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Maternal social environment affects offspring cognition through behavioral and immune pathways in rats

F. Pittet [1,2,3*], N. Van Caenegem [1], A. Hicks-Nelson [1,4], H. P. Santos Jr.[5,6], S. Bradburn[7], C. Murgatroyd[7], B.C. Nephew [1,8]

[1] Department of Biomedical Sciences, Tufts University Cummings School of Veterinary Medicine, North Grafton, Massachusetts, United States
[2] Neuroscience and Behavior Unit, California National Primate Research Center, University of California, Davis, California, United States
[3] School for Human Evolution and Social Change, Arizona State University, Tempe, Arizona, Unites States
[4] Department of comparative Medicine, Stanford University School of Medicine, Stanford, California, United States
[5] School of Nursing, University of North Carolina, Chapel Hill, North Carolina, United States
[6] Institute for Environmental Health Solutions, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, North Carolina, United States
[7] Bioscience Research Centre, Manchester Metropolitan University, Manchester, United Kingdom
[8] Department of Biology and Biotechnology, Worcester Polytechnic Institute, Department of Psychiatry, University of Massachusetts Medical School, Worcester, Massachusetts, United States

*Florent Pittet, Neuroscience and Behavior Unit, California National Primate Research Center. University of California in Davis. One shields Avenue, Davis, CA 95616. florent.pittet@gmail.com

Running title: Social environment during lactation affects offspring cognition

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Abstract

The social environment of lactation is a key etiological factor for the occurrence of postpartum disorders affecting women and their children. Postpartum depression and anxiety disorders are highly prevalent in new mothers and negatively affect offspring's cognitive development through mechanisms which are still unclear. Here, using a rat model, we manipulated the maternal social environment during lactation and explored the pathways through which social isolation (vs. the opportunity for limited social interaction with another lactating female, from 1 day before parturition to postpartum day 16) and chronic social conflict (daily exposure to a male intruder from postpartum day 2 to day 16) affect offspring learning and memory, measured at 40 to 60 days of age. We specifically explored the consequences of these social treatments on two main hypothesized mediators likely to affect offspring neurophysiological development: the quality of maternal care and maternal inflammation factors (BDNF, GM-CSF, ICAM-1, TIMP-1 and VEGF) likely to influence offspring development through lactation.

Maternal rats which had the opportunity to interact with another lactating female spent more time with their pups which, in turn, displayed improved working and reference memory. Social stress affected maternal plasma levels of cytokines that were associated with cognitive deficits in their offspring. However, females subjected to social stress were protected from these stress-induced immune changes and associated offspring cognitive impairment by increased social affiliation. These results underscore the effects of social interaction for new mothers and their offspring and can be used to inform the development of clinical preventative measures and interventions.

Key-words: Cognitive development; ICAM-1; Maternal care; Post-partum depression; Social stress; Social support

Introduction

Ten to twenty percent of women experience depression and anxiety during the early postpartum period (PPD/A), with detrimental effects on their sleep routine, self-gratification of their maternal role, breast-feeding and developing bond with the child [1–3]. Epidemiological studies have consistently identified the importance of psychosocial risk factors in the prevalence of PPD/A[4], including the occurrence of conflicts and the quality of social support in the maternal environment [5,6]. Maternal postpartum disorders have been associated with enduring adverse consequences on child cognitive development through mechanisms which are poorly understood, presenting challenges for the development of preventative strategies and treatments. Postpartum depression is consistently associated with a range of adverse infant cognitive outcomes including deficits in learning skills, attention, language development, and IQ [7,8]. Psychological studies have identified the quality of mother-infant interaction as the main mediator for the influence of maternal depression on infant cognitive development [8]. These studies typically focus on complex social interactions and communication in the dyad (attachment quality, support, synchronicity of affect expression, engagement) and infant language development [9] or composite measures of intelligence associated with further academic achievements [10,11].

The impact of the social environment of lactation on maternal care and primary components of offspring cognition needs to be identified prior to the determination of biological substrates and mechanisms. Multiple etiological pathways need to be considered, as socially induced postpartum disorders are likely to affect several components of mother-infant interaction. Recent works suggest that depression is associated with the dysregulation of maternal immune functioning [5] to which offspring can be directly exposed via lactation [12]. Variation in milk pro-inflammatory cytokine levels affect offspring neurocognitive development, particularly hippocampal development and memory [13]. However, to our

knowledge, no study has investigated maternal immune factors as potential mediators for the effects of the maternal social environment of lactation on offspring cognitive development. In addition, PPD/A etiology involves diverse social factors, and it is likely that social isolation and social conflict act through distinctive pathways to affect mothers and offspring. Therefore, it is necessary to develop manipulative paradigms to disentangle the specific and interactive effects of these different social factors which contribute to maternal mood disorders.

Several studies have investigated the influence of social environment during lactation on maternal care and offspring development in animal models, with a particular focus on social conflict. The chronic social stress (CSS) procedure, consisting of the daily exposure of lactating females to a novel male intruder, induces direct and inter-generational behavioral and physiological changes similar to PPD/A symptoms in mothers and their children (depressed maternal care, increased anxiety and altered offspring neurophysiological development and behavior)[14–20]. In contrast, the effects of social isolation during lactation has received far less attention. Traditional laboratory housing for rats and mice results in social isolation of female from before parturition until weaning. However, lactating female rats (and mice) do not naturally care for their young in total social isolation. Collective care of pups is common [21] and social isolation during lactation may be perceived as stressful for lactating mothers, affecting both their physiology and behavior with potential intergenerational consequences. Positive effects of social housing have been demonstrated by using the "communal rearing" paradigm, where several lactating females and their pups are housed together. As compared to isolated mothers, lactating females housed communally exhibit increased levels of care and their offspring express reduced social anxiety and improved maternal care [21]. However, in this paradigm, both mothers and offspring are exposed to social enrichment and it is impossible to determine whether direct and intergenerational effects result from enrichment in the maternal and/or offspring environment. A novel paradigm exposing mothers to social cues from other

lactating females without altering the offspring environment is needed to address this challenge.

The overall objective of the present study was to investigate the effects of the maternal social environment of lactation on maternal care, immune factors, and offspring cognition in a rat model. The aims of our study were fourfold: 1- develop a novel paradigm of maternal social enrichment without directly altering the offspring environment (hereafter referred to as "social exposure" or "SE") 2- measure the effects of both CSS and SE during lactation on maternal behavior and immune factors, 3- assess how CSS and SE affect offspring cognitive development, and 4- identify the respective roles of maternal care and immune factors in the effects of the social environment of lactation on offspring cognitive development. To model social conflict, we used the robust CSS procedure consisting of the daily exposure of mothers to a male intruder throughout lactation [19,20,22]. To model social exposure, we developed a novel paradigm which allows increased olfactory, visual, and somatosensory interaction between familiar lactating females without altering the social environment of their offspring.

We measured the consequences of these social manipulations on maternal care at mid-lactation. We also measured their impact on maternal plasma levels of immune markers of depression, including Brain Derived Neurotrophic Factor (BDNF) and 4 cytokines (GM-CSF, ICAM-1, TIMP-1, VEGF) at the end of the lactation period. Only variation in maternal plasma Tumor Necrosis Factor α (TNF) has been formally investigated for its consequence on milk immune signaling and associated offspring neurocognitive development[13]. Further research is needed to identify other immune factors likely to mediate the effects of postpartum maternal stress on offspring development. Variation in BDNF, ICAM-1, GM-CSF, TIMP-1, VEGF are associated with human depression[23–27], and plasma levels of these factors are altered in mothers and offspring in previous studies of the CSS model [14,15,19]. The organizational impact of early exposure to varying levels of these immune factors is yet unknown, but they have been

implicated in neuroplasticity and cognition [28–31] and are also suspected to be involved in the effects of the maternal social environment on offspring neurocognitive development. Offspring cognitive abilities were assessed using a spatial memory task which allowed for the separate assessment of working and reference memory [32]. We focused on male cognitive performance due to previous studies of the greater impact of socially induced maternal disorders on male development in both rodents and humans [33]. Due to reported buffering effects of social interactions on neurophysiological and behavioral responses to stress [34], we predicted that SE would reduce the adverse consequences of CSS on both maternal care and immune factors. We also considered the lack of social exposure (social isolation) as a stressor and consequently predicted that, independent from social stress, social exposure would improve maternal care and reduce indicators of inflammation. Finally, we predicted that the social environment of lactation would impact offspring cognition through both maternal behavioral and immune pathways.

