The gene environment aetiology of freezing and its relationship with internalizing symptoms during adolescence

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Summary

Background The freezing response is a universal response to threat, linked to attentive immobility and action preparation. It is relevant for acute stress coping in animals and humans, and subtle deviations in toddler freezing duration (absence of, or excessively long reactions) have been linked to higher risk for internalizing symptoms in adolescence. Yet, while individual freezing tendencies are relatively stable throughout life, little is known about their gene-environment aetiology.

Methods We investigated the heritability of toddler freezing in the Quebec Newborn Twin Study (QNTS; n=508 twins) by fitting behavioural genetic models to video-coded freezing responses during a robot confrontation. Furthermore, we examined the predictive associations between toddler freezing and internalizing symptoms (anxiety and depressive symptoms), as they unfold during adolescence (ages 12–19 years) using linear mixed-effects models.

Findings Freezing was found to be moderately heritable (45% of the variance accounted for by genetic factors). The remaining variance was explained by unique environmental factors, including measurement error. No significant contribution of shared environmental factors was noted. Additionally, shorter freezing was associated with more internalizing symptoms in adolescence at trend level, a pattern that was significant for depressive but not anxiety symptoms.

Interpretation Freezing is an adaptive coping mechanism in early childhood, which is partly driven by genetic factors. Crucially, the absence or shorter duration of these behaviours may signal vulnerability to depressive problems later in life.

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Keywords: Behavioural genetic modelling; Adolescence; Defensive stress response; Toddlerhood; Internalizing symptoms; Longitudinal research design

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Introduction

The freezing response is an evolutionary defensive mode, activated under mild to high threat[1-3] presumably helping individuals to assess the situation and to prepare for adequate action.[4-6] As such, it has been

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Research in context

Evidence before this study

Human freezing has shown to play an important role in risk assessment, decision making, and action preparation in situations perceived as stressful or potentially threatening, making it relevant for adaptive coping. Conversely, insufficient or excessive use of freezing have been linked to the onset of internalizing symptoms, highlighting its potential as an early marker for stress-related disorders. Evidence of freezing being relatively stable over time and its link with variations in a gene encoding a serotonin transporter (*SERT*), provided a basis to further investigate its gene-environment aetiology, although the candidate gene approach has been criticized. Moreover, related phenotypes, such as behavioural inhibition have shown to be under moderate genetic influence in previous twin studies. Before planning our research, we have reviewed reference lists of the according studies and review papers. In addition, we searched online data bases for behavioural inhibition, anxiety, depression, internalizing symptoms and genetic modelling.

Added value of this study

This study adds to the current literature in two ways. First, drawing from the QNTS, a large longitudinal twin sample, we document the relative genetic and environmental contributions to individual differences in freezing. Second, we provided evidence for a predictive association between individual differences in freezing in toddlerhood and depressive symptoms during adolescence.

Implications of all the available evidence

Identification of freezing as an early risk marker for stress-related disorders, which is partly under genetic control, has both theoretical and clinical implications. First, it can direct future research towards a promising new candidate for early interventions targeting child responsivity to stress and the reaction of the caregiver. This, in turn, could eventually prevent the occurrence of internalizing symptoms during adolescence.

suggested to represent an adaptive coping mechanism in response to stress, particularly in toddlers who have not yet developed alternative strategies to optimally act upon perceived threats.

