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Increased Temporal Discounting after Chronic Stress in CHL1-Deficient Mice is Reversed by 5-HT2C Agonist Ro 60-0175

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Abstract

Schizophrenia is a neurodevelopmental disorder in which impaired decision-making and goal-directed behaviors are core features. One of the genes associated with schizophrenia is the Close Homolog of L1 (CHL1); CHL1-deficient mice are considered a model of schizophrenia-like deficits, including sensorimotor gating, interval timing and spatial memory impairments. Here we investigated temporal discounting in CHL1-deficient (KO) mice and their wild-type littermates. Although no discounting differences were found under baseline conditions, CHL1-KO mice showed increased impulsive choice following chronic unpredictable stress (fewer % larger-later choices, and reduced area under the discounting curve). Stressed CHL1-KO mice also showed decreased neuronal activation (number of cFos positive neurons) in the discounting task in the prelimbic cortex and dorsal striatum, areas thought to be part of executive and temporal processing circuits. Impulsive choice alterations were reversed by the 5-HT2C agonist Ro 60-0175. Our results provide evidence for a gene×environment, double-hit model of stress-related decision-making impairments, and identify CHL1-deficient mice as a mouse model for these deficits in regard to schizophrenia-like phenotypes.

Keywords

cFos; intertemporal decision making; prefrontal cortex; schizophrenia; serotonin; striatum

INTRODUCTION

Schizophrenia (SZ) is a neurodevelopmental disorder which affects about 1% of the population (Regier et al., 1993). Patients with SZ exhibit a variety of symptoms, such as positive symptoms (hallucinations, delusions), negative symptoms (affective flattening, avolition, asociality), disorganized thinking and speech, and disorganized motor behavior or catatonia (Tandon, 2013). Cognitive symptoms (inattention, poor working memory,

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CONFLICT OF INTEREST

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executive dysfunction) are currently viewed as distinct from the negative symptoms (Kirkpatrick et al., 2006).

Impaired decision-making and goal-directed behaviors are core features in SZ. Recent studies have revealed impairments in the Iowa Gambling Task and the Wisconsin Card Sorting Task (Shurman et al., 2005; Wing et al., 2013), greater temporal discounting (Heerey et al., 2007; Weller et al., 2014), and increased impulsivity measured by the Barratt Impulsiveness Scale (Nanda et al., 2016) in SZ patients compared to controls. Since cognitive dysfunction has been associated with poor outcome (Green, 1996; Green et al., 2000) and propensity toward addictive disorders (Dervaux et al., 2001; Volkow, 2009), it is critical to understand the neurobiological mechanisms underlying these deficits.

The nature of the SZ cognitive deficits matches its neuropathology, which affects prefrontal cortex, anterior cingulate cortex, and the hippocampus (Robbins et al., 2012), in addition to the dysregulation of dopaminergic (DA) neurotransmission (Grace, 2016). Thus, plausible models for SZ phenotypes involve dysfunction of these regions, via developmental dysplasias (Fernando & Robbins, 2011); mice genetically modified to model gene abnormalities found in SZ patients provide construct validity and exhibit relevant neuroanatomical defects in one or more of these regions.

One of the genes recently associated with SZ is Close Homolog to L1 (CHL1) (Chen et al., 2005; Sakurai et al., 2002; Shaltout et al., 2013; Tam et al., 2010). CHL1 is a cell adhesion molecule highly expressed during the development of the nervous system (Hillenbrand et al., 1999) and involved in hippocampal neurotransmission and plasticity (Leshchyns'ka et al., 2006; Montag-Sallaz et al., 2002). CHL1-deficient mice (KO) (Montag-Sallaz et al., 2002) exhibit sensorimotor gating deficits (Irintchev et al., 2004) and impaired spatio-temporal integration (M. Buhusi et al., 2013) reminiscent of SZ. Here we assessed temporal discounting in CHL1-deficient mice under basal and chronic stress conditions, and we investigated the neural circuits underlying this behavior using measures of neuronal activation (cFos positive cell counts) and pharmacological approaches. We have used Ro 60-0175, an agonist for 5-HT2C receptors, specifically because a biochemical interaction was demonstrated between the CHL1 protein and the 5-HT2C receptor (Kleene et al., 2015).

EXPERIMENTAL PROCEDURES

Subjects

Subjects were thirty-two 4–6 mo-old male CHL1-deficient (KO, n=16) mice and their wild-type littermates (WT, n=16), obtained from heterozygous breeders (Montag-Sallaz et al., 2002). The CHL1 colony was maintained in the C57Bl/6J background for more than 10 generations in our lab. Genotype was confirmed by PCR amplification from tail biopsy samples. Mice were housed in a temperature-controlled room under a 12-h light-dark cycle. Mice were maintained at 85% of their *ad libitum* weights by restricting access to food (Teklad Diet 8064, Harlan Laboratories Inc., Indianapolis, IN). Manipulations were approved by Utah State University IACUC committee.