Methods

1.Animals

Animals in this study were maintained in accordance with the guidelines of the committee of the Care and Use of Laboratory Animal Resources, National Research Council, and the research protocol was approved by the Tufts Institutional Animal Care and Use Committee. During the maternal aggression test and CSS protocol, mothers expressed agonistic behaviors towards intruder males, but care was taken to check for injuries and any signs of pain.

Fifty female Sprague Dawley rats (~70 days old, 175-200g) were obtained from Charles River Laboratories (Wilmington, Massachusetts, USA) and maintained at 21-25°C with LD12:12 light conditions with lights on at 0700 in controlled rooms. Food (Purina rat chow)

and water were provided *ad libitum* throughout the study, except for the F1 males involved in the cognitive tests who were slightly food restricted during the three weeks of habituation and testing, with daily monitoring of their growth (see cognitive test section).

2. General Schedule

For 2 weeks following their arrival, females were housed in dyads in standard transparent cages to habituate to their housing conditions. Females were then mated by placing one male with them for 4 days. The male was then removed, and the females remained in the cage until 2 days before parturition when they were housed singly and randomly assigned to either the control or CSS group and to either isolation or social exposure treatment. On the day of parturition, litters were culled to 10 pups (5 males / 5 females). Sex was determined by anogenital distance. Both social treatments ended when offspring were 16 days old and pups were weaned at 23 days of age by removing mothers from the home cages. Offspring remained with their littermates until they were 25 to 28 days old, when one male per litter was randomly chosen and separated from its litter to be tested for spatial cognition. These males were housed in cages (48 x 26.5 x 20 cm) in groups of 3 or 4 individuals containing only offspring of mothers who experienced a similar social treatment and were individually marked to identify their litter of origin. Groups of males remained in these conditions for 2 additional weeks before the spatial cognition protocol started. The general schedule is described in figure 1.

#Figure 1

3. Chronic Social Stress and Social Exposure treatments

The Chronic Social Stress (CSS) group had a novel male placed in their home cage for 1 h each day from days 2 to 16 of lactation (randomized introductions between 0900 and 1500). Following the introduction of the novel male intruder, the resident mother systematically investigated the intruder and then attacked. Intruders then adopted a submissive posture and froze in place. During the hour of exposure, females actively maintained distance between the

intruder male and the nest / their offspring. As in previous studies applying this CSS protocol, the use of smaller male rats ensured that males consistently and rapidly expressed submissive postures in response to maternal aggression [16].

For the social exposure treatment (SE), females were not housed communally in the same cage because this would present a risk of litter exchange and allo-care and allo-nursing confounds [35], and would also expose offspring to social enrichment [21]. Instead, the SE treatment involved a different housing configuration from 1 day prior to parturition to day 16 of lactation: 2 familiar females were housed in paired cages with a common wall allowing for visual inspection of the neighboring cage and mutual sniffing, and potential increases in exposure to general visual, olfactory, and auditory social stimuli between paired dams (figure 2). Their individual space was virtually identical to that of isolated mothers, who were housed in standard cages. The common wall (48 cm long x 20 cm high) was opaque on its lower part (14 cm from the ground) to prevent pups' exposure to the neighboring cage. The upper part of the wall was transparent and had an opening 40 cm long x 1 cm high) in its center (figure 2). Offspring of both treatments are nevertheless exposed to olfactory and auditory cues from other cages, as is typical in most rodent housing facilities. Pairs of cages for SE were occupied by females exposed to similar stress treatment. Exploration of the window was part of the routinely observed behaviors. When the pups were 16 days old, mother and pups were placed in similar cages as that of isolated mothers.

#Figure 2

Following the mating protocol, 2 females did not successfully reproduce. One female gave birth to a <10 pups litter and was not included in the experiment but served as a social stimulus for a SE female with matching social stress treatment. Our final samples included N = 13 control mothers isolated, N = 10 control mothers with social exposure, N = 13 CSS mothers isolated and N = 11 CSS mothers with social exposure.

4. Behavioral observations and testing

All behaviors were videotaped using High Definition cameras with high ISO sensitivity (Canon VIXIA HF R62). Behavioral scoring was conducted by an experimenter blind to the treatment.

4.1 Maternal behavior

Maternal behavior was observed at days 9 and 10 of lactation (mid-lactation). We observed maternal care during this period due to the present focus on the chronic effects of social manipulation. Effects of CSS in the F0 generation have only been observed at mid-lactation, with little or no differences in maternal behavior at either early (day 2) or late (day 16) lactation [20]. On day 9, we conducted maternal care and maternal aggression tests between 0900 and 1200. Pups were removed from the home cage and isolated from the mother in clean cages with bedding for one hour. Pups were then placed back in their home cage in different locations (2 in the center of the cage and 2 at each corner). This procedure is known to stimulate the typical pattern of maternal care that consists of retrieving all the pups to the nest, some nest building activity, licking and grooming of the pups, then nursing [20]. Maternal behavior was then videotaped for 30 minutes. Latency to retrieve all pups to the nest, to lick / groom the pups and to nurse the pups were scored. After these 30 minutes, an unfamiliar male was introduced in the cage to trigger maternal aggression. The mother's behavior was videotaped for 30 minutes and the latency to attack the male was scored. The male was then removed from the cage of control mothers but remained for an additional 30 minutes in the cage of CSS mothers as part of the CSS protocol.

On day 10 of lactation, cages were videotaped for 4 consecutive hours between 1330 and 1830. Cages remained undisturbed for a minimum of 2 hours before videotaping started (i.e. for the CSS exposed females, the male had been removed at least 2 hours before undisturbed behavior was assessed). From these videos, undisturbed maternal behavior was

scored using instantaneous scan sampling with a discrete observation every 3 minutes, resulting in a total of 80 scans. At each observation, the observer noted whether the mother was in the nest or not, nursing or not, as well as her activity: licking and grooming pups, nesting, self-grooming, resting, exploring the cage (extension on walls) and locomotor activity.

4.2 Spatial memory test

Juvenile males were minimally food restricted to ensure sufficient motivation in the cognitive task. The food restriction protocol started the day before habituation to the apparatus (39 to 42 days old) and was maintained for 3 weeks (one week of habituation and two weeks of acquisition). Animals were provided once a day with a specific amount of food so that the growth rate of the restricted animals was 92 ± 2 % of growth in unrestricted rats. Food was provided at 1830 and systematically completely consumed by the next morning.

The hole-board task is a robust and ethologically relevant procedure designed to assess spatial learning by quantifying, through foraging behavior, the ability of individuals to memorize a single pattern of rewarded sites [32]. The apparatus (figure 3) was a square opaque board (60 x 60 cm) with four 45 cm high transparent walls. The board contained 16 holes (4 rows of four 3.5 cm diameter and 3.5 cm depth holes) equally distributed at its surface. Each hole base was perforated, and inaccessible food was placed under this base so that tested rats can't discriminate between rewarded and unrewarded sites based on olfactory cues. The apparatus was placed in a dimly lit room (4 x 4 m). Tested individuals were individually transported in a PVC box (20 x 10 x 10 cm) that was placed against the center of one of the apparatus' walls. After 10 seconds, the door of the box was lifted, and the rat was free to access the hole-board. Once the tested individual was entirely on the board, the door was closed. Food rewards were small pellets (2 mm diameter) of rat chow. After each test, the apparatus was entirely cleaned with ethanol. The order in which individuals were tested was changed daily and defined to homogenize their average time of testing.

