



OPEN From trauma to resilience: psychological and epigenetic adaptations in the third generation of holocaust survivors

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The transmission of trauma across generations, particularly among descendants of Holocaust survivors, presents a complex interplay of psychological and epigenetic adaptations. This study explored the long-term impacts of Holocaust trauma on the third and fourth generations, focusing on the quality of social-emotional ties and psychopathology, as well as the epigenetic variation in the oxytocin system, the Hypothalamic-Pituitary-Adrenal (HPA) stress axis, and the Sympathetic Nervous System (SNA). Involving 371 participants, including 186 third- and fourth-generation descendants of Holocaust survivors (54.8% women, mean age = 29.67), we employed DNA methylation analysis of saliva samples to uncover these dynamics. Our findings revealed that descendants exhibited significantly lower general attachment avoidance, and a DNA methylation pattern associated with stronger activation of the oxytocin system, indicating enhanced social bonding and social emotion regulation. Conversely, they showed distinct DNA methylation patterns in CRH, CRHBP, FKBP5, and NR3C1 genes linked to increased HPA axis activation and more pronounced stress reactivity. Despite the presence of these two DNA methylation patterns, no elevated levels of psychopathology were observed. Our results highlight the dual nature of trauma transmission, with descendants displaying both vulnerabilities and resilience. While stronger oxytocin system activation may support social cohesion and stress reduction, stronger HPA axis reactivity could ensure sustained vigilance and preparedness. Our findings thus underscore the intricate balance between genetic, epigenetic, and environmental factors in shaping resilience and offer insights into the potential for long-term growth following inherited trauma.

"...there was a moment, in the 1980s, when I first began to wonder why certain stories that my parents had told me, or scenes they had evoked about what they always referred to as "the war," were more vibrant and more vivid in my memories than moments I recalled from my own childhood. Their accounts had the textures and qualities of memories for me, but they were clearly not my memories: I had not experienced any of them directly. And I realized then that my experiences were not at all unique. Not only did I share them with other descendants of Holocaust survivors, but they described a larger cultural phenomenon common to my generation – a generation dominated by histories we did not ourselves live through...."

(M. Hirsch, 2012).

Marianne Hirsch, a second-generation Holocaust survivor, used these words to describe the phenomenon of "post-memory" that she coined in relation to second-generation Holocaust survivors¹. According to Hirsch, the trauma experienced by Holocaust survivors is transferred to their offspring in such a powerful way that it can make them feel as if the memories are their own. The transfer of this trauma from the parent to the child is believed to be mediated not through recall but through the child's imagination and creativity, which can help them experience the parent's memories as vividly as if they had been there themselves. In contrast to the second generation of Holocaust survivors, who often carry the trauma of their parents as their own, research on the third generation presents a complex and nuanced picture, reflecting both negative and positive outcomes. For

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instance, grandchildren of Holocaust survivors often exhibit heightened anxiety and altered stress responses due to inherited trauma^{2–6} yet they also demonstrate remarkable resilience and strong community bonds cultivated over generations that are manifested at the psychological and neurobiological levels^{7–10}. In the current study, we aimed to address these disparities by exploring the quality of social-emotional ties and psychopathology of third- and fourth-generation descendants of Holocaust survivors, as well as the epigenetic variation in related biological systems – the oxytocin system, the Hypothalamic-Pituitary-Adrenal (HPA) stress axis, and the Sympathetic Nervous System (SNS) that governs “fight-or-flight” responses.

Studies on the third generation of Holocaust survivors reveal a spectrum of psychological impacts. Grandchildren of Holocaust survivors have been found to be overrepresented in psychiatric services by 300%, suggesting the potential transmission of trauma effects to this generation². Yet, even in the absence of psychological pathology, third-generation survivors are more likely to exhibit higher levels of anxiety and stress when faced with challenging life events³. Greater levels of distress among third-generation survivors during difficult times further support this vulnerability⁴. Analyzing 108 mother-daughter pairs, researchers found that being a third-generation Holocaust survivor is associated with a higher chance of suffering from an eating disorder, which is linked to the extent of Holocaust exposure experienced by their second-generation mothers⁵. This study also highlighted that third-generation females were more exposed to Holocaust-related narratives from their grandparents than the second-generation, emphasizing the importance of understanding familial backgrounds in therapeutic contexts. Finally, a study among second and third-generation Holocaust survivors found that while third-generation survivors exhibited higher levels of secondary trauma compared to controls, these levels remained within the normal range⁸. These findings suggest that while the legacy of trauma is indeed detectable in the grandchildren of survivors, it often manifests as subclinical distress rather than clinical disorder. Such outcomes support a model in which biological or emotional sensitivity to ancestral trauma persists, even as overt psychopathology remains absent.

These nuanced findings raise an important question: how can individuals exhibit subtle yet measurable psychological effects in the absence of overt trauma exposure? One promising avenue of explanation lies in the field of epigenetics. Epigenetics refers to the study of how environmental factors and life experiences can influence gene expression without altering the underlying DNA sequence. Recent epigenetic research offers critical insights into how trauma can leave lasting biological marks that may be transmitted across generations, adding an important layer to our understanding of the psychological outcomes observed among Holocaust survivors' descendants. Epigenetic changes, such as DNA methylation, can suppress or enhance gene expression^{11,12} potentially influencing psychological and physiological outcomes in subsequent generations¹³. For example, the offspring of Holocaust survivors were shown to have altered cortisol levels and enhanced stress reactivity, indicating a biological underpinning for the transmission of trauma effects. Specifically, differences in DNA methylation of the FK506 binding protein 5 (FKBP5) gene, which plays a crucial role in regulating the stress response, have been observed in peripheral blood mononuclear cells (PBMCs) of Holocaust survivors and their offspring⁹. FKBP5 affects the negative feedback loop of glucocorticoid receptors (GRs)^{14,15} resulting in prolonged activation of the hypothalamic-pituitary-adrenal (HPA) axis and contributing to altered stress reactivity. The study indicated that Holocaust survivors exhibited a distinctive DNA methylation signature associated with long-term cortisol suppression—a hormonal profile frequently linked to post-traumatic stress disorder¹⁶. This low cortisol state is considered an adaptive response to chronic trauma, reflecting the body's attempt to mitigate the damaging effects of prolonged cortisol exposure by downregulating the stress system. However, this adaptation may come at the cost of a blunted stress response and impaired emotion regulation, observed in many Holocaust survivors. Conversely, their offspring showed an opposing pattern: reduced capacity to deactivate the stress response and heightened stress reactivity. Importantly, although these findings were based on non-brain tissue, they were meaningfully correlated with hormone levels (e.g., cortisol) and psychological variables, including childhood trauma exposure, underscoring the potential of peripheral markers to index central stress regulatory mechanisms.

Despite these adverse outcomes, research highlights positive outcomes and resilience within the third generation. One study conducted a qualitative thematic analysis using online semi-structured questionnaires and follow-up telephone interviews with 30 third-generation survivors of the Holocaust. It found that, although these individuals might experience heightened anger and guilt, they also demonstrate a strong sense of pride, psychological resilience, and a deep connection to their Jewish identity⁷. This shift from negative emotions to a more positive identification with their heritage suggests significant spiritual growth and resilience. A different study used a community sample and showed that grandchildren of Holocaust survivors had notably higher self-esteem, fewer indicators of severe psychopathology, and lower scores on a measure of “strangeness,” which assessed traits such as clumsiness, social reservation, peculiarity, and lack of cooperation in group settings¹⁷. These findings suggest that third-generation Holocaust survivors are performing better in social-emotional domains than their peers without a direct Holocaust connection.

The current study

Research on the long-term effects of the Holocaust across generations has yielded mixed findings, highlighting the complexity of transgenerational trauma transmission and underscoring the need for further investigation. The varying outcomes, which range from increased psychiatric vulnerability to notable resilience and positive identification with cultural heritage, reflect the diverse ways in which the third generation navigates the legacy of trauma.

On the one hand, research suggests that third-generation descendants of Holocaust survivors exhibit heightened stress reactivity, which is linked to increased anxiety-related symptoms and distress, particularly when facing challenges. However, their levels of psychopathology generally remain within the normative range and do not meet the criteria for clinical diagnoses. Limited epigenetic research on a specific gene of the HPA stress

axis (FKBP5, which suppresses the negative feedback loop of the axis⁹) supports this idea of a more reactive HPA axis, although with a different pattern compared to individuals with PTSD. Accordingly, our first hypothesis was that third- and fourth-generation descendants of Holocaust survivors would exhibit a DNA methylation pattern associated with a more reactive HPA stress axis, while potentially displaying greater psychological symptom severity that remains within subclinical limits and does not reach clinical thresholds. To test the specificity of this hypothesis, we also considered the epigenetic variation within the second stress system – the sympathetic nervous system (SNS) – which is associated with the rapid “fight-or-flight” response in times of emergency¹⁸.

On the other hand, research indicates that in the social-emotional domain, third-generation descendants of Holocaust survivors often demonstrate a shift from negative emotionality toward indicators of psychological strength. These include heightened pride, resilience, stronger identification with their cultural heritage, greater cooperativeness in group settings, and elevated self-esteem compared to their counterparts. One potential explanation for this positive trajectory lies in the parenting practices adopted by the second generation. While some literature suggests that early life stress or trauma can impair caregiving and emotional availability in the next generation, particularly when trauma is unresolved, research on Holocaust families reveals a more differentiated outcome.

Specifically, studies suggest that many second-generation Holocaust survivors adopt what has been termed compensatory parenting: an intentional effort to provide warmth, stability, and emotional support in contrast to the emotional deprivation they may have experienced¹⁹. Rather than repeating cycles of detachment, these parents actively seek to break them. This approach aligns with the Family Resilience Framework, which highlights three core protective processes—meaning-making, organizational flexibility, and emotionally responsive communication—that can transform family trauma into sources of strength²⁰. By acknowledging their history while emphasizing openness and support, second-generation parents often help their children build resilience. This pattern is consistent with the steeling effect, which proposes that moderate adversity, when encountered within a nurturing context, can foster increased emotional regulation and coping abilities²¹. It also reflects the perspective of resilience as a dynamic process, wherein individuals adapt successfully by accessing both relational and cultural resources in the face of adversity²².