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Procedures

Mice were trained in a TD paradigm with Larger-Later (LL) delays 0s, 4s, 16s, 64s, as in (M. Buhusi et al., 2016) (baseline condition), and then subjected for 21 days to *chronic unpredictable stress* (CUS) as in (M. Buhusi et al., 2016; Dias-Ferreira et al., 2009). Following the CUS treatment, mice were re-tested for 4 sessions (stress condition), and then split in two groups: Six mice in each genotype were randomly selected for cFos immunostaining; the remaining mice (CHL1-KO n=10; WT n=10) were re-tested in the TD paradigm under systemic administration of 5-HT2C agonist Ro 60-0175 (0, 0.6mg/kg, and 1.2mg/kg).

TD Paradigm

Mice were trained in a TD paradigm modified after (Adriani & Laviola, 2003; Evenden & Ryan, 1996; Isles et al., 2003). Briefly, mice were presented with two alternatives, Smaller-Sooner (SS), 1 pellet at 0s delay, and Larger-Later (LL), 4 pellets at progressively larger delays. The 1.5-hr sessions consisted of 32 trials broken up into four 8-trial blocks. The beginning of a block was signaled by the house light flashing for 1 min; continuous illumination of the house light signaled that the mice can self-initiate a trial by pressing on the lever. Each block consisted of 6 forced choice trials (3 pairs of forced-choice trials on the SS and LL alternatives), followed by 2 free-choice trials between alternatives, separated by 30-sec blackouts (inter-trial intervals). The position of the SS and LL nosepokes (to the left or to the right of the lever) was counterbalanced among subjects. For each session, the 4 blocks of trials differed by the delay on the LL choice, presented in increasing order of delay during each session. Mice received five sessions with 0s LL delays, five sessions with the LL delays 0s, 1s, 2s, 4s, and five sessions with the LL delays 0s, 1s, 4s, 16s. Mice were then tested for 4 sessions with LL delays 0s, 4s, 16s, 64s under baseline condition (before stress) and 4 sessions after CUS (stress condition). When tested under Ro 60-0175, mice received 6 TD testing sessions (with the 3 drug doses counterbalanced among subjects) with LL delays 0s, 16s, 64s. The %LL choices was averaged over sessions and analyzed. The discounting curve was normalized both in the delay (x-axis) and %LL (y-axis) (Myerson et al., 2001), and the percent area under the normalized discounting curve (%AUC) was computed and analyzed.

Chronic Unpredictable Stress (CUS)

Mice received CUS as in (M. Buhusi et al., 2016), using the following daily randomly-chosen stressors: 30 min restraint, 10 min forced swim, or 10 min exposure to an aggressive Balb/c male mouse.

cFos Immunostaining

At the end of the last TD test session under Stress condition, 6 mice in each genotype were randomly selected for cFos immunostaining, which was performed using standard procedures (M. Buhusi et al., 2016), using a rabbit anti cFos primary antibody (Cell Signaling Technologies, Danvers, CA, Antibody Registry AB_2247211, 1:300 dilution), Alexa488-conjugated goat anti rabbit secondary antibody and NeuroTrace 530/615 (Life Technologies, Carlsbad, CA). NeuroTrace neuronal labeling was used to visualize the

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regions of interest. A Zeiss LSM710 laser scanning confocal microscope was used for image acquisition. Neuronal activation was estimated by counting cFos-positive nuclei in corresponding areas in 2 sections / region of interest / mouse (OFC: bregma 2.10/2.34, PrL: bregma 1.78/2.10, Acb-shell and core: bregma 1.10/1.34, DS-med and lat: bregma 0.98/1.34) (Franklin & Paxinos, 2008), averaged over two independent observers unaware of genotype (inter-observer reliability r=0.36, p<0.01).

Ro 60-0175 (Ro) drug manipulation

After being tested under stress condition, mice (CHL1-KO n=10, WT n=10) were tested under systemic (i.p.) administration of 5-HT2C agonist Ro 60-0175 (in saline solution). Mice were placed in the testing apparatus 15 min after being injected i.p. with Ro 60-0175 (0, 0.6mg/kg, and 1.2mg/kg). Data from 6 drug TD sessions (doses were counterbalanced daily among subjects) with delays 0s, 16s, and 64s were subjected to data and statistical analyses.

Statistical analyses

The %LL choices were analyzed by mixed ANOVAs with between-subjects variable genotype (KO, WT) and within-subject variables stress (baseline and stress) and delay (0s, 4s, 16s, 64s). The %AUC was analyzed by mixed ANOVAs with between-subjects variable genotype and within-subject variable stress. The %LL choices in drug sessions were analyzed by mixed ANOVAs with between-subjects variable genotype and within-subject variables drug dose (0, 0.6mg/kg, 1.2mg/kg) and delay (0s, 16s, 64s). The %AUC in drug sessions was analyzed by mixed ANOVAs with between-subjects variable genotype and within-subject variable drug dose. All ANOVAs were followed by planned and post-hoc analyses. The individual average neuronal activation (cFos+ counts) for each region of interest was submitted to *t*-tests with between-subjects variable genotype. Analyses were conducted in STATISTICA 6.0 (StatSoft, Tulsa OK), with a 0.05 alpha level.

