Winning territorial disputes selectively enhances androgen sensitivity in neural pathways related to motivation and social aggression

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Winning aggressive disputes can enhance future fighting ability and the desire to seek out additional contests. In some instances, these effects are long lasting and vary in response to the physical location of a fight. Thus, in principle, winning aggressive encounters may cause long-term and context-dependent changes to brain areas that control the output of antagonistic behavior or the motivation to fight (or both). We examined this issue in the territorial California mouse (*Peromyscus californicus*) because males of this species are more likely to win fights after accruing victories in their home territory but not after accruing victories in unfamiliar locations. Using immunocytochemistry and real-time quantitative PCR, we found that winning fights either at home or away increases the expression of androgen receptors (AR) in the medial anterior bed nucleus of the stria terminalis, a key brain area that controls social aggression. We also found that AR expression in brain regions that mediate motivation and reward, nucleus accumbens (NAcc) and ventral tegmental area (VTA), increases only in response to fights in the home territory. These effects of winning were likely exclusive to the neural androgenic system because they have no detectible impact on the expression of progestin receptors. Finally, we demonstrated that the observed changes in androgen sensitivity in the NAcc and VTA are positively associated with the ability to win aggressive contests. Thus, winning fights can change brain phenotype in a manner that likely promotes future victory and possibly primes neural circuits that motivate individuals to fight.

aggression | androgen and progestin receptors | behavioral reinforcement | neurobiology | winner effect

Social experiences that individuals accrue throughout life can modify the brain's morphology and hormonal milieu (1, 2). These changes are often thought to adjust future social behavior because they occur in brain areas that control affective state, arousal, and motivation (3). However, less is known about how the environment of a given social encounter modulates its subsequent effects on the brain. We examined this issue here by testing (*i*) whether winning fights modifies neural circuits that control social aggression and motivation and (*ii*) whether a fight's environment, in turn, modulates any of these effects.

Winning aggressive disputes or competitions can affect future behavior (4, 5). For example, individuals that win fights are more likely to win later in life (6). In some species, this so-called winner effect is long lasting and forms only in response to victories in certain locations (6). Although mechanistic studies of the winner effect focus on how postencounter changes in steroid hormones adjust future winning behavior (7), this physiological model is less suitable for species in which the winner effect either persists well after excess hormones are cleared from circulation or depends on a fight's environment (8, 9). Thus, it is possible that the experience of victory induces long-term modifications to neural circuits that control antagonistic behavior and the motivation to fight. Indeed, such effects may result from postencounter steroid hormone action and depend on environmental stimuli, as these two factors contribute to the formation of the winner effect.

Territorial male California mice (*Peromyscus californicus*) show a robust winner effect that lasts at least 2–6 d and forms only after wins in the home territory (8, 9). At a physiological level, this winner effect is mediated in part by postencounter testosterone release, which acts through androgenic pathways (10). Progestins may also influence the winner effect in these mice, because plasma progesterone fluctuates after a fight (11). Based on this research, we speculate that winning social disputes alters androgen and progestin sensitivity in limbic and mesolimbic brain areas to promote future victories. We also speculate that such changes occur only after wins in the home territory and not in unfamiliar locations. These hypotheses are rooted in studies that show that changes in neural steroid receptors are sensitive to social stimuli (12, 13) and that testosterone and progesterone impact expression of androgen and progestin receptors (AR and PR) (14, 15).

We explored the issues described above by providing male California mice with winning experiences in either their home or unfamiliar cages and then using immunocytochemistry to test if these experiences affected neural AR or PR. Specifically, we examined AR and PR in brain regions that control the output of antagonistic behavior and the reward-related and reinforcing properties of social aggression: the nucleus accumbens (NAcc); lateral septum (LS); medial anterior bed nucleus of the stria terminalis (BNSTma); medial amygdala (mAMY); anterior hypothalamus (AH); ventrolateral subnucleus of the ventromedial hypothalamus (VMHvl); ventral premammillary nucleus (PMv); ventral tegmental area (VTA), and the dorsal periaqueductal gray (dPAG). We also subjected males to a test encounter immediately before they were killed to examine if levels of AR and PR in these brain nuclei are related to winning ability. Lastly, because AR and PR can change anywhere between hours or days in response to regulatory stimuli (15, 16), we conducted a second study using real-time quantitative PCR (qPCR) to confirm that changes in cell immunoreactivity reflect increases in receptor expression rather than changes in receptor epitope.

Results

Experiment 1: Winning and Steroid Receptors. We first tested whether winning fights either at home or in unfamiliar locations alters AR and PR immunoreactivity (AR-ir and PR-ir) in select limbic and mesolimbic brain areas (Fig. 1*A*). Immunostaining was mostly nuclear in the examined regions (Fig. 1*B*), but little PR staining was found in the LS and VTA.

