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Rapid Communication

Dimensional assessment of behavioral changes in the cuprizone short-term exposure model for psychosis

Mari A. Kondo[a,1], Daisuke Fukudome[a,1], Dani R. Smith[b], Michela Gallagher[b], Atsushi Kamiya[a], Akira Sawa[a,*]

[a] Department of Psychiatry, Johns Hopkins University School of Medicine, 600N. Wolfe Street, Baltimore, MD 21287, USA
[b] Neurogenetics and Behavior Center, Department of Psychological and Brain Sciences, The Johns Hopkins University, Baltimore, MD 21218, USA

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ABSTRACT

Recent clinical studies have suggested a role for immune/inflammatory responses in the pathophysiology of psychosis. However, a mechanistic understanding of this process and its application for drug discovery is underdeveloped. Here we assessed our recently developed cuprizone short-term exposure (CSE) mouse model across behavioral domains targeting neurocognitive and neuroaffective systems. We propose that the CSE model may be useful for understanding the mechanism associating inflammation and psychosis, with applications for drug discovery in that context.

Recent studies have suggested a role for immune/inflammatory responses in the pathophysiology of psychosis, including schizophrenia (Hayes et al., 2014; Miller et al., 2011). A popular model for studying schizophrenia is maternal immune activation (MIA) which uses immunological insults such as the viral mimic polyriboinosinic–polyribocytidilic acid [poly(I:C)] or the bacterial endotoxin lipopolysaccharide (LPS), to induce postnatal alterations in the immune/inflammatory response, neurocircuitry, and behavior of the adult offspring (Borrell et al., 2002; Reul et al., 1994; Zuckerman et al., 2003). We sought to develop a higher throughput model in which inflammatory processes underlie neurochemical and behavioral changes relevant to psychosis.

We recently reported that systemic exposure to cuprizone for 1 week could elevate expression of interleukin-6 (IL-6) and gliosis markers [glial fibrillary acidic protein (GFAP) and ionized calcium-binding adaptor molecule 1 (Iba1)] in the hippocampus, frontal cortex, and striatum, leading to behavioral abnormalities, including hypersensitivity to psychostimulants and deficits in some memory tasks (Tezuka et al., 2013). In contrast to prolonged cuprizone exposure (4–8 weeks), which elicits robust demyelination (Matsushima and Morell, 2001; Xu et al., 2011), 1-week exposure to the chemical does not cause overt white matter pathology (Tezuka et al., 2013). Based on the findings we proposed that cuprizone short-term exposure (CSE) may be a promising method to study inflammation-associated psychosis. Since a systematic assessment of behaviors in the CSE model was not included in the original study, here we present a more comprehensive assessment, integrating new data with those recently reported (Tezuka et al., 2013), to assess multiple behavioral domains associated with psychiatric disorders. The results have been organized into eight broad dimensions of neurobehavioral function: motor system, memory, executive function, social cognition, anxiety, depression, psychosis, and information processing.

Eight-week-old C57BL/6J male mice were randomly assigned to two groups. For 1-week, mice were fed either a diet containing 0.2% cuprizone (Sigma–Aldrich), or a control diet consisting of standard chow obtained from Harlan Teklad. Unless otherwise stated, experiments were performed on separate cohorts on the 7th day of diet

Abbreviations: CON, control; CSE, cuprizone short-term exposure; DSM, Diagnostic and Statistical Manual of Mental Disorders; ED, exposure day; FST, forced swim test; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium-binding adaptor molecule 1; IL-6, interleukin-6; LPS, lipopolysaccharide; MIA, maternal immune activation; poly(I:C), polyriboinosinic-polyribocytidilic acid; RDoC, Research Domain Criteria; TST, tail suspension test.

* Corresponding author.
E-mail address: asawa1@jhmi.edu (A. Sawa).
[1] These authors contributed equally to this work.

