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Authors

BELBLIDIA, HASSINA
Sadi-Guettaf, Hassiba

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Adolescent Timing Shapes Behavioral and Peripheral Responses to Social Isolation Stress in Male NMRI Mice

Hassina Belblidia[1] and Hassiba Sadi-Guettaf[1]

[1]Université M'hamed Bougara UMBB, Faculté des Sciences, département de biologie, 35000 Boumerdès, Algeria

Psychosocial stress exposure during sensitive periods of development affects brain circuits involved in emotional regulation and contributes to the emergence of mood disorders as well as alterations in peripheral organ function. Most experimental studies have used post-weaning social isolation (SI) spanning the entire adolescent period to model early-life adversity. However, adolescence comprises heterogeneous developmental phases, marked by region-specific brain maturation and dynamic endocrine changes. Exposure to adverse environments during these distinct windows may therefore produce divergent behavioral and physiological outcomes. Yet, the role of developmental timing in determining the susceptibility of peripheral organs to stress remains poorly understood. Here, we assessed the immediate effects of a 10-day acute SI protocol, starting in mid-adolescence (postnatal day [PND] 35) in male NMRI mice, a stage where adolescence overlaps with puberty. We evaluated a on broad range of behavioral outcomes and on peripheral physiology, including renal and hepatic function through blood biochemistry analysis, in order to determine how stress exposure during a specific developmental window impacts both central and peripheral physiological processes. Our results revealed that mice housed in SI exhibited a hyperactivity phenotype and increased anxiety-like and depressive-like states, while cognitive function remained intact. At the peripheral level, SI induced early signs of hepatic stress under basal conditions, reflected by elevated plasma alanine aminotransferase (ALT) levels, without evidence of renal injury. Together, these results highlight the critical importance of developmental timing in shaping stress-related behavioral and peripheral outcomes and suggest that mid-adolescence constitutes a window of heightened vulnerability.

Keywords: mid-adolescence, mood disorders, NMRI mice,peripheral organs, social isolation

オスの NMRI マウスにおいて、思春期のタイミングが社会的隔離ストレスに対する行動的および末梢の反応を形成する

発達の感受性期における心理社会ストレスへの曝露は、感情調節に関する脳の回路に影響を与え、気分障害の発現や末梢器官機能の変調をもたらす。ほとんどの実験的な研究では、幼少期の逆境をモデル化するために、思春期の全般にわたる離乳後の社会的分離（SI）が用いられてきた。しかし、思春期は、脳の領域特異的な成熟やダイナミックな内分泌変異を特徴とする、多様な発達段階で構成されている。そのため、これらそれぞれの発達の時期において有害な環境への曝露は、異なる行動的および生理的な結果を生じさせる可能性がある。しかし、ストレスに対する末梢器官の感受性を決定するうえでの発達タイミングの役割は未だにほとんど理解されていない。本研究では、オスの NMRI マウスにおいて、思春期と性成熟期が重複している思春期中期（生後 35 日）に隔離を開始する、10 日間の短期社会的隔離プロトコルの即時的な効果を評価した。我々は、特定の発達の時期におけるストレス曝露が、中枢および末梢の生理プロセスにどのように影響を与えるかを特定するために、幅広い行動学的結果および血液生化学分析による腎機能、肝機能を含む末梢生理の結果を評価した。結果として社会的隔離（SI）下で飼育されたマウスは、認知機能は正常に保たれていた一方で、多動表現型を示し、不安様状態及びうつ様状態が増大していることが明らかになった。末梢レベルでは、SI は基礎状態下において、腎障害の証拠を伴わない血漿アラニンアミノトランスフェラーゼ（ALT）値の上昇に反映されるような、初期の肝臓ストレス兆候を誘発した。それらの結果は、ストレスに関連する行動的・末梢的結果を形成するうえでの発達タイミングの高い重要性を浮き彫りにしており、思春期中期が脆弱性の高まる時期に相当することを示唆している。

キーワード：思春期中期、気分障害、NMRI マウス、末梢器官、社会的隔離

Please send correspondence toHassina Belblidia, Faculté des Sciences. Route de la Gare Ferroviaire - Boumerdes 35000, Algérie.
Tel: 00 213 5 44 01 75 69. Email:h.belblidia@univ-boumerdes.dz

La Etapa de la Adolescencia Influye en las Respuestas Conductuales y Periféricas al Estrés por Aislamiento Social en Ratones NMRI Machos

