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Chronic Social Isolation Enhances Reproduction in the Monogamous Prairie Vole (*Microtus ochrogaster*)

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Abstract

Chronic stressors are generally considered to disrupt reproduction and inhibit mating. Here we test the hypothesis that a chronic stressor, specifically social isolation, can facilitate adaptive changes that enhance/accelerate reproductive effort. In general, monogamous species display high levels of prosociality, delayed sexual maturation, and greater parental investment in fewer, higher quality offspring compared with closely related polygynous species. We predicted that chronic social isolation would promote behavioral and neurochemical patterns in prairie voles associated with polygyny. Male and female prairie voles were isolated for four weeks and changes in mating behavior, alloparental care, estrogen receptor (ER) α expression and tyrosine hydroxylase (TH) expression in brain regions regulating sociosexual behavior were examined. In males, isolation accelerated copulation, increased ER α in the medial amygdala (MEApd) and bed nucleus of the stria terminalis (BSTpm), and reduced TH expression in the MEApd and BSTpm, but had no effect on alloparental behavior. In females, isolation resulted in more rapid estrus induction and reduced TH expression in the MEApd and BSTpm, but had no effect on estradiol sensitivity or ER α expression. The results support the hypothesis that ER α expression in the MEApd and BSTpm is a critical determinant of male copulatory behavior and/or mating system. The lack of change in alloparental behavior suggests that changes in prosocial behavior are selective and regulated by different mechanisms. The results also suggest that TH in the MEApd and BSTpm may play a critical role in determining mating behavior in both sexes.

Keywords

prairie vole; isolation; reproduction; prosocial behavior; estrogen receptor alpha; tyrosine hydroxylase

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1. Introduction

Chronic social stressors generally interfere with reproduction and inhibit prosocial behavior (Hawkley et al., 2012; Rivier and Rivest, 1991; Tilbrook et al., 2000). Prosocial behavior consists of positive social interactions that benefit other individuals (Penner et al., 2005). In highly social species, isolation represents a significant stressor, whereas group-housing is a stressor for territorial and solitary-living species (Brain and Benton, 1979; deCatanzaro and Gorzalka, 1979). The majority of studies have focused on the maladaptive consequences of social stressors. However, adverse social conditions may also provide adaptive signals allowing individuals to maximize reproduction. For example, social isolation may mimic limited social contact after dispersal and provide cues about population densities and probabilities of finding a mate. Thus, we propose the novel hypothesis that social stressors can produce adaptive shifts in behavior, particularly in species with alternative reproductive strategies comprising different degrees of prosocial behavior and mating effort.

Prairie voles (*Microtus ochrogaster*) are socially monogamous with most males and females forming pair bonds and rearing offspring together (Carter et al., 1995). However, some seek extra-pair copulations, while others adopt a polygynous “wandering” strategy, or rear offspring alone (McGuire and Getz, 1998; Solomon and Jacquot, 2002). Adoption of these various strategies necessarily involves tradeoffs between the highly prosocial behaviors associated with monogamy and the more rapid copulatory patterns and increased mating effort associated with polygyny.

Chronic isolation is a robust stressor in prairie voles, which results in altered neuroendocrine reactivity, increased anxiety- and depressive-like behaviors, and cardiovascular dysfunction (Bosch et al., 2008; Grippo et al., 2007a; 2007b). However, here we hypothesize that social isolation will also produce adaptive shifts in reproduction and site-specific changes in estrogen receptor alpha (ER α) and tyrosine hydroxylase (TH) expression consistent with patterns seen in polygynous species.

ER α is essential for mating and maternal behavior, and plays a critical role in determining prosocial behavior in males, as demonstrated by a number of intra- and inter-species comparisons. Prairie voles were classified as socially monogamous based upon voles originating from Illinois (IL). However, Kansas (KN) females are less prosocial, display sexual receptivity in response to lower doses of estrogen, display more rapid estrus induction following exposure to males, and are less alloparental than IL females (Cushing et al., 2001; Roberts and Carter, 1997). KN males also display many characteristics associated with polygyny, including lower levels of prosocial behavior, greater sensitivity to testosterone and more robust copulatory behavior (Cushing et al., 2004; Roberts et al., 1998). The behavioral differences between KN and IL males are associated with different patterns of ER α expression in the medial amygdala (MEApd) and bed nucleus of the stria terminalis (BSTpm), with KN males displaying greater expression in both regions. Females from the two populations also differ in that KN females display greater ER α expression than IL females in the medial preoptic area (MPOA) and enhanced sensitivity to estradiol. A comparison of polygynous and monogamous species shows this to be a general pattern (Cushing and Wynne-Edwards, 2006; Wu et al., 2010). Additionally, increasing ER α

