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Aggressive Experience Increases Dendritic Spine Density within the Nucleus Accumbens Core in Female Syrian Hamsters

Nancy A. Staffend and Robert L. Meisel

Department of Neuroscience, University of Minnesota, 321 Church Street SE, Minneapolis, MN, 55455, USA

Abstract

Activity within the mesolimbic dopamine system is associated with the performance of naturally motivated behaviors, one of which is aggression. In male rats, aggressive behavior induces neurochemical changes within the nucleus accumbens, a key structure within the mesolimbic dopamine system. Corresponding studies have not been done in females. Female Syrian hamsters live as isolates and when not sexually responsive are aggressive towards either male or female intruders, making them an excellent model for studying aggression in females. We took advantage of this naturally expressed behavior to examine the effects of repeated aggressive experience on the morphology of medium spiny neurons in the nucleus accumbens and caudate nucleus, utilizing a DiOlistic labeling approach. We found that repeated aggressive experience significantly increased spine density within the nucleus accumbens core, with no significant changes in any other brain region examined. At the same time, significant changes in spine morphology were observed in all brain regions following repeated aggressive experience. These data are significant in that they demonstrate that repeated exposure to behaviors that form part of an animal's life history will alter neuronal structure in a way that may shift neurobiological responses to impact future social interactions.

Keywords

Nucleus accumbens; Caudate; Dendritic morphology; Spine density; Spine morphology; Social behavior

1.1

The mesolimbic dopamine system has long been associated with the regulation of motivated behaviors (Mogenson et al., 1980). One approach taken to link motivated behaviors with mesolimbic dopamine utilized microdialysis to measure increases in extracellular dopamine while animals were engaged in such diverse behaviors as feeding (Bassareo and Di Chiara, 1999; Kaneyuki and Wilson, 1998; Martel and Fantino, 1996; Taber and Fibiger, 1997; Yoshida et al., 1992), drinking (Yoshida et al., 1992; Young et al., 1992), male copulatory behavior (Curtis et al., 2003; Damsma et al., 1992; Pfaus et al., 1990; Pleim et al., 1990; Wenkstern et al., 1993), female copulatory behavior (Becker et al., 2001; Jenkins and

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Corresponding Author: Robert L. Meisel, meisel@umn.edu, Phone: 1-612-625-6796, Fax: 1-612-626-9201, Department of Neuroscience, University of Minnesota, 6-145 Jackson Hall, 321 Church Street SE, Minneapolis, MN 55455.

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Becker, 2003; Kohlert et al., 1997; Kohlert and Meisel, 1999; Meisel et al., 1993; Mermelstein and Becker, 1995; Pfaus et al., 1995), maternal behavior (Afonso et al., 2009; Hansen et al., 1993) and aggression (Ferrari et al., 2003; van Erp and Miczek, 2000). With repeated exposure, the nucleus accumbens exhibits an augmented (i.e., sensitized) response to these motivated behaviors (e.g., Frohmader et al., 2010; Meisel and Mullins, 2006). Such a sensitized response suggests that the nucleus accumbens undergoes a pattern of neuronal plasticity consequent to experience with motivated behaviors.

Studies of male and female sexual behavior have identified a constellation of cellular and molecular changes in the nucleus accumbens resulting from repeated sexual experience. One notable finding is that neuronal structure, as measured by dendritic spine density, is altered following repeated sexual experience (Meisel and Mullins, 2006; Pitchers et al., 2010a). Male rats given a set of daily sexual behavior tests had increased dendritic spine density in nucleus accumbens medium spiny neurons one week following the last sexual behavior test (Pitchers 2010a). Female Syrian hamsters given a single sexual behavior test each week (a cyclic pattern of hormone treatments to bring females into heat precludes daily testing) for six weeks had a similar pattern of increased dendritic spine density in the nucleus accumbens (Meisel and Mullins, 2006). One interesting aspect of Syrian hamsters is that the males and females live in isolation in the wild and defend individual territories (Gattermann et al., 2001). For the females, this means that during three days of the four day reproductive cycle in which the females are not in heat, they are highly aggressive towards either male or female conspecifics (e.g., Floody and Pfaff, 1977; Meisel et al., 1988; Takahashi and Lisk, 1983; Wise, 1974). By extension, removing the ovaries from female hamsters, resulting in low levels of ovarian steroid hormones, leaves them in a perpetual aggressive state (e.g., Meisel et al., 1988). Because sexual behavior experience increases nucleus accumbens dendritic spine density in female hamsters, we were curious whether aggressive experience would produce a comparable pattern of structural changes. For this study, we modeled the behavioral testing regimen from studies of male sexual behavior (Pitchers et al., 2010a) and gave the female hamsters five daily aggression tests, sacrificing them one week later. Utilizing DiOlistic labeling we demonstrate here that repeated aggressive experience also provokes structural changes in the nucleus accumbens of female Syrian hamsters.