La exposición al estrés psicosocial durante períodos sensibles del desarrollo afecta los circuitos cerebrales implicados en la regulación emocional y contribuye a la aparición de trastornos del estado de ánimo, así como a alteraciones en la función de órganos periféricos. La mayoría de los estudios experimentales han utilizado el aislamiento social (AS) posterior al destete, abarcando todo el período de la adolescencia, como modelo de adversidad temprana. Sin embargo, la adolescencia comprende fases heterogéneas del desarrollo, caracterizadas por una maduración cerebral específica de cada región y por cambios endocrinos dinámicos. La exposición a ambientes adversos durante estas ventanas diferenciadas puede producir resultados conductuales y fisiológicos divergentes. No obstante, el papel del momento del desarrollo en la determinación de la susceptibilidad de los órganos periféricos al estrés sigue siendo escasamente comprendido. En este estudio, se evaluaron los efectos inmediatos de un protocolo agudo de AS de 10 días de duración, iniciado en la adolescencia media (día posnatal 35) en ratones macho NMRI, una etapa en la que la adolescencia se superpone con la pubertad. Se evaluó una amplia gama de resultados conductuales y parámetros de fisiología periférica, incluyendo la función renal y hepática mediante análisis bioquímicos sanguíneos, con el fin de determinar cómo la exposición al estrés durante una ventana específica del desarrollo afecta tanto los procesos fisiológicos centrales como los periféricos. Los resultados encontrados mostraron que ratones mantenidos en condiciones de AS exhibieron un fenotipo de hiperactividad y un incremento de los comportamientos de tipo ansioso y depresivo, mientras que la función cognitiva permaneció intacta. A nivel periférico, el AS indujo signos tempranos de estrés hepático en condiciones basales, reflejados por niveles plasmáticos elevados de alanina aminotransferasa (ALT), sin evidencia de lesión renal. En conjunto, estos resultados destacan la importancia crítica del momento del desarrollo en la configuración de las respuestas conductuales y periféricas relacionadas con el estrés, y sugieren que la adolescencia media constituye una ventana de vulnerabilidad aumentada.

Palabras clave: adolescencia media, trastornos del estado de ánimo, ratones NMRI, órganos periféricos, aislamiento social

Stress is a major environmental risk factor underlying the increasing burden of psychiatric disorders. In particular, early life stress such as social isolation (SI) disrupts brain maturation and contribute to the development of mental illnesses (Heim & Nemeroff, 2001; Maccari et al., 2014). Importantly, the impact of stress strongly depends on the developmental stage at which stress exposure occurs. As a result, deficits in the social environment during critical developmental windows are relevant to an increased risk for neuropsychiatric disorders including depression, anxiety, attention-deficit/hyperactivity disorder, and schizophrenia (Gomes et al., 2020; Meyer-Lindenberg et al., 2012). Accordingly, adolescence represents a transitional period from childhood to adulthood, characterized by dynamic and asynchronous maturation of key brain regions (e.g., the prefrontal cortex, hippocampus, and amygdala) as well as the endocrine system (particularly the hypothalamic–pituitary–adrenal (HPA) axis), along with associated behavioral changes across species. These features make adolescence particularly sensitive to socio-environmental stressors (Bell, 2018; Burke et al., 2017; Romeo et al., 2006; Spear, 2000).

Importantly, the consequences of adolescent stress are not confined to the central nervous system but extend to systemic homeostasis, notably metabolic regulation. This is evidenced by alterations in hepatic and renal function biomarkers, accompanied by dysregulated inflammatory profiles. This systemic dysregulation is likely driven by chronic stress through sustained activation of the HPA axis, coupled with oxidative stress and inflammatory pathway engagement (Feng et al., 2025).

Stress represents a state of threatened homeostatic balance that activates adaptive physiological responses, centrally coordinated by the brain through the HPA axis and the sympathetic nervous system (Leistner & Menke, 2020). Acute stress activates these systems, leading to a rapid and transient increase in cortisol secretion that mobilizes energy stores, enhances alertness, and promotes coordinated adaptations across multiple organs to support the response to immediate challenges. However, when stress exposure becomes prolonged or repetitive, these initially protective mechanisms may become maladaptive. Chronic activation of the HPA axis is often associated with glucocorticoid receptor desensitization and impaired negative feedback regulation, resulting in persistently dysregulated cortisol signaling. Such alterations disrupt the function of multiple organs and systems. Ultimately, such changes compromise systemic homeostasis and highlight the transition from adaptive responses to pathological outcomes (Borges et al., 2023; Feng et al., 2025).

This differential impact of acute versus chronic stress may be partly explained by developmental timing. Adolescence is not a unitary sensitive period, but rather comprises multiple sensitive windows of heightened plasticity during which environmental factors, including stress, may differentially influence neurodevelopmental outcomes (Fuhrmann et al., 2015).

In rodents, however, the definition of adolescence varies across studies, which complicates comparisons unless precise postnatal ages are specified. In mice, the juvenile period extends from weaning (postnatal day [PND] 21) to approximately PND 28-30, whereas adolescence spans from PND 28-30 to PND 60 (Andersen, 2003; Bell, 2018; Lukkes, 2009). Adolescence is defined as the developmental period beginning with the onset of puberty and ending at sexual maturity, encompassing major behavioral and neurobiological changes (Bell, 2018; Eiland & Romeo, 2013). This period can be further subdivided into early (PND 28–34), mid- (PND 35–47), and late adolescence (PND 48–60) (Brust et al., 2015; Burke et al., 2017).

