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Chronic Mild Stress Impairs Latent Inhibition and Induces Region-Specific Neural Activation in CHL1-Deficient Mice, a Mouse Model of Schizophrenia

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Abstract

Schizophrenia is a neurodevelopmental disorder characterized by abnormal processing of information and attentional deficits. Schizophrenia has a high genetic component but is precipitated by environmental factors, as proposed by the 'two-hit' theory of schizophrenia. Here we compared latent inhibition as a measure of learning and attention, in CHL1-deficient mice, an animal model of schizophrenia, and their wild-type littermates, under no-stress and chronic mild stress conditions. All unstressed mice as well as the stressed wild-type mice showed latent inhibition. In contrast, CHL1-deficient mice did not show latent inhibition after exposure to chronic stress. Differences in neuronal activation (c-Fos-positive cell counts) were noted in brain regions associated with latent inhibition: Neuronal activation in the prelimbic/infralimbic cortices and the nucleus accumbens shell was affected solely by stress. Neuronal activation in basolateral amygdala and ventral hippocampus was affected independently by stress and genotype. Most importantly, neural activation in nucleus accumbens core was affected by the interaction between stress and genotype. These results provide strong support for a 'two-hit' (genes x environment) effect on latent inhibition in CHL1-deficient mice, and identify CHL1-deficient mice as a model of schizophrenia-like learning and attention impairments.

Keywords

accumbens; c-Fos; chronic mild stress; close homolog to L1; latent inhibition; schizophrenia

1. Introduction

Schizophrenia (SZ) is a neurological disorder characterized by delusions, hallucinations, disorganized behavior and speech, and attentional control deficits that can lead to severe impairments in adaptive function and social integration. Schizophrenia is affecting 1% of the

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population and has a large economic impact on the affected individual as well as society as a whole, with an estimated economic cost of over $32 billion in the United States alone [1]. Genetic factors affect the individual's susceptibility to schizophrenia, the heritability of SZ being estimated to be as high as 81% [2]; men show a higher incidence of SZ than women [3]. Additionally, individuals living in urban areas show a higher incidence of SZ than individuals in suburban or rural areas [4] and immigrant populations have a higher incidence rate than native populations [4]. This suggests that environmental factors also play a role in the onset of schizophrenia. In the absence of a singular genetic or environmental pathogenic agent for schizophrenia, disease models involving multiple factors were developed, starting from a 'two-hit' hypothesis [5] to a more recent 'multi-hit' model [6].

One of the human genes found to be associated with increased risks of developing schizophrenia is the Close Homolog of L1 (CHL1) [7–9], coding for a neuronal cell adhesion molecule. Three separate studies [8, 10, 11] have identified an association between a functional polymorphism Leu17Phe (rs2272522) in the signal peptide region of the CHL1 gene and SZ; although no experimental data exist to date, the CHL1 Leu17Phe polymorphism could alter protein trafficking and recruitment to the membrane. Another study has identified genomic copy number variations (CNVs) of the CHL1 gene in Scottish SZ patients [9]; CNVs involve mainly loss (e.g. deletions) or gain (e.g. duplications) of up to several million base pairs of DNA sequence [12] and thus can alter gene dosage and expression (e.g., a deletion of a gene or part of a gene would leave the individual with only one functional allele, i.e., heterozygous). CHL1 and other cell adhesion molecules of the immunoglobulin superfamily are highly expressed during the development of the nervous system [13], have multiple functions in the formation of appropriate neuronal connections during development [14] and in synaptic function and plasticity in the adult [15], processes which are thought to be disrupted in intellectual disabilities and schizophrenia [16]. CHL1-deficient mice [17] exhibit behavioral alterations suggestive of those found in schizophrenic patients [18–23], such as impaired sensorimotor gating (prepulse inhibition) [24, 25] and reduced exploratory behavior in novel environments [17, 26]. Timekeeping is disrupted in schizophrenic patients and individuals at risk for schizophrenia [22, 27–31], as well as CHL1-deficient mice [32]. Deficits in spatial-temporal integration were reported both in schizophrenics [18, 33], and in CHL1-deficient mice [32].

Latent Inhibition (LI) is a measure of attention and learning, defined as the loss of future associability by a stimulus that has been repeatedly presented without consequence [34]; the loss of associability results in slower learning of a new *conditioned stimulus* (CS) – *unconditioned stimulus* (US) relationship if the *pre-exposed* (PE) stimulus is presented with consequences in the future. LI is attenuated in schizophrenics [35, 36], which learn faster about stimuli previously presented with no consequence, and is thought to be related to the positive symptoms of SZ [37].

