Behavioural Brain Research 200 (2009) 106–112

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Prior sexual experience increases hippocampal cell proliferation and decreases risk assessment behavior in response to acute predator odor stress in the male rat

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ARTICLE INFO

Article history:
Received 5 September 2008
Received in revised form 27 December 2008
Accepted 3 January 2009
Available online 8 January 2009

Keywords:
Sexual behavior
Risk assessment
Stress
Cell proliferation
Hippocampus
Dentate gyrus
Predator odor
TMT

ABSTRACT

Acute exposure to the predator odor trimethylthiazoline (TMT) induces defensive behavior in the male rat, and this response is associated with a decrease in cell proliferation within the dentate gyrus of the hippocampus. Sexual experience appears to be protective, as it exerts anxiolytic-like effects and sustains gonadal function in the face of stress. To examine the influence of sexual experience on subsequent stress-induced defensive behavior and cell proliferation in the hippocampus we exposed adult male rats to TMT odor with or without prior exposure to sexually receptive female rats. A subset of rats was injected with the DNA-synthesis marker bromodeoxyuridine (BrdU; 200 mg/kg) during TMT exposure and perfused 24 h later to provide an index of cell proliferation within the dentate gyrus. In response to TMT, sexual experience reduced the duration of stretched attend postures, but had no significant effect on defensive burying. Furthermore, TMT induced a significant increase in cell proliferation in the dentate gyrus, but only in males with sexual experience. The results demonstrate an influence of socio-sexual experience on the magnitude of the behavioral and neural responses to predator odor stress.

1. Introduction

It is well established that exposure to stressors can inhibit reproductive physiology and behavior [39,48,49,53]. Clinical studies have shown that sexual dysfunction among men is comorbid with anxiety disorders [8,62] and depression [58,68]. Less understood are the potential effects of sexual experience upon subsequent physiological and behavioral responses to stressors. Many of the same brain regions that are activated by acute stressors are also activated by sexual interactions, suggesting potential links. For example, the medial preoptic area (mPOA), amygdala, and bed nucleus of the stria terminalis (BNST) are all activated by both predator odor stress and by sexual experience among male rats [7,9,15,44]. Furthermore, sexual experience appears to exert anxiolytic effects. For example, anxiolytic effects can be induced among male rodents by pair-housing with a female [67], ejaculation [17,37,50], or exposure to female odors [30]. Following a sexual interaction, male rodents display increased circulating levels of testosterone [28,29], and testosterone exerts anxiolytic actions [12]. Stress-induced elevations in glucocorticoids typically inhibit the gonadal production of testosterone [22,48,65], but fail to reverse the gonadal hyperactivity induced by sexual stimulation [34,60]. Hence, sexual experience may afford some level of protection against the detrimental effects of stress.

Previous studies have used predator odor exposure as an ecologically relevant stimulus that reliably and effectively induces physiological and behavioral changes in rodents. Specifically, acute exposure of rats to 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), an odorant extract of fox feces, induces significant increases in plasma corticosterone [9,40,59] as well as defensive burying [26], risk-assessment [14,25] and freezing behaviors [66]. Wild rat species avoid fox odors more than do shrews [11,23] and rats have a much lower olfactory detection threshold for TMT than do primates [33], suggesting that evolution has shaped the ability of rats to detect and respond to TMT. Importantly, Perrot-Sinal et al. [43] determined that acute TMT exposure causes reduced exploratory behavior among reproductively active, but not reproductively inactive, male meadow voles, suggesting that changes in testosterone level may influence the behavioral response to predator stress.

The hippocampus is an important focal point for the effects of TMT. TMT exposure induces cellular activation of the dentate gyrus, CA1, and CA3 subregions of the hippocampus [9], elicits fast wave bursts in the dentate gyrus [24], and rapidly decreases cell proliferation in the dentate gyrus [26,59]. The dentate gyrus pos-

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doi:10.1016/j.bbr.2009.01.003

sesses progenitor cells along the subgranular zone that retain the ability to divide and differentiate into neurons throughout adulthood [31]. Adult male rats given acute exposure to TMT show a decrease in cell proliferation within the dentate gyrus, at either 2 h [26,59] or 24 h after TMT exposure [14,27]. Curiously, the TMT-induced suppression in cell proliferation does not occur in female rats [14], suggesting that sex steroid hormones might contribute to the magnitude of the cellular response to TMT.

