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Social isolation increased ADAR1 expressions leading to cognitive deficits of mice

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A lot of literature show that social isolation stress could be a key reason that leads to cognitive deficits for both humans and rodent models; however, the detailed mechanisms are still not clear completely. ADAR1 (Adenosine deaminase acting on RNA) is an enzyme involved in RNA editing that has a close relation to cognitive function. We hypothesize that social isolation stress may impact the expression of ADAR1, leading to cognitive deficits. To prove our hypothesis, we evaluated the cognition ability of the mice isolated for different durations (2, 4, and 8 weeks) using object recognition and object location tests; we also measured ADAR1 expressions in hippocampus and cortex using immunohistochemistry and western blot. Our study showed that social isolation stress significantly induced spatial and non-spatial cognition deficits. In addition, social isolation significantly increased both the immuno reactivity and protein expressions of ADAR1 in the hippocampus and frontal cortex. Furthermore, we found that adolescent re-socialization recovered not only the cognition deficits but also the increased ADAR1 protein expression in hippocampus and the increased number of ADAR1 positive cells in frontal cortex of the isolated mice. In conclusion, social isolation stress significantly increased ADAR1 expressions in the hippocampus and cortex, leading to cognitive deficits.
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Key words: social isolation; cognitive ability; ADAR1

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Social isolation is a kind of psychosocial stressor (O’Keefe et al., 2014; Barratt et al., 2011) and is defined as an objective reduced social contact (Khodaie et al., 2015). Investigations on both humans (Grant et al., 2009) and rodent models (Fone and Porkess, 2008) indicate that social isolation can lead to cognitive dysfunction (Yusufishaq & Rosenkranz, 2013). Many studies have explored the related mechanisms on social isolation stress-induced cognitive deficits, including the alterations of glutamate receptors (Araki et al., 2014), neurotransmitter systems (Baarendse et al., 2013), ion channel (Quan et al., 2010), Neural cell adhesion molecule (Pereda-Pérez et al., 2013), and the function of hypothalamo-pituitary-adrenal (HPA) axis (Sandstrom & Hart, 2005).

ADAR1 (Gene ID:ADAR) is belonging to ADAR family, which catalyzes the process of conversing adenosine to inosine (A-to-I) in pre-mRNA. A-to-I RNA editing takes place in 5-hydroxytryptamine 2C receptor (5-HT2CR), B subunit of α-amin-3-hydroxy-5-methyl-4-isoxazoleproionic acid (AMPA) receptor, α-3 subunit of the gamma-aminobutyric acid (GABA) A receptor, and KV1.1 potassium channel. ADAR family is well known to be implicated in regulation of cognitive ability (Bombail et al., 2014) with a wide distribution in the center nervous system (Yang JH et al; 2003). So far, no literature has reported whether ADAR1 expression changes in social isolation stress-induced cognitive deficits. We hypothesize that social isolation stress may impact the expression of ADAR1, leading to cognitive deficits. To prove the hypothesis, post-weaning Kunming (KM) mice were isolated for 2, 4 and 8 weeks, respectively. Object recognition and object location tests were used to evaluate the cognition of the isolated mice. Moreover, ADAR1 expressions in frontal cortex and hippocampus were measured using immunohistochemistry and western blot. Furthermore,
the changes of ADAR expressions in hippocampus and frontal cortex for behavioral deficits recovery were evaluated for the mice with re-socialization.

Materials and methods

Animals

Male KM mice (15±5g) at the age of 21 days old were purchased from Laboratory Center of Dalian Medical University (ID: 0003746). They were housed in the plastic cage (Beijing Heli Technology Development Co. Ltd. China, 290×178×160 mm) with 5 mice for each cage reared in conditions of 21±1°C, 55±5% humidity and 12-h rhythm of day/night cycle. The mice were fed with food and water ad libitum. They were divided into 7 groups randomly with 10 mice for each group, as shown in Figure 1A. The mice were housed individually for 2, 4 and 8 weeks respectively, and labeled as SI 2W group (isolation for 2 weeks), SI 4W group (isolation for 4 weeks), and SI 8W group (isolation for 8 weeks). In addition, recovery of behavioral deficits by re-socialization (rearing with their littermates at adolescence period) was also examined, and labeled as SI 2WR group (re-socialization for 2 weeks after isolation for 2 weeks). Control groups were age matched group-housed mice (C 2W, C 4W, and C 8W). All experimental procedures were approved by the Administration of Affairs Concerning Experimental Animals.