Here we explored a 'two-hit' hypothesis in LI expression, using CHL1-deficient mice (genetic component) under no-stress and *chronic mild stress* (CMS) conditions (environmental component). We also comparatively evaluated neuronal activation (c-Fos positive cell counts) during the LI paradigm in brain regions previously shown to be relevant to LI [38–43], in CHL1-deficient mice and their *wild-type* (WT) littermates.

2. Materials and Methods

2.1 Subjects

The subjects were 93 male CHL1 mice in a C57Bl/6J background, 6–8 weeks old at the beginning of the experiment, subdivided by genotype as follows: CHL1-deficient (KO, n=22), heterozygous (HET, n=36), and wild-type littermate controls (WT, n=35). CHL1 KO mice were originally developed by Montag-Sallaz et al. [26]. The subjects for this experiment were generated at Utah State University in a mouse colony maintained on C57Bl/6J background for more than 10 generations. The genotype of the subjects was confirmed by PCR genotyping from tail biopsy samples. The mice were housed in groups of three or four in a climate-controlled room under a 12-h light-dark cycle. The mice were further divided into *Stress* (S, n=52) and *No-Stress* (NS, n=41) groups. Water was provided *ad libitum*, while weight was maintained at 85% of the *ad libitum* weight by restricting food access (Teklad rodent diet 8604, Envigo, Denver, CO) during behavioral training and testing. Mice were tested during the light period of the cycle. All experimental procedures were conducted in accordance with the standards for the ethical treatment and approved by Utah State University IACUC Committee.

2.2 Chronic Mild Stress (CMS)

We have chosen a prolonged chronic mild stress paradigm to parallel exposure to mild stressors in daily life (being stuck in traffic, shift work, changes in living conditions etc). Stress mice received 6 weeks of CMS beginning at 6 weeks of age, while no-Stress mice were kept in standard laboratory conditions. The CMS regimen was modeled after [44] and consisted of three different stressors each day, with each stressor lasting a minimum of 2 hours. The different stressors were: 1) the water bottle removed from the home cage; 2) mice housed into a cage that had housed other mice; 3) mice housed into a clean home cage; 4) food removed from the home cage; 5) wet bedding in the home cage; 6) mice restrained into a small plastic container; 7) home cages placed at a 45-degree angle; 8) rat bedding spread evenly in the home cage; 9) light on during the dark cycle. Due to light-dark cycle manipulations, stress mice were housed in a different colony room from the no-stress mice, but using identical laboratory conditions except when the stress condition dictated otherwise. Behavioral testing for all mice occurred at 12–14 weeks of age, as described below.

2.3 Apparatus

The apparatus consisted of 8 standard mouse operant chambers housed inside sound-attenuating cubicles (Med Associates, St. Albans, VT) equipped with a house light, a fan, two nosepokes on the front wall and one nosepoke on the back wall, a programmable audio generator, a shocker/scrambler module, a lever, and a standard mouse 20-mg pellet feeder. The *pre-exposed* (PE) and *non-pre-exposed* (NPE) conditioned stimuli were a 80-dB tone and a 10-Hz click. The unconditioned stimulus was a 1-s 0.5mA footshock.

2.4 Latent inhibition (LI)

Latent inhibition was assessed using an “on baseline” *conditioned emotional response* (CER) procedure consisting of baseline, pre-exposure, conditioning, rebaseline and test

phases (i.e. allowing the mouse to eat during the all stages of the LI paradigm). Mice were assigned to either a PE tone/NPE click (n=47) or PE click/NPE tone (n=46) in a counterbalanced manner. Mice were shaped to nosepoke for food pellets on an FR1 schedule throughout the LI task, which consisted of four daily sessions as follows: During the 60-min *pre-exposure session* mice received forty 30-s presentations of the PE stimulus separated by a 60-s inter-stimulus interval (ISI). During the 30-min *conditioning session*, the PE and NPE stimuli were presented for 30-sec three times, separated by a 240-s ISI. The last presentation of the PE and NPE stimuli was paired with a 1-s, 0.5-mA footshock. On the next day mice were given a 60-min *rebaseline session* during which mice were reinforced for nosepoking on an FR1 schedule. During a 30-min *test session*, mice were presented with 3-min PE and NPE stimuli with an 8-min ISI. Mouse behavior was video recorded and the duration of freezing behavior was estimated using FreezeScan software (CleverSys Inc., Reston, VA) [45].

2.5 c-Fos immunostaining

Minutes upon neuronal activation, a specific subset of immediate early genes is transcribed. Basal levels of brain expression for these genes (in home cage conditions) are very low; the expression of their protein products peaks at 90–120 min after neuronal activation in behavioral tasks. As previously shown in [46], we have assessed neuronal activation during the LI behavioral paradigm through analyses of expression (positive cell counts) of the immediate early gene c-Fos in brain regions known to be relevant to LI through lesion or pharmacological studies [38–43].