#Figure 3

Before training started, animals were habituated to the hole-board. They were exposed to the hole-board for 10 minutes between 0900 and 1300 for five consecutive days. For the first two first days of habituation, the holes remained non-baited and the individuals explored the apparatus. Over the next 3 days, individuals were habituated to explore the holes and every hole contained a reward. On the last day of habituation, we recorded the number of different sites visited and the total number of sites visited to assess differences between groups in responses to the apparatus before training.

Over the following two weeks (2 series of 5 days), rats were trained to learn a specific pattern of 4 rewarded sites between 1500 and 1900. Two symmetrical patterns were generated (figure 3) to present similar difficulty but prevent any effect of laterality on measured performance [32]. None of the cognition variables differed between animals exposed to pattern 1 and pattern 2 (all $P > 0.05$). Every training test was videotaped from the opening of the door until the rat found the fourth food reward or after the rat spent 10 minutes in the apparatus. A site was considered as visited when the nose of the animal penetrated the hole to the minimum of the eye level. The observer noted the number of visits to each site, as well as the time required to find the 4 rewards (with a maximum of 600 s if the last reward was not found in less than 10 minutes).

5. Measures of serum markers

At the end of lactation (day 23), F0 mothers were euthanized and trunk blood was collected for the analysis of serum marker levels. Assay panels of the immune markers TIMP-1 (RCI1MAG-87K-01), ICAM-1 (RV2MAG-26K-01) GM-CSF and VEGF (RECYTMAG-65K-03) from Millipore (U.K.) were measured using a Luminex 200 Bio-Plex Platform. Immediately prior to the initiation of the study, the Bio-Plex platform underwent a complete on-site maintenance cycle. Samples were thawed directly on the day of analysis. Working wash

solutions and protein standards were prepared within 1 hour of beginning the assay by reconstituting the standard in assay diluent and performing serial dilutions according to manufacturer specifications. A magnetic plate washer was utilized during the plate washing stages. Following processing, protein concentrations were calculated and analyzed with the xPONENT software (Luminex, v.3.1.871). Samples were run in duplicate in an individual assay to eliminate inter-assay variation, and intra-assay variability was of 3–7%. Serum BDNF levels were quantified using the Human/Mouse BDNF DuoSet ELISA from R&D Systems (U.K.). Absorbance levels were read using the Synergy HT plate reader (Bio-Tek) and concentrations determined from the standard curve using GraphPad Prism (v.6). Samples were run in individual assays for each cytokine/growth factor to eliminate inter-assay variability, and the intra-assay variability was 3-6%.

6. Data preparation and statistical analyses

6.1 Data preparation

A Principal component analysis (PCA) including the maternal behavior variables from the different observations (maternal care following separation with pups, maternal aggression and undisturbed maternal care) was run to identify correlations between the different variables and summarize variability in composite measures of maternal care. These variables included latencies to retrieve pups and express maternal behaviors following mother/pup separation, and latency to attack male intruder (in seconds) as well as the proportion of time devoted to the different maternal activities when undisturbed (from scan sampling observations, in percentage). We used factor analysis with varimax rotation to interpret the components revealed by PCA and calculate factor loadings. Maternal behavior was summarized by four dimensions accounting for a cumulated percentage of 63.49% of the variance (Supplementary figure 1). The first factor (“time in nest”) had high positive loadings for the time spent in nest, nursing and resting and negative loadings for self-grooming and exploration. The second factor

("maternal reactivity") assessed variability in the maternal care test following mother-pup separation and had strong positive loadings for the latency to retrieve all pups and lick, groom, and nurse them. The third factor ("Licking/grooming") had positive loadings for pup licking and grooming and for self-grooming, and negative loadings for resting. Finally, the fourth factor ("Nesting") had positive loadings for nesting and locomotor activity.

Three variables were extracted from the spatial memory test using robust indices of performance and memory [32] during the acquisition phase. The first variable was the time needed to finish the trial (latency to fourth reward after entering the apparatus or maximum time of 600 s.). Working memory score reflects the ability of an animal to avoid revisits to same sites within a trial (*i.e.* short-term memory) and was calculated as the ratio between the number of visits to rewarded sites and the total number of visits (and revisits) to rewarded sites. The reference memory score reflects the ability of animals to discriminate between baited and un-baited sites (*i.e.* long-term memory) and was calculated as the ratio between the total number of visits (and revisits) to the rewarded sites and the total number of visits (and revisits) to all the sites. Additionally, to control for potential treatment-related differences in the motivation of animals to find rewards, we assessed visit frequency throughout the acquisition phase (number of all visits / time spent in the apparatus).

6.2. Statistical analyses

Statistical analyses were conducted using R.3.4.4. Multiple comparisons outputs were adjusted using Bonferroni method. Statistical significance was accepted at alpha ≤ 0.05, although we discuss interaction terms with alpha < 0.10.

We initially explored the effect of CSS, social exposure and their interaction on maternal care scores (from PCA) and plasma levels of cytokines and BDNF using two-way ANOVAs. Following the detection of a significant effect of social treatment on maternal care PCA scores, associated raw behavioral variables were compared using Mann Whitney U-test

(since normality assumptions were not met). Student's T tests were used for post-hoc comparisons of cytokine and BDNF plasma levels between subsets. We then explored the effects of the social treatments (CSS and social exposure) and their interaction on F1 cognitive variables through ANOVAs on repeated measures. Interactions between social treatments and training day were systematically investigated to detect their potential impact on learning. Day by day comparisons between subsets were explored using Student's T test.

Finally, to understand how maternal social treatment affects juvenile spatial memory, we built linear mixed models with juvenile ID as a random factor and included CSS, social exposure and their interaction as well as hypothesized mediators (maternal behavior scores and physiological variables). Final models were selected following comparison using Akaike Information Criteria (AICc) to measure goodness of fit after the forward and backward stepwise inclusion of predictors. Continuous data involved in interactions in the different models were systematically scaled. We considered these variables to be mediators of the effects of social treatments on F1 working and reference memory if, after their inclusion, the model did not retain social treatment variables anymore.

Results

1. Social treatment affects maternal behavior and plasma cytokines

Females exposed to another lactating female (SE) scored lower on the PCA factor reflecting "nesting" (score on the factor "nesting": $F = 8.66$, $\beta = -0.33 \pm 0.11$, $P = 0.005$). When undisturbed (*i.e.* with fully formed nests and no environmental perturbation), the proportion of time devoted to nesting activity was three-fold time lower for SE mothers compared to isolated mothers (isolated = 4.8 ± 0.8 %, SS = 1.5 ± 0.4%, $U = 436.5$, $P < 0.001$, figure 4.a). This variable was not affected by CSS or by the interaction between CSS and SE (respectively $F = 3.17$, $P = 0.09$ and $F = 0.006$, $P = 0.9$). SE additionally tended to increase the score of mothers

on the PCA factor reflecting the time spent in nest, nursing and resting (score on the factor "time in nest": $F = 3.52$, $\beta = 0.27 \pm 0.14$, $P = 0.06$). This factor was not affected by CSS or the interaction between CSS and SE (respectively $F = 0.25$, $P = 0.6$ and $F = 1.63$, $P = 0.2$). Compared to mothers isolated during lactation, SE mothers spent ~10% more time in the nest (percentage of time in nest: isolated = 64.3 ± 3.6%, SS = 74.8 ± 4.5%, $U = 171$, $P = 0.05$, figure 4.b), ~10% more time nursing (percentage of time nursing: isolated = 62.5 ± 3.6%, SS = 72.8 ± 4.5%, $U = 170$, $P = 0.05$, figure 4.c) and ~5% more time resting (isolated = 62.8 ± 2.1%, SS = 68.1 ± 3.7%, $U = 160$, $P = 0.03$, figure 4.d). The other dimensions of maternal care ("maternal reactivity" and licking/grooming") were not affected by SE, CSS or interactions between SE and CSS ("maternal reactivity": SE: $F = 1.49$, $P = 0.22$, CSS: $F = 0.58$, $P = 0.44$, SE * CSS : $F = 0.43$, $P = 0.5$); "licking/grooming": SE: $F = 0.32$, $P = 0.57$, CSS: $F = 0.84$, $P = 0.36$, SE * CSS: $F = 0.07$, $P = 0.8$).