Growing evidence suggests that individual differences in freezing already present early in life can have important informative value for child development. Subtle deviations, in the form of both lower and higher duration of freezing have been associated with higher risk for internalizing problems, such as anxious, and depressive symptoms.[7-9] Specifically, while prolonged toddler freezing has previously been shown to predict higher internalizing symptoms in early adolescence (age 12 years,[8] see also Henderson, Pine and Fox[10]), it was the absence of freezing that predicted a constant pattern of higher risk for internalizing symptoms from childhood to late adolescence (larger symptom increase up until age 17[8]). These findings point to the possibility that developmental trajectories of internalizing symptoms from childhood to the end of adolescence vary according to freezing behaviour, especially later in adolescence. Prolonged freezing behaviour may bias towards preferential threat processing and passive responding, which may potentiate risk for internalizing difficulties,[8] particularly anxiety and social phobia in childhood.[11] In the same vein, however, shorter and absence of freezing might point at a lack of coping repertoire, which may also prompt more internalizing problems in the long run. Indeed, freezing has shown to be adaptive in moderately stressful situations[11] and the traditional view of freezing as a marker for psychopathology might undermine more complex or bivalent associations.[2,5,6,12] This is consistent with prior evidence showing that freezing-related heart rate deceleration was linked to optimized visual processing and faster subsequent flight responses,[13,14] and to support value integration[15] as well as faster perceptual decision-making[16,17] (see also Ribeiro & Castelo-Branco[18]). This suggests that lower or absence of freezing may point to a higher subsequent risk of internalizing problems (for reviews see Skora, Livermore, & Roelofs[19]; Roelofs[6]; Roelofs & Dayan[5]; Livermore et al.[12]).

Moreover, while freezing has often been described as one component of, or as similar to, behavioural inhibition, which has been linked to fear regulation itself,[20] we and others have argued that this early phenotype might have unique explanatory value.[8,11] Indeed, whereas both concepts include parasympathetically dominated silencing of motion (attentive immobility), behavioural inhibition also encompasses active sympathetically-driven signs of distress, including crying, and avoidance when confronted to novelty or other salient clues.[20] Importantly, however, while freezing tendencies in animals and humans have been shown to arise early in life and to be relatively stable over time[21] and across the life span,[22] little is known about their genetic and environmental aetiology.

The goal of this project was two-fold. First, we aimed to investigate the genetic and environmental aetiology of freezing using data of a moderately large sample of the Quebec Newborn Twin study (QNTS). Building on exploratory analyses of Niermann and colleagues[8] and previous research identifying an association with a polymorphism located in the serotonin transporter gene (*SERT*) in animals,[23] and humans (translational approach[24]), we expected the freezing response in 18-month-old toddlers to be under partial genetic influence. Yet, since complex phenotypes such as freezing likely arise from many genetic influences of rather small

magnitude, molecular candidate genes may not yet capture the full range of variance under genetic influences and uniquely based genetic approaches overlook environmental factors that may also contribute to variance of this behaviour. As such, twin studies represent a key complementary design to describe the relative contributions of genetic and environmental factors, a necessary step to guide future investigations aimed at identifying the specific genes and environments involved. Our second goal was to describe freezing in toddler twins and to replicate the predictive associations with internalizing symptoms, i.e., anxiety and depressive symptoms, in adolescence.[8] The QNTS used a similar stress inductive situation as Niermann et al.,[8] namely a robot confrontation at 18 months. This situation represents a novel situation for toddlers that reliably induces freezing responses in some toddlers, to varying degree, which can be coded according to the frequency and duration of the body's immobility.[8,11] Building on this study, we hypothesized lower freezing to be predictive of increasing internalizing symptoms from age 12, and that this association becomes more apparent later in adolescence.

Methods

Sample

The QNTS is a cohort of twins recruited at birth between 1995 and 1998 in the Greater Montreal area, Canada, and followed-up longitudinally to assess a wide range of individual, social, family, and school information. From all the families contacted, 662 (67%) agreed to participate when the twins were 5 months old. This sample was representative of the general population in terms of sociodemographic characteristics (recruiting and sampling procedures are described in more details by Boivin and colleagues).[25] The present study is based on data collected in a subsample at 18 months (n=508), 12 (n=365), 13 (n=356), 14 (n=337), 15 (n=329), 17 (n=328), and 19 (n=388) years of age and sample size was determined based on data availability at each time point. Participants were, by and large, representative of the full sample, with only one detected mean difference between the two groups, pertaining to the father's education status. These attrition analyses conducted on demographic variables, such as sex or socioeconomic status, are presented in Appendix S1 of the Supplementary Material.