EXPERIMENTAL PROCEDURES

2.1 ANIMALS

Adult male and female Syrian hamsters (Charles Rivers Laboratories, Wilmington, MA, USA) approximately 60 days old were housed individually (females) or in pairs (males) in polycarbonate cages (50.8-cm long × 40.6-cm wide × 20.3-cm high for the females; 43.2-cm long × 22.9-cm wide × 20.3-cm high for the males) in a temperature and humidity controlled vivarium under a 14:10 light:dark cycle, with lights out at 13:00 hours. Food and water were available to the animals *ad libitum*. All animal procedures were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the University of Minnesota IACUC. Female hamsters were bilaterally ovariectomized under Nembutal anesthesia (7.5 mg/100 g body weight, i.p.), with postsurgical Butorphanol (10 mg, s.c.) and antibiotic (0.1 ml Baytril, 2.27% solution s.c.).

2.2 AGGRESSIVE EXPERIENCE

Syrian hamsters are solitary animals with both males and females defending individual territories. Across their reproductive cycle, the females are aggressive towards both male and female conspecifics (with no attenuation of aggression against familiar opponents) except for their brief period of sexual receptivity. Consequently, we paired ovariectomized female Syrian hamsters, in the absence of ovarian hormone administration, with intruder

male hamsters for our aggression tests. Without the concern of fluctuating levels of ovarian hormones we were able to test the females for aggression daily. As such, the pattern of aggression testing was modeled after that of Pitchers et al. (2010a) who showed that 5 daily sexual behavioral encounters increased dendritic spine density in the nucleus accumbens of male rats one week (but not one day) after their last sexual encounter. Therefore in our study, an adult male hamster was placed in the home cage of an ovariectomized female hamster (n=8) for 5 min each day on 5 consecutive days. Each female pairing was with a different male to minimize the likelihood that a male would show submission to a familiar dominant female (Huhman et al., 2003) and each session was video recorded for later behavioral scoring. Female controls (n=8) remained alone in their home cages for the duration of the experiment.

2.3 DATA ANALYSIS – AGGRESSIVE BEHAVIOR

Female aggressive behavior was videotaped and later scored by recording the attack latency and counting the number of attacks, on backs, and upright attacks for each 5 min session with a male. A repeated measures ANOVA was used in combination with Newman-Keuls post-hoc tests to evaluate statistical differences in aggressive behavior among the aggression sessions for each measure monitored.

2.4 TISSUE PREPARATION

One week following the last pairing for aggressive behavior, female animals were anesthetized with an i.p. injection of 0.2 ml Sleepaway (26% sodium pentobarbital, 7.8% isopropyl alcohol, 20.7% propylene glycol, distilled water; Fort Dodge Animal Health, Fort Dodge, IA, USA) and transcardially perfused with 25 mM phosphate buffered saline (PBS, pH = 7.2) for 3 min at a flow rate of 25 ml/min, followed by 1.5% paraformaldehyde in 25 mM PBS for 20 min. After perfusion, brains were removed, blocked coronally at the level of the cerebellum and post-fixed for 1 h in 1.5% paraformaldehyde in PBS. Brains were Vibratome (Lancer Series 1000, St. Louis, MO, USA) sectioned in 300 μ m serial, coronal sections through the rostral-caudal length of the nucleus accumbens. As medium spiny neurons have extensive dendritic arbors, we used these thick sections to ensure that we would capture the extent of the dendritic branching from an individual neuron within a single section. Following sectioning, slices were placed in 25 mM PBS until labeled with DiI.