#Figure 4

Plasma ICAM-1 level was dependent on an interaction between CSS and SE (CSS * SE: $F = 6.14$, $\beta = -1.6 \pm 0.6$, $P = 0.02$, figure 5.a). More specifically, the CSS treatment reduced plasma ICAM-1 in isolated individuals ($t = -2.49$, $P = 0.03$) but had no significant effect for SE mothers ($t = 1.11$, $P > 0.5$, figure 5). Plasma levels of TIMP-1 were higher in mothers exposed to CSS than in control mothers (control: 1092.92 ± 175.60, CSS: 1603.03 ± 171.74 ng/ml, $F = 4.14$, $\beta = 510.1 \pm 245.6$, $P = 0.04$, figure 5.b). TIMP-1 levels were not different between isolated and SE mothers ($F = 0.27$, $P = 0.60$) and were not affected by an interaction between CSS and SE ($F = 0.52$, $P = 0.47$). Plasma levels for BDNF, VEGF and GM-CSF were not affected by CSS, SE or interaction between the treatments (BDNF: CSS: $F = 0.30$, $P = 0.59$, SE : $F = 0.41$, $P = 0.53$, CSS * SE: $F = 1.78$, $P = 0.19$; VEGF: CSS: $F = 0.003$, $P = 0.95$, SE: $F = 0.17$, $P = 0.68$, CSS * SE: $F = 0.18$, $P = 0.67$; GM-CSF: CSS: 0.98, $P = 0.33$, SE: $F = 1.44$, $P = 0.24$, CSS * SE: $F = 0.0002$, $P = 0.99$).

#Figure 5

2. Social environment during lactation affects offspring spatial cognition through maternal behavior and immune factors

On the last day of the habituation period, there was no significant differences between groups in terms of number of different sites visited or the total number of visits (all Ps > 0.05, supplementary table 1). During the hole-board acquisition period, the site visit frequency did not differ between sets (CSS: $F = 0.05$, $P = 0.83$, SE: $F = 2.03$, $P = 0.16$, CSS * SE: $F = 2.37$, $P = 0.13$) indicating that differences in cognitive measures in the hole-board are not due to differences in activity level or motivation to find the rewards. The training successfully reduced the time needed to solve the task and reduced the memory errors, indicating that juveniles learned the task and improved their performance over time (see below).

SE enhanced the performance of male offspring by improving working and reference memory. Training gradually reduced the time animals needed to complete the task, and each day of training reduced the resolution time by ~17 sec. ($F = 64.51$, $\beta = -16.77 \pm 2.70$ s., $P < 0.0001$). Controlling for this learning effect, males of SE mothers resolved the spatial memory task ~1 minute faster than their counterparts whose mother was isolated ($F = 9.37$, $\beta = 60.34 \pm 19.33$ s., $P = 0.002$, figure 6.a). Exposure of mothers to CSS and the interaction between CSS and SE did not significantly affect offspring resolution time (CSS: $F = 0.09$, $P = 0.76$; CSS * SE: $F = 0.30$, $P = 0.58$).

Controlling for the positive effect of training on working memory score ($F = 33{,}30$, $\beta = 0.02 \pm 0.003$, $P < 0.0001$), males of SE mothers exhibited better working memory indexes than males of isolated mothers ($F = 4.98$, $\beta = 0.05 \pm 0.022$, $P = 0.03$, figure 6.b, table 1). Maternal exposure to CSS and interaction between CSS and SE did not influence significantly offspring working memory indexes (CSS: $F = 0.008$, $P = 0.93$; CSS * SE: $F = 0.01$, $P = 0.92$).

Controlling for the positive effect of training on reference memory scores ($F = 183.10$, $\beta = 0.03 \pm 0.004$, $P < 0.0001$), offspring of SE mothers had better reference memory ($F = 32.86$, $\beta = 0.11 \pm 0.05$, $P < 0.0001$, figure 6.c, table 2). The model also retained an interaction between social exposure treatment and training, indicating a faster reduction in reference memory errors in the offspring of SE mothers (i.e. faster learning of the pattern with training, $F = 7.76$, $\beta = 0.015 \pm 0.005$, $P = 0.006$, table 2). This later effect tended to be additionally mitigated by CSS, as revealed by a third level interaction retained by the model (CSS * SE * training: $F = 3.30$, $\beta = -0.02 \pm 0.01$, $P = 0.08$). CSS delayed the learning of the rewarded pattern if the mother was isolated ($\beta = -0.01 \pm 0.007$, $P = 0.1$), but did not affect learning in offspring of SE mothers ($\beta = 0.006 \pm 0.008$, $P > 0.5$). There was no general effect of CSS ($F = 0.09$, $P = 0.92$) or interaction between CSS and SE ($F = 0.03$, $P = 0.87$) on reference memory indexes.

#Figure 6

We investigated the pathways through which the social environment of lactation influences working memory and reference memory by building linear mixed models which included maternal care scores and maternal cytokine and BDNF levels.

Offspring working memory was negatively influenced by the "nesting" score of mothers. The more time mothers devoted to nesting activity, the lower the working memory score of their offspring ($\beta = -0.027 \pm 0.013$, $P = 0.04$, table 1). By including nesting behavior in the model, the effect of SE on working memory was no longer retained as an explanatory factor ($P > 0.5$). In addition, the model retained a negative influence of maternal plasma BDNF on offspring working memory score ($\beta = -0.0002 \pm 0.00006$, $P = 0.02$, table 1).

#Table 1

Reference memory was negatively influenced by mothers' "nesting" score ($\beta = -0.03 \pm 0.01$, $P = 0.05$, table 2). Additionally, the time mothers spent in nest, as well as their levels of ICAM-1, both interacted with time and accentuated the daily benefits of training (Time in

nest*training: $\beta = 0.02 \pm 0.008$, $P = 0.005$; ICAM-1*training: $\beta = 0.02 \pm 0.008$, $P = 0.004$, table 2). After inclusion of these factors in the model, the model did not retain the interaction between social exposure and training, nor the third level interaction between CSS * SE * training, indicating that time in nest and ICAM-1 mediated the effects of SE and CSS on learning speed. Nevertheless, even after inclusion of all potential mediators, the model still retained a main positive effect of SE on reference memory score ($\beta = 0.11 \pm 0.05$, $P < 0.0001$), indicating that the mediators for the beneficial effect of SE on general reference memory remain unidentified.

#Table 2

Discussion

Our study investigated the effects of maternal social environment during lactation on maternal behavior, immune factors and offspring cognitive development. The novel social exposure paradigm we developed, which allowed limited social interaction between lactating mothers, impacted maternal care and offspring cognition. Compared to socially isolated mothers, mothers exposed to another lactating female spent more time with their offspring and expressed decreased restlessness (more rest, less nest-adjustment and locomotor activity). In turn, their offspring exhibited improved overall cognitive performance and better working and reference memory. In contrast, social stress affected maternal immune factors and slightly delayed spatial learning of offspring, but only when mothers were socially isolated during lactation.