RESULTS

Chronic stress increased impulsive choice in CHL1 KO mice

To evaluate differences in discounting between genotypes the %LL choices were submitted to statistical analyses under baseline (no-stress) conditions (Fig.1 Baseline) and following CUS (Fig.1 Stress). Under baseline (no-stress) conditions, analyses indicated a main effect of delay (F(3,90)=20.97, p<0.01), but failed to identify discounting differences between genotypes (all Fs(1,30)<2.66, p>0.05). The main effect of delay suggests that all mice acquired the TD paradigm. Interestingly, following CUS (Fig.1 Stress), CHL1-KO mice discounted reliably more at the 64-s delay (F(1,30)=6.42, p<0.05), but not at shorter delays (all Fs(1,30)<3.08, p>0.05). Interestingly, at zero delay, the %LL choice was only 70–80%, suggesting imperfect discrimination between the choice alternatives, but no difference between genotypes. Thus, results failed to support baseline differences in TD between genotypes, but indicated that CHL1-KO mice were more affected by stress and discounted more steeply than WT controls after CUS. As both genotypes received the same number of training sessions (before stress) and the same limited number of testing sessions (four) both

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before and after stress, the differences between KO and WT are unlikely to be due to extended training, although this possibility cannot be ruled out entirely.

The discounting curves were further normalized both in the %LL and delay axes (Myerson et al., 2001), as shown in Fig.2A, and normalized %LL choices were submitted to analyses that indicated reliable main effects of delay ($F(3,90)=30.73$, $p<0.01$) and stress ($F(1,30)=11.97$, $p<0.01$), as well as a marginal stress×genotype interaction ($F(1,30)=3.90$, $p=0.057$), suggesting that mice acquired the TD task and discounted in a delay-dependent fashion, and that CHL1-KO mice discounted more than WT controls after stress. Indeed, under baseline conditions (Fig.2A left panel) analyses failed to indicate discounting differences between genotypes (all Fs$(1,30)<1.29$, $p>0.05$), except at the 12.5% (4s) delay ($F(1,30)=4.92$, $p<0.05$). However, following CUS (Fig.2A right panel), CHL1-KO mice discounted more at the 12.5% (4s) delay ($F(1,30)=5.13$, $p<0.05$), and at 100% (64s) delay ($F(1,30)=6.20$, $p<0.05$), but not at the other delays (all Fs$(1,30)<0.25$, $p>0.05$).

Fig.2B shows the %AUC in CHL1-KO and WT mice in the baseline and stress conditions; a small %AUC is taken to be indicative of steeper discounting, and a more impulsive individual (Myerson et al., 2001). Although the effect of stress was highly reliable ($F(1,30)=12.09$, $p<0.01$), stress appeared to be more detrimental in CHL1-KO mice ($F(1,30)=12.29$, $p<0.01$), than in WT controls ($F(1,30)=1.99$, $p>0.05$). Together with analyses of the %LL choice (Fig.1) and analyses of the normalized %LL choices (Fig.2A), these results suggest that stressed CHL1-KO mice showed increased choice impulsivity (reduced %LL choices, steeper discounting rate, reduced %AUC) relative to their stressed WT controls.

Decreased neural activation in CHL1-KO in the prelimbic cortex and dorsal striatum

Despite some limitations, cFos immunoreactivity is a well-known marker of neuronal activation, useful for the identification of brain regions specifically activated during behavioral tasks (da Costa Araujo et al., 2010; Kovacs, 2008; Robertson, 1992). Neuronal activation during TD was evaluated in CHL1-KO (n=6) and WT controls (n=6) in PrL, OFC-med, OFC-vlat, Acb-core, Acb-shell, DS-med, and DS-lat, regions with relevant roles in decision making (Bailey et al., 2016; Floresco et al., 2008), and particularly in TD (da Costa Araujo et al., 2010). Fig.3 indicates a reliable decrease in neuronal activation (number of cFos+ cells) in CHL1-KO mice relative to WT controls in PrL ($t(10)=5.65$, $p<0.01$), DS-med ($t(10)=4.96$, $p<0.01$) and DS-lat ($t(10)=4.05$, $p<0.01$), but no reliable changes in the other brain regions (all ts$(10)<1.63$, $p>0.05$). Although under the current design the cFos marker cannot differentiate the activation specific to sub-processes involved in delay discounting in the stress condition (e.g., reward valuation, working memory, anxiety etc), it is interesting to note that post-stress alterations in neuronal activation were found in regions involved (among other processes) in intertemporal decision-making. Since cFos expression analysis was performed only in stressed animals, the observed differences may be due to either genotype or the genotype×stress interaction; however, since no differences in behavior were noted under baseline conditions, it is less plausible that the genotype alone would be responsible for the differences in cFos expression in our study.