Methods

Object Recognition Test (ORT)

The minor modified protocol (Figure 1B) as published in the literature (Või kar et al., 2005) was used in this study to measure the non-spatial cognitive ability. The test was performed in the behavior procedure room using the behavior observation apparatus (XR-XX117, Shanghai Xinruan Technology Co. Ltd. China). The observed box was 40×40×35 cm. Woody block A and B (Black, 5×5×5 cm) and block C (Black and white pattern, 5×5×5 cm) were used as recognition objects, with each object heavy enough to be stable. The process for ORT included both sample trial and test trial. Firstly, the mouse was trained to be in the empty box for 5 minutes to acclimate to the new environment. Then,
during the sample trial, object A and object B were placed oppositely with a distance of 14 cm between them. The exploring time for each mouse was 5 minutes. The mouse was placed in the middle of two objects in the test. After the sample trial, the mouse returned back to its home cage for 4 hours. During the test trial, object B was replaced with object C that was a novel one for the mouse. After each trial, the objects and the box were cleaned in order to avoid olfactory cues using 75% ethanol. The behavior of the mouse was recorded by videotapes.

The discrimination index (DI) was calculated as follows: \( DI = (Tn - Tf)/(Tf + Tn) \): Tn and Tf were the time taken for the mouse to recognize a new and familiar object, respectively.

Object Location Test (OLT)

The performance of OLT was similar with that of ORT (Võikar et al., 2005). This test is used to measure the spatial cognitive ability. Object A and B used in this study were the same as those used in the ORT. The acclimation was carried out in the same way as in the ORT. In the sample trial, object A and B were put in the same location as that in the ORT. The mouse explored each object for 5 minutes and then was returned to the home cage for 4 hours. After that, in the test trial, object B was moved to the opposite direction toward the object A, then the mouse was left to explore object A and object B with a novel location for 5 minutes. The behavior of the mouse was recorded in the same way as that used in ORT.

Immunohistochemistry Staining

The mice were injected with 4% chloral hydrate for anesthesia (400 mg/kg, i.p.), followed by perfused transcardially with 1% and 4% paraformaldehyde respectively. The mice brains were put into 4% paraformaldehyde for 24 hours, and then in phosphate buffer saline (PBS) with 20% sucrose at 4°C overnight. Then the slices cut by microtome-cryostat were 16 μm thick. The selected slices rinsed with PBS for 3 times with 10 min for each time were incubated in 1% bovine serum albumin. After that, the slices were covered with ADAR1-Ab (P110) (1:100, Proteintech, USA) and put at 4°C overnight. Then the sections were rinsed
with PBS for 3 times with 10 minutes for each time. Then the slices were cultivated with biotinylated second antibody (ZSJQ-BIO Company, China) at room temperature for 1.5 hours. After rinsed with PBS, the slices were treated with avidin-biotin complex at room temperature for 2 hours. Subsequently, diaminobenzidine was used to treat the slices for coloration. Negative control slices were incubated with PBS without the antibody, followed by stained with 1% thionine (sigma), a kind of nissl staining for marking the neurons.

Western Blot

The frontal cortex and hippocampus of the mice were examined in this study. Protein extraction of frontal cortex and hippocampus was performed according to the protocol of extraction kit (Keygen Biotech, China). The tissues were homogenized in 1 ml cold lysis buffer containing 10 μl phosphates inhibitor, 1 μl protease inhibitors, and 5 μl phenylmethylsulfonyl fluoride (PMSF). Then the samples were centrifuged at 10,000 g for 5 minutes at 4℃, after that, the supernatant was removed to a fresh tube and was stored at -80℃. The protein content was assessed using a BCA protein assay kit (Keygen Biotech, China). 30 mg protein was added in 7.5% sodium dodecyl sulfate-polyacrylamide (SDS) gel, and then, the protein was transferred into nitrocellulose membranes. ADAR1-Ab (1:1000, Proteintech, USA) was assessed by western blot analysis. GADPH-Ab (1:1000, Beyotime Company, China) was used as an internal control. Horseradish peroxidase-coupled rabbit anti-goat (1:5000, ZSJQ-BIO Company, China) and anti-mouse (1:5000, ZSJQ-BIO Company China) antibodies were used. The grey values of ADAR1 and GADPH protein expressions were detected by BIO-RAD (Hercules, CA) gel analysis software.

