Published in final edited form as:

J Inherit Metab Dis. 2014 January ; 37(1): 63–68. doi:10.1007/s10545-013-9619-x.

Female mice heterozygous for creatine transporter deficiency show moderate cognitive deficits

Emily R. Hautman, Amanda N. Kokenge, Kenea C. Udobi, Michael T. Williams, Charles V. Vorhees, Matthew R. Skelton

Division of Neurology, MLC 7044 Cincinnati Children's Research Foundation, 3333 Burnet Ave., Cincinnati, OH 45229-3039, USA

Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH 45229, USA

Abstract

Creatine transporter (CrT) deficiency (CTD) is an X-linked disorder characterized by intellectual disability and speech delay. There have been reports that show female carriers have clinical symptoms. We have created CrT knockout ($\text{CrT}^{-/y}$) mice in which males show severe cognitive deficits as a model of this disorder. The purpose of this study was to examine if the female carrier mice show cognitive deficits. Reductions in Cr levels as well as CrT transcript were observed in the brains of the female $\text{CrT}^{+/-}$ mice. $\text{CrT}^{+/-}$ mice show hyperactivity and increased latency to find the cued platform in the Morris water maze (MWM). $\text{CrT}^{+/-}$ female mice showed deficits in MWM hidden platform acquisition but not during reversal testing. Memory deficits on probe trials were observed during both phases. Novel object recognition memory and contextual fear memory were not affected in female $\text{CrT}^{+/-}$ mice. Female $\text{CrT}^{+/-}$ mice show moderate cognitive deficits, which is consistent with some of the human data. Female $\text{CrT}^{+/-}$ mice could prove to be beneficial in further understanding CTD and testing therapeutic approaches.

Introduction

Creatine (Cr) is a guanidino compound that is responsible for replenishing ATP levels in cells with a high-energy demand (Wyss and Kaddurah-Daouk 2000). The loss of Cr, through either the lack of synthesis or transport of Cr has a significant effect on the central nervous system. Cr deficiency syndromes (CDS) lead to severe intellectual disability (ID) with speech delay (Schulze 2003). In addition, there is an increase in the incidence of epilepsy. The most prevalent CDS is caused by mutations of the Cr transporter (CrT) gene. According to prevalence estimates, CrT deficiency (CTD) is another relatively common cause of X-linked intellectual disability (ID) (Rosenberg et al 2004; Lion-Francois et al 2006). Clinical hallmarks of CTD include the aforementioned ID, lack of brain Cr on magnetic resonance spectra, and a high urinary Cr/creatinine (Crn) ratio (Cecil et al 2001; DeGrauw et al 2002; 2003). It should be noted that elevated urinary Cr/Crn is not a conclusive diagnostic and

genetic screening is required to confirm diagnosis. Unlike deficits of the Cr synthesis enzymes arginine:glycine amidinotransferase (AGAT) and guanidinoacetate N-methyltransferase (GAMT), CTD cannot be treated by supplementing with Cr (Cecil et al 2001; Poo-Arguelles et al 2006).

In order to investigate this disorder, we generated CrT knockout ($\text{CrT}^{-/y}$) mice (Skelton et al 2011). The male $\text{CrT}^{-/y}$ mice have no brain Cr and show severe learning and memory deficits (Skelton et al 2011). Similar deficits have been observed in a brain specific CrT knockout mouse (Kurosawa et al 2012), suggesting that the deficits seen are not attributable to motor deficits. These data suggest that the CrT deficient mouse is an accurate model of CTD.