Even though it allowed for limited interactions between maternal females, our social exposure paradigm during lactation had an ameliorative effect on maternal care, regardless of the social stress treatment. The result suggests that, in addition to a buffering effect of social exposure during lactation, social isolation itself is a major stressor substantially affecting

mothers and offspring. Considering the limited nature of the current paradigm, it is likely that more substantial beneficial effects could identified using a less restrictive social exposure protocol and / or a more restrictive social isolation protocol (e.g. preventing olfactory and auditory social cue exposures). The mechanism underlying the effect of social environment on maternal care has been proposed to involve maternal levels of oxytocin (OXT), a neuropeptide associated with the onset and maintenance of maternal care [36]. OXT secretion is regulated by social interactions [37] and OXT (along with maternal care) are downregulated by an increase in peripheral glucocorticoids[36], a reported consequence of social isolation in adult female rats [38]. Limited sensorial interactions with a familiar conspecific have been only investigated for their buffering effect on acute stress in adult rats with conflicting results [39,40] and the consequences of chronic social isolation vs. limited interaction remain unknown. Additionally, the specific pattern of behavioral differences between socially exposed and isolated mothers suggest a difference in maternal thermoregulation. In rats, maternal thermoregulation mediates pup contact time, the length of nesting activity bouts, and nest building [41]. Vagal tone is associated with social well-being and social support seeking [42] and affects metabolic and cardiovascular functions, directly impacting thermoregulation [43]. We suggest that social isolation impaired vagal and metabolic functioning, leading to faster hyperthermia when mothers are in contact with pups, subsequently decreasing time spent with pups and increasing the time spent nesting.

Our results indicate that the variation in maternal care is involved in the beneficial effects of social exposure during lactation on offspring spatial cognition. Parental behaviors towards developing offspring are known to affect the organization of offspring neural and behavioral systems [44]. Studies in rodents report persistent beneficial effects of mother-offspring contact and tactile stimulations on offspring cognition through changes in prefrontal cortex and hippocampus synaptogenesis [45]. Additionally, “fragmented care” (*i.e.* decreased time spent in nest associated with more frequent phases of neglect), matching the pattern isolated mothers’

care in the present study, impairs cognition in offspring and is also associated with structural and molecular changes within their hippocampus [46]. Our results also highlight that the beneficial effects of maternal social exposure on offspring cognitive development are not mediated by the peripheral levels of immune factors targeted in this study and only partially explained by variation in the maternal care we measured. These findings encourage further exploration of the consequences of social exposure on mother-offspring interactions and biochemical communication throughout lactation. These behavioral results also highlight substantial effects of social isolation and raise the question of social environment as a substantial factor and/or confound in rodent studies of maternal care and offspring behavioral and cognitive development. For example, changes and/or variation in housing cage density and cage design (clean vs. opaque, traditional or individually ventilated) could introduce confounds or variation into experiments and be important factors to consider when comparing results from different housing paradigms and/or rodent facilities.

In contrast, we did not find any significant effect of CSS on the maternal activity budget during undisturbed periods through scan sampling method. Previous results in intergenerational studies using the CSS paradigm [20] reported reduced duration of maternal care in stressed females using continuous focal observation immediately following mother-pups separation. Even though these different findings cannot be formally compared due to discrepancies in observation method, they suggest that CSS and SE affect different aspects of maternal care, with CSS impairing ability of mothers to deal with disturbance-related parental challenges while social exposure affects primary components of care in the absence of any disturbance. These contrasts in the impact of CSS and SE treatments on maternal care supports our hypothesis that specific components of the social environment differentially affect mothers. CSS, in comparison with SE, affected maternal immune factors, increasing TIMP-1 and ICAM-1, and slightly impaired offspring cognition. Our results support previous studies reporting

increased plasma TIMP-1 following social stress in the rat [47] and upregulated TIMP-1 mRNA expression in the prefrontal cortex of mice exposed to chronic social stress [48]. Elevated glucocorticoids can inhibit ICAM-1 expression [49]. Interestingly, we found that social exposure protected females from the effects of CSS on peripheral levels of ICAM-1. It is possible that this effect is mediated by the buffering effect of social exposure on the HPA responsivity, thus reducing peripheral levels of glucocorticoids and their inhibitory effect on ICAM-1 [50]. Our results additionally suggest that this immune factor is involved in the slower learning of the spatial pattern for offspring of stressed and isolated mothers. This result supports the role of the maternal immune system in offspring cognitive development [13,51] as well as our hypothesis that the social environment during lactation at least partially affects offspring behavior via changes in maternal plasma (and potentially milk) immune markers. ICAM-1 plays an important role in immune mediated cell-cell adhesion, affecting blood-brain-barrier permeability [52] and neural plasticity, [53] but the early organizational effects of varying levels of ICAM-1 on neurocognitive development remain, to our knowledge, unknown to date. It is possible that early exposure to low levels of ICAM-1 produce similar down regulation of this factor in offspring. Lower ICAM-1 was reported as an intergenerational consequence of CSS in previous rodent studies [14,19] and is strongly associated with maternal maltreatment in humans [54]. Further research is required to identify the consequences of maternal ICAM-1 downregulation on offspring behavioral functions, and to determine if it exerts its effects through maternal care impairment or if this immune alteration is directly transmitted from deficient mothers to offspring through alternative pathways, notably lactocrine.

We developed and demonstrated the effectiveness of a novel and robust paradigm illustrating the critical importance of social interactions with a familiar adult conspecific in lactating females, offering promising opportunities for further exploration of the effects of social factors on maternal care and physiology and associated intergenerational consequences.

The enhanced maternal care observed in socially exposed females is beneficial in the context of the established adverse effects of chronic social stress on maternal care and social and anxiety behavior in F0, F1, and F2 females in the CSS paradigm, in addition to the present F1 male cognition data.

Taken together, our results have significant translational potential by illustrating relative and interactive effects of social isolation and social conflict during the postnatal phase, as these social factors are difficult to dissociate in human social histories. The behavioral and physiological consequences of the adverse social environment of lactation evaluated in this study parallel the adverse consequences of depression in humans, including reduced mother-offspring physical interactions [33], increased restlessness [5] and reduced ICAM-1 immunoreactivity [49] as well as impaired cognitive development in offspring of mothers with PPD/A [7,8]. In particular, our findings highlight the need to use a multidimensional approach to comprehensively evaluate social interactions in new mothers [55,56], which includes both the level of social support and social isolation, as one could potentially buffer the effect of the other and thus affect self-efficacy. Both changes in maternal care and the resulting offspring effects support the conclusion that enriching the social environment is a valuable intervention to apply to various pathological models of maternal behavior, such as investigations of the adverse effects of neglect, PPD/A, or other stressors on mothers and offspring. While it is clear that social stress is a key risk factor for both mother and child, detailed animal studies of social enrichment models may identify key factors mediating the beneficial effects and/or facilitate the optimization of social support protocols/interventions. Results from this study can help advance mechanistic studies aiming at targeting social support as a preventative measure or intervention to improve maternal care and child development.

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Data availability

Behavioral, cognitive and immune data collected for the present research are available from the corresponding author on reasonable request.