Statistical Analysis

All data expressed as the mean ± SEM were analyzed statistically with Graph-Pad Prism (GraphPad Software Inc.) and SPSS 21.0. T test was used to analyze the variance between social isolation groups and control groups; ANOVA was used to analyze the differences among groups. P<0.05 was considered statistically significance.
Results

Decreased DI of cognition by social isolation and its recovery by re-socialization

In the ORT and OLT, the DI of the mice isolated for 2, 4, and 8 weeks respectively significantly decreased as compared to the age matched group-housed mice (Figure 2A). The decreased DI showed the decreased spatial and non-spatial cognition ability for the isolated mice. Moreover, no obvious difference was observed between the re-socialization group (S12WR) and the control group (C4W). This result (Figure 2B) suggested that social isolation stress induced abnormal spatial and non-spatial cognition abilities, which however, could be recovered by re-socialization.

Increased ADAR1 immunoreactivity of social isolated mice and its recovery by re-socialization

As seen arrow 1 and 2 in Figure 3B, ADAR1 (p110) was expressed in only a part of neurons. Compared to the control, the immunoreactivity of ADAR1 (p110) significantly increased in hippocampus and frontal cortex of the mice isolated for 2, 4, and 8 weeks, respectively (Figure 3B and 3C). As shown in Figure 4A, the numbers of ADAR1 (p110) immunoreactive positive cells in frontal cortex of the mice isolated for 2, 4, and 8 weeks were significantly increased, compared to age matched group-housed mice. However, the numbers of ADAR1 (p110) immunoreactive positive cells of CA1 and hilus were only increased obviously in the mice isolated for 2 and 8 weeks, compared to age matched group-housed mice (Figure 4B and 4C). Furthermore, re-socialization mice recovered the increased ADAR1 immunoreactivity in both frontal cortex and hippocampus of the isolated mice (Figure 4D).

Increased ADAR1 protein expressions of social isolated mice and its recovery by re-socialization.

Western blot results of ADAR1 (p110) were consistent with those of immunohistochemistry staining results mostly. The protein expressions of ADAR1 (p110) significantly increased in not only the frontal cortex but also the hippocampus of the mice isolated for 2, 4, and 8 weeks, compared to age matched group-housed mice as shown in Figure 5 and Figure 6A and B. The
above results suggested that social isolation increased ADAR1 (p110) protein expressions.

In addition, the protein expression of ADAR1 (p110) of SI2WR in hippocampus was no difference with age matched group-housed mice. The results suggested that re-socialization recovered the social isolation stress-induced increased expression of ADAR1 (p110) in hippocampus. Actually, we were also focused on the alterations of ADAR1 (p150) and ADAR2 protein expressions, however, no obvious results were found on the basis of very weak protein expressions in all groups (data not shown).

Discussion

More and more studies demonstrate that social isolation stress causes the abnormal cognition ability in human beings (Shankar et al., 2013). Social engagement including enough social connections and participation in social activities can prevent cognitive decline (Green et al., 2008; Crooks et al., 2008). Moreover, Behavior studies in social isolation rodent models (Yusufishaq et al., 2013) have proved that social isolation leads to cognition deficits (Benner et al., 2014), including reversal-learning impairment in Wistar rats (Quan et al., 2010) and impaired fear conditioning in C57BL/6J and DBA/2 strains (Voïkar et al., 2005). Our study showed that the isolated KM mice showed spatial and non-spatial cognitive deficits as shown in OLT and ORT results. The similar results were reported by Khodaie et al when the rats were used in their study (Khodaie et al., 2015).

So far, it has been widely believed that cognition decline resulted from social isolation stress is associated with the hippocampus and frontal cortex related neuron circuits. Social isolation can damage frontal cortex and hippocampus of animals (Djordjevic et al., 2010) leading to abnormal function of neurotransmission (Shao S et al., 2014), synaptic plasticity (Pereda-Pérez et al., 2013), apoptosis (Khodaie et al., 2015), potassium ion channel currents change (Quan MN et al., 2010), the accumulation of oxidative products (Djordjevic et al., 2010), regeneration (Pereda-Pérez et al., 2013), and the dysfunction of HPA axis (Sandstrom & Hart, 2005). However, the mechanisms of cognitive deficits created by social isolation stress and the recovery from the re-socialization are not very clear. Our study
showed that social isolation stress impacted the expressions of ADAR1, leading to cognitive deficits. Furthermore, we found that re-socialization could recover cognitive deficits and increased ADAR1 (p110) expressions for the isolated mice.