expression in the MEApd or BSTpm of IL males significantly reduced prosocial behavior (Cushing et al., 2008; Lei et al., 2010), whereas down-regulation of hypothalamic ER α expression reduces sexual activity in female rats (Spiteri et al., 2012). A role for TH neurons in the MEApd and BSTpm in sociosexual organization has also been inferred, as polygynous species possess fewer TH neurons in these regions than monogamous species, which project primarily to the MPOA and are activated by sociosexual stimuli (Cavanaugh and Lonstein, 2010; Northcutt and Lonstein, 2011; Northcutt et al., 2006).

We predict that social isolation will enhance reproduction in males and females and reduce prosocial behavior and be associated with increased ER α expression, in the MEApd and BSTpm (males) and MPOA (females), and decreased TH expression in the MEApd and BSTpm (both sexes). Collectively, these changes will shift neurochemistry and behavior away from socially monogamous patterns and prepare individuals for more rapid reproduction associated with polygyny.

2. Methods

2.1. Animal husbandry

Animals in this study were laboratory-reared prairie voles that originated from wild stock trapped near Urbana, IL. Animals were housed under a 14:10 h light/dark cycle (lights on at 0600 h) and provided food (Purina high fiber rabbit chow; Purina, St. Louis, Missouri) and water *ad libitum*. Offspring remained in their natal group in large polycarbonate cages (25 $\times$ 45 $\times$ 60 cm) with cotton nesting material until weaning at 21 days of age. At this time, animals were housed in same-sex sibling pairs in smaller cages (12 $\times$ 18 $\times$ 28 cm) until the commencement of the study. All procedures were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were preapproved by the University of Illinois at Chicago Institutional Animal Care and Use Committee.

2.2. Social Isolation

Adults (approximately 60 to 90 days of age) were randomly assigned to isolated or the control pair-housed groups. Isolation involved removing the experimental animal from the home cage and placing it into an individual cage for 4 weeks (12 $\times$ 18 $\times$ 28 cm). Pair-housed animals were also moved into new cages at the same time as the isolated animals, and then were continually housed with their same-sex siblings for the duration of the study. The two groups were matched on handling and cage changing throughout the experimental period.

2.3. The Effects of Isolation on Male Copulatory Behavior

After 4 weeks, male copulatory behavior was examined across three 30-minute tests, each separated by 48 hours. Experimental males (n = 10 per group) were placed in a testing chamber (12 $\times$ 18 $\times$ 28 cm) and allowed to habituate for approximately 10 min after which a sexually-receptive female was placed in the cage. Stimulus females were primed by injection with 10 μ g/day (s.c.) of estradiol benzoate (EB) for 2 days before testing, which is a standard procedure for inducing receptivity in female prairie voles (Cushing et al., 2004). All

stimulus females were verified to display receptivity with a sexually experienced male prior to each test.

The copulatory behavior of male prairie voles is similar to other rodents and consists of mounts, intromissions with repetitive pelvic thrusting and ejaculations (Gray and Dewsbury, 1973). The following parameters were analyzed for each test: latencies for the first mount (ML) and intromission (IL), the ejaculation latency (EL) for the first copulatory bout (i.e., the time from the first intromission until ejaculation), the number of intromissions per ejaculation (I/E) and the post-ejaculatory interval (PEI) after the first bout (i.e., the amount of time between the first ejaculation and the next mount or intromission).

2.4. Female Sexual Receptivity

Sexual receptivity was examined in two groups of experimental females. In the first group, pair-housed and isolated females were placed in a cage with a novel male for 72 hours ($n = 10$ per group). Female prairie voles are induced ovulators and do not display a spontaneous estrous cycle, but are induced into estrus following extended exposure to male chemosignals (Carter et al., 1987). In general, a majority of females display sexual receptivity within 24–68 hours of pairing with a novel male (Roberts et al., 1998; Witt et al., 1988), which provides the rationale for the design of the male-induced estrus experiment. The latency for initiation of sexual receptivity following cohabitation was determined using time-lapsed video recordings (12:1 ratio, which condensed the 72-hour test into 6 hours). Uterine weights were collected from all females after the cohabitation period and compared to uteri from reproductively naïve females to verify reproductive activation, as uterine weight is a sensitive proxy for estrogen exposure (Carter et al., 1987).

