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ARTICLE

Interactive effects between hemizygous 15q13.3 microdeletion and peripubertal stress on adult behavioral functions

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15q13.3 microdeletion is one of several gene copy number variants (CNVs) conferring increased risk of psychiatric and neurological disorders. This microdeletion gives rise to a variable spectrum of pathological phenotypes, ranging from asymptomatic to severe clinical outcomes. The reasons for these varying phenotypic outcomes remain unknown. Using a mouse model of hemizygous deletion of the orthologous region of 15q13.3, the present study examined whether exposure to stressful life events might interact with hemizygous 15q13.3 microdeletion in the development of behavioral dysfunctions. We show that hemizygous 15q13.3 microdeletion alone induces only limited effects on adult behaviors, but when combined with psychological stress in pubescence (postnatal days 30-40), it impairs sensorimotor gating and increases the sensitivity to the psychostimulant drug, amphetamine, at adult age. Stress exposure in adolescence (postnatal days 50–60) did not induce similar interactions with 15q13.3 microdeletion, but led to impaired emotional learning and memory and social behavior regardless of the genetic background. The present study provides the first evidence for interactive effects between hemizygous 15q13.3 microdeletion and exposure to stressful life events, and at the same time, it emphasizes an important influence of the precise timing of postnatal stress exposure in these interactions. Our findings suggest that hemizygous 15q13.3 microdeletion can act as a "disease primer" that increases the carrier's vulnerability to the detrimental effects of peripubertal stress exposure on adult behaviors.

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INTRODUCTION

Gene copy number variants (CNVs) are genetic variations manifesting as deletions or duplications of specific DNA sequences [1, 2]. Genome-wide association studies (GWAS) suggest that CNVs are strongly implicated in the genetic etiology of psychiatric and neurological disorders [3, 4]. The 15q13.3 microdeletion is one of several CNVs conferring increased risk of these disorders, with strong associations revealed for developmental and intellectual disability, autism spectrum disorder, schizophrenia, and epilepsy [4–8]. This microdeletion encompasses a genomic region harboring seven genes (AN1, MTMR10, TRPM1, miR-211, KLF13, OTUD7A, and CHRNA7) and can give rise to a variable spectrum of pathological phenotypes [9], ranging from non-pathogenic to severe clinical outcomes [10]. Hence, some carriers with the 15q13.3 microdeletion are asymptomatic, suggesting that the presence of this CNV alone is not sufficient to cause overt pathologies in some (or even most) individuals [10].

The reasons for the unpredictable and variable phenotypic outcomes induced by the 15q13.3 microdeletion remain elusive [11, 12]. It has been hypothesized that the pathogenicity of this CNV may be influenced by gene dosage, such that hemizygous 15q13.3 microdeletion causes less pronounced phenotypes than homozygous microdeletion. Even though humans with a homozygous deletion of the 15q13.3 region are viable [13], such homozygosity is rare. Hence, most subjects carrying a 15q13.3 microdeletion are hemizygous and present greatly variable phenotypes [9–12]. Besides gene dosage, the nature and/or severity of phenotypes associated with the 15q13.3 microdeletion may also be shaped by a second "hit", be it either genetically or environmentally driven. Such second "hits" may include the presence of an additional CNV, mutation, ethnic background, and history of adverse life events [11, 12].

Against these backgrounds, the present study tested the hypothesis that the phenotypes of hemizygous 15q13.3 microdeletion are influenced by psychological stress across postnatal development. The latter is a recognized environmental risk factor for various psychiatric illnesses, most notably psychotic and affective disorders [14, 15]. Since exploring interactive effects between hemizygous 15q13.3 microdeletion and psychological trauma in human subjects is technically and ethically highly challenging, we took advantage of a recently established mouse model that recapitulates the hemizygous 15q13.3 microdeletion syndrome in humans [16]. Mice with a hemizygous deletion of the orthologous region of 15q13.3 (Df[h15q13]−/+mice) are characterized with neurophysiological changes relevant to the human 15q13.3 microdeletion syndrome [16–18]. In addition, they show

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abnormalities in functional brain connectivity and impairments in sustained attention [17, 19]. Some of these abnormalities are more pronounced in 15q13.3 homozygous knockout mice (i.e., in Df[h15q13]−/− mice) [20], supporting a gene-dosage dependency in the 15q13.3 microdeletion syndrome.

We hypothesized that mice with a hemizygous deletion of the orthologous region of 15q13.3 (Df[h15q13]−/+mice) are more vulnerable to the detrimental effects brought on by stress exposure across postnatal development. To examine the possible influence of the precise timing of postnatal stress exposure [21–23], we subjected Df[h15q13]−/+ or wild-type mice to psychological stress between postnatal days (PNDs) 30 and 40, or between PNDs 50 and 60. These periods cover peripubertal (also referred to as early-to-mid adolescent) and late adolescent stages of development in mice and correspond to age 8–11 and 13–16 years in humans, respectively [22]. Hence, they span distinct pre- and post-pubertal stages, which in turn have been associated with distinct sensitivities to stress-induced neuronal and behavioral adaptations [21–23]. Behavioral outcomes were then examined once the animals reached adulthood using a comprehensive behavioral test battery, which allowed us to identify functional impairments in multiple neuropsychological and cognitive domains independently of predefined nosologic boundaries [24]. Our behavioral phenotyping strategy thus complies with the Research Domain Criteria (RDoC) system, which capitalizes on biological determinism to explain the pathogenesis of distinct psychiatric symptoms and focuses on endophenotypes rather than nosologic entities [25].