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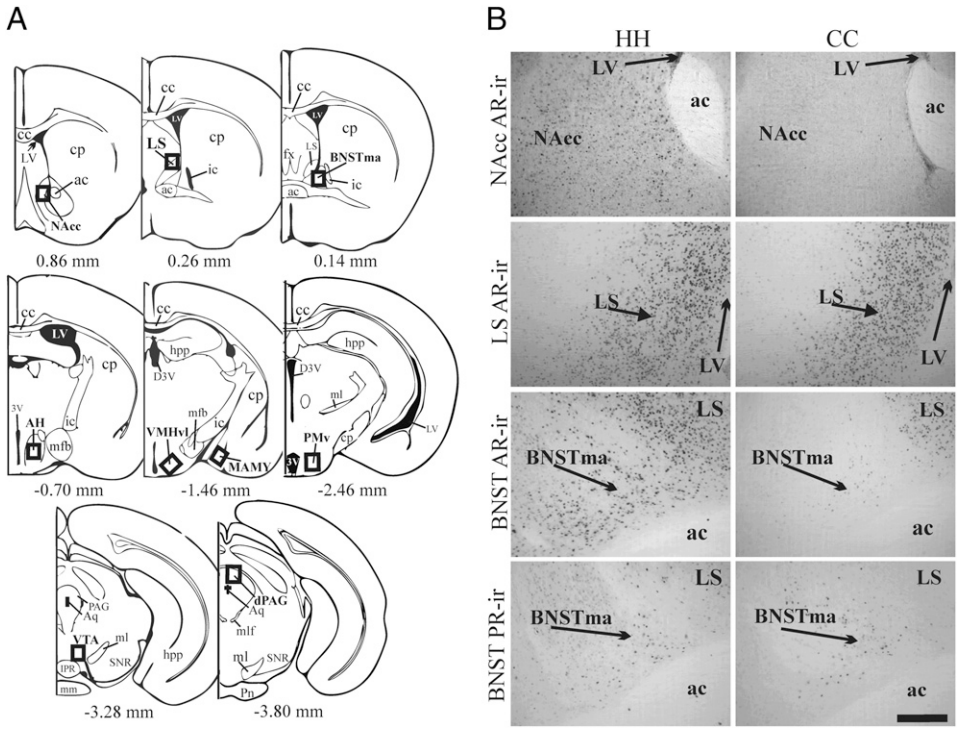

Fig. 1. (*A*) Coronal brain sections from which AR-ir and PR-ir were quantified (*Left* to *Right*, *Top* to *Bottom*: NAcc, LS, BNSTma, AH, VMHvl, mAMY, PMv, VTA, and dPAG). Stereotaxic coordinate of each section (bregma) is provided below each illustration. Boxes depict areas of -ir quantification. (3V) third ventricle, (ac) anterior commissure, (AH) anterior hypothalamus, (Aq) aqueduct, (BNSTma) medial anterior bed nucleus of the stria terminalis, (cp) caudate putamen, (cc) corpus callosum, (D3V) dorsal third ventricle, (fx) fornix, (hpp) hippocampus, (ic) internal capsule, (IPR) rostral interpeduncular nucleus, (LS) lateral septum, (LV) lateral ventricle, (mAMY) medial amygdala, (mfb) medial forebrain bundle, (ml) medial lemniscus, (mm) medial mammillary nucleus, (NAcc) nucleus accumbens, (dPAG) dorsal periaqueductal gray, (PAG) periaqueductal gray, (PMv) ventral premammillary nucleus, (Pn) pontine nuclei, (SNR) reticular part of substantia nigra, (VMHvl) ventrolateral subnucleus of the ventromedial hypothalamus, (VTA) ventral tegmental area. (*B*) Photomicrographs of AR-ir and PR-ir cells in the NAcc, LS, and BNSTma. *Left-most* images are animals that received winning experience and a test encounter in the home cage (HH). *Right-most* images are handled control animals (no fights, CC). (Scale bar, 150 μm.)

Using a series of one-way ANOVAs, we found that the average level of AR-ir differed significantly among treatment groups in the NAcc, BNSTma, and VTA (Fig. 2*A*; NAcc: $F_{3,27} = 9.07$, $P < 0.001$; BNSTma: $F_{3,27} = 5.49$, $P = 0.004$; VTA: $F_{3,18} = 13.71$, $P < 0.001$), but not in the other examined nuclei (Fig. 2*A*; LS: $F_{3,27} = 0.12$, $P = 0.95$; mAMY: $F_{3,25} = 2.60$, $P = 0.073$; AH: $F_{3,22} = 0.22$, $P = 0.88$; VMHvl: $F_{3,24} = 0.69$, $P = 0.57$; PMv: $F_{3,24} = 0.87$, $P = 0.47$; dPAG: $F_{3,21} = 2.55$, $P = 0.083$). Tukey honestly significant difference (HSD) post hoc tests revealed that the effect of winning on AR-ir in the NAcc and VTA depends on a fight's physical location. That is, AR-ir in these two regions increased in response to wins accrued in the home cage (Tukey HSD; $P < 0.05$) but not in response to wins accrued in unfamiliar cages (Tukey HSD; $P > 0.05$). In contrast, Tukey post hoc tests showed that AR-ir in the