A separate group of experimental females were tested for sexual receptivity following daily subcutaneous injections of $0.5\mu\text{g}$ EB in $50\mu\text{l}$ sesame oil for 7 days ($n = 12$ per group). This low dose was chosen in an attempt to pull apart group differences in estrogen sensitivity (Roberts et al., 1998; Cushing and Carter, 1999). During treatment, experimental females remained in their respective housing treatments (i.e., pair-housed or isolated). Twenty-four hours after the second and seventh injections, females were placed in a test chamber with a sexually experienced male for 15 min and observed for sexual receptivity. If the female did not display lordosis during this time, she was placed with a different male for an additional 15 min. Upon displaying lordosis, females were immediately removed and returned to their home cage. Two weeks after the final test and cessation of EB treatment, females were paired with a novel male for 72 hours and verified to display reproductive activation.

2.5. Alloparental Behavior in Males

Alloparental behavior was examined in a separate group of males following 4 weeks of pair-housing or isolation ($n = 10$ per group). The effects of chronic social isolation on female alloparental behavior have already been reported, in which it was shown that 4 weeks of isolation significantly increased pup-directed aggression (Grippe et al., 2008). Therefore, only males were examined in the present study. On the basis of preliminary evidence, additional groups of males were isolated or pair-housed for 8 weeks prior to alloparental testing ($n = 10$ per group) to examine if extended isolation would produce more robust

effects on alloparental behavior, as has been shown for corticotropin-releasing hormone expression in the hypothalamus (Grippe et al., 2007; Grippe et al., 2007). Experimental males were placed into the testing apparatus, which consisted of two inter-connected chambers (each $12 \times 18 \times 28$ cm) and allowed to habituate for approximately 10 minutes. A single prairie vole pup (1–2 days of age) was then placed in the center of the unoccupied chamber and the 10-minute test began when the experimental subject entered the chamber with the pup. Tests were observed for the presence of pup-directed aggression, which when it occurred resulted in the immediate removal of the pup and early termination of the test. Subjects displaying pup-directed aggression were designated as attackers and only tests from non-attackers were scored for the amount of time spent in the chamber containing the pup and the total duration of time spent in direct physical contact with the pup, which included huddling over the pup, licking and grooming.

2.6. Tissue Collection, Immunohistochemistry and Image Analysis

An additional group of experimentally naïve animals (i.e., had not undergone any behavioral testing) were pair-housed or isolated for 4 weeks ($n = 8$ per group). These animals were then deeply anesthetized and their brains were removed following transcardiac perfusion with a fixative solution consisting of 4% paraformaldehyde and 2.5% acrolein (pH 7.4). Brains were post-fixed for 24 hours in 4% paraformaldehyde, and sunk in 25% sucrose prior to being cut into a series of $30 \mu\text{m}$ sections on a freezing sliding microtome. Sections were stored at -20°C in a cryoprotectant antifreeze solution until processed for immunohistochemistry.

Serial brain sections representing one-sixth of the brain were single-labeled for either ER α or TH using standard avidin:biotinylated enzyme complex (ABC) immunohistochemistry on free-floating sections using anti-ER α IgG (Santa Cruz Biotechnology, MC-20, diluted 1:7,500) or anti-TH IgG (Novus Biologicals, NB300-109, diluted 1:3,000), which were both generated in rabbit. Sections were treated with 1% sodium borohydride and 0.014% phenylhydrazine to quench unreacted aldehydes and inactivate endogenous peroxidases, respectively. Sections were then incubated in primary antibody for 1 hour at room temperature, and then approximately 60 hours at 4°C . Sections were then incubated in anti-rabbit IgG (Vector Laboratories, BA-1000, diluted 1:600) for 1 hour at room temperature, followed by incubation in ABC solution (Vector Laboratories, Vectastain Elite PK-6100, prepared according to manufacturer's instructions) for 1 hour at room temperature. ER α was visualized by incubation in nickel-enhanced diaminobenzadine (Ni-DAB) dissolved in sodium-acetate for 15 min at room temperature, whereas TH was visualized with DAB in Tris-buffered saline. Stained sections were mounted on slides, air-dried, dehydrated through an ascending ethanol series, cleared with xylene and coverslipped using Enetellan rapid mounting medium. Images were captured using a Nikon Eclipse E 800 microscope, Sensi-cam camera and immunoreactivity was quantified with IPLab software (Scanalytics, Inc., Fairfax, VA). The number of ER α - and TH-immunoreactive (-ir) cells was determined in the regions of interest using a 40x objective according to procedures described previously by our laboratory (Perry et al., 2009). Two to three brain sections were analyzed for each region, and counts were performed separately for each hemisphere, and the results were averaged between hemispheres. TH-ir neurons were manually counted, whereas ER α -ir cells were

