Maternal stress and vulnerability to depression: coping and maternal care strategies and its consequences on adolescent offspring

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Depressive mothers often find mother-child interaction to be challenging. Maternal stress may further impair mother-child attachment, which may increase the risk of negative developmental consequences. We used rats with different vulnerability to depressive-like behavior (Wistar and Kyoto) to investigate the impact of stress (maternal separation-MS) on maternal behavior and adolescent offspring cognition. MS in Kyoto dams increased pup-contact, resulting in higher oxytocin levels and lower anxiety-like behavior after weaning, while worsening their adolescent offspring cognitive behavior. Whereas MS in Wistar dams elicited higher quality of pup-directed behavior, increasing brain-derived neurotrophic factor (BDNF) in the offspring, which seems to have prevented a negative impact on cognition. Hypothalamic oxytocin seems to affect the salience of the social environment cues (negatively for Kyoto) leading to different coping strategies. Our findings highlight the importance of contextual and individual factors in the understanding of the oxytocin role in modulating maternal behavior and stress regulatory processes.

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INTRODUCTION
Depressive mothers often display higher difficulty in interacting with their child, increasing the liability of child abuse and negligence [1]. In addition, maternal adverse events cause increased maternal stress, which may translate to a higher rate of depressive mothers, and rise of the worldwide number of children growing up under chronic stress [2]. Thus, maternal stress may have long-lasting effects on mothers, their children, and their families. Despite the importance of vulnerability to depression and its relationship with maternal stress, there is a lack of research combining the impact of such risk factors on mother's behavior and its consequences for the offspring.

In rodents, maternal behavior allows a proper mother/pup attachment, adequate nutrition, and temperature regulation [3]. It includes a complex variety of behaviors and its quality/quantity has been linked to the development of emotional and cognitive processes in the offspring [4]. Maternal behavior is regulated by oxytocin [5], a neuropeptide released from the paraventricular nucleus and the supraoptic nucleus of the hypothalamus [6]. While oxytocin activates brain areas related to maternal behavior, its blockade leads to maternal care deficits [7, 8]. Furthermore, the developing oxytocinergic system is impacted by mother’s absence [9]. The maternal separation paradigm (MS), used as a maternal adverse event, is the most widely used animal model to study early-life stress and disruption of the infant-mother relationship [10]. In a recent systematic review, we found that MS induces changes in dam’s emotional behavior by increasing anxiety and depressive-like symptoms [4], aggression, and impacting on maternal care [11]. MS seems to increase the total time spent in maternal behavior [4, 12], which was suggested to likely represent an attempt to overcompensate for the time off-nest [12]. Under MS, pups increase solicitation calls [13], compelling mothers to increase their maternal behavior as a response to the pup needs [4], which has been associated with increased expression of oxytocin receptors (OXTR) [14].

The existing literature seems to indicate that MS induces changes in the hippocampus of the offspring [15, 16], leading to a maladaptive brain development and cognitive impairment in adulthood [17]. The impact of maternal separation on the hypothalamic-pituitary-adrenal (HPA) axis is well-recognized [18, 19]. However, other factors are increasingly recognized as also relevant in triggering MS-induced behavioral alterations, like anxious and depressive-like behaviors [20]. Early-stress life events, including MS, have been associated with changes in immune activation and seem to affect microglia proliferation, morphology and phagocytic activity [21], priming them to contribute to later
neuropsychiatric disorders [22]. The potential interplay between the immune system and depression, however, was yet barely investigated in MS models.

Psychosocial stressors can lead to increased microglial reactivity in the hippocampus, which may also contribute to cognitive impairment with neuroinflammation as a crucial mediator [23, 24]. Neuroinflammation is also linked to depression [25], primarily by its association with pro-inflammatory cytokines increased expression, such as the tumor necrosis factor (TNF), interleukin (IL)-6 and IL-1β [26–28].

In the present study, we used a depressive-like animal model (Wistar-Kyoto rat strain) combined with the MS paradigm to investigate the interplay between the role of pre-existing vulnerability to depression and environmental/social risk factors. The Wistar-Kyoto (from now on referred as Kyoto) rat strain is a validated animal model of endogenous depression, which allows studying vulnerability to genetic depression [29]. The Kyoto strain was obtained by successive selective breeding resulting in rats that present: (i) excessive immobility in the forced swim test (FST), (ii) reduced activity in the open field (OF), (iii) increased anxiety-like behavior, (iv) behavioral despair, (v) lower social interaction and exploration, and (vi) anhedonia [30, 31]. Upon these behavioral characteristics, Kyoto display alterations in the HPA axis, characterized by decreased corticosterone (CORT)-mediated responses to acute stress and deficits in the serotonergic, dopaminergic and neurotrophic systems, thus exhibiting a behavioral and neurobiological phenotype with well-accepted face validity in depression [30, 32]. Emerging studies suggest also the involvement of neuroinflammation [31]. Thus, we studied the MS impact on maternal care and mother’s anxiety/depressive-like behaviors focusing on the oxytocinergic system and neuroinflammatory processes interactions. We predicted that depressive-like mothers, under maternal stress, would worsen their maternal behavior linked to a reduction in oxytocin. Furthermore, we expected that the mother’s maternal-stress-response impact on the adolescent offspring cognition would be worse in the depressive-like group and that it would be related to the pro-inflammatory cytokine levels.

**METHODS**

**Animals**

Wistar and Kyoto rats were obtained from Charles River (Barcelona/Spain). Rats were kept under standard conditions (21 ± 1°C; 60 ± 5% humidity; 12h/12h light cycle – off at noon) with ad libitum food/water. Pregnant females were individually housed on pregnancy day 16. Animal behavior analyses were performed with the software Observer XT version10 (Noldus information technology, Netherlands) and Smart Video Tracking Software (Panlab) version 3.0, following the timeline in Fig. 1. Brain areas were collected 24 h after behavioral tests. All experiments involving animals were approved by the Portuguese regulatory agency Direcção Geral de Alimentação e Veterinária (DGAV) and the ethics committee of iBMC-i3S. The facility and people directly involved in animal experimentation were certified. All animal experiments followed the European guidelines for animal welfare (2010/63/ EU Directive).