2.5 PREPARATION OF DiI-COATED “BULLETS”

Coating of tungsten particles with lipophilic dye DiI was performed as previously described (Gan et al., 2000; Staffend and Meisel, 2011). Briefly, 2 mg of the carbocyanine fluorescent dye, DiI (Molecular Probes, Carlsbad, CA, USA), was dissolved in 75 μ l methylene chloride and applied to 90 mg of 1.3 μ m tungsten particles (Bio-Rad, Hercules, CA, USA) spread evenly on a glass slide. Following application, tungsten particles were allowed to dry, then scraped from the slide and collected into 10 ml of 10 mg/ml polyvinylpyrrolidone (PVP; Sigma-Aldrich, St. Louis, MO, USA) dissolved in deionized water. The suspension was sonicated for 10 min with intermittent vortexing. Tefzel tubing (Bio-Rad) was pre-coated with 10 mg/ml PVP and dried under 0.4 liters per min (LPM) nitrogen gas flow. The DiI/PVP suspension was quickly drawn into the Tefzel tubing and allowed to settle for 3 min. The PVP solution was withdrawn slowly from the tubing making certain not to disturb the tungsten. The Tefzel tubing was slowly rotated 360° and dried for 20 min under 0.4 LPM nitrogen gas flow. After drying, the tubing was cut into 1.3 mm segments (bullets) and stored with desiccant at 4°C in the dark until use.

2.6 DELIVERY OF DiI-COATED TUNGSTEN PARTICLES

A Helios Gene Gun (Bio-Rad) with a modified barrel (O'Brien et al., 2001) was used for delivery of DiI-coated tungsten particles. A 40 mm spacer was attached to the modified barrel to establish a consistent distance between the Gene Gun and brain section. A 70- μ m nylon mesh filter (Plastok Associates Ltd., Birkenhead, Merseyside, UK) was secured at the head of the barrel to prevent large clusters of tungsten particles from reaching the tissue. Immediately prior to labeling, PBS was removed from the well containing the sections. One bullet was shot per brain section at 100 pounds per square inch for delivery of DiI coated tungsten particles. Labeled sections were re-suspended in PBS and dye was allowed to diffuse through neuronal membranes for 24 h in the dark at room temperature. Slices were post-fixed for 1 h in 4% paraformaldehyde in PBS, and then placed in PBS until mounted on Superfrost slides (Brain Research Laboratories, Newton Highlands, MA, USA) using 5% *n*-propyl-gallate in glycerin. Coverslips were sealed to prevent dehydration of tissue.

2.7 CONFOCAL IMAGING

On the same day the tissue slices were mounted on slides, a Leica TCS SPE confocal microscope (Leica, Mannheim, Germany) was used to image DiI impregnated medium spiny neurons, as identified by their characteristic size and dendritic branching pattern (Chang and Kitai, 1985). DiI was imaged with excitation and emission specified to the manufacturer's spectral characteristics (Molecular Probes, Carlsbad, CA, USA). The complete dendritic profile of each DiI impregnated neuron was captured using a 20 $\times$ lens and XY pixel distribution of 512 $\times$ 512 at a frequency of 400 Hz. The neuron was scanned at 1.0 μ m increments along the Z-axis and reconstructed using Leica LAS AF software to determine distance from the soma to the branch level of target dendrites prior to dendrite/spine imaging. Imaged dendritic segments of medium spiny neurons from the nucleus accumbens core (NAc Core), shell (NAc Shell), and caudate/putamen (CPu) were located 70–200 μ m from the soma (Shen et al., 2009). After distance from soma was determined, magnification was increased to 63 $\times$ oil immersion. Frame size was maintained at 512 $\times$ 512 and an optical zoom of 5.61 was utilized to allow for maximum distribution of pixel size (60 nm) to tissue dimensions (60.91 nm) without over sampling. Z-stacks of dendritic segments were taken at 0.12 μ m steps, with a maximum of 200 steps. Images of three DiI impregnated cells were captured per brain region (NAc Shell, NAc Core, CPu) per animal, as well as three high power dendritic segments from each cell, yielding a total of nine dendritic segments per brain region per animal. Within each treatment group onethird of the neurons (essentially one neuron per rostral-caudal level on average) came from the rostral, middle or caudal level of the nucleus accumbens.

