American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Research Article

Effects of Peer Social Interaction on Relative mRNA Expression Ratios of IL-1β and NF-κB in Hair Follicle Cells of Developing

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To Cite This article: *Tokuko Kawasaki, Aya Watarai-Senoo, Hayato Sakurai, Rie Suge and Mamiko Koshiba*, Effects of Peer Social Interaction on Relative mRNA Expression Ratios of IL-1β and NF-κB in Hair Follicle Cells of Developing. Am J Biomed Sci & Res. 2025 29(4) AJBSR.MS.ID.003830,*

DOI: 10.34297/AJBSR.2025.29.003830

Received: December 15, 2025; **Published:** January 08 ,2026

Abstract

This study aimed to visualize the effects of peer social interactions during early development by evaluating neuroimmune system markers in hair root cells, which can be easily and minimally invasively collected from children. Using Quantitative Real-Time PCR (qRT-PCR), we conducted a preliminary small-scale preclinical assessment of mRNA expression for Cytokine Interleukin-1β (IL-1β) and transcription factor Nuclear Factor-Kappa B (NF-κB) in the common marmoset (*Callithrix jacchus*), a primate model. As our previous research suggested a susceptible period for peer social learning between 1 and 4 months of age, young marmosets aged 1 to 6 months were enrolled in a kindergarten-model environment simulating a playground, which has shown efficacy in promoting collaborative social function development in human children. To control the initial background states for parent-infant social development, human surrogate parents provided comprehensive care, including feeding and tactile interaction, to facilitate attachment formation. After weaning (from 3 months onward), mRNA expression levels of NF-κB and IL-1β in hair root cells were measured in individuals who experienced peer social interaction learning. The results indicated that the ratio of IL-1β to NF-κB exhibited characteristic trends correlated with the presence or absence of social experience between 3 and 5 months of age. Specifically, early exposure to peer interaction was associated with lower IL-1β/NF-κB ratios immediately after weaning, followed by increased expression during subsequent development. In contrast, individuals without peer interaction showed higher ratios initially, which decreased over time. These findings suggest that measuring NF-κB and IL-1β mRNA expression in hair root cells may serve as a neuroimmune test marker for visualizing peer social development status. Further investigation is warranted to verify the applicability of this approach in human children and to explore its potential for early diagnosis and intervention in social developmental disorders.

Keywords: Critical period learning, Susceptible period learning, Mutual interaction, Social interaction, Kindergarten, Nursery school, Peer social development, Psychology, Psychological development, Immune, Neuroimmune, Follicle cells, mRNA, qRT-PCR

Abbreviations: NF-κB: Nuclear Factor-Kappa B; IL-1β: Interleukin-1 Beta; IL-6: Interleukin-6; TNF-α: Tumor Necrosis Factor-Alpha; IFN-γ: Interferon-Gamma; ROS: Reactive Oxygen Species; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; qRT-PCR: Quantitative Real-Time Polymerase Chain Reaction; MI: Mutual Interaction (peer social interaction); PD: Postnatal Day

Introduction

The development of cognitive and psychological functions in childhood is expected to be nurtured within a rich social environment. Human development begins with the formation of attachment between parent and child during infancy, followed by the next stage in early childhood and continuing into elementary school, where interactions among children of the same and different generations foster communication, empathy, conflict, competition, collaboration, and cooperation through diverse social experiences. These experiences cultivate adaptive skills for living in society [1]. The psychological functions assembled by networks of information exchanged within and across social groups are increasingly supported by large-scale computational tools modelled after human neural networks, suggesting a future where such technologies may provide meaningful assistance [2]. Given the necessity of developing quantitative diagnostic techniques for assessing the growing steps of social and psychological functions, this study hypothesized and investigated molecular markers that reflect psychologically dependent dynamics in biological samples easily collected from children. Specifically, hair samples from the scalp were collected, targeting hair papilla cells and perifollicular cells [3]. The genes of interest included those involved in innate and adaptive immune responses and inflammation, such as Interleukin-1β (IL-1β), which is secreted by hair follicle dermal papilla cells and is known to increase in response to acute stress in rodents [4], and this immune system adjustable transcription factor NF-κB, which is mutually activated via the IL-1β receptor and is involved in neuronal development, neurogenesis, neurite formation, and synaptic plasticity [5-7]. Focusing on the neuroimmune system, which has been frequently reported in child psychiatry as being associated with psychological dependence and neurodevelopmental regulation [8], we utilized Quantitative Real-Time PCR (qRT-PCR) to measure mRNA expression. The common marmoset (Callithrix jacchus) was selected as a preclinical primate model for evaluating developmental trajectories after weaning [9]. This preliminary small-scale study of preclinical evaluation was designed to target the development of a primate peer socializing model around and after weaning 2-3 months on the social interactions between same-age peers with our interests of susceptible learning [10-14], it was necessary to align the initial controlled background state of parent-child social experience through artificial rearing. This was expected to ensure uniform attachment formation generally with human caregivers, similar to that in human companion animal rapport, using our established social neurobehavioral functional learning model system [15]. We examined an effect of social interactions among same-age peers. Referencing the hypothesis of a susceptible period induced by light cycle disruption [16-18] and a report of a susceptible periods adapting to environmental conditions [19], we hypothesized that the social learning highly sensitive period occurs around weaning, between 1 and 4 months of age and enrolled young marmosets aged 1 to 6 months, a period fully encompassing this presumed susceptible phase, into a model environment designed after the Playground. This environment has demonstrated high effectiveness in fostering collaborative social function development in human children through diverse peer interactions involving risk, cooperation, and competition [20,21]. This paper reports results from neuroimmune mRNA quantification for age-dependent visualization of experienced effects in the peer group.

