AR antiserum, and that this band was selectively regulated by androgen. Regional differences in AR regulation were not observed, at least qualitatively. This should be viewed in the context of the 24-h sampling point used in the study. Caution is necessary before generalizing this finding, however, because other groups have found regional variation in up-regulation at shorter or longer intervals using different measurement systems and when comparing animals before and after puberty (5, 17, 47, 48). Future work that examines multiple time points and uses directly comparable analytical methods can help clarify this issue. Third, cytoplasmic staining was not detected in intact or T-treated males and females and was negligible in the castrated groups. This observation indicated that the decreased intensity of nuclear immunoreactivity seen 24 h after castration was due to a loss of AR, a conclusion strongly supported by the Western blot results. In this context, increased cytoplasmic immunostaining has been reported 2 weeks or more postcastration (10, 11), and others have noted some staining in this compartment (5, 49). Although the former is not inconsistent with the present results because of the intervals employed, the latter may be due to species, tissue, or methodological considerations.

In closing, a pronounced, androgen-dependent sexual dimorphism in endogenous AR populations was described in four regions of the mouse brain. This sexual dimorphism was not due to an inability of PG-21 to recognize unliganded AR, because Western blot analyses showed effects on AR expression that were fully consistent with the ICC findings. The central role of androgen as a determinant of AR expression was demonstrated in both male and female neural tissue. Finally, the virtually identical effects of androgen administration on the level of immunoreactivity in both sexes indicate that there is a common mechanism of AR regulation in mouse brain.

Acknowledgments

Dr. Gail Prins generously provided the PG-21 AR antiserum. The assistance of J. Burton with the image analysis protocol, the technical support of Y. Wang, and the clerical help of J. Hardy-Todero are appreciated.

References

1. **Kemppainen JA, Lane MV, Sar M, Wilson EM** 1992 Androgen receptor phosphorylation, turnover, nuclear transport, and transcriptional activation. Specificity for steroids and antihormones. J Biol Chem 267:968–974