Ethics

Ethical approval was obtained by the Sainte-Justine UHC Research Ethics Committee (reference number 2009-202, 2764) and approval of this study was granted by the Sainte-Justine UHC. Parental informed consent and child assent were obtained annually and approved by the ethics review board at Université Laval, Quebec.

Robot confrontation

The robot confrontation used in the QNTS is a novel situation during which 18-month-old toddlers were confronted with a noisy robot.[26] In this paradigm, each toddler was placed in a room with their mother who was sitting on a chair in one corner of the room and was instructed to hold the child while the experimenter placed the robot in the opposite corner. After the experimenter left the room, the mother was asked to let go of the child and not to intervene for 140 seconds, the time window during which freezing was assessed.

Primary measures

Freezing. Following previous research in animals and humans, the main variable of interest was the total duration of freezing (hence the sum of the duration of all incidences per toddler) as an age appropriate indicator.[8,11] Freezing behaviours (incidences of at least 3s with no to little bodily movement and absence of vocalisation), in addition to secondary behaviours proceeding and preceding each episode, were video-recorded during the robot confrontation and were independently coded by three research assistants and the first author according to an adapted version of the coding scheme developed by Niermann and colleagues.[8] All videos were coded blind to zygosity status, and the members of a twin pair were never coded by the same person. The freezing duration was divided by the actual duration of the robot confrontation to account for possible deviations from the protocol. Out of 554 18-month-old toddlers, the behaviour of 46 could not be coded due to the absence of a video or their participation in the novel situation (n=31) or the impossibility to code their behaviours according to the scheme (n=15, e.g., due to close-to-zero baseline in movement, toddlers disappearing under the chair or from the camera, use of objects, mothers intervening too much or imposing too many movement restrictions on the child). The final sample with available freezing information thus consisted of 508 toddlers. We computed average score one way intraclass correlations (using the irr package[27] between judges' mean ratings. These interrater reliability statistics suggested strong agreement for the duration (r=0·84, 95% CI 0·80–0·87) and the number of episodes (r=0·97, 95% CI 0·95–0·98). More information on the coding is provided in Appendix S2. To reduce the impact of potential outliers (2% or n=12, with a value > 3 standard deviations (SD) above or below the mean), all freezing durations were winsorized to the 95th percentile for all subsequent analyses.

Internalizing symptoms. Depressive symptoms were self-reported at 12, 13, 14, 15, 17, and 19 years of age using an adaptation from the Children's Depression Inventory (CDI)-Short Form[28] and the Achenbach System of Empirically Based Assessment (ASEBA)[29] as an additional measure at 19 years of age. Anxiety

symptoms were reported according to a short version of the Children's manifest anxiety scale (CMAS)[30] at 12, 13, and 14 years of age, and with the ASEBA at 19 years of age. Of the 508 toddlers with complete freezing data, information on depressive and anxiety symptoms was at least partly available for 436 (86%). Mean scores were calculated separately for depressive and anxiety symptoms according to all available items at each time point, and then standardized. The standardized mean scores of depressive and anxiety symptoms were averaged into one internalizing symptom score at each time point, considering the moderate-to-strong Pearson's product moment correlation between these scales (r=0·42−0·75, 95% CI 0·33−0·79, for time points for which both scales were available). More detailed information is provided in Appendix S3.

