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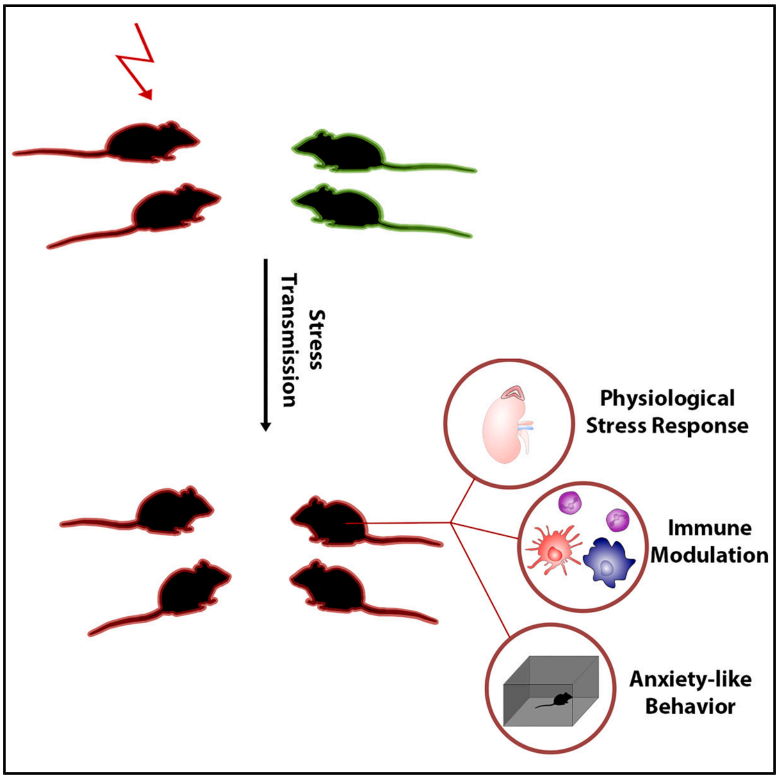
Stress transmission in social groups of mice: unveiling physiological responses, behavioral patterns, and immune dynamics

Graphical abstract



Highlights

- Establishment of a paradigm for social stress transmission in groups of mice
- Elevated anxiety-like behavior in mice experiencing stress transmission
- Stress transmission enhanced innate immune activity
- Stress transmission does not influence adaptive immune responses

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In brief

Rodent behavior; Rodent immunology; Rodent physiology

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Stress transmission in social groups of mice: unveiling physiological responses, behavioral patterns, and immune dynamics

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SUMMARY

In modern societies, stress is pervasive, requiring sophisticated physiological mechanisms for stability and survival, primarily through the sympatho-adrenal medullary (SAM) and hypothalamo-pituitary adrenal (HPA) axes. Chronic stress is linked to a range of mental and physical health problems and has been shown to affect immune function. In this study, a paradigm for social stress transmission in groups of mice was established, based on a restraint stress model to study how stress spreads among individuals. Mice exposed to indirect stress exhibited HPA-axis activation, elevated corticosterone (CORT) levels, enlarged adrenal glands, and anxiety-like behaviors in light-dark-box tests. Notably, female mice were more susceptible to stress transmission. While stress transmission enhanced innate immune responses, it did not affect adaptive immunity following vaccination with a poly(lactic-co-glycolic acid) (PLGA)-based vaccine. In contrast, direct stress impaired both immune responses and the effectiveness of immunotherapy in a melanoma model.

INTRODUCTION

In today's highly dynamic human societies, coping with stressful situations has become an integral part of our daily lives. The physiological stress response plays a vital role in overcoming such challenges, maintaining homeostasis, and ensuring survival. This response is primarily mediated by the activation of the sympatho-adrenal medullary system (SAM) and the hypothalamic-pituitary-adrenal (HPA) axis. From an evolutionary perspective, an acute stress response is an essential mechanism to ensure survival. However, in modern societies, individuals are increasingly exposed to chronic stress, which poses significant health risks. Chronic stress contributes to mental disorders, such as depression and anxiety,[1,2] and physical illnesses including cardiovascular disease and malignancies.[3,4] Importantly, the impact of chronic stress is not confined to humans; environmental changes and shrinking natural habitats create persistent stressors for animals as well. In rodents, numerous studies have confirmed parallels with human stress-related diseases, providing valuable insights into the dynamics and mechanisms of stress-related disorders.[5,6] For example, chronic stress has been shown to increase susceptibility to infectious diseases[7] and impair viral clearance in mice.[8] These findings highlight the complex interplay between physiological stress responses and the immune system. Chronic stress refers to prolonged or repeated exposure to stressors that challenge the body's ability to maintain homeostasis. While short-term stress responses can be adaptive, enabling organisms to cope with immediate threats, sustained activation of stress pathways can lead to physiological dysregulation. Over time, this maladaptive response can impair the immune system, resulting in impairments in both innate and adaptive immunity.[9] While cells of the innate immune system adopt a pro-inflammatory phenotype under chronic stress,[10,11] the adaptive immune response suffers in efficacy, with both cellular and humoral immunity adversely affected.[8,12,13]

Despite intensive research on the effect of acute and chronic stress on physiology and behavior, the phenomenon of transmission of physiological stress has only recently gained attention in research. In humans, stress contagion has been investigated by examining stress responses in individuals observing stressful situations.[14,15] The transmission of stress to observer individuals is strongly influenced by their capacity for empathy.[16] While stress contagion has become a prominent research topic in humans, studies on animal collectives remain limited.[17] Although empathy continues to be a central focus in studies involving non-human primates,[18] research on lower animals within collective groups is still in its early stages. Existing research has primarily focused on pairs of animals, exploring how stress spreads from one individual to another.[19,20] These experiments have provided valuable insight in the dynamics of stress transmission between individuals but underscore the need for expanded research on groups and collectives of animals to understand the influence of social structures and collective behavior. For instance, experiments in groups of rodents have used models adapted from human research, such as observing social defeat in conspecifics (reviewed by Carnevali et al. in 2020[21]).

In the present study, we established a paradigm that enables the investigation of stress transmission within groups of mice. The model is based on the social contagion of physiological stress induced by restraint stress. Recipient mice exposed to this secondary, socially transmitted stress consistently exhibited a physiological stress response. Specifically, we demonstrated activation of the HPA axis in these animals, accompanied by alterations in anxiety-like behavior and immune modulation. This paradigm thus provides a valuable foundation for studying the mechanisms of stress transmission within social contexts and its effects on physiological and behavioral outcomes.

RESULTS

Stress transmission impacts physiology and immune parameters within social groups of mice

Transmission of physiological states within social groups of animals is a frequently observed yet poorly understood phenomenon. Here, we investigate whether transmission of physiological stress in a group of mice and the resulting consequences on the respective animals can be detected. Mice were subjected to 4 h of restraint stress and subsequently co-housed with stress naive mice for 16 h prior to the analysis as described in Figures S1 and S2A. When comparing the co-housed mice that experienced socially transmitted stress (TS) from acutely stressed individuals (ARS) with control mice (CTRL), a significant increase in adrenal gland weight was detected (Figure 1B). Although no significant impact on the thymic mass and corticosterone (CORT) levels were measured in TS and ARS mice compared to CTRL mice, a clear tendency toward reduced thymic mass and increased CORT levels could be observed in both ARS and TS mice (Figures 1A and 1C). Next, we investigated behavior of mice subjected to restraint stress and stress transmission in a light-dark-box test (Figure 1D). Although not significantly different, compared with CTRL mice, TS mice showed a similar trend toward more anxiety-like behavior as ARS mice.

The immune system is closely linked to the stress axis and in particular, innate immune parameters were reported to be increased upon subjection to stressors.[10] Hence, secretion of the pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor (TNF) were quantified by ELISA. Splenocytes of CTRL, chronic restraint stress (CRS), and TS mice were stimulated overnight with lipopolysaccharide (LPS) and cytokine levels in the supernatant were determined (Figures 1E and 1F). While IL-6 secretion in response to LPS stimulation was not altered in the TS group (Figure 1E), the detected levels of TNF in the supernatant of TS mice were significantly higher than CTRL levels (Figure 1F). These data suggest that stress can be socially transmitted among mice, as even a single incident of stress exposure led to significant physiological and immunological changes detectable within the social group. However, the observed effects of stress transmission mediated by a singular event were minor. Hence, a more robust stress induction was required for deeper investigations of the effects of stress transmission in mice.

Chronic transmission of stress manifests HPA axis impairments but does not alter thymic development

Guided by our findings on social transmission of stress in an acute setting, our data suggest the necessity for further investigations on the dynamics of stress transmission in mice. Given that pathological responses to stress predominantly emerge from prolonged exposure, we anticipated more definitive outcomes regarding the impact of stress transmission when individuals are subjected to extended periods of social interaction with stressed conspecifics. Therefore, an experimental setup for chronic stress transmission over 10 days was established (see Figure S2B). Interestingly, the examination of HPA axis activation revealed notable differences in activation strength between the sexes. Female mice seemed to be more susceptible to stress transmission than their male counterparts. While both, male and female mice that were subjected to CRS lost weight over the course of the 10-day stress procedure, only male mice that experienced TS did show a normal development of body weight, similar to controls (Figure 2A). For female TS mice on the other hand, the body weight increase over 10 days was significantly lower compared to the control group with several individuals experiencing weight loss during the stress procedure interval (Figure 2C). Sex differences were also observable in adrenal hypertrophy development. Both, male and female mice experiencing stress transmission showed a significant increase in adrenal gland mass compared to control mice. However, the severity in male TS mice was significantly lower compared to the CRS group (Figure 2B). In contrast, adrenal gland hypertrophy in female TS mice was similar to that in mice which were chronically stressed through restraint (Figure 2D). In response to the hypertrophy of the adrenal glands, an increase in plasma CORT levels was detected in CRS and TS mice. The elevations in plasma CORT levels, quantified by ELISA, were similarly increased in CRS and TS groups, affecting both sexes equally (Figures 2E and 2G). Interestingly, although thymocytes are sensitive to stress hormone-mediated apoptosis,[22] no thymus atrophy was detected in TS mice. While both, male and female mice subjected to CRS showed a drastic decrease in thymus weight, the thymuses in male as well as female TS mice were completely unaffected regarding weight loss (Figures 2F and 2H). This was confirmed by flow cytometric analysis of thymocyte populations. Figure 2I reveals significant declines across all populations (double negatives, single positives and double positives) in CRS mice relative to CTRL and TS groups between which no alterations were detectable. Despite the significant reduction of all thymic populations in CRS mice, a shift toward a diminished double-positive population relative to double negatives and single positives was observed in these mice (Figures 2I and 2J). This was not evident for TS mice.