MATERIALS AND METHODS

Animals

Df[h15q13]−/+ mice were originally generated at TaconicArtemis (Köln, Germany) by hemizygous deletion of the orthologous region of the human 15q13.3 chromosomal segment as described in detail elsewhere [16]. To obtain Df[h15q13]−/+ (HE) and wild-type (WT) littermates, female WT mice on a C57BL/6N background (Charles Rivers, Sulzfeld, Germany) were bred with male Df[h15q13]−/+ mice maintained on a C57BL/6N background (Taconic MB A/S, Lille Skensved, Denmark). This breeding scheme was used to avoid any possible effects of the hemizygous Df[h15q13] deletion on post-partum maternal behavior. All animals were kept in temperature- and humidity-controlled (21 ± 1 °C, 55 ± 5%) holding facilities under a reversed light-dark cycle (lights off: 7:00 A.M. to 7:00 P.M.) and had ad libitum access to standard rodent chow (Kliba 3430, Klibamuehlen, Kaiseraugst, Switzerland) and water unless specified otherwise. All procedures described in the present study were approved by the Cantonal Veterinarian's Office of Zurich, Switzerland. All efforts were made to minimize the number of animals used and their suffering.

Stress exposure in pubescence or adolescence

HE and WT littermates were weaned and earmarked on PND 21. After determination of the genotype, littermates were caged such that 2 HE and 2 WT animals of the same sex were kept per cage. Due to insufficient numbers of female offspring (Suppl. Table 1), only male littermates were kept for all subsequent manipulations and investigations. All animals of a particular housing cage underwent the same postnatal stress exposure (see below). Littermates distributed to different housing cages were assigned to different postnatal treatment conditions in order to minimize potential confounds associated with litter effects [26]. Different cohorts of animals were used to ascertain functional impairments in multiple behavioral domains. The number of animals used in each cohort, as well as the order and age of testing, are summarized in Suppl. Table 2.

HE and WT mice were left undisturbed (=no stress, nS) or exposed to variable and unpredictable stress during pubescence (between PND 30 and 40, =pS) or late adolescence (between PND 50 and 60, =aS). Based on our previous work assessing stress effects in other neurodevelopmental disruption models [21], we used a variable and unpredictable stress protocol that included exposure to five distinct stressors (1. electric foot shock; 2. restraint stress; 3. swimming stress; 4. food deprivation; 5. repeated home cage changes) applied on alternate days. A detailed description of the stress protocol is given in the Supplementary Information.

Assessment of behavioral functions in adulthood

After exposure to the last stressor in pubescence or late adolescence (see above), the animals were left undisturbed (with the exception of weekly home change changes) for 4 weeks before behavioral testing began. Hence, behavioral assessment of pS animals and corresponding age-matched nS controls was carried out between PND 70 and 95, whereas aS and age-matched nS controls were tested between PND 90 and 115 (Suppl. Table 2).

The behavioral characterization of stressed and non-stressed HE and WT mice involved the assessment of anxiety-like and anhedonic behavior, emotional learning and memory, social behavior, sensorimotor gating, and sensitivity to the psychostimulant drug amphetamine (Amph). A detailed description of the apparatuses and procedures used for each test is provided in the Supplementary Information.

Anxiety-like behavior and emotional learning and memory were examined in order to study the single and combined effects of hemizygous 15q13.3 microdeletion and stress exposure on phenotypes relevant to anxiety disorders and depression [27, 28]. Anxiety-like behavior was measured using a standard open field procedure, whereas classical fear conditioning and active avoidance learning served as tests for emotional learning and memory. Anhedonic behavior was investigated using a standard sucrose preference test, which indexes central reward processing relevant to depressive disorders [28]. Social behavior and sensorimotor gating were measured using a social approach test and the paradigm of prepulse inhibition (PPI) of the acoustic startle reflex, respectively. They were selected to explore the single and combined effects of hemizygous 15q13.3 microdeletion and stress on core behavioral phenotypes implicated in neurodevelopmental disorders, including schizophrenia and autism spectrum disorders [29, 30]. The sensitivity to the psychostimulant drug Amph was assessed by measuring the animals' locomotor reaction to the drug in an open field. This test was selected in relation to the clinical findings showing that patients with schizophrenia, especially those with marked positive symptoms, display a potentiated behavioral and neurochemical response to acute Amph administration [31].

Statistical analysis

All statistical analyses were conducted using SPSS Statistics (version 22.0, IBM, Armonk, NY, USA) and Prism (version 7.0; GraphPad Software, La Jolla, CA, USA). Statistical significance was set at $P < 0.05$. A detailed description of the statistical analyses used for each test is provided in the Supplementary Information.