Statistics

Twin models. Potential differences in variance and means of the freezing duration between sexes and zygosity were tested by means of F-tests and t-tests. To assess differences in the association of freezing between MZ and DZ twins, we computed two way intraclass correlations. Then, additive genetic (A), shared environmental (C) or dominance genetic (D), and unique environmental contributions (E) to freezing were estimated through structural equation modelling of variance and covariance patterns among monozygotic (MZ) and dizygotic (DZ) twin pairs using OpenMx[31] for R.[32] To determine the best fitting and parsimonious model, we estimated both ACE and ADE models, and used nested χ2-difference tests, to compare the full ACE (or ADE) model to the saturated and more restrictive models. Moreover, we compared Akaike Information Criterion (AIC), and Bayesian Information Criterion (BIC), where lower values indicate good model fit and parsimony. More detailed information on the genetic modelling method is provided in Appendix S4.

Mixed-Effects-Models Analysis. We ran three Bayesian linear mixed-effects models, using brms[33] in R.[32] In the main model, we predicted internalizing symptom scores (depressive and anxiety combined), and in the two follow-up (sensitivity analysis) models, we predicted depressive and anxiety symptoms scores separately. In each model, we included the main effects of toddler freezing (continuously distributed and centred around 0) to assess the positive or negative valence of its association with symptom scores. We also included the polynomial (linear and quadratic effects) of age to assess linear and curvilinear development of symptoms during adolescence (i.e., linear and quadratic time effects). Lastly, we included the interaction term of freezing with the polynomial age effect to assess whether differences in freezing are associated with distinct developmental patterns of internalizing symptoms over time, and whether this association accentuates in late adolescence. We included per-subject random adjustments to the fixed intercept as well as random adjustments to the age effects and included all possible random correlation terms among the random effects.[34] This allowed random variations of the effects across subjects (and twin pairs). To infer existence of an effect, we obtained the probability of direction (probability that an effect is of the same sign as the median).[35] Since Bayesian models do not provide traditional p-values, we inferred significance of effects based on the posterior probabilities (pp), i.e., the proportion of the posterior parameter distributions that lie above or below 0.[15] Additional information on model fitting is reported in S5.

Role of funders

The funding sources had no role in planning and conducting the study and writing the report.

Data statement

All data is coordinated by the research unit on children's psychosocial maladjustment (GRIP). It is not available publicly but can be shared upon request via the data access form available on the institutional website: http://www.gripinfo.ca/grip/public/www/Etudes/en/dadprocedures.asp.

Results

Descriptive statistics

Freezing. For the 508 toddlers with available freezing data, the average duration of each freezing incidence (grand average of within-subject means) was 5·73s, and the mean number of episodes per child was 2·61. A total of 136 toddlers (27%) showed no behavioural signs of freezing during the robot confrontation. The average total duration per toddler (corrected for the total duration of the robot confrontation) was 22·48s. The average scores did not differ in the subsample with available information on internalizing symptoms (n=436, see Appendix S6). Means and standard deviations for sex and zygosity status are displayed in Table 1. Freezing data was complete for 100 MZ pairs (n=41 male, n=59 female) and 141 DZ twin pairs (n=42 male, n=35 female, n=64 opposite sex). There were no significant sex differences in variance (F(1, 506)=0·05, p=0·82), and means (t(506)=0·01, p=0·99) and no significant differences in variance (F(1,506)=0·22, p=0·64) and means (t(506) =0·39, p=0·70) between DZ and MZ twins. The percentages of other behaviours shown before and after all freezing episodes, such as approaching or avoiding the robot, are presented in Appendix S7. They suggest that freezing does not rule out alternative behaviours.

	Mean	SD	N	Min	Max	Skewness	Kurtosis
Zygosity							
MZ	21·04	21·83	211	0	76·97	1·01	0·14
DZ	21·84	23·42	297	0	76·97	1·07	0·06
Gender							
Males	21·50	23·19	245	0	76·97	1·06	0·02
Females	21·51	22·38	263	0	76·97	1·05	0·20

***Table 1*: Freezing duration per group in seconds corrected for the duration of the robot confrontation.**
MZ: Monozygotic pairs, DZ: Dizygotic pairs, SD: Standard deviation, n: number of twins.