Within this developmental framework, mid-adolescence represents a period of high neurobiological and behavioral plasticity, during which environmental perturbations such as stress may exert pronounced effects. This stage is characterized by ongoing synaptic pruning and circuit refinement, particularly within prefrontal and limbic regions (Eiland & Romeo, 2013). It coincides with pubertal maturation, which begins around PND 30–35 in male mice and overlaps with mid-adolescence (Bell, 2018). Social behavior also undergoes marked reorganization during this period, with social play peaking between PND 30 and 40 (Burke & Miczek, 2014). In parallel, the HPA axis progressively transitions toward an adult-like profile, with corticosterone responses maturing earlier (P30–P40) than ACTH regulation (P50–P60). This developmental pattern likely reflects an incomplete maturation of negative feedback mechanisms required for effective HPA axis regulation, contributing to increased stress sensitivity during mid-adolescence (Foilb et al., 2011; Romeo et al., 2016).

SI is a widely used experimental paradigm to model psychosocial stress in rodents. Standard protocols typically begin at weaning (PND 21) and may extend into adulthood (Burke et al., 2017; Cattane et al., 2022). This manipulation prevents direct physical interaction with peers while preserving indirect sensory cues (olfactory, auditory, and visual) (Burke et al., 2017; Weiss et al., 2004).

In the present study, a 10-day SI protocol was initiated at PND 35 in male NMRI mice, targeting mid-adolescence. NMRI mice were selected due to their outbred genetic background, which provides greater behavioral variability compared to inbred strains such as C57BL/6, making them potentially more suitable for modeling the diverse stress responses and behavioral outcomes observed in humans (Griebel et al., 2000). Only males were included to reduce variability associated with estrous cycle-dependent hormonal fluctuations, as sex differences in stress responsiveness emerge during puberty and continue to develop across adolescence (Romeo et al., 2016).

The aim of the present study was to determine whether short-term SI during mid-adolescence is sufficient to induce early behavioral and peripheral physiological alterations in male NMRI mice. To address this question, behavioral outcomes and peripheral markers of organ function were assessed, including creatinine (renal function) and alanine and aspartate transaminases (ALT and AST; hepatic function). This integrative approach provides insight into brain–periphery interactions following adolescent stress exposure.

Method

Animals and Housing Conditions

Male NMRI mice ($N = 24$) were purchased from the Pasteur Institute (Algiers, Algeria) at PND 28 and housed in standard laboratory cages under a 12 h light/dark cycle (lights on 07:00 AM) and at a temperature (22 ± 1 °C). Food and water were available *ad libitum.*

One week after arrival, mice were randomly assigned to either social isolation (SI; n = 12, one mouse per cage) or group-housed (GH; n = 12, four per cage) conditions for 10 days, starting at PND 35. In order to limit handling and social interaction with the experimenter, cages for both groups were changed once a week. Olfactory, visual, and auditory contact between the cages was maintained. Nesting materials were provided equally across groups. All experiments were conducted in accordance with the ethical guidelines of the National Agency of Health Research Development (ANDRS) and were approved by the Animal Experimentation Sciences Committee (AASEA, agreement number: 5/DGLPAG/DVA.SDA.14).

Behavioral Assays

At the end of isolation period (PND 45),animals underwent a battery of behavioral tests in the following order, with at least a 24 h interval between tests: open field, object-location memory (including habituation phase) and sucrose preference. For all behavioral tests, testing was conducted during the light phase between, 09:00 and 15:00. Animal cages were placed in the experimental room 30 min before the beginning of the experiments. Each task was conducted by the same experimenter. Behavioral apparatus was cleaned with 70% ethanol between trial and after each experiment. Mice were returned to their home cage following completion of each behavioral task.

Open Field Test

Locomotor activity and anxiety-like behaviors (Prut & Belzung, 2003) were assessed in a white squared (50 × 50 × 50 cm) open field arena (OF). Each mouse was placed in the center of the arena and allowed to explore freely for 30 min. Total distance travelled (cm), time (s) spent in the central and periphery zone were recorded and analyzed using EthoVision software XT 15 (Noldus®).