RESULTS

Genotype-independent effects of stress on anxiety-related behavior and emotional learning and memory

Innate anxiety-like behavior was indexed by the distance travelled and the time spent in the center of an open field arena [27]. Stress exposure in pubescence (pS) similarly increased anxiety-like behavior in mice with the hemizygous 15q13.3 microdeletion (HE) and controls (WT), as supported by the main effect of stress in the analysis of percent center zone distance ($F_{[1,50]} = 5.6$, $P < 0.05$) and percent center zone time ($F_{[1,50]} = 5.6$, $P < 0.05$) (Fig. 1a). The total distance moved in the entire arena did not differ between groups (Fig. 1a), suggesting that the effects of pS on innate

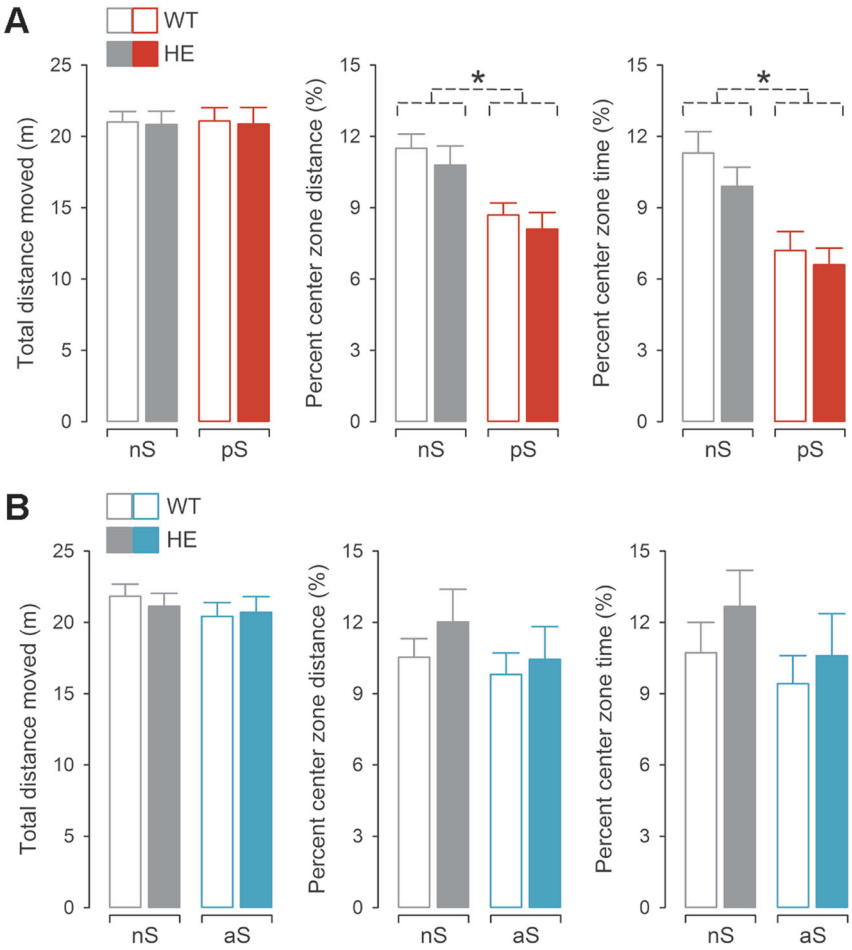

Fig. 1 Single and combined effects of hemizygous 15q13.3 microdeletion and postnatal stress exposure on innate anxiety-like behavior. Wild-type mice (WT) and mice with hemizygous 15q13.3 microdeletion (HE) were exposed to stress in pubescence (pS) or adolescence (aS), or they were left non-stressed (nS) during the corresponding maturational stages. **a** Total distance moved, percent center zone distance, and percent center zone time in the open field test for WT and HE mice exposed to pS or nS. *$P < 0.05$; N(WT/nS) = 15, N(WT/pS) = 15, N(HE/nS) = 12, and N(HE/pS) = 12. **b** Total distance moved, percent center zone distance, and percent center zone time in the open field test for WT and HE mice exposed to aS or nS. N(WT/nS) = 10, N(WT/aS) = 10, N(HE/nS) = 9, and N(HE/aS) = 9. All data are means ± s.e.m

anxiety-like behavior emerged without concomitant changes in basal locomotor activity. In contrast to the effects of pS, stress exposure in late adolescence (aS) did not affect indices of innate anxiety-like behavior in the open field test (Fig. 1b). It also did not change basal locomotor activity as indexed by total distance moved in the entire arena (Fig. 1b).

We used classical fear conditioning, in which a tone served as the conditioned stimulus (CS) and electric foot shock as the unconditioned stimulus (US), as a first test to explore emotional learning and memory [32]. No group differences were detected during the initial CS-US conditioning phase (day 1; Fig. 2a, b), indicating that neither 15q13.3 microdeletion nor pS or aS affected the development of the conditioned fear response. One day after conditioning, the animals were placed back to the same conditioning chamber without presentation of any discrete stimulus. This served as a test for conditioned fear expression towards the context, in which conditioning took place [32]. Again, no differences were evident during this phase of the test (day 2; Fig. 2a, b), suggesting that the retention of contextual information was not affected by the genotype or by the postnatal stress exposures. One the third day, the animals were placed into a novel context and presented with the tone-CS without subsequent US exposure. This served as a test for CS-cued conditioned fear expression [32]. The amount of conditioned freezing towards the tone-CS was significantly reduced in animals exposed to aS but not pS (day 3; Fig. 2a, b). The effect of aS on CS-cued fear expression emerged similarly in WT and HE animals, as supported by the main effect of stress ($F_{[1,34]} = 6.6$; $P < 0.05$) and absence of main effect of, or interaction with, genotype (P's > 0.2)

Exposure to aS but not pS also impaired two-way active avoidance learning, which is another test commonly used to assess emotional learning [33]. In this test, the animals were required to shuttle from one side of the chamber to the other in response to the presentation of a tone-CS. If they failed to do so, they received an electric foot shock (US). All animals were capable of acquiring this instrumental learning rule, and consequently, the number of shocks avoided generally increased as conditioning progressed (Fig. 2c, d). Exposure to aS, however, led to an overall decrease in the number of shocks avoided, and this effect emerged similarly in WT and HE animals (Fig. 2d; main effect of stress: $F_{[1,34]} = 8.5$; $P < 0.01$).