Our investigations reveal a highly significant physiological response to stress transmission in mice in the form of HPA axis activity, manifested in adrenal hypertrophy, increased plasma CORT levels as well as an alteration in body weight

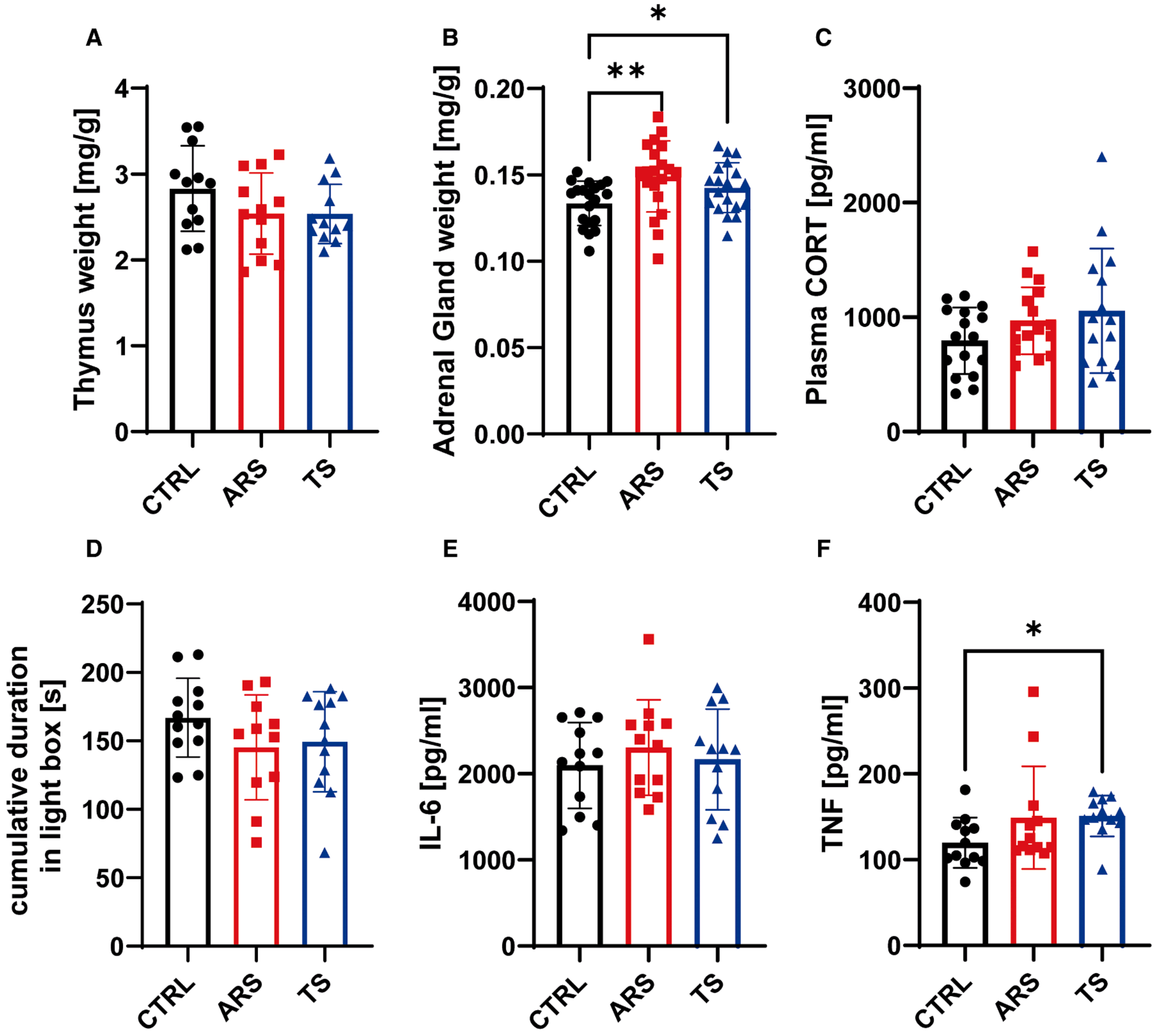

Figure 1. Effects of acute stress transmission on physiology, anxiety-like behavior, and immune parameters

(A and B) Female C57BL/6 mice (*n* = 12–16 per group) were subjected to acute restraint stress (ARS, red squares), stress transmission (TS, blue triangles), or control treatment (CTRL, black dots). 1 day after the stress procedure, thymuses (A) and adrenal glands (B) were harvested, and the organ mass was determined relative to the body weight.

(C) Corticosterone levels were analyzed in the blood plasma via ELISA.

(D) 4 h after the stress procedure, mice were tested for exploratory behavior in a light-dark box using an automated tracking device. Cumulative duration in light box is depicted.

(E and F) 1 day after the stress procedure, splenocytes were harvested and incubated for 72 h with 1 μg/mL LPS. Supernatants were analyzed for secreted IL-6 (E) and TNF (F) via ELISA. Pooled data from three independent experiments are presented as means ± SD.

Statistics: one-way ANOVA followed by Tukey's multiple comparison test $^{*}p < 0.05$ and $^{**}p < 0.01$.

development. Notably, female mice displayed a greater susceptibility to stress transmission compared to their male conspecifics. Yet, stress transmission appeared to have no impact on thymus weight or the composition of thymocyte populations.

Chronic stress transmission fosters anxiety-like behavior in mice

The strong induction of a physiological stress response in mice, co-housed with conspecifics that were subjected to chronic restraint stress suggests an impact on animal behavior. In particular, the highly elevated stress hormone levels measured in TS mice have the potential to contribute to anxiety and reduced exploratory behavior in mice. For quantitative investigation of these behavioral shifts, we performed light-dark-box and open field tests. Quantification of the anxiety-like behavior revealed an impact of stress transmission (Figure 3). The study indicates that while stress transmission did induce anxiety-like behavior in mice, only modest effects on exploration were detected. In a light-dark-box test, CRS and TS mice spent significantly less time exploring the light box but stayed within the dark box for a longer duration relative to controls (Figures 3A–3C). This tendency was less pronounced in mice experiencing stress

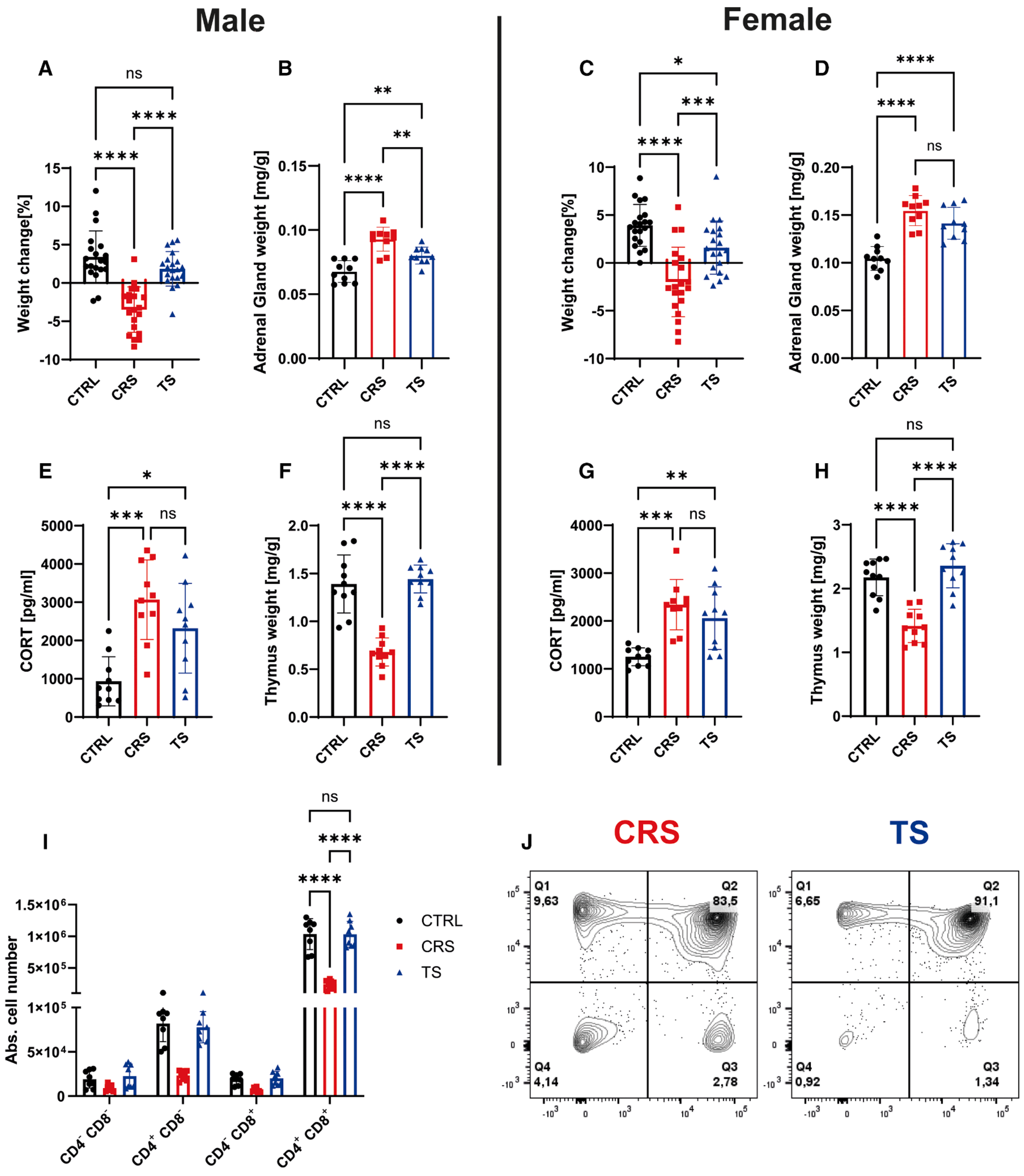

Figure 2. Impacts of chronic stress transmission on physiology

(A and C) Male (n = 10 per group) (A) or female (n = 10 per group) (C) C57BL/6 mice were subjected to chronic restraint stress (CRS, red squares), stress transmission (TS, blue triangles), or control treatment (CTRL, black dots) and the change in body weight was monitored from day 1 to day 10 of the stress procedure. Pooled data from five independent experiments are presented as means ± SD.