Object Location Memory

Object Location Memory (OLM) was carried out in a black wooden box (32 × 32 × 20 cm) (Rochais et al., 2020). On days 1 and 2, each mouse was habituated to the empty box for 10 min. On the experiment day (day 3), the test consisted of two sessions (5 min each; inter-session interval: 5 min). During the presentation session, the mouse was free to explore two identical objects (constructed from plastic toys), placed 5 cm away from the walls, before being returned to its home cage. In the test session, the mouse was placed with two objects: one located in a previously occupied position during the presentation session (stationary object), and the other placed in a new location (displaced object). Object exploration time during each session was manually measured from video recordings by an experimenter blind to the experimental condition. Exploration of an object was considered when the mouse was oriented toward the object at a distance of 1 cm or less by revealing interest: touching or sniffing the object. The discrimination index was calculated as follows: [(exploration time of the displaced object) – (exploration time of the stationary object)]/[(exploration time of the displaced object) + (exploration time of the stationary object)].

Sucrose Preference Test

Sucrose preference test has been extensively used to assess anhedonia in chronic stress models. The test was performed as previously described (Pozzi et al., 2014). After measurement of water intake on a 24 h period, mice were given a free choice between two bottles; one containing sucrose solution (2%) and the other containing water for a 72 h period. The position of the bottles was alternated to avoid potential side-preference bias. The water and sucrose intake were measured every 24 h by weighing the bottles containing the solution. No previous food or water deprivation was applied before testing. For GH mice, the total volume of sucrose and water was divided by the number of mice per cage, as the mice were not separated during testing to avoid the potential effects of periodic SI. Sucrose preference was calculated with the following formula: (sucrose solution intake / total intake) × 100.

Blood Biochemistry

At the end of the behavioral tests, animals were anesthetized with chloral hydrate (400mg/kg; ip). After confirmation of deep anesthesia, the cardiac puncture was used to collect the blood of mice. It was collected into sterile tubes and immediately centrifugated at 3000 rpm for 10 min to extract plasma. This fraction was stored at -20 °C until analyzed. Biochemical parameters analyzed in our study were alanine aminotransferase (ALT), aspartate transaminase (AST) and creatinine.

Statistical Analysis

Data analyses were conducted using Statview® (Version 5.0; SAS Institute Inc) and results presented using GraphPad Prism® (Version 8.02; GraphPad Software). Cage effects were systematically assessed for all variables to examine potential clustering effects due to group housing. Paired group comparisons were conducted with either a repeated-measures ANOVA (for multiple comparisons) or Paired t-test. Unpaired comparisons were assessed using unpaired Student's t-test. Comparisons to a reference value were performed using a univariate t-test. When data were not normally distributed, Wilcoxon test was used for paired comparisons and Mann Whitney test was used for unpaired comparisons. Results were expressed as mean ± standard error of the mean (SEM). A significant difference was considered when $p < .05$.

Results

Assessment of Cage Effects

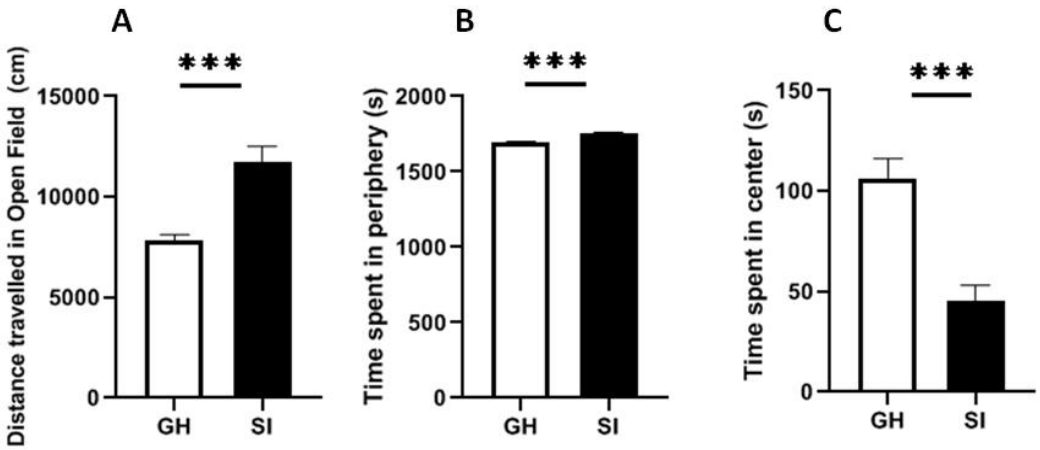
Prior to the main comparisons, potential cage effects were assessed to evaluate possible clustering due to group housing. One-way ANOVA revealed no significant differences between the three control cages for all variable measured (all $p > .05$), suggesting a limited influence of cage-related clustering. Accordingly, individual mice were considered as independent experimental units for subsequent analyses.

Locomotor Activity and Anxiety-like Behavior in OF

SI mice showed significantly greater total distance travelled in the OF compared to GH mice (Mann Whitney test, $p < .001$; Figure 1A) suggesting a hyperactivity phenotype. Moreover, SI mice spent significantly more time in the periphery (Mann Whitney test, $p < .001$; Figure 1B) and less time in the center area than GH mice (Mann Whitney test, $p < .001$; Figure 1C), suggesting an anxiogenic phenotype.