counted using density thresholds and cell size limits to estimate the total cell number. ER α -ir cells were examined within the anteroventral periventricular nucleus (AVPV), MPOA, BSTpm, MEApd, and ventromedial nucleus of the hypothalamus (VMH), whereas TH-ir cells were examined within the BSTpm, MEApd and zona incerta (ZI). The regions analyzed for ER α have all been implicated in male and/or female reproduction and social behavior (Cushing et al., 2008; E. Hull and J. Dominguez, 2007; Spiteri et al., 2010). The TH neurons in the MEApd and BSTpm have also been implicated in prairie vole social behavior (Northcutt et al., 2006; Northcutt and Lonstein, 2009), whereas the ZI served as a control region. Each of these regions could be discretely identified on the basis of their immunolabeling pattern- that rarely or never spread into adjacent regions- and the relative position of adjacent neuroanatomical landmarks. The AVPV was a discrete ER α -positive region surrounding the third ventricle (3V), sitting on top of the optic chiasm (och) rostral to the joining of the anterior commissure (ac). The MPOA was a discrete ER α -positive block surrounding the 3V, bounded above and below by the ac and the och and extending laterally the same as the ac. The BSTpm was a triangular/crescent shaped region of ER α /TH-ir cells bounded by the stria terminalis (st) above and extending the length of the stria medullaris (sm) and fornix (fx), and excluding the adjacent immuno-negative intermediate and lateral subdivisions of the posterior BST. The MEApd was an ER α /TH-ir region delimited by the entire length of the optic tract (ot) and surrounding immuno-negative regions. There was a clear distinction between ER α -ir in the MEApd and adjacent posteroventral subdivision of the MEA (MEApv), which was not as apparent with TH-ir. Therefore, TH-ir counts may include a small number of cells within the MEApv. The VMH was an ER α -ir crescent/sphere at the ventrolateral base of the otherwise immuno-negative tilted oval of the entire VMH, adjacent to the 3V and below the level of the fx and clearly separate from the ER α -ir in the more medial arcuate. The TH-ir neurons in the ZI comprised the A13 dopamine cells ventral and medial to the mamillothalamic tract and extending laterally into the ZI.

2.7. Data Analysis

Analyses were performed with SPSS (IBM, v21.0). Male copulatory behavior was analyzed with repeated measures ANOVA with housing condition as between subject factor and test day as within subject factor. ML, IL, EL and PEI were log-transformed prior to analysis. Group differences in latencies for male-induced estrus were analyzed using unpaired *t*-test. Due to unequal group variance uterine weights were analyzed by Kruskal-Wallis test for overall differences and if significant a Mann-Whitney U test was used for pair-wise comparisons. Fisher's exact test was used to analyze the proportion of females displaying lordosis following EB treatment and the proportion of males that attacked the pup. McNemar's test was used to analyze the change in the number of receptive females over the days of EB treatment. The duration of total contact time with the pup was analyzed by ANOVA with housing condition and duration of treatment as independent variables. Individual ANOVA were used to analyze ER α - and TH-ir cell numbers for each brain region with sex and housing condition as independent variables. Repeated-measures ANOVA were conducted with sphericity assumed modeling, and Greenhouse–Geisser and Huynh–Feldt adjustments were applied as appropriate. *Post hoc* comparisons were conducted using Fisher's test of least significant difference. A probability value of $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effects of isolation on male copulatory behavior

One paired and one isolated male failed to show a complete ejaculatory series during all three tests and were excluded from all analyses ($n = 9$ per group).

There was a significant effect of test day on ML ($F_{2,32} = 27.68$, $p < 0.0001$) and IL (Figure 1A.; $F_{2,32} = 21.01$, $p < 0.0001$); however, neither of these parameters was affected by housing condition and there was no significant interaction between housing condition and test day. ML were significantly reduced across the three days of testing in both pair-housed and isolated voles (test 1 vs. test 2: $p = 0.0057$; test 1 vs. test 3: $p < 0.0001$; test 2 vs. test 3: $p = 0.0054$). IL were also reduced across the three test days in both pair-housed and isolated voles (test 1 vs. test 2: $p = 0.012$; test 1 vs. test 3: $p < 0.0001$; test 2 vs. test 3: $p = 0.005$).