In the case of third-generation Holocaust descendants, resilience is often facilitated by second-generation caregivers who create safe environments in which intergenerational trauma can be processed without becoming overwhelming. Empirical findings support this view. Third-generation individuals have reported greater resilience when their second-generation parents acknowledged the family’s traumatic past without catastrophizing it and modeled adaptive coping strategies^{23,24}. Similarly, the post-traumatic growth framework suggests that adversity can become a catalyst for growth, meaning, and purpose, outcomes often observed when trauma is contextualized within supportive family narratives²⁵. Additional cultural resources may also play a role. Jewish cultural values—such as collective memory, continuity, and education—can help reframe trauma as a source of strength rather than a cause of helplessness²⁶. These cultural narratives may interact with parenting practices to further enhance resilience. The Family Adjustment and Adaptation Response (FAAR) model suggests that families can successfully adapt to stress when the demands they face are balanced by available resources²⁷.

In this population, second-generation caregivers may introduce Holocaust-related themes gradually, supported by emotional tools and stability, keeping the level of challenge within a tolerable and adaptive range. Taken together, while early life stress often poses risks for intergenerational emotional unavailability, the case of Holocaust descendants provides a compelling counterexample. Here, trauma appears to be met with intentional caregiving practices, cultural meaning-making, and emotionally attuned parenting—all of which contribute to the development of resilience in the third generation.

The above pattern of resilience could be manifested in greater attachment security among third-generation survivors. Secure attachment refers to a socio-emotional tendency to form and perceive relationships as stable and benevolent and to see close others as sources of a safe haven and a secure base for exploration and growth²⁸. It is characterized by trust, a sense of safety, and the ability to rely on caregivers and other attachment figures for comfort and support²⁹ and to handle distress by social allostasis^{30,31}. One major way to assess secure attachment in adulthood is through self-report questionnaires, although narrative-based methods such as the Adult Attachment Interview (AAI) are also widely used³². In self-report measures, secure attachment is typically defined by low levels of both attachment anxiety and attachment avoidance²⁸. Attachment avoidance reflects discomfort with emotional intimacy, while attachment anxiety involves fear of rejection and preoccupation with a partner’s responsiveness. Thus, low scores in attachment avoidance and anxiety are conceptually and empirically consistent with higher attachment security.

Secure attachment fosters a positive self-concept, leading to sensations of pride and resilience^{33,34}. Individuals with secure attachment often feel a strong sense of connectedness to others³⁵ enhancing their social interactions and emotional well-being. Additionally, securely attached individuals tend to be more cooperative and collaborative in group settings^{36,37} which facilitates effective teamwork and positive social interactions. Secure attachment is also linked to higher and more stable self-esteem³⁸ as individuals feel valued and important, which boosts their confidence and self-worth. Thus, our second hypothesis was that the traits observed in third-generation Holocaust survivors—such as higher self-esteem, resilience, and connectedness—would align with the characteristics of secure attachment (i.e., low scores in attachment avoidance and anxiety), suggesting that their ability to thrive despite potential vulnerabilities is supported by a foundation of attachment security.

The predicted higher attachment security described above could also be manifested neurobiologically by a more active oxytocin system. Oxytocin is known to promote prosocial behaviors^{39,40} including bonding^{41,42} trust⁴³ empathy⁴⁴ and social interaction⁴⁵. Additionally, oxytocin plays a critical role in social allostasis, facilitating the maintenance of social stability and adaptability in response to social stressors through changes in neurobiological processes⁴⁶ as well as promoting “tend-and-befriend” behaviors that are linked to attachment⁴⁷. These behaviors involve seeking out and nurturing social connections to mitigate stress via co-regulation. Oxytocin also reduces

anxiety and stress⁴⁸ promoting social approach behaviors, especially under threat. By acting on various brain regions involved in social behavior, such as the amygdala and nucleus accumbens⁴⁹ oxytocin amplifies the effects of social experiences, fostering a sense of security and connectedness. These tendencies are specifically tuned to close others and in-group members, because oxytocin was also found to induce aggression towards outgroup members, reflecting a “tend-and-defend” response⁵⁰.

Secure attachment is closely tied to the function of oxytocin in various ways. Studies have shown that a single dose of intranasally administered oxytocin can enhance the experience of attachment security in adults previously classified as insecure. In these studies, individuals who were initially insecure in their attachment showed a significant shift towards ranking “secure attachment” phrases as more appropriate for attachment-related scenarios after receiving oxytocin⁵¹. Other research indicates that the effects of oxytocin are influenced by individuals’ existing attachment patterns⁵². Less anxiously attached individuals reported their mothers as more caring and closer after oxytocin administration, whereas more anxiously attached individuals remembered their mothers as less caring and close after receiving oxytocin. These findings challenge the popular belief that oxytocin is exclusively linked with universally positive social perceptions. Instead, they align with animal studies suggesting that oxytocin plays a broader role in encoding social memories and in assigning emotional or motivational value to those interactions. Specifically, oxytocin appears to shape how social experiences—whether rewarding or threatening—are remembered and integrated into future relational behavior⁵³. Neurobiologically, oxytocin attenuates amygdala responses to social stimuli, reducing uncertainty about the predictive value of social cues and influencing brain regions associated with pair bonding and attachment, such as the striatum⁵¹. Furthermore, epigenetic research ties attachment security with the expression of genes in the oxytocin system. For instance, Thaler and colleagues found that women with anorexia nervosa had higher DNA methylation levels of the oxytocin receptor gene, which was linked to insecure attachment and social avoidance⁵⁴. Ein-Dor and colleagues found that elevated DNA methylation at the oxytocin receptor promoter region in young adults was selectively related to attachment avoidance⁵⁵. Additionally, studies by Fujisawa and colleagues⁵⁶ and Robakis and colleagues⁵⁷ linked higher oxytocin receptor gene DNA methylation to early adverse experiences. Therefore, the neurobiological underpinnings of secure attachment, heavily influenced by oxytocin, are hypothesized to play a crucial role in the enhanced social and emotional traits observed in third-generation Holocaust survivor. In summary, we predicted that third and subsequent generations of Holocaust survivors would exhibit a shift in how trauma is transferred across generations. Instead of displaying post-traumatic symptoms, they would be likely to show biobehavioral tendencies that resonate with their grandparents’ resolve to “never again” endure a trauma as severe as the Holocaust. Specifically, these descendants may demonstrate more reactive survival responses through a more active HPA axis (hypothesis 1). This heightened reactivity would enable them to better perceive and respond to threats. Alongside this, they would exhibit higher attachment security associated with altered oxytocin system activity (hypothesis 2). These traits would foster a stronger ability to trust and cooperate with others in times of need. Together, these characteristics would enhance their collective resilience and survival across generations, utilizing the strength of numbers in overcoming adversity.

Results

Psychopathology, HPA axis, and SNS (hypothesis 1)

Results are presented in Supplementary Table 1 (psychopathology), 2 (HPA axis), and 3 (SNS). In these analyses, we controlled for biological sex, age, batch effect, the estimated frequency of epithelial and fibroblast cells in the saliva samples, and adverse childhood experiences. Analyses did not reveal any credible differences between third- and fourth-generation descendants of Holocaust survivors and non-descendants in symptom severity across measures of anxiety, depression, somatization, or general distress, nor in the DNA methylation levels of genes within the sympathetic nervous system that regulate “fight-or-flight” responses. Because our assessment tools were not designed to yield clinical diagnoses, findings should be interpreted in terms of symptom severity rather than diagnostic status.

Conversely, we found four credible differences in DNA methylation levels of genes in the HPA stress axis (see Fig. 1). First, 3rd- and 4th-generation descendants of Holocaust survivors had significantly less DNA methylation on FKBP5’s cg08915438 TC21 site compared to non-descendants and more DNA methylation on NR3C1’s cg21979215 BC21 site. NR3C1 encodes the glucocorticoid receptor gene that governs the negative feedback loop of the HPA axis by repressing the production of CRH and ACTH. FKBP5 interferes with this negative feedback loop by acting as a co-chaperone that binds to several components of the steroid receptor complex. This interaction reduces the sensitivity of the glucocorticoid receptor by inhibiting its binding to the transport protein dynein, delaying the nuclear translocation of the GR, and decreasing its transcriptional activity.

According to the JASPAR 2024 database⁵⁸ these DNA methylation sites are located within binding sites of transcription factors associated with gene activation. This suggests that increased DNA methylation on NR3C1 is likely linked to reduced NR3C1 expression. Furthermore, lower DNA methylation on FKBP5 is likely linked to increased expression. Additionally, we found that 3rd- and 4th-generation descendants of Holocaust survivors had significantly less DNA methylation on CRH’s cg03405789 BC21 site and more DNA methylation on CRHBP’s cg26196496 BC21 site compared to non-descendants. CRH encodes the corticotropin-releasing hormone that facilitates the activation of the HPA axis and ultimately promotes the production of ACTH. Conversely, CRHBP inhibits the function of CRH by binding to free CRH molecules in the extracellular space, effectively insulating them and preventing them from binding to and activating CRH receptors.

According to the JASPAR 2024 database⁵⁸ these DNA methylation sites are located within binding sites of transcription factors associated with gene activation. As indicated in Supplementary Table 2, the EWAS Open Platform⁵⁹ confirms that higher DNA methylation levels on CRHBP’s DNA methylation site correlate with decreased CRHBP RNA expression (the platform lacks data on other HPA-related genes). Taken together, these findings suggest that descendants of Holocaust survivors show a pattern of increased methylation in genes that