Stress-independent effects of hemizygous 15q13.3 microdeletion on social behavior

We investigated whether hemizygous 15q13.3 microdeletion alone, or in combination with pS or aS, might induce anhedonic behavior toward a natural reward. To this end, we compared stressed and non-stressed WT and HE animals in a two-bottle sucrose preference test, which is frequently used to assess depression-like anhedonic behavior in rodents [28]. We found

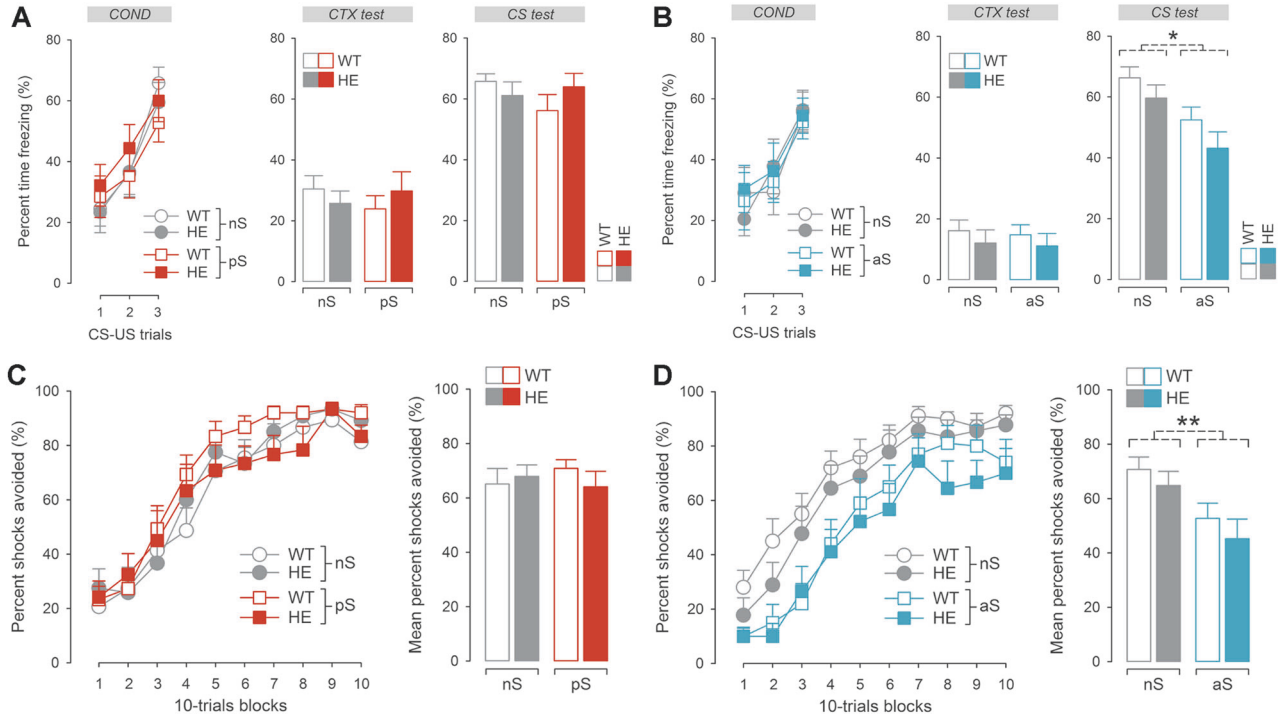

Fig. 2 Single and combined effects of hemizygous 15q13.3 microdeletion and postnatal stress exposure on classical fear conditioning and active avoidance learning. Wild-type mice (WT) and mice with hemizygous 15q13.3 microdeletion (HE) were exposed to stress in pubescence (pS) or in adolescence (aS), or they were left non-stressed (nS) during the corresponding maturational stages. **a** Fear conditioning in WT and HE mice exposed to pS or nS. The graphs show percent time freezing during the initial conditioning phase (COND; day 1) and subsequent test phases assessing conditioned fear expression towards the context (CTX test; day 2) and tone-CS (CS test, day 3). N(WT/nS) = 10, N(WT/pS) = 10, N(HE/nS) = 10, and N(HE/pS) = 9. **b** Fear conditioning in WT and HE mice exposed to aS or nS. The graphs show percent time freezing during the COND (day 1), CTX test (day 2) and CS test (day 3) phases. *$P < 0.05$; N(WT/nS) = 10, N(WT/aS) = 10, N(HE/nS) = 10, and N(HE/aS) = 10. **c** Two-way active avoidance learning in WT and HE mice exposed to pS or nS. The line plot shows percent shocks avoided as a function of 10-trial blocks, and the bar plots depicts the mean percent shocks avoided. N(WT/nS) = 15, N(WT/pS) = 15, N(HE/nS) = 12, and N(HE/pS) = 12. **d** Two-way active avoidance learning in WT and HE mice exposed to aS or nS. The line plot shows percent shocks avoided as a function of 10-trial blocks, and the bar plots depicts the mean percent shocks avoided. **$P < 0.01$; N(WT/nS) = 10, N(WT/aS) = 10, N(HE/nS) = 9, and N(HE/aS) = 9. All data are means ± s.e.m

that neither the genetic nor the environmental manipulations affected percent sucrose intake (Suppl. Figure 1), indicating that reward-related behavior toward a natural reinforcer was intact in all groups.