Statistical significance was analyzed by ANOVA followed by Tukey's multiple comparison test; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$; ns, not significant.

(B and D–H) Male or female C57BL/6 mice (n = 10) were subjected to chronic restraint stress (CRS, red squares), stress transmission (TS, blue triangles), or control treatment (CTRL, black dots). One day after the last stress procedure, adrenal glands (B and D) and thymuses (F and H) were harvested, and the organ mass was determined relative to the body weight. (E and G) Corticosterone levels were analyzed in the blood plasma via ELISA. (B, D, E, F, and G) Pooled data from three independent experiments are presented as means ± SD.

Statistics: ANOVA followed by Tukey's multiple comparison test; $^{*}p < 0.05$, $^{**}p < 0.01$ $^{***}p < 0.001$, and $^{****}p < 0.0001$; ns, not significant.

(legend continued on next page)

transmission, indicating a similar outcome as observed in the open field test. Here, only chronically stressed mice (CRS group) spent significantly less time exploring the inner zone of the arena compared to the control group. This shift in exploratory behavior was not visible to a significant extent in TS mice. However, no significant differences in exploratory behavior between chronically stressed mice (CRS) and mice that experienced stress transmission (TS) was detected either (Figures 3D and 3E).

In addition to anxiety-like and exploratory behavior, an effect of stress and stress transmission on the cognitive capacity of the animals was investigated. The data revealed no significant impact of stress or its social transmission on short-term memory and recognition capabilities in a novel object recognition test. All groups, regardless of stress exposure, displayed a similar interest in exploring the novel object (Figure 3G). Notably, despite the observed sex differences in HPA axis activation, no significant differences were found between the sexes in behavioral assessments.

Elevated cytokine secretion to LPS stimulation in mice experiencing chronic stress transmission

Stress has been shown to interact with the immune system in a complex manner. Cytokine secretion by innate immune cells upon chronic stress exposure is known to be increased and shifted toward a pro-inflammatory milieu. The effect of stress transmission on innate immunity, in particular cytokine secretion, was assessed in LPS stimulated splenocytes. Cell culture supernatants were analyzed for various cytokines using flow cytometry based multiplex screening. Most pro-inflammatory cytokine levels produced by splenocytes of chronically stressed mice as well as stress transmission mice were upregulated relative to controls (Figure 4A). TNF and IL-6 levels were additionally quantified by ELISA. Relative to control mice, significantly elevated secretion of TNF (Figure 4B) and IL-6 (Figure 4C) was detected in CRS and TS mice whereas no significant difference between the latter was evident for neither of the cytokines.

Efficacy of the adaptive immunity is not affected by stress transmission

Elevated cytokine levels and an overactivation of innate immunity as a consequence of chronic stress are often correlated with immunopathology. In contrast, the adaptive immunity is suppressed in its efficacy upon a chronic stress exposure. Therefore, we conducted a series of experiments based on vaccinations with a poly(lactic-co-glycolic acid) (PLGA)-microparticle (MP) based vaccine platform to analyze the antigen-specific immune response upon exposure to chronic stress and stress transmission. CRS, TS, or control mice were immunized subcutaneously with PLGA MP carrying ovalbumin (Ova) and the TLR-3/RIG-I ligand Riboxxim on day 5 of the stress procedure (see Figure S2C). On day 21, the bone marrow was analyzed by FLUOROSPOT for Ova-specific IgG secreting plasma cells. No significant difference in the amount of spot forming units was detected between all groups, regardless of stress exposure (Figure 5A). Confirmatory, no significant differences in serum antibody titers were found between all three groups. The EC50 of Ova-specific IgG antibody titers revolved around similar levels as depicted in Figure 5B. Next, Ova specific T cell responses were analyzed via intracellular cytokine staining for interferon gamma (IFNγ) of CD8$^+$ cytotoxic T lymphocytes (CTLs). Mice were immunized on day 5 of the stress procedure and 6 days later, single cell suspension of splenocytes were re-stimulated with SIINFEKL peptide (see Figure S2D). IFNγ production was measured by flow cytometry. Mice exposed to chronic restraint stress showed a significant reduction in IFNγ^+ cells of about 50% relative to controls. In contrast, the CTL response of mice experiencing stress transmission was unaffected, showing similar levels as the control group (Figure 5C). PLGA MP anti-tumor vaccination has been used as a tool for T cell driven immunotherapy.[23] We employed a B16-Ova melanoma mouse model for lung metastasis formation to investigate the impact of stress and stress transmission on applied cancer vaccination therapy. Mice were double-route immunized (subcutaneously and intranasally) with PLGA MP Ova/Riboxxim or empty control particles on day 5 of the stress procedure (see Figure S2E). 6 days later, mice were inoculated with 1×10^5 B16-Ova cells intravenously. The analysis of lung tissue for metastasis 21 days post-tumor cell application revealed a similarly significant reduction in metastasis count in both control and TS mice treated with the PLGA MP vaccine, compared to those receiving empty control MP. This therapeutic effect was not observed in chronically stressed mice, which showed a high metastasis count similar to the empty control vaccination (Figure 5D). Histological examination further confirmed the reduced metastasis in TS mice and highlighted the contrast with the high metastasis burden in the chronically stressed group (Figure 5E).

DISCUSSION

The phenomena of stress transmission or emotional contagion have been described in humans and non-human primates.[24–27] Despite expanding research on stress transmission into diverse animal collectives,[17] the exploration of these phenomena remains nascent. Specifically in the context of rodents, investigation of stress transmission remains a widely underexplored topic. While emotional contagion of pain and fear between individuals was reported in mice and rats,[28] little is known on the transmission of the physiological state of stress within social groups of rodents. A primary limitation in this area of research is the lack of adequate stress models. Recent advancements on understanding the dynamics of stress transmission in mice were achieved by applying chronic social defeat stress models.[21] Here, one individual is invading the home cage of a physically superior dominant male, leading to social defeat and the induction of a stress response. The model depends on social hierarchies, which makes it suitable for effectively inducing stress

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(I) Thymocytes were analyzed by flow cytometry for CD4 and CD8 populations (*n* = 8 mice per group (4 female, 4 male)). Absolute cell numbers pooled from two independent experiments are presented as means ± SD.

Statistics: two-way ANOVA, followed by Tukey's multiple comparison test; ****$p < 0.0001$; ns, not significant.

(J) Representative flow cytometry plot showing thymocyte compositions of CRS and TS mice.

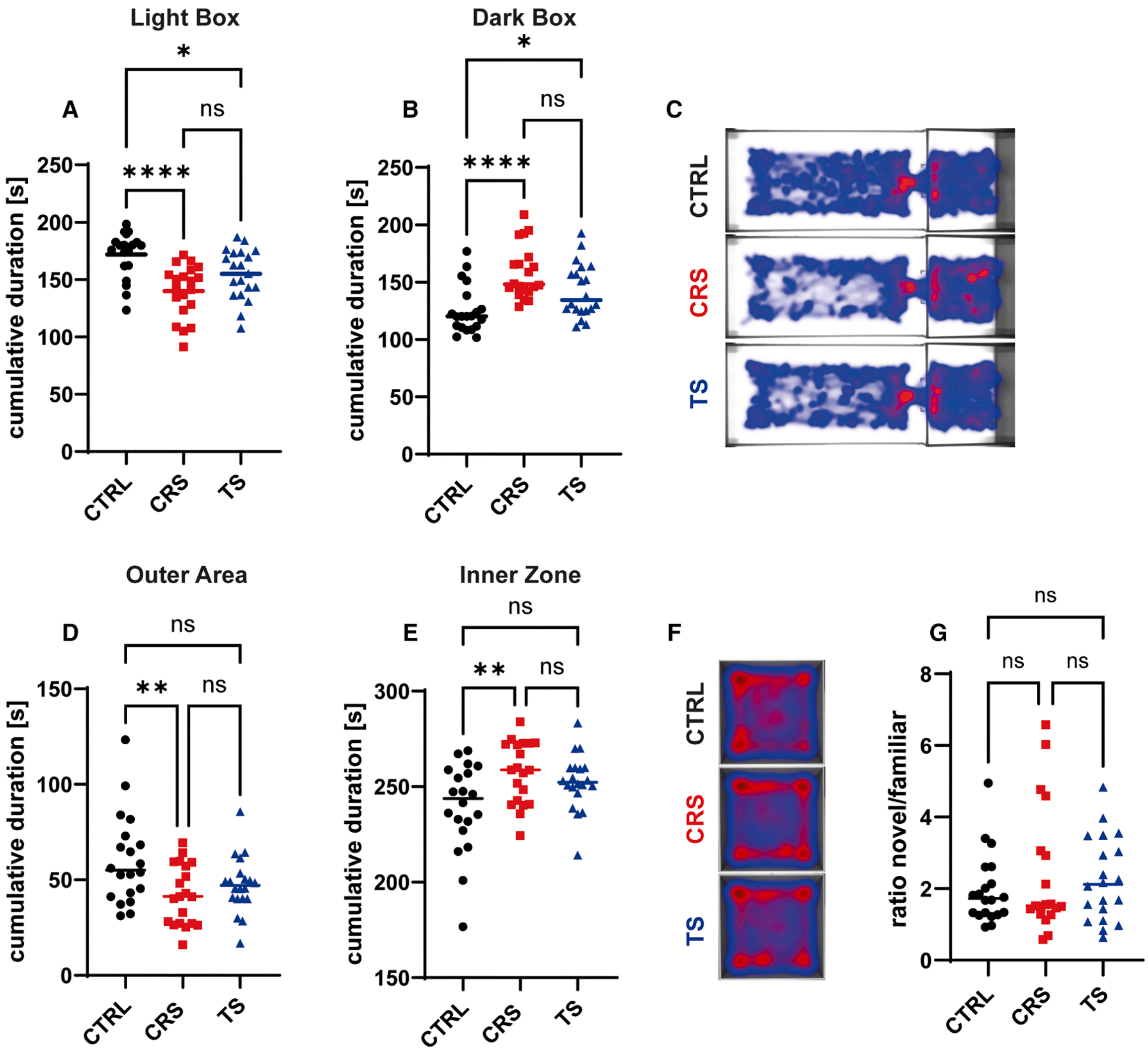

Figure 3. Chronic stress transmission reduces exploratory behavior in mice

(A and B) Male (*n* = 10 per group) and female (*n* = 10 per group) C57BL/6 mice were subjected to chronic restraint stress (CRS, red squares), stress transmission (TS, blue triangles), or control treatment (CTRL, black dots) for 10 consecutive days. On day 10 of the stress procedure, mice were placed in a light-dark box arena and tested for exploratory behavior for 5 min. The cumulative duration spent in the light box (A) or dark box (B) was quantified by automated video tracking.

