Relationship between Sexual Satiety and Brain Androgen Receptors

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Abstract

Recently we showed that 24 h after copulation to satiety,
there is a reduction in androgen receptor density (ARd) in
the medial preoptic area (MPOA) and in the ventromedial
hypothalamic nucleus (VMH), but not in the bed nucleus of
the stria terminalis (BST). The present study was designed to
analyze whether the ARd changes in these and other brain
areas, such as the medial amygdala (MeA) and lateral sep-
tum, ventral part (LSV), were associated with changes in sex-
ual behavior following sexual satiety. Males rats were sacri-
ficed 48 h, 72 h or 7 days after sexual satiety (4 h ad libitum
copulation) to determine ARd by immunocytochemistry; ad-
ditionally, testosterone serum levels were measured in inde-
pendent groups sacrificed at the same intervals. In another
experiment, males were tested for recovery of sexual behav-
ior 48 h, 72 h or 7 days after sexual satiety. The results showed
that 48 h after sexual satiety 30% of the males displayed a
single ejaculation and the remaining 70% showed a com-
plete inhibition of sexual behavior. This reduction in sexual
behavior was accompanied by an ARd decrease exclusively
in the MPOA-medial part (MPOM). Seventy-two hours after
sexual satiety there was a recovery of sexual activity accom-
panied by an increase in ARd to control levels in the MPOM
and an overexpression of ARd in the LSV, BST, VMH and MeA.
Serum testosterone levels were unmodified during the post-
satiety period. The results are discussed on the basis of the
similarities and discrepancies between ARd in specific brain
areas and male sexual behavior.

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Introduction

In males, androgens, principally testosterone and 5α-
dihydrotestosterone, are involved in a wide variety of
physiological and developmental responses and are spe-
cially important for male reproductive function [1]. Most
of the actions of androgens are exerted via an intracellu-
lar mechanism involving the nuclear/cytoplasmic andro-
gen receptor (AR), which belongs to a family of proteins
that function as transcription factors to regulate the ex-
pression of target genes [2]. The AR is an autoregulated
protein as its concentrations largely depend on circulat-
ing levels of androgens [3, 4].

The AR is expressed in several tissues, such as the
prostate [5] and brain [6]. It is documented that in the
male rat brain [7, 8] as well as in the brain of other species
such as the Syrian hamster [9], Brazilian opossum [10],

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several songbird species [11] and even humans [6], AR neurons are basically localized in limbic areas such as the hypothalamus. Limbic areas participate in the control of endocrine function and in the expression of sexual behavior; for example a medial preoptic area (MPOA)/anterior hypothalamus lesion eliminates copulation permanently. Males with this lesion do not recover sexual behavior after high doses of testosterone, suggesting that the mating deficits are not associated with testicular function, but specifically with the neural control of copulation. Moreover, AR blockade within the MPOA results in complete inhibition of masculine sexual behavior [12], stressing the importance of the AR in the brain areas that participate in this behavior. Indeed, colocalization of AR immunoreactivity (AR-ir) and Fos-ir was found in 80% of MPOA neurons, suggesting the activation of AR-sensitive neurons by copulation [13]. Using electrical stimulation, electrolytic or neurotoxic lesions, immunocytochemical procedures and pharmacological manipulations, it has been proven that there are other limbic areas, besides the MPOA and its specific nuclei, that participate in the expression of male sexual behavior, such as the bed nucleus of the stria terminalis (BST), the ventromedial hypothalamic nucleus (VMH), the medial amygdaloid nucleus (MeA), the lateral septal nucleus and the nucleus accumbens [for review, see 14, 15].

Sexual satiety is a phenomenon common to males of many species; it appears after repeated ejaculation and is characterized by a long-term inhibition of sexual activity [16, 17]. A particular sexual satiety paradigm was established in which sexually experienced male rats were allowed to mate ad libitum with a single receptive female during a 4-hour period [18]. During this time, the males executed an average of seven ejaculatory series before ceasing copulation. The recovery from sexual satiety is slow: males need at least a 15-day resting period to completely recover their full mating capacity [17]. Following this sexual satiety paradigm, it is clear that 24 h later, two thirds of the population show a complete inhibition of sexual activity while the remaining third is able to execute a single ejaculatory series without recovery [18]. A previous study found that precisely 24 h after sexual satiety there was a drastic reduction in AR density (ARd) in a very circumscribed anterior part of the MPOA, nucleus accumbens and VMH, whereas there was no such change in the BST. Such ARd reductions did not seem to depend upon the levels of circulating androgens, since they were unmodified at this interval [7]. These results suggest that sexual behavior reduces ARd in certain brain regions. These data, however, did not specifically relate

ARd changes to sexual behavior. On this basis, in the present study we evaluated whether the recovery of sexual behavior at different intervals after sexual satiety (48 h, 72 h, and 7 days) concurred with changes in ARd in several brain areas: the medial part of the medial preoptic nucleus (MPOM, a specific area of the anterior MPOA), the MPOA (considered in all its anteroposterior extension), the BST (medial division, posteromedial and posterointermediate), the VMH (dorsomedial and central parts); the MeA (anterodorsal, anteroventral and posteroventral parts), and the LSV (lateral septal nucleus, ventral part). These brain areas were selected because of: (a) their clear role in the mediation of rat sexual behavior [12, 19–24]; (b) modifications in ARd 24 h after sexual satiety [7], and (c) changes in neuronal activity (reflected as variations in c-Fos-ir) related to sexual behavior in general, and specifically to sexual satiety [21, 25]. In addition, since ARd importantly depends upon androgen circulating levels, we measured the serum levels of testosterone in control and sexually satiated males sacrificed 48 h, 72 h or 7 days after sexual satiety.