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5-HT2C agonist Ro 60-0175 decreased impulsivity in stressed CHL1-KO mice and WT controls

CHL1-KO mice (n=10) and WT control (n=10) were further tested in the TD paradigm under systemic administration of 5-HT2C agonist Ro 60-0175 (0.6mg/kg and 1.2mg/kg) and saline control (SAL) with the 0s, 16s, and 64 delays. The discounting curves were normalized both in the delay and %LL axes in order to compute the %AUC (Myerson et al., 2001) (Fig.4A). Analyses indicated a main effect of drug dose ($F(2,36)=10.27$, $p<0.01$) and genotype ($F(1,18)=5.83$, $p<0.05$) but no interactions, suggesting that the drug affected both genotypes similarly. Post-hoc analyses indicated a significant increase in %AUC under Ro 0.6mg/kg relative to SAL ($F(1,18)=10.45$, $p<0.01$ for CHL1-KO mice, and $F(1,18)=10.69$, $p<0.01$ for WT controls), but no difference in %AUC between SAL and Ro 1.2mg/kg ($F(1,18)=0.27$, $p>0.05$ for CHL1-KO mice, and $F(1,18)=1.67$, $p>0.05$ for WT controls).

To further visualize the effect of Ro 60-0175 on choices in the TD paradigm, Fig.4B shows the normalized %LL choices under SAL and Ro 0.6mg/kg in WT and CHL1-KO mice. Fig. 3B indicates that in both genotypes systemic administration of Ro significantly decreased discounting at the larger delay (100% delay, 64s). Analyses indicated a main effect of delay ($F(2,36)=30.33$, $p<0.01$), suggesting mice discounted, a main effect of drug ($F(2,36)=7.58$, $p<0.01$), suggesting that Ro 60-0175 decreased discounting in both genotypes, and a main effect of genotype ($F(1,18)=6.52$, $p<0.05$), and a genotype×delay interaction ($F(2,36)=3.33$, $p<0.05$), suggesting CHL1-KO mice discounted more than WT controls, particularly at the 100% (64 s) delay. Indeed, Ro administration reliably increased %LL choices in CHL1-KO mice at the 100% (64s) delay ($F(1,18)=4.56$, $p<0.05$) and in WT controls at 25% (16s) and 100% (64s) delays (all $Fs(1,18)>5.00$, $p<0.05$), but not at the other delays (all $Fs(1,18)<4.15$, $p>0.05$). No reliable differences in %LL choices were found between SAL and Ro 1.2mg/kg ($F(1,18)=0.80$, $p>0.05$). Also, no reliable differences in %LL choices were found between CHL1-KO under Ro 0.6mg/kg and WT mice under SAL ($F(1,18)=0.08$, $p>0.05$), suggesting that 5-HT2C agonist Ro 60-0175 reversed impulsivity in stressed CHL1-KO to levels found in WT controls. Taken together, these results suggest that 5-HT2C agonist Ro 60-0175 decreased discounting in a dose-dependent manner in both CHL1-KO mice and WT control mice.

DISCUSSION

Our current study investigated decision making in CHL1-deficient mice, a genetic model of SZ. Using a TD procedure developed in our laboratory (M. Buhusi et al., 2016) we evaluated the effects of genetic inactivation of the CHL1 gene in comparison to WT mice under baseline (no-stress) conditions as well as after exposure to chronic unpredictable stress (CUS). Although CHL1-deficient mice did not discount differently from their littermates under no-stress conditions, CHL1-KO mice were more impulsive (showed decreased %LL choices and %AUC) than WT mice after CUS. Surprisingly, neuronal activation was decreased only in PrL and DS (regions involved in delay processing), but not in OFC, Acb-core or Acb-shell (regions involved in value processing). 5-HT2C agonist Ro 60-0175 reversed impulsivity in stressed CHL1-KO and WT controls, supporting a role for 5-HT2C receptors in future therapies of schizophrenia.

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Neural correlates of temporal discounting

Intertemporal decision making, such as TD, relies on both reward magnitude and reward delay (temporal processing) (Bailey et al., 2016; Cardinal, 2006; Floresco et al., 2008). A recent comparative study of neuronal activation (cFos expression) in adjusting delay and adjusting reward magnitude paradigms (Da Costa Araujo, 2010) reported that exposure to the adjusting-delay schedule was associated with enhanced cFos expression in both the OFC and Acb, whereas exposure to the adjusting-magnitude schedule was associated with enhanced Fos expression in the OFC but not the Acb. In our current TD paradigm, we failed to identify differences between genotypes in neuronal activation in OFC and Acb, regions known to be involved in value computation (Galtress & Kirkpatrick, 2010; Schmajuk et al., 1997; van Duuren et al., 2008; Zeeb et al., 2010) and TD (Baruch et al., 1988; Cardinal et al., 2001; Valencia-Torres et al., 2012; Wright et al., 2007). Interestingly, in our study mice showed imperfect discrimination between the choice alternatives (at zero delay, the %LL choice was only 70–80%), but there were no differences between genotypes in regard to reward magnitude, regardless of condition. Taken together, our results suggest that steeper TD in stressed CHL1-KO mice is not explained by alterations in perception of reward magnitude.