(C–E) Merged trials per group of one representative experiment are shown as heatmaps. On day 8 of the stress procedure, mice were placed in an open field arena and tested for exploratory behavior for 5 min. The cumulative duration spent in the inner zone (D) or outer area (E) of the maze was quantified by automated video tracking.

(F) Merged trials per group of one representative experiment are shown as heatmaps. (A, B, D, and E) Pooled data of five independent experiments are presented as means ± SD.

Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison test; *$p < 0.05$, **$p < 0.01$, and ****$p < 0.0001$; ns, not significant.

(G) On day 9 of the stress procedure, mice were tested for cognitive performance in a novel object recognition test. Mice were trained for the familiar objects for 10 min. After 5 min, mice were tested for the novel object recognition for 5 min. Data of five independent experiments are presented as ratio of the cumulative durations exploring the novel object and the familiar object is presented. Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison test; ns, not significant.

in male mice. Nevertheless, inducing stress in female individuals has proven to be more challenging, although several groups reported successful attempts in females.[29–31] In the present study, we established a paradigm for stress transmission in a group of mice based on restraint stress. Applying restraint stress allows for reproducible induction of physiological stress responses in a selected number of individuals of the group independent of sex differences. Furthermore, stress induction via immobilization in restraint tubes avoids inevitable injuries from bite wounds in dominance-based stress models. Bite wounds can potentially influence the scientific outcomes,[32] which is of special relevance in studies involving immune modulation. With the applied model,

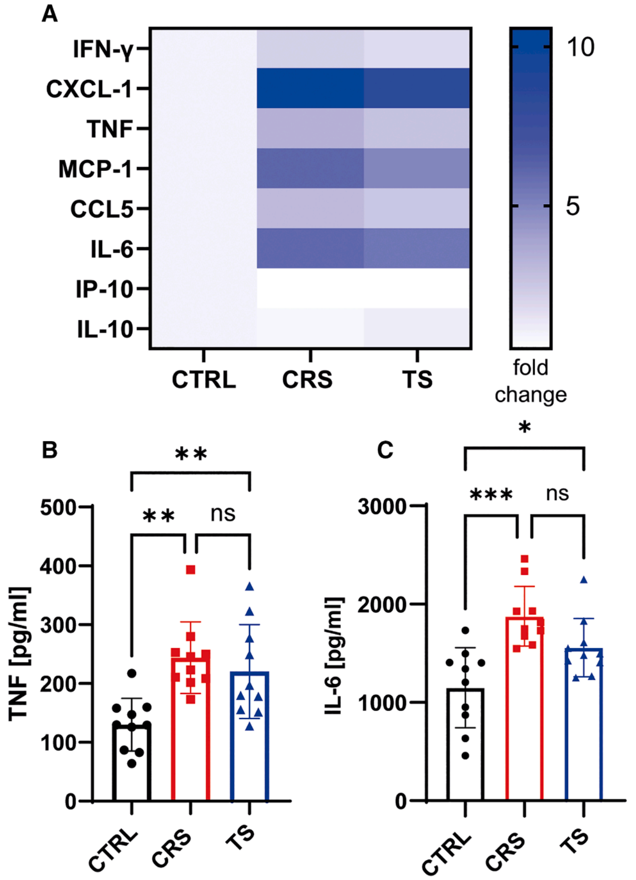

Figure 4. Chronic stress transmission induces elevated cytokine secretion upon LPS stimulation

Male ($n = 5$ per group) and female ($n = 5$ per group) C57BL/6 mice were subjected to chronic restraint stress, stress transmission, or control treatment for 10 consecutive days. On day 11, splenocytes were harvested and stimulated *in vitro* for 75 h with LPS. Supernatants were screened for cytokine secretion using a flow cytometry based multiplex assay (A). Pooled data of two independent experiments are presented as heatmap plot of fold change relative to controls. TNF (B) or IL-6 (C) levels in the supernatants of chronically restraint stressed (CRS, red squares, $n = 10$) mice or mice subjected to stress transmission (TS, blue triangles, $n = 10$) or control treated mice (CTRL, black dots, $n = 10$) were quantified by ELISA. Data of three independent experiments are presented as means ± SD.

Statistical significance was analyzed by one-way ANOVA followed by Tukey's multiple comparison test; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$; ns, not significant.

reproducible evidence for stress transmission in groups of mice could be found on a physiological, behavioral, and immunological level.

The physiological stress response has been shown to have a major impact on physiology, behavior, and immune function. Extensive research on stress responses in mice has offered insights with potential relevance to human physiology.[33] Developing a mouse model for investigating stress transmission within groups could provide a better understanding of how physiological states, notably stress, are contagiously spread. Our findings demonstrate a significant impact of stress transmission following a single incidence of stress exposure. Acute, short-term stress has been shown to induce adrenal gland hyperplasia and thymus atrophy[34] alongside pro-inflammatory activation within innate immunity.[35] Although we were able to demonstrate a significant impact of transmitted stress in an acute, short-term setting, the overall effects observed were relatively modest. This was true not only for the mice exposed to transmitted stress but also for those subjected to direct stress. To investigate the phenomenon of stress transmission in greater depth and to amplify the physiological effects observed, we therefore transitioned to a chronic restraint paradigm designed to induce prolonged stress exposure and, consequently, chronic stress transmission. Indeed, within a chronic stress transmission paradigm, both the direct effects of stress and those of transmitted stress were markedly amplified. This approach allowed us to delineate clear differences in the stress responses of individuals exposed to direct stress versus those experiencing stress indirectly through transmission. Chronic stress is well known to be associated with substantial negative health outcomes.[33] A mouse model of chronic stress transmission thus offers a valuable tool for understanding—and potentially mitigating—the negative effects of stressful social environments.

By applying the developed paradigm for chronic stress transmission, we were able to demonstrate a transmission of stress on a physiological level. Manifestations such as adrenal hypertrophy and elevated plasma CORT levels are hallmarks of a stress response in mice. These effects were significantly more pronounced in mice subjected to stress transmission compared to controls and were similar in magnitude to values reported in previous studies.[36] Notably, we observed sex-based differences in the response to transmitted stress. While stress transmission was evidenced in both sexes, females seemed to be more susceptible to the transmitted stress, showing similarly increased adrenal glands as chronically stressed mice and displayed a reduced weight gain compared to controls. In contrast, male TS mice did not show significant changes in body weight development and the adrenal gland hypertrophy, although noticeable higher compared to controls, did not match the levels of the CRS group. Indeed, minor sex differences in susceptibility to stress have been highlighted in previous studies in mice.[37] Furthermore, it has been reported that female mice display more empathy-like behaviors than their male counterparts,[38,39] potentially increasing their vulnerability to stress transmission. Empathy plays a crucial role in the spread of stress and its effects on the well-being of conspecifics. For example, in non-human primates, elevated body temperatures have been observed in response to the vocalizations of stressed individuals or the observation of social conflict.[40,41] Despite the pronounced physiological effects of stress transmission-reflected in increased adrenal gland weight and elevated plasma CORT levels-its impact did not extend to thymic mass or thymocyte development. Interestingly, while directly stressed mice exhibited significant thymic atrophy and a shift in thymocyte populations characterized by a reduction in $CD4^{+}CD8^{+}$ double-positive cells, mice exposed to transmitted stress remained unaffected. This is particularly notable given the high sensitivity of thymocytes, especially the double-positive population, to glucocorticoids.[42] Although, measured plasma CORT levels of TS mice were not significantly