While the primary focus of CTD research is on the affected males it is important to understand the effect of this mutation on female carriers. Female carriers show a wide range of cognitive function with a majority of those examined show some cognitive deficits that range from mild learning deficits to significant intellectual disability (DeGrauw et al 2002; van de Kamp et al 2011a). Additionally, CTD carriers show reductions in brain Cr levels (van de Kamp et al 2011a). A case of epilepsy in a CTD carrier female was successfully treated by administration of Cr and the Cr precursors L-arginine and glycine, though cognitive impairments remained throughout treatment (Mercimek-Mahmutoglu et al 2010). This would suggest that female carriers who show symptoms might benefit from treatments developed for CTD. In order to determine if the heterozygous female CrT carrier ($\text{CrT}^{+/-}$) mouse is a model of the affected human females, we tested female $\text{CrT}^{+/-}$ mice in behavioral tests that were affected in the $\text{CrT}^{-/y}$ mice.

Methods

Generation of CrT knockout mice

The CrT knockout mice were created as was previously described (Skelton et al 2011). Testing was done on wildtype ($\text{CrT}^{+/+}$) and heterozygous ($\text{CrT}^{+/-}$) females. No $\text{CrT}^{-/-}$ females were used since no human $\text{CrT}^{-/-}$ females exist. Further, to date, $\text{CrT}^{-/y}$ mice have failed to breed. The vivarium is fully accredited by AAALAC and all protocols were approved by the Institutional Animal Care and Use Committee.

Behavioral testing

Forty ($n=21 \text{ CrT}^{+/+}$, $19 \text{ CrT}^{+/-}$) adult female mice were used in this experiment. Mice were weighed on the first and last day of testing. The testing order consisted of: locomotor behavior (1 day), Morris water maze (MWM) cued (6 days) and hidden platform trials (14 days), novel object recognition (5 days), and conditioned fear (3 days). To minimize circadian effects, animals were tested during at the same time interval day (1000–1400 h). Animals were tested in two separate cohorts consisting of approximately equal numbers.

Locomotor activity

The task assesses spontaneous locomotor activity and exploration (Brooks and Dunnett 2009). Animals were tested in an automated activity chamber, Photobeam Activity System

(PAS) – Open Field (San Diego Instruments, San Diego, CA) for 1 h. Locomotor chambers were 41cm (W) × 41cm (D) × 38 cm (H) with 16 photobeams in both the X and Y planes. Photobeams were spaced 2.5 cm apart. Animals were placed in the chamber and allowed to explore for 1 h. The dependent measure was number of photobeam interruptions.

Morris water maze

The MWM is a test of spatial learning and reference memory (Vorhees and Williams 2006). Animals were tested as described previously (Skelton et al 2003; Schaefer et al 2009). Prior to hidden platform testing, cued platform training (cued learning) was conducted for 6 days as a test of proximal cue learning. During this phase, a 10 cm diameter platform with an orange ball mounted above it on a brass rod was placed in a predetermined quadrant. On the first day, six trials (90 s) were administered with the platform and start in the same position to teach the basic test requirement. Next, mice were given two trials per day on subsequent days with the start and platform positions randomized.

Hidden platform trials were conducted in two phases consisting of four trials per day for 6 days for animals to learn the location of the hidden platform followed by a single probe trial (no platform) on day 7 (Vorhees and Williams 2006). The tank was 122 cm in diameter filled with room temperature (21 ± 1 °C) water. Platforms were 10 cm for acquisition and 7 cm for reversal (located in the opposite quadrant). The time limit per trial was 90 s with an intertrial interval of ~10 s. Mice that failed to reach the platform within the trial limit were placed on the platform. Performance was measured using AnyMaze software (Stoelting Company, Wood Dale, IL).

Novel object recognition

Novel object recognition (NOR) is a test of short-term memory (Clark et al 2000). Mice were tested in the Anybox apparatus (Stoelting Company, Wood Dale, IL). Mice were habituated to an empty arena (41 × 41 cm) for 2 days (10 min/day). Mice were habituated to two identical objects in the chamber (10 min/day) for the following 2 days. On the fifth day, animals were presented with two new identical objects until 30 s of cumulative observation time between the objects was obtained. One hour later, memory was tested by presenting the animal with an identical copy of one of the familiar objects along with a novel object and tested identically to the familiarization trial. Time exploring the object was defined as entry into a 2 cm zone around the object. Performance was measured using AnyMaze software.