Twin models

Because there were no significant sex differences and due to insufficient power to run sex limitation models, DZ twin pairs of opposite sex (DZO) were included in the main analyses. We have also rerun the same analyses excluding DZO pairs. They are reported in S8, resulting in the same selected model with comparable estimates, suggesting that the inclusion of DZO pairs did not inflate the estimation of the genetic effects in the larger sample. The difference in two way intraclass correlations of the freezing scores between the MZ twins (r=0·47, 95% CI 0·30−0·61) and the DZ twins (r=0·16, 95% CI -0·00−0·32) suggested a substantial genetic contribution to freezing behaviours. After fitting the nested univariate genetic analyses, the AIC, BIC and χ^2-difference tests suggested that the ADE model fit the data slightly better than the ACE model (Δ AIC=1·29), but neither the ACE, nor the ADE model significantly differed from the more parsimonious AE model, that had the lowest AIC and BIC (see Table 2). While the AIC and BIC of the DE model were smaller, a pure dominance effect in absence of additive genetic effects is unlikely. In addition to the interaction of alleles within specific genes, a dominance effect could also result from interaction of alleles across genes (i.e., epistasis). In this relatively modest sample, however, we cannot robustly distinguish additive from dominance effects. Nonetheless, both models underline moderate genetic contribution to freezing. Indeed, the best fitting AE model indicated that 45% (95% CI 34%−54%) of the variance underlies genetic influence while the remaining variance is attributable to unique environmental contributions and measurement error (55%; 95% CI 49%−61%). Power analysis on the significant parameter A, following Verhulst[36] revealed 66% power which limits certainty over the true effect existence.

Mixed-effects models

First, we tested the predictive contributions of freezing, and age (linear and non-linear) to an integrated internalizing symptoms score (mean scores of depressive and anxiety symptoms combined). In line with previous findings by Niermann and colleagues,[8] we noted a tendency of lower (or the absence of) freezing in toddlerhood predicting more internalizing symptoms during adolescence (i.e., 11−19 years). However, for the combined anxiety and depressive symptoms measure this did not reach significance (91·82% probability [pd] of a negative effect, Median= -0·06, 89% CI -0·11−0·01, $pp_{>0}$=0·08); see Figure 1 for internalizing symptom scores of high vs. low freezers and Appendix S9 for model estimates of all predictors, including standard errors and 95% CIs). There was no main contribution of age indicating the relative stability of internalizing symptoms over time, and no interaction between freezing and the polynomial effect of time (linear and quadratic), indicating that a distinct developmental

	A^2 (95% CI)	C^2 or D^2 (95% CI)	E^2 (95% CI)	AIC	BIC	Δ -2LL(np)	χ^2	Δdf	P
ACE	45% (34%−54%)	<1% (0%−0%)	55% (49%−61%)	3580·72	1258·29	4588·72(4)	3·73	6	0·71
AE	**45% (34%−54%)**	..	**55% (49%−61%)**	**3578·72**	**1251·68**	**4588·72(3)**	**3·73**	**7**	**0·81**
CE	..	27% (17%−36%)	73% (69%−76%)	3586.49	1259.45	4596·49(3)	11·50	7	0·12
ADE	11% (0%−40%)	38% (0%−52%)	51% (44%−58%)	3579·43	1257·00	4587·43(4)	2·44	6	0·87
DE	..	50% (39%−57%)	50% (44%-57%)	3577·55	1250·51	4587·55(3)	2·56	7	0·92
E	..	..	100%	3603·37	1271·72	4615·37(2)	30·38	8	<0·01

***Table 2*: Model estimates of the univariate genetic models.**
Note. P significance value of the likelihood ratio chi-square test, Δ -2LL: difference in -2lnL (negative 2 log-likelihood); df: degrees of freedom; AIC: Akaike information criterion. BIC: Bayesian Information criterion. The best fitting model is in bold.