Methods

Subjects

Sexually experienced male Wistar rats (250–300 g) and receptive female rats (200–300 g) were used in this study. Food and water were provided ad libitum. The animals were housed 5/cage under a reversed 12-hour light/12-hour dark cycle, lights off at 10.00 h. All experiments and sacrifices were performed during the dark phase of the cycle.

Male Sexual Behavior

Animals were trained for sexual behavior in 3–4 sessions previous to the experimental test. Males were individually placed in cylindrical arenas and 5 min later exposed to females brought into sexual receptivity by the sequential administration of estradiol benzoate (4 μg/rat s.c., –48 h) and progesterone (2 mg/rat s.c., –4 h) [18]. If a male did not intromit within 20 min the test was ended; otherwise all the classical sexual behavior parameters were recorded [for definition see 14]. Only sexually active males were used: those that had ejaculation latencies (time from first intromission to ejaculation) of less than 15 min in the last two training sessions.

Sexual Satiety

Sexually experienced male rats were allowed to copulate ad libitum during 4 h with the same receptive female. The criterion to establish that a male reached sexual satiety was that it did not show sexual activity for 90 min after repeated ejaculations. Previous data from our laboratory showed that 24 h after copulating ad libitum, around 67% of males show complete inhibition of sexual behavior, while the rest is able to execute a single ejaculatory series from which they do not recover [18]. In the present study, we eval-

uated the recovery of sexual satiety at different intervals post-satiety: 48 h, 72 h and 7 days. In these tests the percentage of animals able to ejaculate was registered. Independent groups of males were used for each recovery interval in order to prevent sexual behavior aftereffects of one test over the next one (i.e. males tested 48 h after sexual satiety were not retested 24 h later to determine the level of recovery at 72 h). A group of rested sexually experienced males exposed to receptive females and allowed to copulate ad libitum was used as a control group. The number of animals in each of these groups was 10.

Androgen Receptor Density
Animals were randomly divided into 4 groups of 6–8 subjects each: sexually active males that did not copulate for at least 4 days previous to sacrifice were used as controls, and the 3 experimental groups included sexually satiated rats sacrificed and perfused at 48 h, 72 h or 7 days after sexual satiety.

Brain Perfusion and Fixation
All animals were sacrificed during the dark phase and between 11.00 and 13.00 h. Males were anesthetized with ketamine (100 mg/kg i.p.) and xilacine (20 mg/kg i.p.) and perfused intracardially with a phosphate-buffered saline solution (PBS, pH 7.2) and heparine (0.3 ml) followed by 4% paraformaldehyde in PBS (pH 7.2). Brains were removed and post-fixed for 4 h in 4% paraformaldehyde, then cryoprotected with a 30% sucrose and 0.1% thimerosal solution. Before cutting, brains were rinsed with PBS. Brains were coronally cut (50 μm) using a cryostat (–20°C).

Immunocytochemistry
AR immunocytochemistry was carried out simultaneously in at least 1 animal from each of the 4 groups (48 h, 72 h and 7 days post-satiety and controls). Free-floating sections that included the MPOA, BST, VMH, MeA and LSV, were rinsed (3 × 15 min) with TBS (0.9% NaCl, Tris ultrapure 0.05 M, pH 7.6). All the antibodies were diluted in TBS plus 0.5% Triton X-100 (pH 7.6).

The primary antibody (PG-21) was an IgG-purified polyclonal rabbit antibody targeting the first 21 amino acids of the AR [5]. The sections were incubated with PG-21 (Sigma Chemical Laboratories) diluted at 1:250, left for 1 h at room temperature and subsequently at 4°C overnight. The next day, after rinsing with TBS, the sections were incubated for 1 h with a goat anti-rabbit biotinylated secondary antibody (Vector Laboratories, cat. No. BA-1000) diluted at 1:200. Sections were rinsed with TBS and then incubated for 1 h in an avidin-biotin complex (Vector Laboratories, ABC kit) diluted at 1:800. After washing 3 times with TBS, the immunoreactivity was visualized using 3,3′-diaminobenzidine, 0.0001% hydrogen peroxide and 0.03% nickel ammonium sulfate, all diluted in distilled water (Vector Laboratories). The sections were then mounted on microscope slides, dehydrated in ethanol and xylene and cover-slipped.

We recently reported that the bilateral distribution of AR-ir neurons was similar [26]. On this basis, in the present study we analyzed AR-ir on both brain sides.

Quantitative Analysis of ARd
For all analyses the observer was unaware of the animal's condition, i.e., whether the sections belonged to control or experimental animals sacrificed at different intervals after sexual satiety. The observations were made at two different microscopic magnifications: 5× panoramic view to determine the area of interest and 10× for quantification. The selected brain area was photographed with a Pixera Viewfinder camera (Pixera Co., Los Gatos, Calif., USA). Hi-fidelity colored photomicrographs of the selected areas were taken with a resolution of 1,260 × 960 pixels. All photographs were adjusted for contrast (–10 to 10), brightness (–12 to 12) and gamma (0.75 to 1.15) (using the program Pixera Studio, Version two 1996–8, Pixera Co.) before analysis to optimize AR-ir detection. For ARd analyses the photographs were converted to black and white (grayscale) and not further modified. Objects were considered AR-positive when their immunoreactive intensity varied in a range between 43–100% black. Previous reports using a similar range have demonstrated clear changes in ARd [7] and estrogen-α receptor density [27] associated with sexual satiety. Since the program labels each identified particle, double counts were not possible.