2.8 QUANTITATION AND ANALYSIS OF DENDRITIC SPINE DENSITY AND SPINE MORPHOLOGY

Dendritic Z-stacks were reconstructed using the Surpass module of the Imaris software package (Version 7.0, Bitplane Inc., St Paul, MN, USA). Intensity and contrast of the Z-stack images were adjusted as needed to optimize the visual contrast prior to dendritic spine analysis. Dendritic shafts and spines were manually traced in the XY plane using the Auto Depth function of the Filament module. After tracing, accurate 3-dimensional reconstruction of the diameter of the dendritic shaft, spine neck, and head was made possible using the diameter function with a contrast threshold appropriate to the individual image, generally between 0.1–0.5. Average dendrite diameter was generated automatically from the 3-dimensional rendering of the dendritic segment. Spine head classifications of stubby, filopodial, long thin, and mushroom were completed through the Classify Spines wizard in the Imaris software package. Criteria for spine head classifications have been described elsewhere (Harris et al., 1992; McKinney et al., 1999). Branch number data for the spines were also generated within the Classify Spines wizard.

2.9 DATA ANALYSIS - SPINES

Spine density was calculated by summing the total number of spines per dendritic segment length and calculating average number of spines/10 μm . These values were then averaged over the three dendritic segments to yield the number of spines/10 μm for each animal. Spine branch number was calculated by summing the total number of spine branch points per dendritic segment length and averaging those values across all segments per brain region per animal. Student's *t*-test was used to evaluate statistical differences between treatment groups for the spine measurements. Total spine population and counts of each spine class (stubby, filopodial, long thin, and mushroom) were summed for each treatment group. A χ^2 test was used to determine significant differences in distribution of spine morphology.

RESULTS

3.1 CHANGES IN AGGRESSIVE BEHAVIOR OVER TIME

Repeated exposure to a male resulted in a significant increase in the number of attacks female hamsters displayed during the 5 min test session ($F(4,28) = 6.09$, $p < 0.002$). Newman Keuls post-hoc test indicated significant increase in female attacks on days 2 ($p < 0.001$), 3 ($p < 0.05$) and 5 ($p < 0.05$) when compared to day 1 (Fig.1).

3.2 CHANGES IN DENDRITIC SPINE DENSITY FOLLOWING REPEATED AGGRESSIVE EXPERIENCE

Fig. 2 shows representative dendritic segments from medium spiny neurons of the nucleus accumbens core from naïve females (Fig. 2A) and females who experienced 5 consecutive days of aggressive experience (Fig. 2B). Female aggressive experience (Fig. 3A) resulted in a significant increase in spine density of medium spiny neurons within the nucleus accumbens core ($t(14) = 3.73$, $p < 0.01$, two-tailed). No effect of aggressive experience on spine density was observed in the nucleus accumbens shell (Fig. 3B) or caudate (Fig. 3C).

3.3 ALTERATIONS TO DENDRITIC SPINE MORPHOLOGY FOLLOWING REPEATED AGGRESSIVE EXPERIENCE

Spines were classified into one of four categories in maturing order: stubby (least mature), filopodial, long thin or mushroom (most mature). Summation of all four spine categories for a given treatment group provided normative values for the total spine number per treatment group, population counts for each spine subtype in a given brain region, as well as the percentage of total spine population.