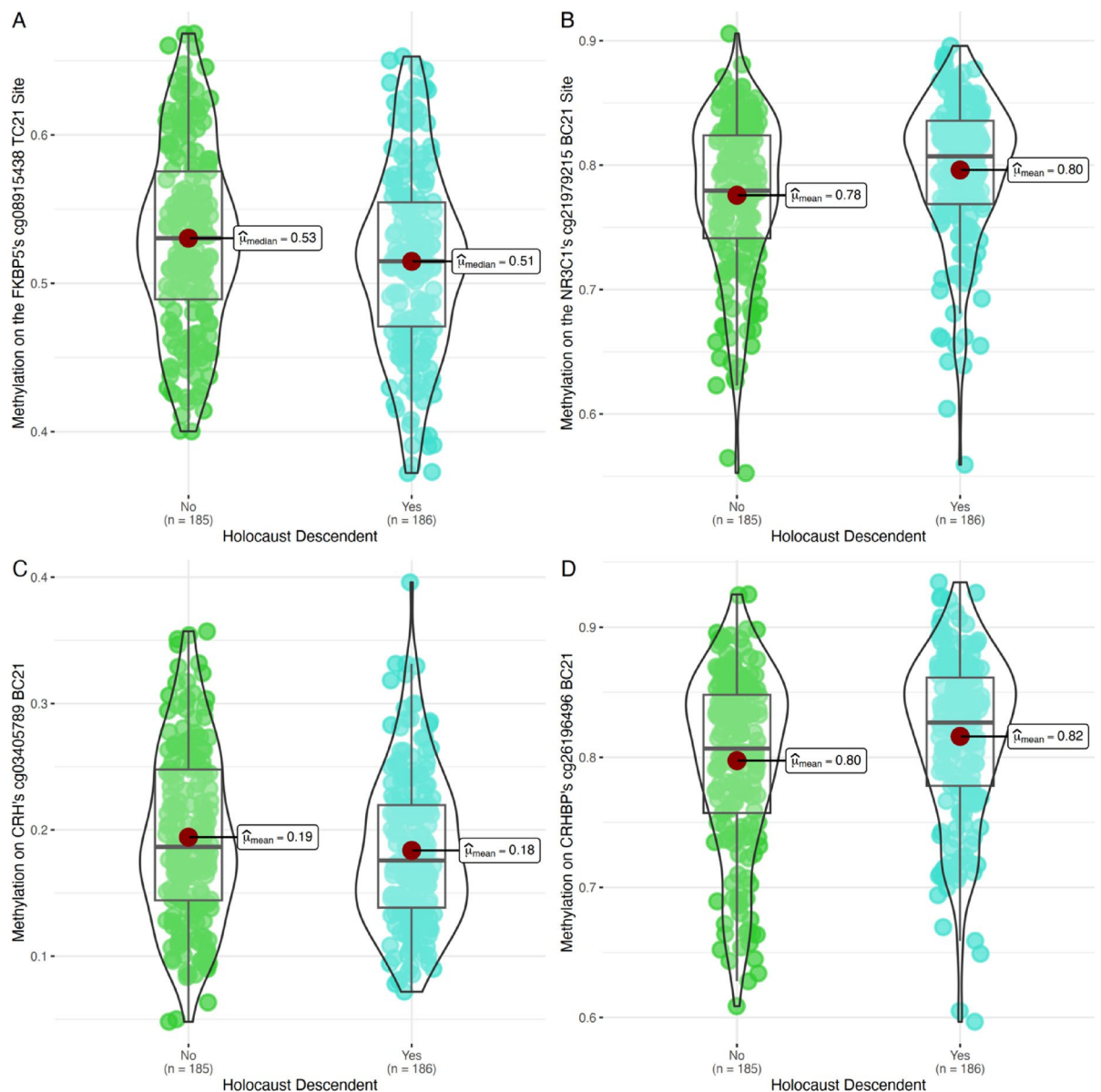


Fig. 1. Credible differences in DNA methylation levels between 3rd—and 4th-generation descendants of Holocaust survivors and non-descendants on FKBP5's cg08915438 TC21 site (A), on NR3C1's cg21979215 BC21 site (B), on CRH's cg03405789 BC21 site (C), and CRHBP's cg26196496 BC21 site. Descendants of Holocaust survivors exhibited a DNA methylation pattern associated with higher activation of the HPA axis (i.e., lower FKBP5 and CRH, but higher NR3C1 and CRHBP DNA methylation).

downregulate HPA axis activity (NR3C1 and CRHBP), and decreased methylation in genes that upregulate it (CRH and FKBP5). According to external reference databases, higher methylation at these sites is typically associated with lower gene expression. In contrast, we found no significant differences in DNA methylation within genes related to the sympathetic nervous system (SNS), which controls the rapid “fight-or-flight” response. Finally, no group differences were observed in symptom severity of anxiety, depression, somatization, or general distress (GSI).

Attachment and oxytocin (hypothesis 2)

We found that 3rd - and 4th -generation descendants of Holocaust survivors had credibly lower self-reported scores of general attachment avoidance (Median=3.33) compared to their non-descendant counterparts (Median=3.67; see Supplementary Table 1 and Fig. 2). No credible differences were found in attachment anxiety. These findings suggest that descendants are not necessarily more securely attached overall, but score lower on the avoidance dimension specifically.

Several credible differences in the level of DNA methylation of oxytocin-related genes corroborated the above-described difference in general attachment avoidance. Specifically, we identified differences in DNA methylation

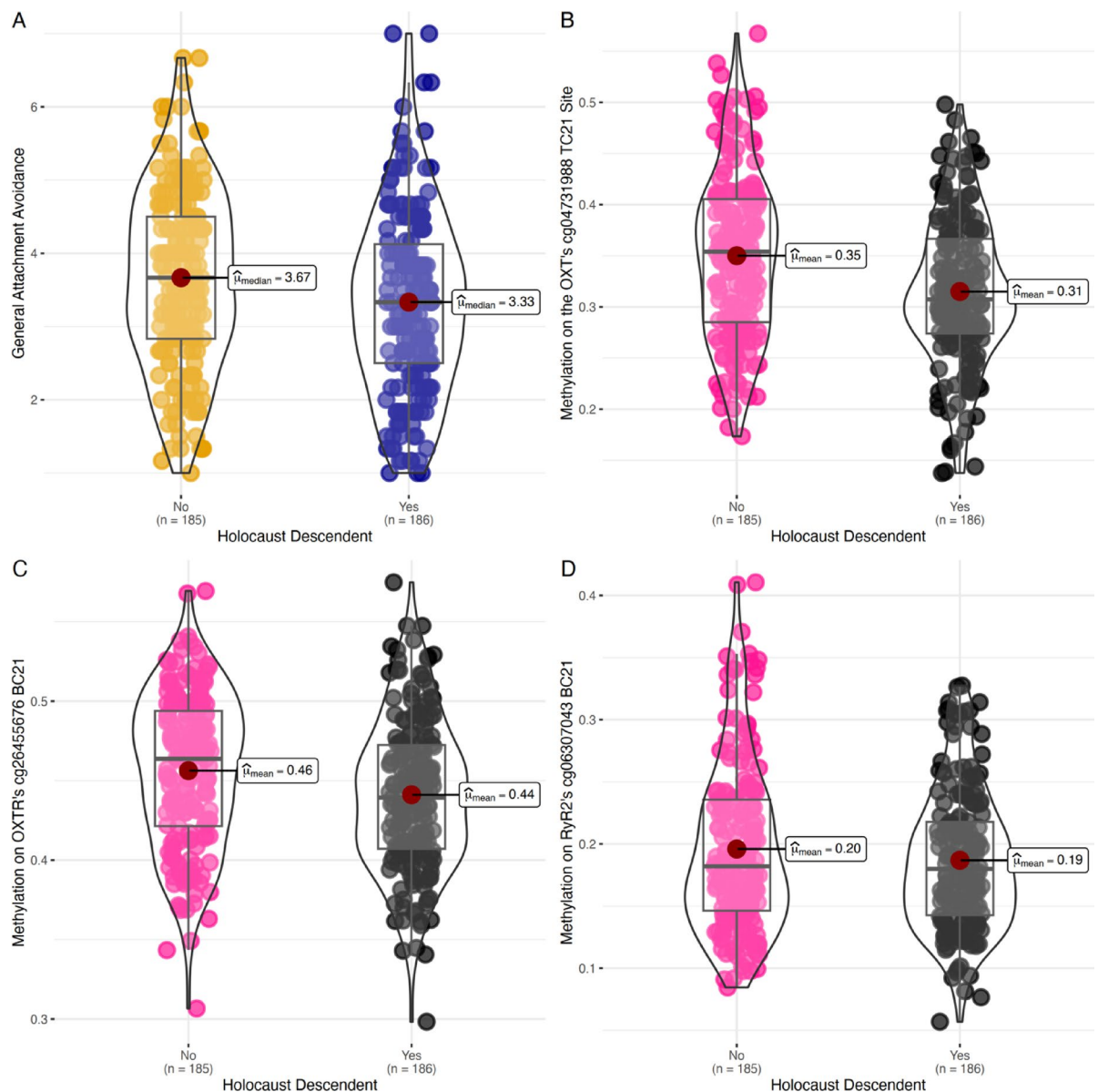


Fig. 2. Credible differences between 3rd- and 4th-generation descendants of Holocaust survivors and non-descendants in general attachment avoidance (A), DNA methylation level on OXT's cg04731988 TC21 site (B), DNA methylation level on OXTR's cg26455676 BC21 site (C), and RyR2's cg06307043 BC21 site. Descendants of Holocaust survivors exhibited lower general attachment avoidance and a DNA methylation pattern associated with higher oxytocin expression (i.e., lower DNA methylation).

levels at 10 CpG sites on the oxytocin gene (out of a total of 11 sites analyzed) and at 1 CpG site each on the oxytocin receptor, ryanodine receptor 1, and ryanodine receptor 2 genes (see Supplementary Table 4). The results were consistent across all sites, indicating that 3rd- and 4th-generation descendants of Holocaust survivors had significantly less DNA methylation compared to non-descendants. Exemplary results are presented in Fig. 2.

According to the JASPAR 2024 database⁵⁸ all these DNA methylation sites are located within the binding sites of transcription factors associated with gene activation. As indicated in Supplementary Table 4, the EWAS Open Platform⁵⁹ confirms that higher DNA methylation levels on all CpGs associated with the oxytocin gene correlate with decreased oxytocin RNA expression (note that the platform does not include data for the ryanodine receptor genes). These findings suggest that descendants of Holocaust survivors exhibit a pattern of decreased methylation in genes that enhance activity within the oxytocin pathway—specifically, OXT and OXTR, which code for oxytocin and its receptor, and RyR1 and RyR2, which support the positive feedback loop involved in oxytocin secretion.