The location of the brain areas analyzed for ARd were established according to cresyl violet-stained sections (selected 50 μm apart from the stained ARd sections) and a rat brain atlas [28]. Quantification of ARd was carried out with the aid of image analysis software (LabWorks, 4.5 for Windows, UVP Bioimaging Systems, Cambridge, UK) for the following brain structures throughout the anteroposterior range (in reference to bregma according to the atlas [28]): MPOM (including its central part) –0.80 to –0.92 mm; MPOA (from its most anterior to its most posterior portion) –0.3 to –1.30 mm; BST (medial division, posteromedial and posterointermediate) –0.80 to –0.92 mm; VMH (dorsomedial and central parts) –2.30 to –2.80 mm, MeA (anterodorsal, anteroventral and posteroventral parts) –2.30 to –2.80 mm, and LSV 0.20 to –0.40 mm. Thus, a single animal provided several ARd values for a given brain region; these values were averaged to obtain a mean value. The evaluated area within each brain structure was kept constant for all determinations as follows: LSV, 0.50 mm^2; MPOM, 0.26 mm^2; MPOA, 0.26 mm^2; BST, 0.63 mm^2; VMH, 0.36 mm^2, and MeA, 0.36 mm^2 (fig. 2–5). ARd is expressed as the percentage of stained area, i.e., the sum of the stained areas divided by the total evaluated area. The statistical analyses were based on the number of animals and not on the number of sections evaluated. A one-way ANOVA followed by Dunnet's test was run to analyze putative statistical differences of ARd in a given brain structure depending upon the animals' condition: controls and sexually satiated males sacrificed 48 h, 72 h or 7 days after satiety.

Circulating Levels of Androgens
Serum testosterone levels were measured in sexually experienced rested control rats that did not copulate (n = 7) and sexually satiated males sacrificed 48 h (n = 9), 72 h (n = 8) or 7 days (n = 8) after sexual satiety. Males were sacrificed by decapitation and the trunk blood collected in cold tubes. The blood was centrifuged (5,000 rpm for 30 min at 0°C) to obtain plasma samples that were stored at –4°C.

Total plasma testosterone concentrations were measured by radioimmunoassay using a commercial kit (TKTT1, Diagnostic Product Corporation). The procedure used antibody-coated tubes in which ^{125}I-labelled testosterone competed with testosterone in the sample for antibody sites. After incubation, separation of bound testosterone was achieved by decanting. The tubes were

Fig. 1. Quantification of androgen receptor density (ARd) expressed as the percentage of stained area in the MPOM (medial preoptic nucleus, its medial and central parts), MPOA (from its most anterior to its most posterior portion), BST (bed nucleus of the stria terminalis, medial division, posteromedial and posterointermediate), VMH (ventromedial hypothalamic nucleus, dorsomedial and central parts), MeA (medial amygdaloid nucleus, anterodorsal, anteroventral and posteroventral parts) and LSV (lateral septal nucleus, ventral part) of controls or males sacrificed 48 or 72 h or 7 days after sexual satiety. One-way ANOVA (see text) followed by Dunnett's test: * $p \leq 0.05$; ** $p \leq 0.01$.
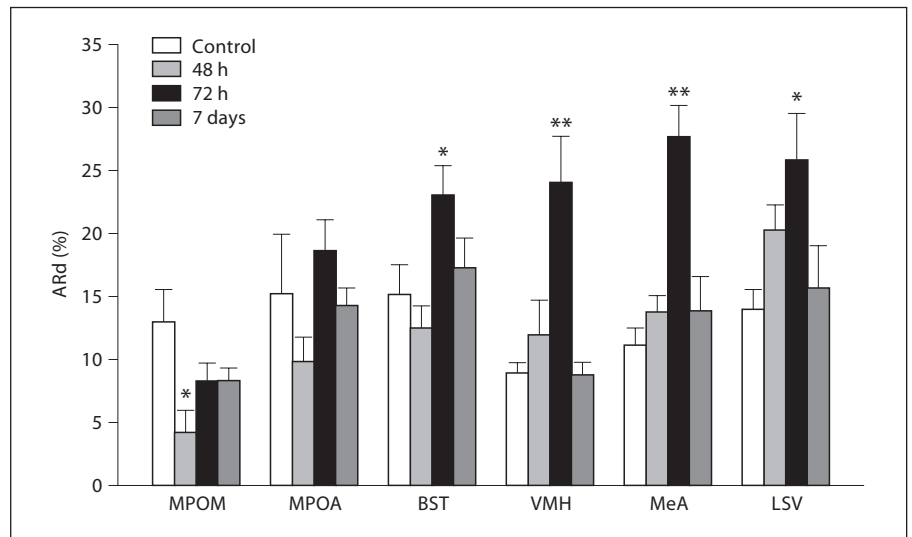
then counted in a gamma counter, the counts being inversely related to the amount of testosterone present in the serum. The total quantity of testosterone (ng/ml) was determined by comparing counts to a calibration curve. The specific activity was 4 μCi. The inter-assay and intra-assay variabilities were 7.65 and 6.85, respectively.

Since values for testosterone serum determinations did not pass the normality test, these data were analyzed using a Kruskal-Wallis ANOVA.

Results

Our analysis revealed that AR-ir was specifically nuclear (i.e., none was cytoplasmatic, data not shown).

Figure 1 shows the quantitative analysis of ARd expressed as percentage of stained area. As previously reported, 24 h after satiety there was a noteworthy decrease in ARd in the anterior part of the MPOA [7]. In the present study, the MPOM had a significant decrease in ARd 48 h post-satiety; when compared to the control group ($F_{3,22} = 3.90$; $p = 0.022$). ARd recovered 72 h and 7 days after sexual satiety; at these intervals no differences in AR-ir were observed between the groups. When considering the MPOA in all its anteroposterior extension (and not exclusively its medial or anterior nuclei), the changes in ARd in sexually satiated males retained a tendency to decrease that did not reach statistical significance ($F_{3,24} = 1.61$; $p = 0.212$).

