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CD44 is required for spatial memory retention and sensorimotor functions

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Abstract

CD44 is a transmembrane receptor for the glycosaminoglycan hyaluronan, a component of the extracellular matrix. CD44 is expressed by neural stem/progenitor cells, astrocytes, and some neurons but its function in the central nervous system is unknown. To determine the role of CD44 in brain function, we behaviorally analyzed CD44-null (KO) and wild-type (WT) mice. KO mice showed increased activity levels in the light-dark test and a trend towards increased activity in the open field. In addition, KO mice showed impaired hippocampus-dependent spatial memory retention in the probe trial following the first hidden-platform training day in the Morris water maze: WT mice showed spatial memory retention and spent more time in the target quadrant than any other quadrant, while KO mice did not. Although there were no genotype differences in swim speeds during the water maze training sessions with the visible or hidden platform, sensorimotor impairments were seen in other behavioral tests. In the inclined screen and balance beam tests, KO mice moved less than WT mice. In the wire hang test, KO mice also fell off of the wire faster than WT mice. In contrast, there was no genotype difference when emotional learning and memory were assessed in the passive avoidance test. These data support an important role for CD44 in locomotor and sensorimotor functions, and in spatial memory retention.

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Keywords

CD44; mouse; locomotor; sensorimotor; memory

1. Introduction

The CD44 transmembrane glycoprotein has been implicated in the regulation of numerous cellular activities including proliferation, differentiation and cell migration [1]. Multiple forms of CD44 are generated by both alternative mRNA splicing and post-translational modifications that include N- and O-linked glycosylation, and the addition of heparin sulfate and chondroitin sulfate side chains. CD44 functions as a receptor for hyaluronan, a glycosaminoglycan found in most extracellular matrices, including perineuronal nets and to a lesser extent in white matter [2, 3]. The contribution of CD44 to nervous system development and how CD44 influences nervous system function is not understood.

In the central nervous system (CNS), CD44 is weakly expressed by astrocytes [4], Müller glial cells and their progenitors in the retina [5], and Bergmann glia in the cerebellum [6]. Some subpopulations of neurons are also at least transiently CD44-positive, including Purkinje and granule neurons in the cerebellum [6, 7]. CD44 is also expressed by a number of progenitor cell populations including oligodendrocyte progenitor cells [6, 8]. Chronically elevated CD44 inhibits oligodednrocyte differentiation and prevents myelination, an effect that likely depends on interactions with hyaluronan [8, 9]. Following a variety of insults including ischemia [10], inflammatory demyelination [11], and traumatic brain and spinal cord injuries [12, 13], CD44 expression is elevated in astrocytes, microglia, oligodendrocyte progenitors and some neurons. Following seizures, CD44 is elevated in the hippocampus and may contribute to mossy fiber sprouting [14]. Hyaluronan accumulates in CNS lesions where CD44 is elevated, especially in areas where there is reactive astrogliosis [8], and has been implicated in blocking oligodendrocyte maturation and remyelination in inflammatory demyelinating disease [8].

CD44 is also expressed by neural stem cells [6, 15]. In the subgranular zone (SGZ) of the hippocampal denate gyrus, most neural stem cells differentiate into dentate granule cells that migrate into the inner granule cell layer then functionally integrate into hippocampal neural circuits. Mice with reduced numbers of adult-born dentate granule cells have cognitive dysfunction characterized by deficiencies in forming hippocampus-dependent long-term spatial memory and impaired performance in contextual fear extinction [16]. Given that CD44 is expressed in numerous CNS cell types including neural stem cells implicated in spatial memory, we postulated that CD44 is required for a wide range of neurological functions. We therefore compared the performance of wild type (WT) and CD44-null (KO) mice in a battery of sensory, motor, and cognitive assays.

2. Methods

2.1. Animals

Six-month old female CD44-null (KO) mice, generated as described [17], and matched C57BL/6J wild-type (WT) mice were used. All mice were bred in the rodent animal care

facility at the Oregon National Primate Research Center. The mice were kept on 12:12 hr light-dark schedule (lights on at 6 AM) with chow (PicoLab Rodent Diet 20, #5053; PMI Nutrition International, St. Louis, MO) and water given ad libitum. All the experiments reported here were conducted in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee at the Oregon Health & Science University.