Conditioned fear

Cued and contextual fear were assessed as described previously with modification (Peters et al 2010). On day 1, mice were placed in the chamber for 10 min before exposure to three tone-footshock pairings (82 dB, 2 kHz, 30 s on/off cycle). Each pairing consisted of the 30 s tone accompanied during the last second by a scrambled footshock (0.3 mA for 1 s) delivered through the floor. On the second day, animals were returned to the chamber with no tone or shock presented as a test of contextual fear. The next day, animals were placed in the chamber with a novel lattice floor. Following 3 min acclimation, the tone was presented continuously and freezing behavior was scored. Freezeframe software and Coulbourn test

chambers were used (Coulbourn Instruments, Allentown, PA). Percent time freezing was analyzed. Due to equipment malfunction, data for cohort 2 was not captured.

Real-time qPCR and creatine determination

Following behavioral testing a subset of animals were sacrificed by decapitation. Brains were divided into hemispheres and flash frozen. RNA was isolated from one hemisphere using RNABee® Reagent (Amsbio, Lake Forest, CA). Reverse transcription was performed using Superscript III reagent (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. qPCR was performed using the CrT (SLC6A8) Taqman Gene Expression Assay with Taqman Universal PCR Master Mix on an AB7500 Real Time PCR System from Applied Biosystems according to the manufacturer's instructions. PCR reaction (95 °C for 10 s, 60 °C for 60 s) was performed for 40 cycles with fluorescent reading following each cycle. CrT transcript was calculated from the linear phase of the reaction using AB7500 software. 18s RNA was used to normalize the reaction.

The second hemisphere was used for Cr determination. Cr was determined using a fluorescent assay as described previously (Conn 1960). Briefly, samples were homogenized in NP-40 buffer (150 mM NaCl, 1.0 % NP-40, 50 mM Tris), boiled for 5 min and centrifuged for 10 min at 12,000×g. The supernatant was added (1:2) to 0.3 N BaOH and 5 % ZnSO₄. Following centrifugation (3 min at 12,000×g), samples were mixed (2:1) with 1.0 % ninhydrin and 10 % KOH. Samples were incubated protected from light for 8–10 min and read on a SpectraMax M3 spectrophotometer (Molecular Devices, Sunnyvale, CA) at 525 nm.

Statistical analysis

Novel object, conditioned fear, body weight, Cr and CrT levels were analyzed using 2-tailed *t*-tests for independent samples. Equality of variance was tested and the appropriate *t*-test utilized. For measures with repeated measures (locomotor activity and MWM), repeated measures ANOVA was used (Proc MIXED; SAS Institute, Cary, N.C.). In order to determine if any effects in MWM cued platform carried over to the hidden platform, planned comparisons of each day of testing were performed using slice ANOVAs on each day. Sample sizes were determined based on power analysis based on data for male CrT mice.

Results

Body weight

In order to determine effects of CrT haploinsufficiency on body weight, animals were weighed on the first and the last day of behavioral testing. No differences in body weight were observed between genotypes.

Locomotor activity

Spontaneous locomotor activity was examined in female CrT^{+/-} mice as male CrT^{-/-} mice show a transient hypoactivity compared to WT. Female CrT^{+/-} mice showed an increase in activity compared to controls ($F(1,66.6)=18.07$, $p<0.001$; Fig. 1).