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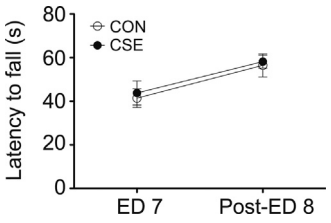

Fig. 1. Motor function in cuprizone short-term exposure (CSE) mice.
CSE mice demonstrated normal motor coordination and motor learning on the rotarod. Assessments were made on two consecutive days [exposure day (ED) 7 and post-ED 8]. Data are presented as mean ± S.E.M. ($n = 8$ for each group). CON, control

exposure (exposure day 7; ED 7). All experimental procedures followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and the Johns Hopkins University Animal Care and Use Guidelines. Data were assessed for normal distribution using the Shapiro–Wilk test, and homogeneity of variance using the *F*-test. Outliers were identified using a Grubbs test with $\alpha = 0.01$. Where assumptions were met, parametric statistical comparisons were performed using a two-tailed unpaired Student's *t*-test, except for the rotarod motor learning, reversal learning test, fear conditioning extinction, social interaction, and amphetamine-induced hyperactivity, which were analyzed with two-way analysis of variance (ANOVA) or two-way repeated measures ANOVA. An α value of $p < 0.05$ was considered statistically significant.

Our previous study reported that CSE mice show no difference in baseline locomotor activity (Tezuka et al., 2013). Because longer-term exposure to cuprizone (5 weeks) results in poor motor coordination (Ye et al., 2013), we first conducted an assessment of motor function using the rotarod test to ensure that motor coordination and motor learning are not confounding other behavioral tasks. There was no significant difference in baseline motor coordination or motor learning between control (CON) mice and CSE mice (Fig. 1). Briefly, mice were placed on a rotarod (Rotamex-5; Columbus Instruments) that accelerated from 4 to 40 rpm over 120 s and the latency to fall onto a platform below was recorded. Motor learning was assessed with four trials per day for two consecutive days (ED 7 and post-exposure day 8).

In our previous study CSE mice displayed decreased preference for the novel object in the novel object recognition test and decreased spontaneous alternation in the Y-maze test, indicating deficits for some types of memory dependent on the medial temporal lobe (Tezuka et al., 2013). To test additional forms of learning and memory, in the current study we used a fear-conditioning procedure to assess associative learning and memory (Tovote et al., 2015). CON and CSE mice showed similar levels of baseline freezing (data not shown). There was no significant difference in freezing between CON mice and CSE mice during tests for the acquisition of learned fear to either the context or associative cue used during training, indicating that retrieval of fear memories is intact in CSE mice (Fig. 2A, top). We also examined extinction of associative learning by testing extinction to the context and cue but found no significant difference in rate of extinction between the groups (Fig. 2A, bottom). Fear conditioning and extinction were conducted as follows. On ED 5, mice were habituated to the training chamber for 10 min. On ED 6, mice were returned to the training chamber and after 120 s, presented with a 20 s tone that co-terminates with a 2 s foot-shock (0.5 mA). The tone-shock pairing presentation was repeated 3 times, with an intertone interval of 10 min. On ED 7, mice were returned to the training chamber and allowed to move freely for 5 min (no presentation of tone or shock). Mice were then placed in a novel context. Following a 120 s acclimation period, the tone is presented for 20 s. The tone presentation is repeated 20 times with an intertone interval of 180 s. Data was analyzed in five blocks of