Two hours after the start of the test session 6–9 mice in each group were deeply anesthetized and transcardially perfused with a paraformaldehyde solution (4% in 0.1M phosphate buffer, pH 7.4). Brains were collected and sectioned on a vibrating microtome (VT1200S, Leica, Germany). Brain sections were prepared for immunohistochemistry using a Vectastain Elite ABC kit (Vector Labs, Burlingame, CA), according to manufacturer's protocol. Briefly, 50um sections were treated with 0.3% hydrogen peroxide to inhibit endogenous enzymes, then permeabilized and blocked (5% goat serum, 0.5% triton x-100 in PBS solution) at room temperature for 2 hrs. Sections were incubated with a rabbit anti c-Fos antibody (pc-38, Calbiochem, San Diego, CA; diluted 1:20,000), then with a biotinylated goat anti-rabbit secondary antibody (Vector Labs, Burlingame, CA; diluted 1:200), and then with a avidin-biotinylated horseradish peroxidase complex (Elite ABC reagent). Staining was developed using 3,3′-Diaminobenzidine (DAB Substrate Kit SK4100, Vector Labs, Burlingame, CA), after which sections were washed in order to stop the reaction. Sections were then mounted on slides, left to dry, then dehydrated, and coverslipped with Permount (Fisher Scientific, Pittsburgh, PA).

2.6 Neural activation analysis

Image acquisition and neuronal activation analysis were performed on a Zeiss AxioImager M2 motorized research microscope with an imaging system. Analysis of neuronal activation was performed by counting c-Fos-positive nuclei, in corresponding areas in 2 sections/region of interest/mouse in the following areas of interest: *prelimbic cortex* (PrL: bregma 2.1 to 1.54), *infralimbic cortex* (IL: bregma 1.94 to 1.42), *basolateral amygdala* (BLA: Bregma

−1.34 to −1.94), *ventral hippocampus* (vHipp: bregma −2.92 to −3.52), *nucleus accumbens shell* (Acb-shell: bregma 1.78 to 1.1), and *nucleus accumbens core* (Acb-core: bregma 1.78 – 1.10) [47], by two independent observers unaware of genotype. Neuronal activation in each region was averaged over observers and subjected to statistical analyses.

2.7 Statistical analyses

The estimated duration of freezing behavior in the first 30-s of the presentation of the PE and NPE stimuli, and the latency to freeze in the conditioning and test sessions were subjected to mixed ANOVAs with between-subjects variables stress (S, NS) and genotype (KO, HET, WT), and within-subjects variable pre-exposure (PE, NPE) followed by posthoc analyses. The number of rewards and nosepokes during the test session were subjected to mixed ANOVAs with between-subjects variables stress (S, NS) and genotype (KO, HET, WT), and within-subjects variable pre-exposure (PE, NPE) followed by posthoc analyses. Neuronal activation (counts of c-Fos+ cells in each brain region) was subjected to 2-way ANOVAs with factors stress (S, NS) and genotype (KO, HET, WT). All statistical analyses were conducted at an alpha level 0.05.

3. Results

3.1 Latent inhibition

The average freezing duration during the PE and NPE stimuli in the test session is shown in Fig. 1. Analyses indicated a main effect of pre-exposure ($F(1,87)=86.86$, $p<0.01$), showing that mice froze longer during the NPE stimulus than during the PE stimulus (LI). However, LI was not expressed equally in all stress conditions: Analyses indicated a reliable pre-exposure x stress interaction ($F(1,87)=8.62$, $p<0.01$), showing that NS mice expressed more LI (larger difference in freezing to NPE and PE) than S mice. Furthermore, analyses indicated a reliable 3-way interaction pre-exposure x genotype x stress ($F(2,87)=3.93$, $p<0.05$). Post-hoc Tukey HSD and Bonferroni tests indicated that all unstressed mice as well as the stressed WT mice showed reliable LI (all ps<0.01), while neither HET nor KO stressed mice showed LI (all ps>0.05). These results provide support for a ‘two-hit’ under which environmental factors (stress) potentiate the effect of genetic background to reveal schizophrenia-like symptoms (lack of LI) only in stressed CHL1 HET and KO mice.