Morris water maze

During the cued platform trials female CrT^{+/-} mice had longer latencies ($F(1,60.7)=21.32$, $p<0.001$) to reach the platform compared with female CrT^{+/+} mice (Fig. 2, top). However, no differences between the groups were observed on the final day of cued platform testing ($p<0.20$). On hidden platform trials, latency, path length, and cumulative distance from the platform were analyzed as learning variables. These variables were very similar; therefore path length is presented. In the acquisition phase of the MWM, female CrT^{+/-} mice showed an increase in path length compared with CrT^{+/+} ($F(1,49.8)=9.53$, $p<0.01$; Fig. 2, middle). During the probe trial, female CrT^{+/-} mice had a higher average distance from the platform site ($t_{37}=-2.54$, $p<0.05$, Fig. 2 bottom). Similar effects were observed for time and distance travelled in the target quadrant. During reversal, there were no differences ($F(1,51.5)=0.42$; Fig. 2, middle) in the path length. During the reversal probe trial, female CrT^{+/-} mice showed an increase in average distance to the platform site ($t_{37}=-2.23$, $p<0.05$; Fig. 2, bottom), with time and distance in the target quadrant showing similar results. No changes in swim speed were observed (LS Mean $\pm$ SEM (m/s): CrT^{+/+}=0.166 $\pm$ 0.007; CrT^{+/-}=0.145 $\pm$ 0.008, ns) in the hidden platform phase of the MWM. No interactions of test day with genotype were observed in any phases of MWM testing.

Novel object recognition

There was no difference between the percent of time spent exploring the novel object compared to the familiar object between (CrT^{+/-} and CrT^{+/+} female mice ($t_{37}=0.22$, ns, Mean $\pm$ SEM: CrT^{+/+}=57.2 $\pm$ 1.8 %; CrT^{+/-}=56.5 $\pm$ 2.8 %).

Conditioned fear

There was no difference in freezing between CrT^{+/+} mice and CrT^{+/-} female mice during contextual ($t(18)=-0.29$, $p<0.80$) or cued retention ($t(18)=-0.47$, $p<0.70$, Fig. 3).

Creatine and CrT levels

Brain Cr levels were decreased in female CrT^{+/-} mice ($t(17)=7.2$, $p<0.001$, Fig. 4) as were CrT transcript levels ($t(15)=2.5$, $p<0.05$) compared to CrT^{+/+}.

Discussion

Genes that have been implicated in intellectual disability are three times more frequent on the X-chromosome than on autosomes (Zechner et al 2001). With a few exceptions such as Rett syndrome, males are more affected by the loss of these genes compared with females. Mutations in the CrT show a typical X-linked inheritance with males severely affected. However, it has been reported that some females have learning disabilities (DeGrauw et al 2003). If CrT carriers have inadequate transfer of Cr into the brain, they could benefit from treatments developed for affected males. We generated CrT^{-/-} mice in order to further understand this disorder and to test potential treatments (Skelton et al 2011). We hypothesized that since the male mice appear to be a high fidelity model of CTD that female carrier mice would show effects similar to those reported in human CTD carriers. This study tested whether the female CrT^{+/-} mice model the learning disability seen in human female carriers. Female CrT^{+/-} mice showed a reduction in brain Cr levels that corresponded to a

similar reduction seen in transcription of the CrT gene. The reduction of brain Cr is consistent with human female carrier data (Cecil et al 2003).

Female CrT^{+/-} mice have some spatial learning and memory deficits however show no object recognition or fear conditioning memory. This is evidenced by deficits during the acquisition phase, but not during the reversal phase of the MWM. Female CrT^{+/-} mice show deficits during probe trials that reflect spatial memory impairments. The deficit observed in the female mice is not as severe as in male CrT^{-/-} mice, which is consistent with CTD patients. Male CrT^{-/-} mice showed severe deficits during both phases of MWM testing. Further, the magnitude of the deficit during the acquisition phase was larger in the males compared to the females (100 % vs 30 % increase in path length compared with control). With male mice having severe cognitive impairments and females having mild to moderate deficits, it appears that the corresponding CrT KO mice show a high degree of similarity to the affected and carrier CTD patients. This makes the CrT KO mouse ideal for drug development and further understanding mechanisms underlying this disorder.

