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Immediate Early Gene Activity-Regulated Cytoskeletal-Associated Protein Regulates Estradiol-Induced Lordosis Behavior in Female Rats

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Abstract

Sensory feedback is an important component of any behavior, with each instance influencing subsequent activity. Female sexual receptivity is mediated both by the steroid hormone milieu and interaction with the male. We tested the influence of repeated mating on the level of sexual receptivity in ovariectomized rats treated with estradiol benzoate (EB) once every fourth day to mimic the normal phasic changes of circulating estradiol. Females were divided into two groups: naïve, which were tested for lordosis behavior once, and experienced rats, which were tested for lordosis after each EB injection. To monitor the effect of mating, the number of neurons expressing the immediate early gene activity-regulated cytoskeleton-associated protein (Arc) were counted in the mediobasal hypothalamus. Females were unreceptive following the first EB treatment, but the mating induced Arc expression. In naïve rats, each subsequent EB injection increased the levels of sexual receptivity. This ramping was not observed in experienced rats, which achieved only a moderate level of sexual receptivity. However, experienced females treated with EB and progesterone were maximally receptive and did not have Arc expression. To test whether the expression of Arc attenuated lordosis, Arc antisense oligodeoxynucleotides (asODN) were microinjected into experienced females' arcuate nuclei. Arc expression was attenuated, and the experienced EB-treated females achieved maximal sexual receptivity. These results demonstrate that Arc expression in the hypothalamus might influence future sexual receptivity and provides evidence of learning in the arcuate nucleus. The loss of Arc results in unrestrained sexual receptivity.

Keywords

arcuate nucleus; estradiol; sexual receptivity; Arc

The central nervous system (CNS) regulation of female sexual behavior requires integration of steroid hormone information from the gonads and somatosensory stimuli in a circuit within the limbic system and hypothalamus (Kow et al., 1979; Micevych and Dewing, 2011). The estradiol induction of sexual receptivity has been well documented and requires the expression of a large number of neuropeptides, neurotransmitters, synthetic enzymes,

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and receptors in a limbic–hypothalamic lordosis-regulating circuit, which consists of the arcuate nucleus of the hypothalamus, the medial preoptic nucleus, and the ventromedial nucleus (VMH). The VMH, the common output of this “module,” sends descending efferent projections to innervate the periaqueductal gray and the vestibular nucleus, which provide input to spinal motoneurons innervating muscles responsible for the lordotic posture for which the behavior is named. Induction of receptive behavior requires estrogen receptor- α in the arcuate nucleus, which rapidly acts via estradiol membrane signaling. Less well understood is the importance of ascending tactile information from the flanks and the perineum reaching the VMH and arcuate nucleus (Flanagan-Cato et al., 2001; Pfau et al., 2006). Tactile information increases expression of immediate early genes (IEG; Tetel et al., 1993; Polston and Erskine, 1995; Calizo and Flanagan-Cato, 2003; Flanagan-Cato et al., 2006).

The IEG activity-regulated cytoskeleton-associated protein (Arc) has been correlated with sensory stimuli (Matsuoka et al., 2002a). Arc is widely distributed throughout the CNS and is considered to be a marker of neuronal plasticity (Plath et al., 2006). It is induced by high-frequency electrical stimulation and by mitogen-activated protein kinase, which is activated by a constellation of extracellular signals including glutamate, dopamine, brain-derived neurotrophic factor, and nerve growth factor (Lyford et al., 1995; Wallace et al., 1998; Guzowski et al., 2001; Brackmann et al., 2004; Giorgi et al., 2007). Flanagan-Cato and colleagues (2006) are the only group thus far to explore the actions of Arc in the hypothalamus. They showed that cells in the ventrolateral VMH are preferentially activated after a bout of mating, with no activation in naïve animals. Additionally, they demonstrated that mating experience correlates with a decrease in spine density, which might involve Arc activation. Little is known about whether and how sensory stimuli influence the limbic–hypothalamic circuit upstream of the VMH, especially the arcuate nucleus of the hypothalamus, the location of the initial estradiol activation of lordosis-regulating circuits (Mills et al., 2004; Micevych and Dewing, 2011; Sanathara et al., 2011; Micevych and Sinchak, 2013). Thus, we were especially interested in the response of Arc to sensory feedback in the arcuate nucleus.