In contrast, hemizygous 15q13.3 microdeletion led to robust impairments in a social interaction test, in which the animals were allowed to concomitantly explore an inanimate dummy object and an unfamiliar mouse of the same sex and genotype. In this test, the relative time spent with the latter is typically taken as an index of social approach behavior [30]. As depicted in Fig. 3, HE mice without additional stress exposure displayed a marked reduction in the relative time spent with the unfamiliar mouse (main effect of genotype in the pS cohort of animals: $F_{[1,35]} = 9.5$, $P < 0.01$; $P < 0.01$ between WT and HE mice in the aS cohort of animals, based on post hoc tests following a significant interaction between genotype and stress: $F_{[1,36]} = 6.5$, $P < 0.05$). The microdeletion-induced reduction in the relative time spent with the unfamiliar mouse was attributable to a significant decrease in the absolute time spent with the unfamiliar mouse (main effect of genotype in the pS cohort of animals: $F_{[1,35]} = 18.7$, $P < 0.001$; $P <$ 0.01 between WT and HE mice in the aS cohort of animals, based on post-hoc tests following a significant interaction between genotype and stress: $F_{[1,36]} = 8.1$, $P < 0.01$). In addition to the genetic effects on social approach behavior, we found that exposure to aS (Fig. 3b), but not to pS (Fig. 3a), led to marked deficits in the social interaction test. Hence, WT mice exposed to aS also spent significantly less time interacting with the unfamiliar mouse compared to non-stressed WT controls ($P < 0.01$, post hoc comparison following a significant interaction between genotype and stress: $F_{[1,36]} = 8.1$, $P < 0.01$). This deficit was also evident in the analysis of the relative time spent with the unfamiliar mouse (Fig. 3b): WT mice exposed to aS displayed a significant reduction in the percent time interacting with an unfamiliar mouse ($P < 0.01$, post hoc comparison following a significant interaction between genotype and stress: $F_{[1,36]} = 6.5$, $P < 0.05$).

Interactive effects between hemizygous 15q13.3 microdeletion and peripubertal stress on sensorimotor gating and amphetamine sensitivity

To study the single and combined effects of hemizygous 15q13.3 microdeletion and stress on sensorimotor gating, we used the paradigm of PPI of the acoustic startle reflex. PPI of the acoustic startle reflex refers to the reduction of startle reaction in response to a startle-eliciting acoustic stimulus when it is shortly preceded by a weak prepulse stimulus [34, 35]. Neither hemizygous 15q13.3 microdeletion alone, nor stress exposure alone, was sufficient to affect PPI (Fig. 4). Most interestingly, however, pS combined with 15q13.3 microdeletion led to a significant reduction in % PPI scores (Fig. 4a), demonstrating interactive effects between the genetic background and peripubertal stress exposure on sensorimotor gating in adulthood (interaction between genotype and pS: $F_{[1,50]} = 6.3$, $P < 0.05$; post-hoc comparisons: $P < 0.01$ for HE/pS versus HE/nS or WT/pS, and $P < 0.05$ for HE/pS versus WT/nS). In contrast to pS, aS did not interact with 15q13.3 microdeletion to modulate PPI (Fig. 4b). Hemizygous 15q13.3 microdeletion reduced the startle reaction to pulse-alone trials, but this effect