Similar to male CrT^{-/-} mice, female CrT^{+/-} mice showed a deficit reaching the cued platform during MWM testing. The increase in latency is not likely due to changes in swim speed; as there were no differences in swim speed observed during hidden platform trials. It has been shown that pretraining in the MWM using the cued platform will eliminate non-spatial deficits in the MWM (Cain et al 1996). By the final day of cued testing, there were no differences in latency between the CrT^{+/-} mice and the CrT^{+/+} mice. This would suggest that pretraining the mice in the cued platform of the maze may have prevented non-spatial deficits that would have exaggerated the hidden platform deficits. It could be that the cued platform deficits are evidence of motivational problems; however, this is unlikely due to the CrT^{+/-} mice successfully learned each phase of the task. More likely is that CrT^{+/-} mice have a deficit in task-specific skills such as learning that there is a platform, that it is not near the perimeter and that remaining on the platform leads to removal from the tank.

The type of deficit observed in CrT^{+/-} mice could provide insight into areas of the brain that are sensitive to reductions in Cr. It appears that the hippocampus is preferentially impaired from the loss of Cr. The hippocampus is sensitive to metabolic insults such as hypoglycemia (Yamada et al 2004) and is preferentially damaged during hypoxic injury (Hossmann 1999). It is well known that efficient performance in the MWM requires an intact hippocampus (Morris et al 1982). The amygdala is predominate in conditioned fear memory (Parkes and Westbrook 2010). Using lesion studies it has been shown that greater damage to the hippocampus is required to generate object recognition deficits than are required for MWM reference memory deficits (Clark et al 2000; Broadbent et al 2004). Taken together, it appears that the hippocampus is the most sensitive to the loss of Cr. Future studies will be designed to test how the hippocampus is damaged in these animals compared with the male CrT^{-/-} mice. The findings from this study show that the CrT^{+/-} female mice have the potential to provide valuable insight into this disorder.

It is possible that X-inactivation plays a role in the results observed. The nature of X-inactivation in the brain is not well understood. While X-inactivation did not prevent the transport of Cr into the brain, the reductions in Cr could be likely due to skewed activation

of the CrT. The maternal X-chromosome, which carries the deleted CrT, is preferentially expressed in glutamatergic neurons of the hippocampus and prefrontal cortex (Gregg et al 2010). This could explain the differences seen; however, there could be transcriptional activation of the inactive X-chromosome in these cells as well. Female CrT^{+/-} mice show hyperactivity that was not observed in the male CrT^{+/-} mice. This difference in activity could be due to X-inactivation. It is possible that an imbalance in neurotransmitter systems that control locomotor behavior has been created by selective loss of Cr. Both male and female CTD patients have been diagnosed with ADHD (Mercimek-Mahmutoglu et al 2010; van de Kamp et al 2011b). It is possible that the phenotype observed in the male mice are due to some unknown effect that does not match the human phenotype, such as the reductions in body size of the CrT^{-/-} mice. It would be of interest to examine the role of X-inactivation on the cognitive deficits observed in the female mice. The recent validation of a CrT-specific antibody (Wong et al 2012) will allow for the further determination of CrT expression in the brain of CrT^{+/-} mice.

In conclusion, the data presented in this study show that female CrT^{+/-} mice have reductions in Cr and selective cognitive deficits, supporting the value of CrT knockout mice as an appropriate model of CTD. In addition, the female CrT^{+/-} mouse will be a valuable tool for understanding the mechanisms underlying CTD.