2. **Sar M, Lubahn DB, French FS, Wilson EM** 1990 Immunohistochemical localization of the androgen receptor in rat and human tissues. Endocrinology 127:3180–3186
3. **Syms AJ, Norris JS, Panko WB, Smith RG** 1985 Mechanism of androgen-receptor augmentation. Analysis of receptor synthesis and degradation by the density-shift technique. J Biol Chem 260:455–461
4. **Tan J, Joseph DR, Quarmby VE, Lubahn DB, Sar M, French FS, Wilson EM** 1988 The rat androgen receptor: primary structure, autoregulation of its messenger ribonucleic acid, and immunocytochemical localization of the receptor protein. Mol Endocrinol 2:1276–1285
5. **Freeman LM, Padgett BA, Prins GS, Breedlove SM** 1995 Distribution of androgen receptor immunoreactivity in the spinal cord of wild-type, androgen-insensitive and gonadectomized male rats. J Neurobiol 27:51–59
6. **Iqbal J, Swanson JJ, Prins GS, Jacobson CD** 1995 Androgen receptor-like immunoreactivity in the Brazilian opossum brain and pituitary: distribution and effects of castration and testosterone replacement in the adult male. Brain Res 703:1–18
7. **Simon NG, McKenna SE, Lu SF, Cologer-Clifford A** 1996 Development and expression of hormonal systems regulating aggression. Ann NY Acad Sci 794:8–17
8. **Zhou L, Blaustein JD, DeVries GJ** 1994 Distribution of androgen receptor immunoreactivity in vasopressin- and oxytocin-immunoreactive neurons in the male rat brain. Endocrinology 134:2622–2627
9. **Choate JVA, Resko JA** 1992 Androgen receptor immunoreactivity in intact and castrate guinea pig using antipeptide antibodies. Brain Res 597:51–59
10. **Wood RI, Newman SW** 1993 Intracellular partitioning of androgen receptor immunoreactivity in the brain of the male syrian hamster: effects of castration and steroid replacement. J Neurobiol 24:925–938
11. **Clancy AN, Whitman C, Michael RP, Albers HE** 1994 Distribution of androgen receptor-like immunoreactivity in the brains of intact and castrated male hamsters. Brain Res 33:325–332
12. **Herbison AE** 1995 Sexually dimorphic expression of androgen receptor immunoreactivity by somatostatin neurones in rat hypothalamic periventricular nucleus and bed nucleus of the stria terminalis. J Neuroendocrinol 7:543–553
13. **Lumbroso S, Sandillon F, Georget V, Lobaccaro JM, Brinkmann AO, Privat A, and Sultan C** 1996 Immunohistochemical localization and immunoblotting of androgen receptor in spinal neurons of male and female rats. Eur J Endocrinol 134:626–632
14. **Michael RP, Clancy AN, Zumpe D** 1995 Distribution of androgen receptor-like immunoreactivity in the brains of cynomolgus monkeys. J Neuroendocrinol 7:713–719
15. **Lu S, Simon NG, Nau G, Burton J** 1994 Testosterone enhances androgen receptor immunoreactivity in mouse brain. Soc Neurosci Abstr 20:376
16. **Kashon ML, Arbogast JA, Sisk CL** 1996 Distribution and hormonal regulation of androgen receptor immunoreactivity in the forebrain of the male European ferret. J Comp Neurol 376:567–586
17. **Menard CS, Harlan RE** 1993 Up-regulation of androgen receptor immunoreactivity in the rat brain by androgenic-anabolic steroids. Brain Res 622:226–236
18. **Greco B, Edwards DA, Michael RP, Clancy AN** 1996 Androgen receptor immunoreactivity and mating-induced Fos expression in forebrain and midbrain structures in the male rat. Neuroscience 75:161–171
19. **Meisel RL, Sachs BD** 1994 The physiology of male sexual behavior. In: Knobil E, Neill JD (eds) The Physiology of Reproduction. Raven Press, New York, vol 2:3–105
20. **Prins J, Birch L, Greene G** 1991 Androgen receptor localization in different cell types of the adult rat prostate. Endocrinology 129:3187–3199
21. **Slotnick BM, Leonard CM** 1975 A Stereotaxic Atlas of the Albino Mouse Forebrain. DHEW, Washington DC
22. **Laemmli V** 1970 Cleavage of structural proteins during the assembly of bacteriophage T4. Nature 227:680–685
23. **Mora GR, Prins GS, Mahesh VB** 1996 Autoregulation of androgen receptor protein and messenger RNA in rat ventral prostrate is protein synthesis dependent. J Steroid Biochem Mol Biol 58:539–549
24. **Prins G, Birch L** 1993 Immunocytochemical analysis of androgen receptor along the ducts of the separate rat prostate lobes after androgen withdrawal and replacement. Endocrinology 132:169–178
25. **Grino PB, Griffin JE, Wilson JD** 1990 Testosterone at high concentrations interacts with the human androgen receptor similarly to dihydrotestosterone. Endocrinology 126:1165–1172