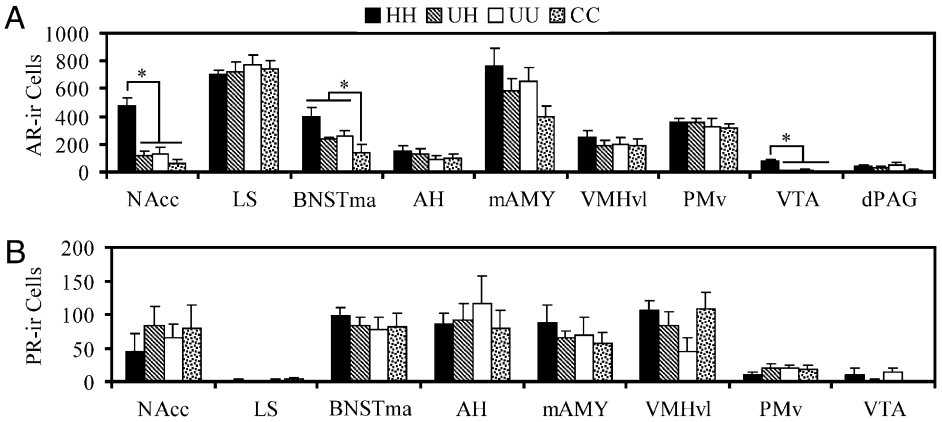

Fig. 2. Amount of (*A*) AR-ir and (*B*) PR-ir in limbic and mesolimbic brain areas [HH = three wins and a test encounter in the home cage; UH = three wins in unfamiliar cages and a test encounter in the home cage; UU = three wins and a test encounter in unfamiliar cages; CC = four handling experiences (no-fight controls)]. Certain regions in a single brain were sometimes damaged, causing sample sizes (*n*) to range from 5–8/group. Data are presented as mean ± SEM **P* < 0.05, Tukey HSD post hoc test.

BNSTma increased in response to victories accrued in both the home cage and unfamiliar cages (Tukey HSD; $P < 0.05$). In all three of these brain regions (NAcc, BNSTma, VTA), post hoc tests did not detect a difference in AR-ir in mice that acquired wins in unfamiliar cages and had a test encounter in either their home cage or another unfamiliar cage (Fig. 2*A*) [home cage (UH) vs. unfamiliar cages (UU); Tukey HSD; $P > 0.05$]. These particular results therefore imply that the three prior winning experiences and their associated environments are the main factors that trigger site-specific changes in AR-ir, rather than the test encounter and its environment. To this end, we also confirmed that animals that accrued winning experiences in their home cage exhibited similar levels of aggressive behavior (as measured by attack latency) compared with animals that accrued winning experiences in unfamiliar cages (mixed-design ANOVA; location as between-subjects factor: $F_{1,30} = 1.41$, $P = 0.25$). Together, these data suggest that the experience of winning predominantly drives the changes to AR-ir in the NACC, BNSTma, and VTA.

We found no difference in the average level of PR-ir among treatment groups in the examined brain nuclei (Fig. 2*B*; one-way ANOVAs; NAcc: $F_{3,26} = 6.04$, $P = 0.62$; LS: $F_{3,26} = 0.16$, $P = 0.92$; BNSTma: $F_{3,24} = 0.70$, $P = 0.56$; mAMY: $F_{3,17} = 0.46$, $P = 0.72$; AH: $F_{3,23} = 0.88$, $P = 0.46$; VTA: $F_{3,20} = 0.28$, $P = 0.84$; VMHvl: $F_{3,23} = 0.82$, $P = 0.50$; PMv: $F_{3,21} = 1.15$, $P = 0.35$).

To explore the functional consequences of our results, we tested if the level of AR-ir in the NAcc, BNSTma, or VTA is associated with the ability to win fights (Fig. 3). For each focal mouse, we calculated a winner index score based on behavior displayed during the test encounter (*Methods*). This index provided a continuous dependent measure of the likelihood of winning (17), with scores closer to 1 representing a higher likelihood of victory and scores closer to −1 representing a higher likelihood of loss. Winner index scores were positively associated with AR-ir in the NAcc ($r = 0.53$, $P = 0.008$) and VTA ($r = 0.45$, $P = 0.027$), but not in the BNSTma ($r = 0.19$, $P = 0.37$). These data indicate that individuals with abundant AR-ir in the NAcc and VTA are more likely to win aggressive encounters.

We examined possible cellular mechanisms through which AR changes in response to winning by testing if AR-ir in the NAcc, BNSTma, or VTA is associated with postencounter levels of testosterone or progesterone (9, 11). Postencounter testosterone

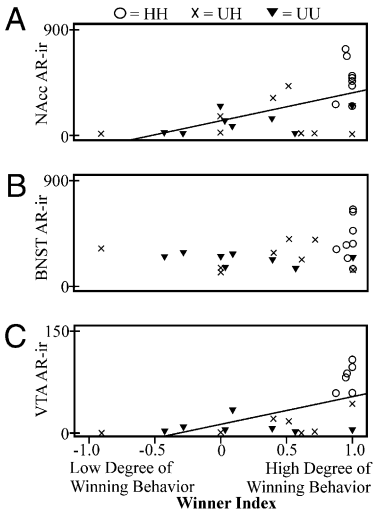

Fig. 3. Relationships between scores on the winner index and the amount of AR-ir in the (*A*) NAcc, (*B*) BNSTma, and (*C*) VTA. HH, three wins and a test encounter in the home cage; UH, three wins in unfamiliar cages and a test encounter in the home cage; UU, three wins and a test encounter in unfamiliar cages. Best-fit lines depict significant correlations, $P < 0.05$, Pearson's correlation coefficient.