2.2 Behavioral Analysis

WT and KO mice were tested in the open field, elevated plus maze, elevated zero maze, and light-dark tests in week 1; the Morris water maze in week 2; then the rotorod, wire hang, inclined screen and balanced beam tests in week 3. Finally, emotional learning and memory were assessed using the passive avoidance test. Performance in the open field, elevated zero maze, elevated plus maze, water maze and passive avoidance tests was analyzed as previously described by us [18]. The rotorod, inclined screen, and wire hanging tests were performed as previously described [19]. In the light-dark test, mice were placed in the open-field enclosure containing black plastic inserts covering from the sides and the top 50% of the open field (Hamilton-Kinder, Poway, CA). A single opening in the wall of the insert adjacent to the open area allowed the mice to enter or exit the more anxiety-provoking light area of the maze. Active times, distance moved and rest time were recorded for a single 10-min session. Breaks in the photo beams were used to calculate path length, active times and rest time in the open and closed compartments of the enclosure. Mice with increased levels of anxiety in this enclosure spend less time in the lighted compartment of the maze.

For the balance beam test, mice were placed in the middle of a horizontal beam (88.5 cm long, 1.8 cm in diameter, 30.5 cm high). Total distance moved and mean velocity of movement in two trials of 2 min each were recorded using a Noldus Instruments' EthoVision video tracking system.

2.3 Statistical Analyses

The data are expressed as means ± SEM. Differences among means were evaluated by ANOVA, followed by Student's *t*-test or Tukey-Kramer posthoc tests, as indicated, using GraphPad Prism (San Diego, CA) and SPSS (Chicago, IL) software. For all analyses, the null hypothesis was rejected at the 0.05 level.

3. Results

Given the cell types that express CD44 in the brain, loss of CD44 might be expected to have significant effects on neurological function. We therefore performed a battery of behavioral and cognitive tests over three weeks to determine if CD44-null mice display cognitive or other neurological disturbances. Mice were tested for exploratory behavior and measures of anxiety in the open field, elevated plus maze, elevated zero maze, and light-dark tests in the first week. Then mice were then tested for spatial learning and memory in the Morris water maze in the second week and for sensorimotor function using the rotorod, wire hang, inclined screen and balanced beam tests in week three. Finally, emotional learning and memory were assessed using the passive avoidance test.

In the open field, there was a trend towards increased activity levels in the KO mice as compared to controls but it did not reach significance (pokes into the periphery: $t_{(14)}$ = 1.990, p = 0.0665; pokes into the center: $t_{(14)}$ = 1.863, p = 0.0836; total pokes: $t_{(14)}$ = 2.121, p = 0.0523; and entries into the center: $t_{(14)}$ = 2.009, p = 0.0642). There were no genotype differences in percent time spent in the more anxiety-provoking center of the open field ($t_{(14)}$ = 1.767, p = 0.0990). In the elevated plus maze and elevated zero maze, there were no genotype differences in activity levels or measures of anxiety (Table 1). However, consistent with the open field data, in the light-dark test, activity levels were higher in KO mice than in controls ($t_{(14)}$ = 4.120, p = 0.001; pokes into the dark compartment: $t_{(14)}$ = 3.473, p = 0.0037; total pokes: $t_{(14)}$ = 2.121, p = 0.0523; and entries into the light compartment: $t_{(14)}$ = 3.941, p = 0.0015).

In the Morris water maze, the visible and hidden platform learning curves were first analyzed using time to reach the platform location as a performance measure (Fig. 1A). CD44-null mice demonstrated no alterations in swim speeds during the visible (WT: 10.95 ± 1.15 cm/s; KO: 12.82 ± 1.04 cm/s) or hidden (WT: 15.00 ± 0.60 cm/s; KO: 13.00 ± 1.31 cm/s) platform training sessions. Both genotypes learned to locate the visible platform location (effect of session: F(3,42) = 22.977, p < 0.0001) but there was a genotype x session interaction (F(3,42) = 3.859, p = 0.016). This interaction was driven by a trend towards a genotype difference in the second visible platform training session (F(1,14) = 4.377, p = 0.055). As the hidden platform data were not normally distributed, they were log transformed first. Both genotypes learned to locate the hidden platform location (effect of session: F(5,70) = 3.994, p = 0.003) with no significant effect of genotype. Learning curves were also analyzed using distance moved as a performance measure (Fig. 1B). Both genotypes learned to locate the visible (effect of session: F(3,42) = 12.464, p < 0.0001) and hidden (effect of session: F(5,70) = 3.994, p = 0.003) platform locations, also with no significant effect of genotype. However, there was a profound genotype difference when spatial memory retention was assessed in the probe trial following the first hidden platform training day. While WT mice showed spatial memory retention and spent more time in the target quadrant than any other quadrant, KO mice did not (Fig. 1C). This impairment could be overcome with additional training. KO mice did show spatial memory retention in the probe trial following the second hidden platform training day (Fig. 1D).