Figure 1

Effect of Social Isolation During Mid-Adolescence on Locomotor Activity and Anxiety-like Behavior Investigated in the Open Field Test



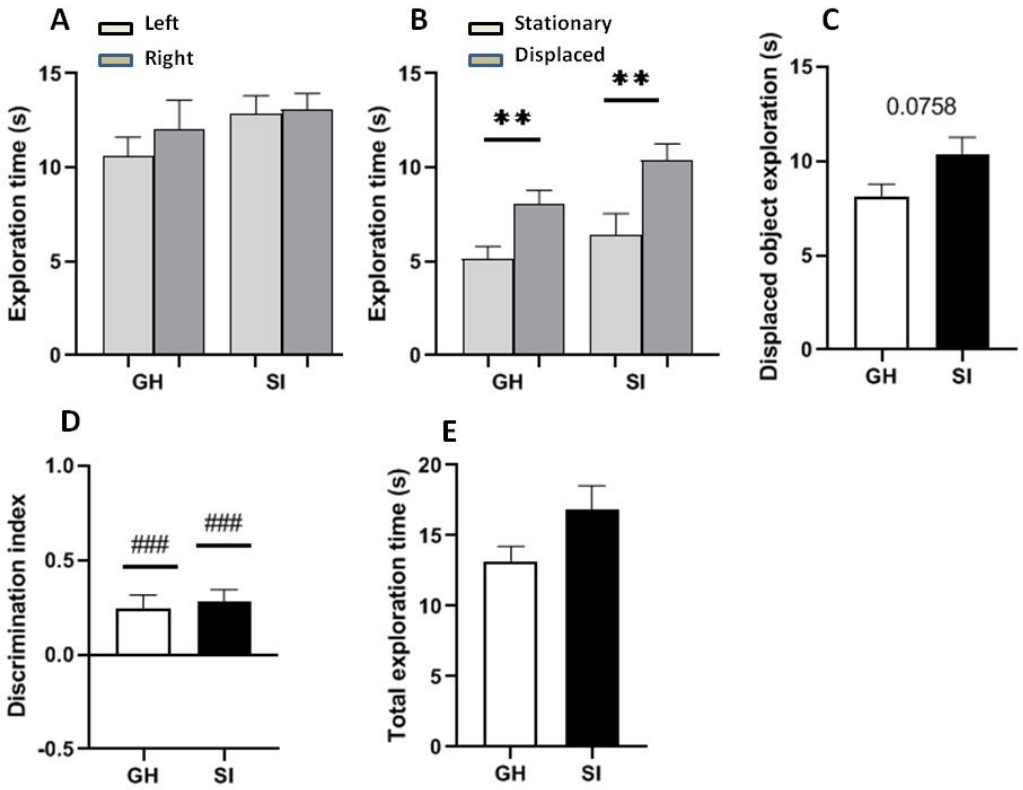
Note.(A) Total distance (cm) travelled during 30 min. Time spent in the periphery area for 30 min. (C) Time spent in the center area for 30 min: Mann Whitney test: ***p < .001. (n = 12 per housing condition groups). Data are expressed as mean ± SEM.

Memory Assessment in OLM

In OLM test,during the presentation session (Figure 2), two-way repeated-measures ANOVA demonstrated that two-way repeated-measures ANOVA demonstrated that mice presented neither differences due to the housing conditions nor differences due to the objects positions ($F(1, 22) = 1.795$, $p = .194$ and $F(1, 22) = 0.738$, $p = .399$, respectively), and no interaction ($F(1, 22) = 0.431$, $p = .518$; Figure 2A). However, during test session both groups showed a significant preference for the displaced object compared to the stationary one (Paired t-test : GH mice $t(11) = -3.26$, $p = .007$; Wilcoxon test: SI mice, $p = .0049$; Fig. 2B). Interestingly, SI mice tended to explore the displaced object more than GH groups(Mann-Whitney test, $p = .0758$; Figure 2C). The discrimination index in both groups (Figure 2D), differed from 0 (univariate *t*-tests: GH mice $t(11) = 10.769$, $p < .001$; SI mice $t(11) = 10.948$, $p < .001$, respectively), reflecting intact spatial memory in both groups. Total exploration time during the test session did not differ between groups (Mann-Whitney test, $p = .160$; Figure 2E), suggesting that memory performance was not due to an increase in exploratory activity.