To delve deeper into the DNA methylation results, we conducted a network analysis to identify the associations between CpGs in the oxytocin system. Specifically, we employed the Extended Bayesian Information Criterion (EBIC) with a Graphical Lasso, followed by refitting without the Lasso penalty to get more accurate

A. Network Among Significant CpGs

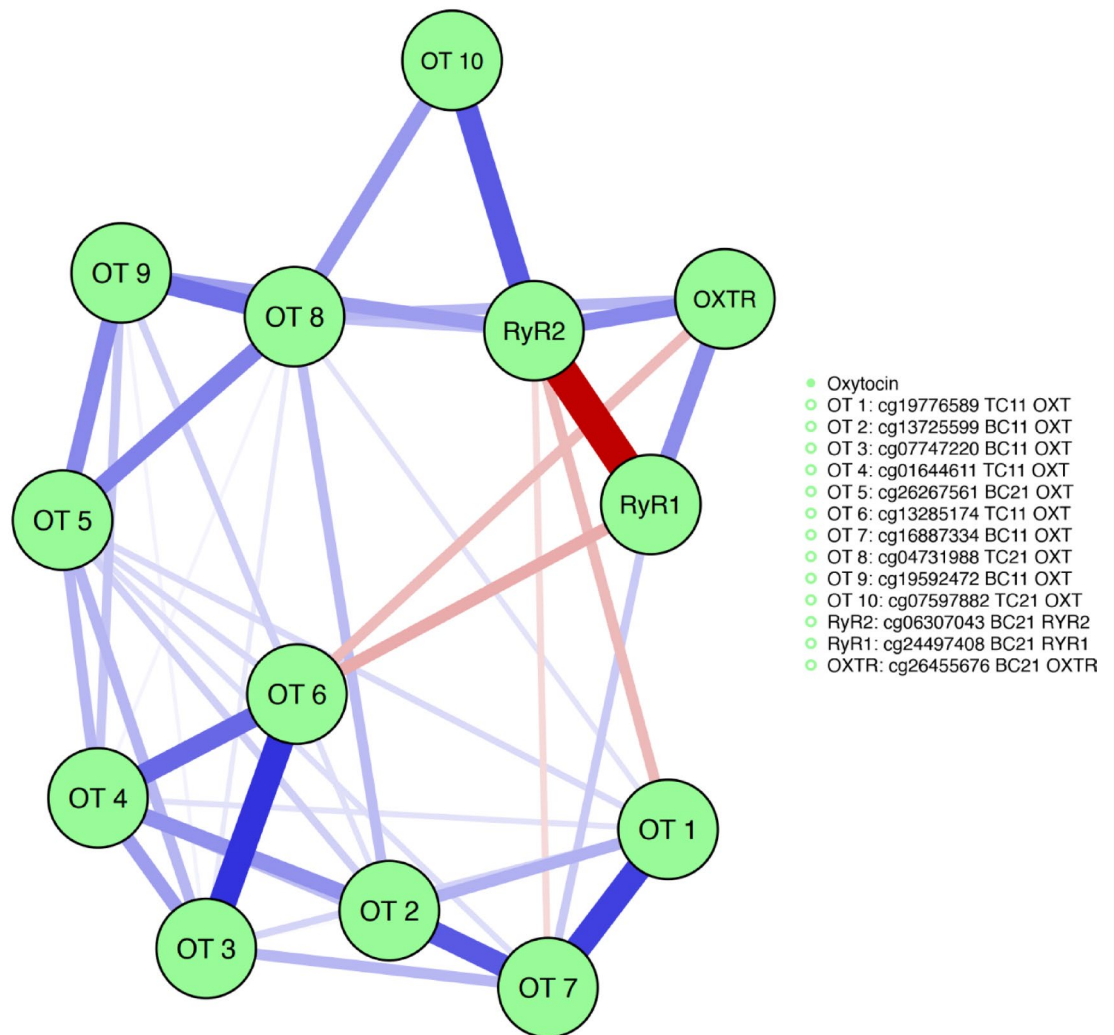


Fig. 3. Network analysis among the credible CpG sites within the oxytocin system. **(A)** The network analysis with blue edges (i.e., paths) reflecting positive associations and red edges reflecting negative associations. The edges' distance, width, and color intensity represent their relative strength. **(B)** The centrality scores of each of the nodes.

(non-shrunk) estimates of the partial correlations. The correlations employed in this process were based on polychoric and polyserial correlations. The estimation process was performed with the *estimateNetwork* function of the *bootnet* R package. The network is presented in Fig. 3a, and the centrality measures in Fig. 3b. Centrality refers to a series of measures to evaluate the function of each node (i.e., variable) within the network, and centrality invariance tests allow distinguishing nodes of different networks using the centrality scores. We focused on the following scores: (i) *Closeness* – Indicates how quickly a node can connect to others in the network; (ii) *Betweenness* – Shows how often a node acts as a key connector between other nodes; (iii) *Strength* – Reflects the overall influence of a node based on its connections; and (iv) *Expected Influence* – Represents the overall impact a node has within a network, considering both its outgoing and incoming connections.

The network analysis revealed important insights into the potential functional roles of oxytocin-related CpG sites and their interrelationships. CpG sites associated with the oxytocin gene exhibited higher expected influence values, indicating their significant role in potentially shaping the DNA methylation profile of the oxytocin system. In contrast, the RyR2 site emerged as a central hub in the network, exhibiting the highest scores for betweenness, closeness, and strength centrality. This indicates that RyR2 serves as a key connector (betweenness), enabling efficient communication across the network (closeness), and maintains strong and numerous associations with other nodes (strength). These centrality patterns underscore RyR2's pivotal role within the network, suggesting that its DNA methylation status may significantly influence the coordination and regulation of other genes in the oxytocin system. Overall, the centrality analysis highlights the distinct yet interconnected roles of oxytocin-related CpG sites, positioning RyR2 as a potential regulatory node within the broader epigenetic architecture of the oxytocin pathway.

B. Centrality of the Network's Nodes

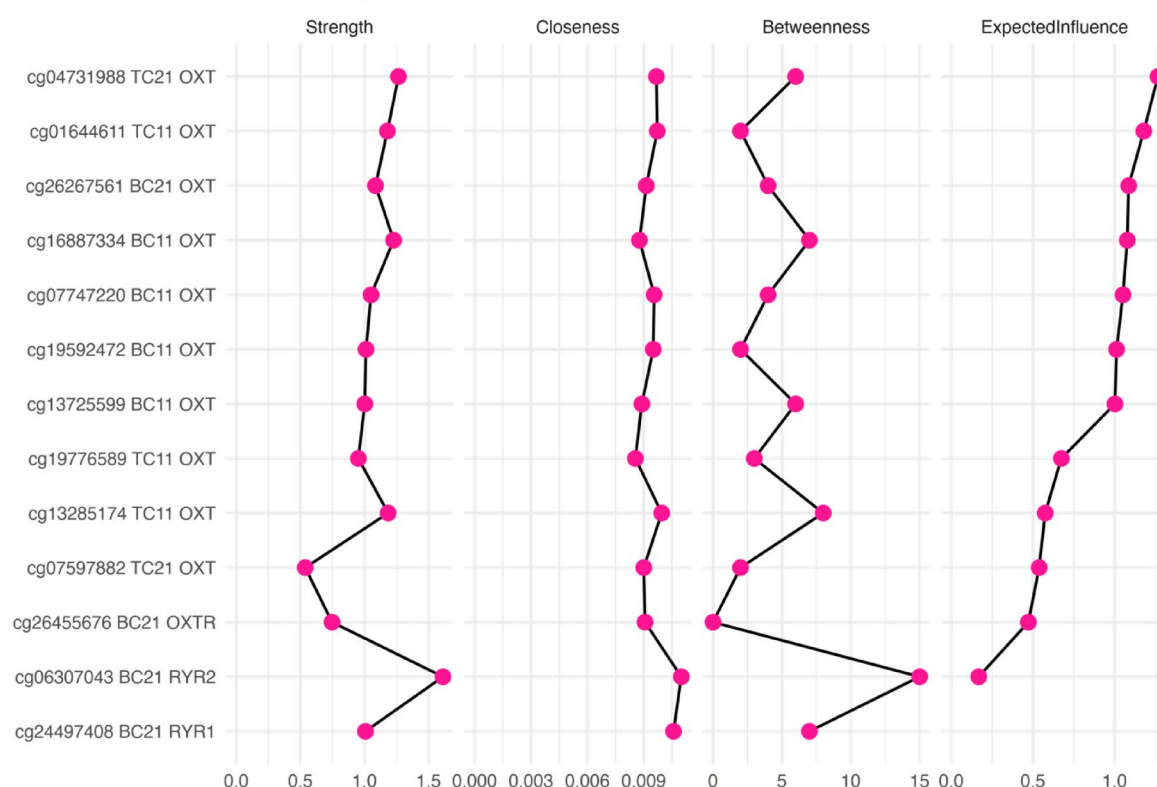


Fig. 3. (continued)

Next, we compared the oxytocin network of 3rd- and 4th-generation descendants of Holocaust survivors and non-descendants by employing edge and centrality invariance tests using the *NetworkComparisonTest* R package. In network analysis, edges are equivalent to partial correlations between two variables (known as nodes). Results are presented in Fig. 4 (the networks) and Fig. 5 (the centrality indices). The comparison revealed that although the global network strengths were not significantly different (6.60 for the descendants and 6.34 for non-descendants, $S=0.26$, $p=.718$), the interconnections between the DNA methylation sites differed significantly in eight edges (see Supplementary Table 5). Six of these edges (i.e., 75%) were stronger among 3rd- and 4th-generation descendants of Holocaust survivors, with four involving associations with the RyR genes. This pattern suggests that DNA methylation in components of the oxytocin system, particularly those regulating the positive feedback loop of oxytocin secretion, may be more tightly interconnected in Holocaust descendants. Additional differences were observed in 13 centrality indices (see Supplementary Table 6), with seven being stronger among 3rd- and 4th-generation descendants of Holocaust survivors and six among non-descendants. These results indicate that while the overall network strength was comparable across groups, the internal structure of the oxytocin methylation network differed meaningfully. Together, these findings provide a more nuanced perspective: third- and fourth-generation descendants of Holocaust survivors not only exhibit altered DNA methylation patterns but also demonstrate differences in how these methylation sites interact within the broader oxytocin system.

In a subsequent analysis, we examined whether general attachment avoidance was associated with DNA methylation levels of oxytocin-related genes, and whether these associations differ between third- and fourth-generation descendants of Holocaust survivors and non-descendants. Our aim was to explore whether the observed pattern—lower attachment avoidance and lower DNA methylation in oxytocin-related genes among descendants—might be associated with a shared underlying mechanism. To test this, we ran additional models predicting participants' general attachment avoidance scores from their DNA methylation levels at oxytocin-related genes, Holocaust descendency, and their interaction. Covariates were consistent with all prior analyses, and the full results are presented in Supplementary Table 7.