Materials and Methods

Social Interaction Experiment, Hair follicle cell Sampling, and RNA Extraction

The experimental protocols were approved by the Animal Experiment Committees of Saitama Medical University and Tokyo University of Agriculture and Technology, Japan (Approval No. 3356-4026), and were conducted in accordance with the guidelines. Four common marmosets (Callithrix jacchus), two males and two females, were reared in our specialized primate experimental facility from the neonatal stage to adulthood (approximately one year of age) according to the protocol [22,23]. For the present study, animals were observed up to 8 months of age. The rearing room was located within a thermally insulated building. Infants were initially raised by their parents until one week of age, after which they were artificially reared with milk feeding until the weaning period (around 3 months of age). Caretakers acted as surrogate parents, providing toilet training and grooming to establish attachment. Animals were housed individually in light-shielded incubators (fluorescent lighting, constant temperature 32-28°C). After weaning, they were transferred to standard home cages, where they had free access to food and water until the end of the experiment. The average illumination per cage was maintained at 750-930 lux.

To establish social interaction conditions, animals were exposed once per week for 10 minutes to either:

1) MI+ (Mutual Interaction): social experience with age-matched peers (Figure 1), or

2) MI- (No Interaction): solitary exposure in the same cage environment without peers (Figure 1).

This procedure was repeated weekly (upward arrows in Figure 2). Two condition groups were designed that MI early group experienced social interaction during the early period (Postnatal Days PD30-P190; Figure 2), whereas the MI late group experienced social interaction later in development (PD190-P230; Figure 2). Food pellets (CMS-1M, CLEA Japan, Inc.) and water of their home cage were replaced daily, and the home cages were cleaned between 10:00-11:00 a.m. The MI "playground" cage experiments were conducted during this daily maintenance period.

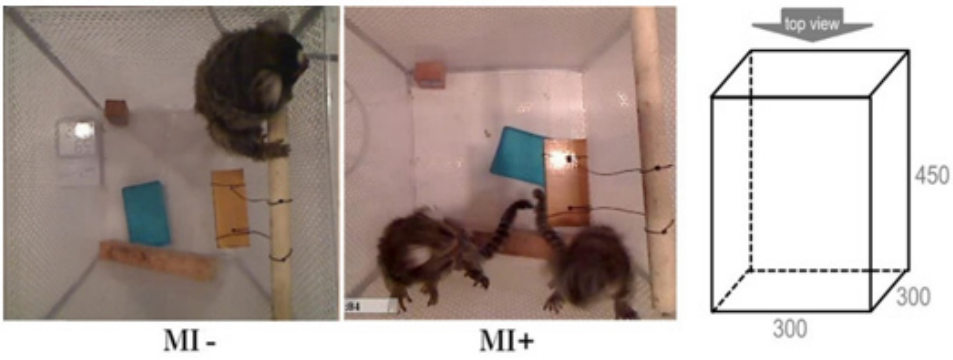


Figure 1: The scenes of infant marmosets spending a "kindergarten playground" without (MI-) or with (MI+) socio-Mutual Interaction between peers. The two photographs show young animals inside a cage set with play equipment-swings, boxes, branches, and interior walls for free climbing-as seen from the ceiling.