Figure 2

Effect of Social Isolation During Mid-Adolescence on Object-Location Memory Test



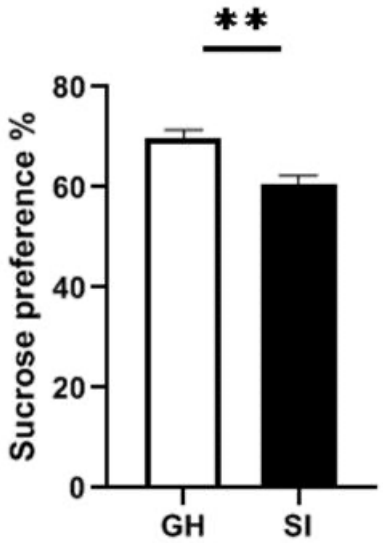
Note.(A-E) Object-location recognition test with 5 min exploration sessions and 5 min intersession interval. (A) Time spent by mice exploring left and right objects during presentation session or (B) Stationary and displaced objects during test session. (C) Displaced object exploration. (D) Discrimination index. (E) Total exploration time during the test session. Comparisons between in each housing condition groups were performed using Paired t-test or Wilcoxon test: $^{**}p < .01$ and comparison to a reference value was performed with univariate t-test: $^{###}p < .001$. (n = 12 per housing condition groups). Data are expressed as mean ± SEM.

Depressive-like Behavior in Sucrose Preference Test

In the sucrose preference test (Figure 3), no group difference was observed in total water intake (Mann Whitney test, $p = .148$). However, GH mice showed greater sucrose preference than SI mice (Mann Whitney test,$p = .002$), suggesting an anhedonic phenotype in SI mice.

Figure 3

Effect of Social Isolation During Mid-Adolescence on Depressive-Like Behavior Investigated in Sucrose Preference Test



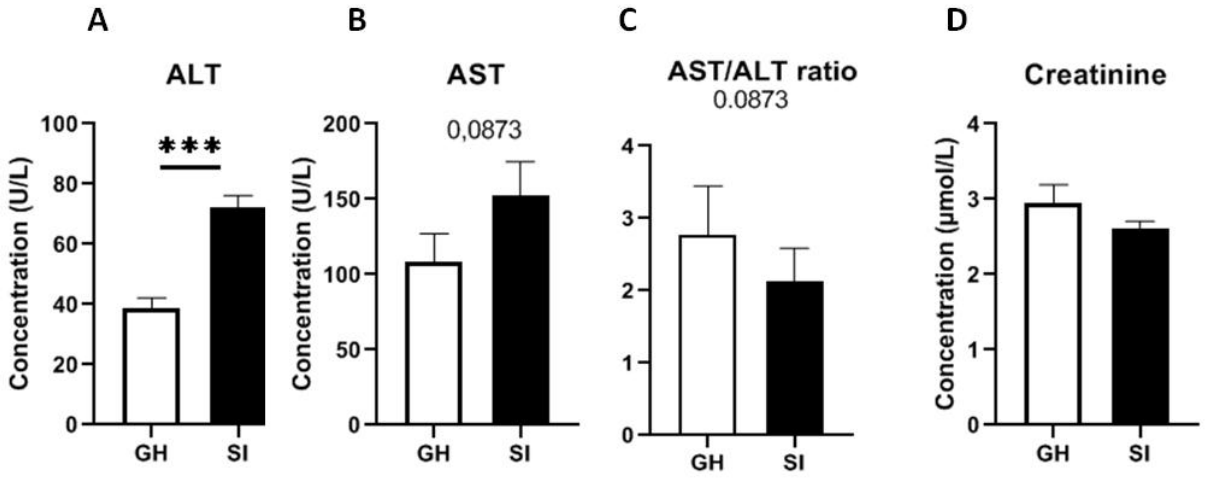
Note. Anhedonia analysis in sucrose preference test (Mann Whitney test: $^{**}p < .01$) ($n = 12$ per housing condition groups). Data are expressed as mean ± SEM.

Blood Biochemistry

We next assessed the impact of SI stress on the circulating levels of ALT, AST and creatinine as biomarkers of organ damage (Figure 4). Plasma ALT levels were significantly higher in SI mice compared to GH group (Unpaired Student's test,$t(8) = 6.022$, $p < .001$; Figure 4A). A tend toward an increase was observed in plasma AST levels (Mann Whitney test, $p = .087$; Figure 4B) as well as in the AST /ALT ratio (Mann Whitney test, $p = .087$; Figure 4C). However, plasma creatinine levels were comparable between the two housing conditions (Unpaired Student's test,$t(8) = 1.260$, $p = .243$; Figure 4D), suggesting that 10 days of stress exposure during mid-adolescence are sufficient to induce liver, but not kidney damage.

Figure 4

Effect of Social Isolation During Mid-Adolescence on Blood Biochemistry



*Note.*Levels of ALT, AST, their ratio and the creatinine (A-D) in the blood (Unpaired Student's test: ***$p < .001$) (n = 5 per housing condition groups). Data are expressed as mean ± SEM.