The analysis showed that lower general attachment avoidance was significantly associated with lower methylation on the RyR2 gene, a central hub in the oxytocin methylation network. Notably, the six most credible CpG sites associated with attachment avoidance were all located on RyR genes, further underscoring their key role in the network. However, CpG sites on the oxytocin (OXT) and oxytocin receptor (OXTR) genes—despite showing robust methylation differences between descendants and non-descendants—were not significantly associated with general attachment avoidance. This suggests that while lower avoidance may be linked to the methylation of RyR-related genes within the oxytocin system, this association cannot fully explain the group-level differences observed between Holocaust descendants and non-descendants. In short, the epigenetic

A. Network of Significant CpGs Among Descendents

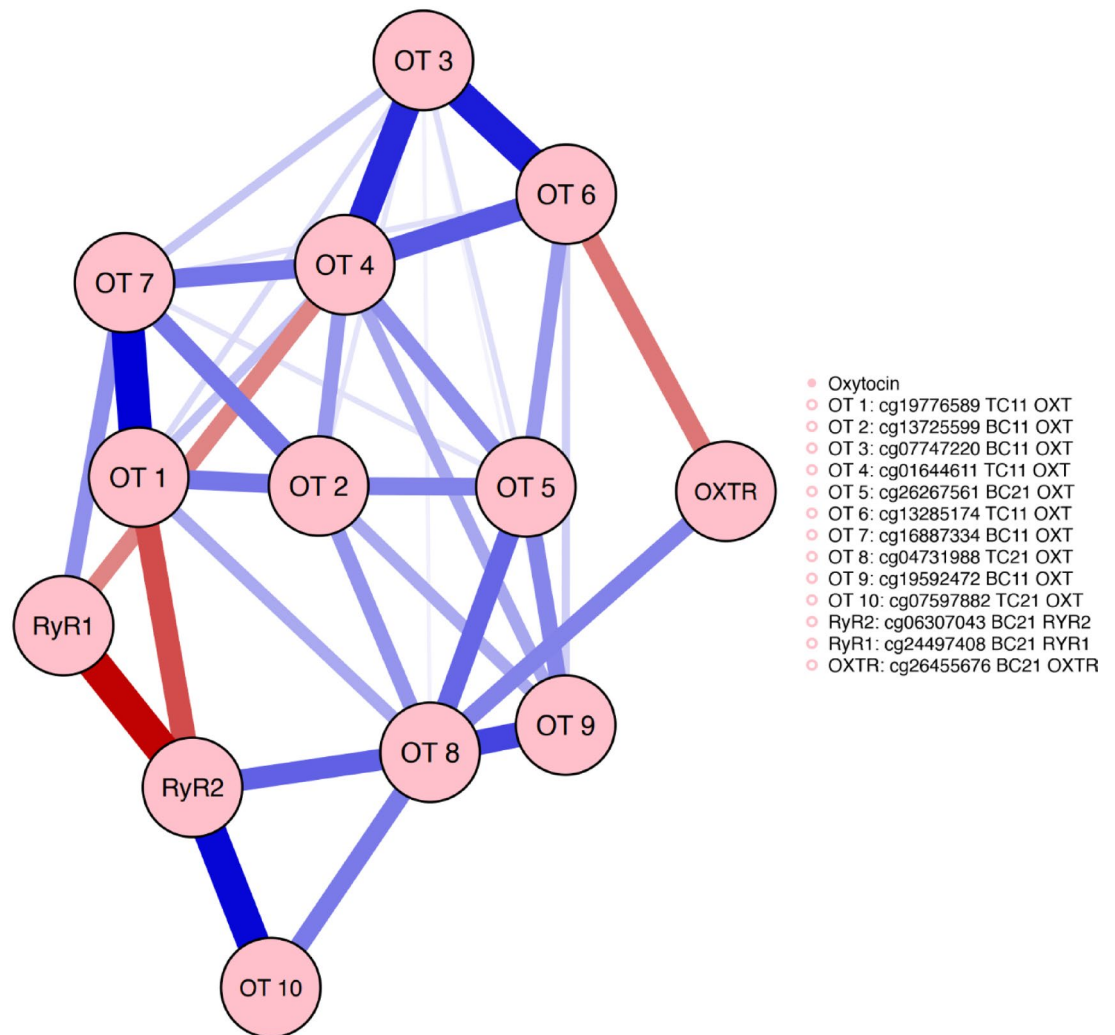


Fig. 4. Network analysis among the credible CpG sites within the oxytocin system. **(A)** The network among 3rd—and 4th-generation descendants of Holocaust survivors. **(B)** The network among non-descendants. Blue edges (i.e., paths) reflect positive associations, whereas red edges reflect negative associations. The edges' distance, width, and color intensity represent their relative strength.

correlates of attachment avoidance appear to overlap partially, but not entirely, with the methylation differences distinguishing the two groups.

In the final step of the analysis, we aimed to examine the stability of the differences between 3rd- and 4th-generation descendants of Holocaust survivors and non-descendants when considering the effects of participants' adverse childhood experiences. This examination would allow us to appraise whether the source of differences could be attributed to Holocaust descent and not to events in one's own life history. To this end, we reran the models while also controlling for the number of participants' adverse childhood experiences. Results are presented in Supplementary Tables 8 to 11. The addition of adverse childhood experiences did not change the pattern of results.

Discussion

The intergenerational transmission of trauma is a complex phenomenon, with the experiences of Holocaust survivors' descendants offering a unique lens through which to examine its long-term effects. Our study aimed to elucidate the enduring psychological and epigenetic consequences of Holocaust trauma in the third and fourth generations. We investigated whether these descendants, rather than primarily displaying post-traumatic symptoms, might instead exhibit biobehavioral patterns indicative of resilience and adaptation. Specifically, we examined whether their DNA methylation profiles may point to a more reactive survival response via the HPA axis, alongside greater attachment security supported by methylation patterns potentially linked to a more expressive oxytocin system. These traits may reflect an inherited legacy—rooted in their grandparents' determination to “never again” endure such trauma—that promotes trust and cooperation in times of need. Our

B. Network of Significant CpGs Among Non-Descendents

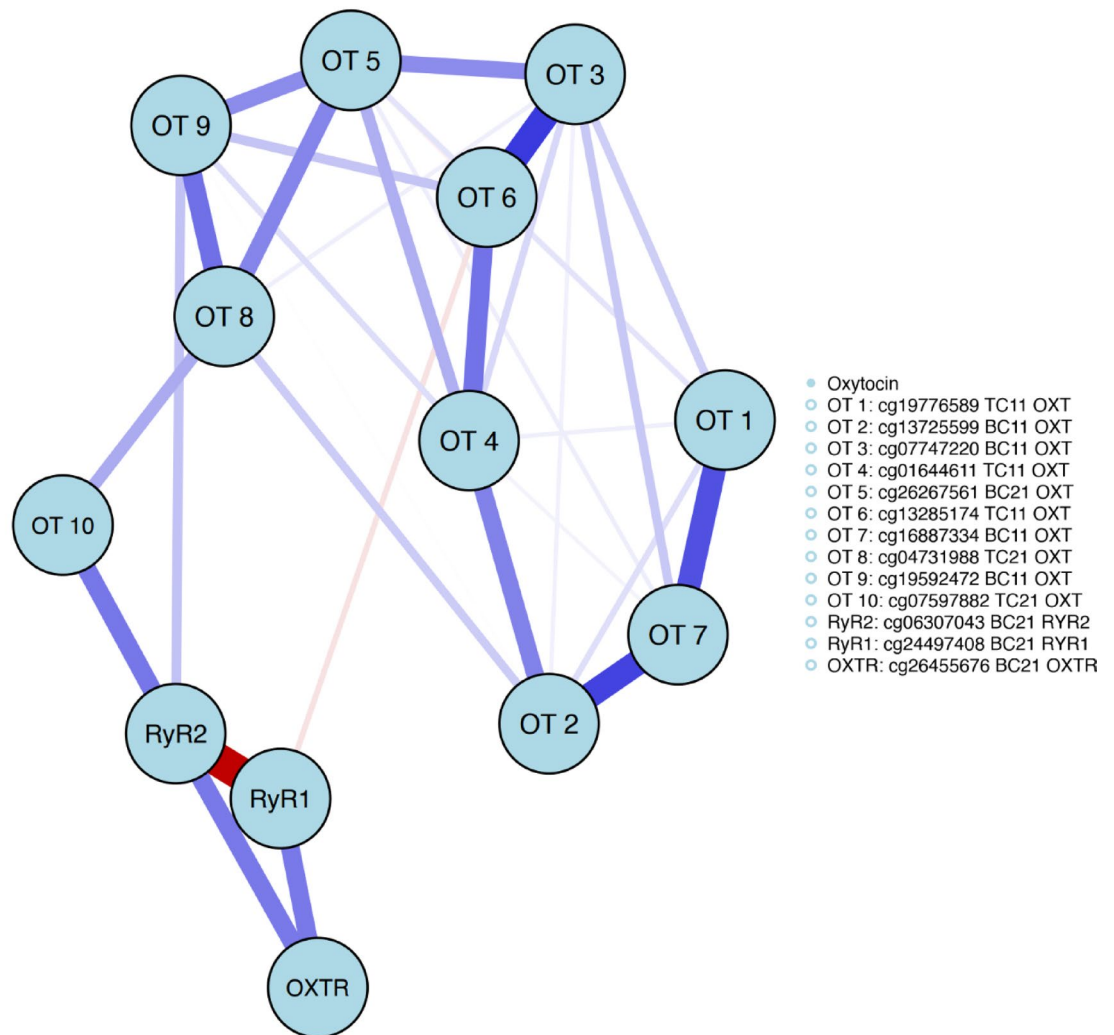


Fig. 4. (continued)

findings reveal both vulnerabilities and strengths, offering a nuanced understanding of how trauma may shape resilience across generations.

With respect to the HPA stress axis, we found that third-generation descendants of Holocaust survivors exhibited distinct DNA methylation patterns in several related genes. Specifically, there was lower DNA methylation on the CRH gene and higher DNA methylation on the CRHBP gene among descendants compared to non-descendants. CRH is a pivotal regulator of the HPA axis, initiating the body's central stress response. When encountering a stressor, CRH is released from the hypothalamus and stimulates the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland, which in turn promotes cortisol production in the adrenal glands^{60,61}. CRHBP modulates this activity by binding to CRH, thereby preventing excessive activation of the HPA axis⁶¹. Based on established associations from external expression databases, the DNA methylation pattern we observed suggests a tendency toward increased expression of CRH and reduced expression of CRHBP, which could indicate a more reactive HPA axis. Such a pattern may reflect an adaptive mechanism—a biological preparedness for heightened vigilance—that echoes the survival needs of previous generations. However, this interpretation remains inferential and should be corroborated by future studies using RNA expression data.