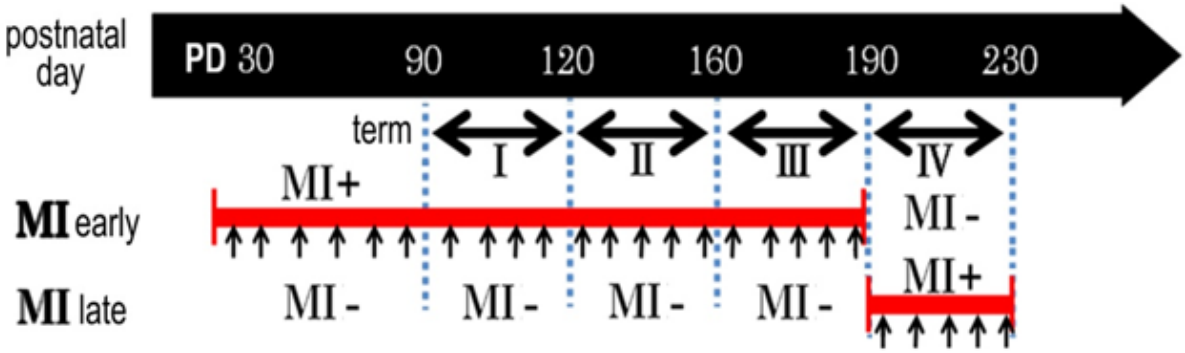


Figure 2: The scenes of infant marmosets spending a kindergarten cage without (MI-) or with (MI+) socio-Mutual Interaction. Upward arrows indicate the timing of the 10-minute MI+ sessions with MI- of either group, MI early or MI late, conducted once per week.

Hair Sampling, Total RNA Extraction from Follicular Cells, cDNA Synthesis, and Real-Time PCR

Approximately up to ten hairs were plucked from the head region at two time points: the day before the Mutual Interaction (MI) test at the same time of day, and immediately after the test. Preliminary examinations suggested that hair roots and perifollicular regions may contain multiple cell types, including dermal papilla cells, keratinocytes, and Langerhans cells. However, in this study, we did not attempt to identify specific cell types; instead, all cells obtained from the samples were analyzed collectively to detect gene expression changes dependent on peer social learning. After collection, hair samples were placed in plastic tubes, snap-frozen in liquid nitrogen, and stored at -80°C. Total RNA was extracted using the RN Easy Mini Kit (QIAGEN) according to the manufacturer's protocol and diluted with RNase-free water. cDNA was synthesized using the Prime Script® RT reagent Kit with gDNA Eraser (Perfect Real Time; TaKaRa Bio), which included a genomic DNA removal step, following the manufacturer's instructions [24]. Based on preliminary assessments of cDNA concentration, the experimental design allowed for two samples per individual to be analyzed statistically. Four developmental terms (I-IV) were defined for measurement following weaning. Terms I-III corresponded to age ranges previously identified as susceptible periods in our previous behavioral evaluation system, which used circadian rhythm manipulation to detect heightened susceptibility [17]. Term IV was designed as a confirmatory stage under reverse conditions, extending beyond the predicted susceptible period. Multiple hair samples collected within each term under the same condition were pooled and analyzed as replicates. Relative quantification of gene expression ratios between target genes was performed across these developmental stages (terms I-IV) (Figure 2).

Quantification of Gene Expression in Hair Follicle Cells: Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Real-Time PCR

Gene expression in hair follicle cells was quantified using real-time PCR with SYBR Green dye, which fluoresces upon binding to double-stranded DNA. Fluorescence intensity was used to estimate the amount of amplified DNA. Each PCR cycle consisted of three steps: DNA denaturation, primer annealing, and extension by DNA polymerase. The number of cycles required to amplify target gene

fragments extracted from biological samples was used to infer the original template quantity.