**ADARs expressions characters**

ADAR1 is one of ADAR family and catalyzes the conversion process of adenosine to inosine (A-to-I) in post transcription level (Buechel et al., 2014). Three ADARs have been described in mammalian cells (ADARs 1-3), ADARs highly express in the nervous system and lay in the nuclear of the neuron chiefly (Chen et al., 2000). The expression level of ADAR1 mRNA is constant in the development, whereas ADAR2 expression level increases markedly (Chen et al., 2000). ADAR3 is only expressed in the brain as a regulatory role inhibiting the activities of ADAR1 and ADAR2. There are two predominant isoforms including p110 and p150 for ADAR1 (Figure 7). p150 is composed of multiple RNA-binding motifs (21, 24), Zα and Zβ (two copies of a Z-DNA-binding motif) in the N-terminal region of the protein, the constitutive p110 lacks the N-terminal 295 amino acids of the Z-binding motif. p110 and p150 are generated through transcription by alternative promoters (George et al., 2005).

Because both prefrontal cortex and hippocampus are cognitive-related brain areas and are vulnerable to social isolation stress (Buechel et al., 2014), we were focused on these two brain areas in this study. For morphology level, we found that ADAR1 (p110) immunoreactivity was predominantly detected in the pyramidal cell layer of the frontal cortex and hippocampus in both control and social isolation mice, in the meanwhile, the number of detectable ADAR1 (p110) immunoreactivity positive cells significantly increased in the social isolation stress groups, compared to age matched group-housed mice. In frontal cortex, the ADAR1 (p110) immunoreactivity positive cells displayed almost all layers from molecular layer to multiform layer. On the basis of the closed role of pyramidal cells in the cognition ability (Elston et al., 2001), we counted ADAR1 (p110) positive immunoreactivity cells in layer 5. Our findings showed that the numbers of ADAR1 (p110) positive immunoreactivity cells in both frontal cortex and hippocampus significantly increased in the isolated mice,
compared to those in age matched group-housed control mice. In hippocampus, we also found that ADAR1 (p110) positive cells distributed widely in CA1, dentate gyrus and hilus, especially in the pyramidal cells layer. For protein level, our findings showed that ADAR1 (p110) protein expression obviously increased in both frontal cortex and hippocampus of the mice isolated for 2, 4, and 8 weeks, compared to the control mice. However, ADAR1 (p150) protein expressions were hard to be detectable in both control and social isolation groups because of relatively low expressions (data not shown). That is in agreement with the reported that ADAR1 (p150) expression is extremely low in the brain (George et al., 2005).

The function of morphological distribution character of ADAR1 in the hippocampus and frontal cortex in the social isolation stress is still unknown. The deletion and mutation of ADAR gene influence the biological phenotype exceedingly, including ataxia, seizure, and neuron degeneration. ADAR deficient mice exhibit defects in nervous system and decreased tolerance to stress (Tseng et al., 2013). All of above published reports combined with our findings supported that ADAR1 got involved in modifying social isolation stress-induced cognitive deficits.

**The role of ADAR1 (p110) in adolescent recovery of cognition deficits induced by isolation**

Because that re-socialization is a therapeutic procedure to recover the isolation-induced aberrations in locomotor reactivity, pain sensitivity and anxiety-like behavior (Maisonnette et al. 1993), we also set and analyzed re-socialization group for evaluating the role of ADAR1 in behavioral deficits recovery. Interestingly, we found that both cognitive dysfunction and increased ADAR1 (p110) protein expression in hippocampus were recovered by re-socialization in adolescence. Those results may be related to the strong plasticity in the adolescence, because that environment stress produces much more strong and lasting effects on the behavior and neurophysiology character in young animals than those in adult animals (Forbes & Dahl, 2005). Our findings suggested that adolescent re-socialization was considered to be an efficient cognitive enhancer capable to compensate for increased expression of ADAR1 caused by isolation.
Conclusions

Our findings were summarized that social isolation resulted to spatial and non-spatial cognitive deficits, moreover, ADAR1 (p110) expressions increased in hippocampus and frontal cortex of the isolated mice, furthermore, adolescent re-socialization recovered not only the cognitive deficits but also the increased protein expression in hippocampus and the increased number of ADAR1 positive cells in frontal cortex of the isolated mice. In the future, our study will be focused on understanding how ADARs family members maintain their homeostasis for regulating the RNA editing in the cognitive dysfunction caused by social isolation stress, which are the basis of the studies on social environment and body-mind healthy in human beings.