One of the interesting aspects of Arc is that its induction is dependent on stimuli relevant to a particular region of the nervous system. For example, in male rats, sexually salient olfactory input increased Arc in the accessory olfactory bulb but not the main olfactory bulb (Matsuoka et al., 2002a,b). Arc induction has been associated with both inhibition and facilitation in the CNS. In the hippocampus, Arc is involved in long-term depression through its association with, and removal of, AMPA receptors from the membrane and with long-term potentiation (LTP) by regulating the actin core of dendritic spines, assisting their growth and maturation. We hypothesized that, in the arcuate nucleus as in the VMH (Flanagan-Cato et al., 2006), sensory feedback from mating would activate Arc to modulate sexual receptivity. We used behavioral analyses and antisense oligodeoxynucleotides (asODNs) to test this theory.

MATERIALS AND METHODS

Animals

Male and ovariectomized (ovx; by the supplier) female (200–250 g body weight) Long-Evans rats were purchased (Charles River, Portage, MI). Upon arrival, rats were housed in a climate-controlled room, two per cage, under a 12-hr light–dark cycle (lights on at 0600 hr) and provided food and water ad libitum. All experimental procedures were approved by the Chancellor's Animal Research Committee at the University of California, Los Angeles.

Steroid Priming

Animals were allowed to recover for 2–3 weeks after ovx, prior to steroid treatment. For all experiments, 17 β -estradiol benzoate (EB) and progesterone (P) were dissolved in safflower oil and injected subcutaneously (sc) in a volume of 0.1 ml per rat. Animals tested for lordosis behavior received an injection of 5 μ g EB (sc) once every 4 days between 0900 and 1000 hr to mimic the rise and fall of estradiol over the natural estrous cycle (Micevych et al., 1994). The EB injection and subsequent 4 days is referred to as a *cycle* (Fig. 1A). Females that were tested for lordosis behavior after each cycle were designated *experienced*, and those that were tested once were designated *naïve*. When P (500 μ g; sc) was used, it was injected 24–26 hr after EB injections to mimic the surge of P in a naturally cycling female, as previously described (Sinchak and Micevych, 2001).

Behavioral Testing

Lordosis testing began 30 hr after EB injection, either after every injection or once after the last injection. Stimulus males were acclimated to the testing arenas for at least 15 min. Sexual receptivity was measured by placing a female rat in a Plexiglas testing arena with a sexually experienced male. Males were allowed to mount females 10 times, and the number of times the female displayed lordosis (lifting of the head, arching of the back, movement of the tail to one side) was recorded. For each female, the level of sexual receptivity was quantified as a lordosis quotient (LQ), the number of lordosis displays/10 mounts $\times$ 100. A moderately receptive female will receive 10 mounts from a stimulus male in approximately 30 min.

Immunohistochemistry

Females were perfused immediately after the conclusion of the behavioral test (social interaction, olfactory stimulation of lordosis behavior). Animals were anesthetized and transcardially perfused with chilled 0.9% saline, followed with 4% paraformaldehyde dissolved in 0.2 M Sorenson's phosphate buffer (pH 7.4). Brains were removed and placed in fixative, stored overnight at 4°C, followed by replacement with 20% sucrose in phosphate buffer for cryoprotection. Brains were blocked, sectioned (20 μ m) on a cryostat (Leica CM 1800; Leica Microsystems, Bannockburn, IL), and collected in chambers filled with phosphate-buffered saline (PBS). Sections from the arcuate nucleus were incubated overnight with a mouse antibody directed against Arc (1:250; Santa Cruz Biotechnology, Santa Cruz, CA). Immunoreactivity was visualized with a diaminobenzidine (DAB) histochemistry kit (Vectastain Elite ABC kit; Vector, Burlingame, CA). Sections were

mounted on SuperFrost/Plus slides (ThermoFisher Scientific, Pittsburgh, PA), air dried, and dehydrated before being coverslipped with Krystalon (EMD Chemicals, Gibbstown, NJ).