Real-time PCR reactions were performed using SYBR® Premix Ex Taq™ (Tli RNaseH Plus; TaKaRa Bio) on an ABI PRISM® 7100 system under the following conditions:

1) Step 1: 95°C for 30 seconds (1 cycle)

2) Step 2: 95°C for 5 seconds and 60°C for 31 seconds (50 cycles)

3) Step 3: 95°C for 15 seconds, 60°C for 60 seconds, and 95°C for 15 seconds (1 cycle)

Relative quantification was performed using the ΔCt method, with the Cycle Threshold (Ct) defined as the number of cycles at which fluorescence exceeded the instrument-recommended threshold line. The relative expression of target genes was calculated as 2^-ΔCt, allowing comparison across developmental stages. Target genes included NF-κB and IL-1β, with GAPDH serving as the housekeeping reference gene. Primer sequences were designed using the Primer3Plus web application (*http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi*). Preliminary experiments confirmed that amplification efficiencies of all primer sets were equivalent.

1) Primer sequences:

The primer sequences used for amplification were as follows:

a) Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)

Forward:5′-cagaacatcatccctgcctct-3′-Reverse: 5′-acgcctgcttcaccacct-3′

b) Interleukin-1β (IL1B)

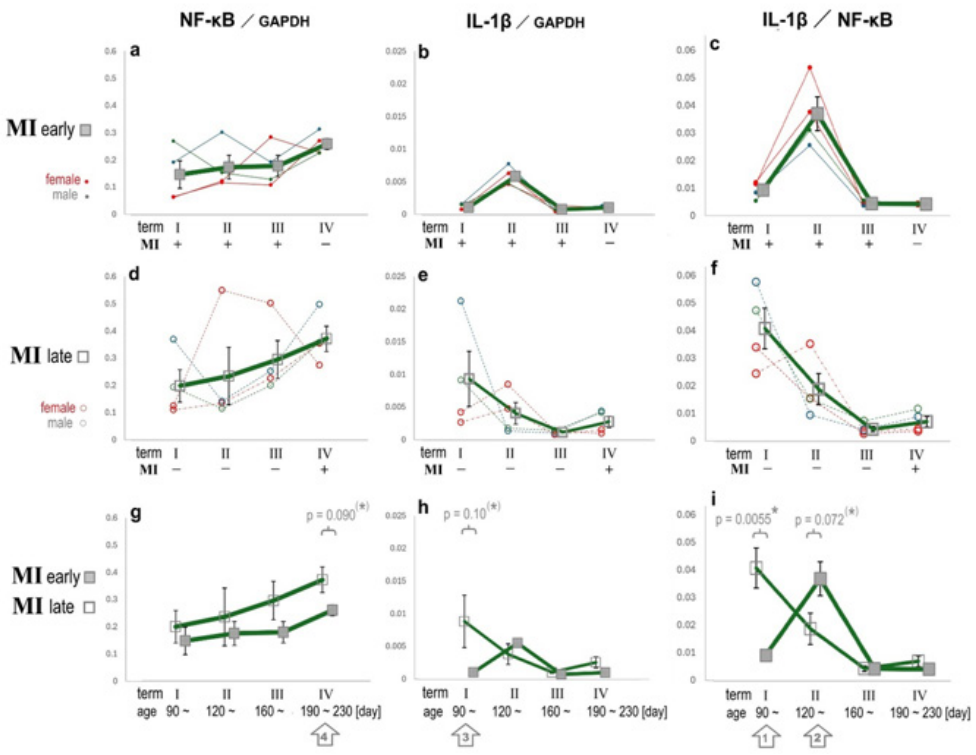
Forward: 5′-cagcaccttctttcccttca-3′ Reverse: 5′-tgcacaaaggacatggagaa-3′

c) Nuclear Factor-Kappa B (NF-κB)

Forward: 5′-tgattatgggcaggaaggac-3′-Reverse: 5′-gaaccaagaaaggaagccaag-3′

Results and Discussion

The relative expression ratios of NF-κB and IL-1β in hair follicle cells, analyzed according to the presence (MI+) or absence (MI-)of peer Mutual Interaction (MI) and developmental stage, are shown in Figure 3.



Note*: Panels a–c: sample trajectories (thin line) and averages (thick line) for the MI early group; panels d–f: sample trajectories and averages for the MI late group; panels g–i: comparison of group averages. (a, d, g) Relative expression ratio of NF-κB to GAPDH; (b, e, h) relative expression ratio of IL-1β to GAPDH; (c, f, i) relative expression ratio of IL-1β to NF-κB. Error bars represent standard error. Statistical evaluation was performed using Student's t-test (*p < 0.05; (*) p < 0.1).

Figure 3: Developmental age-related changes in relative mRNA expression levels of immune system molecules within hair follicle cells, and comparison between same-age individuals with and without social interaction experience.