In addition to significant increases in spine density, repeated aggressive experience resulted in a significant decrease of long thin spines ($\chi^2(1) = 10.20$, $p < 0.01$, two-tailed) with a concurrent significant increase of mushroom spines ($\chi^2(1) = 5.42$, $p < 0.05$, two-tailed) in the nucleus accumbens core (Figure 4A). Despite the fact that no significant changes in spine density were observed in the nucleus accumbens shell or caudate following repeated aggressive experience, significant increases in mushroom spines were also observed in the nucleus accumbens shell ($\chi^2(1) = 20.02$, $p < 0.0001$, two-tailed; Figure 4B) and in the caudate ($\chi^2(1) = 5.18$, $p < 0.05$, two-tailed; Figure 4C) following repeated female aggressive experience.

3.4 EFFECTS OF AGGRESSIVE EXPERIENCE ON DENDRITIC DIAMETER AND SPINE BRANCHING

In contrast to other measures taken, no significant changes in dendrite diameter or dendritic spine branching were observed in any brain region analyzed as a result of repeated aggressive experience (data not shown).

DISCUSSION

4.1

The goal of this experiment was to determine if aggressive experience could induce structural changes in a key reward area of the brain, the nucleus accumbens. Here we demonstrate that repeated aggressive experience indeed has the ability to persistently alter the spine density of medium spiny neurons specifically within the nucleus accumbens core. The increase in spine density adds aggression to a growing list of experiences, such as environmental housing, salt appetite, and male or female sexual behavior that impact dendritic structure in the nucleus accumbens (Kolb et al., 2003a; Kolb et al., 2003b; Meisel and Mullins, 2006; Pitchers et al., 2010a; Roitman et al., 2002). Such data are significant in that they demonstrate that previous life experience has the capacity to induce presumably long-lasting plastic changes within the mesolimbic dopamine circuitry.

In our study of aggression, we modeled the timing of the behavioral experiences and sacrifice on studies of male sexual behavior first characterized by Pitchers et al. (2010a). In their work, five consecutive days of copulatory experience increased dendritic spines in the nucleus accumbens when the animals were sacrificed one week, but not one day, after their last behavioral test (Pitchers et al., 2010a). The molecular mechanisms driving dendritic spine formation certainly are initiated rapidly (O'Donnell et al., 2011; Penzes and Rafalovich, 2012) and increases in dendritic spines can occur within minutes (Matsuzaki et al., 2004). This means that the time course of the consolidation of spine formation following sexual or aggressive experience does not stem from molecular time constraints.

In addition to changes in spine density, we analyzed spine morphology based on observations that spine shape is strongly correlated with synaptic efficacy (Kasai et al., 2003; Matsuzaki et al., 2001). Repeated aggressive experience resulted in what could be considered an amalgamation of synapses within the core of the nucleus accumbens with a decrease in long-thin spines giving way to increases in mushroom spine types. The general conclusion from the literature is that larger, more mature spine heads (a defining feature of mushroom spines) make a greater functional contribution to the excitability of the cell than do small, immature spine heads (Kasai et al., 2003). Perhaps these presumed increases in intrinsic excitability form part of the basis for the decrease in attack latency with repeated aggressive experience.

As this is the first study of its kind on aggression there are important relationships between the components of the aggressive experience and structural changes that need to be discovered. We believe that the effects on dendritic structure do not simply arise from nonspecific social interactions or locomotor activity arising from the aggressive interactions. We conducted a microdialysis study in which female hamsters interacted socially with males, but did not attack or display sexual responsiveness (Meisel et al., 1993). Under those conditions, extracellular dopamine in the nucleus accumbens was not elevated as it is during sexual encounters in female hamsters (Kohlert and Meisel, 1999; Kohlert et al., 1997; Meisel et al., 1993). We also know that during sexual behavior the nucleus accumbens is activated without a corresponding increase in locomotor activity. Female hamsters are immobile for upwards of 90% of a sexual behavior test (Meisel et al., 1988), making the increase in dialysate dopamine and dendritic spine densities in the nucleus accumbens following sexual experience independent of changes in locomotor activity. Though we can not rule out locomotor activity as a mediating variable, certainly it is not a requirement for nucleus accumbens activation.