3.2 Unconditioned freezing

On the other hand, the difference in freezing to PE and NPE stimuli in Fig. 1 could be due not only to LI, but also to intrinsic (unconditioned) differences in freezing to the two stimuli. To evaluate this hypothesis we performed analyses of freezing behavior to the PE and NPE stimuli in the conditioning session, before these stimuli were paired with footshock. Analyses failed to indicate any main effects of stimulus (PE/NPE) ($F(1,87)=0.03$, $p>0.05$), genotype ($F(2,87)=1.11$, $p>0.05$), stress ($F(1,87)=0.77$, $p>0.05$), or any interactions (all Fs<2.21, $p>0.05$), suggesting no differences in unconditioned freezing to the PE and NPE stimuli, irrespective of genotype and stress condition. This result further suggests that the differences in freezing between genotypes/groups in Fig. 1 are not due to differences in unconditioned freezing, but rather due to differences in conditioned freezing (associability/learning), thus describing true differences in LI.

3.3 Reactivity to shock

Alternatively, the differences in LI between groups could be due to CHL1 HET and KO mice becoming more reactive to shock following stress. To evaluate this hypothesis we followed three lines of evidence: First, posthoc Tukey HSD and Bonferroni tests of the duration of freezing failed to indicate differences between genotypes in duration of freezing to the NPE stimulus (all ps>0.05) (see Fig. 1); same analyses also failed to indicate differences in duration of freezing to the NPE stimulus between unstressed and stressed mice for each genotype (all ps>0.05) (see Fig. 1). Taken together, these analyses suggest that all mice learned similarly about the NPE stimuli, thus making it unlikely that they had different reactivity to shock. Second, analyses of the number of rewards earned and number of nosepokes performed during the test session failed to indicate any effects of genotype (all Fs(2,87)<1.31, all ps>0.05), stress (all Fs(1,87)<2.99, all ps>0.05), or interactions (all Fs(2,87)<0.69, all ps>0.05), suggesting that mice poked and were rewarded similarly irrespective of stress and genotype, thus making it unlikely that stress and genotype affected reactivity to shock. Finally, analyses of the latency to freeze in the conditioning session (before stimuli being paired with shock) and in the test session (after stimuli were paired with shock), failed to indicate any effects of genotype (F(2,87)=0.43, p>0.05), stress (F(1,87)=1.81, p>0.05), session (F(1,87)=2.06, p>0.05) or any interactions (all Fs<1.96, all ps>0.05), suggesting that mice's propensity to freeze in the given context did not change before and after stress, irrespective of genotype, thus making it unlikely that mice differed in their reactivity to shock. In summary, in spite of mice performing similarly in the FR1 task, learning similarly about the NPE stimulus, and also learning similarly about the context, they differed only in their freezing to the PE stimulus: Freezing to the PE stimulus was reliably smaller than freezing to the NPE stimulus in all unstressed mice and in the WT stressed mice, but increased (to levels not reliably different than freezing to the NPE stimulus) only in the stressed CHL1 HET and KO mice, suggestive of impaired LI.

3.4 Neuronal activation

Neuronal activation during LI testing was evaluated in PrL, IL, Acb-core, Ach-shell, vHipp, and BLA, brain regions with relevant roles in latent inhibition [38–43, 46, 48, 49]. Fig. 2A indicates three different patterns of neuronal activation: First, neuronal activation in PrL, IL, and Acb-shell was affected only by stress (F(1,25)=33.11, p<0.01 for PrL; F(1,25)=47.60, p<0.01 for IL, and F(1,21)=25.72, p<0.01 for Acb-shell), but not by genotype (all Fs<2.71, p>0.05), or interactions (all Fs<2.34, p>0.05). Second, BLA and vHipp were independently affected by stress (F(1,23)=19.74, p<0.01 for BLA; F(1,24)=7.15, p<0.05 for vHipp), and genotype (F(1,23)=12.31, p<0.01 for BLA; F(1,24)=5.03, p<0.05 for vHipp), but not by stress x genotype interactions (all Fs<0.18, p>0.05). Third, Acb-core activation was reliably affected by genotype (F(1,21)=5.74, p<0.05), marginally affected by stress (F(1,21)=3.80, p=0.065), and reliably affected by a stress x genotype interaction (F(1,21)=5.93, p<0.05). These results indicate that various brain regions relevant to LI are differentially affected by stress, genotype, and their interaction, thus supporting a complex 'two-hit' stress x genotype model.

To further understand the effect of stress and genotype on these brain regions, we evaluated the patterns of neuronal activation in the nuclei that control the behavioral output [48, 49],

Acb-shell and Acb-core, as shown in Fig. 2BCD. Fig. 2A indicates that Acb-shell activation increases after exposure to stress. Consistent with previous studies [46], Fig. 2B indicates that LI (the difference in freezing duration to NPE and PE stimuli) correlates positively with Acb-shell activation (number of c-Fos+ cells) in WT mice ($R^2(13)=0.24$, $p<0.05$); in contrast, in CHL1 KO mice LI correlates negatively with Acb-shell activation ($R^2(12)=0.31$, $p<0.05$).