Statistical evaluation was performed using F-tests followed by Student's t-tests. In Term I, the relative expression ratio of IL-1β to NF-κB (IL-1β/NF-κB) was suppressed in the MI early group, which experienced peer interaction, whereas the MI late group, which remained in the kindergarten model cage without peer interaction, exhibited significantly higher expression (Figure 3i, arrow 1). In Term II, the pattern generally reversed: IL-1β/NF-κB ratios were higher in the MI early group and lower in the MI late group (Figure 3i, arrow 2). In Terms III and IV, IL-1β/NF-κB ratios were reduced in both groups (Figure 3i). For the relative expression ratio of IL-1β to GAPDH (IL-1β/GAPDH), the MI early group showed suppressed expression in Term I, whereas the MI late group displayed greater variability (Figure 3h, arrow 3). In Term IV, the relative expression ratio of NF-κB to GAPDH tended to be slightly higher in the MI late group, reflecting age-related increases (Figure 3g, arrow 4). These results, interpreted in reference to previous studies, suggest that IL-1β expression relative to NF-κB-whose overall variation was limited (Figure 3g)-provides a meaningful index of peer social interaction effects across developmental stages. A schematic summary of these findings is presented in Figure 4.

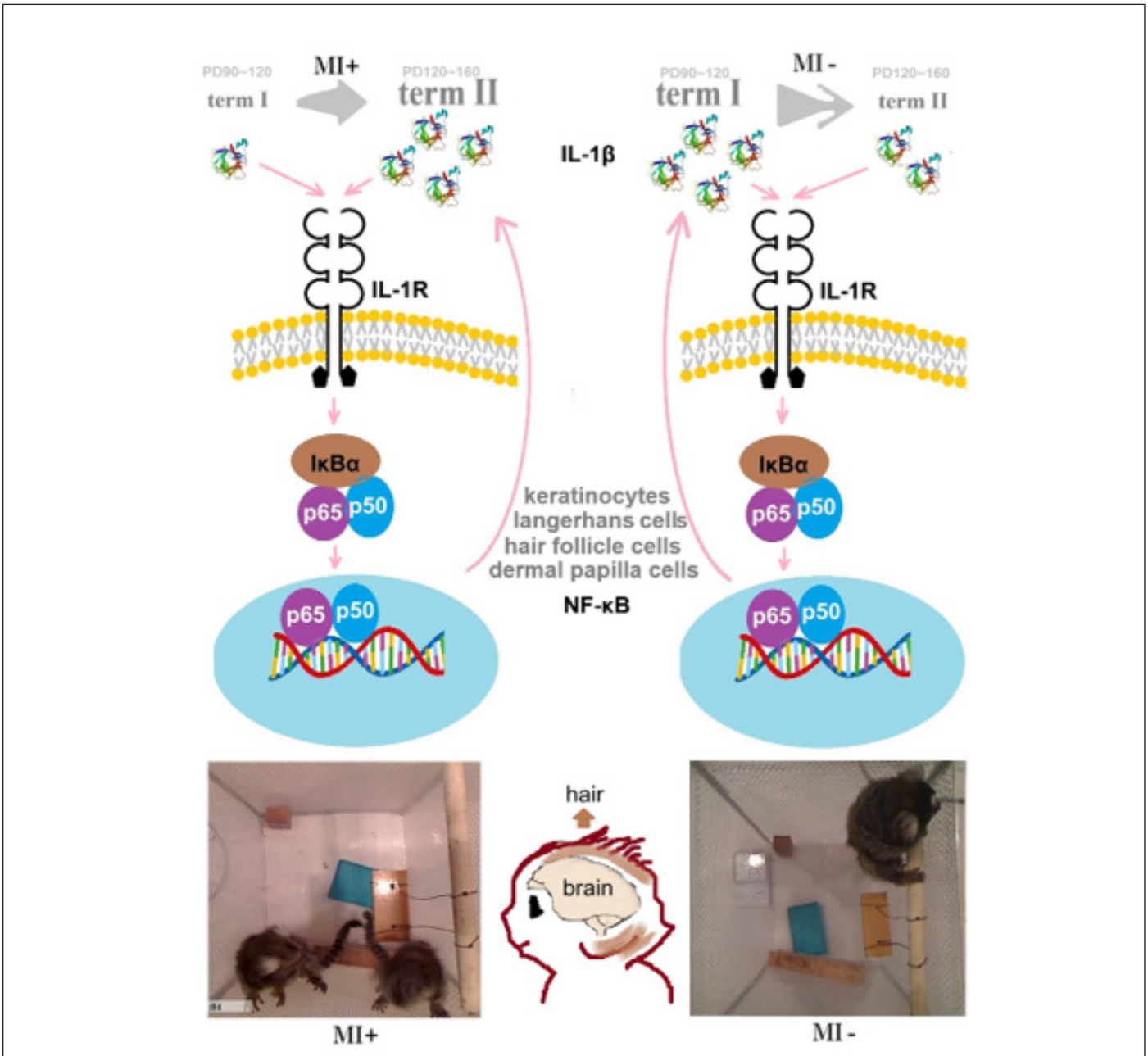


Figure 4: Hypothetical pathway cycle illustrating NF-κB and IL-1β expression in hair follicle cells of young common marmosets (term I (PD 3 months)–term II (PD 4 months) around the predicted susceptible period of peer-social learning at the "Playground Kindergarten".

Schematic diagram of the hypothesized pathway cycle for NF-κB and IL-1β expression in hair follicle cells following peer social interaction learning in the Play Park Kindergarten Model of 3-4-month-old common marmoset pups. Based on literature references, this study compared the social interaction experience groups (MI+) and non-experience groups (MI-) starting from before weaning. PD denotes postnatal days.

The schematic summarizes developmental changes associated with peer social interaction learning, highlighting the proposed cascade of neuroimmune activation and stress-related modulation during the susceptible post-weaning period.

Based on the present findings and in reference to previous literature, we compared groups of common marmosets that experienced peer social interaction (MI+) with those that did not (MI-). In the MI early group, which began weekly 10-minute sessions of peer interaction in the indoor "play-park kindergarten" model from approximately PD30 (prior to the weaning period at 2-3 months of age), hair follicle cells exhibited suppressed relative mRNA levels of the cytokine IL-1β compared to NF-κB during Term I (90-120 days). By Term II (120-160 days), however, IL-1β expression increased. The elevated IL-1β/NF-κB ratio at this stage may reflect the impact of social interaction learning, suggesting that psychosocial and physical stress loads activated the immune inflammatory cascade within follicular cells [25].