Image Analysis

Sections were examined via a Zeiss Axioskop 2 Axiocam digital camera and AxioVision digital image analysis system (Carl Zeiss North America, Thornwood, NY). Images were adjusted for brightness and contrast in Zeiss LSM-PC and PhotoShop (version 10.0; Adobe, San Jose, CA). DAB sections were viewed with brightfield illumination. Every fourth section from the arcuate nucleus was analyzed for Arc immunoreactivity. Cells were considered Arc-positive if their cell body showed intense positive staining. The arcuate nucleus and VMH from only one side of each section was counted in an alternating pattern, including both the left and the right sides of the brain in the final count. Sections in which the arcuate nucleus or VMH from both sides were damaged during immunohistochemistry were not used for analysis. Because only the anterior half of the arcuate nucleus showed positive staining for Arc, only the staining from these sections was considered here.

Social Interaction

To test the effects of social interaction on Arc expression, females were transferred to a new, clean cage (just as when they were tested for lordosis) with a novel female without access to food or water. The animals were allowed to explore one another for 30 min (duration of the lordosis testing). One animal from the cage was perfused as described below. The other remained in the cage and was perfused 30 min later. There was no difference in the upregulation of Arc at the two time points, so the results were pooled.

Olfactory Stimulation

Behavioral testing was not performed in the female's home cage. To determine whether olfactory stimuli from a male would induce Arc, soiled male bedding from a single male was placed into a neutral cage. The experimental female was allowed to explore this cage without access to food or water for 30 min and then perfused.

Western Blotting

Animals were anesthetized and decapitated, and their brains were removed and rinsed with cold PBS. The arcuate nucleus was dissected out on ice and placed in radioimmuno-precipitation assay lysis buffer with protease inhibitors (Santa Cruz Biotechnology). The tissue was sonicated and centrifuged. The supernatant, including all cellular fractions, was collected, and 50 µg of protein was heated for 5 min at 95°C in Laemmli sample buffer (Bio-Rad, Hercules, CA) and run on a 10% acrylamide gel. The protein was transferred overnight to a Hybond-P polyvinylidene fluoride membrane (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom). The membrane was blocked in 5% milk in 0.1% Tris-buffered saline (TBS)-Tween for 1 hr before being transferred to primary mouse antibody raised against Arc (1:250; Santa Cruz Biotechnology) made in 1% bovine serum albumin. The membrane was incubated in primary antibody overnight at 4°C. On the next day, membranes were washed and incubated in peroxidase-conjugated secondary antibody (1:2,000; Vector) for 1 hr. After another 1-hr wash, the membranes were developed with

enhanced chemiluminescence (GE Healthcare) and exposed to film for 2 min. The membrane was then stripped of antibody by using stripping buffer (60 mM Tris-HCl, 2.4 mM Tris base, 2% sodium dodecyl sulfate, 0.7% β -mercaptoethanol) at 55°C for 5–7 min, rinsed in 500 ml ddH₂O, blocked in 5% milk for 1 hr, incubated in primary antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:2,000; Millipore) in 5% milk overnight, and exposed to film for 5–10 sec.

Guide Cannulae Implantation Surgery

Bilateral guide cannulae (22 gauge; Plastics One, Roanoke, VA) directed at the arcuate nucleus (coordinates from bregma: anterior –2.8 mm, lateral 0.8 mm, ventral –7.4 mm from dura; tooth bar: –3.3 mm) were implanted by using standard stereotaxic procedures while female rats were anesthetized with isoflurane (2–3% in equal parts oxygen and nitrous oxide). Cannulae were secured to the skull with dental acrylic and stainless-steel bone screws. Obturators (Plastics One), which protruded less than 0.5 mm beyond the opening of the guide cannulae, were placed in the cannulae. Animals were individually housed after surgery, received oral antibiotics (trimethoprim and sulfamethoxazole, 0.4 mg/ml; Hi-Tech Pharmacal, Amityville, NY) in the drinking water, and were allowed to recover for 6–7 days before microinfusion. Rats with cannulae that were not positioned in the arcuate nucleus (i.e., located above, lateral to the arcuate nucleus, or where microinjections had compromised the wall of the third ventricle) were excluded from the study.