Furthermore, our study identified differences in DNA methylation patterns of FKBP5 and NR3C1, genes that play pivotal roles in the negative feedback regulation of the HPA axis. FKBP5, which interferes with glucocorticoid receptor (GR) sensitivity^{62,63} showed lower DNA methylation levels in descendants of Holocaust survivors compared to non-descendants, implying increased expression and a delayed shutdown of the stress response. Conversely, NR3C1, encoding the glucocorticoid receptor, had higher DNA methylation levels in descendants of Holocaust survivors compared to non-descendants, suggesting reduced expression and a decreased ability to terminate the stress response⁶¹. The finding regarding FKBP5 replicates previous research, which has shown that the offspring of Holocaust survivors exhibit DNA methylation patterns associated with increased HPA axis

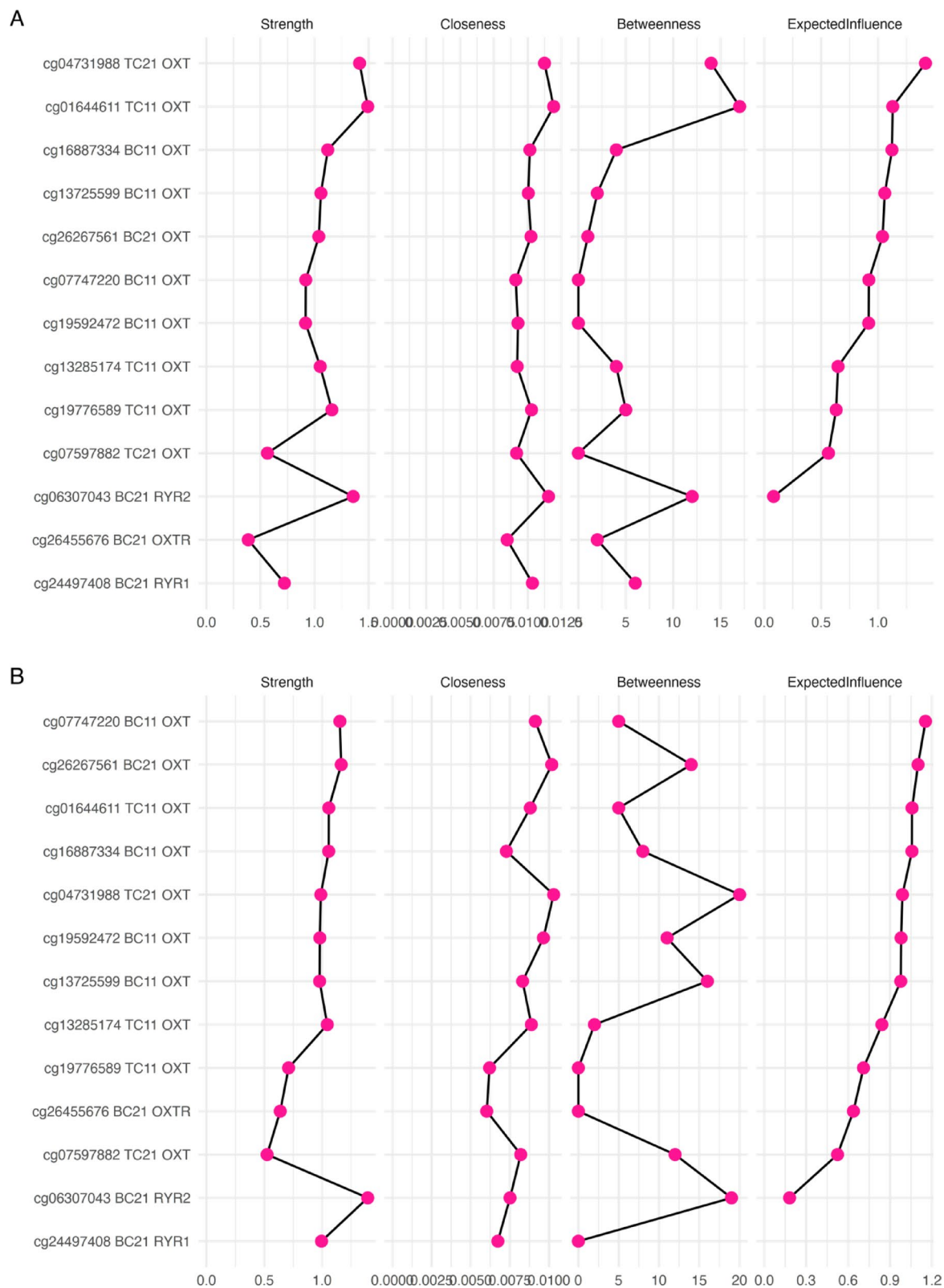


Fig. 5. The centrality scores of each node among descendants (A) and non-descendants (B). Each panel is sorted by the nodes' relative expected influence.

activity. Specifically, these patterns are linked to a more chronically reactive stress response, without evidence of long-term cortisol blunting effects⁹.

It should be noted here that these epigenetic changes are not solely attributable to transgenerational trauma but have also been linked to individuals' own history of traumatic experiences. For example, a study reported increased DNA methylation at specific sites of NR3C1's promoter region in hippocampal tissues among suicide victims with a history of child abuse⁶⁴. Similarly, a study examining the association between childhood abuse and PTSD found that those exposed to childhood trauma and carrying an FKBP5 risk allele (rs1360780) had, on average, a 12.3% decrease in DNA methylation of a specific FKBP5 region⁶⁵. These findings reflect the dual role of CRH and CRHBP, alongside NR3C1 and FKBP5, in balancing immediate stress responses with the long-term regulation of the HPA axis, thereby highlighting the influence of personal trauma in shaping stress reactivity. Crucially, however, in our study, the reported differences in HPA axis DNA methylation remained unchanged when controlling for the number of participants' adverse childhood experiences. Thus, although the current findings on the third and fourth generation of Holocaust survivors share similarities with the well-studied field of adverse childhood experiences, they reflect variance uniquely attributable to intergenerational trauma above and beyond personal adversity.

Interestingly, the observed DNA methylation pattern potentially associated with increased HPA reactivity among third- and fourth-generation descendants of Holocaust survivors was not accompanied by heightened symptom severity in the domains we assessed—namely depression, anxiety, somatization, and general psychological distress. This finding aligns with previous studies suggesting that, despite elevated biological sensitivity to stress, these descendants often function within non-clinical psychological ranges. For example, one study found that while third-generation individuals reported higher levels of secondary traumatic stress (STS) compared to controls, the majority still fell within non-clinical levels of severity⁸. One plausible explanation for this pattern lies in the accumulation of psychosocial resources that buffer against emotional dysregulation^{20–22}. Many second-generation caregivers appear to have cultivated emotionally secure environments through what has been described as parental compensation—a deliberate effort to counterbalance the emotional deprivation they may have experienced by emphasizing warmth, stability, emotional availability, and openness about the family's traumatic past^{19,23,24,27}. These parenting strategies likely enhanced emotional regulation capacities in their children, supporting more adaptive psychological outcomes²⁵. Jewish cultural values may also play a significant role in this adaptive trajectory²⁶. Themes such as collective memory, intergenerational continuity, education, and a strong emphasis on meaning-making may serve to recontextualize trauma as part of a shared narrative of survival and resilience, rather than as an enduring psychological burden. These cultural frameworks can strengthen identity and offer existential anchors that promote coping and growth. Notably, we did not identify any DNA methylation patterns associated with genes directly regulating sympathetic nervous system (SNS) activity. Given that the SNS is typically responsible for acute “fight-or-flight” responses, the absence of such methylation differences may suggest that this system is not persistently altered in these descendants. However, this interpretation remains speculative, as our study did not include gene expression data, and further research is needed to clarify the functional implications of these methylation patterns. Taken together, our findings portray a complex interplay between biological sensitivity and psychosocial modulation, suggesting that inherited stress responsivity does not inevitably result in psychological dysfunction. Instead, it may be buffered—or even redirected—by intentional caregiving practices and cultural meaning systems that foster resilience across generations.

Alongside higher DNA methylation in genes involved in downregulating the HPA stress axis and lower methylation in genes involved in upregulating it, we observed significantly lower levels of general attachment avoidance among third- and fourth-generation descendants of Holocaust survivors. Attachment avoidance refers to the degree to which individuals are uncomfortable with emotional closeness, dependence, and interpersonal vulnerability²⁸. Those scoring lower on this dimension are more open to intimacy, more willing to depend on others and to be depended upon, and more inclined to seek emotional support in times of need. Although lower attachment avoidance is typically associated with greater attachment security, we found no corresponding differences in attachment anxiety. Thus, the observed pattern does not necessarily reflect a heightened sense of interpersonal safety or emotional confidence overall, but rather a greater willingness to face challenges with others rather than alone. This distinction is meaningful. Lower avoidance facilitates proximity-seeking and interpersonal engagement under stress—behaviors that are well established as adaptive in both developmental and evolutionary contexts. In fact, the human capacity for social cooperation under threat has deep evolutionary roots. Evidence suggests that *Homo sapiens* survived environmental, intergroup, and existential threats not through solitary action, but through coordinated group efforts supported by advanced social-cognitive and emotional mechanisms^{66–70}. The ability to band together, to signal distress, and to elicit help from others was—and remains—a core survival strategy. From this perspective, the observed reduction in attachment avoidance among descendants of collective trauma may reflect not only an individual-level adaptation but also an evolutionarily adaptive message encoded epigenetically: in the aftermath of shared catastrophe, survival favors those who reach out, not those who retreat. This possibility aligns with the broader literature on social allostasis, which highlights the regulatory benefits of coregulation within close relationships. In challenging or threatening environments, individuals with lower attachment avoidance may be more likely to draw upon relational resources that help conserve metabolic energy⁷¹ dampen physiological stress responses, and enhance long-term resilience. In short, their openness to others may function not simply as a relational trait but as a survival-enhancing mechanism shaped by both social experience and evolutionary history.

At the epigenetic level, we observed significant differences in DNA methylation patterns in genes associated with the oxytocin system, including the genes encoding oxytocin, the oxytocin receptor, and ryanodine receptor types 1 and 2. Based on converging evidence from external expression databases, these methylation profiles may point to increased oxytocin system activity in third-generation descendants of Holocaust survivors.

Although we did not directly assess gene expression in this study, existing research suggests that lower DNA methylation in these regions is typically associated with increased gene transcription, which has been linked to enhanced social bonding, reduced anxiety, and improved emotional regulation. However, this interpretation remains tentative and should be corroborated by transcriptomic analyses in future research. Nonetheless, our findings are particularly relevant in light of oxytocin's role in social allostasis—the process by which social relationships regulate internal physiological states, especially during stress⁴⁶. Oxytocin modulates the hypothalamic–pituitary–adrenal (HPA) axis by inhibiting the release of corticotropin-releasing hormone from the hypothalamus and adrenocorticotrophic hormone from the pituitary gland, thereby reducing cortisol output and dampening the physiological stress response⁷². Beyond its endocrine effects, oxytocin facilitates proximity, trust, and interpersonal co-regulation. In emotionally supportive interactions, increased oxytocin release not only promotes bonding but also further attenuates stress responses, reinforcing a feedback loop that enhances both psychological and physiological stability.

