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Maternal behavior and offspring resiliency to maternal separation in c57bl/6 mice ☆

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ABSTRACT

Adverse early life experience, such as childhood abuse, neglect, and trauma, increases lifetime risk for mental illness. To investigate underlying mechanisms, the maternal separation (MS) paradigm was developed and validated as an animal model of early adversity in rats, reliably effecting long-term changes to anxiety, gene expression, and stress response. However, across-species validation of core findings in mice has met with limited success. To re-visit parameters governing the effectiveness of MS in mice, this study investigated the effect of MS on maternal care, offspring behavior, and offspring stress-induced corticosterone response in the c57bl/6 mouse strain. The results from this study suggest that: (i) levels of maternal care increase as a function of separation duration immediately after daily MS, but long-term care remains unchanged; and (ii) c57bl/6 mice are resilient to MS, exhibiting subtle decreases in anxiety and unchanged stress-induced corticosterone response as adults, irrespective of separation duration.

Introduction

Adverse early life experiences, such as child abuse, neglect, or trauma increase lifetime risk for mental illness (Teicher, 2000), and are associated with long-term changes in brain development (De Bellis et al., 1999; Ito et al., 1998). A widely-used animal model for the investigation of molecular and behavioral responses to early life stress is the maternal separation (MS) paradigm. Developed as a rat model, pups are treated to either a mild (15 min, i.e. MS15) or prolonged (180 min, i.e. MS180) daily maternal separation from postnatal day 2–14. As adults, relative to animal facility reared (AFR) animals, MS180 animals exhibit increased anxiety, elevated stress hormone levels (Aisa et al., 2008; Daniels et al., 2004; Ladd et al., 2004), and impaired negative feedback (Maciag et al., 2002; Plotsky and Meaney, 1993). Conversely, MS15 animals exhibit decreased anxiety, reduced stress hormone levels (Korosi et al., 2010), and enhanced negative feedback (Plotsky and Meaney, 1993).

Efforts have been made to adapt the paradigm to mice, provided advantages in lower husbandry costs and advances in mouse genetics. However, attempts at replication in mice have met with mixed success. Anxiety and stress response in MS180 mice have been reported to be increased (Bhansali et al., 2007; Parfitt et al., 2004; Romeo et al., 2003; Veenema et al., 2007), decreased (Parfitt et al., 2007; Savignac et al., 2011), or unchanged (Millstein and Holmes, 2007; Venerosi et al., 2003; Wang et al., 2011). Further, omissions of the MS15 or AFR condition in comparisons against MS180 (Bhansali et al., 2007; Navailles et al., 2010; Romeo et al., 2003; Savignac et al., 2011; Veenema et al., 2007; Wei et al., 2010) complicates across-study comparisons, making it difficult to determine whether discrepancies between studies are due to handling, separation, strain, or protocol differences. Of the more comprehensive studies in mice, results suggest either a resistance to the MS paradigm (Millstein and Holmes, 2007) or a marginal "stress-resilient" phenotype in both MS15 and MS180 conditions under certain circumstances (Parfitt et al., 2007). Because of inconsistencies, an extensive behavioral and physiological re-evaluation of MS as an early life stress paradigm is necessary to review its effectiveness in mice. By including additional behavioral measures and assessing dam maternal behavior during MS, this study aimed to clarify the role of dam care on offspring behavior and stress response following MS in the c57bl/6 mouse strain.

☆ Author contribution: Lawrence S. Own planned, performed, and oversaw animal husbandry, behavioral testing, immunoassays, statistical analysis, and interpretation of data. Dr. Paresh D. Patel supervised experimental design, theoretical and technical guidance, statistical analysis, and interpretation of data.

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Materials and methods

Animal husbandry

A total of 28 female and 14 male c57bl/6 mice, 7 weeks old, were ordered from Jackson Laboratory. Mice were habituated to the breeding facility for 2 weeks prior to mating. Each male was housed with 2 females, maintained in a Plexiglas cage (22 cm × 16 cm × 14 cm) filled with ~400 cm^3 of Corncob bedding, and kept under standard housing conditions (room temperature ~ 22 °C, 55% humidity) in a 12-h light/dark cycle (lights on 0700–1900). Visibly pregnant dams were moved to individual cages and checked daily for litters. If birth occurred <10 AM, age was designated PND0, and a 3/4 in. cotton square added for nesting material. The 1st litter was culled after 7 days, dams re-bred 2 weeks after culling, and the 2nd litter used for MS studies. All animals were cared for in compliance with national guidelines.