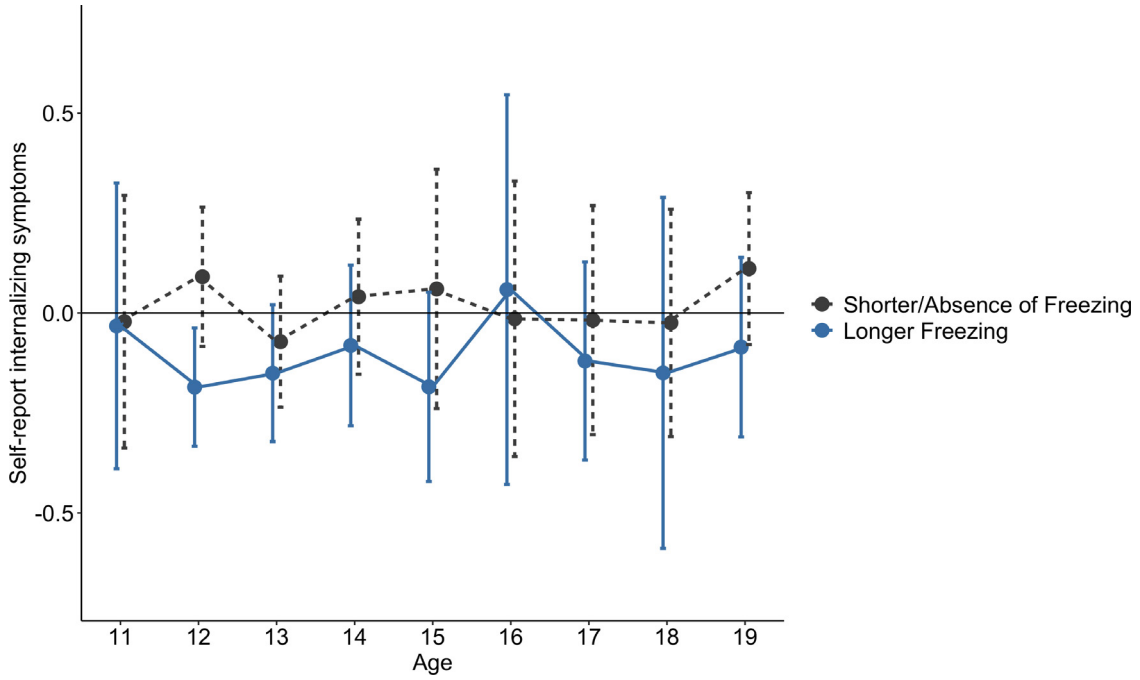

Figure 1. For illustration purpose, we plotted the internalizing symptom scores at each time collection for participants with higher and lower (or no) freezing behaviours as based on quartiles (n_{high}=115, n_{low}=114). The vertical lines represent the 95% confidence intervals around the means.

symptomatology did not emerge during adolescence according to freezing behaviour. Importantly, planned separate analyses for depressive and anxiety symptom scores indicated that the main effect of freezing to internalizing symptoms during adolescence was mainly driven by its significant contribution to depressive symptoms, (95·15% probability [pd] of a negative effect, Median= -0·06, 89% CI -0·12– -0·00, $pp_{>0}$=0·05]) as compared to anxiety symptoms (85·64% probability [pd] of a negative effect, Median= -0·04, 89% CI -0·10–0·02, $pp_{>0}$=0·14, see Appendix S10–S11). In line with previous findings by Niermann et al.,[8] lower (or the absence of) freezing was linked to more depressive symptoms. Patterns of findings did not change when controlling for sex (see Appendix S12).