Interestingly, important differences in ARd were revealed in the VMH ($F_{3,22} = 10.45$; $p < 0.001$), LSV ($F_{3,23} = 3.73$; $p = 0.026$), MeA ($F_{3,21} = 12.64$; $p = 0.001$) and BST

($F_{3,21} = 4.54$; $p = 0.013$). In these brain areas, there was a statistically significant ARd increase in the group sacrificed 72 h after sexual satiety (fig. 1). For example, MeA ARd was almost three times higher in rats assayed 72 h post-satiety compared to controls (27.69 ± 2.60 vs. 11.13 ± 1.28, respectively). This augmentation was circumscribed to 72 h after sexual satiety, since no significant differences were observed in the groups sacrificed 48 h or 7 days after sexual satiety (fig. 1).

Figures 2–5 show photomicrographs of sections stained with cresyl violet (panels b) and AR-ir (panels a) illustrating the different brain nuclei and the sites of ARd analysis within them. In these same figures, the changes in AR-ir at different times after sexual satiety are illustrated in representative photomicrographs (fig. 2–5, panels c and d). Clearly, there is a drastic decrease in ARd 48 h after sexual satiety in the MPOM; while there is an important increase in ARd 72 after sexual satiety in the LSV, VMH and MeA.

Testosterone serum levels in controls and in males tested 48 h, 72 h and 7 days after sexual satiety are shown in table 1. Clearly, circulating testosterone levels were similar between controls and males tested at different intervals after sexual satiety. These data are in line with previous findings [7, 29].

Table 2 shows the percentage of rats ejaculating 1–6 successive times at different intervals after sexual satiety. All control males (previously not subjected to sexual satiety) showed, as a minimum, 6 ejaculations before reaching sexual satiety. Clearly, 48 h after sexual satiety only a few males ejaculated (30%) and none of these animals re-
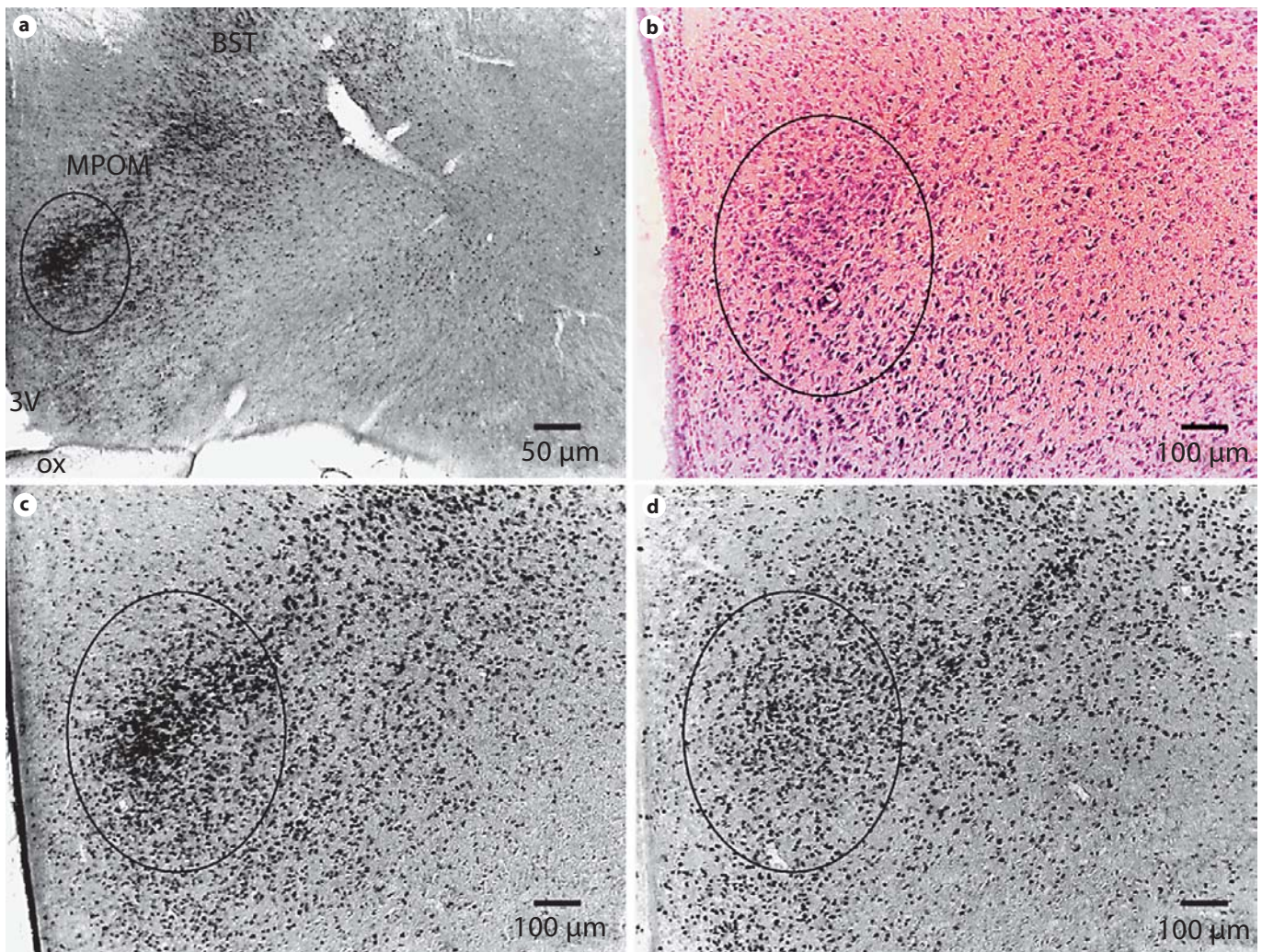
Fig. 2. Representative photomicrographs showing changes in the androgen receptor density (ARd) of the MPOM (medial preoptic nucleus, medial and central parts) after sexual satiety. **a** Panoramic view of AR-ir in a control male at 5× magnification. 3V = Third ventricle; ox = optic chiasm; BST = bed nucleus of the stria termi-nalis. **b** Cresyl violet MPOM of a control male at 10× magnification. **c** MPOM AR-ir of a control male rat. **d** MPOM AR-ir 48 h after sexual satiety. Note the drastic ARd reduction 48 h after sexual satiety compared to control (**c**, **d**).

initiated copulation. Interestingly, 72 h after sexual satiety a high percentage (70%) of animals ejaculated once and 50% recovered to show a second ejaculation. Seven days after sexual satiety all males were able to ejaculate twice and 30% ejaculated 6 times.

