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Rapid Communication

Increased stereotypy in conditional Cxcr4 knockout mice

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ABSTRACT

Chemokines play important roles in the central nervous system, including mediating neuroinflammation and guiding the intracortical migration of interneurons during development. Alteration in parvalbumin-positive interneurons is a key neuropathological hallmark of multiple mental conditions. We recently reported a significant reduction in the expression of CXCL12 in olfactory neurons from sporadic cases with schizophrenia compared with matched controls, suggesting a role for CXCR4/CXCL12 signaling in mental conditions. Thus, we depleted the chemokine receptor Cxcr4 from mice using the parvalbumin-2A-Cre line. The conditional knockout mice exhibited a unique behavioral phenotype involving increased stereotypy. Stereotypy is observed in many psychiatric conditions, including schizophrenia, autism, and dementia. Thus, the *Cxcr4* conditional knockout mice may serve as a model for this symptomatic feature.

Chemotactic cytokines, or chemokines, play several important roles in the central nervous system (Adler et al., 2005; Mithal et al., 2012). They mediate the infiltration of leukocytes into the central nervous system during neuropathology (Jaerve and Muller, 2012) and may also be involved in neurogenesis, neuroprotection, and neurotransmission (Edman et al., 2008; Guyon, 2014). During development, the chemokine CXCL12 (also called stromal cell derived factor-1 or SDF-1) and its receptor CXCR4 guide the tangential intracortical migration of GABAergic interneurons to their correct laminar positions (Stumm et al., 2003; Lopez-Bendito et al., 2008). In addition to its role in the pathology of multiple sclerosis, Alzheimer's disease, and HIV-associated dementia, we have previously reported altered expression of the CXCR4/CXCL12 cascade in olfactory neurons from sporadic schizophrenia patients (Toritsuka et al., 2013).

Cxcr4 is constitutively expressed throughout the brain in neurons, astrocytes, and to a lesser extent microglia (Banisadr et al., 2002). Among cortical interneurons, Cxcr4 is especially critical for the migration of PV-positive interneurons (Zhao et al., 2008; Wang et al., 2010; Meechan et al., 2012). However, the postnatal expression of Cxcr4 in PV neurons may be relatively low (Stumm et al., 2007). As far as we are aware, the influence of CXCR4 on behavior has been addressed only with *Sox1*-Cre;*Cxcr4*$^{fl/fl}$ mice, in which *Cxcr4* is deleted in neural stem and progenitor cells, including populations in the lateral ventricle walls, dentate gyrus, and Purkinje cell layer of the cerebellum. That study focused on the cerebellum and detected abnormal motor behaviors (Huang et al., 2014). In contrast, here we address the influence of *Cxcr4* on a variety of behaviors relevant to multiple dimensions of mental disorders (Cuthbert and Insel, 2013). We thus aim to examine the specific effect of *Cxcr4*-mediated PV-positive neuron deficits on behavior.

We intended to genetically deplete *Cxcr4* in PV-positive neurons by crossing floxed *Cxcr4* mice [B6.129P2-*Cxcr4*^{tm2Yzo}/J mice (Jax 008767)] (Nie et al., 2004) with B6.Cg-*Pvalb*$^{tm1.1(cre)Aibs}$/J mice (Jax 012358) (Madisen et al., 2010). Experiments were performed with homozygous conditional *Cxcr4* knockout males and their wild-type littermate controls. All animal experiments were carried out in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 823) and were approved by the Johns Hopkins IACUC.

We tested adult males (at least 3 months old) in the following sequence of behavioral tests: open field, Y maze, three-chamber sociability, prepulse inhibition, and forced swim. Tests were performed from less to more stressful and approximately 1 week apart to minimize inter-trial interference.

In the open field paradigm, each mouse was allowed to roam freely in a novel open field box (40 cm × 40 cm; San Diego Instruments, San Diego, CA) for 2 h. Horizontal and vertical locomotion and fine (stereotypic) movements were automatically recorded by an infrared activity monitor (San Diego Instruments). Single beam breaks are reported as "counts." PV-*Cxcr4*$^{-/-}$ mice showed hyperlocomotion over the whole 2 h (Fig. 1A, left) and their horizontal activity was higher than that of controls at all time points