On the other hand, Fig. 2C indicates that in WT controls LI does not correlate with Acb-core activation ($R^2(13)=0.08$, $p>0.05$). In contrast, in CHL1 KO mice LI correlates negatively with Acb-core activation ($R^2(12)=0.232$, $p<0.05$). Indeed, stress determines an increase in Acb-core activation only in CHL1 KO mice (stress x genotype interaction) (see Fig. 2A), such that stressed CHL1 KO mice, but not stressed WT mice, show impaired LI (Fig. 1).

Finally, Fig. 2D shows that the correlation between LI and Acb-core activation differs drastically in no-stress (NS) and stress (S) mice: In NS mice LI does not correlate with Acb-core activation ($R^2(12)=0.05$, $p>0.05$), while in S mice LI correlates negatively with Acb-core activation ($R^2(15)=0.31$, $p<0.05$). Indeed, there is no reliable difference in Acb-core activation in S and NS WT mice (Fig. 2A), which show LI (Fig. 1), while stressed CHL1 KO mice show an increase in Acb-core activation (Fig. 2A), and fail to show LI (Fig. 1). In summary, all three patterns in Fig. 2BCD contribute to the reliable LI in stressed WT mice, and to the lack of LI in stressed CHL1 KO mice, as shown in Fig. 1.

4. Discussion

Using an “on baseline” within-subject CER LI procedure developed in our lab, the current study found that WT mice in a C57BL/6J background showed LI, consistent with previous findings [50]. Additionally, results indicate that both CHL1 HET and KO mice in C57BL/6J background showed LI under no-stress conditions. However, after exposure to a CMS regimen in adolescence/young adulthood, both CHL1 HET and KO mice failed to show LI, while WT littermates continued to show LI. These results are unlikely to be due to differences in unconditioned freezing to the two stimuli, to changes in auditory generalization for all cues as a result of stress, or to differences in reactivity to shock, as all mice froze similarly to the two stimuli (before they were paired with shock), learned similarly about the NPE stimulus and context, nosepoked similarly, and were rewarded similarly in the FR1 task.

Neuronal activation analyses in brain regions proposed to be involved in LI suggested that in some brain regions activity was affected solely by stress (PrL, IL, Acb-shell), while in others it was affected by both stress and genotype (BLA, vHipp) or their interaction (Acb-core). Our results are in accord with current neurobiological [48] and neuro-computational models [49, 51, 52] of LI (Fig. 3, discussed below), which propose that, in regard to LI, Acb receives input from multiple brain regions (cortex, amygdala, hippocampus) and participates in the selection and integration of motivationally relevant cortico-limbic information that governs behavioral output. However, our data cannot differentiate whether the increased Acb-core activation is the underlying cause of decreased LI or a compensatory effect.

Moreover, current results should be considered with caution since other brain regions not investigated in this study may contribute to the LI behavior, and to the current results.

4.1 Neural substrates of latent inhibition

Latent inhibition was introduced by Lubow and Moore [34] as the phenomenon by which repeated presentation of a neutral stimulus without consequences reduces its future associability relative to learning about novel stimuli; this phenomenon is similar in humans and other species [reviewed in 53]. LI promotes stimulus selectivity required for rapid, efficient learning [54], learning that is different from habituation [55] or conditioned inhibition [56]. Most theories explaining LI focus on attentional mechanisms, i.e. during pre-exposure of an inconsequential CS the animal or participant learns not to attend to it [49, 54, 57]. Weiner and Feldon [58] suggested a 'switching' theory; the CS-noUS association is learned during pre-exposure, the hippocampus is responsible for detecting mismatches, while switching from the dominant CS-noUS strategy to the new CS-US one is controlled by the core of the nucleus accumbens (with the shell having a modulatory role) [48, 59]. Since LI is disrupted by a change in context between pre-exposure and conditioning [60], Lubow and Gerwitz [54] proposed that the context serves as an occasion setter; since the context is also processed in the hippocampus, this theory does not contradict 'switching'.

LI prominently depends on Acb, BLA, frontal cortex, and the hippocampus. The role of the hippocampus in LI is underlined by pharmacological and lesion studies showing maintenance of LI, but loss of context specificity of the CR and LI, in rats with specific hippocampal lesions [61–63]. LI is disrupted after ventral hippocampal (vHipp)/ventral subiculum (vSub) NMDA receptor activation [42, 64]. On the other hand, lesions to the BLA produce an abnormally persistent LI in rats, even with a low number of pre-exposures, but not with context shift [39, 65]. Lesions of the orbitofrontal cortex but not of the medial prefrontal cortex also produce an abnormally persistent latent inhibition in rats [39]. Acb is a central structure for the acquisition and expression of LI: lesions of the Acb-shell disrupt LI [66], while lesions of Acb-core or Acb-shell+core are associated with persistent LI [41, 66].