On the other hand, in our study we found changes in PrL and DS, regions with known roles in processing of delays (C. V. Buhusi & Meck, 2005; C. V. Buhusi et al., 2016; Kim et al., 2013; Matell et al., 2003; Xu et al., 2014). In a previous study (M. Buhusi et al., 2013) we examined time perception and production in CHL1-deficient mice and WT controls in the peak-interval paradigm. In the same session, trials in which subjects are reinforced at the criterion duration are randomly interspersed with non-reinforced peak trials, in which subjects respond with a characteristic bell curve which peaks at a duration subjectively equal to the reinforced duration (C. V. Buhusi et al., 2009). While in peak trials WT mice respond maximally at the criterion duration, CHL1-KO mice respond maximally before the criterion duration, such that at the criterion duration their response is lower than that of WT controls (M. Buhusi et al., 2013, pp. 27–28, Figs.2 and 3), indicating that CHL1-KO mice respond as if the same objective duration (criterion) is subjectively longer than in WTs. Similarly, in our current TD study, CHL1-KO mice may take the delay to the LL reward to be longer compared to WT controls, thus valuing the LL option less than controls (for a similar interpretation see Cardinal, 2006). This possibility is further supported by the reduced neuronal activation in stressed CHL1 KO mice comparatively to stressed WT in PrL and DS, structures known to be involved in time perception and timed behaviors (Matell et al., 2003; Mello et al., 2015; Xu et al., 2014).

A role of the striatum in TD is revealed by a recent study in rats (Tedford et al., 2015) showing that DA lesions of the DS were followed by steeper TD. In primates, activity in both the DS and Acb is modulated by temporally-discounted values (Cai et al., 2011). Caudate activation is also found in human imaging studies (Benningfield et al., 2014; Massar et al., 2015). Temporary inactivation of the rat PrL/IL (Churchwell et al., 2009) using muscimol revealed changes in the TD function, with a decrease in %LL choices. Excitotoxic lesions of the rat PrL (Cardinal et al., 2001) also changed TD functions, with a flattening of the curve, suggestive of an altered sensitivity to delay. Moreover, recent time-

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based behavioral interventions have been successful in decreasing impulsive choices in rats (Peterson & Kirkpatrick, 2016; Smith et al., 2015). Therefore, our previous findings of impaired time perception and production in CHL1-deficient mice (M. Buhusi et al., 2013) could be relevant to an altered perception of delay intervals in TD, associated with decreased % LL choices in our present study.

Although functional changes in the prefrontal cortex and striatum seem plausible causes for the stress-induced decision-making dysfunction in stressed CHL1 mice, we cannot exclude the role of other developmental neuroanatomical changes. For example, CHL1-deficient mice exhibit morphological and functional changes in the hippocampus (Leshchyns'ka et al., 2006; Montag-Sallaz et al., 2002; Morellini et al., 2007), a brain region which is also thought to play a role in time perception (Howard & Eichenbaum, 2015) and TD (Cardinal, 2006; Cheung & Cardinal, 2005). This interpretation is compatible with the suggestion that the hippocampal-prefrontal pathway may be a weak link in psychiatric disorders (Godsil et al., 2013).

Stress, psychosis, and executive dysfunction

Stress induces adaptive processes to promote survival when faced with real or perceived threat. While acute stress triggers immediate, time-limited responses to maximize organismal adaptation (Hermans et al., 2014), long lasting structural and functional changes are generated following chronic stress, particularly neuroendocrine modulation of executive functions (Anderson et al., 2016; Cook & Wellman, 2004; Dias-Ferreira et al., 2009). In humans, such maladaptive reactivity to chronic stress includes improper decision making, and impulsivity (Dias-Ferreira et al., 2009; Fields et al., 2014; George & Koob, 2010; Wang et al., 2014), but also changes of the timekeeping mechanisms. Indeed, time seems to stop (durations are perceived as longer) under stress, or when facing negative emotions (Brown et al., 2007; Matthews et al., 2012).

In vulnerable individuals, stress precipitates psychosis (Aiello et al., 2012; Holtzman et al., 2012; Holtzman et al., 2013), anxiety and mood disorders (Bale, 2006; Deppermann et al., 2014), and substance abuse (Lijffijt et al., 2014; Sinha, 2008). The behavioral alterations may be the result of changes in DA tone and DA processing (Ahmad et al., 2010; Belujon & Grace, 2015; Wanat et al., 2013), changes in serotonin synthesis and neurotransmission (Donner et al., 2016) in multiple key areas of the brain, or increases in GABA release onto medial prefrontal cortex pyramidal neurons and prefrontal inhibition (McKlveen et al., 2016) induced by stress through elevated corticosteroid and CRF signaling, or through other mechanisms.