Discussion

The present findings indicate that exposure to 10-day period of SI during mid-adolescence (PND 35–45) is sufficient to induce both behavioral and peripheral alterations in male NMRI mice. This developmental window is characterized by ongoing pubertal maturation and continued reorganization of neuroendocrine systems involved in stress regulation, suggesting a period of heightened sensitivity to environmental perturbations (Bell, 2018; Romeo et al., 2016).

In addition, brain regions implicated in stress processing, including prefrontal and limbic circuits, remain highly plastic, while the HPA axis continues to mature toward adult-like regulatory patterns (Eiland & Romeo, 2013; Romeo et al., 2016). In this context, even a relatively short period of SI may be sufficient to disrupt the ongoing balance between neural and peripheral homeostatic systems.

At the behavioral level, SI during mid-adolescence (PND 35–45) in male NMRI mice resulted in increased locomotor activity in the open field test. However, previous studies have reported inconsistent findings, likely reflecting differences in strain, developmental timing, and duration of isolation, all of which critically modulate stress-related behavioral outcomes (Hu et al., 2023; Rivera-Irizarry et al., 2020).

Most studies have used male C57BL/6 mice with isolation initiated during the juvenile period (PND 21–28) and maintained for prolonged durations (typically more than four weeks), reporting either increased locomotor activity (Hu et al., 2023; Medendorp et al., 2018) or no significant changes (Rivera-Irizarry et al., 2020). In contrast, Lander et al. (2017) applied SI starting at PND 38 (mid-adolescence) for three weeks in male C57BL/6 mice and observed no changes in baseline locomotor activity, although increased activity emerged during behavioral testing (Lander et al., 2017).

The present study, which applied a short 10-day period of SI restricted to mid-adolescence in male NMRI mice, also revealed increased locomotor activity. Taken together, these findings indicate that the locomotor response to SI is highly dependent on the developmental timing and duration of isolation exposure, although strain-related differences in stress responsivity may contribute to the variability reported across studies.

However, in the present study, male NMRI mice showed preserved spatial recognition memory during mid-adolescence in the OLM task, as both GH and SI groups spent significantly more time exploring the displaced object. This finding contrasts with previous studies reporting impairments in hippocampal-dependent tasks following adolescent SI, which are often more pronounced than those induced by other adolescent stress paradigms (Green & McCormick, 2013; Lu et al., 2003). The preserved spatial recognition memory in the present study may be due to the short retention interval (5 min). Green and McCormick (2013) previously reported that social instability stress from early to mid-adolescence (PND 30–45) in male Long-Evans rats impaired object-location memory at long delays (240 min) but not at short delays (15 or 60 min). This suggests that SI stress during mid-adolescence may preferentially affect long-term memory.

Interestingly, SI mice exhibited increased exploration of the displaced object compared to GH controls, while both groups showed similar exploration during the presentation session. This pattern may reflect an enhanced sensitivity to spatial novelty in SI mice rather than a deficit in memory performance (Silva-Gómezet al., 2003). Consistent with this interpretation, the increased locomotor activity observed in the open field test may reflect a heightened behavioral reactivity to novel environments, suggesting that during mid-adolescence may enhance novelty-driven exploration rather than impair cognitive function per se.

Moreover, SI during mid-adolescence in male NMRI mice induced anxiogenic and depressive-like phenotypes. SI mice spent significantly less time in the center area of the open field test and showed reduced sucrose preference, indicating increased anxiety-like behavior and anhedonia, respectively. These findings are consistent with previous studies reporting anxiety-like and depressive-like behaviors following adolescent SI in mice (Borsini et al., 2023; Du Preez et al., 2020; Grigoryan et al., 2022; Han et al., 2019).

These behavioral alterations are consistent with features of several psychiatric disorders observed in humans, particularly in contexts of social exclusion or reduced social contact during sensitive developmental periods (Gazelle & Rudolph, 2004).

However, important methodological differences exist across studies, particularly in terms of developmental timing, duration of isolation, and strain. Most studies reporting anxiogenic effects have employed prolonged isolation protocols extending across large portions of adolescence or into early adulthood. For instance, Du Preez et al. (2020) observed increased anxiety-like behavior in male BALB/c mice following 9 weeks of isolation initiated at PND 42–49, corresponding to mid-to-late adolescence. Similarly, Lopez and Laber (2015) reported anxiogenic effects in male C57BL/6J mice after isolation spanning from PND 21 to PND 60, covering both the juvenile and entire adolescent periods. Võikar et al. (2005) also found increased anxiety-like behavior in C57BL/6J and DBA/2 mice following 7 weeks of isolation starting at PND 28, corresponding to early adolescence.