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References


Figure Legends

Figure 1: Treatment of divided mice groups with isolation and re-socialization followed by evaluating cognition behavior of the treated mice

A: The mice were housed individually for 2, 4, and 8 weeks respectively, and labeled as SI 2W group (isolation for 2 weeks), SI 4W group (isolation for 4 weeks) and SI 8W group (isolation for 8 weeks). In addition, recovery of behavioral deficits by re-socialization (rearing with their littermates at adolescence period) was also examined, and labeled as SI 2WR group (re-socialization for 2 weeks after isolation for 2 weeks). Control groups were the age matched group-housed mice (C 2W, C 4W, and C 8W).

B: Objection Recognition Test

The diagram shows the apparatus and recognition objects used in this test. Woody block A and B (Black, 5×5×5 cm) and block C (Black and white pattern, 5×5×5 cm) were used as recognition objects. Objects A and B were identical. The process for ORT included both sample trial and test trial. During the sample trial, object A and object B were placed oppositely with a distance of 14 cm between them. In the test trial, object B was replaced by a novel one, object C.

C: Objection Location Test

The apparatus and objects A and B were the same ones used in the ORT. In the sample trial, objects A and B were put in the same location in the ORT. In the test trial, object B was moved to be located at the opposite direction toward the object A.

Figure 2: Decreased DI of cognition by social isolation and its recovery by re-socialization

A: Decreased DI of spatial and non-spatial cognition by social isolation

2 weeks social isolation (SI2W) resulted in decreased discrimination index (DI) compared to the control group (C2W). Similarly, 4 and 8 weeks social isolation also showed decreased DI
(SI4W vs. C4W and SI8W vs. C8W).

Data is presented as mean ± SEM (n=10/group). *P < 0.05 (C2W vs. SI2W; C4W vs. SI4W; C8W vs. SI 8W).

B: Recovery of decreased DI of Spatial and non-spatial cognition by re-socialization

Re-socialization (SI2WR) recovered the decreased DI of isolated mice (SI 4W).

Figure 3: Increased ADAR1 immunoreactivity in frontal cortex and hippocampus of social isolated mice and its recovery by re-socialization

ADAR1 (p110) immunoreactivity was predominantly detected in the pyramidal cell layer of the frontal cortex and hippocampus in both control and social isolation mice, in the meanwhile, the numbers of detectable ADAR1 immunoreactivity positive cells significantly increased in the social isolation stress groups as shown in Fig 3 B and C, compared to age matched group-housed control mice. In frontal cortex, the ADAR1 immunoreactivity positive cells displayed almost all layers from molecular layer to multiform layer. In hippocampus, the wide distribution of ADAR1 positive cells existed in CA1, dentate gyrus, and hilus, especially in the pyramidal cells. Adolescent re-socialization recovered the increased ADAR1 immunoreactivity in frontal cortex of the isolated mice (Fig 4D).

A: The brain areas were analyzed on the basis of mice brain atlas of Paxinos and Watson (1997). Black boxes represented the brain areas magnified and presented as following figure B and C. Scale bar = 50 μ m.

B and C: Double staining was performed using 1% thionine for marking neurons and anti-ADAR1-Ab (P110) for marking ADAR1 (p110) immunoreactive positive cells. Arrow 1 was pointed at the ADAR1 (p110) immunoreactive positive neurons. Interestingly, not all neurons expressed ADAR1 (p110), as seen in arrow 2 and glia cells also expressed ADAR1 (p110), as seen in arrow 3.

Figure 4: Increased ADAR1 immunoreactive positive cells in frontal cortex and hippocampus of the isolated mice in statistical analysis
(A) The numbers of ADAR1 (p110) positive cells in frontal cortex (Fr) significantly increased in the mice isolated for 2, 4, and 8 weeks, compared to age matched group-housed control mice (C2W vs. SI2W, C4W vs. SI4W, C8W vs. SI8W).

(B) The number of ADAR1 (p110) positive cells in CA1 significantly increased in the mice isolated for 2 and 8 weeks, compared to the group house control mice (C2W vs SI2W, C8W vs SI8W).