The effects of chronic stress at the behavioral level in our study are especially relevant since CHL1-deficient mice are considered a model of SZ-like impairments. Genetic associations between CHL1 gene polymorphisms and schizophrenia (Chen et al., 2005; Sakurai et al., 2002; Shaltout et al., 2013) were found in Asian populations and rare copy number variants of this gene were identified in a Scottish study (Tam et al., 2010). Prepulse inhibition of the acoustic startle response is impaired in CHL1-KO mice, which can thus model sensorimotor gating impairments found in schizophrenic patients (Irintchev et al., 2004). Our current results show that other SZ-like deficits, such as impulsivity, can be revealed in these mice by

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stress, supporting a ‘double hit’ hypothesis (Bayer et al., 1999). The enhanced effects of stress in CHL1-KO mice may be the result of functional changes in neuronal signaling or may be due to developmental neuroanatomical alterations.

Serotonin and impulsivity

In our study, increased choice impulsivity after CUS was reversed in CHL1-KO mice by systemic administration of a 5-HT2C agonist, Ro 60-0175. This is compatible with the proposal that serotonin plays a role in patience, impulsivity (Dalley & Roiser, 2012; K. Miyazaki et al., 2012) and time perception (C. V. Buhusi & Meck, 2007). Activation of dorsal raphe serotonergic neurons using optogenetic techniques increases patience for delayed consequences (K. W. Miyazaki et al., 2014), while agonists for the 5-HT1A autoreceptors increase impulsivity (Winstanley et al., 2005). On the other hand, there is extensive evidence for a role of 5-HT2C receptors in the perception of and response to reinforcer value: 5-HT2C receptor agonists reduce responding for both food (Higgins et al., 2013; Rapoport et al., 2005), cocaine (Fletcher et al., 2004; Fletcher et al., 2008) and nicotine (Higgins et al., 2012).

Of particular interest is a recently identified interaction between CHL1 and the serotonin receptor 5-HT2C (Kleene et al., 2015). Abnormal behaviors (such as a reduced reactivity to novelty) in CHL1-deficient mice are thought to result from abnormal signaling through constitutively active 5-HT2C receptor isoforms (Kleene et al., 2015), and can be rescued with 5-HT2C antagonists, which increase DA neuronal activity and striatal DA levels (Alex et al., 2005). Stress increases sensitivity of 5-HT2C receptors in the striatum (Strong et al., 2011), and alters expression of other serotonin receptors as well (Chang et al., 2016). However, the roles of 5-HT2C receptors are not easy to understand, due to the great diversity of isoforms, generated not only through alternative splicing but also through post-transcriptional editing, and their expression on multiple types of neurons; as an example, both 5-HT2C receptors antagonists and agonists have antidepressant properties (Chagraoui et al., 2016). The decrease in impulsive choice observed under 5-HT2C agonist Ro 60-0175 in stressed mice in our study could be explained by the differential effects of the agonist in the VTA and SN: acute or chronic 5-HT2C agonists reduce the number of spontaneously active DA neurons in the ventral tegmental area, but not in the substantia nigra, and selectively reduce DA levels in the Acb relative to striatum (Di Matteo et al., 2000). Since 5-HT2C receptors are currently thought to have a crucial role in disorders such as SZ (Chagraoui et al., 2016), substance abuse or obesity (Higgins et al., 2013), our results add to the expanding preclinical evidence emphasizing the potential for 5-HT2C agonists as therapeutic agents for these conditions.

CONCLUSIONS

Here we characterized stress-induced impulsive choice in a genetic model of SZ, the CHL1-deficient mice. Although CHL1-deficient mice did not discount differently from their littermates under no-stress conditions, they showed increased impulsivity (decreased %LL choices and %AUC) after stress. Neuronal activation (number of cFos+ cell) decreased only in PrL and DS (regions involved in temporal processing), but not in OFC, Acb-core or Acb-

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shell (regions involved in valuation). 5-HT2C agonist Ro 60-0175 reversed impulsivity in both stressed CHL1-KO and WT controls. Although the effects of Ro 60-0175 on TD was not genotype-specific, our results show that targeting the 5-HT2C receptor may be a valuable treatment strategy for disorders of impulsivity and SZ. Further studies should investigate whether the behavioral changes observed in CHL1-deficient mice are present in other models of SZ, and whether they stem from similar or different neural mechanisms and pathology.