The mean number of I/E was significantly affected by housing condition (Figure 1B; $F_{1,16} = 16.75$, $p = 0.001$), but not test day, and there was no interaction between these factors. Isolated males had significantly fewer I/E compared to pair-housed males during all 3 tests (test 1: $p = 0.003$; test 2: $p = 0.003$; test 3: $p = 0.009$). There were also significant effects of housing condition ($F_{1,32} = 6.00$, $p = 0.026$), test day ($F_{2,32} = 3.61$, $p = 0.043$) and an interaction between these factors ($F_{2,32} = 9.46$, $p = 0.002$) on EL (Figure 1C). Isolated males initially had shorter EL compared to pair-housed males (test 1: $p = 0.005$; test 2: $p = 0.021$); however, their EL were similar by the third test. The EL of pair-housed males became shorter as they gained sexual experience (test 1 vs. test 3: $p = 0.011$), whereas EL did not change significantly from the first test in isolated males (except test 2 vs. test 3: $p = 0.004$).

Finally, Only 4 out of 9 paired males and 7 out of 9 isolated males initiated multiple ejaculatory bouts during all three tests allowing for the examination of PEI. Similar to EL, there was a significant effect of test day ($F_{2,18} = 5.35$, $p = 0.019$) and interaction between test day and housing condition ($F_{2,18} = 4.89$, $p = 0.025$) on PEI (Figure 1D). PEI decreased in pair-housed males as they gained sexual experience (test 1 vs. test 3: $p = 0.005$; test 2 vs. test 3: $p = 0.02$), whereas PEI did not change with repeated testing in isolated males. Isolated males also had significantly shorter PEI relative to pair-housed males on test 1 ($p = 0.049$) and a non-significant trend on test 2 ($p = 0.054$), whereas the PEI of pair-housed and isolated males were not significantly different on test 3.

3.2. Effects of isolation on female sexual receptivity

Isolation significantly reduced the latency for male-induced estrus (Figure 2A; $t_{1,18} = 2.98$, $p = 0.008$). Mean latencies ($\pm$ SEM) for pair-housed and isolated females were: 33.3 ± 1.9 and 24.7 ± 2.2 hours, respectively. There were significant group differences in uterine weights ($H_{3,36} = 30.00$, $p < 0.0001$), which was due to uterine hypertrophy following cohabitation in both pair-housed ($p = 0.003$) and isolated females ($p = 0.00025$). There were no significant differences in uterine weights between pair-housed and isolated females either before or after cohabitation. Mean uterine weights ($\pm$ SEM) were as follows: 0.035 ± 0.001 g (pair-housed females, naïve), 0.037 ± 0.003 g (isolated females, naïve), 0.071 ± 0.002 g (pair-housed females, after cohabitation) and 0.076 ± 0.002 g (isolated females, after cohabitation).

Compared to pair-housed females, isolation did not change the proportion of females displaying lordosis following either 2 or 7 days of exogenous EB treatment (Figure 2B); however, there was an overall increase in the total proportion of females showing sexual receptivity between the two test days (McNemar test: $p < 0.001$), indicating that the estrogen treatments were effective. All the females in both groups became sexually receptive and mated during a subsequent 72-hour cohabitation with a novel male.

3.3. The effects of isolation on male alloparental behavior

There was no significant treatment effect on male alloparental behavior, with the majority of males displaying high levels of alloparental behavior. Pup directed aggression was also relatively infrequent and was unaffected by housing conditions. Extending isolation for an additional 4 weeks (i.e., 8 weeks total) had no significant effect on alloparental behavior in male prairie voles. Table 1 displays the alloparental behavior of paired and isolated animals.

3.4. Effects of isolation on ER α and TH expression

There was a significant main effect of sex on the number of ER α -ir cells within all the brain regions examined (Figure 3; MEApd: $F_{1,28} = 158.54$, $p < 0.0001$; BSTpm: $F_{1,28} = 102.99$, $p < 0.0001$; AVPV: $F_{1,28} = 144.84$, $p < 0.0001$; MPOA: $F_{1,28} = 48.91$, $p < 0.0001$; VMH: $F_{1,28} = 5.82$, $p = 0.023$). The sex differences in ER α -ir were present in all regions in both pair-housed and isolated males, as males in each group had significantly less ER α -ir in the MEApd ($p < 0.0001$ for both), BSTpm ($p < 0.0001$ for both), AVPV ($p < 0.0001$ for both), MPOA ($p < 0.0001$ for both) and VMH ($p < 0.05$ for both), relative to their female counterparts. There was no main effect of housing condition or interaction between housing condition and sex in any brain region. Pre-planned comparisons demonstrated that social isolation significantly increased ER α -ir in the MEApd ($p = 0.036$) and the BSTpm ($p = 0.038$) of males, but not in the AVPV, MPOA or VMH. Social isolation had no effect on ER α -ir in any of the brain regions examined in females.