References

- Broadbent NJ, Squire LR, Clark RE (2004) Spatial memory, recognition memory, and the hippocampus. *Proc Natl Acad Sci U S A* 101(40):14515–14520 [PubMed: 15452348]
- Brooks SP, Dunnett SB (2009) Tests to assess motor phenotype in mice: a user's guide. *Nat Rev Neurosci* 10(7):519–529 [PubMed: 19513088]
- Cain DP, Saucier D, Hall J, Hargreaves EL, Boon F (1996) Detailed behavioral analysis of water maze acquisition under APV or CNQX: contribution of sensorimotor disturbances to drug-induced acquisition deficits. *Behav Neurosci* 110(1):86–102 [PubMed: 8652076]
- Cecil KM, DeGrauw TJ, Salomons GS, Jakobs C, Egelhoff JC, Clark JF (2003) Magnetic resonance spectroscopy in a 9-day-old heterozygous female child with creatine transporter deficiency. *J Comput Assist Tomogr* 27(1):44–47 [PubMed: 12544242]
- Cecil KM, Salomons GS, Ball WS Jr et al. (2001) Irreversible brain creatine deficiency with elevated serum and urine creatine: a creatine transporter defect? *Ann Neurol* 49(3):401–404 [PubMed: 11261517]
- Clark RE, Zola SM, Squire LR (2000) Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci* 20(23):8853–8860 [PubMed: 11102494]
- Conn RB Jr (1960) Fluorimetric determination of creatine. *Clin Chem* 6:537–548 [PubMed: 13695044]
- DeGrauw TJ, Cecil KM, Byars AW, Salomons GS, Ball WS, Jakobs C (2003) The clinical syndrome of creatine transporter deficiency. *Mol Cell Biochem* 244(1–2):45–48 [PubMed: 12701808]
- DeGrauw TJ, Salomons GS, Cecil KM et al. (2002) Congenital creatine transporter deficiency. *Neuropediatrics* 33(5):232–238 [PubMed: 12536364]
- Gregg C, Zhang J, Butler JE, Haig D, Dulac C (2010) Sex-specific parent-of-origin allelic expression in the mouse brain. *Science* 329(5992):682–685 [PubMed: 20616234]
- Hossmann KA (1999) The hypoxic brain. Insights from ischemia research. *Adv exp med biol* 474:155–169 [PubMed: 10635000]
- Kurosawa Y, DeGrauw TJ, Lindquist DM et al. (2012) Cyclocreatine treatment improves cognition in mice with creatine transporter deficiency. *J Clin Invest* 122(8):2837–46 [PubMed: 22751104]