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Abbreviations

5-HT	5-hydroxytryptamine (serotonin)
Acb-core	nucleus accumbens core
Acb-shell	nucleus accumbens shell
ANOVA	analysis of variance
AUC	area under the normalized TD curve
CHL1	close homolog to L1
CUS	chronic unpredictable stress
DA	dopamine, dopaminergic
DS-med	dorsomedial striatum
DS-lat	dorsolateral striatum
KO	knock-out
LL	larger-later
OFC	orbitofrontal cortex
PrL	prelimbic cortex
Ro	Ro 60-0175
SZ	schizophrenia
SS	smaller-sooner
TD	temporal discounting

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WT wild-type

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Highlights

- Temporal discounting was evaluated in Close-Homolog to L1 (CHL1) deficient mice
- CHL1-KO mice and WT controls showed similar basal levels of discounting / impulsivity
- CHL1-KO mice showed increased vulnerability to stress, becoming more impulsive than WT
- Neuronal activation (cFos counts) was reduced in prelimbic cortex, dorsal striatum in KO mice
- 5-HT2C agonist Ro 60-0175 reversed choice impulsivity in CHL1-KO mice to levels in WT controls

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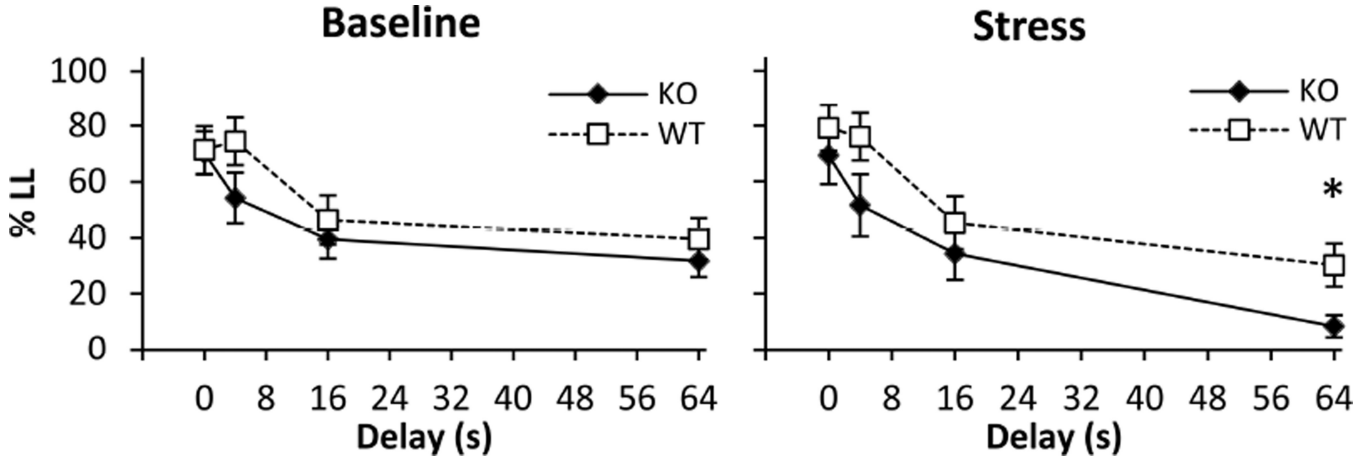

Fig.1. Increased discounting after chronic stress in CHL1-deficient mice

Average %LL choices (± SEM) in CHL1- KO mice (n=16) and WT controls (n=16) under baseline conditions (left) and after chronic unpredictable stress (right). * $p<0.05$, ** $p<0.01$.

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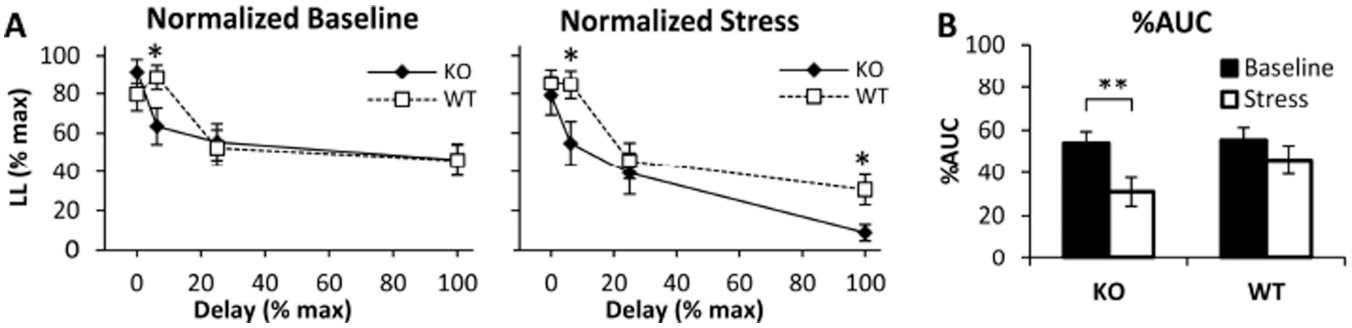

Fig.2. Increased impulsivity after chronic stress in CHL1-deficient mice

(A) Average normalized %LL choices (± SEM) in CHL1-KO mice (n=16) and WT controls (n=16) under baseline conditions (left) and after chronic unpredictable stress (right). (B) Average %AUC (± SEM) in CHL1-KO mice and WT controls under baseline and stress conditions. * $p<0.05$, ** $p<0.01$.