The number of TH-ir neurons was sexually dimorphic within the MEApd and BSTpm (Figure 4; MEApd: $F_{1,28} = 50.89$, $p < 0.0001$; BSTpm: $F_{1,28} = 139.97$, $p < 0.0001$). Pair-housed and isolated males had significantly more TH-ir than females in the MEApd ($p < 0.001$ for both) and BSTpm ($p < 0.001$ for both). There was a significant effect of housing condition (MEApd: $F_{1,28} = 23.24$, $p < 0.0001$; BSTpm: $F_{1,28} = 12.43$, $p = 0.0015$), but no interaction between sex and housing condition in these regions. *Post-hoc* comparisons revealed that isolation significantly reduced TH-ir in the MEApd and BSTpm in males ($p < 0.01$ for both) and females ($p < 0.05$ for both). There were no effects of sex, housing condition or interaction between these factors on TH-ir in the ZI.

4. Discussion

The results supported our hypothesis that the stressor of chronic isolation would produce shifts in reproductive behavior, pushing males and females towards more rapid copulation and estrus induction. Alloparental behavior was similar in isolated and pair-housed males, suggesting that mating behavior and this type of prosocial behavior may be independently regulated. Isolation also shifted the organization of ER α and TH expression in the MEApd

and BSTpm towards the patterns found in less prosocial and polygynous species/populations (Cushing et al., 2004; Cushing and Wynne-Edwards, 2006; Horton et al., 2014; Northcutt et al., 2006). Therefore, these neurochemical changes may directly contribute to the determination of reproductive strategy in males and females.

4.1. Mating Behavior and Estrus Induction

The finding of adaptive changes in reproductive activity in prairie voles in response to social isolation is in stark contrast with most studies in laboratory species where stress antagonizes sexual behavior (deCatanzaro and Gorzalka, 1979; Thor, 1980). The acceleration of reproductive activity in male voles (i.e., reduced EL and PEI) is most likely adaptive for several reasons: 1) more rapid copulation was also observed in pair-housed males as they gained sexual experience, which suggests that the accelerated pace of isolates is not a “dysfunction”, but actually represents the end point that “normal” pair-housed males also reach- it’s just the latter require more time and experience, 2) more rapid patterns of copulation are consistent with those displayed by polygynous congeners (e.g., montane voles) (Dewsbury, 1973; 1976; Gray and Dewsbury, 1973), and 3) male prairie voles in the field display multiple reproductive strategies from strictly monogamous, to less monogamous with large home ranges that overlap other females with which they ostensibly mate, to “wandering” males that do not form pair bonds (McGuire and Getz, 1998; Ophir et al., 2008; Solomon and Jacquot, 2002). More rapid copulation would be beneficial to less monogamous and wandering males, as they must be able to mate rapidly before the partners of resident females chase them away and be able to respond quickly to multiple mating opportunities. Therefore, it is likely that these changes represent pre-adaptations that would facilitate a more polygynous mating strategy in isolated males.

More rapid estrus induction in isolated females may also represent a shift towards a polygamous mating strategy. Pair bond formation is a time-dependent process with both males and females requiring extensive exposure to one another in order to display a subsequent partner preference (DeVries and Carter, 1999). Protracted estrus induction followed by extended mating likely affords sufficient time for the initiation of pair bond formation. However, more rapid estrus induction could result in the completion of mating before one or both individuals have firmly established a pair bond.

An important caveat of this work is that it is unclear whether social isolation would have the same effects in juveniles or pair-bonded adults. In nature, the vast majority of prairie voles remain in the natal nest well into adulthood, and those that do disperse are usually adults that spend a significant amount of time wandering before settling into a new social group (McGuire et al., 2013; 1993). Therefore, our results in naïve adults are highly relevant to the demographic that is most likely to need and/or use environmental cues to determine their reproductive strategy following dispersal. In contrast, pair-bonded individuals have already committed to a strategy. Thus, chronic isolation after bonding would comprise a significant stressor (Bosch et al., 2008), and most likely without providing the same adaptive cue that we have hypothesized occurs in naïve adults.