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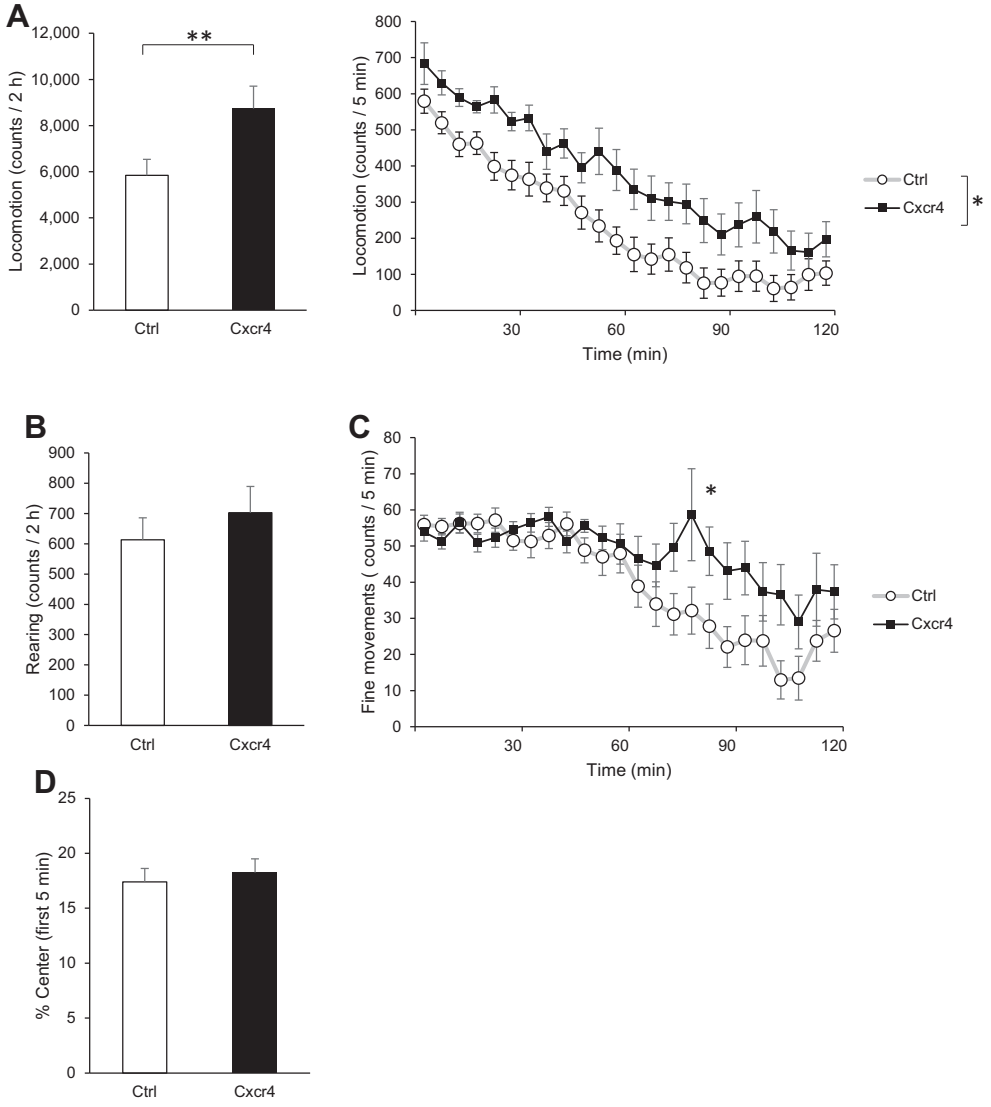

Fig. 1. Behavior of PV-*Cxcr4*$^{-/-}$ mice in a novel open field. (A) Cxcr4 mice were hyperactive in the open field as measured by horizontal locomotion. Left, total counts over the 2 h, $^{**}p < 0.01$. Right, locomotion over time, two-way repeated measures ANOVA showed a significant effect of the genotype: $F(1,26) = 6.26$, $^{*}p < 0.05$. (B) Cxcr4 mice did not differ significantly from control mice in rearing over the 2 h. (C) Cxcr4 mice made more fine/stereotypic movements during the second hour in the open field. Two-way repeated measures ANOVA: significant genotype × time interaction, $F(23,598) = 2.24$, $^{***}p < 0.001$. Bonferroni post hoc analysis indicated a significant difference in the fine movements in the 5 min interval 80–85 min, $^{*}p < 0.05$. (D) Cxcr4 mice did not differ from control mice in percentage time spent in the center of the open field during the first 5 min, suggesting no difference in anxiety.

(Fig. 1A, right). Both PV-*Cxcr4*$^{-/-}$ mice and controls showed normal habituation of horizontal activity throughout the test period. Although vertical movements (rearing) were slightly increased in the PV-*Cxcr4*$^{-/-}$ mice, there was no significant difference from controls (Fig. 1B). PV-*Cxcr4*$^{-/-}$ mice showed increased fine/stereotypic movements compared to controls during the second hour, suggesting abnormally slow habituation (Fig. 1C). Both groups spent a similar amount of time in the anxiogenic center of the open field vs. the safer periphery during the first 5 min (Fig. 1D).

In the Y-maze paradigm, we recorded arm entries for each mouse over a 5 min free exploration period in a Y-shaped maze. Spontaneous alternation was calculated as the percentage of triads of successive arm entries containing entries into all three arms. PV-*Cxcr4*$^{-/-}$ mice had a higher number of arm entries, supporting our conclusion of hyperactivity seen in the open field test (Fig. 2A, left). However, they did not differ from the controls in the percentage of alternations between the three arms of the maze, suggesting normal short-term spatial memory (Fig. 2A, right).

Following the open field and Y-maze, we conducted the three-chamber sociability test. Mice were habituated to the three-chamber apparatus for three consecutive days prior to the experiment by being allowed to freely roam the apparatus for 10 min. The experiment consisted of a 5 min habituation period followed by a 10 min trial to measure sociability. A young, unfamiliar mouse of the same background and sex as the experimental mice was placed in an enclosure in one of the side chambers while the enclosure in the other side chamber was left empty. Mice will normally interact with the enclosure containing the unfamiliar mouse more than the empty enclosure. In contrast to control mice, PV-*Cxcr4*$^{-/-}$ mice did not significantly prefer to interact with the stranger over the empty enclosure, suggesting reduced sociability (Fig. 2B).

We next examined prepulse inhibition of the startle response. Acoustic startle and prepulse inhibition responses were measured in a startle chamber (San Diego Instruments). Each mouse was subjected to six pseudorandomly distributed sets of three trial types: pulse-alone trials, prepulse-pulse trials, and no-stimulus

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