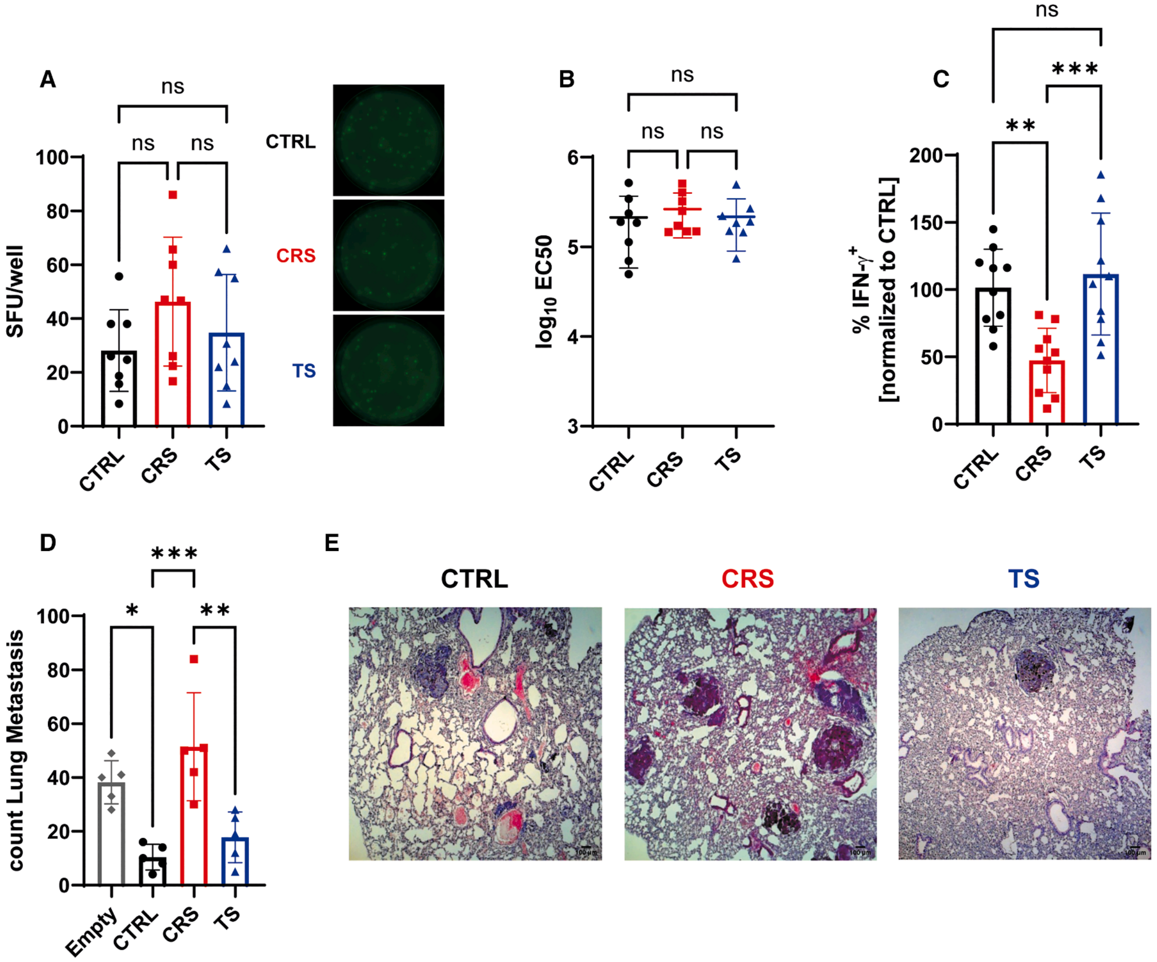

Figure 5. Adaptive immune response is not affected by stress transmission

(A) C57BL/6 mice ($n = 8$ per group [4 female, 4 male]) were subjected to chronic restraint stress (CRS, red squares), stress transmission (TS, blue triangles), or control treatment (CTRL, black dots) for 10 consecutive days. On day 5, mice were immunized subcutaneously with 5 mg PLGA MP Ova/Riboxxim. On day 21, bone marrow was harvested and the amount of Ova-specific IgG secreting cells was analyzed by FLUOROSPOT assay (A). Representative wells are shown per group. Pooled data of two independent experiments are presented as means ± SD.

(B) Blood serum was analyzed for Ova-specific antibody titers by ELISA. Pooled data of two independent experiments are presented as geometric means ± geometric SD.

(C) C57BL/6 mice ($n = 10$ per group [5 female, 5 male]) were subjected to chronic restraint stress (CRS, red squares), stress transmission (TS, blue triangles), or control treatment (CTRL, black dots) for 10 consecutive days. On day 5, mice were immunized subcutaneously with 5 mg PLGA MP Ova/Riboxxim. On day 11, splenocytes were harvested and Ova-specific CTL responses were analyzed by intracellular cytokine staining of CD8$^+$ for IFNγ followed by flow cytometry.

(D) Male C57BL/6 mice ($n = 5$ per group) were subjected to chronic restraint stress (CRS, red squares), stress transmission (TS, blue triangles), or control treatment (CTRL, black dots) for 10 consecutive days. On day 5, mice were double-route immunized subcutaneously with 4 mg and intranasally with 1 mg PLGA MP Ova/Riboxxim or empty PLGA MP (empty, gray diamonds). On day 11, mice were inoculated with 1×10^5 B16-Ova tumor cells intravenously. 21 days post-tumor cell administration, lungs were harvested and formed metastasis were counted. Pooled data of two independent experiments are presented as means ± SD.

(E) Representative H&E-stained tissue slides of lungs. Scale bars represent 100 μm.

Statistical significance was analyzed by one-way ANOVA followed by Tukey's multiple comparison test; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$; ns, not significant.

lower than those of CRS mice at the endpoint of blood sampling, the highly elevated CORT levels during or shortly after the stress procedure in the CRS mice is not accounted for. Arguably, these high peak concentrations over the limited time spans when mice are actively stressed by restraint and rapidly decline within minutes to few hours, are the limiting factors for thymus atrophy.[43] Furthermore, the release of catecholamines may contribute to the CORT sensitivity in CRS mice compared to TS mice in a direct or indirect manner via the induction of glucocorticoid receptor upregulation.[44,45] We therefore propose that stress transmission induces a low-grade, persistent stress state in recipient mice. The hypothesis of a mild physiological stress response as consequence of stress transmission is supported by the observed subtle changes in body weight and the impact on anxiety-like behavior. Here, the effects were noted to be intermediate when comparing to control mice on the one hand and mice that were subjected to chronic stress on the other. Recently, a study on stress contagion in humans yielded similar findings. Observers of participants undergoing the Trier social stress test (TSST) exhibited a secondary stress response, showing a mild yet significant elevation in saliva corticosterone levels and heart rate. These increases, while notable, did not achieve the levels observed in individuals directly exposed to TSST.[27] Taken together, these findings suggest that stress transmission induces a mild yet sustained physiological stress response, characterized by distinct endocrine alterations and measurable behavioral changes. Notably, despite these effects, thymic development remained unaffected.

This raises the question of whether a low-grade stress state is sufficient to modulate immune function, particularly in consideration of the well-established sensitivity of the immune system to physiological stress. On the one hand, stress can be beneficial for an individual's defense against pathogens, chronic exposure to stress, however, is often associated with immunopathology.[46] Chronic inflammation and inflammatory disease progression, mediated by monocytes, were shown in stress exposed mice and humans. This is underpinned by a transition toward an inflammatory gene expression profile and enhanced sensitivity to toll-like receptor activation.[10] We could show that even mild physiological stress by the phenomenon of chronic stress transmission is sufficient to induce elevated cytokine secretion in response to TLR-4 stimulation by LPS. Remarkably, the effect was on a similar level as that observed in mice subjected to chronic restraint stress. This suggests that stress transmission could play a role in the development and progression of systemic inflammatory diseases, emphasizing its implications for public health. In contrast, no effect of stress transmission on the generation of an antigen-specific immune response was observed. The PLGA-based vaccine platform employed in this study has been shown to induce robust and consistent T-helper I (Th-1) biased antigen specific immune responses, demonstrating effective immunotherapeutic potential as cancer vaccine.[47] While chronic restraint stress led to a 50% decrease in OVA-antigen specific CTL responses generated by the vaccine, the response in TS mice remained unaffected. To investigate the effect of stress and stress transmission on the effector function of the generated T cell response, we applied a challenge with B16 cancer cells expressing OVA in a model for metastasis formation. The efficacy of the responses in the immunotherapeutic mouse model for B16-OVA melanoma metastasis formation remained unaffected in mice experiencing stress transmission. Notably, the cancer vaccine displayed no therapeutic benefit in mice subjected to chronic restraint stress, confirming a study from our group outlining the impact of chronic stress on the efficacy of cancer vaccine-based immunotherapy.[48] Interestingly, no impact on antigen-specific antibody responses to the vaccine, both in mice subjected to stress transmission and those under active stress was found in our study. This outcome diverges with previous studies on chronic stress and vaccination efficacy.[13,49] The adjuvant Riboxxim, employed in this study stimulates Th-1 biased immune responses. Previous findings suggest that under chronic stress conditions, immune responses are prone to shift toward Th-2 dominated responses, typically associated with enhanced antibody production and diminished cellular immunity.[50] Further investigations on the immune response elicited under stressed conditions need to be conducted in order to shed light onto the dynamics of chronic stress, stress transmission, and adaptive immunity.

A limitation of our study is the absence of long-term follow up beyond the active stress or stress transmission period. While our findings demonstrate robust physiological and behavioral changes during and immediately after the 10-day stress procedure, it remains to be determined whether these effects persist or normalize over time. This question is particularly relevant since chronic stress-related pathologies in humans can involve sustained dysregulation even after the stressor has been removed.[51] Although our study was not designed to fully capture such long-term effects, future studies examining the duration and reversibility of stress transmission will help to clarify whether this paradigm induces lasting physiological or behavioral alterations.

In conclusion, we have established a paradigm for the investigation of transmission of stress in groups of mice, uncovering strong evidence of stress contagion across physiological, behavioral, and immunological dimensions. These results show potential health implications of stress transmission, drawing parallels with observed outcomes in human studies.

Limitations of the study

While our findings demonstrate clear physiological and immune effects of stress transmission in mice, our study is limited by the lack of long-term follow up beyond the 10-day stress procedure. It remains unclear whether the observed effects persist or resolve over time. Additionally, while we identify sex-specific differences, further mechanistic studies are needed to clarify the underlying biological mechanisms. Finally, the study focuses on one specific model of stress transmission, and its generalizability to other social stress paradigms remains to be determined.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Michael Basler (michael.basler@uni-konstanz.de).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report new code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

D.H., conceptualization, methodology, validation, formal analysis, investigation, writing—original draft, visualization, and project administration; D.M., conceptualization, methodology, validation, formal analysis, investigation, and visualization; K.S., investigation; K.I., investigation, and writing—reviewing and editing; P.H.W., validation and supervision; M.B., validation, supervision, and writing—reviewing and editing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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SUPPLEMENTAL INFORMATION