**Maternal separation**

Each litter was sexed and adjusted to 8–10 pups in PND1 (PND0 = delivery day). Dams from each strain were randomly assigned to control (n = 8/strain) or MS group (n = 7/strain). Control group followed animal facility rearing (1 cage change/week). The MS dams were separated from their offspring at 9:00 am, 180 min/day, from PND2-14, following the MS protocol described by Alves et al. [33]. This study used 88 pups and the remaining were used in a different study [33]. Rats were weaned and housed (2-3/cage) at PND21 and 8 groups were established (n = 11/group): control Wistar male; control Wistar female; control Kyoto male; control Kyoto female; MS Wistar male; MS Wistar female; MS Kyoto male; and MS Kyoto female. Each group contained rats from all litters. Pup’s weight was measured at PND2, 12, 33.

**Mothers evaluation**

*Mature behavior*. Maternal behavior toward offspring was scored immediately after pup reunion, at PND 2, 6, 10, 14, for 10 min. Animals were not disturbed during behavioral observations. The following behavior categories were observed: total maternal behavior (affiliative plus non-affiliative behavior); affiliative behavior (grooming/licking and nursing time); non-affiliative behavior (dam in contact with the offspring but not in grooming/licking and nursing).

*Pup retrieval test (PRT)*. At PND4, pups were returned to the original cage after MS away from the nest, followed by the return of the mother. Control group mothers were removed for 3 min and returned, while their pups were moved to the opposite-nest side. The observation ended when mothers retrieved all pups, or the 10 min observation limit was achieved [34]. First-contact latency; first-pup-retrieval latency; and full litter retrieval time were quantified.

*Elevated plus maze (EPM)*. Mother anxiety-like behavior was measured during the MS period (PND 7). The apparatus consisted of four connected arms (44 cm × 14 cm) in a plus-shaped structure and elevated 72 cm above the floor. Two arms were an open platform (no sidewalls) and two were enclosed within 22 cm-high sidewalls. Arms were positioned so opposing arms were of the same type. Each mother was placed in the central platform (the intersection of the four arms - 14 × 14 cm area) facing an open arm and left undisturbed for 5 min. Testing was conducted in the dark phase of the light/dark cycle. The apparatus was cleaned between animals with neutral, odor free soap [35]. The following behaviors were measured: (a) open-arms time; (b) open-arm entries; (c) center-area time; (d) immobility time; (e) total arm entries; (f) rearing; and (g) head dipping.

*Open-field (OF)*. On weaning day (PND21), mothers were tested in the OF to measure anxiety-like symptoms. The apparatus consisted of an empty rectangular arena (80 × 60 cm). Mothers were placed in the corner of the apparatus and left to explore the arena for 10 min. The following behaviors were recorded: (a) center time; (b) center entries; (c) center latency; (d) total distance traveled; and (e) rearing.

*Forced swimming test (FST)*. Mother passive coping behavior was assessed as a depressive-like behavior indicator with the FST at PND22 according to the protocol described by Aguggia et al. [34]. Dams were placed in a transparent container filled with water (25 ± 2°C) for 10 min (pre-test session to reduce acute stress) and tested 24 h later for 5 min in the same container. Swimming, climbing, and immobility duration were recorded.

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**Fig. 1** Schematic timeline summarizing the experimental procedures. PND = Postnatal day.
**Spatial learning and reference memory.** After cued learning, rats were subjected to the spatial learning task (PND39–42, four trials/day). Extra maze cues were placed in the room and the platform was submerged in a fixed location. Latency to reach the platform was recorded. On probe day (PND43), the reference memory was evaluated (1 trial). The platform was removed, and rats were released in the quadrant opposite the platform’s previous location. Rats could swim freely during 30 s. The following measures were quantified: (a) target mean distance; (b) total distance traveled; (c) target quadrant time percentage; (d) target quadrant latency; (e) target crossings.

**Spatial working memory.** At PND44–47, the platform and rat starting positions were the same in each day but changed every day (four trials/day, adapted from Tata et al. [37]). Maze cues were kept as in the spatial learning task. Latency to reach the platform was recorded. Only latency to reach the platform within the day was expected to decrease, but not between days.

**Mothers and offspring biomarkers**

**Protein extract preparation.** Dams were sacrificed at PND24, 24 h after the FST. The adolescent offspring were sacrificed at PND48, 24 h after the spatial working memory test.

For dams, the amygdala (Bregma −1.60 to −2.30) [38], the prefrontal cortex (PFC; Bregma 2.70 to 5.20) [38], and the whole hippocampus were homogenized in a buffer containing 50 mM Tris Base, 10 mM EDTA, 150 mM NaCl, 0.1% Tween 20 and protease inhibitors [39]. The offspring’s hippocampus was homogenized using the same buffer. Samples were sonicated and centrifuged for 15 min at 2271 × g. The supernatant was centrifuged for 5 min at 13500 g. The protein concentration was estimated using Pierce BCA Protein Assay kit (Thermo Scientific), following the manufacturer’s instructions. After centrifugation, total protein concentration was determined in the supernatant using a BCA kit (23227, Thermo Fisher Scientific).

**Enzyme-linked immunosorbent assay (ELISA).** Oxycotin was measured by ELISA (ADI-900-153A, Enzo Life Sciences – Detection range: 15.6 to 1000 pg/ml) in mother’s hypothalamus protein homogenates obtained by dissecting the hypothalamic region between Bregma −0.26 and −1.40 [38], which encompass the medial preoptic area (MPOA), the paraventricular and supraoptic (suprachiasmatic) nuclei. BDNF (mediator of neuronal plasticity [40]) levels were measured in offspring’s hippocampus homogenates by ELISA (CYT306, Millipore). The quantification of IL-1β, IL-6, and TNF in the mother’s hippocampus and of IL-1β and IL-6 in the offspring’s hippocampus were also performed by ELISA (900-M91, 900-M86, 900-M73, PeproTech).