All these features, including the role of the hippocampus and nucleus accumbens are captured by a computational model of LI [49] which addresses not only the effect of hippocampal lesions [52], the complex LI pharmacology [51], and 'switching' [49], but also predicts that LI is may be altered by a 'two-hit' environment x neurobiology interaction [52]. For example, [52] predicts that hippocampal lesions (neurobiological component) may have vastly different (sometimes contradictory) effects depending on the setting of the LI paradigm (environmental component), ranging from facilitated LI to impaired LI. This model predicts that genetic manipulations which affect the hippocampus (e.g., CHL1 deficiency [26]) may have different effects depending on environmental factors, as in our study.

4.2 Latent inhibition and schizophrenia

On a neuroanatomical level, schizophrenia is characterized by many changes including: enlarged ventricles [67], localized decreases in the density of cortical gray matter [68], decreases in the size of the thalamus [69] and of the caudate nuclei [70]. Additionally,

abnormalities are often reported in the medial temporal lobe [71], particularly in the entorhinal cortex [72, 73], the hippocampus and the amygdala [74–76]. Many of the neuroanatomical changes observed in SZ brains are thought to be related to neurodevelopmental processes [16, 77], although some may be the result of treatments with neuroleptics [78, 79]. Neuroanatomical changes observed in the cortex, thalamus, hippocampus and amygdala could be linked to the impairments in LI.

LI is absent or much reduced in patients with SZ [36, 80]. According to LI theories this may be explained by SZ patients not stopping attending the familiar inconsequential stimuli [53], having a hyperactive 'switching' mechanism [81], or not being able to use context as an occasion setter [54]. Most interestingly, our results are compatible with a computational model suggesting that LI is affected by the interaction between environmental stimuli and brain insults [52]. Under this model, LI is absent in SZ patients because some environmental conditions trigger their abnormally-developed hippocampus to transition from processing stimuli in an automatic mode, to processing stimuli in a controlled mode [49]. This is the only current LI model that incorporates at its core an interaction between biological and environmental factors, as detailed below [5].

In the Buhusi et al. [5] neuro-computational model, LI depends on the novelty of the PE and NPE stimuli relative to the environment, which depends on learned associations between stimuli, which in turn depend on normal hippocampal function. Under these assumptions, novelty depends both on the environment and on the state of the hippocampus, and the model is, at its core, a 'two-hit' LI model, while the hippocampus is a 'vulnerability' region. Indeed, simulations ([5], Fig. 21 and Fig. 22, pages 243–344) indicate that alterations in hippocampal function (e.g., due to lesions, drugs, genetic factors etc) are predicted to have variable effects on LI depending on environmental factors. Interestingly, CHL1-deficient mice show alterations of hippocampal circuitry and function, and behavioral anomalies such as impairment of novelty detection and altered exploratory behavior in novel environments [17, 26, 82]. Thus, according to the model in [5], current data could be explained by genetically-induced alterations in hippocampal function combined with environmental factors, which interact to alter novelty computation, and impair LI in stressed CHL1 KO mice.

4.3 CHL1 and schizophrenia

The Close Homolog to L1 cell adhesion molecule is highly expressed in the brain during development [13, 83] and regulates important processes such as neuronal migration, neurite outgrowth and axonal pathfinding, through interactions with other cell adhesion molecules, integrins [84, 85] and guidance receptors [86–88]. Recently, CHL1 was found to regulate neurite outgrowth through interactions with another protein linked to SZ – Disrupted in Schizophrenia (DISC1) [89]. CHL1 deficiency in mice is associated with neuroanatomical anomalies reminiscent of those found in SZ patients, such as enlarged ventricles [26], abnormal thalamocortical projections [86] and altered positioning and morphology of deep-layer cortical neurons [90]. CHL1 KO mice also exhibit many hippocampal anomalies, such as ectopic mossy fiber synapses in the lateral CA3 region, outside the trajectory of the infra-

pyramidal mossy fiber bundle [26], and abnormal synapses with enhanced perisomatic inhibition and altered long-term potentiation in the CA1 region [91].

4.4 Stress and latent inhibition

Chronic stress affects gene expression, as well as neuronal morphology and function in many brain regions. For example, after stress, pyramidal neurons in the cortex and hippocampus exhibit altered dendritic and spine morphology, decreases in spine density [92–94], and changes in neurotransmission [95, 96]. Stress induces alterations in dopamine neurotransmission [95, 97, 98], which are particularly important for the acquisition and expression of LI [37, 99].