Discussion

The present results show that ARd is reduced in the MPOM 48 h after animals copulated to reach sexual satiety. This ARd reduction coincides with a drastic inhibi-tion of sexual behavior subsequent to sexual satiety. A reduction in ARd and in mating was also found 24 h after sexual satiety [7]. ARd analysis in all the length of the MPOA did not reveal differences between sexually sati-ated and control males, although 48 h after sexual satiety ARd tended to reduce. The most surprising observation of the present experiments is that 72 h after sexual satiety there is a drastic increase in ARd in the VMH, LSV, MeA and BST. This ARd increase was restricted to this time interval and could be associated with the recovery of sex-ual behavior, reflected as an increased percentage of ejac-ulating males.
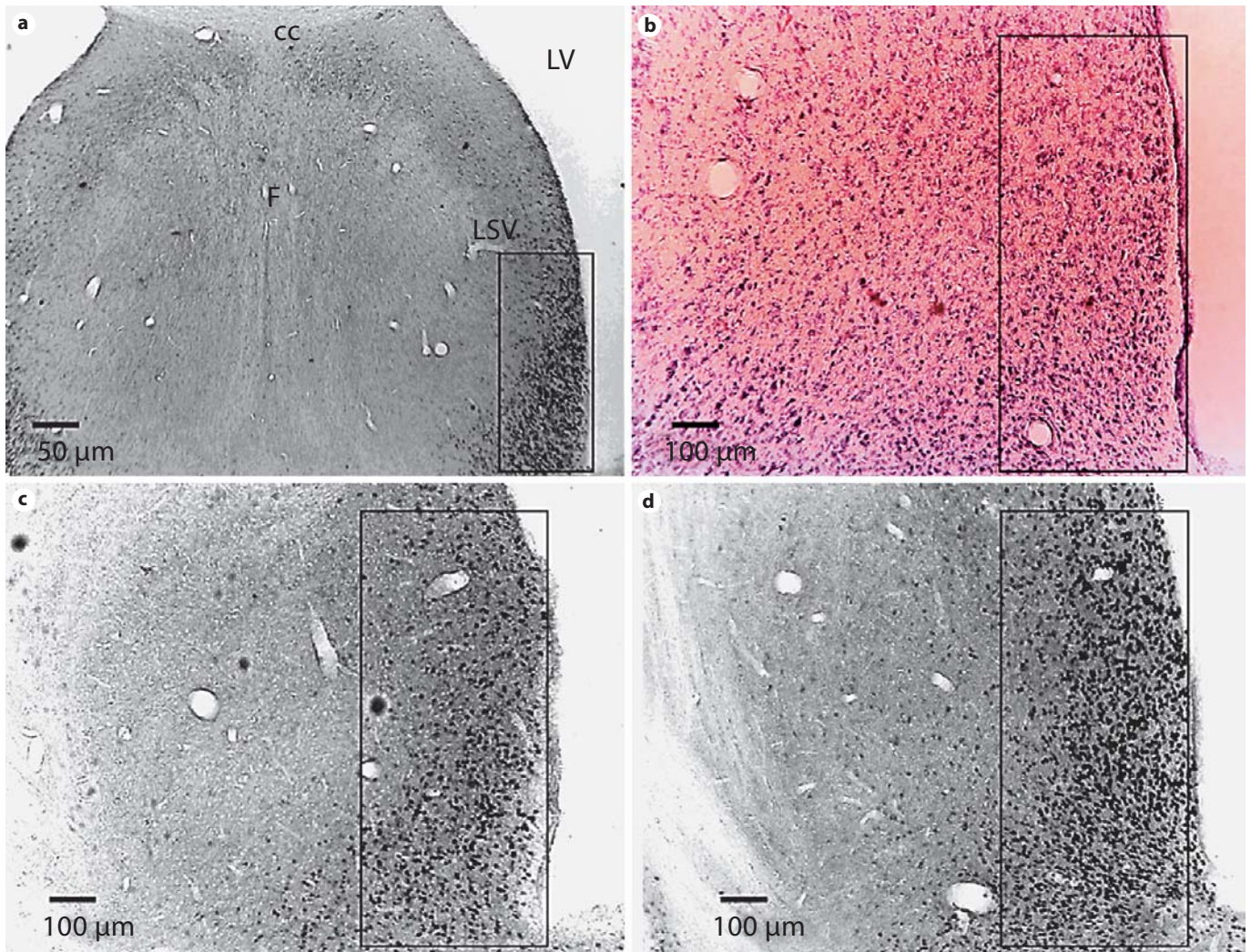
Fig. 3. Representative photomicrographs showing changes in the androgen receptor density (ARd) of the LSV (lateral septal nucleus, ventral part) after sexual satiety. **a** Panoramic view of AR-ir in a control male at 5× magnification. LV = Lateral ventricle; cc = corpus callosum; F = fornix. **b** Cresyl violet LSV of a control at 10× magnification. **c** LSV AR-ir of a control male. **d** LSV AR-ir 72 h after sexual satiety. Note the drastic ARd increase 72 h after sexual satiety compared to control (**c, d**).