**Western blot.** We determined the expression of (i) OXTR on mother’s PFC, hippocampus (100 μg protein), and amygdala (70 μg protein); (ii) postsynaptic density protein 95 (PSD95, abundant scaffold protein present in the glutamatergic postsynaptic terminal [41]) and vesicular glutamate transporter 1 (vGlut1, a presynaptic transporter protein that mediates glutamate uptake into synaptic vesicles, linked to the efficacy of glutamatergic neurotransmission [42]) on offspring’s hippocampus (using 30 μg protein). Samples were denatured at 95 °C for 5 min and loaded into a 10% SDS-page gel, transferred to a nitrocellulose membrane, processed with blocking solution, and incubated with primary antibody overnight, at 4°C (OXTR, 1:400 (Thermo Fisher Scientific); PSD95, 1:2000 (ThermoFisher Scientific); vGlut1, 1:1000 (Synaptic Systems); GADPH, 1:100,000 (HyTest). Primary antibodies were visualized with HRP-conjugated anti-rabbit or HRP-conjugated anti-mouse (1:10,000, Jackson ImmunoResearch). Signals were enhanced using SuperSignal™ West Pico PLUS Chemiluminescent Substrate (ThermoFisher Scientific). Data was quantified using ImageJ software [43].

**RESULTS**

**Mothers**

Wistar dams presented higher-quality maternal behavior than Kyoto dams in response to MS. MS impact on mothers and their offspring occurs due to the separation period but more importantly, due to changes in their interaction afterward. Thus, we started by investigating the MS effect on maternal behavior. Total maternal behavior was higher in both MS groups ($F(1,26) = 15.7, p < 0.001$). MS dams spent more time in maternal behavior than control dams of the same strain (Wistar: $p < 0.01$; Kyoto: $p < 0.05$) (Fig. 2A). Within Wistar dams, affiliative behavior was higher in MS group ($p < 0.01$), but not for Kyoto (Fig. 2B). In contrast, MS Kyoto dams had more non-affiliative contact than their control ($p < 0.001$) and MS Wistar ($p < 0.001$) (Fig. 2C). The MS impact on non-affiliative contact was strain-dependent (Strain X MS: $F(1,26) = 4.95, p < 0.001$; MS: $F(1,26) = 4.95, p < 0.05$). Pup weight was only affected by strain ($p < 0.001$) (Fig. 2D). We also performed the Pup Retrieval Test (PRT), revealing that Kyoto dams present a higher latency to the first mother/pup contact (Strain: $F(1,26) = 4.55, p < 0.05$) (Fig. 2E). However, the first retrieval latency and the total retrieval time had no differences (Fig. 2F, G). Taken together, control Wistar and Kyoto dams spent similar time in maternal behavior. However, under MS conditions, the strains increased differently the time spent in maternal behavior, with higher affiliative behavior for Wistar and higher non-affiliative contact for Kyoto.

**Increased exploration in Wistar dams during the MS period and decreased anxiety-like behavior in Kyoto dams after the weaning period.** We hypothesize that an animal that experienced maternal-parental stress is expected to alter its emotional state, and thus, mothers’ exploratory and anxiety-like behaviors were also assessed. The EPM was conducted during the MS protocol period while the OF was conducted after weaning. In the EPM, no strain or MS differences were found for the time or entries in the open arms (Fig. 3A, B). However, Kyoto dams spent more time in the EPM center than Wistar, independently of MS (Strain: $\chi^2 = 16.6, p < 0.001$) (Fig. 3C) and that the Kyoto increased the total arm entries due to MS (MS: $F(1,26) = 7.55, p < 0.05$) (Fig. 3D). In contrast, Wistar dams displayed more rearing (Strain: $F(1,26) = 50.4, p < 0.001$, MS: $F(1,26) = 4.22, p < 0.05$), with MS further increasing the Wistar rearing ($p < 0.05$) (Fig. 3E). Head dipping followed the same pattern, with Wistar dams performing more head dipping (Strain X MS: $\chi^2 = 7.32, p < 0.01$; Strain: $\chi^2 = 10.2$, $p < 0.001$).
Maternal Behaviour Observations

Fig. 2 Maternal separation impacts maternal behavior of mothers with different vulnerabilities to depression. A MS increased maternal behavior in both strains. However, Wistar MS dams increased B affiliative behavior (high-quality behaviors) and Kyoto MS dams increased C non-affiliative contact time. D Wistar showed higher weight gain than Kyoto strain, but it was not impacted by MS. Data were analyzed by two-way ANOVA (2 × 2 factorial design) with Bonferroni comparisons, except for D which was analyzed with a linear mixed model (strain and MS as fixed effects and litter as random effect). E Latency to first contact was higher in the Kyoto strain. 

After weaning, exploratory and anxiety-like behaviors were studied using the OF (Fig. 3G). Kyoto dams spent more time in the center (Strain X MS: \( F_{(1,26)} = 5.07, p < 0.05 \); Strain: \( F_{(1,26)} = 12.1, p < 0.01 \)), with MS Kyoto dams spending more time in the center area than the control Kyoto (\( p < 0.05 \)) and MS Wistar dams (\( p \leq 0.001 \)) (Fig. 3H). Total distance was similar in all groups (Fig. 3I) and the exploratory behavior, assessed as rearing occurrence, was different between the two strains (Strain: \( F_{(1,26)} = 11.4, p < 0.01 \)). Kyoto dams displayed fewer rearing, yet MS Kyoto dams performed more rearing than their control (\( p < 0.05 \)) (Fig. 3J).

Altogether, the results obtained in the EPM suggest that anxiety-like behavior was not affected during the MS protocol. However, Wistar dams displayed more exploratory behaviors than Kyoto dams and MS further increased exploration in Wistar. The OF results suggest that Kyoto dams present less anxiety-like behavior after weaning than the Wistar and MS resulted in less anxiety-like behavior in the Kyoto dams. Although MS increased exploration only in Kyoto, Wistar dams displayed more exploratory behavior.

**MS did not influence depressive-like behavior in dams.** As expected due to their strain characteristics, Kyoto dams spent more time immobile in comparison with Wistar dams in the FST (\( F_{(1,26)} = 36.3, p < 0.001 \)) (Fig. 3K). Consistently, Kyoto dams also spent less time climbing (\( F_{(1,26)} = 43.5, p < 0.001 \)) (Fig. 3L). Kyoto dams presented more depressive-like behaviors than Wistar, with no observed effect from MS, which may be due to a ceiling effect since the Kyoto control already spent most of the test immobile.