Rotorod performance assesses a combination of balance and muscle strength. There were no significant genotype differences in rotorod performance between WT and KO mice (Table 1). However, in the inclined screen test, KO mice moved less than WT mice (Fig. 2A, $t_{(14)}$ = 3.454, p = 0.0039). In the balance beam test, KO mice also moved less than WT mice (Fig. 2B, $t_{(14)}$ = 4.022, p < 0.0014). In the wire hang test, KO mice fell off the wire faster than WT mice (Fig. 2C, $t_{(14)}$ = 3.818, p < 0.002). These data and our analyses of swimming speeds in the Morris water maze indicate that although motor function is generally intact in the KO mice, they have some sensorimotor deficits.

The passive avoidance test has been used to study learning and memory in the context of a stressful experience. In contrast to the spatial memory deficits we observed in the Morris water maze, there were no genotype differences in trials to criterion in passive avoidance

learning or memory retention 24 hrs later (Table 1). These data suggest that the cognitive phenotypes of KO mice are limited to spatial memory retention deficits.

4. Discussion

We are the first group to identify sensorimotor and memory retention phenotypes in CD44 null animals. Mice lacking CD44 are viable, fertile, and do not exhibit gross physical or behavioral abnormalities [17]. A small number of previous studies examined CD44 null mice in some sensory and motor assays, including in the context of brain injury studies [e.g. 20, 21], but failed to find any deficits. We find that CD44 null animals have deficits in one behavioral test and one cognitive test: the light-dark test, which measures anxiety based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviors of rodents in response to mild stressors, and the Morris water maze, which measures spatial learning and memory. Interestingly, there were no differences between genotypes in the passive avoidance test, a test of emotional learning and memory, or in the elevated or plus maze tests. Similarly, while we found that the KO mice had deficits in some sensorimotor tests (the inclined screen, balanced beam, and wire hang), they did not have deficits in swimming speed or in rotorod performance. These findings suggest that the behavioral, cognitive and sensorimotor deficits we observed in the KO mice are not due to widespread CNS abnormalities but rather subtle changes in brain development and function.

The cognitive phenotype of the CD44-null mice is consistent with disturbances in hippocampal function. Combined with the fact that CD44 is expressed by neural stem cells [6, 15] and that neural stem cells undergoing neurogenesis are implicated in hippocampal learning and memory [16], our data raise the possibility that CD44 may influence adult hippocampal neurogenesis. Some components of contextual fear conditioning involve hippocampal neurogenesis [22] and spatial memory in the Morris water maze is also sensitive to alterations in adult neurogenesis [23]. Given the contributions of CD44 and hyaluronan to regulating cell proliferation and differentiation, it will be interesting to test how their disruption influences cells within neural stem cell niches.

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- CD44 null mice exhibited memory retention deficits in the Morris water maze task
- CD44 null mice exhibited sensorimotor deficits in some tests
- CD44 is necessary for normal nervous system development and function

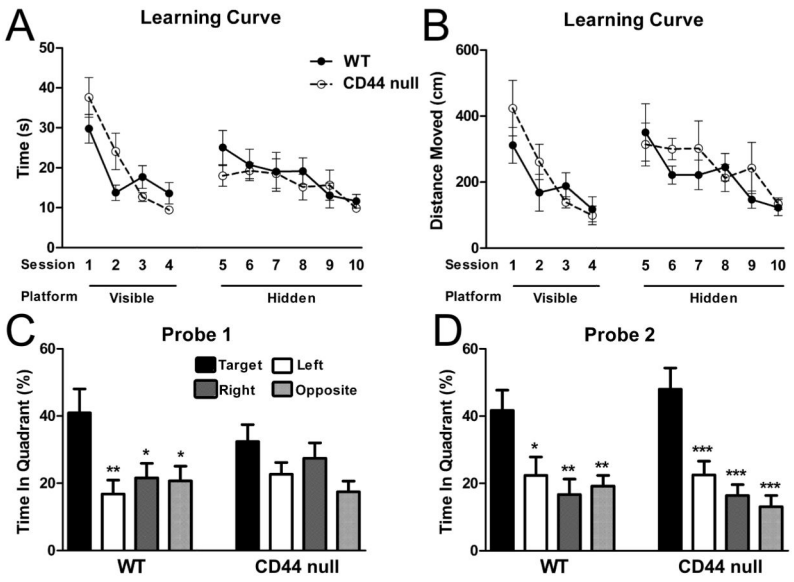

Fig. 1.