It is well documented that AR-ir in various brain areas may change depending upon the circulating levels of androgens in males and females of several species, including humans [30–32]. Thus, in contrast with the intense nuclear AR-ir observed in intact male rats, after castration AR-ir is pale in the nucleus and occasionally present in the cytoplasmic compartment [9, 30]. Treatment with testosterone or non-aromatizable androgens, but not with estrogens, restores AR in the cell nucleus within minutes [8, 9, 10, 33]. Moreover, chronic treatment of castrated or intact male rats with a mixture of anabolic androgenic steroids upregulates ARd in various brain areas associated with male sexual behavior [8, 34]. On these bases, the changes in ARd in various brain areas after different intervals following sexual satiety may be due to changes in androgen levels. Arguing against this association, previous [7] and present data demonstrate that circulating androgen levels are unmodified in sexually satiated animals sacrificed 24 h, 48 h, 72 h or 7 days later. However, immediately after sexual satiety there is a drastic increase in serum testosterone levels [29] that may account for the increase in ARd observed in the present study. This finding disagrees with the decrease in ARd observed 24 [7] and 48 h after sexual satiety. Further-
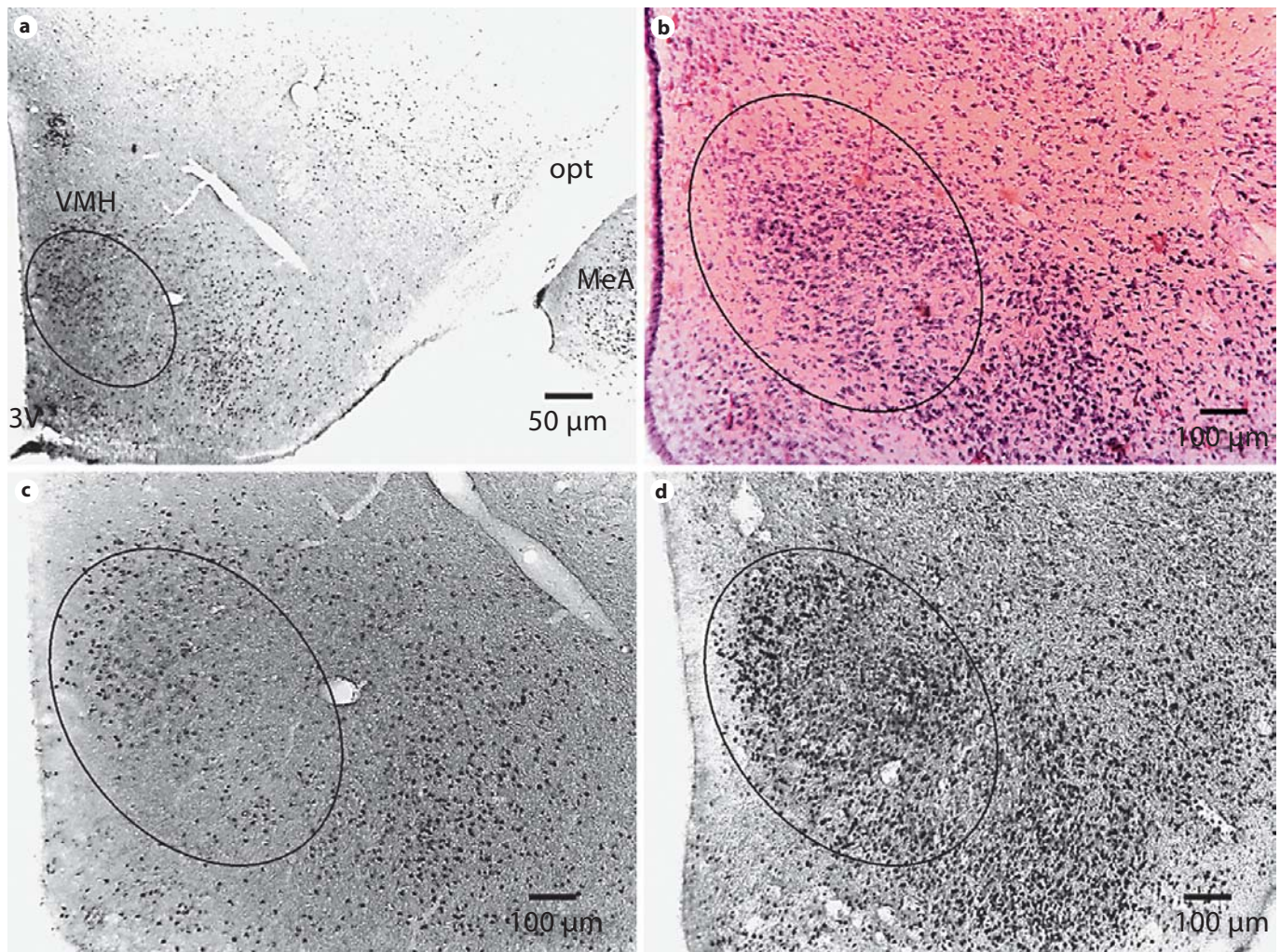
Fig. 4. Representative photomicrographs showing changes in the androgen receptor density (ARd) of the VMH (ventromedial hypothalamic nucleus, dorsomedial and central part) after sexual satiety. **a** Panoramic view of AR-ir in a control at 5× magnification. 3V = Third ventricle; opt = optic tract; MeA = medial amygdala. **b** Cresyl violet VMH of a control male at 10× magnification. **c** VMH AR-ir of a control male rat. **d** VMH AR-ir 72 h after sexual satiety. Note the drastic ARd increase 72 h after sexual satiety compared to control (**c, d**).

more, there is a time course delay (a 72-hour interval) between the augmentation in serum testosterone and AR overexpression that usually occurs within a few hours (see below). It must also be considered that male sexual behavior importantly depends upon the presence or activity of enzymes that participate in androgen metabolism in specific brain nuclei [for review, see 14]. Therefore, the proposition that very localized changes in steroid metabolism, which influence androgen levels within specific brain nuclei, could underlie the alterations in ARd after sexual satiety cannot be discarded. The possibility that MPOM ARd decreases, observed 24 and 48 h after sexual satiety, are mediated by AR re-compartmentalization and subsequent dilution to the cytoplasmic fraction, with a consequent decrease in the ability of the antibody to recognize the unoccupied AR, was previously discussed and discarded [7].