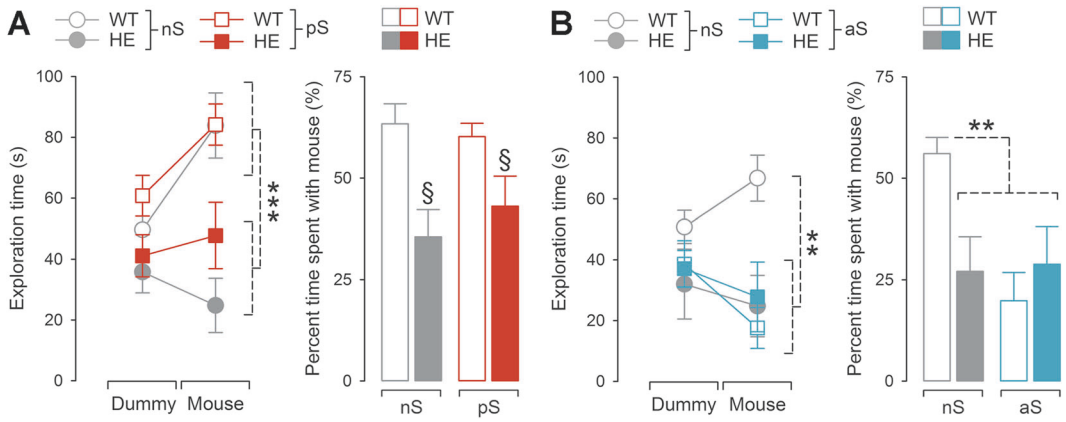

Fig. 3 Single and combined effects of hemizygous 15q13.3 microdeletion and postnatal stress exposure on social approach behavior. Wild-type mice (WT) and mice with hemizygous 15q13.3 microdeletion (HE) were exposed to stress in pubescence (pS) or in adolescence (aS), or they were left non-stressed (nS) during the corresponding maturational stages. **a** Social approach behavior in WT and HE mice exposed to pS or nS. The line plot shows the absolute time (s) spent with an inanimate dummy object (dummy) and an unfamiliar mouse of the same sex and genotype (mouse), and the bar plot depicts the relative (%) time spent with the unfamiliar mouse. ***$P < 0.001$ and $^{§}P < 0.01$, based on the main effect of genotype; N(WT/nS) = 10, N(WT/pS) = 10, N(HE/nS) = 10, and N(HE/pS) = 9. **b** Social approach behavior in WT and HE mice exposed to aS or nS. The line plot shows the absolute time (s) spent with an inanimate dummy object (dummy) and an unfamiliar mouse of the same sex and genotype (mouse), and the bar plot depicts the relative (%) time spent with the unfamiliar mouse. **$P < 0.01$, based on post hoc comparisons; N(WT/nS) = 10, N(WT/pS) = 10, N(HE/nS) = 10, and N(HE/pS) = 10. All data are means ± s.e.m

was only clearly evident in the aS cohort of animals (see Suppl. Figure 2). No significant group effects were detected in the analysis of the reaction to prepulse-alone trials (data not shown), suggesting that the experimental manipulations did not affect hearing capacities.

Marked interactive effects between hemizygous 15q13.3 microdeletion and pS also emerged in the Amph sensitivity test. We assessed the sensitivity to the drug in terms of measuring the animals' locomotor reaction to acute treatment with Amph (2.5 mg/kg, i.p.) in an open field apparatus. As shown in Fig. 5a, we found that pS in HE mice increased the locomotor response to Amph compared to nS/HE animals. In marked contrast, pS in WT mice led to the opposite effect, that is, it decreased the locomotor response to Amph compared to nS/WT animals (Fig. 5a). This led to a significant interaction between genotype, pS and bins ($F_{[15,525]} = 1.98$, $P < 0.05$) and significant group differences at individual bins (see Fig. 5a). Intriguingly, aS did not induce these effects (Fig. 5b). Hence, aS did not decrease the locomotor reaction to Amph in WT animals, nor did it interact with hemizygous 15q13.3 microdeletion to modulate the drug-induced locomotor hyperactivity response. For both stress conditions, there were no group differences in the distance moved during the initial habitation phase or subsequent saline injection phase (Fig. 5a, b).

DISCUSSION

The present study provides the first evidence suggesting that hemizygous 15q13.3 microdeletion can interact with postnatal stress exposure to disrupt adult behavioral functions. Using a comprehensive behavioral test battery, we revealed that such gene-environment interactions are dependent on the precise postnatal timing of stress exposure and predominantly affected sensorimotor gating and the sensitivity to the psychostimulant drug, Amph. More specifically, we found that the combination between hemizygous 15q13.3 microdeletion and stress during the peripubertal period was required to disrupt PPI and to potentiate Amph sensitivity. Such alterations recapitulate features of schizophrenia and related psychotic disorders [34–36] and represent key phenotypes in existing animal models of these disorders [29, 31]. Hence, the interaction between hemizygous 15q13.3 microdeletion and peripubertal stress appears particularly relevant for etiological processes underlying the development of psychosis-related dysfunctions.

In a broader clinical context, our findings may also help to explain the variable phenotypic outcomes induced by the hemizygous 15q13.3 microdeletion in humans. Indeed, it is known that some 15q13.3 microdeletion carriers show overt signs of neurodevelopmental and/or neurological abnormalities, whereas others do not [9–12]. The present data suggest that hemizygous 15q13.3 microdeletion is associated with latent abnormalities, which can be unmasked by additional environmental adversities such as postnatal stress. When back-translated to the clinical context in humans, our findings of gene-environment interactions may also encourage the inclusion of specific environmental factors such as stress in attempts to elucidate the role of 15q13.3 microdeletion in neurodevelopmental disorders. This may help to explore how (hemizygous) 15q13.3 microdeletion may interact with the environment to shape the risk of developing overt psychiatric and/or neurological conditions.

The phenotypic outcomes of the interactions between hemizygous 15q13.3 microdeletion and postnatal stress exposure, as well as their dependency on the precise timing of stress exposure, are strikingly similar to our previous findings demonstrating neuropathological interactions between prenatal immune activation and peripubertal stress in mice [21]. In this environmental "two-hit" model, several psychosis-related behavioral dysfunctions, including PPI disruption and increased psychostimulant drug sensitivity, emerged when the initial prenatal insult was combined with exposure to unpredictable stress in pubescence [21]. No interactions occurred, however, when prenatal immune activation was combined with stress in late adolescence [21]. It thus appears that the peripubertal period is particularly sensitive for environmentally or genetically predisposed subjects to develop psychosis-related abnormalities in response to repeated stress exposure. This impression is also supported by a recent human epidemiological study indicating that a similar window of vulnerability exists with respect to the risk of developing psychotic disorders following exposure to traumatizing experiences [23]. The mechanisms that determine such vulnerable windows remain largely elusive and are currently the subject of intense investigations [22, 37].