4.2. ER α Expression and Behavior

Our findings provide strong support for the hypothesis that social isolation produced adaptive changes in male mating behavior through site-specific re-organization of ER α expression in the MEApd and BSTpm- two critical nodes in the sociosexual neural network (Newman, 1999). In socially monogamous prairie and pine (*M. pinetorum*) voles, ER α expression in the MEApd and BSTpm is sexually dimorphic (Cushing et al. 2004, Cushing and Wynne-Edwards 2006) and low levels are “necessary” for the expression of prosocial behavior in males (Cushing et al. 2008, Lei et al 2010). Males of these species also display relatively longer EL and PEI compared to the closely-related polygynous montane (*M. montanus*) and meadow (*M. pennsylvanicus*) voles (Dewsbury, 1973; 1976; Gray and Dewsbury, 1973). As chronic isolation has been shown to have no effect on circulating testosterone levels in male prairie voles (Klein et al., 1997), these changes in ER α expression are likely to enhance the effects of testosterone and/or estradiol on the MEApd and BSTpm. Estradiol increases spine density, soma size and excitatory neurotransmission in the MEApd (Castilhos et al., 2008; Cooke et al., 2003; Schiess et al., 1988) and facilitates male mating behavior (Huddleston et al., 2003; 2006). Therefore, the isolation-induced increase in ER α expression in the MEApd may have increased sensitivity to genital sensory inputs allowing males to reach ejaculatory thresholds more quickly and with fewer intromissions (Dominguez and Hull, 2001; Hull and Dominguez, 2006).

We predicted that isolation, which hastened male-induced estrus in females, would be associated with increased ER α expression in the MPOA (and/or AVPV) and VMH, as KN females display more rapid male-induced estrus, increased sensitivity to estradiol and have greater ER α expression in the MPOA and VMH than IL females (Cushing et al., 2004; Roberts et al., 1998). Additionally, female offspring of rat dams displaying low maternal care (ostensibly analogous to KN dams) possess greater ER α in the AVPV and enhanced sexual receptivity as adults (Cameron et al., 2008). Isolated females did not display greater sensitivity to estradiol or alterations in ER α expression in any of the brain regions examined, including the MPOA, AVPV and VMH. Chronic isolation does not affect peripheral estradiol concentrations in female prairie voles (Klein et al., 1997), and uterine weights (a proxy for estradiol exposure) confirmed that isolated females were not spontaneously in estrus. Therefore, the mechanisms underlying the effects of isolation on male-induced estrus appear to be independent of changes in estradiol and ER α expression. Therefore, hypothalamic ER α expression may be more sensitive to social influences during early development, particularly in females (Cameron et al., 2008; Champagne et al., 2003).

4.3. TH Expression and Behavior

The functions of the TH-ir neurons in the MEApd and BSTpm of prairie voles are currently unknown. However, they are responsive to the social environment and activated by mating (Cavanaugh and Lonstein, 2010; Northcutt and Lonstein, 2009). Additionally, these TH-ir neurons lack the other requisite enzymes for dopamine biosynthesis, suggesting that their projections to the MPOA may preferentially release L-DOPA or other trace amines (Ahmed et al., 2011; Northcutt et al., 2006; Northcutt and Lonstein, 2011; Ugrumov, 2009). Trace amine receptors inhibit dopamine neurotransmission (Lindemann et al., 2008), which in the MPOA would suppress sexual motivation and copulation in males (Hull and Dominguez,

2006) and block cyclicity in females (MacKenzie et al., 1988). Thus, we propose the novel hypothesis that TH neurons in the MEApd and BSTpm function to delay and extend the mating process in male and female prairie voles, which may provide time for individuals to become familiar with one another and establish pair bonds (DeVries and Carter, 1999). Thus, the reduction in TH expression following isolation may allow males and females to mate before pair bond formation can occur, which would facilitate polygamous strategies in both sexes (e.g., wandering males and females rearing offspring alone).

4.4. Alloparental Behavior

Chronic isolation had no apparent effect on male alloparental behavior, which suggests that the mechanisms controlling mating and prosocial behavior are not the same- although they may still share some commonality. Alloparental behavior is almost impossible to disrupt in juvenile and adult males. Manipulations that increase ER α expression in the MEApd (and possibly BSTpm) are among the most effective at disrupting male alloparental behavior, including neonatal castration, postnatal ER α activation, and viral vector over-expression of ER α in the MEApd of adults (Cushing et al., 2008; Cushing and Kramer, 2005; Lei et al., 2010; Lonstein and De Vries, 2000; Lonstein et al., 2002; Perry et al., 2015; Roberts et al., 1996). The increase in ER α in the MEApd and BSTpm of isolated males was relatively modest compared to the “female-like” expression patterns observed after early developmental manipulations. Therefore, it is possible that ER α expression was only affected in a subset of the circuits within the MEApd and BSTpm, such as those receiving genital sensory inputs as opposed to chemosensory cues. Taken together the implication is that ER α expression within prosocial behavior circuits may be established relatively early (i.e., before or during adolescence) and then become relatively inflexible in adulthood, whereas ER α expression within mating circuits may retain some plasticity. Given the social structure of voles, there would appear to be no selective advantage to the suppression of alloparental behavior in males- nor a need for it to be linked to their reproductive strategy.