26. **Kaufman M, Pinsky L, Schwartz A, Long-Simpson L** 1983 Possible implications of the comparative dissociative behavior of testosterone- and 5α-dihydrotestosterone-receptor complexes formed by human skin fibroblasts. J Steroid Biochem 19:561–565
27. **Chen X, Simon NG** 1990 Genetic variation in hypothalamic methyltrienolone (R1881) binding in male mice. Physiol Behav 47:589–592
28. **Clark CR, Nowell NW** 1979 Binding properties of testosterone receptors in the hypothalamic-preoptic area of the adult male mouse brain. Steroids 33:407–427
29. **Ginsburg M, Shori DK** 1978 Are there distinct dihydrotestosterone and testosterone receptors in brain? J Steroid Biochem 9:437–441
30. **Kerr JE, Allore RJ, Beck SG, Handa RJ** 1995 Distribution and hormonal regulation of androgen receptor (AR) and AR messenger ribonucleic acid in the rat hippocampus. Endocrinology 136:3213–3221
31. **McGinnis MY, Dreifuss RM** 1989 Evidence for a role of testosterone-androgen receptor interactions in mediating masculine sexual behavior in male rats. Endocrinology 124:618–626
32. **McGinnis MY, Davis G, Meaney MJ, Singer M, McEwen BS** 1983 In vitro measurement of cytosol and cell nuclear androgen receptors in male rat brain and pituitary. Brain Res 275:75–82
33. **Galligani N, Renck A, Hansen S** 1996 Personality profile of men using anabolic androgenic steroids. Horm Behav 30:170–175
34. **Choi PYL, Parrot AC, Cowan D** 1990 High-dose anabolic steroids in strength athletes: effects upon hostility and aggression. Hum Psychopharmacol 5:349–356
35. **Pope HG, Katz DL** 1988 Affective and psychotic symptoms associated with anabolic steroid use. Am J Psychiatry 145:487–490
36. **Melloni RH, Connor DF, Hang PT, Harrison RJ, Ferris CF** 1997 Anabolic androgenic steroid exposure during adolescence and aggressive behavior in golden hamsters. Physiol Behav 61:359
37. **Gandelman, R** 1980 Gonadal hormones and the induction of intraspecific fighting in mice. Neurosci Biobehav Rev 4:130–140
38. **Simon NG, Lu SF, McKenna SE, Chen X, Clifford AC** 1993 Sexual dimorphisms in regulatory systems for aggression. In: Haug M, Whalen R, Aron C, Olsen KL (eds) The Development and Expression of Sex Differences in Behavior. Kluwer Academic Press, Amsterdam, pp 389–408
39. **Simon N, Whalen R, Tate M** 1985 Induction of male-typical aggression by androgens but not by estrogens in adult female mice. Horm Behav 19:204–212
40. **Janne OA, Crozat A, Palvimo J, Eisenberg L** 1991 Androgen-regulation of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase genes. J Steroid Biochem Mol Biol 40:307–315
41. **Meek LR, Romeo RD, Novak CM, Sisk CL** 1997 Actions of testosterone in prepubertal and postpubertal male hamsters: dissociation of effects on reproductive behavior and brain androgen receptor immunoreactivity. Horm Behav 31:75–88
42. **Lubahn DB, Joseph DR, Sar M, Tan J, Higgs HN, Larson RE, French FS, Wilson EM** 1988 The human androgen receptor: complementary deoxyribonucleic acid cloning sequence analysis and gene expression in prostate. Mol Endocrinol 2:1265–1275
43. **Puy L, MacLusky NJ, Becker L, Karsan N, Trachtenberg J, Brown TJ** 1995 Immunocytochemical detection of androgen receptor in human temporal cortex: characterization and application of polyclonal androgen receptor antibodies in frozen and paraffin-embedded tissues. J Steroid Biochem Mol Biol 55:197–209
44. **Brinkmann AO, Jenster G, Kuiper GGJM, Ris C, van Laar JH, van der Korput JAGM, Degenhart HJ, Trifiro MA, Pinsky L, Romalo G, Schweikert HU, Veldscholte J, Mulder E, Trapman J** 1992 The human androgen receptor: structure/function relationship in normal and pathological situations. J Steroid Biochem Mol Biol 41:361–368
45. **Wilson CM, McPhaul MJ** 1994 A and B forms of the androgen receptor are present in human genital skin fibroblasts. Proc Natl Acad Sci USA 91:1234–1238
46. **Young CYF, Johnson MP, Prescott JL, Tindall DJ** 1989 The androgen receptor of the testicular-feminized (Tfm) mutant mouse is smaller than the wild-type receptor. Endocrinology 124:771–775
47. **Kashon ML, Sisk CL** 1994 Pubertal maturation is associated with an increase in the number of androgen receptor-immunoreactive cells in the brain of male ferrets. Dev Brain Res 78:237–242
48. **Kashon ML, Hayes MJ, Shek PP, Sisk CL** 1995 Regulation of brain androgen receptor immunoreactivity by androgen in prepubertal male ferrets. Biol Reprod 52:1198–1205
49. **Balthazart J, Foidart A, Wilson EM, Ball GF** 1992 Immunocytochemical localization of androgen receptors in the male songbird and quail brain. J Comp Neurol 317:407–420