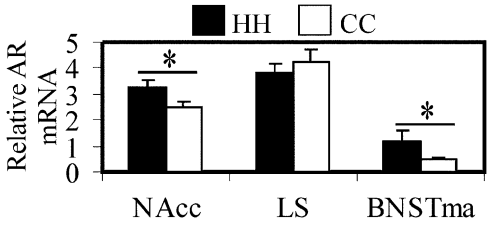

Fig. 4. Relative amounts of AR mRNA in the NAcc, LS, and BNSTma. HH/black bars = three wins and a test encounter in the home cage; CC/white bars = four handling experiences (no fight controls). Sample sizes (*n*) ranged from 5–7/group because one individual was selected at random and used to standardize the other values. Data presented as mean± SEM. *$P < 0.05$, *t* test.

was positively associated with AR-ir in the NAcc ($r = 0.50$, $P = 0.003$) and VTA ($r = 0.42$, $P = 0.048$), but not in the BNSTma ($r = 0.30$, $P = 0.10$). There was no association between postencounter progesterone and AR-ir in these nuclei (all $P > 0.05$). Thus, AR regulation may be androgen-dependent in some brain regions and androgen-independent in others.

Experiment 2: Confirmation and Characterization of Results. In a second study, we sought to confirm the effects of winning on AR and test if these effects result from localized up-regulation of AR transcription. Thus, we used real-time qPCR to measure AR mRNA in response to winning in a subset of brain regions examined in experiment 1: the NAcc, LS, and BNSTma (Fig. 4). Mice that won fights in their home cage had significantly more AR mRNA in the NAcc ($t_{10} = 2.52$, $P = 0.034$) and BNSTma ($t_{10} = 2.95$, $P = 0.017$) compared with handled controls. However, we found no difference in AR mRNA between these two groups in the LS ($t_{10} = -1.23$, $P = 0.25$). These results suggest that site-specific changes in AR-ir in response to victories result from increased AR expression.

Discussion

Using a territorial mammal, we demonstrated that winning multiple fights modifies neural phenotype and that this effect is gated in part by the environmental context of such victories. Thus, male mice that won disputes in their home territory experienced increases in AR expression in select regions of the mesolimbic system (NAcc, BNSTma, VTA), whereas males that won the same number and type of fights in unfamiliar locations experienced increases in AR expression in only one of these areas (BNSTma). The effects of winning are likely specific to the neural androgenic systems because the data presented here suggest that winning has no effect on PR, and prior studies imply that victories are unlikely to enhance future winning via estrogenic activity (10).

Context-Dependent Effects of Winning on AR Expression in Brain Regions That Mediate Reward and Reinforcement. Perhaps our most intriguing finding is that a fight's location modulates how victory affects AR expression in the brain's so-called reward pathway. Prior studies in rats show that ejaculation during sex, itself a rewarding stimulus, decreases AR-ir in the NAcc, which likely suppresses sexual motivation and thus contributes to sexual satiety (18). However, this work does not explore whether the environment in which sex occurs impacts changes in accumbens AR, even though neuronal activity within the mesolimbic system is known to be highly sensitive to extrinsic stimuli (19). For example, the environment in which mice receive psychostimulants markedly impacts resultant immediate early gene expression in the NAcc core and shell (20). Our results are therefore provocative because they suggest a mechanism through which environmental context modulates socially induced changes to the functional properties of neural circuits that control behavioral motivation and reinforcement.

Our experiments also reveal the cues associated with a fight's environment that impact site-specific AR expression the brain, namely olfactory and visual information. This hypothesis is based on the fact that scent and the spatial arrangement of objects within a given cage comprised the main differences between home and unfamiliar contexts. Further studies will be required to tease apart the effects of these two stimuli and determine which has a more potent impact on the brain when it is paired with an aggressive encounter.

Less understood are the cues directly associated with winning that impact the brain. Although displays of aggression during a fight may in theory contribute to changes in AR expression, most research suggests that the perception of winning itself is likely the prominent factor driving this process (21, 22). Indeed, mice exhibit similar levels of aggression, as measured by attack latency, when they accrue wins in their home cages as they do when they accrue wins in unfamiliar locations (23). Thus, differences in aggressive behavior between environments do not likely account for the subsequent effects on the nervous system. Further, other work shows that as California mice gain winning experience and their overall winning ability increases, they attack future opponents less frequently during a fight (9). This too suggests that the effect of victory, rather than the output of aggressive behavior, modifies the ability to win fights and the neural circuitry associated with this ability.

Functional Significance of Increased AR in the Limbic and Mesolimbic Systems. A likely functional consequence of elevated AR in the NAcc, BNSTma, and VTA is an increased ability to win aggressive encounters. That is, mice with abundant AR in the NAcc and VTA attain higher scores on the winner index during the test encounter, indicating that these individuals have an above-average chance of winning social disputes (17, 24). Thus, the experience of victory itself likely modifies the neural machinery controling social aggression (25, 26), which in turn suggests that the winner effect is to some degree regulated at the level of the brain. One possibility is that increased androgen sensitivity in the NAcc and VTA primes the neuronal "reward circuitry," such that the reinforcing properties of aggressive encounters develop more readily and the resulting motivation to fight in the future becomes more pronounced. This hypothesis is anchored in prior research that suggests that androgen action in the mesolimbic system mediates dopamine release in the NAcc (27, 28) and that this effect during or after a fight increases the desire to seek future aggressive interactions (29, 30). Ethologically, such fine-tuning of endogenous reinforcement-related circuitry may be a way in which aggressive encounters modify future territorial behavior by increasing the motivation to patrol territory boundaries and evict unwanted intruders (31).