**MS increased oxytocin expression in the hypothalamus of Kyoto dams.** Since maternal behavior is strongly influenced by
oxytocin, we analyzed the expression of oxytocin in the hypothalamus of dams. Surprisingly, MS Kyoto dams presented higher oxytocin expression (Strain X MS: $F_{(1,16)} = 6.60, p < 0.05$) (Fig. 4A). Because altered levels of oxytocin may result in altered expression of OXTR in oxytocin projection regions, we evaluated the expression of this receptor in oxytocin target regions that are recognized as relevant for regulation of maternal behavior, such as the PFC, the hippocampus, and
Fig. 3 Maternal separation affects mothers’ emotional behavior. During MS period (using EPM), A, B no differences were found regarding anxiety. MS increased D Kyoto total arm entries and increased Wistar F number of rearing and F number of head dipping. G example of Wistar and Kyoto control groups tracking in OF (Smart v3.0, Panlab, Barcelona, Spain; https://www.panlab.com/en/). After weaning (using OF), MS increased Kyoto H time in center area and J number of rearing, indicating decreased anxiety levels. CF confirmed Kyoto depressive profile since Kyoto strain showed higher time K immobility and lower L climbing comparing with Wistar. MS did not impact depression-like behaviors. Data were analyzed by two-way ANOVA (2x2 factorial design) with Bonferroni comparisons. A, C, H, K, L Data were arc sine transformed. B, D, E, F Data were log-transformed. I, J Data were square-root transformed. n = 8 (control and MS Kyoto). Results are expressed as mean ± SEM. *p <0.05, **p < 0.01, ***p < 0.001. MS = maternal separation.

the amygdala. No differences were observed in the OXTR expression (Fig. 4B–D).

Kyoto dams present higher TNF in the hippocampus than Wistar dams. As pro-inflammatory cytokines can influence anxiety- and depressive-like behaviors, we analyzed the expression of TNF, IL-6, and IL-1β in the hippocampus. Kyoto dams presented higher expression of TNF (Wald χ² = 18.3, p <0.001) (Fig. 4E). Concerning IL-6 (Fig. 4F) and IL-1β (Fig. 4G) no significant differences were observed.

Offspring
MS worsened the spatial learning and the reference memory in Kyoto adolescents. We evaluated the offspring cognitive ability using the MWM. Kyoto adolescents displayed reduced spatial learning when compared to the Wistar group (represented by a reduction in latency over the learning days) (Fig. 5A). MS Kyoto performed poorly than the Kyoto, with MS Kyoto female adolescents presenting longer latency to the target (Strain Chi-Square(1) = 157.26, p <0.0001, MS:Chi-Square(1) = 10.12, p <0.01, Strain X MS:Chi-Square(1) = 11.59, p <0.01, Sex: Chi-Square(1) = 6.11, p <0.05, Strain X Sex: Chi-Square(1) = 5.28, p <0.05, Strain X Days:Chi-Square(1) = 57.78, p <0.0001) (Fig. 5A). During the probe test Kyoto performed poorly than Wistar, and a main sex effect was only observed for the total traveled distance (Fig. 5C-G). The mean distance to the target was higher for the Kyoto strain and MS worsened both strains (Strain, Chi-Square(1) = 60.85, p <0.0001; MS, Chi-Square(1) = 7.74, p <0.01) (Fig. 5C). Kyoto adolescents also spent less time in the target quadrant (strain, Chi-Square(1) = 246.00, p <0.0001), with the MS Kyoto group spending even less time in that quadrant (Strain X MS Chi-Square(1) = 20.64, p <0.0001; z value = 4.19, p <0.0001) (Fig. 5D). Regarding the latency to enter the target quadrant, Kyoto presented higher latency to enter it (Chi-Square(1) = 387.79, p <0.0001), with the MS Kyoto showing even higher latency (Strain X MS Chi-Square(1) = 7.81, p <0.01; z value = −4.16, p <0.0001) (Fig. 5E). A strain effect was also observed for target crossings, which were decreased in Kyoto adolescents (Chi-Square(1) = 38.72, p <0.0001) (Fig. 5F). For total traveled distance we found main effects of strain, MS and sex, plus interactions between them (Strain: Chi-Square(1) = 6772.47, p <0.0001, MS:Chi-Square(1) = 458.24, p <0.0001, Sex: Chi-Square(1) = 105.69, p <0.0001, Strain X Sex: Chi-Square(1) = 6.64, p <0.01, MS X Sex: Chi-Square(1) = 5.55, p < 0.05; Strain X MS X Sex Chi-Square(1) = 133.54, p <0.0001), with the Kyoto strain traveling a shorter distance, and with MS groups traveling less than their respective controls (Wistar vs MS Wistar, z value = 6.41, p <0.0001; Kyoto vs MS Kyoto, z value = 21.26, p <0.0001). Upon that, we also found sex differences within both strains (Male Wistar vs Female Wistar, z value = −11.69, p <0.0001; Male Kyoto vs Female Kyoto, zvalue = −6.60, p < 0.0001), and between MS within each strain, with males traveling less than females (Control Male Wistar vs Control Female Wistar, z value = −14.48, p <0.0001; MS Male Wistar vs MS Female Wistar, z value = −2.26, p = 0.0318; Control Male Kyoto vs Control Female Kyoto, z value = −2.95, p = 0.0634; MS Male Kyoto vs MS Female Kyoto, z value = −8.42, p <0.0001) (Fig. 5G).

Under the work memory protocol, the Kyoto presented a worse performance, with MS-induced changes resulting in an even poor performance (Strain: Chi-Square(1) = 357.68, p <0.0001, MS: Chi-Square(1) = 21.47, p <0.0001, Trial Chi-Square(1) = 146.64, p <0.0001; Strain X MS: Chi-Square(1) = 10.81, p <0.0001; Strain X Trial: Chi-Square(1) = 68.71, p <0.0001; Strain X MS X Trial: Chi-Square(1) = 8.72, p <0.05) (Fig. 5H). For this test we also found sex differences for the Kyoto strain, where MS males performed better than MS females, independently of the trial (Sex, Chi-Square(1) = 14.93, p <0.0001, Strain X Sex: Chi-Square(1) = 8.40, p <0.01, MS X Sex: Chi-Square(1) = 9.39, p <0.01; Strain X MS X Sex, Chi-Square(1) = 20.36, p <0.0001; MS Male Kyoto vs MS Female Kyoto, z value = 7.24, p <0.0001) (Fig. 5H).