Arc Antisense

Arc asODNs were designed to knockdown Arc mRNA and protein. Lordosis quotients were compared between asODN and nonsense ODN (nsODN) infused rats. The asODNs were a mix of two sequences: 5'-ATGGTCCAGCTCCATCTG-3' and 5'-GGAGGCCCGCGGTCGTCAT-3'. The scrambled nsODN cocktail was composed of two random sequences with no known mRNA targets: 5'-GGTGCATTCCCGAGTCCA-3' and 5'-GCGGACGGTCGCGCTACGT-3' (Life Technologies, Grand Island, NY). All ODNs had ends modified with phosphorothioate to protect against degradation. ODNs were dissolved in artificial cerebral spinal fluid to a final concentration of 1 μ g/ μ l.

An infusion pump (Harvard Apparatus, Holliston, MA) was used to microinfuse 1 μ l Arc asODN or scrambled nsODN into the arcuate nucleus at a rate of 0.25 μ l/min, 90 min prior to the start of each behavior test. Microinjection needles (28 gauge) protruded not more than 1 mm beyond the opening of the guide cannula. The injectors were allowed to remain in place for 1 min after infusion to allow for diffusion away from the injector tip. After microinfusion, the obturators were reinserted into the guide cannulae, and animals were returned to their home cages prior to behavior testing.

Statistical Analysis

All data are expressed as mean $\pm$ SEM and were analyzed by two-tailed *t*-tests or one-way ANOVA, as specified in Results. Statistical analysis was conducted in GraphPad Prism (version 5.02; GraphPad Software, La Jolla, CA). The number of animals used in each experiment is also specified in Results. Differences were considered significant at $P < 0.05$.

RESULTS

Naïve Females Were More Sexually Receptive Than Experienced Females

Females were injected with 5 µg EB once every 4 days. Each injection and the following 4 days were considered a cycle (Fig. 1A). naïve females were injected the requisite number of times and tested once for lordosis behavior 30 hr after the final EB injection. naïve animals showed that as the number of EB injections increased so did the LQ, and maximum receptivity was reached by cycle three (Fig. 1B). Experienced females, tested 30 hr after each EB injection (i.e., once per cycle), did not reach maximal receptivity and instead plateaued at moderate LQs (~50), despite having the same number of EB injections (i.e., amount of estradiol) as their naïve counterparts (Fig. 1; *t*-test; cycle 4 $P = 0.04$, $t = 2.269$, $df = 19$; cycle 5 $P = 0.03$, $t = 2.446$, $df = 11$; $n = 4-32$).

Sexual Behavior Upregulated Arc in the Arcuate Nucleus

To determine whether steroid treatment regulated Arc in the arcuate nucleus, animals were injected with oil, EB, or EB followed by P and sacrificed 1 hr later. None of the treatments, without a behavioral bout, was sufficient to upregulate Arc in the arcuate nucleus or the VMH (Fig. 2A; one-way ANOVA, arcuate nucleus $P = 0.80$, VMH $P = 0.76$; $n = 3-8$).

Naïve animals injected for four cycles with estradiol and tested for sexual behavior had a significantly increased number of Arc-immunoreactive cells in the arcuate nucleus (Fig. 3A–D; one-way ANOVA, $P = 0.0032$; Newman-Keuls multiple-comparisons test, naïve vs. experienced $P < 0.05$, naïve vs. naïve + P $P < 0.05$, naïve vs. experienced + P $P < 0.05$, naïve vs. experienced + P + EB $P < 0.05$; $n = 4-5$) and VMH (Fig. 3D; one-way ANOVA, $P = 0.002$; Newman-Keuls multiple-comparisons test, naïve vs. experienced $P < 0.005$, naïve vs. naïve + P $P < 0.005$, naïve vs. experienced + P $P < 0.005$, naïve vs. experienced + P + EB $P < 0.005$; $n = 4-5$).

A single injection of oil followed by a mating test also induced Arc expression in the arcuate nucleus that was comparable to that seen in EB-treated naïve females (Fig. 3D; Newman-Keuls multiple comparisons test, oil vs. experienced $P < 0.05$, oil vs. naïve + P $P < 0.05$, oil vs. experienced + P $P < 0.05$, oil vs. experienced + P + EB $P < 0.05$; $n = 4-6$). These oil-treated females were mounted 10 times by the stimulus males and their LQ was 0, indicating that the level of sexual receptivity was not associated with increased Arc expression.