Elevated AR expression in the BNSTma may contribute to the winner effect as well, even though AR-ir in this region is not directly associated with winner index scores. The BNST integrates social and emotional information from various limbic and hypothalamic nuclei and relays this information to efferent targets that include the descending control column for social aggression (VMHvl, PMV, dPAG) and the VTA, which then sends dopamine fibers to the NAcc (32, 33). It is possible that increased AR in the BNSTma alters not only how this brain area responds to the perception of a social threat, but also how it conveys this information to the neural circuits that control motor activity in aggressive bouts and behavioral reinforcement. This concept is consistent with the winner effect literature, in that this phenomenon is thought to result from changes in how individuals perceive fights, rather than changes in actual motor performance during battle (6).

The neurobiological relationship between territoriality and the properties of reinforcement must be considered with caution because they are poorly understood and likely complex. For instance, androgens may not always induce NAcc dopamine release (34). The lack of clarity between these two areas of biology may be due to the fact that animals used to study reinforcement are often deprived of social experience, which affects AR expression in mesolimbic nuclei (18). Our data support this idea in that post-encounter testosterone was positively related to AR density in the NAcc and VTA but not in the BNSTma. Thus, androgens might promote AR expression in the NAcc and VTA (14), whereas social experiences and other androgen-independent mechanisms might contribute to AR expression in the BNSTma.

Winning-Induced Changes to AR in Neural Networks That Guide Sociality. The effects of winning on neural androgen sensitivity might impact other elements of animal sociality. The NAcc, BNST, and VTA are either part of, or provide input to, the social behavior network, a conserved group of brain areas that mediate many aspects of social behavior (32, 35). This network is thought to influence behavioral output by weighing neural activity among different network loci and then inducing a patterned release of neurochemicals (32). Thus, winning might alter the network's functional connectivity and neurochemical release by adjusting how specific network nodes detect and respond to androgen hormones (see ref. 36 for example). Indeed, activation of AR in adult animals plays a critical role in aspects of reproduction and parenting (37, 38), which implies that these behaviors might be adjusted in winners as a result of changes to neural AR expression.

Effects of Winning on Neural Progestin Signaling Systems. As noted above, winning had no appreciable effect on PR-ir in the examined brain regions, suggesting that winning specifically modifies androgenic signaling systems. However, this does not rule out the possibility that progestins influence aggression. For example, other work shows that progesterone changes after a fight and thus might help shape future aggression (11). One possibility that has received little attention is whether progestins affect behavior during and after an aggressive bout by suppressing natural levels of contest-related anxiety (39, 40).

Conclusion. In sum, winning fights increased androgen sensitivity in distinct brain regions (NAcc, BNSTma, VTA), and some of these changes (NAcc, VTA) were modulated by a fight's environmental context. Thus, we demonstrated a way in which the environment regulates social control of select brain pathways important for behavioral motivation and reinforcement. Also, we showed that androgen sensitivity in areas of the mesolimbic system likely promote future winning ability. Together, these data suggest that there is a neural basis for the winner effect, because winning itself likely causes AR in these brain nuclei to increase.

Methods

Animals. California mice were from a laboratory colony at the University of Wisconsin at Madison established from individuals captured in the Santa Monica Mountains of California. Individuals were housed in same-sex groups of 3–4/cage (48 × 27 × 16 cm), provided food and water ad libitum, and kept under a 14:10 h light-dark cycle. The colony was maintained according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the University of Wisconsin, Madison Institutional Animal Care and Use Committee approve of the research.

Experiment 1: Winning and Steroid Receptors. This study tested not only whether winning fights alters AR-ir and PR-ir in select brain nuclei (Fig. 1), but also whether the environmental context of a fight mediates these effects. Brains were collected from 32 sexually experienced males (focal mice, n = 8/group) that received either (*i*) three wins and a test encounter in their home cage (HH); (*ii*) three wins in unfamiliar cages and a test encounter in their home cage (UH); (*iii*) three wins and a test encounter in unfamiliar cages (UU); or (*iv*) four handling experiences in the home cage in lieu of aggressive encounters (no-fight controls, CC). Behavioral treatment occurred under dim-red light at least 1 h after the dark cycle's onset.

On day 1, each focal mouse was paired with a female in a standard cage. On day 10, each pair was moved into a polycarbonate home cage (30 × 50 × 30 cm) that contained aspen bedding, nesting material, a nest box, and food and water ad libitum.

On days 13, 15, and 17, focal mice received winning or handling experiences. Wins in the home cage followed a resident–intruder paradigm described previously (8): the female was removed from the home cage; an opaque divider was inserted into the cage and isolated the focal mouse on one side; an opponent was placed in the cage's opposite side; the mice were given 2 min to acclimate; the divider was removed and the mice were given 10 min to freely interact. Winning experiences in unfamiliar cages followed the same paradigm, but focal mice were taken from the home cage and placed in the unfamiliar cage with the divider already in position. Unfamiliar cages were constructed identically to home cages and contained the same materials. Unlike home cages, unfamiliar cages were washed and lined with fresh bedding before each encounter and the objects were arranged in a spatial layout that was different to the focal mouse. During these encounters, focal mice fought randomly chosen, unfamiliar opponents ($n = 40$ opponents) that were smaller, sexually inexperienced, and losers of at least one prior fight. These contest asymmetries ensured that focal mice won fights on days 13, 15, and 17 (the one mouse that did not win all three fights was replaced by another that did). Contest winners were individuals that initiated at least three consecutive attacks toward their opponent, which each elicited losing behavior (8, 9). Handled mice experienced the same treatment as mice subjected to fights (i.e., removal of female, insertion of divider, etc.), but an opponent was not placed in the home cage. In other work, similar "no fight" treatments are used as controls to provide baseline measures of neural or physiological state, as other types of social experience (i.e., losing) can induce separate physiological or behavioral changes (6, 7).