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Figure legends

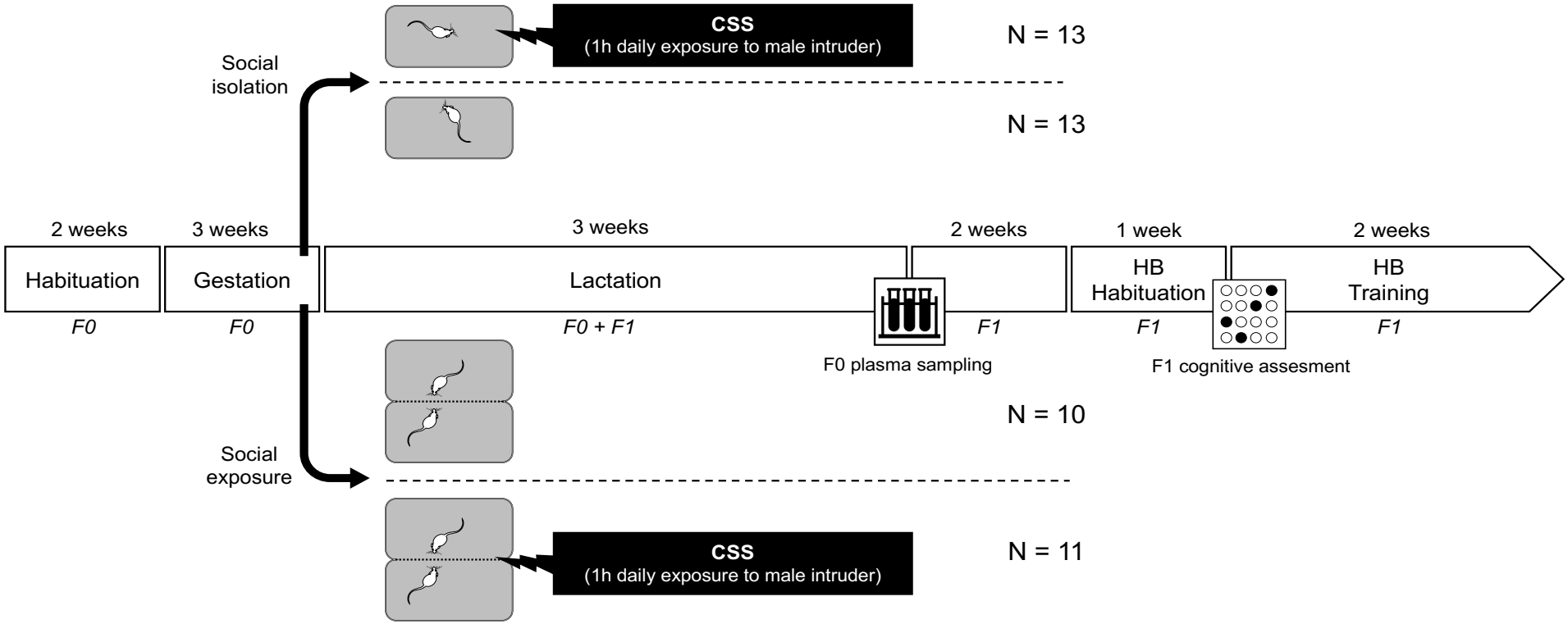

Figure 1. General experimental schedule. CSS: Chronic social stress procedure. HB: Hole-board spatial cognitive task.

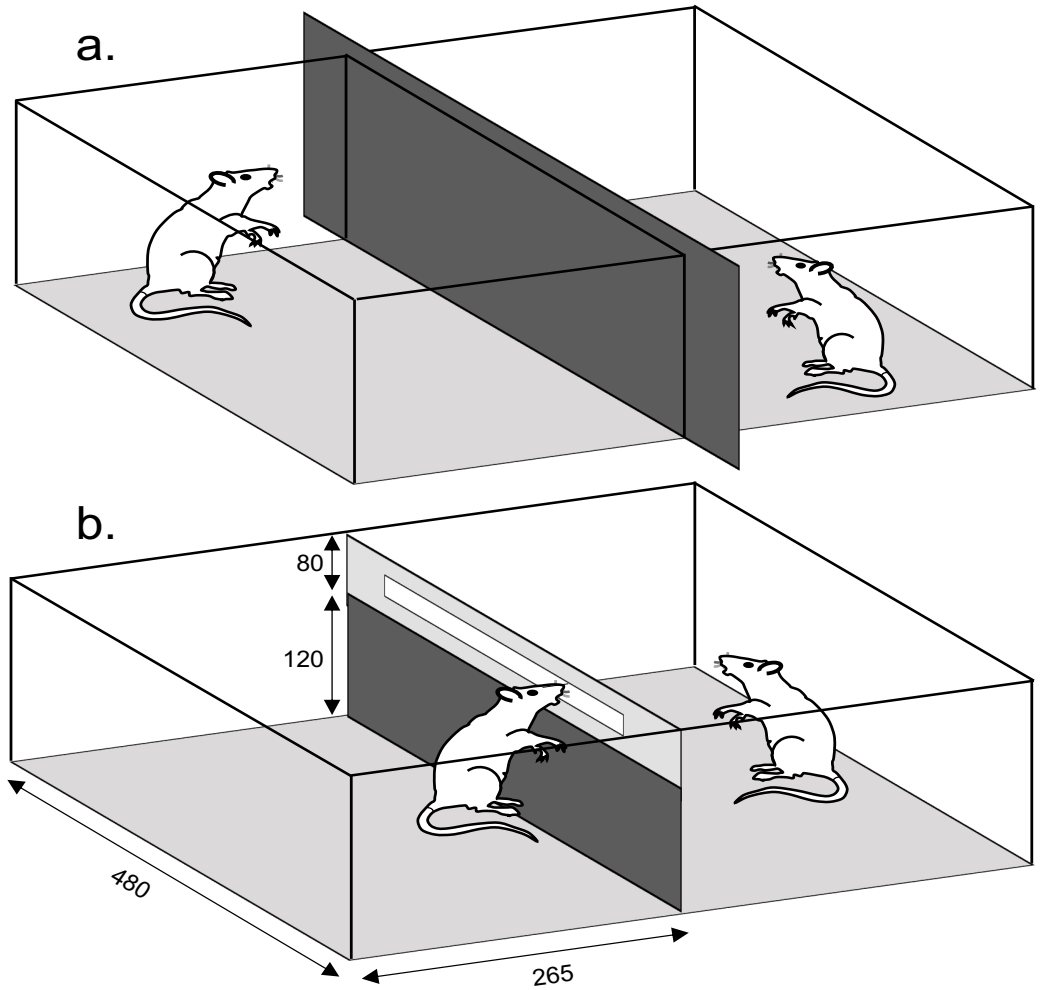

Figure 2. Schematic representation of housing conditions for social isolation (a) and social exposure (b). Dimensions are given in mm.