Because aggression has rewarding consequences (Meisel and Joppa, 1994) it may be that the particular behaviors displayed during the aggressive interactions are less important than their

post-test consequences for nucleus accumbens activation. This possibility is highlighted by a study of nucleus accumbens dopamine dynamics associated with aggression in male rats (van Erp and Miczek, 2000). Surprisingly, in this study there were no changes in dialysate dopamine in the nucleus accumbens during a 10 min aggression test. Instead, during the hour following the aggressive encounter dialysate dopamine was significantly elevated over pre-aggression baseline levels. It is interesting that male rats with aggression experience show an anticipatory increase in accumbens dopamine prior to the time of their daily aggressive encounter (Ferrari et al., 2003). The female hamsters in our study were tested at the same time each day raising the possibility that anticipatory processes or the consequences of the aggressive encounter contributed to the changes in spine density. A clear goal of our future studies will be to uncover the critical behavioral mechanisms underlying the structural changes seen in this study.

Structural changes in the nucleus accumbens, particularly as a response to repeated exposure to psychostimulants, are thought to represent a heightened (i.e., sensitized) response of the nucleus accumbens to further activation (Samaha and Robinson, 2005). In fact we know that different kinds of social experiences can produce long term alterations of receptor signaling and cellular physiology in the nucleus accumbens (Aragona et al., 2003; Aragona et al., 2006; Aragona and Wang, 2007, 2009; Bradley and Meisel, 2001; Bradley et al., 2004; Bradley et al., 2005; Hedges et al., 2009; Meisel and Mullins, 2006; Pitchers et al., 2010b). Accompanying this plasticity in the nucleus accumbens is a cross-sensitized response to an initial exposure to drugs of abuse (Bradley and Meisel, 2001; Pitchers et al., 2010a), suggesting that nucleus accumbens plasticity may impact both its normal and pathological responses.

We acknowledge that female rodents are not common subjects in studies of aggression. This is unfortunate, as aggression is clearly within the domain of social behaviors among females of many species (Stockley and Bro-Jørgensen, 2011). We are so used to thinking about sex differences in aggression that we lose sight of the fact that there may be neural mechanisms for aggression common to both males and females. The nucleus accumbens may be one such site for the regulation of aggression in both males and females, highlighting the importance of conducting these studies in both sexes.

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HIGHLIGHTS

Repeated exposure to males facilitates female aggressive behavior.

Aggressive experience increases dendritic spine density in the nucleus accumbens core.

Aggressive experience does not affect spine density in the nucleus accumbens shell or dorsal striatum.

Aggressive experience increases mushroom spines throughout the ventral and dorsal striatum.

Fig 1.

Female aggressive behavior over time. (A) Attacks are plotted for each of the 5 consecutive days of testing. Newman-Keuls post-hoc test revealed significant increases in attack behavior on days 2 (** $p < 0.001$), 3 (* $p < 0.05$) and 5 (* $p < 0.05$) when compared to day 1. (B) Newman-Keuls post-hoc test revealed significant decreases in attack latency on days 2 (** $p < 0.01$), 3 (** $p < 0.001$), 4 (** $p < 0.001$) and 5 (** $p < 0.01$) when compared to day 1.

Fig 2.
Representative dendritic segments from medium spiny neurons of (A) naive or (B) female hamsters with aggressive experience. Scale bar = 5 μ m.

Fig 3. Spine density of medium spiny neurons significantly increased in the (A) core of the nucleus accumbens (NAcCore) of females who experienced 5 days of consecutive aggressive experience (** $p < 0.01$) with no significant changes in the (B) nucleus accumbens shell (NAcShell) or (C) caudate (CPu).

Fig 4.

Aggressive experience results in a shift in spine morphology across the ventral and dorsal striatum. Aggressive experience significantly decreased the number of long thin spines (** $p < 0.01$) while significantly increasing the number of mushroom spines (* $p < 0.05$) within (A) the nucleus accumbens core (NAcCore) of female hamsters. Aggressive experience significantly increased the number of mushroom spines in the (B) nucleus accumbens shell (NAcShell, *** $p < 0.001$) and (C) caudate (CPu, * $p < 0.05$).