Altogether, we observed that Kyoto adolescents presented reduced spatial learning, reference and working memory in comparison with age-matched Wistar, with MS worsening their performance, particularly in females.

Kyoto adolescents presented higher levels of vGlut1 expression. To better understand the observed learning and memory deficits, we analyzed the expression of two different glutamatergic synaptic markers the postsynaptic density protein 95 (PSD95), which is a well-characterized and abundant scaffold protein present in the glutamatergic postsynaptic terminal, and the vesicular glutamate transporter 1 (vGlut1) that is a presynaptic transporter protein, mediating glutamate uptake into synaptic vesicles, the expression levels of these transporter determines the vesicle load in glutamate, shaping the efficacy of glutamatergic neurotransmission.

For PSD95 hippocampal expression levels, no differences between strains, or resulting from MS were observed (Fig. 5I). Regarding the expression levels of vGlut1, we found only strain-related differences, with Kyoto adolescents presenting increased vGlut1 levels (Strain, Chi-Square(1) = 4.23, p < 0.05) (Fig. 5J). These changes in vGlut1 may be associated with strain differences reported for the MWM assessments.

MS increased brain-derived neurotrophic factor (BDNF) expression in Wistar but not in Kyoto adolescents. BDNF is a crucial mediator of neuronal plasticity and a key factor for synapse formation/maintenance during development, as well as for memory processes. BDNF expression was impacted by MS (Chi-Square(1) = 16.26, p <0.0001) and an interaction between Strain and MS (Chi-Square(1) = 14.37, p <0.0001), which translates in MS-induced increase in BDNF in Wistar adolescents when compared with their control (z-value = −5.93, p <0.001) (Fig. 5K). This BDNF increase was previously linked to early aversive experiences that may trigger adaptive processes, allowing better adaptation to later events [44]. However, it is also possible that the increase in BDNF may result from improved maternal care (Fig. 2).

MS decreased IL-1β expression in Kyoto adolescents. Because BDNF levels may associate with altered inflammatory markers, and pro-inflammatory cytokines can influence learning, memory and shape the neuronal circuit, we decided to evaluate IL-6 and IL-1β expression. Kyoto adolescents presented lower IL-6 levels (strain: Chi-Square(1) = 19.44, p <0.0001), which were further decreased by MS in females (MS X Sex: Chi-Square(1) = 4.65, p < 0.05, z-value = 2.88, p < 0.05) (Fig. 5L). For IL-1β, we found differences due to strain, MS and sex M (Strain, Chi-Square(1) = 28.35, p
pups, performing more affiliative behavior, in both strains, in response to MS. Interestingly, each strain displayed different strategies for handling maternal stress: males (Wistar) spent more time directing their behavior towards nursing. These affiliative behaviors are the most common pup-oriented maternal behaviors [12] and they crucial for mother-pup attachment [47]. In contrast, during simple physical contact (skin contact), mothers lie over their pups, but without offspring-oriented behavior. The increased contact of Kyoto dams with offspring suggests that the mother keeps the offspring close, while keeping a watch on the environment around her, likely because she does not know when the pups will be taken from her again. This behavior in depressive-like rat dams seems to be a form of maternal defense to protect pups from unpredictable stress (MS). In fact, contact behavior was previously reported as an alternative maternal defense behavior towards pups when a threat was present [48]. These differences in maternal behavior seem to be due to distinct maternal experiences and/or distinct internal states of the dams. Our results showed that maternal depressive-like traits played an important role regarding how each dam adjusted its maternal behavior in response to maternal stress. In line with these results, a recent study, in humans, showed that mothers with personality disordered traits do not perceive themselves as having bonding impairments with their infants, although they are less sensitive during interactions [49]. This highlights the relevance of genetic components in the expression of maternal behavior, but further studies are needed to elucidate gene-environment interactions in depression/maternal behavior.

DISCUSSION AND CONCLUSION

This study explored the impact of MS on the maternal behavior of dams with different vulnerabilities to depressive-like behavior and explored its consequences on adolescent cognitive performance. Considering the high comorbidity between mothers’ depression and low maternal care [1], one could predicted that MS would reduce maternal behavior in depressive-like Kyoto mothers. However, confirming the results reported in publications that reviewed this issue [4, 46], we observed an increase in maternal behavior, in both strains, in response to MS. Interestingly, each strain displayed different strategies for handling maternal stress: depressive-like mothers (Kyoto) spent more time in contact (simple physical contact) with pups, whereas non-depressive mothers (Wistar) spent more time directing their behavior towards pups, performing more affiliative behaviors (licking, grooming and nursing). These affiliative behaviors are the most common pup-oriented maternal behaviors [12] and they crucial for mother-pup attachment [47]. In contrast, during simple physical contact (skin contact), mothers lie over their pups, but without offspring-oriented behavior. The increased contact of Kyoto dams with offspring suggests that the mother keeps the offspring close, while keeping a watch on the environment around her, likely because she does not know when the pups will be taken from her again. This behavior in depressive-like rat dams seems to be a form of maternal defense to protect pups from unpredictable stress (MS). In fact, contact behavior was previously reported as an alternative maternal defense behavior towards pups when a threat was present [48]. These differences in maternal behavior seem to be due to distinct maternal experiences and/or distinct internal states of the dams. Our results showed that maternal depressive-like traits played an important role regarding how each dam adjusted its maternal behavior in response to maternal stress. In line with these results, a recent study, in humans, showed that mothers with personality disordered traits do not perceive themselves as having bonding impairments with their infants, although they are less sensitive during interactions [49]. This highlights the relevance of genetic components in the expression of maternal behavior, but further studies are needed to elucidate gene-environment interactions in depression/maternal behavior.

Of note, MS-related differences in maternal behavior did not impact pups’ weight gain (a measure of growth efficiency), which further supports a previous study that did not find differences between Wistar and Kyoto offspring’s physical development after MS [50]. It was also reported that offspring of low-licking mothers do not differ in weaning weight and survival rate [51]. Thus, even though the Kyoto maternal behavior appears to lack quality, these mothers exhibit awareness towards their pups and do not compromise the offspring’s physical health.