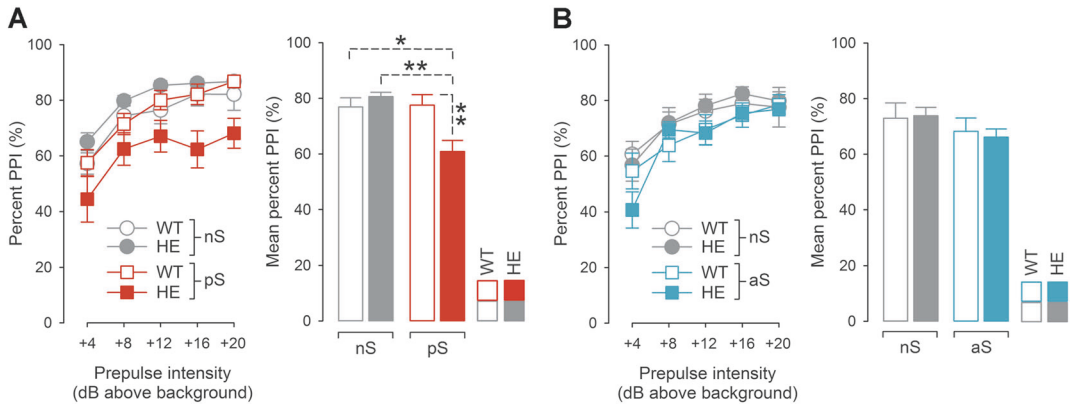

Fig. 4 Single and combined effects of hemizygous 15q13.3 microdeletion and postnatal stress exposure on prepulse inhibition (PPI) of the acoustic startle reflex. Wild-type mice (WT) and mice with hemizygous 15q13.3 microdeletion (HE) were exposed to stress in pubescence (pS) or in adolescence (aS), or they were left non-stressed (nS) during the corresponding maturational stages. **a** PPI in WT and HE mice exposed to pS or nS. The line plot shows % PPI as a function of prepulse intensity (dB above background of 65 dB), and the bar plot depicts the mean % PPI across all prepulse levels. $^{*}P < 0.05$ and $^{**}P < 0.01$, based on post hoc comparisons; N(WT/nS) = 15, N(WT/pS) = 15, N(HE/nS) = 12, and N (HE/pS) = 12. **b** PPI in WT and HE mice exposed to aS or nS. The line plot shows % PPI as a function of prepulse intensity (dB above background of 65 dB), and the bar plot depicts the mean % PPI across all prepulse levels. N(WT/nS) = 10, N(WT/aS) = 10, N(HE/nS) = 9, and N (HE/aS) = 9. All data are means ± s.e.m

Our view emphasizing a vulnerable window in pubescence does not imply that exposure to stressful life events in late adolescence (or during earlier postnatal phases such as the neonatal period) is inconsequential with regards to subsequent brain functioning. In fact, we found that stress exposure in late adolescence led to a number of adult behavioral deficits, both in WT and HE mice. For example, mice exposed to aS displayed diminished retention of CS-cued conditioned fear and impaired two-way active avoidance learning, suggesting that adolescent stress exposure effectively disrupts emotional learning and memory in adulthood. In addition, stress exposure in late adolescence per se was sufficient to impair social approach behavior in adulthood. Together, these findings are consistent with rodent models of chronic unpredictable stress exposure, which are widely used to study depression-like behavior and behavioral dysfunctions relevant to posttraumatic stress disorders (PTSD) [38, 39]. Interestingly, whilst patients with depression and PTSD typically show increased fear responses to previously experienced traumatic cues [39, 40], they show impairments in de novo emotional learning, such as fear conditioning to novel CS-US contingencies [41]. The latter deficits are consistent with the behavioral impairments emerging in mice exposed to aS.

Another main finding of our study was that hemizygous 15q13.3 microdeletion alone (that is, in the absence of additional stress exposure) induced consistent deficits in social approach behavior. These findings are consistent with the recent findings obtained in another mouse model recapitulating the human 15q13.3 microdeletion syndrome [42]. Given that non-stressed HE and WT did not differ in the open field test, or in other tests measuring anxiety-like behavior [16], the deficits in social approach behavior are unlikely to be driven by altered states of anxiety or general deficits in exploratory behavior, but instead, could represent decreased interests in social contacts. Social interaction impairments are a core feature of autism spectrum disorder [30, 43] and are also frequently present in patients with schizophrenia, especially in those with marked negative symptoms [44]. Notably, the low sociability scores in mice with a hemizygous 15q13.3 deletion could have masked possible interactions with postnatal stress exposure. More sensitive social interaction assays (such as social interaction tests investigating novel peer interaction for unconstrained novel dyads) may be needed to reveal possible interactive effects between hemizygous 15q13.3 deletion and postnatal stress exposure. The lack of implementing multiple social interaction tests is a limitation of our study.