The number of Arc-positive neurons was not increased in EB-primed females treated with P, despite the fact that the mating experience was novel (Fig. 3D). Experienced females treated with P 4 hr before their final behavior test were maximally receptive (LQ of ~100; Fig. 3). Furthermore, experienced animals treated with P on the third cycle and tested for lordosis behavior after an additional cycle of EB priming (experienced + P + EB) were maximally receptive with very low numbers of Arc-immunoreactive cells (Fig. 3; $P > 0.05$).

Similarly, Arc protein, determined by Western blot, was also increased only in naïve compared with untested or experienced females (Fig. 3E; one-way ANOVA, $P = 0.02$; Newman-Keuls multiple-comparisons test, untested vs. naïve $P < 0.05$, experienced vs. naïve $P < 0.05$; $n = 3-4$).

Other Factors Involved in Arc Upregulation

To test whether mating or other similar stimuli induced Arc, females were given several novelty tests. In the first test, females were housed for 30 min (the length of a typical LQ test for a moderately receptive female) with an unknown female to test whether socializing with a new animal upregulated Arc. Compared with naïve animals that had been tested for sexual behavior once, the numbers of the Arc-positive neurons in the arcuate nucleus were not increased. Similarly, male-soiled bedding did not induce Arc in the arcuate nucleus (Fig. 2B; one-way ANOVA, $P = 0.006$; Newman-Keuls multiple-comparisons test, naïve vs. novel female $P < 0.005$, naïve vs. male bedding $P < 0.005$; $n = 4-5$). In the VMH, however, both male-soiled bedding and novel females induced a statistically significant increase in Arc-expressing neurons that was similar to that of females tested for lordosis behavior (Fig. 2B; one-way ANOVA, $P = 0.11$; $n = 4-5$). These results suggest that olfactory input might be more important to the regulation of Arc in the VMH than in the arcuate nucleus.

Knockdown of Arc Restored Sexual Receptivity in Experienced Animals

To test formally whether Arc was involved in the attenuation of sexual receptivity, experienced females were infused 90 min before each behavioral test with either Arc asODNs or scrambled nsODN (control). In the arcuate nucleus, Arc asODN-treated females had 50% less Arc protein compared with nsODN controls (Fig. 4A; t -test, $P = 0.04$, $t = 2.823$, $df = 5$; $n = 3-4$). More-over, Arc asODN prevented the mating-induced Arc increase in the arcuate nucleus. Arc asODN-treated experienced females had significantly higher LQs compared with nsODN-treated experienced females. Indeed, the Arc asODN-treated rats and naïve rats had similar LQs (Fig. 4B; t -test, cycle two $P = 0.04$, $t = 2.317$, $df = 12$; cycle three $P = 0.005$, $t = 3.545$, $df = 11$; cycle four $P = 0.0004$, $t = 5.026$, $df = 11$; $n = 6-7$).

DISCUSSION

The major finding of these experiments is that the behavioral induction of Arc in the arcuate nucleus of the hypothalamus is associated with reduced sexual receptivity in subsequent tests. Estradiol-treated experienced females had significantly attenuated sexual receptivity compared with naïve rats that received similar estradiol treatments but were tested only once. Blocking Arc expression with asODN prevented the blunting of lordosis behavior in experienced, EB-only-treated rats. Because repeated treatments with moderate doses of EB (5 μ g) produce a ramping of sexual receptivity that is maximal after three doses, we initially predicted that pairing a mating test with each EB treatment would result in an accelerated attainment of maximal receptivity. The results were not in agreement with our original hypothesis. Rather, the results were in agreement with the idea that repeated lordosis testing after every EB priming results in attenuated sexual receptivity.