Maternal care variations are associated with differences in oxytocin and OXTR expression in the brain [52]. However, we did not find changes in OXTR in the analyzed regions, but we showed, for the first time, that when a depressive-like dam is exposed to maternal stress (MS), the hypothalamic expression of oxytocin is
augmented. In accordance, previous findings reported that skin contact between mother and pups, but not maternal licking or grooming, was positively related to pups’ hypothalamic oxytocin [53, 54]. A similar mechanism can be happening in mothers and help explaining our observations. Oxytocin release seems to promote social cohesion in the mother-offspring dyad, serving as a defensive behavior toward potential and unpredictable threats. Considering the social salience hypothesis of oxytocin [55], the
oxytocin increase in depressive mothers could enhance social sensibility [56] to negative social cues (offspring absence), eliciting a more protective behavior from depressive mothers, as a defense against a potentially dangerous intruder. The mechanism by which oxytocin affects social behavior depends both on contextual and individual factors [57], which seems to influence the sensitivity and interpretation of the emotional meaning or relevance of an event.

In the present study, MS Kyoto dams, in combination with higher levels of hypothalamic oxytocin, displayed decreased anxiety-like behavior decrease in the OF and increased arm entries in the EPM, suggesting exploratory behavior disinhibition, likely because of lower anxiety. This outcome supports reports indicating an oxytocin anxiolytic effect [58, 59] and an oxytocin increase in response to conditioned fear and restraint [60, 61]. In agreement, Light and colleagues (2004) [62] suggested that oxytocin release during stressful situations alleviates physiological stress. The decreased anxiety-like behavior observed in depressive-like mothers can be a result of increased oxytocin. Of note, according to a recent publication, that reported that more resilient rats (as the Wistar strain) no longer display FST effects in corticosterone, BDNF or IL-1β levels, 24 h after the test, while more susceptible rats (such as the Kyoto strain) still display increased corticosterone and IL-1β at the same time point, is also possible that the FST performed before dams’ sacrifice, may somehow contribute to differences in oxytocin [63].

Data from human studies support that, under great psychosocial stress, mothers with higher oxytocin levels present fewer anxiety symptoms, suggesting that oxytocin may protect women in stressful situations from developing major depression [64, 65]. Moreover, human studies share a common assumption that mothers with higher oxytocin levels present fewer depressive symptoms, compared to mothers with lower oxytocin levels, and this supports a model where low oxytocin levels represent a causal effect of Postpartum Depression (PPD) rather than a consequence [66]. However, there are also inconsistent findings in this issue, that may be due to discrepancies in sample size, differences in assessment timepoints and in the methodologies used to measure both oxytocin and depressive symptoms. Our and other studies [67] are beginning to shed light on the complex nature of oxytocin’s effect in depression, bringing a new perspective in which the oxytocin’s role is dependent on the individual experience, such as lack of social support and teenage parenting, or single parenthood. In this context, the role of oxytocin on PPD needs further investigation.

Wistar mothers increased licking, grooming, and nursing behaviors due to MS, with no impact on the oxytocinergic system. These results suggest that Wistar dams increased pro-social behavior to counteract MS effects, exhibiting a more positive emotional regulation in coping with maternal stress. These dams did not reveal changes in anxiety-like behavior due to MS, but interestingly increased exploration (represented by rearing and head dipping). Moreover, the lack of increased oxytocin expression in the hypothalamus of MS Wistar mothers corroborates the idea that Wistar mothers were more emotionally regulated, given that increased oxytocin levels in the hypothalamus are part of the physiological response to social stress [62]. Of note, attenuation of the anxiety response by oxytocin occurs in individuals with poor coping, but not in individuals with adequate coping [68, 69], which is in accordance with our observations for the Kyoto and Wistar strains.

In the absence of other comorbidities, depressive disorders are related with increased levels of in central and peripheral pro-inflammatory cytokines, particularly with TNF [26]. In agreement, TNF was higher in Kyoto dams than in Wistar. Studies report that oxytocin reduces TNF production, inducing an anti-inflammatory state, however, the mechanisms involved are elusive and in vitro results seem to indicate that oxytocin does not directly regulate TNF [70]. Maternal care critically affects offspring’s brain maturation and cognitive and emotional behaviors development in mammalian species [71]. We observed that depressive-like adolescents (Kyoto) had significant learning and memory deficits. MS had higher impact in the depressive-like offspring, which suggests that MS effects on cognition depend on the interaction of both genetic (depressive trait) and environmental factors (maternal care quality and early-life stress, here represented by MS), which may explain the smaller MS impact on cognitive performance of non-depressive-like offspring raised with more licking and grooming. It is also important to note that the learning deficits observed in Kyoto exposed to MS were more pronounced in females, while working memory deficits were more pronounced in males. Considering the major role of synaptic proteins in learning and memory, the observed higher vGlut1 expression in Kyoto adolescents, seems to be consistent with the memory deficits shown in the behavioral test. Our data highlight the relevance of vGlut1 in memory and reinforces previous studies [72]. On the other hand, Wistar adolescents presented increased BDNF in response to MS, which could had a protective role, preventing possible MS-induced learning deficits. Besides, high levels of licking and grooming have been associated with higher offspring BDNF levels [73], as such, the lack of this affiliative maternal behavior may contribute to explain the worse cognition observed in MS Kyoto adolescents.

Pro-inflammatory cytokines are critical factors for memory and cognition. Notably, IL-6 is important for spatial learning and reference memory formation [74, 75]. In agreement, the worse cognitive performance of the Kyoto adolescents was associated with reduced IL-6 expression. In agreement, the observed sex difference in learning in the Kyoto strain (MS Kyoto females presented a worse learning performance) was also associated with a decrease in IL-6. Further studies will be necessary to determine the nature of such effect. Adequate levels of IL-1β are required for learning and memory processes [76]. IL-1β expression decreased in the hippocampus of MS Kyoto adolescents, particularly in females, which performed poorly in the MWM. This is in accordance with previous studies, showing poorer performances in mice lacking the IL-1 receptor [45] or after the IL-1 receptor antagonist (IL-1ra) administration [77].