Stress may attenuate LI in humans [100] or rats [101], while, in some cases, it may potentiate it [102]. For example, a recent study revealed a disrupted LI in highly stress reactive mice [103], supporting our own observation that genetic factors are major contributors to the effects of stress: Only stressed CHL1 HET and KO mice, but not wild-type littermates, failed to show LI. Although some neuronal cell adhesion molecules of the immunoglobulin superfamily, such as NCAM and L1, are considered important mediators of the effects of stress on the brain [104, 105], to date nothing is known of the interaction between stress and CHL1 expression. It is possible that the effect is indirect, for example through effects on interneurons [106] or through effects on serotonergic circuits [107].

5 Conclusions

Our results show that a SZ-related behavioral deficit – impaired LI – may be uncovered in mice by the interaction of a genetic vulnerability to SZ (CHL1 deficiency) and environmental stressors (CMS). These results provide strong evidence for a ‘two-hit’ hypothesis of schizophrenia, and support CHL1-deficient mice as a valid model to study environment x genetic interactions on SZ-behavioral phenotypes. The neural dysfunction causing the loss of LI may be the result of interactions between the genetic background supporting changes in neuronal excitability and regional changes in neuronal activation due to stress.

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Abreviations

Acb-core	nucleus accumbens core
Acb-shell	nucleus accumbens shell

ANOVA	analysis of variance
BLA	basolateral amygdala
CER	conditioned emotional response
CHL1	Close Homolog of L1
CMS	chronic mild stress
CR	conditioned response
CS	conditioned stimulus
DISC1	Disrupted in Schizophrenia
HET	heterozygous
IL	infralimbic cortex
ISI	inter-stimulus interval
KO	knock-out
LI	latent inhibition
NPE	non-pre-exposed
NS	no-stress group
PE	pre-exposed
PrL	prelimbic cortex
S	stress group
SZ	schizophrenia
US	unconditioned stimulus
vHipp	ventral hippocampus
vSub	ventral subiculum
WT	wild-type

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Highlights

- Latent inhibition (LI) was evaluated in CHL1 KO mice under chronic mild stress
- Stressed CHL1 KO and HET mice failed to show LI, while all other mice showed LI
- Neuronal activation demonstrated regional differences relevant for LI
- Some regions were affected by stress only, others by stress, genotype, and their interaction
- Stressed CHL1-deficient mice are a two-hit model for schizophrenia-like phenotypes

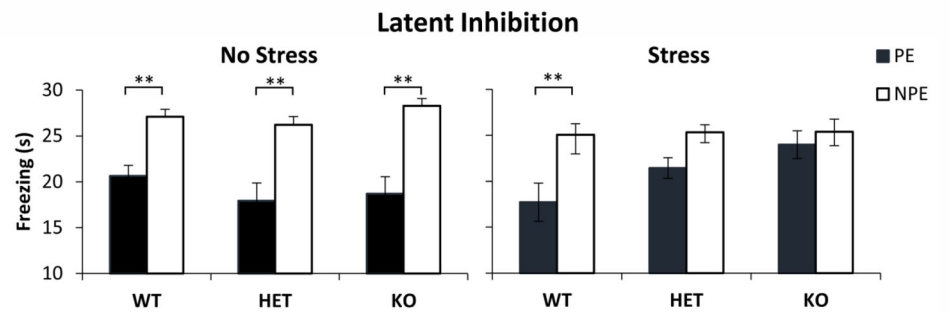

Fig. 1. Latent inhibition by stress and genotype

Average duration of freezing (± SEM) to the pre-exposed (PE) and non-pre-exposed (NPE) stimulus in CHL1 knock-out (KO), heterozygotes (HET) and wild type littermate controls (WT) under no-stress (left) and chronic mild stress (right). A reliably latent inhibition (larger freezing to NPE than PE) was observed in all groups except in stressed CHL1 HET and KO mice. * = $p<0.05$; ** = $p<0.01$.