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REFERENCES

1. Seo, J.S., Wei, J., Qin, L., Kim, Y., Yan, Z., and Greengard, P. (2017). Cellular and molecular basis for stress-induced depression. Mol. Psychiatry *22*, 1440–1447. https://doi.org/10.1038/mp.2016.118.
2. Yan, L., Yang, F., Wang, Y., Shi, L., Wang, M., Yang, D., Wang, W., Jia, Y., So, K.F., and Zhang, L. (2024). Stress increases hepatic release of lipocalin 2 which contributes to anxiety-like behavior in mice. Nat. Commun. *15*, 3034. https://doi.org/10.1038/s41467-024-47266-9.
3. Song, H., Fang, F., Arnberg, F.K., Mataix-Cols, D., Fernández de la Cruz, L., Almqvist, C., Fall, K., Lichtenstein, P., Thorgeirsson, G., and Valdimarsdóttir, U.A. (2019). Stress related disorders and risk of cardiovascular disease: population based, sibling controlled cohort study. BMJ *365*, l1255. https://doi.org/10.1136/bmj.l1255.
4. Dai, S., Mo, Y., Wang, Y., Xiang, B., Liao, Q., Zhou, M., Li, X., Li, Y., Xiong, W., Li, G., et al. (2020). Chronic Stress Promotes Cancer Development. Front. Oncol. *10*, 1492. https://doi.org/10.3389/fonc.2020.01492.
5. Tran, I., and Gellner, A.K. (2023). Long-term effects of chronic stress models in adult mice. J. Neural Transm. *130*, 1133–1151. https://doi.org/10.1007/s00702-023-02598-6.
6. Biltz, R.G., Sawicki, C.M., Sheridan, J.F., and Godbout, J.P. (2022). The neuroimmunology of social-stress-induced sensitization. Nat. Immunol. *23*, 1527–1535. https://doi.org/10.1038/s41590-022-01321-z.
7. Cohen, S., Janicki-Deverts, D., Doyle, W.J., Miller, G.E., Frank, E., Rabin, B.S., and Turner, R.B. (2012). Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. Proc. Natl. Acad. Sci. USA *109*, 5995–5999. https://doi.org/10.1073/pnas.1118355109.
8. Sommershof, A., Basler, M., Riether, C., Engler, H., and Groettrup, M. (2011). Attenuation of the cytotoxic T lymphocyte response to lymphocytic choriomeningitis virus in mice subjected to chronic social stress. Brain Behav. Immun. *25*, 340–348. https://doi.org/10.1016/j.bbi.2010.10.016.
9. Morey, J.N., Boggero, I.A., Scott, A.B., and Segerstrom, S.C. (2015). Current Directions in Stress and Human Immune Function. Curr. Opin. Psychol. *5*, 13–17. https://doi.org/10.1016/j.copsyc.2015.03.007.
10. Barrett, T.J., Corr, E.M., van Solingen, C., Schlamp, F., Brown, E.J., Koelwyn, G.J., Lee, A.H., Shanley, L.C., Spruill, T.M., Bozal, F., et al. (2021). Chronic stress primes innate immune responses in mice and humans. Cell Rep. *36*, 109595. https://doi.org/10.1016/j.celrep.2021.109595.
11. Heidt, T., Sager, H.B., Courties, G., Dutta, P., Iwamoto, Y., Zaltsman, A., von Zur Muhlen, C., Bode, C., Fricchione, G.L., Denninger, J., et al. (2014). Chronic variable stress activates hematopoietic stem cells. Nat. Med. *20*, 754–758. https://doi.org/10.1038/nm.3589.
12. Mills, P.J., Yu, H., Ziegler, M.G., Patterson, T., and Grant, I. (1999). Vulnerable caregivers of patients with Alzheimer's disease have a deficit in circulating CD62L- T lymphocytes. Psychosom. Med. *61*, 168–174. https://doi.org/10.1097/00006842-199903000-00008.
13. Segerstrom, S.C., and Miller, G.E. (2004). Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychol. Bull. *130*, 601–630. https://doi.org/10.1037/0033-2909.130.4.601.
14. Blons, E., Arsac, L.M., Grivel, E., Lespinet-Najib, V., and Deschodt-Arsac, V. (2021). Physiological Resonance in Empathic Stress: Insights from Nonlinear Dynamics of Heart Rate Variability. Int. J. Environ. Res. Public Health *18*, 2081. https://doi.org/10.3390/ijerph18042081.
15. Park, J., Carrillo, B., and Mendes, W.B. (2021). Is vicarious stress functionally adaptive? Perspective-taking modulates the effects of vicarious stress on future firsthand stress. Emotion *21*, 1131–1143. https://doi.org/10.1037/emo0000963.
16. Dimitroff, S.J., Kardan, O., Necka, E.A., Decety, J., Berman, M.G., and Norman, G.J. (2017). Physiological dynamics of stress contagion. Sci. Rep. *7*, 6168. https://doi.org/10.1038/s41598-017-05811-1.
17. Brandl, H.B., Pruessner, J.C., and Farine, D.R. (2022). The social transmission of stress in animal collectives. Proc. Biol. Sci. *289*, 20212158. https://doi.org/10.1098/rspb.2021.2158.
18. Fraser, O.N., Stahl, D., and Aureli, F. (2008). Stress reduction through consolation in chimpanzees. Proc. Natl. Acad. Sci. USA *105*, 8557–8562. https://doi.org/10.1073/pnas.0804141105.
19. Sterley, T.L., Baimoukhametova, D., Füzesi, T., Zurek, A.A., Daviu, N., Rasiah, N.P., Rosenegger, D., and Bains, J.S. (2018). Social transmission and buffering of synaptic changes after stress. Nat. Neurosci. *21*, 393–403. https://doi.org/10.1038/s41593-017-0044-6.
20. Furtbauer, I., and Heistermann, M. (2016). Cortisol coregulation in fish. Sci. Rep. *6*, 30334. https://doi.org/10.1038/srep30334.
21. Carnevali, L., Montano, N., Tobaldini, E., Thayer, J.F., and Sgoifo, A. (2020). The contagion of social defeat stress: Insights from rodent studies. Neurosci. Biobehav. Rev. *111*, 12–18. https://doi.org/10.1016/j.neubiorev.2020.01.011.
22. Gruver, A.L., and Sempowski, G.D. (2008). Cytokines, leptin, and stress-induced thymic atrophy. J. Leukoc. Biol. *84*, 915–923. https://doi.org/10.1189/jlb.0108025.