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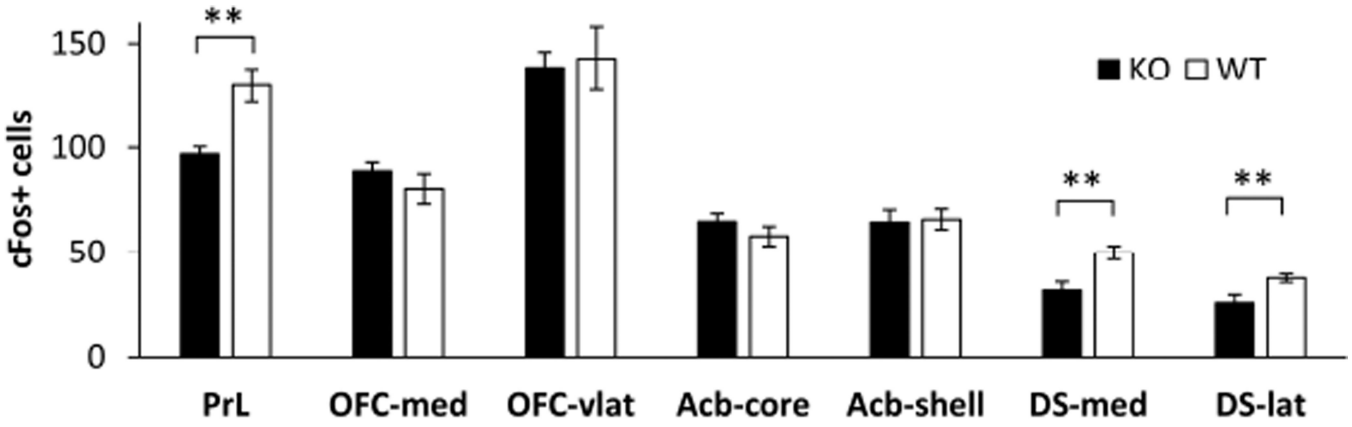

Fig.3. Neural activation during TD in stressed CHL1-deficient mice and stressed WT controls

Average number of cFos+ neurons (± SEM) in CHL1-KO (n=6) and WT controls (n=6) in the stress condition in prelimbic cortex (PrL), medial OFC (OFC-med), ventrolateral OFC (OFC-vlat), nucleus accumbens core (Acb-core), nucleus accumbens shell (Acb-shell), dorsomedial striatum (DS-med), and dorsolateral striatum (DS-lat). * $p<0.05$; ** $p<0.01$.

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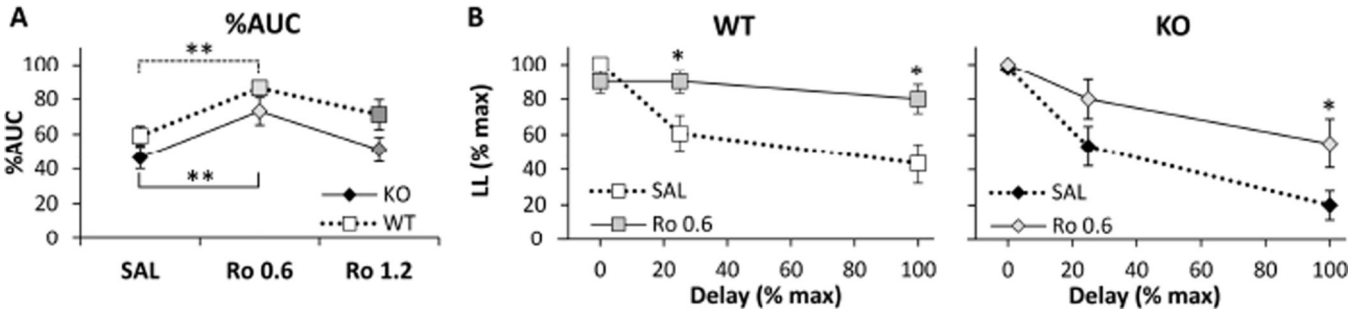

Fig.4. Systemic administration of Ro 60-0175 reverses impulsivity in stressed CHL1-deficient mice and WT controls

(A) Average %AUC (± SEM) in CHL1-KO mice (n=10) and WT controls (n=10) under Ro 60-0175 (0.6mg/kg, 1.2mg/kg) and saline (SAL) control. (B) Average normalized %LL choices (± SEM) in CHL1-KO mice (n=10, right) and WT controls (n=10, left) under Ro 60-0175 0.6mg/kg and saline (SAL) control. * $p<0.05$, ** $p<0.01$.