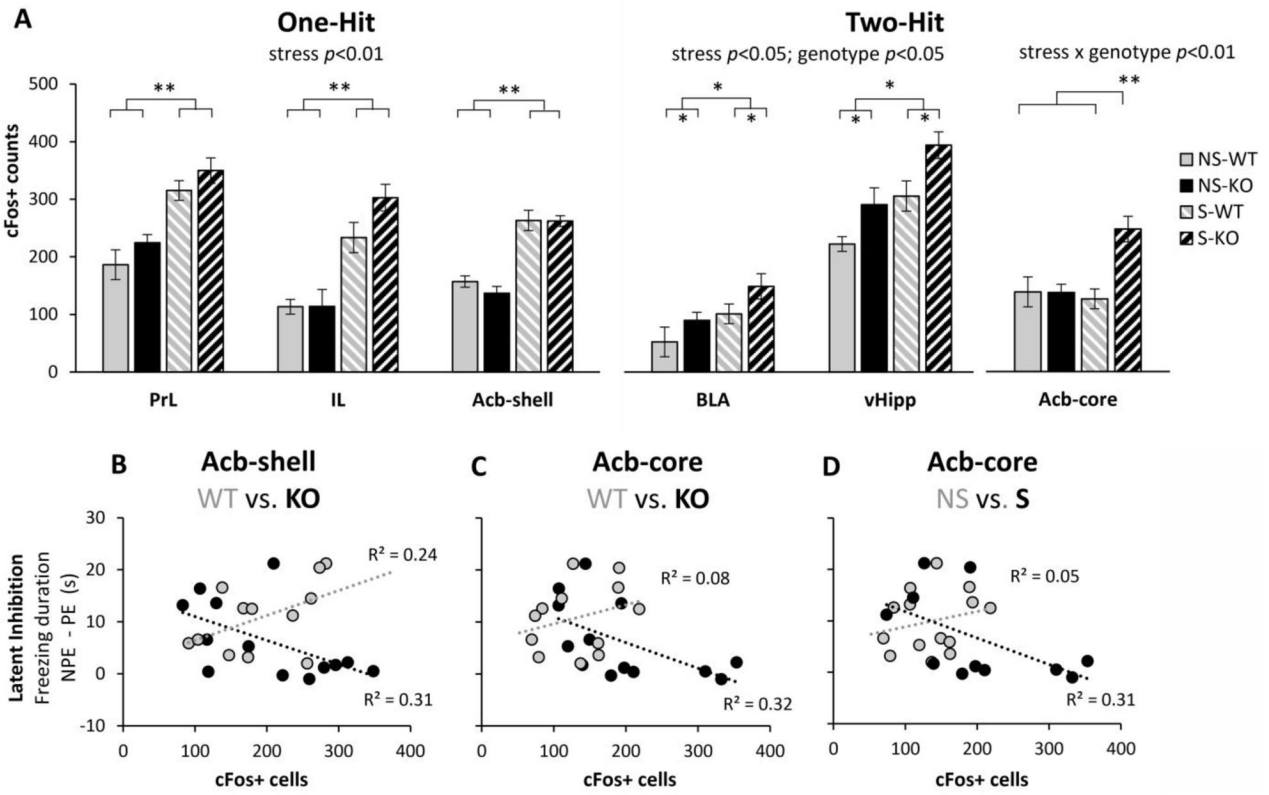

Fig. 2. Neural activation during latent inhibition testing

A: Average c-Fos+ cell counts (± SEM) in prelimbic cortex (PrL), infralimbic cortex (IL), nucleus accumbens shell (Acb-shell), basolateral amygdala (BLA), ventral hippocampus (vHipp), and nucleus accumbens core (Acb-core) in the stress (S) and no-stress (NS) CHL1-deficient mice (KO) and wild-type littermate controls (WT). **BCD**: Correlations between latent inhibition (difference in freezing duration to the non-pre-exposed, NPE, and pre-exposed, PE, stimuli) and neural activation in Acb-shell (**B**) and Acb-core (**CD**). * = $p<0.05$.

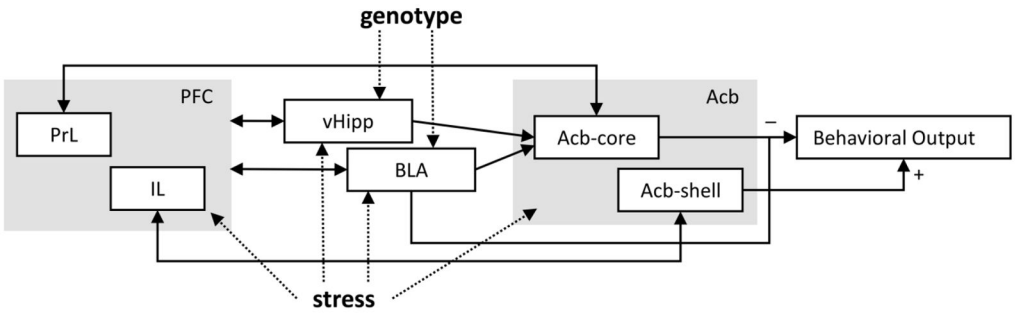

Fig. 3. Modulation of a putative latent inhibition circuit by stress or the CHL1 genotype

A putative circuit for latent inhibition (modified after [38–39]) indicating the brain regions where activity was affected by stress and/or CHL1 genotype. PFC = prefrontal cortex; PrL = prelimbic cortex; IL = infralimbic cortex; BLA = basolateral amygdala; vHipp = ventral hippocampus; Acb = nucleus accumbens; Acb-core = nucleus accumbens core; Acb-shell = nucleus accumbens shell.