No previous studies have systematically examined the effects of sexual experience on neurogenesis in adult males, but there is evidence to suggest that social and sexual experiences and associated hormone changes can influence adult neurogenesis. For example, socially isolated male rats show reduced cell proliferation in the hippocampus relative to group-housed males [35,57]. Sexual experience can induce an increase in cell proliferation, at least in the subventricular zone. Specifically, female mice that engaged in mating display increased neurogenesis in the subventricular zone compared to non-mated females [55]. Testosterone levels increase among males following sexual interactions, and chronic injections of testosterone have been shown to increase hippocampal neurogenesis [56], suggesting that prolonged exposure to elevated testosterone levels during sexual stimulation could have an impact on cell proliferation.

Taken together, previous studies suggest that sexual interactions protect against the detrimental effects of stress on behavior as well as on cell proliferation in the hippocampus. The current study examined the influence of sexual stimulation in adult male rats on the behavioral and neurological response to acute predator odor exposure. Due to anxiolytic effects, sexual interactions were expected to decrease defensive and risk assessment behaviors. Sexual interactions were also expected to influence the magnitude, and possibly direction, of the TMT-induced suppression of cell proliferation previously documented within the dentate gyrus.

2. Methods

2.1. Animals

Forty-eight adult male Long–Evans rats (approximately 60 days old, 250–275 g) were obtained from Charles River Canada as experimental subjects. In addition, sixteen adult female Sprague–Dawley rats (220–270 g), obtained from the University of British Columbia Animal Care Facility, were used for sexual interactions. The difference in strain allowed easy distinction between the sexes during behavioral observations. Furthermore, males and females readily engaged in sexual interactions and different strains have been used previously to examine male sexual behavior [16,21]. Males and females were housed in separate colony rooms in opaque polyurethane bins with aspen-chip bedding. Males were pair-housed and females were housed individually. Rooms were temperature controlled ($21 \pm 1\,^{\circ}C$) with a 12:12 h light/dark cycle (lights on at 07:00 h). Tap water and rodent chow (Lab Diet #5012; Jamieson) were provided *ad libitum* throughout the experiment. All animal procedures were approved by the animal care committee at the University of British Columbia and were carried out in accordance with ethical guidelines set by the Canada Council for Animal Care.

2.2. Screening for sexual competence

All male subjects were handled daily for at least 5 days prior to the first day of screening for sexual competence. For both sex screening and experimental trials, behavioral estrus was induced among ovarectomized females by s.c. injections of 17β-estradiol benzoate (10 μg) 48 h prior to testing and progesterone (500 μg) 4 h prior to testing. All sexual testing was conducted in bi-level Plexiglas chambers (15 cm × 50 cm × 70 cm) with a platform (40 cm in length) centered 28 cm above the floor and ramps between levels [44,45]. Chambers were cleaned with 70% ethanol after each trial.

Each male received 4 sex-screening trials separated by 3-day intervals, and no male was exposed to the same female more than once. For each trial, a receptive female was placed into a bi-level chamber 5 min prior to adding a single male, and the pair was allowed to interact for 30 min. The third and fourth screening trial for each male was video recorded and scored for frequency of mounts, intromissions, and ejaculations [45]. Twelve males were screened per day, and four batches of 12 males were screened in all. The four males showing the least sexual behavior in each batch were eliminated from further testing. BestCollection software (ver. 7.0, Educational Consulting Inc.) was used to score the frequency of mounts, intromissions, and ejaculations [52] for each day of screening. Of the males that were eliminated, none ejaculated and 2 males exhibited intromission during the third and fourth screening trials only. All males retained for experimental testing engaged in frequent mounts and intromissions during screening and most (20 of 32) ejaculated at least once during the third and fourth screening trials.

2.3. Experimental protocol

Experimental testing started 5 days after the completion of male screening. A 2 × 2 experimental design was used in which males were divided into four treatment groups of 8 males per group: no-sex/water, no-sex/TMT, sex/water, and sex/TMT. Males in the sex treatment groups were exposed to receptive females in bi-level chambers for 30 min over 5 consecutive days. No male was exposed to the same female more than once. In a separate room, males in the no-sex treatment groups were placed in an empty, clean bi-level chamber for 30 min over 5 consecutive days. Two males were tested at a time in each of the two rooms (i.e., sex room and no-sex room) using bi-level chambers that were visually isolated from each other. All trials were video recorded for behavioral analysis.