The increase in ARd in various brain areas 72 h after sexual satiety seems puzzling and, to our knowledge, represents the first evidence of a physiologically mediated increase in neuronal ARs. As aforementioned, overexpression of brain ARs occurs after prolonged treatment with anabolic androgens [34], likely due to continuous receptor occupation that prevents receptor breakdown
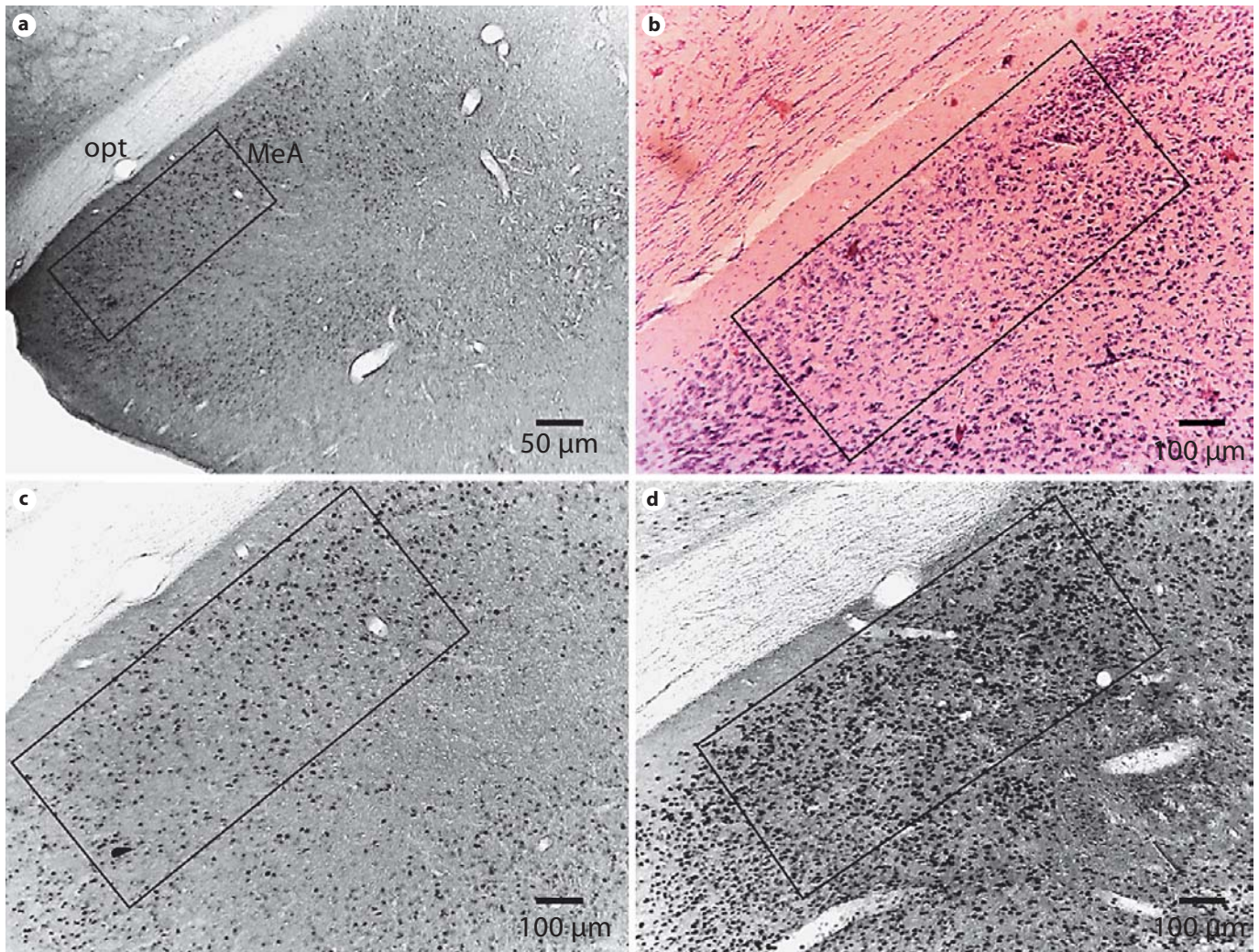
Fig. 5. Representative photomicrographs showing changes in the androgen receptor density (ARd) of the MeA (medial amygdaloid nucleus, anterodorsal part) after sexual satiety. **a** Panoramic view of AR-ir in a control male at 5× magnification. opt = Optic tract; MeA = medial amygdala. **b** Cresyl violet MeA of a control male at 10× magnification. **c** MeA AR-ir of a control male. **d** MeA AR-ir 72 h after sexual satiety. Note the drastic ARd increase 72 h after sexual satiety compared to control (**c**, **d**).

[3]. Additionally, progesterone-receptor knockout mice have facilitated masculine sexual behavior associated with an increase in AR-ir in the MPOA and BST [35]. According to these authors, the increase in AR could, at least partly, explain the enhancement of copulatory behavior. Finally, there is a wide body of evidence indicating that high AR levels are present in recurrent forms of prostate cancer; indeed, high AR levels may contribute to the etiology of the disease [33]. The high levels of AR in these cells are due to either AR gene amplification or AR upregulation after prolonged periods of androgen deprivation and antiandrogen therapy [36, 37]. From the present

results, the mechanisms underlying AR overexpression 72 h after sexual satiety cannot be deduced. However, the increase in AR expression in these brain structures provides a putative explanation for the recovery of sexual behavior in sexually satiated males, most likely mediated by a more robust effect of androgens via AR. In this regard, we have recently shown that AR overexpression, induced by chronic administration of anabolic androgens, produces a drastic recovery of sexual behavior after sexual satiety [Phillips-Farfán et al., unpublished results].