In summary, our study shows that beyond pro-social behaviors, oxytocin is involved in stress and mood disorder regulation (Fig. 6). We demonstrated that when a depressive mother is...
exposed to maternal stress, the release of endogenous hypotha-
lamic oxytocin is triggered and leads to an altered perception of
the environment (as insecure). Our study, in combination with
previous studies [53, 54, 78], suggests that oxytocin not only
increases sensitivity to environmental cues, but it may also amplify
the social stress communication contributing to a social synchrony
bidirectional intensification. This social synchrony in an adverse
situation may contribute to better interactions, enabling the
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nement of social communication and consequently promoting
social cohesion as a defensive behavior toward potential threats.
Finally, the different maternal stress-coping styles affect the
offspring’s behavior, providing important clues about how early-
life stress increases the risk for poor cognitive performances and
how maternal care is a possible target for intervention.

REFERENCES
1. Righetti-Veltema M, Conne-Perreard E, Bousquet A, Manzano J. Postpartum
depression and mother-infant relationship at 3 months old. J Affect Disord.
2002;70:291–306.
2. Vedam S, Stoll K, Rubashkin N, Martin K, Miller-Vedam Z, Hayes-Klein H, et al. The
Mothers on Respect (MOR) index: measuring quality, safety, and human rights in
childbirth. SSM - Popul Health. 2017;3:201–10.
3. Fleming AS, O’Day DH, Kraemer GW. Neurobiology of mother–infant interactions:
experience and central nervous system plasticity across development and gener-
ations. Neurosci Biobehav Rev. 1999;23:673–85.
4. Alves RL, Portugal CC, Summaville T, Barbosa F, Magalhaes A. Maternal
separation effects on mother rodents’ behavior: a systematic review. Neurosci
Biobehav Rev. 2020;117:98–109.
5. Insel TR, Young LJ. The neurobiology of attachment. Nat Rev Neurosci.
2001;2:129–36.
6. Meyer-Lindenberg A, Domes G, Kirsch P, Heinrichs M. Oxytocin and vasopressin
in the human brain: social neuropeptides for translational medicine. Nat Rev
Neurosci. 2011;12:524–38.
7. Pedersen CA, Boccia ML. Oxytocin antagonism alters rat dams’ oral grooming
and upright posturing over pups. Physiol Behav. 2003;80:233–41.
8. Ferris CF. Functional magnetic resonance imaging and the neurobiology of
vasopressin and oxytocin. In: Neumann ID, Landgraf R, editors. Progress in Brain
Research, Vol. 170. Elsevier; 2008, p. 305–20.
9. Amini-Khoei H, Mohammadi-Asl A, Amiri S, Hosseini M-J, Momeny M, Hassani-
pour M, et al. Oxytocin mitigated the depressive-like behaviors of maternal
separation stress through modulating mitochondrial function and neuroin-
flammation. Prog Neuro-Psychopharmacol Biol Psychiatry. 2017;76:169–78.
10. Newport DJ, Stove ZN, Nemeroff CB. Parental depression: animal models of an
adverse life event. Am J Psychiatry. 2002;159:1265–83.
11. Mundorf A, Bölükbas I, Freund N. Maternal separation: does it hold the
potential to model consequences of postpartum depression? Dev Psychobiol.
2022;64:e22219.
11. Orso R, Creutzberg KC, Wearick-Silva LE, Wendt Viola T, Tractenberg SG, Benetti F, et al. How early life stress impact maternal care: a systematic review of rodent studies. Front Behav Neurosci. 2019;13:197.

12. Kaidbey JH, Ranger M, Myers MM, Anwar M, Ludwig RJ, Schulz AM, et al. Early life maternal separation and maternal behaviour modulate acoustic characteristics of rat pup ultrasonic vocalizations. Sci Rep. 2019;9:19012.

13. Champagne F, Dion J, Sharma S, Meany MJ. Naturally occurring variations in maternal behavior that is reversed by recombinant IL-10. PLoS ONE. 2013;8:e58488.

14. Champagne F, Dion J, Sharma S, Meany MJ. Naturally occurring variations in maternal behavior that is reversed by recombinant IL-10. PLoS ONE. 2013;8:e58488.

15.抑うつ症状を改善するための環境因子的有效性。Biopsychosocial Med. 2018;5:100.

16. Alves RL, Oliveira P, Lopes IM, Portugal CC, Alves CJ, Barbosa F, et al. Early-life stress affects drug abuse susceptibility in adolescent rat model independently of mother-pup interaction. Sci Rep. 2019;9:19012.

17. Ruiz-Sanchez E, Lopez-Ramirez AM, Ruiz-Chow A, Calvillo M, Resendiz-Albornoz A, Anguiano B, et al. Variability in behavioral phenotypes after forced swim stress: importance of maternal care. Neurosci Biobehav Rev. 2020;105:1.

18. Björklund A, Lindvall O, Hokfelt T, Svenningsson P, Schousboe A. Oxytocin in central amygdala allows alternative defensive behaviors and mother-pup interaction. Proc Natl Acad Sci USA. 2001;98:12736.

19. Holubová A, Lukášková I, Tomášová N, Suhádová M, Šlamberová R. Early postnatal stress impacts cognitive functions of male rats persisting until adulthood. Front Behav Neurosci. 2018;12:176.

20. Roque S, Mesquita AR, Palha JA, Sousa N, Correia-Neves M. The behavioral and endocrine responses to stress: role of the postnatal environment. 2011. In Comprehensive Physiological (ed. R.Terjung), pp. 271–292. Hoboken, NJ: John Wiley & Sons, Inc.

21. Gehrad AL, Hoyneck LB, Jablonis MR, Leonovicz C, Cullinan WE, Raff H. Programming of the adult HPA axis after neonatal separation and environmental stress in male and female rats. Endocrinology. 2018;159:2777-89.

22. Roque S, Mesquita AR, Palha JA, Sousa N, Correia-Neves M. The behavioral and immunological impact of maternal separation: a matter of timing. Front Behav Neurosci. 2014;8:192.

23. Calderi C, Liu D, Sharma S, Diorio J, Francis D, Meaney MJ, et al. Development of maternal separation model and the implications for mental illness. Psychopharmacology. 2016;233:1637.