On day 19, focal mice were subjected to a videotaped test encounter to assess winning ability. In these encounters, focal mice fought randomly chosen, unfamiliar opponents ($n = 32$ test encounter opponents) that were slightly larger (1.4 ± 0.22 g; mean± SEM), sexually experienced, and the winner of one prior fight. This modest opponent advantage refines the sensitivity with which experience-induced changes in behavior can be detected (9). Each opponent was used once. After the test encounter, opponents were removed from the test cage and focal mice remained in the test cage for 45 min, which is the time that postencounter testosterone otherwise peaks in this species (9). Trunk blood was collected and centrifuged. Plasma was stored at −80 °C. Brains were harvested, placed in 4% acrolein in 0.1 M PO_4 buffer overnight, transferred into 30% sucrose solution in 0.1 M PO_4 buffer for 72 h, flash frozen with methylbutane, and stored at −80 °C.

An observer blind to treatment recorded behavior from each focal mouse during the test encounter (as in ref. 8). These data were used to calculate each animal's score on the winner index, which is a continuous dependent variable that represents the likelihood of victory based on displays of winning-typical behavior relative to that of losing-typical behavior. The score is calculated by subtracting the total submissive behaviors (jumps away, freezes, retreats) from the total attacks (bites, chases, initiated wresting bouts), and dividing this number by the combined total submissive and attack behaviors (17, 24). Scores range from 1 to −1: positive scores indicate higher displays of attack behavior and a greater chance of victory, whereas negative scores indicate higher displays of submissive behavior and a greater chance of loss.

Postencounter hormones from focal mice are presented elsewhere (8), but we examined these data here with respect to AR-ir and PR-ir. Plasma testosterone and progesterone were assayed at the Wisconsin Regional Primate Center, using enzyme immunoassay techniques described elsewhere [testosterone antibody R156; University of California, Davis, diluted to 1:35,000; progesterone antibody R4861; UC-Davis, diluted to 1:33,000 (9, 10)]. Intra- and interassay coefficients of variation, respectively, are as follows: testosterone, 2.9% and 4.3% ($n = 2$ plates), and progesterone, 1.3% and 2.3% ($n = 2$ plates).

Brain tissue for immunocytochemistry was cut into coronal sections using a cryostat (angle = 30°) and stored in cryoprotectant at −20 °C. Sections were later rinsed three times in Tris buffered saline (TBS) (5 min/rinse) and then incubated in 0.1% sodium borohydride for 15 min. Sections were again rinsed three times in TBS (5 min/rinse) and then blocked for 60 min in 20% normal goat serum and 1% hydrogen peroxide in TBS. The remaining steps followed protocols described elsewhere (41). Briefly, AR-containing cells were stained with PG-21 antibody (Upstate Biotechnology) diluted to 1:1,000 in 2% goat serum and 0.5% gelatin in 0.3% TBS-Triton X-100 (TBS-T) for 18 h at room temperature. PR-containing cells were stained with PGR antibody (Cat# MBS300415; MyBioSource) diluted to 1:10 in 2% goat serum and 0.5% gelatin in 0.3% TBS-Triton X-100 (TBS-T) for 72 h in the refrigerator. After incubation in either primary antibody, sections were incubated in secondary antibody (BA-1000, goat anti-rabbit biotinylated IgG; Vector Labs) diluted to 1:500 in 0.3% TBS-Triton X-100 (TBS-T) with 0.5% gelatin for 90 min. Sections were again rinsed and incubated for 60 min in Vectastain ABC peroxidase (Vectastain Elite; Vector Laboratories) with half of the concentration recommended by the manufacturer. Sections were rinsed and visualized using Vector SG for 20 min (Vector SG; Vector Laboratories) with half of the concentration recommended by the manufacturer made in 0.3% TBS-Triton X-100 (TBS-T) and 0.5% gelatin.

AR-ir and PR-ir quantification followed procedures outlined elsewhere (42, 43). For each mouse, single sections of the NAcc, LS, BNSTma, mAMY, AH, VMHvl, PMv, VTA, and dPAG were matched using the mouse brain atlas (Fig. 1*A*) (44). PR-ir cells were not counted in the dPAG because PR immunostained tissue from this area was not collected. The NAcc core and shell were not distinguished because the boundaries between these areas are poorly defined in *Peromyscus* mice (45). Brain areas were inspected under bright-field illumination, using an Olympus BX-61 microscope (20× objective) fitted with an Olympus FV II digital camera. A digital image of each area was captured, and AR-ir and PR-ir cells were counted by Olympus MicroSuite software (Soft Imaging Corp.). Foreground threshold and microscope light intensity were kept constant during imaging sessions.

Hormone and receptor data were normalized using natural log transformations and winner index scores were normalized using square root transformations (46). For each brain region, a one-way ANOVA was used to determine if AR-ir or PR-ir differed among treatment groups. ANOVAs that produce significant results were followed by Tukey HSD post hoc analyses, which revealed if the location of past wins influenced site-specific changes in AR-ir or PR-ir. Pearson correlation coefficients were calculated to test if AR-ir in brain regions responsive to winning were associated with postencounter hormones or winner index scores.