We appreciate a number of other limitations associated with our study. First, we included only males in all investigations, leaving the question unanswered as to whether similar pathological effects and interactions would also emerge in females. Although we were unable to detect sex-specific outcomes in our previous studies exploring interactive effects between prenatal immune activation and peripubertal stress exposure [21], other gene-environment or environment-environment interaction models revealed differential outcomes in males and females [45–47]. Hence, gender can be a crucial factor influencing the nature and/or severity of adult brain pathologies arising from exposure to multiple risk factors during development. An extension of our model to female animals is thus cleary warranted so as to identify potential sex-dependent interactions between 15q13.3 microdeletion and postnatal stress exposure. Second, our study does not provide insights into the cellular and molecular mechanisms underlying the emergence of behavioral dysfunctions induced by hemizygous 15q13.3 microdeletion and its interaction with peripubertal stress. It has been hypothesized that the loss of CHRNA7, which encodes for the cholinergic receptor nicotinic alpha 7 (nAChA7) subunit, may be key in causing the 15q13.3 microdeletion syndrome [9, 48]. This hypothesis is indeed tempting in view of the findings implicating genetic variations within the CHRNA7 gene in the etiology of psychiatric disorders with neurodevelopmental components, especially schizophrenia [49]. Additional evidence supporting this hypothesis stems from recent mouse studies demonstrating that Lu AF58801, a nAChA7 positive allosteric modulator, is capable of mitigating altered functional brain connectivity in a hemizygous 15q13.3 microdeletion mouse model (i.e., in Df[h15q13]−/+ mice) [19]. Based on the existing evidence, however, it should be noted that CHRNA7 alone is unlikely to explain the diverse phenotype of the 15q13.3 microdeletion syndrome [16, 50]. Hence, reduced expression of several of the six genes encompassed in the 15q13.3 microdeletion may be key in mediating the pathological consequences of this CNV and its interaction with stress.

In conclusion, the present study provides the first piece of evidence suggesting that hemizygous 15q13.3 microdeletion can interact with peripubertal stress exposure to disrupt behavioral functions, especially those that are implicated in psychosis-related disorders. Our findings thus suggest that hemizygous 15q13.3

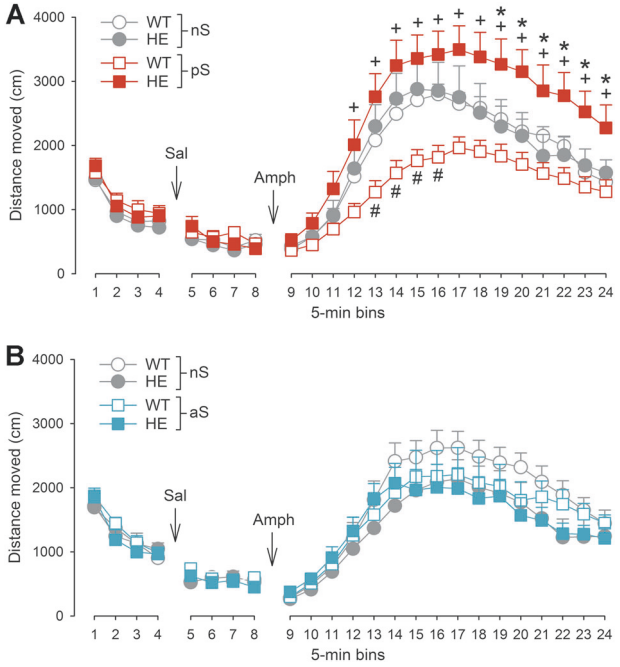

Fig. 5 Single and combined effects of hemizygous 15q13.3 microdeletion and postnatal stress exposure on amphetamine (Amph) sensitivity. Wild-type mice (WT) and mice with hemizygous 15q13.3 microdeletion (HE) were exposed to stress in pubescence (pS) or in adolescence (aS), or they were left non-stressed (nS) during the corresponding maturational stages. **a** Amph sensitivity in WT and HE mice exposed to pS or nS. The line plot shows the distance moved (cm) as a function of 5-min bins during the initial habituation phase (bins 1–4), the subsequent saline (Sal) injection phase (bins 5–8), and the final Amph injection phase (bins 9–24). $^{+}P < 0.001$, reflecting significant differences between WT/pS and HE/pS; $^{\#}P < 0.01$, reflecting significant differences between WT/pS and WT/nS or HE/nS; $^{*}P < 0.05$, reflecting significant differences between HE/pS and WT/nS or HE/nS (all based on post-hoc comparisons). N(WT/nS) = 15, N(WT/pS) = 15, N(HE/nS) = 12, and N(HE/pS) = 12. **b** Amph sensitivity in WT and HE mice exposed to aS or nS. The line plot shows the distance moved (cm) as a function of 5-min bins during the initial habituation phase (bins 1–4), the subsequent saline (Sal) injection phase (bins 5–8), and the final Amph injection phase (bins 9–24). N(WT/nS) = 10, N(WT/aS) = 10, N(HE/nS) = 9, and N(HE/aS) = 9. All data are means ± s.e.m

microdeletion can act as a "disease primer" that increases the carrier's vulnerability to the detrimental neuropathological effects of subsequent stress exposure. The combination of hemizygous 15q13.3 microdeletion and environmental adversities such as peripubertal stress thus likely magnify the risk of developing psychiatric disorders in later life.

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ADDITIONAL INFORMATION

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