The IEG Arc, which is upregulated by salient sensory experience in other parts of the brain (Guzowski et al., 2001), was increased in both the VMH and the arcuate nucleus after an initial mating test. Arc induction was independent of the level of sexual receptivity or EB priming because Arc was upregulated in both oil and EB-treated females that were tested for lordosis. In fact, the level of Arc induction in oil-treated animals was similar to that measured in EB-primed naïve females. Thus, the estradiol-induced Arc expression in vitro

reported by Chamniansawat and Chongthammakun (2009) was not observed in the arcuate nucleus *in vivo*. Our results did confirm that a mating bout was sufficient to activate Arc in the female VMH (Flanagan-Cato et al., 2006) and extended these observations to the arcuate nucleus. In contrast to a previous study, from which Flanagan-Cato and colleagues (2006) reported an increase in Arc in both naïve and experienced animals, Arc in the present experiments increased only in females mated a single time (naïve), regardless of EB priming. Flanagan-Cato and colleagues had used a paradigm significantly different from that used in the present study, obviating a direct comparison. In their study, rats were treated with 10 µg EB (for 2 days) and P before the mating test. In our paradigm, 5 lg EB was given cyclically, every fourth day, designed to mimic the estrus cycle (Micevych et al., 1994). Additionally, we tested behavior 30 hr after EB priming, whereas Flanagan-Cato and colleagues tested behavior after 48 hr. Which of these conditions contributed to the disparate results is not obvious. In this study, Arc was induced in the arcuate nucleus by a single novel mating bout and then blunted lordosis behavior on subsequent mating bouts. Although estradiol did not regulate Arc expression, P treatment prevented Arc expression and reversed the Arc attenuation of sexual receptivity, which was not observed by Flanagan-Cato and coworkers.

The mechanism of Arc induction in the arcuate nucleus remains to be elucidated. Current results indicate that, for EB-only-primed females, a novel mating experience increases Arc expression. Attenuation of lordosis behavior persists, but Arc protein does not. We observed that, after the initial mating experience increase, subsequent tests did not increase the number of Arc positive neurons above the levels found in untested females. We interpret these findings with caution because the immunohistochemical detection of Arc in the arcuate nucleus might underrepresent the levels of Arc protein in arcuate neurons. Other studies have demonstrated that, after induction of Arc, its mRNA is transported to dendritic sites where it is transcribed, leaving the cell bodies with little detectable Arc protein (Steward and Worley, 2001; Rodriguez et al., 2005). This is analogous to the situation with many neuropeptides that are rapidly transported down the axon to the terminal. It is often difficult to visualize cell body staining without blocking axoplasmic transport with colchicine. Although there might be issues with immunohistochemical detection, the Arc knockdown experiment dramatically demonstrated that Arc was restricting sexual receptivity in experienced females.

In the present study, P administered 4 hr before the behavior test inhibited Arc induction, even in naïve females. Because this is the steroid milieu in the intact female rat, our results suggest that mating-induced Arc coincides with an endocrine profile that is not associated with ovulation. Thus, an interaction with a male in which there is no possibility of fertilization can be considered an “empty mating bout.” We hypothesize that Arc expression might act as a molecular gate moderating lordosis behavior; Arc is upregulated if mating occurs in nonovulating females and then acts to attenuate subsequent sexual receptivity. Although estradiol primes the lordosis-regulating circuits, high levels of peripheral P, signaling ovulation, inhibit Arc expression, resulting in sexual receptivity. In this way, Arc modulates the negative sensory feedback designed to prevent sexual receptivity in the absence of ovulation. Further studies are required to test this molecular gate hypothesis.

Importantly, a time period does exist when such a molecular gate might be functional, i.e., puberty.

In the present study, induction of Arc expression by mating inhibited lordosis behavior during future mating bouts. EB-only experienced females had moderate LQs, which became maximally receptive when supplemented with P. Moreover, this effect persisted through the next mating bout. P blocked Arc expression and produced maximal sexual receptivity in subsequent mating bouts, even though the females were treated with EB alone on that next bout. Such a period of elevated estradiol and low P occurs before puberty in the intact female rat. Circulating levels of estradiol rise but are insufficient to induce ovulation and the synthesis of P from the corpus luteum. In such females, mating-induced Arc would prevent further sexual receptivity until mating is likely to be successful (i.e., after ovulation). To determine whether this gating occurs will require additional experimentation, but these data do indicate a mechanism of sensory feedback onto lordosis-regulating circuits that might prove useful for understanding the differences between estradiol-only- and estradiol + P-induced lordosis behavior.