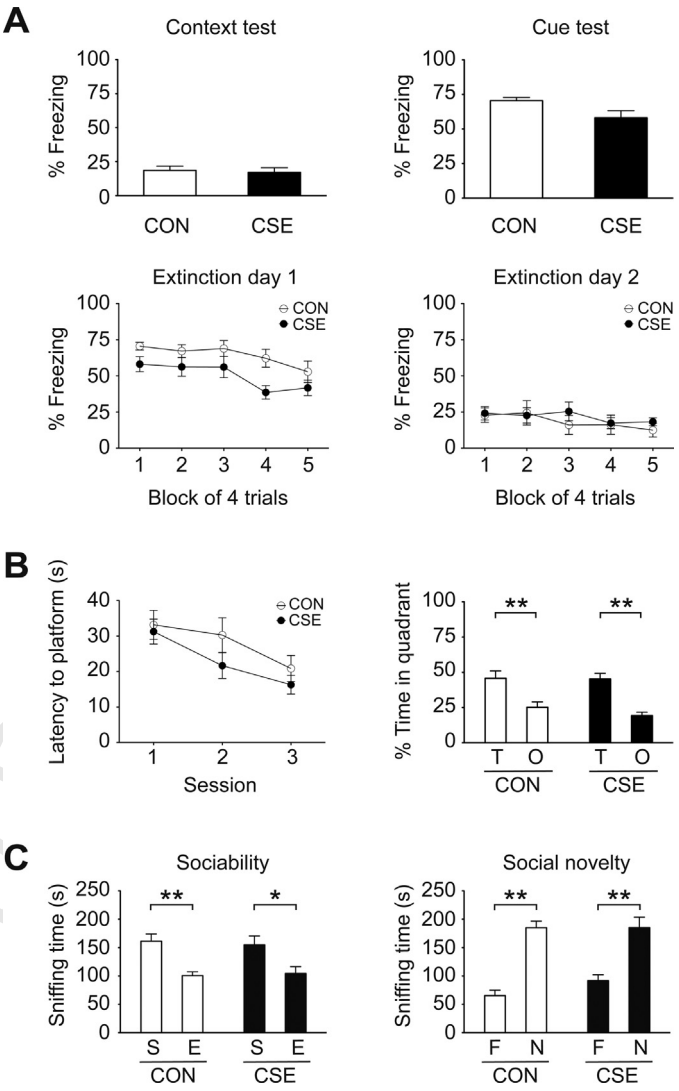

Fig. 2. Memory, executive function, and social cognition in CSE mice.
(A) Fear conditioning: there were no significant differences between CON and CSE mice in freezing behavior during the context and cue tests of fear retrieval (top), or freezing during extinction of fear (bottom; Block 1 of first extinction day corresponds to the cued fear retrieval test, above). **(B)** Reversal learning: CSE mice exhibited normal spatial reversal learning in the Morris water maze based on latency to the hidden platform and time in target quadrant during the probe trial. $^{**}p < 0.01$, as analyzed by 2-way ANOVA with post hoc Bonferroni test (quadrant occupancy $F_{1,42} = 33.11$, $p < 0.0001$). **(C)** Three-chamber social interaction test: both CON and CSE mice preferred sniffing the enclosure with the stranger mouse during the sociability phase and preferred the unfamiliar stranger in the social novelty phase. $^{*}p < 0.05$, $^{**}p < 0.01$, as analyzed by 2-way ANOVA with post hoc Bonferroni test (sociability phase; preference $F_{1,28} = 20.63$, $p < 0.0001$, social novelty phase; preference $F_{1,28} = 68.09$, $p < 0.0001$). Data are presented as mean ± S.E.M. (CON $n = 7$, CSE $n = 8$ for fear conditioning, CON $n = 12$, CSE $n = 11$ for reversal learning, $n = 8$ for each group for social interaction test). T, target; O, opposite; S, stranger; E, empty; F, familiar; and N, novel.

four tone presentations each (the first block on extinction day 1 corresponds to the cued test). On day post-exposure 8, the procedure of ED 7 was repeated.

Behavioral/cognitive flexibility, a neurocognitive function that depends heavily on prefrontal cortical function, was assessed by reversal learning in the water maze. The water maze reversal learning task was designed to test the effect of cuprizone on memory performance and reversal learning. There was no significant difference between CON and CSE mice in latency to reach the hidden platform during the reversal training trials or percent time spent in the target quadrant during the probe trial following reversal