Consistent with these findings, prolonged isolation is often associated with the emergence of anxiety- and depressive-like phenotypes. However, when isolation is restricted to shorter durations during mid-adolescence, behavioral effects are less consistent, with some studies reporting no significant alterations in male Wistar rats (Alshammari et al., 2020). In contrast, Gong et al. (2018) showed that even a brief period of isolation (eight days) initiated at an earlier developmental stage was sufficient to induce depressive-like behaviors in mice. Together, these findings indicate that behavioral outcomes are shaped by multiple factors, including duration, developmental timing, strain, and species.

In the present study, the 10-day isolation protocol targeted mid-adolescence, a restricted developmental window characterized by ongoing pubertal maturation and dynamic social behavioral reorganization. This period precedes the peak of social play, a process known to be essential for the maturation of emotional regulation and socio-sexual behaviors (Li et al., 2021; Marquardt et al., 2023; Walker et al., 2019). Disruption of social interactions during this developmental window, even for a short duration, may therefore interfere with the normal maturation of emotional and social behaviors.

As acute SI stress had elicited behavioral changes, we next sought to investigate whether this brief period of SI could affect systemic physiological functions. Accordingly, we examined peripheral biological markers. In this context, the results of the blood biochemistry analysis revealed a significant elevation in ALT levels, a near-significant trend in AST, and no alterations in creatinine, indicating early hepatic stress dysregulation without evident renal impairment. These results suggest a differential sensitivity of hepatic and renal systems to acute psychosocial stressor.

SI has been identified as a major risk factor for several systemic diseases, including hepatic disorders. Plasma transaminases are well-established biomarkers of hepatic inflammation and toxic hepatic injury. Increased ALT levels are a more sensitive and liver-specific indicator of hepatocellular damage than AST, due to its predominant cytoplasmic localization. Although AST is present in higher total amounts in hepatocytes, its distribution across both cytoplasmic and mitochondrial compartments limits its specificity for hepatic injury (Feldman et al., 2006; Zahir et al., 2021).

The early stage hepatic stress dysregulation most likely reflects activation of the HPA axis in response to the chronic psychosocial stressor, leading to oxidative damage and the release of pro-inflammatory cytokines, thereby establishing a mechanistic link between central stress signaling and peripheral organ effects, particularly in the liver (Chida et al., 2006; Swain, 2000; Zahir et al., 2021).

However, Hamilton et al. (2022) reported that two weeks of adolescent SI in male CD-1 mice did not alter ALT, AST, or creatinine levels under basal conditions, but enhanced bacterial clearance after a bacterial sepsis challenge. This improved clearance was accompanied by elevated levels of pro-inflammatory cytokines, suggesting that SI induces early immune adaptations that protect against organ injury (Hamilton et al., 2022).

In contrast, our study demonstrates that a 10-day isolation protocol in male NMRI mice elicited early signs of hepatic stress (elevated ALT) under basal conditions, in the absence of any infectious or toxic challenge. This indicates that even relatively acute psychosocial stress can impair liver function. While these alterations may reflect an initial adaptive response to the stressor, the observed ALT elevation also raises the possibility that maladaptive processes could already be emerging, particularly given the timing of exposure during mid-adolescence (Feng et al., 2025).

One possible explanation is that mid-adolescence coincides with heightened HPA axis reactivity to psychosocial stressors compared to early or late adolescent developmental windows (Foilbet al., 2011; Romeo et al., 2016). Under this heightened reactivity, exposure to stress may shift the balance from adaptive toward pathological outcomes in the liver. This hypothesis warrants further investigation through comparative studies applying the same isolation protocol across distinct adolescent periods, early, mid-, and late adolescence and where effects are expected to be most pronounced during the mid-adolescent window. Furthermore, these results cannot be generalized to female mice, as sex differences in responses to adolescent SI may lead to distinct physiological and behavioral outcomes (Begni et al., 2020; Holder & Blaustein, 2014).

In summary, our findings indicate that a brief SI during a narrow developmental window can induce early behavioral and peripheral alterations in male NMRI mice, highlighting the critical role of developmental timing in shaping stress-related outcomes.

The current study has some limitations. First, only male NMRI mice were included; therefore, the findings may not generalize to females given known sex differences in stress responsivity (Begni et al., 2020; Holder & Blaustein, 2014; Marquardt et al., 2023). Second, although SI was applied for 10 days during mid-adolescence (PND 35), no parallel comparison with other developmental stages was performed, limiting the ability to determine whether this period represents a particularly sensitive window for the effects of SI. Third, long-term behavioral and physiological outcomes were not assessed; as a result, it remains unclear whether the observed effects persist into adulthood or are reversible after re-socialization. Longitudinal studies would help clarify the temporal dynamics of these effects.

Despite these limitations, the present findings provide novel insights into the impact of SI during mid-adolescence on both central and peripheral physiological processes. Future investigations incorporating female subjects and other developmental windows will be essential to assess the sensitivity of these periods and better capture the developmental impact of stress across sexes and over time.

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