In summary, mating drastically affects hypothalamic nuclei that regulate lordosis behavior. In the arcuate nucleus, a region where estradiol rapidly activates the lordosis-regulating circuit, the IEG Arc, which is known to play a role in synaptic plasticity, was induced after the first bout of sexual behavior. Although the number of Arc-expressing cells was not upregulated after repeated mating tests, experienced animals never reached maximal sexual receptivity. Downregulation of Arc, with P or with Arc asODN, blocked the attenuation of sexual receptivity, indicating that Arc is involved in the negative sensory feedback that restricts the expression of lordosis behavior. The mechanism(s) underlying this regulation will require further investigation.

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Fig. 1.

Experience reduces sexual receptivity. **A:** The paradigm for EB treatment and sexual behavior testing (denoted by LQ). Females were injected every fourth day with 5 μ g EB (one cycle) and tested for sexual receptivity 30 hr later. **B:** naïve females were injected for the stated number of cycles but were tested for sexual behavior only once, 30 hr after the final injection. They showed the characteristic ramping of receptivity. Females reached maximal receptivity after three cycles. Experienced females were tested 30 hr after each injection of EB (experienced $n = 8$ –32/group, naïve $n = 4$ /group). Although both groups of females received the same dose of EB, only naïve females became maximally receptive; experienced females remained moderately receptive (lordosis quotient ~50). Some experienced females were killed after each cycle of EB priming to determine Arc levels. * $P < 0.05$ vs. naïve with the same number of injections.

Fig. 2.

Steroid hormone treatment and novel experiences do not affect Arc levels in arcuate nucleus. **A:** Female rats treated with oil, EB, or EB + P (1 hr) had no change in levels of Arc-immunopositive (Arc⁺) neurons in the arcuate nucleus or VMH. (n = 3–8). **B:** Effect of novelty tests on Arc⁺neurons. In the VMH, Arc⁺ neurons were upregulated by exposure to novel female and male-soiled bedding but not in the arcuate nucleus. The data from these tests were compared with ⁺ neurons in rats with a single lordosis test that had received four cycles of EB injections (4x naïve; from Fig. 3). In the VMH, the number of Arc⁺ neurons was increased by novel females or male bedding scent and was not significantly different from that elicited by sexual behavior. ARH, arcuate nucleus of the hypothalamus; VMH, ventromedial hypothalamus. * $P < 0.05$ vs. number of Arc⁺ neurons in the same brain area 4x naïve (n = 4–5).

Fig. 3.

Induction of Arc⁺ neurons and protein in the mediobasal hypothalamus of naïve females. All groups were mated with stimulus males. Arc immunostaining was localized in cell bodies and dendrites of naïve animals (**A,B**) but was not apparent in experienced animals (**C**). Vehicle-treated, mated (oil), and naïve rats had similar numbers of Arc⁺ neurons (**D**), suggesting that lordosis testing, not EB treatment, induced Arc. The number of Arc⁺ neurons was very low in experienced animals. P treatment of EB-primed naïve (naïve + P) and experienced (experienced + P) females prevented the increase of Arc⁺ neurons (**D**). Additional EB treatment (Exp + P + EB) did not increase Arc⁺ neurons numbers. Results with Arc protein levels (**E**) paralleled the immunohistochemistry. Experienced rats did not have an increase in Arc protein, but in naïve rats Arc was significantly elevated. The dashed line outlines the arcuate nucleus in A,C. Exp, experienced; ARH, arcuate nucleus of the hypothalamus; VMH, ventromedial hypothalamus. * $P < 0.05$ compared with naïve (D; $n = 4-6$); $P < 0.05$ compared with untested (E; $n = 3-4$). Scale bars = 20 μm in B; 100 μm in C (applies to A,C).

Fig. 4.

Arc knockdown restores sexual receptivity. **A:** Levels of Arc protein in the arcuate nucleus were reduced in animals treated with Arc asODNs compared with scrambled nsODN (controls; $n = 3-4$). GAPDH was used as a loading control. **B:** Arc asODNs microinfused into the ARH of experienced animals 90 min before each lordosis test significantly increased lordosis quotients (LQs) compared with nsODN-treated females ($n = 6-7$). * $P < 0.05$ vs. scramble at the same cycle number.