CD44 mutant mice have hippocampal memory deficits. **A.** Water maze learning curves of wild type (WT) and CD44 null mice using time to reach the platform location (latency) as performance measure. **B.** Water maze learning curves analyzed using distance moved as performance measure. **C.** Spatial memory retention in the probe trial following the first day of hidden platform training. The mean time spent in each quadrant of the water maze was analyzed and the time spent in the quadrant containing the platform during the hidden platform training (Target quadrant) was compared to that in any other quadrant. $*p < 0.04$; $**p < 0.01$. **D.** Analysis of mean time spent in each quadrant of the Morris water maze by WT as compared to CD44 null mice during the second probe trial. $*p < 0.04$; $**p < 0.02$; $***p < 0.01$.

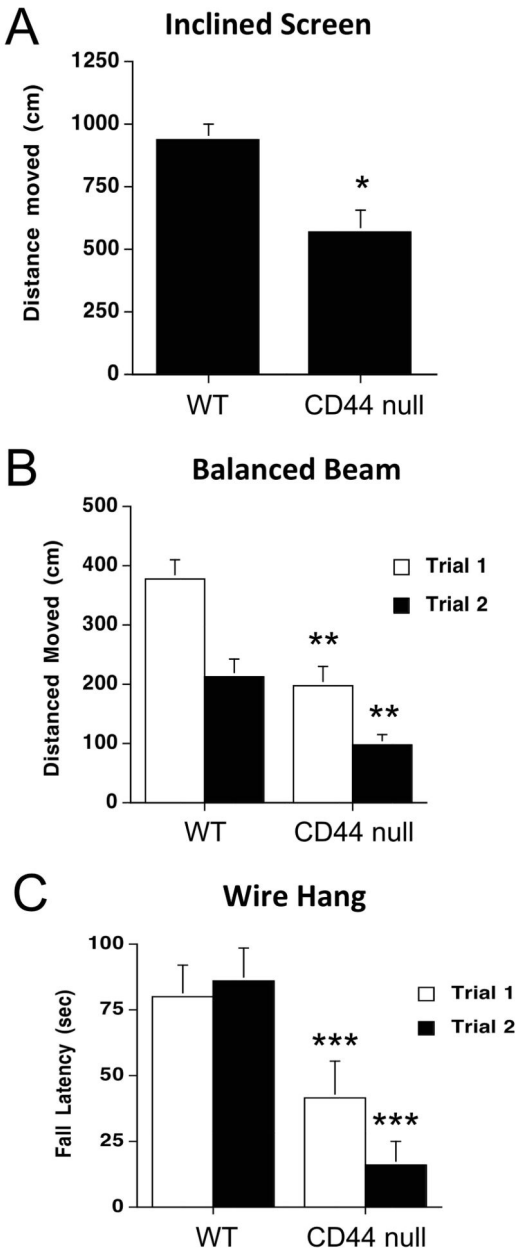

Fig. 2.

A. CD44 null mice show reduced activity in the inclined screen test compared to wild type (WT) mice. There was a significant effect of genotype, $t_{(14)} = 3.454$, $*p = 0.0039$. **B.** CD44 null mice move less than wild type mice in the balanced beam test, $t_{(14)} = 4.022$, $**p < 0.0014$. **C.** CD44 null mice fall off the wire faster than wild type mice in the wire hang test., $t_{(14)} = 3.818$, $***p < 0.002$).

Table 1

Elevated plus maze, elevated zero maze, rotorod performance, and passive avoidance learning and memory of CD44 KO and WT female mice

	WT (*n* = 8)	**KO (*n* = 8)**
Elevated Plus Maze		
% Time in Open Arms	20.7 ± 8.9	13.2 ± 1.4
Distance moved (cm)	2,906 ± 362	2,970 ± 214
Elevated Zero Maze		
% Time in Open Areas	7.6 ± 2.8	5.6 ± 1.9
Distance moved (cm)	1,294 ± 220	1,463 ± 68
Rotorod Performance		
Mean Fall Latency (sec)	40.0 ± 3.7	46.9 ± 4.0
Passive Avoidance		
Trials to criterion Day 1	1.9 ± 0.3	2.3 ± 0.3
Latency to Enter Day 2 (s)[1]	300 ± 0	300 ± 0

[1] None of the mice re-entered the dark compartment.