(C) The numbers of ADAR1 (p110) positive cells in hilus significantly increased in the mice isolated for 2 and 8 weeks, compared to the group house control mice (C2W vs. SI2W, C8W vs. SI8W).

(D) Re-socialization recovered the increased number of ADAR1 (p110) positive cells in frontal cortex of isolated mice (SI 4W vs. SI2WR).

The numbers of ADAR1-immunoreactive positive cells were counted in the sequential cutting sections. The sections analyzed in frontal cortex were from Bregma 1.18mm for 4 sections, and the sections analyzed in hippocampus were from Bregma -2.18mm for 4 sections (16 μ m per section). The brain areas analyzed were focused on the internal pyramidal cell layer 5 of frontal cortex, and the brain areas analyzed were focused on hilus and CA1 of hippocampus. The square analyzed was 10000 μ m².

Data were expressed as the mean ± SEM and were analyzed by two-way ANOVA followed by Tukey’s post hoc testing (n=5/group).

*P<0.05 (C2W vs. SI2W, C4W vs. SI4W, C8W vs. SI8W).

#P<0.05 (SI4W vs. SI2WR).

Figure 5: Increased ADAR1 protein expressions in frontal cortex and hippocampus of isolated mice and its recovery by re-socialization.

The protein expressions of ADAR1 (p110) significantly increased in the social isolation stress groups as shown in Fig 5, compared to age matched group-housed control mice. The detailed
results were that ADAR1 (p110) protein expressions increased in both frontal cortex and hippocampus of the mice isolated for 2, 4, and 8 weeks, in the meanwhile, re-socialization mice recovered the increased ADAR1 (p110) protein expression in the hippocampus of the isolated mice. The analyzation was shown in the following Figure 6.

Figure 6: Increased ADAR1 protein expressions in frontal cortex and hippocampus of isolated mice and its recovery by re-socialization in statistical analysis

The ADAR1 (p110) protein expressions increased in both frontal cortex and hippocampus of the mice isolated for 2, 4, and 8 weeks, in the meanwhile, re-socialization mice recovered the increased ADAR1 (p110) protein expression of the isolated mice in hippocampus. Interestingly, the ADAR1 (p110) protein expressions in both frontal cortex and hippocampus showed an age-dependent manner, which can be seen that ADAR1 (p110) protein expressions of 11 weeks and 7 weeks old mice were less than those of 5 weeks old mice.

A: The ADAR1 (p110) protein expressions of frontal cortex significantly increased in the mice isolated for 2, 4 and 8 weeks, compared to age matched group-housed control mice (SI2W vs. C2W, SI4W vs. C4W and SI8W vs. C8W). Moreover, the ADAR1 (p110) protein expressions of 11 weeks and 7 weeks old mice were less than those of 5 weeks old mice (C2W vs. C4W and C2W vs. C8W).

B: The ADAR1 (p110) protein expressions of hippocampus significantly increased in the mice isolated for 2, 4, and 8 weeks, compared to age matched group-housed control mice (SI2W vs. C2W, SI4W vs. C4W and SI8W vs. C8W). Moreover, the ADAR1 (p110) protein expressions of 11 weeks and 7 weeks old mice were less than those of 5 weeks old mice (C2W vs. C4W and C2W vs. C8W) in hippocampus.

C: Re-socialization did not recover the increased ADAR1 (p110) protein expression of the isolated mice in frontal cortex.

D: Re-socialization mice recovered the increased ADAR1 (p110) protein expression of the isolated mice in hippocampus to normal level (no different between C4W and SI2WR).
The optical density ratio of ADAR1 (p110) divided by GADPH was measured. The data was expressed as the mean ± SEM and analyzed by two-way ANOVA followed by Tukey’s post hoc testing.

*P<0.05 (C2W vs. SI2W, C4W vs. SI4W, C8W vs. SI8W).

^P<0.05 (SI4W vs. SI2WR).

Figure 7: **Diagram for isoforms p110 and p150 of ADAR1**

There are two predominant isoforms including p110 and p150 for ADAR1. p150 is composed of multiple RNA-binding motifs, Zα and Zβ (two copies of a Z-DNA-binding motif) in the N-terminal region of the protein, the constitutive p110 lacks the N-terminal 295 amino acids of Z-binding motif.
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