- Lion-Francois L, Cheillan D, Pitelet G et al. (2006) High frequency of creatine deficiency syndromes in patients with unexplained mental retardation. *Neurology* 67(9):1713–1714 [PubMed: 17101918]
- Mercimek-Mahmutoglu S, Connolly MB, Poskitt KJ et al. (2010) Treatment of intractable epilepsy in a female with SLC6A8 deficiency. *Mol Genet Metab* 101(4):409–412 [PubMed: 20846889]
- Morris RG, Garrud P, Rawlins JN, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. *Nature* 297(5868):681–683 [PubMed: 7088155]
- Parkes SL, Westbrook RF (2010) The basolateral amygdala is critical for the acquisition and extinction of associations between a neutral stimulus and a learned danger signal but not between two neutral stimuli. *J Neurosci* 30(38):12608–12618 [PubMed: 20861367]
- Peters J, Dieppa-Perea LM, Melendez LM, Quirk GJ (2010) Induction of fear extinction with hippocampal-infralimbic BDNF. *Science* 328(5983):1288–1290 [PubMed: 20522777]
- Poo-Arguelles P, Arias A, Vilaseca MA et al. (2006) X-Linked creatine transporter deficiency in two patients with severe mental retardation and autism. *J Inherit Metab Dis* 29(1):220–223 [PubMed: 16601898]
- Rosenberg EH, Almeida LS, Kleefstra T et al. (2004) High prevalence of SLC6A8 deficiency in X-linked mental retardation. *Am J Hum Genet* 75(1):97–105 [PubMed: 15154114]
- Schaefer TL, Vorhees CV, Williams MT (2009) Mouse plasmacytoma-expressed transcript 1 knock out induced 5HT disruption results in a lack of cognitive deficits and an anxiety phenotype complicated by hypoactivity and defensiveness. *Neuroscience* 164(4):1431–1443 [PubMed: 19786075]
- Schulze A (2003) Creatine deficiency syndromes. *Mol Cell Biochem* 244(1–2):143–150 [PubMed: 12701824]
- Skelton MR, Ponniah S, Wang DZ, Doetschman T, Vorhees CV, Pallen CJ (2003) Protein tyrosine phosphatase alpha (PTP alpha) knockout mice show deficits in Morris water maze learning, decreased locomotor activity, and decreases in anxiety. *Brain res* 984(1–2):1–10 [PubMed: 12932834]
- Skelton MR, Schaefer TL, Graham DL et al. (2011) Creatine transporter (CrT; Slc6a8) knockout mice as a model of human CrT deficiency. *PLoS One* 6(1):e16187 [PubMed: 21249153]
- van de Kamp JM, Mancini GM, Pouwels PJ et al. (2011a) Clinical features and X-inactivation in females heterozygous for creatine transporter defect. *Clin Genet* 79(3):264–272 [PubMed: 20528887]
- van de Kamp JM, Pouwels PJ, Aarsen FK et al. (2011b) Long-term follow-up and treatment in nine boys with X-linked creatine transporter defect. *J Inherit Metab Dis* 35(1):141–9 [PubMed: 21556832]
- Vorhees CV, Williams MT (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc* 1(2):848–858 [PubMed: 17406317]
- Wong AC, Velamoor S, Skelton MR, Thorne PR, Vlajkovic SM (2012) Expression and distribution of creatine transporter and creatine kinase (brain isoform) in developing and mature rat cochlear tissues. *Histochem cell biol* 137(5):599–613 [PubMed: 22307408]
- Wyss M, Kaddurah-Daouk R (2000) Creatine and creatinine metabolism. *Physiol Rev* 80(3):1107–1213 [PubMed: 10893433]
- Yamada KA, Rensing N, Izumi Y et al (2004) Repetitive hypoglycemia in young rats impairs hippocampal long-term potentiation. *Pediatr Res* 55(3):372–379 [PubMed: 14681492]
- Zechner U, Wilda M, Kehr-Sawatzki H, Vogel W, Fundele R, Hameister H (2001) A high density of X-linked genes for general cognitive ability: a run-away process shaping human evolution? *Trends genet*: TIG 17(12):697–701 [PubMed: 11718922]

Fig. 1. Hyperactivity observed in CrT^{+/-} mice. Mice were examined for 1h in the locomotor test. CrT^{+/-} mice show an overall increase in activity compared to WT counterparts Data are presented as mean $\pm$ SEM. * $p < 0.05$

Fig. 2.

CrT^{+/-} mice show spatial learning and memory deficits. (*Top*) CrT^{+/-} mice show increased latency to the platform during cued platform trials. Mice were tested in the cued platform for 6 days. Data are collapsed across days. (*Middle*) CrT^{+/-} mice show increased in path lengths during the acquisition phase while no differences were observed in the reversal phase of the MWM. Mice are tested for 6 days during each phase. Data are collapsed across days. (*Bottom*) During the probe trial given on day 7 of each phase, CrT^{+/+} and CrT^{+/-} mice show increased average distance from the platform. Data are presented as mean $\pm$ SEM. * $p < 0.05$

Fig. 3.

$CrT^{+/-}$ mice show normal conditioned and contextual fear memories. Mice were exposed to a tone-footshock pairing. One day following exposure, mice were re-exposed to the floor that administered the shock. On the next day, mice were exposed to the tone that was associated with the shock. Freezing behavior is an indicator of association of the stimulus with a negative event. Data are presented as mean $\pm$ SEM

Fig. 4. Reductions of Cr and CrT levels in the brain of CrT^{+/-} mice. Cr levels were determined in one hemisphere of a group of mice that underwent behavioral testing. The other hemisphere was used to determine CrT transcript levels using RT-qPCR