4.5. Conclusions

Chronic social isolation had a pronounced effect on reproduction in both male and female prairie voles, hastening reproductive activity in both. Social isolation also shifted ER α and TH expression in the MEApd and BSTpm towards the patterns observed in less prosocial and more polygynous species, which may have contributed to their altered mating behavior and function to coordinate reproductive strategies with the social environment. Based on our additional findings that many of these changes represented normal trajectories that were generally accrued with experience, these data support the hypothesis that social stressors can provide adaptive cues allowing individuals to shift their mating behavior and/or strategy. In contrast, organization of prosocial behavior, at least in regards to alloparental care, appears to be less sensitive to environmental cues and may be determined by interactions between genes and early life experiences (Cushing and Kramer, 2005).

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Highlights

- chronic stress has adaptive effects on reproduction and neurochemistry is proposed
- isolation hastened mating and estrus induction in male and female prairie voles
- isolation shifted neurochemistry towards patterns associated with polygamous species

Figure 1. Isolation accelerated copulatory behavior in male prairie voles

A. Pair-housed and isolated males showed a similar reduction in intramission latency (IL) with experience. B. Isolated males required fewer intramissions per ejaculation (I/E) than pair-housed males and this feature was stable across all three tests. C. Isolated males displayed a reduced ejaculation latency (EL); however, pair-housed males achieved similar reductions with experience. D. Isolated males displayed a shorter post-ejaculatory interval (PEI); however, pair-housed males achieved similar reductions with experience. *, $p < 0.05$ compared to pair-housed males on the same test day. #, $p < 0.05$ compared to test day 1 in the same housing condition. ^, $p < 0.05$ compared to test day 2 in the same housing condition. For all measures $N = 9$ per group, except for PEI in which $N = 4$ (pair-housed males) and $N = 7$ (isolated males). Vertical lines represent SEM.

Figure 2. Isolation accelerated estrus induction following exposure to a novel male without affecting sensitivity to exogenous estradiol

A. Following pairing with a novel male, isolated females displayed sexual receptivity with a shorter latency than pair-housed females. B. There were no significant differences in sexual receptivity between groups following exogenous estradiol benzoate (EB) administration. After cessation of EB treatments, all females displayed sexual receptivity following a 72-hour cohabitation (cohab) with a male irrespective of housing condition, confirming that they were all capable of displaying sexual receptivity. *, $p < 0.05$ compared to pair-housed females. #, $p < 0.05$ compared to 2 days of EB treatment. $N = 10$ per group. Vertical lines represent SEM.

Figure 3. Isolation increased ERα Expression in the MEApd and BNST of males

Representative estrogen receptor α (ER α) expression in the posterodorsal medial amygdala (MEApd) of pair-housed females (A), isolated females (B), pair-housed males (C) and isolated males (D). Mean ER α expression in the MEApd (E) and bed nucleus of the stria terminalis (BNST) (F) for each group. *, $p < 0.05$ compared to pair-housed individuals of the same sex. #, $p < 0.05$ compared to females in the same housing condition. $N = 8$ per group. Vertical lines represent SEM, ot = optic tract.

Figure 4. Isolation reduced TH expression in both males and females

Representative tyrosine hydroxylase (TH) expression in the posterodorsal medial amygdala (MEApd) of pair-housed females (A), isolated females (B), pair-housed males (C) and isolated males (D). Mean TH expression in the MEApd (E) and bed nucleus of the stria terminalis (BNST) (F) for each group. *, $p < 0.05$ compared to pair-housed individuals of the same sex. #, $p < 0.05$ compared to females in the same housing condition. $N = 8$ per group. Vertical lines represent SEM, ot = optic tract.

Table 1

Alloparental Behavior of Pair-housed and Isolated Male Prairie Voles

Expeimental Groups		Percent Attacking	Pup Contact in Sec (SEM)	Sec in Pup Chamber (SEM)
4 Weeks:	Paired (n=10)	10%	402.1 (41.2)	566.7 (15.2)
	Isolated (n=10)	10%	342.6 (68.9)	507.6 (48.4)
8 Weeks:	Paired (n=10)	20%	364.4 (56.1)	564.7 (12.1)
	Isolated (n=10)	20%	417.4 (65.2)	548.6 (25.0)