Experiment 2: Confirmation and Characterization of Results. The second study used real-time qPCR to confirm that winning changes neural AR in select brain areas and if these changes reflect increased AR transcription. Brains were collected from 13 sexually experienced males that received either (*i*) three wins and a test encounter in the home cage ($n = 7$, HH) or (*ii*) four handling experiences in lieu of aggressive encounters (no-fight controls, $n = 6$, CC). The protocol of this study was identical to Experiment 1 ($n = 10$ opponents for day 13, 15, 17 encounters; $n = 13$ test encounter opponents), but harvested brains were flash frozen without fixative. Detailed behavioral data were not collected from these focal mice, yet all focal mice won staged fights on days 13, 15, 17, and 19.

Brain tissue used for real-time qPCR was defrosted to −15 °C and cut into 250-μm coronal sections using a cryostat. Micropunches of the NAcc, LS, and BNSTma were collected (Fig. 1*A*) and refrozen in methylbutane for storage at −80 °C. Micropunches of the VTA were not obtained because landmarks do not surround this region that make it easy to identify and collect. DNA and RNA were extracted from the tissue using the AllPrep DNA/RNA Mini Kit (Qiagen), and concentrations of RNA in each sample were measured using the Qubit quantitation platform (Invitrogen). RNA was converted to cDNA using ImProm-II Reverse Transcription System (Promega), and cDNA concentration for each converted sample was also measured using the Qubit quantitation platform. cDNA was amplified and SYBR florescence was measured in a Stratagene Mx3000P real-time PCR system, using Promega Supermix. AR primers (accession no. NM_013476.3; forward: 5′-AAGCAGGTAGCTCTGGGACA-3′, reverse: 3′-GAGCCAGCGGAAAGTTGTAG-5′) and 18S housekeeping gene primers (Invitrogen) were designed using the OligoPerfect tool (Invitrogen). Primer efficiencies were between 90–110%. ROX was used as a passive reference dye to control for baseline florescence within each tube. The PCR consisted of an initial denaturing step at 95 °C (2 min), followed by 40 cycles of a 95 °C denaturing step (30 s), a 58 °C annealing step for (30 s), and a 72 °C elongation step for (30 s). Relative mRNA levels were calculated using the $2^{-\Delta\Delta CT}$ method (47).

For each of the three brain regions examined in the second study, two-tailed *t* tests were used to test if AR mRNA differed between mice with winning experience and handled controls.

