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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 x 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. © **2017 Published by Elsevier Ltd on behalf of IBRO.**

Key words: 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, 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 and 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) (Sakurai et al., 2002; Chen et al., 2005; Tam et al., 2010; Shaltout et al., 2013). 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 (Montag-Sallaz et al., 2002; Leshchyns'ka et al., 2006). CHL1-deficient mice (KO) (Montag-Sallaz et al., 2002) exhibit sensorimotor gating deficits (Irintchev et al., 2004) and impaired spatio-temporal integration (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

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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; WT, wild-type.

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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/6 J 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.

Procedures

Mice were trained in a TD paradigm with Larger-Later (LL) delays 0 s, 4 s, 16 s, 64 s, as in (Buhusi et al., 2016b) (baseline condition), and then subjected for 21 days to *chronic unpredictable stress* (CUS) as in (Dias-Ferreira et al., 2009; Buhusi et al., 2016b). Following the CUS treatment, mice were re-tested for 4 sessions (stress condition), and then split into 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, 0.6 mg/kg, and 1.2 mg/kg).

TD paradigm

Mice were trained in a TD paradigm modified after (Evenden and Ryan, 1996; Adriani and Laviola, 2003; Isles et al., 2003). Briefly, mice were presented with two alternatives, Smaller-Sooner (SS), 1 pellet at 0 s delay, and Larger-Later (LL), 4 pellets at progressively larger delays. The 1.5-h 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-s 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 0 s LL delays, five sessions with the LL delays 0 s, 1 s, 2 s, 4 s, and five sessions with the LL delays 0 s, 1 s, 4 s, 16 s. Mice were then tested for 4 sessions with LL delays 0 s, 4 s, 16 s, 64 s 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 0 s, 16 s, 64 s. 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 (Buhusi et al., 2016b), 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 (Buhusi et al., 2016b), 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 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 and 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, 0.6 mg/kg, and 1.2 mg/kg). Data from 6 drug TD sessions (doses were counterbalanced daily among subjects) with delays 0 s, 16 s, and 64 s were subjected to data and statistical analyses.

Statistical analyses

The %LL choices were analyzed by mixed ANOVAs with between-subject variable genotype (KO, WT) and within-subject variables stress (baseline and stress) and delay (0 s, 4 s, 16 s, 64 s). The %AUC was analyzed by mixed ANOVAs with between-subject variable genotype and within-subject variable stress. The %LL choices in drug sessions were analyzed by mixed ANOVAs with between-subject variable genotype and within-subject variable drug dose (0, 0.6 mg/kg, 1.2 mg/kg) and delay (0 s, 16 s, 64 s). The %AUC in drug sessions was analyzed by mixed ANOVAs with between-subject variable genotype and within-subject variable drug dose.

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