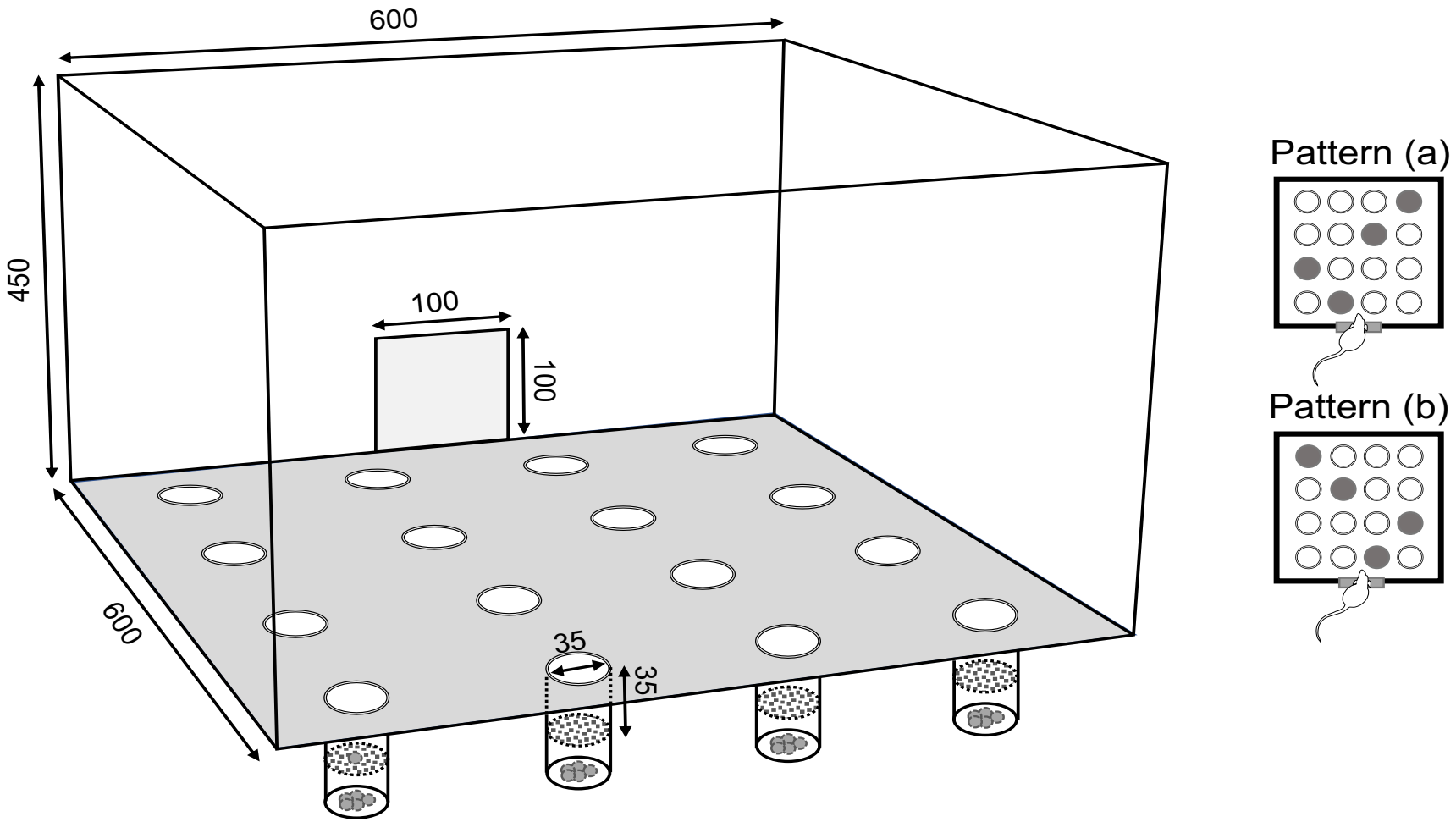

Figure 3. Schematic representation of the hole-board apparatus. Dimensions are given in mm. The two patterns of rewarded sites (in grey) are illustrated on the right side of the figure.

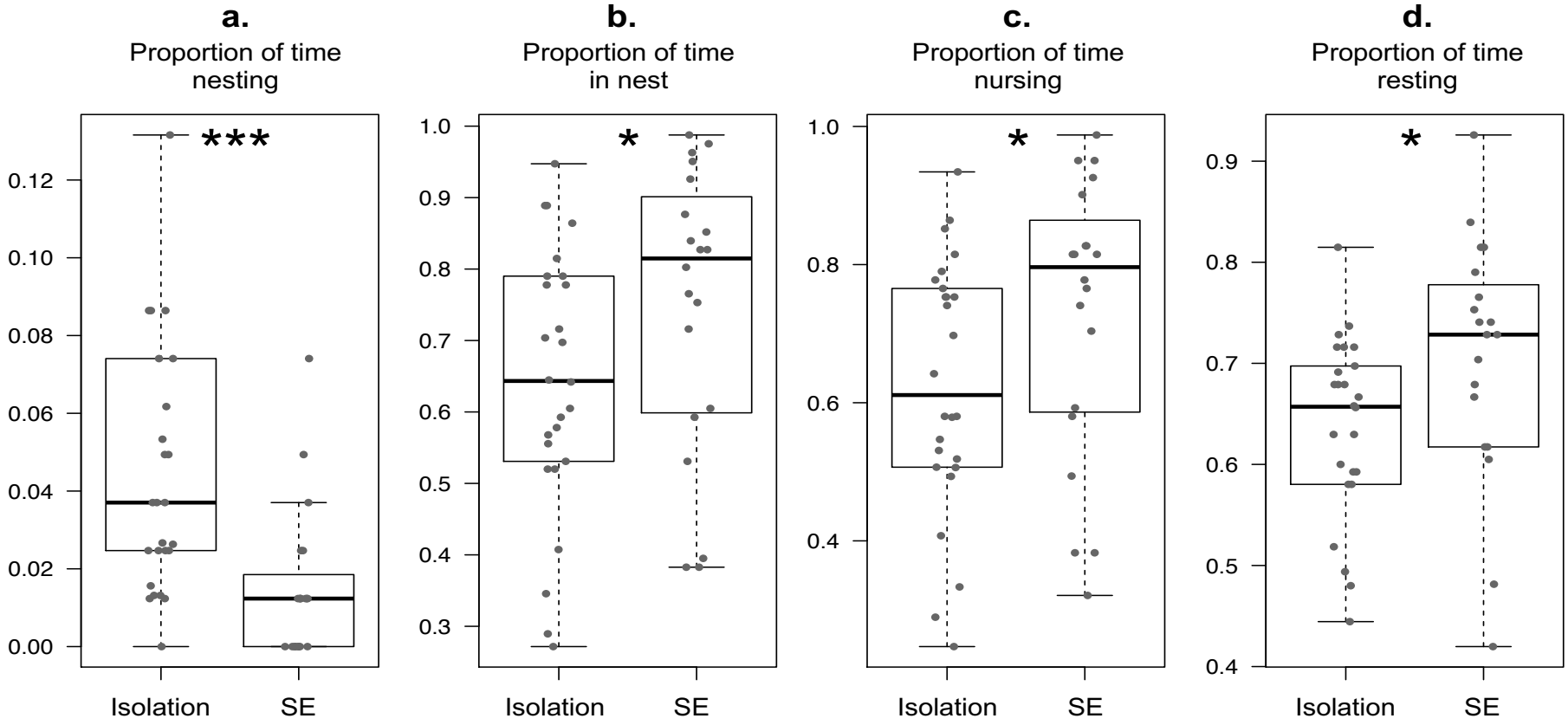

Figure 4. Effects of social exposure on the proportion of time spent nesting (a), in nest (b), nursing (c) and resting (d) at mid-lactation in undisturbed conditions. As compared to isolated females, socially supported females spent less time nesting and more time in nest and nursing. Mann-Whitney U test $*P < 0.05$, $***P < 0.001$. Comparison is between the global populations of isolated and SE mothers since CSS did not influence these behaviors.