In contrast, the MI late group, which remained alone in the same kindergarten cage for weekly 10-minute sessions, showed the opposite pattern. During Term I, IL-1β/NF-κB ratios were elevated, indicating heightened stress responses in the absence of peer interaction. By Term II, expression was suppressed, suggesting that adaptation to the environment gradually reduced stress-induced IL-1β activation.

Although not illustrated in Figure 4, subsequent Terms III and IV revealed no major differences between MI+ and MI- groups in the relative ratios of NF-κB and IL-1β to GAPDH (Figure 3). This indicates that peer social interaction-dependent changes in gene expression were not observed beyond early developmental stages. Taken together, these results suggest that sensitive periods of heightened susceptibility may exist only during Terms I and II, corresponding to the early post-weaning juvenile phase. NF-κB plays multiple roles in neurodevelopment. Under normal conditions, NF-κB promotes neuronal survival by inducing anti-apoptotic genes such as Bcl-2, Bcl-XL, and manganese superoxide dismutase [26]. In microglia, NF-κB activation drives pro-inflammatory responses, leading to the release of cytokines (IL-1β, IL-6, TNF-α, IFN-γ) and Reactive Oxygen Species (ROS) [6, 26]. In astrocytes, NF-κB activation is required for astrocytic differentiation and further enhances inflammatory signaling [26].

The IL-1β/NF-κB signaling pathway has been reported to influence social development and behavior through its regulation of inflammatory responses. Keratinocytes and dermal cells in the skin and perifollicular regions are known to express IL-1β and NF-κB under psychological stress. IL-1β, as a major pro-inflammatory cytokine, activates NF-κB, and this pathway has been linked to psychosocial stress and infection-related conditions that impair social functioning and contribute to neurodevelopmental disorders [27,28]. In stressed rat skin, IL-6 and IL-1β mRNA expression levels were significantly increased [4]. IL-1β has also been shown to inhibit hair follicle growth, and genetic polymorphism analyses have reported elevated IL-1β expression in patients with active alopecia areata [29]. In Autism Spectrum Disorder (ASD), salivary IL-1β levels were found to increase in association with poor sleep quality [30]. Moreover, Cytomegalovirus (CMV) infection in children with ASD has been reported to elevate IL-1β and IL-6 levels, exacerbating restricted eating behaviors, tactile hypersensitivity, and other autism-related symptoms [31]. These findings highlight the relevance of IL-1β and NF-κB in neuroimmune regulation and psychosocial development.