Importantly, this mechanism may carry evolutionary significance in the context of intergenerational trauma. The ability to co-regulate through close social bonds is not merely a psychological comfort but a biologically grounded survival strategy. Following large-scale collective trauma, as experienced by Holocaust survivors, an intergenerational shift toward increased social engagement—reflected here in reduced attachment avoidance and potentially enhanced oxytocin signaling—may serve as an adaptive recalibration. The underlying evolutionary message may be: “in the aftermath of catastrophic threat, survival depends not on self-sufficiency, but on seeking safety in numbers, co-regulation, and mutual reliance.” Given that the survival of *Homo sapiens* has long depended on coordinated collective action—ranging from cooperative foraging to group defense and the sharing of emotional burdens—such intergenerational biological signaling may represent an inherited inclination to reorient toward connection, not withdrawal, when danger looms. It is therefore plausible to hypothesize that the relatively low symptom severity of anxiety, depression, somatization, and general distress observed among third- and fourth-generation descendants may, in part, be buffered by an oxytocin-mediated enhancement of social connectedness and stress modulation^{55,73–75}. These descendants may carry not just the molecular echoes of trauma but also epigenetically transmitted signals that prioritize togetherness in the face of adversity—an echo of what has made our species resilient throughout evolutionary history.

While our study offers valuable insights, there are several limitations to consider. First, because it is a correlational study, we cannot determine causality in the relationships observed among Holocaust descendancy, attachment, psychopathology, the HPA axis, and oxytocin. Second, although we controlled for cell type composition, our use of peripheral tissue from saliva samples may not accurately reflect epigenetic changes in specific brain regions. Third, while both third- and fourth-generation descendants were included in the analyses, the fourth-generation group was relatively small ($n = 19$), and therefore, the conclusions drawn primarily reflect the third generation. Fourth, we did not collect RNA expression data from our participants. Instead, we inferred gene expression associations using established external databases linking DNA methylation to gene expression. While this is a common approach in human genetic and epigenetic research, it does not substitute for direct expression measurements, and as such, conclusions about gene expression remain speculative. Fifth, although we controlled for key covariates—including age, sex, batch effect, and estimated epithelial and fibroblast cell composition—we acknowledge that additional early-life factors, such as prenatal exposures and broader postnatal environmental conditions, may also influence epigenetic outcomes. Among potential confounders, we focused on adverse childhood experiences (ACEs) due to their strong empirical relevance and their particular importance in disentangling the effects of personal life adversity from transgenerational trauma. To address this, we conducted additional analyses adjusting for ACEs, and the observed group differences remained robust. Nevertheless, the absence of direct measures for other early-life exposures limits our ability to fully account for all environmental contributors to the observed methylation patterns. Despite these notable limitations, our research highlights the complex interplay between genetic, epigenetic, and environmental factors in shaping the resilience of third-generation Holocaust survivors. The interplay found between the oxytocin system and the HPA axis underscores the multifaceted nature of resilience. The enhanced oxytocin system supports social bonding and reduces stress, while the reactive HPA axis ensures a vigilant response to potential threats. This combination of social-emotional strengths and physiological preparedness equips third-generation survivors with a robust toolkit for navigating the complexities of their inherited trauma.

Methods

Ethics information

This study is part of a large-scale longitudinal project named “Project Alpha,” pre-registered at <https://osf.io/kcn57/>. It was approved by Reichman University's Institutional Review Board and by the Helsinki Committee of the Israel Ministry of Health (approval number 5-2020), in accordance with ethical guidelines for research involving human participants. All methods were performed in accordance with the ethical guidelines and regulations set forth by the Helsinki Committee. The specific examination discussed here was pre-registered at <https://osf.io/t3ynm/>.

Participants

The study included 384 participants (192 couples). However, the biological samples from 11 participants were of low quality (either 5% of low-quality DNA methylation data points across probes or bisulfite intensity scores lower than 3 standard deviations below the mean), and 2 participants declined to complete the psychological measures. These individuals were excluded from the analyses, resulting in data from 371 participants. Of these, 186 were third ($n = 167$) and fourth ($n = 19$) generation descendants of Holocaust survivors (54.8% women, mean age = 29.67, SD = 3.58), and 185 were non-descendants (46.4% women, mean age = 29.34, SD = 3.10). There were no significant differences in age and gender between the groups. Bayesian hypothesis testing was used, and hence

no power analysis is reported. Instead, a minimum cut-off of bulk and tail effective sample sizes (ESS) > 400 was used to ensure reliable and stable estimates of central tendencies, variability, and credible intervals of parameters.

Measures

DNA methylation

DNA was sampled using the Oragene-DNA OG-600 saliva collection kit, which involves passive drool collection (i.e., spitting) of 2 mL of saliva into a self-contained tube prefilled with stabilizing buffer. This method is non-invasive and self-administered, and it differs from buccal swab techniques, which collect epithelial cells by swabbing the inner cheek. The OG-600 collection format is known to yield significantly higher quantities of high-quality DNA with lower bacterial contamination⁷⁶. On average, approximately 110 µg of DNA was extracted per sample. The kit ensures high sample stability at room temperature for up to five years and is compatible with high-throughput processing. Samples were shipped at room temperature to the Genomics Core Facility of Erasmus Medical Center, Rotterdam, the Netherlands. Upon delivery, DNA was extracted, isolated, and normalized using the internal procedures of the Human Genomics Facility (HuGe-F). An epigenome-wide DNA-DNA methylation scan was then performed using Illumina's Infinium[®] DNA methylationEPIC v2 BeadChip kit, which includes data on 935,000 DNA methylation sites. Raw data was saved in iDAT (Illumina Data) files.

Normalization and correction of DNA methylation values In the comprehensive preprocessing of DNA methylation data derived from the Illumina DNA methylationEPIC v2 kit, a sequence of advanced normalization and correction techniques were employed to ensure the highest quality and comparability of DNA methylation measurements across samples. Initially, the *preprocessENmix* method from the *ENmix* R package was utilized for background correction, leveraging a sophisticated exponential-normal mixture model to differentiate between signal and noise, thus enhancing the clarity and reliability of the DNA methylation signal. This was followed by *norm.quantile*, also from the *ENmix* package, to apply quantile normalization across samples. This is a crucial step in adjusting for batch effects and technical variability by aligning the distribution of probe intensities for all participants, thereby standardizing the data. The third method applied was the Regression on Correlated Probes (RCP) technique using *rcp* of *ENmix*, aimed at correcting probe design type biases by adjusting the DNA methylation data based on expected correlations between proximal probes targeting the same genomic features. Subsequently, the data underwent Beta-Mixture Quantile normalization (BMIQ) using the *wateRmelon* package, specifically tailored to adjust type-2 probe values, ensuring that they match the distribution of type-1 probes and accurately reflect true biological DNA methylation levels. We applied the *ComBat* method to adjust for batch effects using an empirical Bayes framework as a final step in this meticulous combination of normalization and correction procedures (using the *sva* R package). *ComBat*, renowned for its effectiveness in minimizing batch-related variability, further refines the data by correcting for any remaining batch effects that could confound the analysis. This step ensures sample variations reflect genuine biological differences rather than technical artifacts. This comprehensive suite of preprocessing methods — each addressing different aspects of technical variability and bias inherent in DNA methylation data — significantly improves downstream analyses' accuracy and biological relevance, facilitating a deeper understanding of epigenetic patterns and their implications.

Selection of CpGs for analysis Following the preprocessing phase of normalization and correction procedures, we filtered out CpG sites deemed unsuitable for further analysis. Initially, we utilized the *nmode* function from the *ENmix* package to identify such probes (referred to as “gap probes”), which exhibit an apparent multimodal distribution. These gap probes often indicate the presence of SNPs within the CpG probe region or other unknown factors leading to DNA methylation beta values with a multimodal distribution. Probes displaying multimodal distributions (ranging from 2 to 4 modes) were excluded from all subsequent analyses. Next, we eliminated CpG sites exhibiting less than a 7% interquartile range in their beta values, ensuring the selected CpGs possessed sufficient variance for follow-up analyses and that this variance surpassed the level of technical variability (see Supplementary Fig. 1). Furthermore, research has demonstrated that selecting CpGs with adequate variance is also associated with improved reliability indices⁷⁷. Following this phase, 157,909 DNA methylation sites were identified as eligible for further analysis. For the current study, specific CpG sites were selected based on their location within genes implicated in three major physiological systems: the oxytocin system (63 CpGs), the hypothalamic-pituitary-adrenal (HPA) axis (85 CpGs), and the sympathetic nervous system (21 CpGs). The complete list of genes associated with each system is provided in Table 1, while Supplementary Table 12 presents the comprehensive list of CpG sites included in the analyses.

Estimation of cell type composition Research has noted that different cell types have distinct DNA methylation profiles, suggesting that a portion of the variance in DNA methylation values stems from differences in cell type composition between participants^{78,79}. These differences should be accounted for in analyses. To estimate the cell type composition of each sample, we employed the HEpiDish package⁷⁹. The distribution of cell types is presented in Supplementary Fig. 2, and the correlation between the ratio of different cell types is in Supplementary Fig. 3. The most common cell types were neutrophils and epithelial cells. In addition, whereas the correlations between epithelial cells and all immune cell types were exceptionally high (above 0.80), the correlation with fibroblasts was only moderate ($r = .26$). Hence, in the analyses, we controlled for the contribution of the percentage of the epithelial and fibroblast cells to account for the different ratio of cell types across samples.