For TMT exposure, we followed a protocol that was previously shown to induce defensive behaviors and a decrease in cell proliferation within the dentate gyrus of adult male rats [14,26,27]. Immediately after exposure to the female or empty bi-level chamber, each male was placed in a Plexiglas box (29 cm × 30 cm × 46 cm) inside a fume hood for 20 min on the first 4 days of testing to habituate to the testing procedures. The testing boxes were filled with 5 cm of corn cob bedding and a vial with a dry Kimwipe was placed in the corner. Two separate fume hoods were used, one for the TMT group and one for the control group, and two visually isolated chambers were used under each fume hood. On the fifth day, vials under one fume hood were filled with 150 μl TMT and vials under the other fume hood were filled with 150 μl of water. The fume hood prevented diffusion of odor throughout the room. The first 15 min was video recorded to determine the behavioral response to TMT or water. Immediately after video recording, all rats were given an i.p. injection of the thymidine analog BrdU (5-Bromo-2′deoxyuridine; 200 mg/kg body mass) to label dividing cells. Rats were then returned to the testing chambers for an additional 45 min of exposure (1 h total). The strong smell of TMT within the chambers did not dissipate noticeably during testing, indicating that rats were exposed to TMT for the full hour. Twenty-four hours later, rats were anaesthetized with a lethal dose of sodium pentobarbital (Euthanyl; Bimeda-MTC, Cambridge, ON, Canada) and perfused transcardially with 0.9% saline (60 ml) followed by 4% paraformaldehyde (120 ml). The cell cycle in adult male rats has been shown to be 24.7 h [6], and therefore the 24 h time period was used to measure cell proliferation (i.e., cells that have undergone one division) within the dentate gyrus. Brains were extracted and post-fixed with 4% paraformaldehyde (4 °C) for 24 h. Brains were then cryoprotected with 30% sucrose in 0.1 M TBS (0.08 M Tris–HCl, 0.02 M Tris-base, 0.9% saline, pH 7.4) and stored at 4 °C until slicing. Half the brains ($N = 16$) were mistakenly stored at −20 °C and were not viable for immunohistochemistry.

BestCollection software was used to score sexual behaviors and the behavioral response to TMT or water. The frequency of mounts, intromissions, and ejaculations was scored for all 5 days of sexual interactions. The frequency and duration of the following behaviors were scored during the first 15 min of exposure to TMT or water: defensive burying, stretched attend, contact with vial, rearing, and grooming. Defensive burying involved the rat using its forepaws to push and fling bedding toward the vial [47]. Stretched attend is a risk assessment behavior which was characterized by the rat extending the front half of its body toward the vial while its hind paws remained stationary [46]. Rearing involved rats raising their forepaws off the ground and stretching vertically. Grooming included licking any part of the body or vigorously rubbing licked forepaws on any part of the body.

2.4. Immunohistochemistry

Brains were sliced into 40 μm coronal sections through the extent of dentate gyrus in a bath of TBS using a vibratome (Leica VT1000S). Tissue was collected and stored in an antifreeze solution (0.05 M TBS, 30% ethylene glycol and 20% glycerol) at −20 °C until immunohistochemical processing.

Peroxidase immunohistochemistry was performed on free-floating tissue sections at 400 μm intervals (i.e., every 10th section) through the rostrocaudal extent of the hippocampus to visualize BrdU-labeled cells. The sections were rinsed three times for 10 min between steps with 0.1 M TBS (pH 7.4) unless otherwise stated. Tissue was initially incubated for 30 min in 0.6% H_2O_2 to eliminate endogenous peroxidase activity. DNA was denatured by applying 2N HCl for 30 min at 37 °C, and this step was immediately followed by a 10 min incubate in 0.1 M borate buffer (pH 8.5) to neutralize the acid. Sections were blocked for 30 min with 3.0% normal horse serum (NHS; Chemicon, Temecula, CA, USA) in 0.1 M TBS and then incubated 48 h at 4 °C in mouse monoclonal antibody against BrdU (Roche Diagnostics, Laval, Quebec, Canada; 1:200 in 0.1 M TBS, 3% NHS and 0.1% Triton-X). Sections were next incubated for 4 h at room temperature in horse anti-mouse secondary antibodies (1:100 in 0.1 M TBS; Vector Laboratories, Burlington, ON, Canada). Sections were incubated for 2 h in avidin–biotin horseradish peroxidase complex (ABC Elite Kit; 1:50; Vector Laboratories). Sections were reacted for approximately 5 min in a solution of 0.02% diaminobenzidine (DAB; Sigma–Aldrich) and 0.003% H_2O_2 in 0.1 M TBS. The sections were mounted onto slides and dried overnight. Finally, sections were coun-