Discussion

This project aimed to assess the heritability of human freezing and to test the predictive value of toddler freezing behaviours to internalizing symptoms during adolescence. Our findings point at a significant contribution of genetic factors (with a percentage of 45% pointing to a moderate contribution) whereas the shared environment is not associated with individual differences in this behaviour. The remaining variance (55%) was explained by uniquely-experienced environmental factors, including the error estimate of the model. This finding echoes previous views on freezing as a biological and stable threat-response that has co-evolved across animals.[6,9,22] It is also consistent with previous work that has evidenced distinct patterns of freezing behaviours to be associated with DNA-based differences at the level of the serotonin transporter gene in animals (*SERT*),[23,37] and in humans.[24] However, single genetic variants usually explain only a very small part of the variance. To the best of our knowledge, this is the first twin study on human freezing, which shows that individual differences in this behaviour are substantially explained by genetic factors.

At 18 months, this biological-based behavioural response to stressful and potentially threatening situations may be more easily detected, notably because it is less confounded (or masked) by alternative coping strategies acquired over time. Thus, freezing might represent a suitable partly inherited marker for fear-related behaviours in toddlerhood.[11] In line with findings of Niermann and colleagues,[8] we also observed a tendency that a relatively lower duration of freezing behaviours –including the absence of this typical response in this unfamiliar lab-based situation– predicted more internalizing symptoms during adolescence. This trend was mostly driven by its significant contribution to depressive symptoms. In contrast with these earlier findings, however, we did not find that freezing behaviour was associated with distinct developmental courses of internalizing symptoms during adolescence. Thus, there was no indication that the association with freezing

increased over time or faded away during adolescence (i.e., freezing x polynomial effect of age). These findings suggest that the absence of freezing in toddlerhood may signal risk for later internalizing problems, mainly depressive symptomatology. Freezing buys time for risk assessment, advances perception,[14,38,39] has been linked to action preparation,[13,16,17] and integration of outcome value on the basis of action options.[15] If this important primary defensive threat reaction is absent, or blunted, adequate coping may be hindered, especially early in development when coping mechanisms remain primitive and grounded in emotional responses and avoidance. We did not find evidence for deviations in the other direction. In other words, unlike Niermann et al.,[8] we found no evidence that relatively longer freezing to an unfamiliar and potentially threatening situation in toddlerhood predicts internalizing symptoms during adolescence. Perhaps detection of such relation requires to take into account environmental adversity. Indeed, in Niermann and colleagues' study,[8] this effect was moderated by exposure to a highly stressful environment. Unfortunately, we did not assess similar exposure to a stressful environment in this study, and therefore cannot tell if the same pattern of findings signalling an interaction effect might have emerged. It would also be interesting to assess whether such opposite pattern can be found at younger time points, as in Niermann et al.[8] In fact, it might be the case that toddlers who show more freezing - and possibly more internalizing symptoms- at a younger age attract more help from caregivers, and that it is the absence of this support for toddlers who do not show these behaviours that leads to symptoms at later ages. In a similar vein, it would be interesting for future research to assess whether individual differences in freezing measured in toddlerhood are associated with difficulties at a later time point (i.e., beyond the transition to adulthood), when the protective influence of the caretaker and institutions diminishes. It would be interesting for future studies to explore these questions.

Our findings may have implications for early assessment of risk factors related to the onset of depressive symptoms. Depression is one of the main causes for non-fatal health loss and years lived with disability worldwide.[40] An important challenge for researchers and clinicians is to determine for whom early preventive interventions should be prioritized and may be the most efficient, as well as to identify transdiagnostic targets for treatment. Our findings suggest that attentive immobility may be an adaptive response, possibly by allowing toddlers to consider and choose how to react to these unexpected and novel situations or by enhancing the caregivers' support to them. Interventions focused on caregivers' responses during threat, such as taking more supportive roles in identifying emotions, threats, novelty and modelling adaptive prosocial responses may not only increase the child's responsivity but also facilitate the learning of emotion regulation and coping.[9] Future investigations are clearly needed to investigate whether targeting these early markers of primary defensive reactions are relevant targets for intervention to prevent depressive symptomatology later in adolescence. Indeed, while we showed that freezing is to a moderate extent inherited, this does not imply that genetic vulnerability cannot be compensated by environmentally-based interventions.