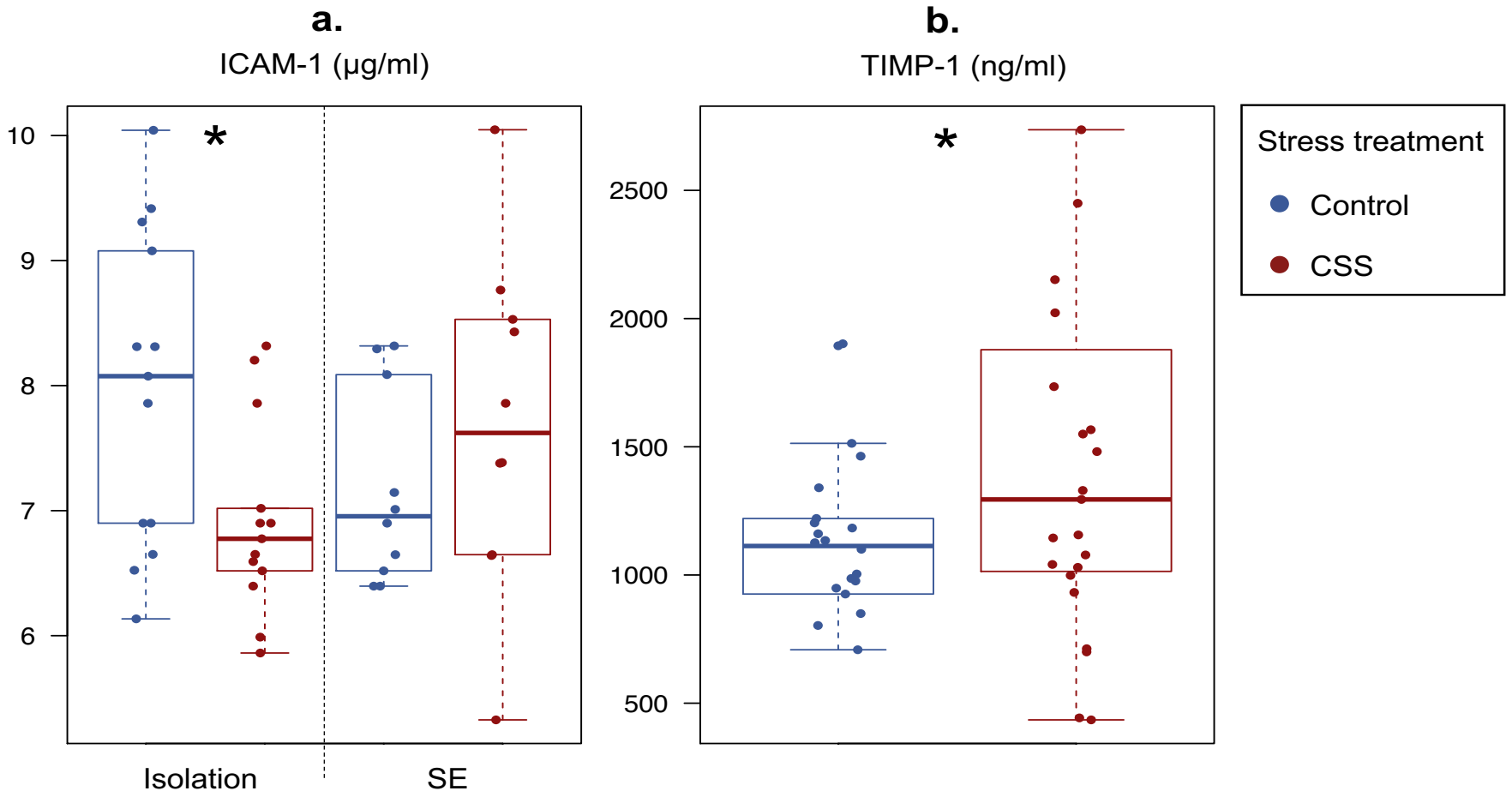

Figure 5. Plasma ICAM-1 levels (µg/ml) based on social stress and social exposure treatments (a) and plasma TIMP-1 level (ng/ml) according to social stress treatment (b). CSS reduces plasma ICAM-1 level in isolated mothers, but not in socially exposed mothers. CSS treatment increases TIMP-1 regardless of social support treatment. Student's T-test *$P < 0.05$.

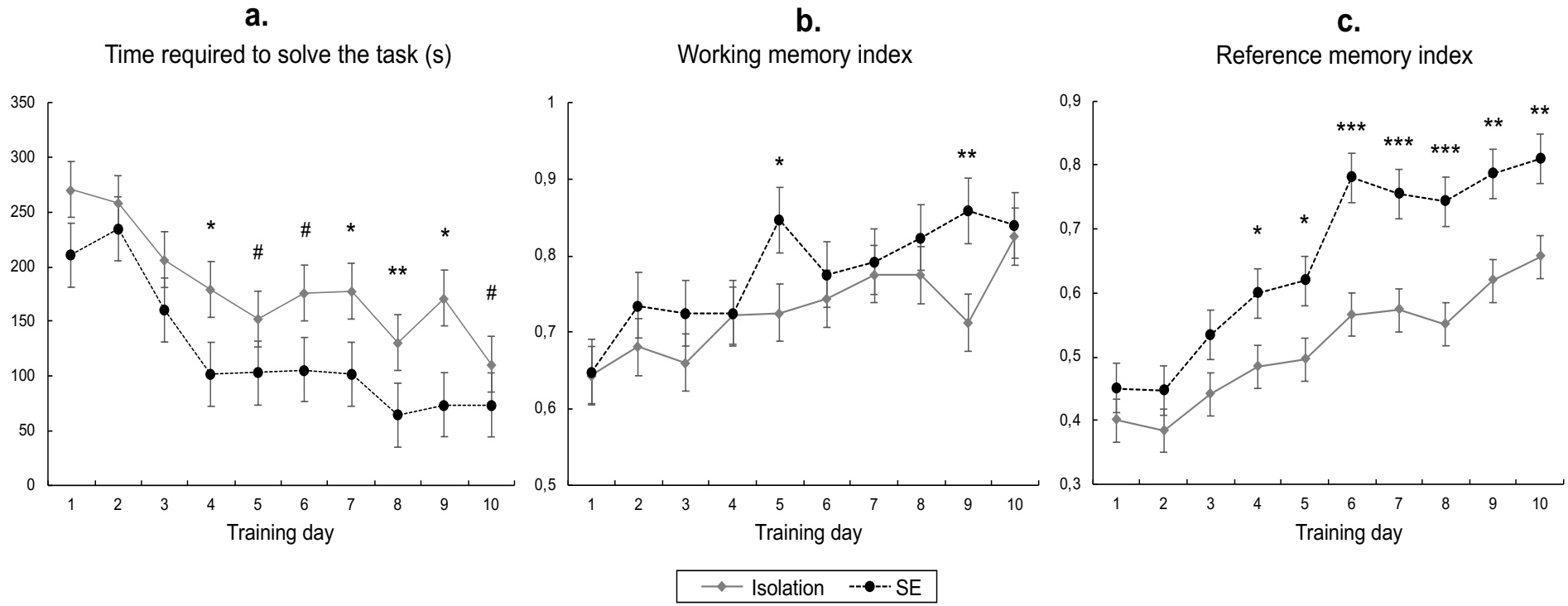

Figure 6. Mean ± SE time required to solve the task (a), working memory (b) and reference memory (c) of F1 juvenile males according to social support treatment of their mothers. Social exposure of the mother improved the general performance of their offspring, their working memory and their reference memory. Student's T-test #p<0.08, *p<0.05, **p<0.005, ***p<0.001.

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Tables

Table 1. Linear models for working memory score.

Initial model

Parameter	Estimate	SE	P
Intercept	0.63	0.02	<0.0001
Social exposure	0.05	0.02	0.03
Training	0.02	0.003	<0.0001
Marginal R^2 = 0.08; Conditional R^2 = 0.13			

Final model with candidate mediators

Parameter	Estimate	SE	P
Intercept	0.7	0.25	<0.0001
Training	0.015	0.003	<0.0001
"Licking grooming"	0.02	0.011	-
"Nesting" score	-0.03	0.013	0.04
BDNF	-0.0002	0.00006	0.02
Marginal R^2 = 0.09; Conditional R^2 = 0.13			

The first model was selected by only including social treatment variables, training and interactions. The final model (bottom) was built by additionally including covariates (maternal care and immune response). (-) indicates marginal *P* value without meaningful interpretation ($P > 0.10$).

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Table 2. Linear models for reference memory score.

Initial model

Parameter	Estimate	SE	P
Intercept	0.43	0.04	<0.0001
Social exposure	0.11	0.05	<0.0001
Training	0.04	0.006	<0.0001
Social exposure*training	0.005	0.008	0.005
Social exposure* CSS * training	-0.02	0.01	0.08
Marginal R^2 = 0.35; Conditional R^2 = 0.43			

Final model with candidate mediators

Parameter	Estimate	SE	P
Intercept	0.61	0.04	<0.0001
Social exposure	0.11	0.02	<0.0001
Training	0.1	0.008	<0.0001
"Nesting score"	-0.03	0.014	0.06
GM-CSF	-0.007	0.004	0.06
TIMP-1	-0.00002	0.00001	-
"Time in nest" score * training	0.02	0.008	0.005
ICAM-1 * training	0.02	0.008	0.004
Marginal R^2 = 0.39; Conditional R^2 = 0.43			

The first model was selected by only including social treatment variables, training and interactions. The final model (bottom) was built by additionally including covariates (maternal care and immune response). (-) indicates marginal *P* value without meaningful interpretation ($P > 0.10$).