Maternal separation

On PND2, pups were selected for an even sex-ratio to minimize the influence of litter sex composition on maternal behaviors (Alleva et al., 1989). To maintain sex ratios, litter size was targeted to 8, but culled to 6 if necessary to maintain the ratio at the start of the paradigm. Average litter size for dams was: AFR = 7.29 ± 0.29, MS180 = 7.43 ± 0.20, MS15 = 7.38 ± 0.26 at the start of the MS paradigm (PND2). From PND2-14, pups were separated daily for 15 min (MS15), 180 min (MS180), or left undisturbed (AFR) between 0900 and 1300 h, based on prior MS protocols (Huot et al., 2001). Dams and litters were placed in separate holding cages and rooms. Litter cages were pre-warmed and maintained at 31 °C using an adjustable heat mat during separations. Ambient cage temperature measured after separation remained within ± 0.5 °C of starting temperature. MS15 and MS180 pups were handled individually during transfers and when returned, placed into the corner opposite of the nest. MS15 and MS180 pup displacement outside of nest was based on a previously established MS protocol (Dr. Paul Plotsky, personal communication, 2009). To control for time-of-day effects on post-separation maternal behavior, MS15 separation began 15 min before the end of MS180 separation. Partial cage cleaning, in which half of the bedding was replaced, occurred on PND2, 8, and 14 during separations. AFR litters were left in the nest during cage cleaning, with bedding cleaned from around the nest to minimize handling effects. On PND21 males were weaned to 3–4/cage and females culled. On PND35, males were further separated to 2–3/cage. Weight was assessed on PND2, 14, 21, 35, and 67.

Maternal care recording/scoring

Pre- and post-separation maternal care levels were recorded for MS15 (n = 9), AFR (n = 7), and MS180 (n = 8) dams. Pre-separation care was assessed at the onset of the light cycle, 0600–0800 and scored at 30 s intervals for 2 h on PND2, 5, 8, 11, and 14. Post-separation care was assessed immediately after separation (MS15 & MS180) or cage cleaning (AFR), scored at 10 s intervals for 15 min. Post-separation recording occurred on PND2, 5, 8, 11, and 14 for MS groups, but only during partial cage changes for AFR on PND2, 8, and 14. For AFR cage cleaning, pups remained undisturbed and bedding around the nest replaced, while MS cage cleaning occurred during separations. Behaviors of pup handling, licking, nursing, covering, and nesting were scored as maternal. Behaviors of movement outside/inside nest, grooming, and eating/drinking were scored as non-maternal. For more information on scoring, refer to Stern (Stern, 1996).

Overview of behavior battery

On PND60, male AFRs (n = 30), MS15s (n = 24), and MS180s (n = 29) were moved to the behavioral suite and separated to individual cages. On PND66, mice were weighed and cages cleaned. To acclimate mice to manipulations, mice were handled on PND66/67 for 5 min. Over the next 2 weeks, mice were tested on the elevated zero maze (PND68), open field test (PND70), light/dark box (PND72), forced swim test (PND74), activity monitor (PND77), and novelty suppressed feeding (PND79). Each test was staggered by a day except following FST, in which 2 days were given. All manipulations were performed between 0900 and 1300 h, with a 30 min pre-acclimation to the behavioral suite prior to testing. On PND78, cages were changed at the start of an 18-hour food deprivation for NSF. The following week, animals were sacrificed (PND90) following an acute-restraint stress.

Elevated zero maze (EZM)

Mice were placed facing the closed arm and tracked for 5 min. The room was illuminated with diffuse light at ~50 lx. Trials were captured and analyzed with Limelight video-tracking software (Actimetrics).

Open-field 5 min [video-tracked] (OFT.5)

Mice were placed in the center of a large field (61 cm diameter) and tracked for 5 min. Room condition, video-tracking, and analysis were identical to EZM.

Light/dark box (L/D box)

Mice were placed in the light compartment facing the wall opposite the dark compartment and tracked for 5 min. The light compartment was illuminated with a directional 60 W bulb at 500 lx. Behavior was analyzed using a computer-assisted scoring program, similar to a prior published EPM program (Patel and Seasholtz, 2006).

Forced swim test (FST)

Mice were placed in a 19 cm diameter cylinder filled with 20 cm of water acclimated to 23–25 °C and tracked for 6 min (Lucki, 1997). The height and volume of water was sufficient to prevent hind paws or tail from coming into contact with the bottom of the tank. Room illumination was at ~50 lx. Behavior from 2 to 6 min, after an initial 0–2 min acclimation period (Castagné et al., 2001), was analyzed using a computer-assisted scoring program (Patel and Seasholtz, 2006) re-coded for the FST (FSTscore). Behavior was scored in 5 s blocks according to the predominant behavior observed within each block (Lucki, 1997).

Open-field 20 min [activity monitor] (OFT.20)

Mice were placed into a 40 × 40 × 35.5 cm square, covered open-field and tracked for 20 min using an activity monitor (Accuscan) with IR sensors. Testing illumination was diffuse at ~50 lx.

Novelty-Suppressed Feeding (NSF)

A 48 × 48 × 72 cm box was layered with ~2 cm of bedding and a pellet of food placed on a 10 cm square piece of filter paper in the center of the box. Testing area was illuminated diffusely at ~50 lx. Mice were food deprived for 24 h prior to testing. Mice were placed facing a wall and assessed for feeding latency, with a max limit of 10 min. Feeding behavior was defined as rearing with visible food consumption. Upon feeding, testing was terminated and animals returned to their home cage. Post-NSF food consumption was assessed by a 5 min period of free-consumption in the home cage after testing and pre-/post-assessment of pellet weight.