24. Champagne F, Diorio J, Sharma S, Meany MJ. Naturally occurring variations in maternal behavior that is reversed by recombinant IL-10. PLoS ONE. 2013;8:e58488.

25. champagne FA, Francis DD, Mar A, Meaney MJ. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav. 2003;79:359-71.

26. Rilling JK, Young LJ. The biology of maternal parenting and its effect on offspring social development. Science. 2014;345:771-7.

27. Alves RL, Oliveira P, Lopes IM, Portugal CC, Alves CJ, Barbosa F, et al. Early-life stress affects drug abuse susceptibility in adolescent rat model independently of mother-pup interaction. Sci Rep. 2019;9:19012.

28. Champagne FA, Francis DD, Mar A, Meaney MJ. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav. 2003;79:359-71.

29. champagne FA, Francis DD, Mar A, Meaney MJ. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav. 2003;79:359-71.

30. Will CC, Aird F, Rodell EE. Selectively bred Wistar-Kyoto rats: an animal model of depression and hyper-responsiveness to antidepressants. Mol Psychiatry. 2003;8:925-32.

31. Millard SJ, Weston-Green K, Newell KA. The Wistar-Kyoto rat model of endo-membrane transport and the implications for mental illness. Psychopharmacology. 2016;233:1637.

32. Bayer L, Davis AM, Auger AP, Doris DM, McCarthy MM. CNS region-specific oxytocin receptor expression: importance in regulation of anxiety and sex behavior. J Neurosci. 2001;21:2154-52.

33. Ring RH, Malberg JE, Potestio L, Ping J, Bolkes S, Luo B, et al. Anxiolytic-like activity of oxytocin in male mice: behavioral and autonomic evidence, therapeutic implications. Psychopharmacology. 2006;185:218-25.

34. Onaka T. Neural pathways controlling central and peripheral oxytocin release during stress. J Neuroendocrinol. 2004;16:308-12.

35. Champagne FA, Francis DD, Mar A, Meaney MJ. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav. 2003;79:359-71.

36.icaidbe JH, Ranger M, Myers MM, Anwar M, Ludwig RJ, Schulz AM, et al. Early life maternal separation and maternal behaviour modulate acoustic characteristics of rat pup ultrasonic vocalizations. Sci Rep. 2019;9:19012.

37. Cattane C, Gironda S, Lo Iacono L, Carola V. Microglial function in the effects of early life stress perturbs the function of microglia in the hippocampus. Neurosci Lett. 2015;584:146.

38. Championships of the Wistar-Kyoto rat model of endo-membrane transport and the implications for mental illness. Psychopharmacology. 2016;233:1637.

39. Champagne FA, Francis DD, Mar A, Meaney MJ. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav. 2003;79:359-71.

40. Champagne FA, Francis DD, Mar A, Meaney MJ. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav. 2003;79:359-71.
65. Zelkowitz P, Gold I, Feeley N, Hayton B, Carter CS, Tulandi T, et al. Psychosocial stress moderates the relationships between oxytocin, perinatal depression, and maternal behavior. Horm Behav. 2014;66:351–60.

66. Moura D, Canavarro MC, Figueiredo-Braga M. Oxytocin and depression in the perinatal period—a systematic review. Arch Women’s Ment Health. 2016;19:561–70.

67. Wong SF, Cardoso C, Orlando MA, Brown CA, Ellenbogen MA. Depressive symptoms and social context modulate oxytocin’s effect on negative memory recall. Soc Cogn Affect Neurosci. 2021;16:1234–43.

68. Cardoso C, Linnen AM, Joober R, Ellenbogen MA. Coping style moderates the effect of intranasal oxytocin on the mood response to interpersonal stress. Exp Clin Psychopharmacol. 2012;20:84–91.

69. Quirin M, Kuhl J, Diizing R. Oxytocin buffers cortisol responses to stress in individuals with impaired emotion regulation abilities. Psychoneuroendocrinology. 2011;36:898–904.

70. Panaro MA, Benameru T, Porro C. Hypothalamic neuropeptide brain protection: focus on oxytocin. J Clin Med. 2020; 19:1534.

71. Bath K, Manzano-Nieves G, Goodwill H. Early life stress accelerates behavioral and neural maturation of the hippocampus in male mice. Hormones Behav. 2016;82:64–71.

72. Ménard C, Quirion R, Vigneault E, Bouchard S, Ferland G, El Mestikawy S, et al. Glutamate presynaptic vesicular transporter and postsynaptic receptor levels correlate with spatial memory status in aging rat models. Neurobiol Aging. 2015;36:1471–82.

73. Liu D, Diorio J, Day JC, Francis DD, Meaney MJ. Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nat Neurosci. 2000;3:799–806.

74. Baier PC, May U, Scheller J, Rose-John S, Schiffelholz T. Impaired hippocampus-dependent and -independent learning in IL-6 deficient mice. Behav Brain Res. 2009;200:192–6.

75. Bialuk I, Taranta A, Winnicka G. IL-6 deficiency alters spatial memory in 4- and 24-month-old mice. Neurobiol Learn Mem. 2018;155:21–29.

76. Brennan FX, Beck KD, Servatus RJ. Low doses of interleukin-1β improve the leverpress avoidance performance of Sprague-Dawley rats. Neurobiol Learn Mem. 2003;80:168–71.

77. Yirmiya R, Winocur G, Goshen I. Brain interleukin-1 is involved in spatial memory and passive avoidance conditioning. Neurobiol Learn Mem. 2002;78:379–89.

78. Kojima S, Alberts JR. Warmth from skin-to-skin contact with mother is essential for the acquisition of filial huddling preference in preweaning rats. Dev Psychobiol. 2011;53:813–27.

AUTHOR CONTRIBUTIONS

R.A. and AM conceived the study and edited the paper. R.A., AM, and CC conceived the experimental approach. R.A., CCP, CJA, and AM conducted the experiments and R.A., PO, and IML analyzed the results. R.A., CCP, IML, FB, TS, and AM co-wrote the manuscript. All authors critically discussed the results and reviewed the final version of the manuscript.

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COMPETING INTERESTS

All authors report no conflicts of interest. The results and discussion here presented were previously posted as a preprint at bioRxiv on February 24, 2021 (https://doi.org/10.1101/2021.02.23.432580).

ADDITIONAL INFORMATION

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