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1. Holmes MM, et al. (2007) Social control of brain morphology in a eusocial mammal. *Proc Natl Acad Sci USA* 104:10548–10552.
2. Francis RC, Soma K, Fernald RD (1993) Social regulation of the brain-pituitary-gonadal axis. *Proc Natl Acad Sci USA* 90:7794–7798.
3. Oliveira RF (2009) Social behavior in context: Hormonal modulation of behavioral plasticity and social competence. *Integr Comp Biol* 49:423–440.
4. Amorim MC, Almada VC (2005) The outcome of male-male encounters affects subsequent sound production during courtship in the cichlid fish *Oreochromis mossambicus*.. *Anim Behav* 69:595–601.
5. Coates JM, Herbert J (2008) Endogenous steroids and financial risk taking on a London trading floor. *Proc Natl Acad Sci USA* 105:6167–6172.
6. Hsu YY, Earley RL, Wolf LL (2006) Modulation of aggressive behaviour by fighting experience: Mechanisms and contest outcomes. *Biol Rev Camb Philos Soc* 81: 33–74.
7. Oliveira RF, Silva A, Canario AVM (2009) Why do winners keep winning? Androgen mediation of winner but not loser effects. *Proc Biol Sci* 276:2249–2256.
8. Fuxjager MJ, Marler CA (2010) How and why the winner effect forms: Influences of contest environment and species differences. *Behav Ecol* 21:37–45.
9. Oyegbile TO, Marler CA (2005) Winning fights elevates testosterone levels in California mice and enhances future ability to win fights. *Horm Behav* 48:259–267.
10. Trainor BC, Bird IM, Marler CA (2004) Opposing hormonal mechanisms of aggression revealed through short-lived testosterone manipulations and multiple winning experiences. *Horm Behav* 45:115–121.
11. Fuxjager MJ, Mast G, Becker EA, Marler CA (2009) The "home advantage" is necessary for a full winner effect and changes in post-encounter testosterone. *Horm Behav* 56: 214–219.
12. Burmeister SS, Kailasanath V, Fernald RD (2007) Social dominance regulates androgen and estrogen receptor gene expression. *Horm Behav* 51:164–170.
13. Holmes MM, Goldman BD, Forger NG (2008) Social status and sex independently influence androgen receptor expression in the eusocial naked mole-rat brain. *Horm Behav* 54:278–285.
14. Lu SF, McKenna SE, Cologer-Clifford A, Nau EA, Simon NG (1998) Androgen receptor in mouse brain: Sex differences and similarities in autoregulation. *Endocrinology* 139: 1594–1601.
15. Turgeon JL, Waring DW (2000) Progesterone regulation of the progesterone receptor in rat gonadotropes. *Endocrinology* 141:3422–3429.
16. Lu S, Simon NG, Wang Y, Hu S (1999) Neural androgen receptor regulation: Effects of androgen and antiandrogen. *J Neurobiol* 41:505–512.
17. Oyegbile TO (2006) The winner effect and related hormones in *Peromyscus* mice. PhD dissertation (Univ of Wisconsin, Madison, WI).
18. Fernandez-Guasti A, Swaab D, Rodríguez-Manzo G (2003) Sexual behavior reduces hypothalamic androgen receptor immunoreactivity. *Psychoneuroendocrinology* 28: 501–512.
19. Badiani A, Robinson TE (2004) Drug-induced neurobehavioral plasticity: The role of environmental context. *Behav Pharmacol* 15:327–339.
20. Badiani A, et al. (1998) Amphetamine-induced behavior, dopamine release, and c-fos mRNA expression: Modulation by environmental novelty. *J Neurosci* 18:10579–10593.
21. Oliveira RF, Carneiro LA, Canário AVM (2005) Behavioural endocrinology: No hormonal response in tied fights. *Nature* 437:207–208.
22. Hirschenhauser K, Wittek M, Johnston P, Möstl E (2008) Social context rather than behavioral output or winning modulates post-conflict testosterone responses in Japanese quail (*Coturnix japonica*). *Physiol Behav* 95:457–463.
23. Bester-Meredith JK, Martin PA, Marler CA (2005) Manipulations of vasopressin alter aggression differently across testing conditions in monogamous and nonmonogamous *Peromyscus* mice. *Aggress Behav* 31:189–199.
24. Eisenberg JF (1962) Studies of the behavior of *Peromyscus maniculatus gambelii* and *Peromyscus californicus parasiticus*. *Behaviour* 19:177–207.
25. Sperry TS, Wacker DW, Wingfield JC (2010) The role of androgen receptors in regulating territorial aggression in male song sparrows. *Horm Behav* 57:86–95.
26. Canoine V, Fusani L, Schlinger B, Hau M (2007) Low sex steroids, high steroid receptors: Increasing the sensitivity of the nonreproductive brain. *Dev Neurobiol* 67:57–67.
27. Dimeo AN, Wood RI (2006) ICV testosterone induces Fos in male Syrian hamster brain. *Psychoneuroendocrinology* 31:237–249.
28. Packard MG, Cornell AH, Alexander GM (1997) Rewarding affective properties of intra-nucleus accumbens injections of testosterone. *Behav Neurosci* 111:219–224.
29. Couppis MH, Kennedy CH (2008) The rewarding effect of aggression is reduced by nucleus accumbens dopamine receptor antagonism in mice. *Psychopharmacology (Berl)* 197:449–456.
30. van Erp AMM, Miczek KA (2000) Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. *J Neurosci* 20:9320–9325.
31. Wingfield JC (1994) Regulation of territorial behavior in the sedentary song sparrow, *Melospiza melodia morphna*. *Horm Behav* 28:1–15.
32. Goodson JL, Kabelik D (2009) Dynamic limbic networks and social diversity in vertebrates: From neural context to neuromodulatory patterning. *Front Neuroendocrinol* 30: 429–441.
33. Jalabert M, Aston-Jones G, Herzog E, Manzoni O, Georges F (2009) Role of the bed nucleus of the stria terminalis in the control of ventral tegmental area dopamine neurons. *Prog Neuropsychopharmacol Biol Psychiatry* 33:1336–1346.
34. Triemstra JL, Sato SM, Wood RI (2008) Testosterone and nucleus accumbens dopamine in the male Syrian hamster. *Psychoneuroendocrinology* 33:386–394.
35. Newman SW (1999) The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network. *Ann N Y Acad Sci* 1999:242–257.
36. Maney DL, Goode CT, Lange HS, Sanford SE, Solomon BL (2008) Estradiol modulates neural responses to song in a seasonal songbird. *J Comp Neurol* 511:173–186.
37. Lea RW, Clark JA, Tsutsui K (2001) Changes in central steroid receptor expression, steroid synthesis, and dopaminergic activity related to the reproductive cycle of the ring dove. *Microsc Res Tech* 55:12–26.
38. Balthazart J, Schumacher M, Malacarne G (1985) Interaction of androgens and estrogens in the control of sexual behavior in male Japanese quail. *Physiol Behav* 35:157–166.
39. Auger CJ, Forbes-Lorman RM (2008) Progestin receptor-mediated reduction of anxiety-like behavior in male rats. *PLoS ONE* 3:1–6.
40. Schino G, Rosati L, Geminiani S, Aureli F (2007) Post-conflict anxiety in Japanese macaques (*Macaca fuscata*) aggressor's and victim's perspectives. *Ethology* 113:1081–1088.
41. Auger CJ, Vanzo RJ (2006) Progesterone treatment of adult male rats suppresses arginine vasopressin expression in the bed nucleus of the stria terminalis and the centromedial amygdala. *J Neuroendocrinol* 18:187–194.
42. Olesen KM, Auger AP (2008) Dopaminergic activation of estrogen receptors induces *fos* expression within restricted regions of the neonatal female rat brain. *PLoS ONE* 3: 1–6.
43. Olesen KM, Auger AP (2005) Sex differences in Fos protein expression in the neonatal rat brain. *J Neuroendocrinol* 17:255–261.
44. Paxinos G, Franklin KBJ (2001) *The Mouse Brain in Stereotaxic Coordinates* (Academic Press, San Diego).
45. Eleftheriou BE, Zolovick AJ (1965) The forebrain of the deermouse in Stereotaxic coordinates. *Technical Bulletin: Kansas State University* 146.
46. Zar JH (1999) *Biostatistical Analysis* (Prentice Hall, Upper Saddle River, NJ).
47. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25:402–408.