Changes in the number of AR-ir neurons have been associated with male sexual behavior. In this regard,

some data show a parallel reduction in brain AR and masculine sexual behavior. However, the time course of such reductions and, in specific cases, inverse associations, deserves attention. Thus, in at least three situations characterized by a low level of masculine sexual behavior (prepubertal subjects [38], old animals [39] and males that have copulated to satiety [7]) there are low AR-ir levels in some brain areas involved in the control of this behavior. In the first two conditions, the reductions in AR-ir and in masculine sexual behavior are likely due to low circulating androgen levels. Thus, to study whether prepubertal and old males could similarly react to exogenous testosterone (with an adult-like response in terms of masculine sexual behavior and AR-ir), several experiments have been performed. Interestingly, testosterone treatment failed to induce sexual behavior in old males that did not ejaculate and in prepubertal hamsters, but increased AR-ir [38, 39]. However, the levels of testosterone-induced AR-ir were lower in old rats than in adult copulating males [39]. Therefore, the authors concluded that the diminished sexual behavior of old subjects was due to a decreased ARd in brain structures like the MPOM, independently of the steroid hormonal milieu. However, prepubertal male hamsters had a lower testosterone effect on masculine sexual behavior and normal

to larger levels of AR-ir in the MPOA and MeA compared to adults [38]. From these data, it was concluded that the AR-ir increases within the brain circuitry that regulates masculine sexual behavior were not sufficient, although they might have been necessary to induce copulation. These data seem divergent with the present findings; however, some factors have to be considered. First, the use of different models of sexual inhibition seems crucial. Thus, the use of prepubertal male hamsters versus adult male rats may not produce comparable results: in addition to the age differences between both species, most striking is the length of time that their nervous system was exposed to endogenous testosterone prior to treatment. Second, sexually inexperienced male hamsters were utilized [38], while in our investigation only sexually experienced rats were used. Finally, adult hamsters were sacrificed 1 h after copulating. Previous data [7] have shown that sexual activity (one ejaculation) reduces ARd in male rats. Therefore, it is possible that the reduced ARd observed in adult hamsters, compared to young, is induced by copulation in adults, rather than a specific increase in young.

The dissociation between the testosterone effects on sexual behavior and on AR-ir is also apparent in adult subjects if two parameters are analyzed: testosterone doses and time course effects. Thus, regarding the former, the testosterone doses that actively restore male sexual behavior in adult rats [40] and hamsters [9] differ from those required to reestablish AR-ir to levels observed in age-matched sham castrates. Additionally, several laboratories have documented that testosterone increases AR-ir in castrated males within minutes or hours [8–10], while sexual behavior appears after various days [14, 40]. Inversely, the decline in AR-ir after castration occurs after some hours or a few days [9, 10], while sexual behavior may last for several days, particularly if males are sexually experienced [14]. These data indicate that, although ARs are important for the expression of masculine sexu-

Table 1. Serum androgen levels in control animals and male rats sacrificed 48, 72 h or 7 days after sexual satiety

Condition	Testosterone (ng/ml)
Control	3.97 ± 1.03 (n = 7)
Sexually satiated, 48 h	2.03 ± 0.28 (n = 9)
Sexually satiated, 72 h	3.80 ± 0.86 (n = 8)
Sexually satiated, 7 days	2.28 ± 0.37 (n = 8)

The Kruskal-Wallis ANOVA did not reveale statistically significant differences between groups ($H_{3,28} = 3.377$, $p = 0.337$).

Table 2. Recovery of sexual behavior after sexual satiety expressed as the percentage of males ejaculating 1–6 successive times at different intervals after satiety

	E1	E2	E3	E4	E5	E6
Control	100	100	100	100	100	100
48 h	30**	0***	0***	0***	0***	0***
72 h	70	50*	30**	0***	0***	0***
7 days	100	100	90	70	50*	30**

E1–E6 = Successive ejaculatory series.
Fisher's F test comparisons between experimental groups (48, 72 h and 7 days after sexual satiety) vs. controls (non-sexually satiated): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

al behavior, other mechanisms not involving androgens and their receptors contribute to the maintenance and reinstallation of this behavior after castration. Pharmacological monoamine manipulations revert sexual satiety once installed [18]. These data, together with previous [7] and present data associating changes in AR with sexual satiety, suggest that concurrent mechanisms, some involving steroid receptors and others independent of these receptors, participate in the regulation of sexual satiety.

Although controversial [22], the MPOA has been implicated in the neural control of the consummatory components of masculine sexual behavior [14, 21]; while the VMH, MeA, BST and LSV have been proposed to participate in motivational aspects of copulatory behavior [19, 20, 24, 41] via the activation of AR [20, 35, 42]. Interestingly, ARd is significantly reduced in the MPOM 48 h after sexual satiety coinciding with a drastic inhibition in sexual behavior. It could be proposed that the reduction in ARd in this area is, at least partly, responsible for the inability of the animal to execute sexual behavior. Conversely, ARd is increased 72 h after sexual satiety in the VMH, MeA, BST and LSV coinciding with the recovery of sexual behavior. Therefore, AR overexpression in brain structures that regulate sexual motivation might be associated with the capacity of males to recover sexual behavior. On the bases of all these data, it seems possible to propose that the changes in ARd could, at least partly, underlie the inhibition and recovery of sexual behavior observed after sexual satiety.

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