Some strengths and limitations of the study need to be considered. First, the ratings of freezing showed high reliability of 0·84 to 0·97, supporting the consistency of our observed measure and underlying construct. Second, the present twin design allowed to investigate heritability of freezing and to replicate previous findings documenting its predictive value in toddlerhood to later internalizing symptoms with increased statistical power. The longitudinal design with repeated measures of symptoms prospectively collected allowed us to test the role of freezing in internalizing symptoms over a longer time span. In terms of limitations, power for our heritability analysis was compromised which may indicate a higher probability of Type I error. However, being, to the best of our knowledge, the only twin modelling conducted on freezing, it nevertheless offers a unique insight for future investigations. Moreover, we only measured freezing in a two-minute-long robot confrontation at a single time point. Although this is similar to Niermann and colleagues,[8] it would be interesting to assess freezing in a variety of situations to explore the context-specificity of the observed effects. It would also be interesting for future research, to assess alternative behaviours in toddlers that do not show any freezing episodes. Regarding the context, it is also important to highlight a crucial distinction between threat anticipatory freezing and tonic immobility as shown, e.g., in patients with posttraumatic stress disorder[41] or toddlers with a disorganized attachment style.[42] Whereas threat anticipatory freezing comes with the beneficial upregulated cognitive processing linked to parasympathetic dominance (as discussed above), tonic immobility has been characterized by distinct physiological processes and to occur to an unconditioned stimulus (actual threat) rather than a conditioned stimulus that signals potential threat.[2,5,6,12] Crucially, our study was deliberately limited to assessing the former at an intermediate threat level, where freezing could have served to resolve conflict between rewarding novelty (approach) and potential punishment (avoidance) elicited by the robot.[43,44] A highly stressful environment on the other hand, might have revealed distinct cases of maladaptive immobility in highly fearful toddlers. Another limitation is that, for some participants, data on internalizing symptoms were missing at certain time points (20%) and not all items were available at each time point which might have partially impeded the reliability of the measure. However, statistics still

indicated acceptable to good reliability. There was also no assessment of anxiety at age 15 and 17 which prevented us from testing our hypotheses according to the full age range for this phenotype. Further relating to the measure, self-report bias may have influenced our results despite being deemed more reliable for internalizing symptoms than parent reports.[45,46] Lastly, the underlying mechanism in the link between freezing and internalizing symptoms remains yet unknown since we did not examine whether common genetic factors are partly explaining this phenotypic association due to the relatively small magnitude of the phenotypic association.

Our findings indicate that individual freezing tendencies seem to be moderately driven by genetic factors. Second, absence of typical freezing behaviours during unfamiliar situations in toddlerhood may be an early indicator of internalizing symptoms during adolescence. Taken together, our findings suggest that freezing might represent a promising early risk marker for stress-related disorders. Future research should examine whether it can be used to identify which children need additional support and may contribute to refine our conceptual theoretical models aiming to explain the onset of internalizing problems.

Contributors

L. K. Held coded the videos, analysed the data, and wrote the paper. K. Roelofs and I. Ouellet-Morin developed the idea to replicate previous work by K. Roelofs. K. Roelofs and I. Ouellet-Morin supervised data analysis together with J. M. Vink. K. Roelofs and I. Ouellet-Morin supervised the writing of the paper. M. Boivin hosted L. K. Held and shared the data on internalizing symptoms and videos. K. Roelofs developed the freezing rating scale that was used for the freezing analyses. L. Provost coded the videos. M. Boivin, F. Vitaro, M. Brendgen, and G. Dionne shared data and commented on the manuscript together with J. M. Vink and L. Provost. All authors have verified the underlying data and read and approved the final version of the manuscript.

Data sharing statement

Upon publication, all data and analysis scripts can be requested via the data access form of GRIP, available on the institutional website: http://www.gripinfo.ca/grip/public/www/Etudes/en/dadprocedures.asp.

Declaration of interests

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104094.

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