Regulatory structure estimation The biological significance of our findings was examined by appraising the possible function of each CpG using the JASPAR 2024 database⁵⁸. JASPAR is an open-access repository of transcription factor (TF) binding profiles derived from high-throughput experiments and curated datasets. Its 2024

System	Short name	Full name	Description
Oxytocin	OXT	Oxytocin	Neuropeptide hormone involved in social bonding, childbirth, and stress regulation.
Oxytocin	OXTR	Oxytocin receptor	Receptor mediating oxytocin's effects on the brain and body, crucial for social and emotional behaviors.
Oxytocin	CD38	CD38 molecule	Enzyme involved in oxytocin secretion and social memory formation.
Oxytocin	RYR1	Ryanodine receptor 1	Involved in the positive feedback of oxytocin secretion through intracellular calcium signalling.
Oxytocin	RYR2	Ryanodine receptor 2	Involved in the positive feedback of oxytocin secretion through intracellular calcium signalling.
Oxytocin	RYR3	Ryanodine receptor 3	Involved in the positive feedback of oxytocin secretion through intracellular calcium signalling.
HPA axis	CRH	Corticotropin releasing hormone	Main hypothalamic hormone that triggers the HPA axis response.
HPA axis	CRHBP	Corticotropin releasing hormone binding protein	Regulates CRH activity by binding and modulating its availability.
HPA axis	CRHR1	Corticotropin releasing hormone receptor 1	Receptor for CRH, initiates the HPA axis stress response cascade.
HPA axis	POMC	Pro-opiomelanocortin	Precursor polypeptide for ACTH, a major driver of cortisol synthesis.
HPA axis	MC2R	Melanocortin 2 receptor (ACTH receptor)	Receptor for ACTH, stimulates cortisol production in the adrenal cortex.
HPA axis	STAR	Steroidogenic acute regulatory protein	Protein that regulates steroid hormone synthesis in the adrenal glands.
HPA axis	FKBP5	FK506 binding protein 5	Co-chaperone regulating glucocorticoid receptor sensitivity and HPA axis reactivity.
HPA axis	NR3C1	Glucocorticoid receptor (NR3C1)	Major receptor for cortisol, key regulator of the stress response and HPA axis feedback.
SNS	DBH	Dopamine beta-hydroxylase	Enzyme converting dopamine to norepinephrine, crucial in sympathetic neurotransmission.
SNS	SLC6A2	Norepinephrine transporter (SLC6A2)	Responsible for reuptake of norepinephrine in sympathetic neurons.
SNS	ADRA1A	Adrenoceptor alpha 1 A	Adrenergic receptor mediating vasoconstriction and sympathetic responses.
SNS	ADRA2A	Adrenoceptor alpha 2 A	Adrenergic receptor inhibiting norepinephrine release, moderates sympathetic tone.
SNS	ADRA2B	Adrenoceptor alpha 2B	Adrenergic receptor subtype, modulates vascular smooth muscle contraction.
SNS	ADRB1	Adrenoceptor beta 1	Beta-adrenergic receptor stimulating heart rate and contractility.
SNS	ADRB2	Adrenoceptor beta 2	Beta-adrenergic receptor involved in smooth muscle relaxation and metabolic regulation.

Table 1. Full list of genes examined in the current study.

update features expanded coverage of TF binding motifs across diverse species, offering a critical tool for investigating regulatory mechanisms linked to DNA DNA methylation. JASPAR's inclusion in DNA methylation-based analyses adds significant depth to the interpretation of CpG site functionality. By integrating TF binding profiles with CpG DNA methylation data, it becomes possible to hypothesize how DNA methylation at specific loci might influence TF binding, gene regulation, and downstream biological processes. For instance, DNA methylation at CpG sites within TF binding motifs can inhibit binding, thereby altering gene expression patterns and contributing to phenotypic variability or disease susceptibility.

DNA methylation-expression association We utilized the EWAS Open Platform⁵⁹ to examine the relationship between the DNA methylation levels observed in our samples and the expression (i.e., RNA levels) of the genes associated with each CpG site. The EWAS Open Platform integrates extensive DNA methylation and transcriptomic datasets, providing a comprehensive framework to explore the regulatory relationship between DNA DNA methylation and gene expression. By focusing on CpG sites located within regulatory elements such as promoters, enhancers, and gene bodies, this platform enables the identification of patterns where DNA methylation status correlates with transcriptional activity. By integrating expression data, the EWAS Open Platform facilitates the generation of mechanistic insights, bridging the gap between epigenetic regulation and functional gene output. The current EWAS Open Platform DNA methylation data is based on Illumina's 450k DNA methylation kit, so it only partially covers the CpGs studied in this research derived from Illumina's DNA methylationEPIC v2 kit.

Holocaust descendency
Participants were asked to indicate whether they were third or fourth-generation descendants of Holocaust survivors.

Attachment patterns
To examine participants' attachment patterns, we employed the Experiences in Close Relationships Structures (ECR-RS) questionnaire in its revised version⁸⁰ a self-report measure designed to assess attachment patterns with respect to 4 targets (i.e., mother, father, romantic partner, and best friend) and a general overall attachment tendency. The same 9 items are used to assess two attachment dimensions, with 6 items appraising attachment avoidance (e.g., "I don't feel comfortable opening up to others"), and 3 assessing attachment anxiety ("I often worry that other people do not really care for me"). Participants are asked to indicate, on a scale from 1 ("strongly disagree") to 7 ("strongly agree"), the extent to which they agree with each item. The average rating score of attachment anxiety and avoidance regarding each target was calculated. The internal consistency values ranged between 0.70 and 0.89 for Cronbach's Alpha and 0.71 and 0.89 for McDonald's Omega, ensuring that the questionnaire demonstrated sufficient reliability in the current sample. Validity was verified by previous research^{32,81}.

Psychopathology

To examine participants' psychopathology, we employed the Brief Symptom Inventory-18 (BSI-18)⁸². This self-report measure contains 18 items rated on a 5-point scale (0 = not at all, 1 = a little bit, 2 = moderately, 3 = quite a bit, and 4 = extremely) to reflect respondents' distress during the previous week. The BSI-18 includes three symptom scales: Somatization (e.g., "Feeling faint or dizzy"), Depression (e.g., "Feeling hopeless about the future"), and Anxiety (e.g., "Nervousness or shakiness inside"), each comprising six items. Participants rate how much they have been distressed or bothered by each symptom in the past seven days. Scores on the 18 items are summarized on the Global Severity Index (GSI), providing an overall measure of psychological distress. The internal consistency values ranged between 0.70 and 0.78 for Cronbach's Alpha and 0.73 and 0.88 for McDonald's Omega, ensuring that the questionnaire demonstrated sufficient reliability in the current sample. Validity was verified by previous research⁸³.

Procedure

Recruiting a large cohort committed to a long-term study presents significant challenges, particularly in minimizing attrition. To address these challenges and recruit a substantial sample of young Israeli couples, we collaborated with a reputable advertising agency that dedicated its resources to promoting the study until we reached the necessary cohort size. Our recruitment efforts included a targeted advertising campaign across various social media platforms. To further encourage participation, we offered monetary compensation of 100 ILS (approximately 25 Euros) per assessment and provided participants access to a specially designed knowledge center tailored for young couples and parents. This knowledge center, developed by our lab's students, offers valuable, simplified, academic-based information on topics such as genetics, epigenetics, social neuroscience, and psychological phenomena. Project information and participant registration were managed through a dedicated website.

Sampling was conducted at locations convenient for the participants, either at university or their homes, based on their preference. Participants were instructed to fast for 30 min prior to sampling to ensure the reliability of the saliva samples. Upon arrival, they provided informed consent and received a briefing on the study's objectives and procedures. Saliva samples were collected using the Oragene-DNA OG-600 kit, which collects 2 ml of saliva, yielding approximately 110 µg of DNA, and guarantees high specimen stability at room temperature for up to 5 years. The samples were then shipped at ambient temperature to the Genomics Core Facility of Erasmus Medical Center in Rotterdam, where DNA was extracted, isolated, and normalized following the internal protocols of the Human Genomics Facility (HuGe-F). An epigenome-wide DNA methylation scan was performed using Illumina's Infinium[®] DNA methylationEPIC v2 BeadChip, providing DNA methylation data across approximately 935,000 sites.

After the sampling, participants engaged in a series of recorded interactions (not within the scope of this study). Following that, they completed a set of questionnaires on the Qualtrics platform, which included the Experiences in Close Relationships–Relationship Structures (ECR-RS), psychopathology (BSI-18), Holocaust Descendancy, and a socio-demographic questionnaire. Each session lasted approximately an hour and a half, and participants were compensated with 100 ILS for their time and involvement.

Data analysis plan

Individual differences in DNA methylation values are not purely indicative of biological differences but are also influenced by measurement variability, known as the "batch effect" (where samples processed with the same kit show more similarity than those processed with different kits). Additionally, differences in cell type composition (as DNA methylation values vary based on the originating cell type or tissue)^{78,79} as well as the participant's biological sex and age, contribute to these variations. Therefore, data analysis models must account for and eliminate these sources of variability to accurately reflect biological differences.

Furthermore, the current study's data analysis must recognize that participants are couples, not independent individuals. Consequently, multilevel analyses were employed to address both individual and couple levels. Two common biases in epigenetic analyses were also considered: (1) outliers (extreme values that can skew results) and (2) heteroscedasticity (unequal distribution of observations along the regression line). To mitigate these issues, robust priors were used in a Bayesian analytical approach.

The research hypotheses were tested using Bayesian Mixed-Effects models with the *brm* function from the *brms* R package. Holocaust descendancy (0 = no, 1 = yes) was the predictor variable. The outcome variables included the psychological constructs of attachment anxiety and avoidance in specific relationships and in general and the psychopathology clusters (anxiety, depression, somatization, and the global severity index). Additional outcome measures were DNA methylation beta-values of CpGs in the oxytocin systems, the HPA axis, and the Sympathetic Nervous System. The frequency of epithelial and fibroblast cells, the participant's biological sex, and age were included as covariates. The batch effect was controlled using the *ComBat* function from the *sva* R package, and couples' interdependence was managed by including the couple's identifier number as a random effect.

The analysis employed Monte Carlo Markov Chains (MCMC), a robust computational method that iteratively samples from probability distributions to approximate the posterior distributions of the parameters. MCMC offers a significant advantage over traditional regression models, as it enables the estimation of complex hierarchical and non-linear relationships while incorporating prior knowledge into the analysis. This approach also provides more accurate uncertainty quantifications through credible intervals rather than relying solely on p-values, thus enhancing the interpretability and reliability of the findings. MCMC is particularly well-suited for high-dimensional data and models with random effects, making it ideal for analyzing the intricate interdependence within couples and the interplay between psychological and epigenetic factors.

In the Bayesian models, two robust priors were set: one for the regression coefficients and one for the group-level standard deviations. These priors were based on Student's *t* distribution, which is more robust than a normal distribution, with degrees of freedom set to 8 and the scale parameter set to 0, resulting in a tighter distribution around the mean. The reported effects have Bayesian 95% credible intervals excluding 0, indicating a high probability of the direction of the effect, often accompanied by a “probability of direction” of 97% or higher.

Data availability

Data is publicly available at <https://osf.io/t3ynm/>.

Code availability

The R code comprising the analytical steps is publicly available at <https://osf.io/t3ynm/>.

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Author contributions

The study is part of G.O.'s master's thesis, which was supervised by T.E. and D.A.S.; G.O. wrote the first draft of the paper, which was edited by T.E., D.A.S., A.S., P.V., and W.V.; T.E. was in charge of the analytical plan and the analyses. N.N.S. provided genetic analysis guidelines and was responsible for the study's ethical approval.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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