23. Horvath, D., and Basler, M. (2023). PLGA Particles in Immunotherapy. Pharmaceutics *15*, 615. https://doi.org/10.3390/pharmaceutics15020615.
24. Davila Ross, M., Menzler, S., and Zimmermann, E. (2008). Rapid facial mimicry in orangutan play. Biol. Lett. *4*, 27–30. https://doi.org/10.1098/rsbl.2007.0535.
25. Engert, V., Linz, R., and Grant, J.A. (2019). Embodied stress: The physiological resonance of psychosocial stress. Psychoneuroendocrinology *105*, 138–146. https://doi.org/10.1016/j.psyneuen.2018.12.221.
26. Mancini, G., Ferrari, P.F., and Palagi, E. (2013). Rapid facial mimicry in geladas. Sci. Rep. *3*, 1527. https://doi.org/10.1038/srep01527.
27. Auer, A., Walther, L.M., Jendryczko, D., Auer, L., and Wirtz, P.H. (2024). Is your stress my stress? A standardized, randomized-controlled paradigm to study physiological stress contagion based on direct stress observation. Psychoneuroendocrinology *162*, 106964. https://doi.org/10.1016/j.psyneuen.2024.106964.
28. Meyza, K.Z., Bartal, I.B.A., Monfils, M.H., Panksepp, J.B., and Knapska, E. (2017). The roots of empathy: Through the lens of rodent models. Neurosci. Biobehav. Rev. *76*, 216–234. https://doi.org/10.1016/j.neubiorev.2016.10.028.
29. Harris, A.Z., Atsak, P., Bretton, Z.H., Holt, E.S., Alam, R., Morton, M.P., Abbas, A.I., Leonardo, E.D., Bolkan, S.S., Hen, R., and Gordon, J.A. (2018). A Novel Method for Chronic Social Defeat Stress in Female Mice. Neuropsychopharmacology *43*, 1276–1283. https://doi.org/10.1038/npp.2017.259.
30. Iniguez, S.D., Flores-Ramirez, F.J., Riggs, L.M., Alipio, J.B., Garcia-Carachure, I., Hernandez, M.A., Sanchez, D.O., Lobo, M.K., Serrano, P.A., Braren, S.H., and Castillo, S.A. (2018). Vicarious Social Defeat Stress Induces Depression-Related Outcomes in Female Mice. Biol. Psychiatry *83*, 9–17. https://doi.org/10.1016/j.biopsych.2017.07.014.
31. van Doeselaar, L., Yang, H., Bordes, J., Brix, L., Engelhardt, C., Tang, F., and Schmidt, M.V. (2021). Chronic social defeat stress in female mice leads to sex-specific behavioral and neuroendocrine effects. Stress (Luxemb) *24*, 168–180. https://doi.org/10.1080/10253890.2020.1864319.
32. Varholick, J.A., Godinez, G., Jenkins, A., Mobin, S., and Maden, M. (2023). Bite Wounds and Dominance Structures in Male and Female African Spiny Mice (Acomys cahirinus): Implications for Animal Welfare and the Generalizability of Experimental Results. Animals. *14*, 64. https://doi.org/10.3390/ani14010064.
33. Mariotti, A. (2015). The effects of chronic stress on health: new insights into the molecular mechanisms of brain-body communication. Future Sci. OA *1*, FSO23. https://doi.org/10.4155/fso.15.21.
34. Reber, S.O., Birkeneder, L., Veenema, A.H., Obermeier, F., Falk, W., Straub, R.H., and Neumann, I.D. (2007). Adrenal insufficiency and colonic inflammation after a novel chronic psycho-social stress paradigm in mice: implications and mechanisms. Endocrinology *148*, 670–682. https://doi.org/10.1210/en.2006-0983.
35. Tang, L., Cai, N., Zhou, Y., Liu, Y., Hu, J., Li, Y., Yi, S., Song, W., Kang, L., and He, H. (2022). Acute stress induces an inflammation dominated by innate immunity represented by neutrophils in mice. Front. Immunol. *13*, 1014296. https://doi.org/10.3389/fimmu.2022.1014296.
36. Laber, K., Veatch, L.M., Lopez, M.F., Mulligan, J.K., and Lathers, D.M.R. (2008). Effects of Housing Density on Weight Gain, Immune Function, Behavior, and Plasma Corticosterone Concentrations in BALB/c and C57BL/6 Mice. J. Am. Assoc. Lab. Anim. Sci. *47*, 16–23.
37. Johnson, A., Rainville, J.R., Rivero-Ballon, G.N., Dhimitri, K., and Hodes, G.E. (2021). Testing the Limits of Sex Differences Using Variable Stress. Neuroscience *454*, 72–84. https://doi.org/10.1016/j.neuroscience.2019.12.034.
38. Langford, D.J., Tuttle, A.H., Brown, K., Deschenes, S., Fischer, D.B., Mutso, A., Root, K.C., Sotocinal, S.G., Stern, M.A., Mogil, J.S., and Sternberg, W.F. (2010). Social approach to pain in laboratory mice. Soc. Neurosci. *5*, 163–170. https://doi.org/10.1080/17470910903216609.
39. Laviola, G., Zoratto, F., Ingiosi, D., Carito, V., Huzard, D., Fiore, M., and Macrì, S. (2017). Low empathy-like behaviour in male mice associates with impaired sociability, emotional memory, physiological stress reactivity and variations in neurobiological regulations. PLoS One *12*, e0188907. https://doi.org/10.1371/journal.pone.0188907.
40. Dezecache, G., Zuberbühler, K., Davila-Ross, M., and Dahl, C.D. (2017). Skin temperature changes in wild chimpanzees upon hearing vocalizations of conspecifics. R. Soc. Open Sci. *4*, 160816. https://doi.org/10.1098/rsos.160816.
41. Kano, F., Hirata, S., Deschner, T., Behringer, V., and Call, J. (2016). Nasal temperature drop in response to a playback of conspecific fights in chimpanzees: A thermo-imaging study. Physiol. Behav. *155*, 83–94. https://doi.org/10.1016/j.physbeh.2015.11.029.
42. Khedri, M., Samei, A., Fasihi-Ramandi, M., and Taheri, R.A. (2020). The immunopathobiology of T cells in stress condition: a review. Cell Stress Chaperones *25*, 743–752. https://doi.org/10.1007/s12192-020-01105-0.
43. Hare, B.D., Beierle, J.A., Toufexis, D.J., Hammack, S.E., and Falls, W.A. (2014). Exercise-associated changes in the corticosterone response to acute restraint stress: evidence for increased adrenal sensitivity and reduced corticosterone response duration. Neuropsychopharmacology *39*, 1262–1269. https://doi.org/10.1038/npp.2013.329.
44. Engler, H., and Stefanski, V. (2003). Social stress and T cell maturation in male rats: transient and persistent alterations in thymic function. Psychoneuroendocrinology *28*, 951–969. https://doi.org/10.1016/s0306-4530(02)00117-8.
45. Schmidt, P., Holsboer, F., and Spengler, D. (2001). Beta(2)-adrenergic receptors potentiate glucocorticoid receptor transactivation via G protein beta gamma-subunits and the phosphoinositide 3-kinase pathway. Mol. Endocrinol. *15*, 553–564. https://doi.org/10.1210/mend.15.4.0613.
46. Dhabhar, F.S. (2014). Effects of stress on immune function: the good, the bad, and the beautiful. Immunol. Res. *58*, 193–210. https://doi.org/10.1007/s12026-014-8517-0.
47. Koerner, J., Horvath, D., Herrmann, V.L., MacKerracher, A., Gander, B., Yagita, H., Rohayem, J., and Groettrup, M. (2021). PLGA-particle vaccine carrying TLR3/RIG-I ligand Riboxxim synergizes with immune checkpoint blockade for effective anti-cancer immunotherapy. Nat. Commun. *12*, 2935. https://doi.org/10.1038/s41467-021-23244-3.
48. Sommershof, A., Scheuermann, L., Koerner, J., and Groettrup, M. (2017). Chronic stress suppresses anti-tumor T(CD8+) responses and tumor regression following cancer immunotherapy in a mouse model of melanoma. Brain Behav. Immun. *65*, 140–149. https://doi.org/10.1016/j.bbi.2017.04.021.
49. Dragos, D., and Tanasescu, M.D. (2010). The effect of stress on the defense systems. J. Med. Life *3*, 10–18.
50. Glaser, R., MacCallum, R.C., Laskowski, B.F., Malarkey, W.B., Sheridan, J.F., and Kiecolt-Glaser, J.K. (2001). Evidence for a shift in the Th-1 to Th-2 cytokine response associated with chronic stress and aging. J. Gerontol. A Biol. Sci. Med. Sci. *56*, M477–M482. https://doi.org/10.1093/gerona/56.8.m477.
51. Juster, R.P., McEwen, B.S., and Lupien, S.J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci. Biobehav. Rev. *35*, 2–16. https://doi.org/10.1016/j.neubiorev.2009.10.002.
52. Inholz, K., Bader, U., Mundt, S., and Basler, M. (2024). The Significant Role of PA28αβ in CD8+ T Cell-Mediated Graft Rejection Contrasts with Its Negligible Impact on the Generation of MHC-I Ligands. Int. J. Mol. Sci. *25*, 5649. https://doi.org/10.3390/ijms25115649.
53. Oliveri, F., Basler, M., Rao, T.N., Fehling, H.J., and Groettrup, M. (2022). Immunoproteasome Inhibition Reduces the T Helper 2 Response in Mouse Models of Allergic Airway Inflammation. Front. Immunol. *13*, 870720. https://doi.org/10.3389/fimmu.2022.870720.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
APC anti-mouse CD8a Antibody	BioLegend	Cat#100712; RRID:AB_2621550
Brilliant Violet 421^{TM} anti-mouse IFN-γ Antibody	BioLegend	Cat#505830; RRID:AB_2563105
PE anti-mouse CD4 Antibody	BioLegend	Cat#100408; RRID:AB_312692
Goat anti-mouse IgG, ALP	MabTech	Cat#3310-4-1000
Biotin Goat anti-mouse IgG (minimal x-reactivity) Antibody	BioLegend	Cat#405303; RRID:AB_315006
BD Pharmingen^{TM} FITC Streptavidin	BD Bioscience	Cat#554060; RRID:AB_10053373
Chemicals, peptides, and recombinant proteins		
Albumin from chicken egg white	Merck	Cat#A2512-1G
OVA (257–264) (H-2K^b) (SIINFEKL)	peptides&elephants	Cat# EP01994_
Critical commercial assays		
Corticosterone ELISA kit	Enzo Life Sciences	Cat#ADI-900-097
Mouse IL-6 ELISA Ready-SET-Go!^{TM} Kit	Invitrogen eBioscience	Cat#12364003
Mouse TNF ELISA Ready-SET-Go!^{TM} Kit	Invitrogen eBioscience	Cat#15591107
LEGENDplex^{TM} Mouse Anti-Virus Response Panel (13-plex)	BioLegend	Cat#740621
Experimental models: Cell lines		
B16-MO5 (Male C57BL/6 mouse)	TU Munich, Germany	RRID:CVCL_WM77
Experimental models: Organisms/strains		
C57BL/6 mice	Charles River	
Software and algorithms		
EthoVision^{TM} XT 15	Noldus	
Prism 10	GraphPad	
FlowJo^{TM} v10.9	BD Bioscience	
SPSS Statistics v29.01	IBM	
Other		
Resomer® RG 502 H, Poly(D,L-lactide-*co*-glycolide)	Evonik	Cat#B6013-1
Riboxxim^{TM}	Riboxx Pharmaceuticals	
Lipopolysaccharides from *Escherichia coli* O111:B4	Sigma	Cat# L2630
Brefeldin A	Merck	Cat#B6542
BD Cytofix/Cytoperm^{TM} Fixation/Permeabilization Kit	BD Bioscience	Cat#554722
pNPP for ALP	MabTech	Cat#3652-P10
FluoroSpot enhancer	MabTech	Cat#3641-F10
Immunospot S6 Ultra M2	C.T.L.	

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice

C57BL/6J mice were originally purchased from Charles River (Sulzfeld, Germany) and further bred in the accredited animal facility of the University of Konstanz. The animals were kept under specific pathogen-free conditions in air-conditioned rooms with controlled temperature of 22°C and 55% relative humidity on a 12-h light/dark cycle with lights switched on at 7 a.m. The room was kept under constant ventilation (17 air changes/h). Animals had *ad libitum* access to standard, autoclaved laboratory animal diet and tap water. Male and female mice were used at 8–12 weeks of age, when their immune system is fully developed. For endpoint analysis, animals were euthanized by CO_2 hypoxia. Euthanasia of mice was performed during the light phase (2–3 h after lights turned on). Behavioral tests were performed during the dark phase of the light/dark cycle (2–4 h after lights turned off). This study examined male and female mice; sex-specific differences are reported.

Randomization and blinding

Mice were housed in groups of four mice per cage, cages were randomly assigned into groups. Behavioral testing and analysis of tissue from mice was done in a blinded manner.

Ethics statement

Animal experiments have been conducted in compliance with ethical standards of German and EU guidelines after approval by the animal experimentation ethics committee of the Review Board of the Governmental Presidium Freiburg, Germany with the approval numbers G-20/113, G-22/075, and G-24/046.

Cell lines

The OVA expressing murine melanoma cell line (MO5, male C57BL/6 mouse) was kindly provided by Dr. Antje Heit (TU Munich, Germany). Cells were cultured in DMEM supplemented with 10% (v/v) FCS, 100 IU/mL Penicillin, and 100 μg/mL Streptomycin. All cells were cultured at 37°C with 5% CO_2 in a humidified atmosphere. For detaching, cells were incubated with 0.05% trypsin-EDTA for 5 min at 37°C. The cell line was not further authenticated beyond source verification but was morphologically consistent with published descriptions. Cells were not tested for mycoplasma contamination.

METHOD DETAILS

Restraint stress/stress transmission procedure

Male and female C57BL/6 mice were randomized into the following groups: control (CTRL), acute restraint stress (ARS), chronic restraint stress (CRS) and stress transmission (TS). Mice were housed in groups of four mice per cage (four CTRL or two ARS with two ATS or two CRS with two CTS). During the stress procedure, restraint stress groups were separated spatially and visually from the stress transmission groups by introducing an opaque acrylic glass wall into the housing cage. CTRL mice were divided into pairs by introducing an opaque acrylic glass wall into the housing cage during the stress procedure. Stress induction was achieved by horizontally immobilizing ARS or CRS mice for 4 h in ventilated restraint tubes during the light cycle. ARS mice underwent the procedure once, while CRS mice were subjected to daily restraint for 10 consecutive days. CTRL and stress transmission mice were left untreated during the stress procedure. Stress procedures were always conducted during the light phase of the light/dark cycle (2–3 h after lights turned on).

Open field test (OFT)

To assess anxiety-like and exploratory behavior in mice, an OFT was conducted on day 8 of the restraint stress/stress transmission procedure. The open field apparatus measures 55 × 55 × 55 cm and is divided into a center area (20 × 20 cm) and a peripheral outer area. A camera was placed 1.5 m above the apparatus for real-time analysis and recordings. Each mouse was introduced individually into the apparatus and their trajectories were recorded and analyzed using EthoVision XT 15 software (Noldus). The experiments were conducted in a quiet environment with male and female animals. During the measurements, at least 2 m distance was kept between the apparatus and the experimenter. To eliminate odors between trials, the apparatus was cleaned with 80% EtOH after each trial.