To quantify such complex information, qRT-PCR technology has been widely applied in child psychiatry and neurodevelopmental research, given its robust capacity for measuring mRNA expression and integrating data into well-established analytical frameworks. Beyond immune signaling, cross-generational interactions have been shown to promote emotional stability and attachment formation via oxytocin release, thereby strengthening social bonds [32]. Cortisol, a stress hormone, is suppressed by oxytocin, enabling stable developmental trajectories [33,34]. Early childhood is recognized as a sensitive period during which social brain functions exert long-term influences across the lifespan [35]. Therefore, improving caregiving environments and interventions requires the development of multidimensional diagnostic systems that integrate behavioral, molecular, and neuroimaging measures of socio-emotional development [10]. In this study, we conducted a preclinical evaluation of socio-emotional and psychological functions based on quantitative mRNA expression analysis in a primate developmental model, the common marmoset (Callithrix jacchus) [9]. To specifically target peer social interaction, we employed our previously established neurobehavioral learning model system, which allowed controlled parental social experience through artificial rearing [36]. Experimental paradigms were designed with reference to our test battery system that identified alterations in social behavior induced by disrupted light cycles, supporting the hypothesis of sensitive developmental periods [16,17], as well as reports of sensitivity to climatic adaptation [37].

Because the body weight of the common marmoset is approximately one one-hundred-fiftieth that of humans [23], sufficient follicular cells had to be collected by repeated sampling from the same head region to obtain adequate mRNA for analysis. However, when extended to human applications, smaller samples are expected to yield sufficient mRNA, enabling higher temporal and spatial resolution in measurement. The limitations of this study include the small sample size, which was constrained by the technical challenges of long-term controlled learning conditions in juvenile animals. As a result, the present work remains a small-scale hypothesis-generating study. Behavioral evaluation systems for acquired traits are currently under development and will be reported in future studies. Future directions should include increasing the number of experimental cases and applying multidimensional evaluation analyses to strengthen preclinical validation. Ultimately, the effectiveness of molecular quantification techniques in hair follicle cells should be examined as a potential biomarker system for optimizing complex learning processes in human children through experiences of peer and intergenerational social interaction.

Conclusion

This study focused on the effects of early peer social interaction on neuroimmune development, utilizing Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) to measure mRNA expression as a molecular marker of psychosocial dynamics in biological samples easily obtainable from children. We examined the expression of NF-κB and IL-1β in hair follicle cells of common marmosets (Callithrix jacchus), a primate model of childhood development, during the sensitive period around weaning (3-4 months of age). Our results demonstrated that in the MI early group, which experienced peer interaction beginning around PD30, the relative mRNA levels of IL-1β in hair follicle cells were suppressed during Term I (90-120 days) but increased during Term II (120-160 days). The elevated IL-1β/NF-κB ratio suggests that social interaction learning may have induced activation of immune inflammatory cascades in follicular cells under psychosocial and physical stress [25]. In contrast, the MI late group, which remained solitary in the kindergarten environment, exhibited higher IL-1β/NF-κB ratios in Term I, reflecting heightened stress responses, but suppressed expression in Term II, indicating adaptation to the environment over time. By Terms III and IV, no significant differences were observed between MI+ and MI- groups in the relative ratios of NF-κB and IL-1β to GAPDH, suggesting that peer social interaction-dependent gene expression changes were restricted to early developmental stages. These findings indicate that sensitive periods of heightened susceptibility may exist only during the early juvenile phase (Terms I and II). Overall, the results highlight the importance of peer and intergenerational social interaction experiences in early childhood learning. Furthermore, the methodological approach of extracting mRNA from hair follicle cells in the common marmoset model provides preliminary evidence for potential application as a non-invasive molecular quantification technique in human developmental studies

Acknowledgements

We appreciate Mr. Koki Shimizu who mainly supported this study. Our research project was sponsored by JSP, JST with grant number kakenhi21200017 and ALCA10104101 and our universities, Yamaguchi University, Saitama Medical University and University of Human Arts and Sciences.

Conflict of Interest

We declare that we have no conflict of interests.

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