Novel object recognition test (nORT)

To test for recognition memory in mice, a nORT was conducted on day 9 of the restraint stress/stress transmission procedure. The experiment is performed in an open field apparatus of 55 × 55 × 55 cm with a camera installed 1.5 m above the apparatus for data collection. On the first day of the experiment, mice are introduced individually for a 5 min habituation phase into the open field apparatus. On day 2, two identical objects were introduced into the apparatus and each animal underwent a 5-min training phase to familiarize themselves with the objects. During a subsequent 5-min standby phase, the animals were transferred into a solitary housing cage and one object was replaced with a novel object of a different shape. In the following testing phase, the mice were reintroduced into the apparatus and exploration and trajectories were recorded and analyzed using EthoVision XT 15 software (Noldus). The experiments were conducted in a quiet environment with male and female animals. During the measurements, at least 2 m distance was kept between the apparatus and the experimenter. To eliminate odors between trials, the apparatus was cleaned with 80% EtOH after each trial.

Light dark box (LDB)

Light dark box tests were performed to assess anxiety-like and exploratory behavior in mice on day 10 of the restraint stress/stress transmission procedure. The LDB apparatus is comparted into a 40 × 20 cm light box and a 20 × 20 cm dark box. The light box was illuminated from the top (~900 lux), whereas the dark box is covered with a lid. An infrared lamp illuminated the bottom to facilitate camera tracking through the lid of the dark box. Both compartments were connected via an entrance gate into the dark box. A camera was placed 1.5 m above the apparatus for real-time analysis and recordings. Each mouse was introduced individually into the apparatus and their trajectories were recorded and analyzed using EthoVision XT 15 software (Noldus). The experiments were conducted in a quiet environment with male and female animals. During the measurements, at least 2 m distance was kept between the apparatus and the experimenter. To eliminate odors between trials, the apparatus was cleaned with 80% EtOH after each trial.

Preparation of PLGA microparticle vaccines

Poly(lactic-co-glycolic acid) microparticles were prepared by spray-drying as previously described.[47] Briefly, 50 mg OVA and 0.5 mg Riboxxim were dissolved in 1 mL of 0.1 M $NaHCO_3$ (aqueous phase) and 1 g PLGA was dissolved in 20 mL dichloromethane (organic phase). A water-in-oil emulsion was prepared with a digital microtip sonicator and immediately spray-dried using the Mini Spray-Dryer 290 (Büchi Labortechnik AG) with inlet/outlet temperatures of 24°C/22°C at a flow rate of 1 mL/min. Spray-dried MP were treated with 0.05% Poloxamer 188 (#P5556, Merck) and dried under vacuum at room temperature. MP were stored at 4°C under desiccation.

In vivo applications

For vaccination studies, female mice were immunized subcutaneously at the base of the tail with 5 mg Poly(lactic-co-glycolic acid) (PLGA) microparticles (MP) OVA/Riboxxim in PBS. For cancer immunotherapy vaccinations, PLGA MP application involved simultaneous subcutaneous and intranasal double route-immunization. Mice were anesthetized with isoflurane, and a subcutaneous injection of 4 mg PLGA MP OVA/Riboxxim along with an intranasal instillation of 1 mg PLGA MP OVA/Riboxxim in PBS was administered. On day 6 post PLGA MP application, mice were challenged intravenously with $1x10^5$ B16-OVA melanoma cells in PBS via the tail vein.

Flow cytometry of thymic populations

Mouse thymuses were isolated, and a single cell suspension was prepared by mechanical disruption. Thymocytes were depleted of erythrocytes and a surface staining was performed using PE-coupled anti-CD4 (clone GK1.5, #100407, BioLegend) and APC coupled anti-CD8α (clone 53-6.7, #17-0081-82 eBioscience) antibodies. Cells were subsequently fixed in 4% paraformaldehyde and analyzed with BD FACSLyric (BD Bioscience) or CytoFLEX (Beckman Coulter) instruments. Data were analyzed using FlowJo v10.9 software (BD Bioscience).

Intracellular cytokine staining (ICS) for IFNγ

Intracellular detection of antigen-specific IFNγ production of cytotoxic T lymphocytes was performed by ICS as previously described.[52] Shortly, freshly isolated splenocytes were incubated with or without 10^{-6} M SIINFEKL peptide for 5 h in the presence of 10 μg Brefeldin A (#B6542, Merck). Cells were subsequently stained with APC coupled anti-CD8α (clone 53-6.7, #17-0081-82, eBioscience) for 30 min at 4°C. Cells were fixed and permeabilized using a Cytofix/Cytoperm Kit (#554722, BD Bioscience) according to the manufacturer's protocol. Intracellular staining was performed with BV421 coupled anti-IFNγ (clone XGM1.2, #505829, BioLegend) overnight. $CD8^+IFN\gamma^+$ cells were analyzed by flow cytometry using a BD FACSLyric instrument. Data were analyzed using FlowJo v10.9 software (BD Bioscience). Gates were set using FMO-controls and unstimulated samples were used as a negative control, intra-assay CV was 0.08%.

Enzyme-linked immunosorbent assay (ELISA)

Antibody titers: OVA specific serum antibody titers were determined by ELISA. High-binding microplates (Greiner) were coated with 100 μg/mL OVA protein (Merck) in PBS overnight. Serial dilutions of mouse sera were prepared and loaded on the precoated plates at room temperature for 2 h. For detection, goat anti-mouse IgG, alkaline phosphatase (ALP) antibodies (MabTech) were used according to the manufacturer's instructions followed by colorimetric conversion of pNPP substrate (MabTech) for 1 h at room temperature. OD_{405nm} was determined using an infinite M200PRO plate reader (TECAN). Endpoint titers were defined as highest sample dilution exceeding the signal of the mean of the MP Empty control group + 3x standard deviation (SD). Antibody ELISA was performed without technical replicates.

CORT levels: Plasma corticosterone (CORT) levels were determined by competitive ELISA using a corticosterone ELISA kit (ADI-901-097, EnzoLifesciences) according to the manufacturer's instructions. Briefly, mouse plasma samples were diluted with Steroid Displacement Reagent to enable measurement of total CORT. Samples were loaded together with ALP conjugated CORT and sheep anti-CORT antibody onto precoated plates and incubated at room temperature for 2 h followed by colorimetric conversion of pNPP substrate for 1 h at room temperature. OD_{405nm} was determined using an infinite M200PRO plate reader (TECAN). Intra-assay coefficient of variability (CV) was calculated at 8.4%.

Cytokine levels: quantification of *in vitro* released IL-6 (intra-assay CV = 7.6%) and TNF (intra-assay CV = 8.2%) was performed by ELISA according to the manufacturer's instructions (ELISA Ready-SET-Go!, Invitrogen eBioscience). Briefly, freshly isolated splenocytes were depleted of erythrocytes and $1x10^6$ cells per well were stimulated in 96 well plates with 1 μg/mL LPS (E. coli O111:B4, Sigma) overnight. Supernatants were collected and loaded on precoated plates. Bound cytokines were then detected by the addition of biotin-conjugated detection antibodies, followed by incubation with a streptavidin-horseradish peroxidase (HRP) conjugate. TMB substrate was added for colorimetric conversion and stopped after 10 min by adding 1 M H_2SO_4. OD_{450nm} was determined using an infinite M200PRO plate reader (TECAN).

FLUOROSPOT assay

IgG production by OVA specific plasma cells was analyzed using FLUOROSPOT. Freshly isolated bone marrow derived cells were depleted of erythrocytes and $2x10^5$ cells per well were incubated in pre coated MultiScreenHTS PVDF Filter Plates (Merck Millipore) in triplicates at 37°C in a humidified CO_2 atmosphere for 16 h. Following the incubation with a biotinylated secondary antibody specific for IgG (Biolegend), a streptavidin–FITC conjugate was added (BD bioscience). Following a 1 h incubation at RT, a 15-min incubation step with FluoroSpot enhancer (MabTech) was performed. Plates were dried overnight, and automated spot analysis and quantification of the plates were performed using the ImmunoSpot S6ULTRA analyzer and ImmunoSpot software version 7.0.33.1 (C.T.L. Europe).

Multiplex cytokine screening

For the screening of cytokines released *in vitro*, multiplex screening (LEGENDplex, BioLegend) was performed according to the manufacturer's instructions. In brief, freshly isolated splenocytes were depleted of erythrocytes and $1x10^6$ cells per well were stimulated in 96 well plates with 1 μg/mL LPS (E. coli O111:B4, Sigma) overnight. Supernatants were collected and incubated with a capture antibody-bead cocktail for the corresponding cytokines for 2 h at room temperature. Subsequently, after 1 h incubation with biotin-labeled detection antibodies, an incubation step with streptavidin-PE for 30 min at room temperature was performed. Cytokine-levels were analyzed by flow cytometry using a BD FACSLyric instrument, no technical replicates were assessed. Data were analyzed using FlowJo v10.9 software (BD Bioscience).

H&E staining

Lungs were excised and immediately fixed in 10% neutral-buffered formalin for 24 hours followed by paraffin-embedding and sectioning to 5-μm-thick slices. H&E staining was performed according to standard protocols as previously described.[53] Results of three random fields per section were recorded with AxioObserver or AxioImager microscopes and ZEN 3.2 (blue edition) imaging software (Carl Zeiss Imaging Inc.) and analyzed in a blinded manner.

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were performed using GraphPad Prism 10 (GraphPad Software) and SPSS Statistics v29.01 (IBM). Statistical tests used included unpaired Student's t test for comparisons between two groups, and one-way or two-way ANOVA followed by Tukey's or Sidak's post-hoc tests for comparisons among multiple groups. The specific test used for each comparison is indicated in the figure legends.

Data are presented as means ± standard deviation (SD) unless otherwise noted. The definition of n is provided in the figure legends and represents the number of biologically independent animals per group or pooled technical replicates from independent experiments. The center of each data distribution is defined as the mean, and dispersion/precision is indicated by SD. All statistical details can be found in the results section, figure legends, and figures.