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Social Behavior Assay in Mouse Model of Autism  
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**Social Behavior Assay in Mouse  
Model of Autism Spectrum Disorder**

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## **Abstract**

# **Social Behavior Assay in Mouse Model of Autism Spectrum Disorder**

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Autism spectrum disorder (ASD) is very heterogeneous disease that symptoms widely vary as its name suggests and exact etiology is not yet found. Clinical diagnosing criteria of ASD are defined thoroughly by behavioral phenotype (American Psychiatric Association, 2013) because ASD has no specific biomarker or imaging-marker (Buxbaum and Hof, 2012). Therefore, investigating ASD with animal models also has to rely largely on behavioral assays.

Researchers have been developing mouse models of psychiatric diseases, including ASD, to provide testing ground for investigating molecular mechanism of the disease and to find curing drugs. However, developing appropriate behavioral test method is another challenge since many laboratories find it hard to reproduce the same behavioral results even though they use the same mouse line and behavioral test scheme (Crabbe et al., 1999; Wahlsten et al., 2003). Reliable behavioral experimental method is especially important for ASD study because with no biomarker, behavioral phenotype would work as final gate for confirming applied treatments, drugs etc.

In this study, we utilized three-chamber apparatus and pup retrieval test scheme to assess C57BL/6 mice first and then assess established ASD mouse models, CD38 knockout (CD38KO) mice and Shank2 knockout (Shank2KO) mice. C57BL/6 mouse line shows high level of social characteristics and widely used as a genetic background for many mutant mouse lines, which make them as a good comparison group. Both CD38KO and Shank2KO mice have genetic background of C57BL/6 and their autism-like behavior are reported in previous studies (Jin et al., 2007; Won et al., 2012).

CD38KO mice exhibited social behavior deficit in three-chamber test compared to their wild type (WT) littermates and to C57BL/6. Shank2KO mice showed tendency of impairment in pup retrieval test compared to their WT littermates and to C57BL/6 mice.

*Keywords : Social behavior, autism spectrum disorder, ASD, three-chamber test, pup retrieval test*

*Student number : 2012-20318*

# Introduction

## *Autism Spectrum Disorders*

Autism spectrum disorder (ASD) is a neuro-developmental psychiatric disease initially described as a distinct disorder by Leo Kanner in 1943. As ASD is very heterogeneous disease, showing different levels, severity, spectrum of symptoms, the clinical definition of ASD has changed and reformulated several times through history. Most up-to-date definition of ASD is by the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) and it describes ASD in two broad types of impairment. i) Impairment in social communication and interaction, ii) restricted repetitive patterns of behaviors, interests or activities (American Psychiatric Association, 2013). ASD patients are diagnosed in childhood as early as 1 to 4 year-old. ASD children miss the milestones of language, attention and gestural behaviors of social or communicative cues and show long lasting repetitive interests.

ASD is worth noting from societal and industrial aspect as well since prevalence of ASD is very high nowadays. 1 of 88 children in the US (Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators Centers for Disease Control and Prevention, 2012), 2.6% in South Korea (Kim et al., 2011) are diagnosed as ASD. Furthermore, the number of ASD patients has been increasing drastically in last twenty years. According to a study from the Centers for Disease Control and Prevention (CDC), prevalence of autism increased 289.5% between 1997 and 2008 in the

US (Boyle et al., 2011). Such sudden increase of ASD patient number is becoming a burden to families of patients and the whole society. It depends on the severity of symptoms but usually ASD patients find it difficult carrying out everyday life on their own. Extra aid is required for the patients in education and medication. Medical-care cost and education-cost altogether is burden upon patient's family and society.

Researchers have been long trying to find out the etiology of this disorder through various aspects, including molecular, electrophysiological, imaging, behavioral approaches, yet, the exact cause of ASD is still unclear. From genetic approach, many alleged genes are being discovered from human ASD patients by advanced sequencing technology (Pinto et al., 2010). Such sequencing studies are providing clues for therapeutics for ASD. Concordance of ASD symptoms in monozygotic twins is very high (~70%), male to female ratio of patients is four-to-one (Buxbaum and Hof, 2012). These observations strongly suggest that there is genetic component in developing this disorder.

Indeed, 'synaptic theory of autism' has been strongly supported by many groups. This theory claims that each component of synaptic pathways is responsible for abnormal symptoms in ASD patients and each element is being intensively investigated. For instance, in Fragile X syndrome (FXS) and mGluR theory, insertion of CGG repeats in a single gene encoding fragile X mental retardation protein (FMRP1) is pointed as a cause of the syndrome (Laggerbauer et al., 2001; Verkerk et al., 1991). FMRP is a protein translation inhibitor that operates by binding RNA. FMRP knockout mice have increased mGluR-dependent long-term depression in hippocampus (Huber et al., 2002) and impaired long-term depression in the amygdala (Suvrathan et al., 2010; Zhao et al., 2005). It is shown that the synaptic-level symptoms in these mice

are caused by increased sensitivity to mGluR5 signaling and ERK1/2 (Osterweil et al., 2010). As previously stated, among ASDs, fragile X syndrome is relatively well known of its etiology; CGG repeats in *FMRPI* gene.

The Shank proteins are in the center of this synaptic map of autistic pathology. They bind to mGluRs indirectly via Homer, and to NMDARs and NLGNs via GKAP and PSD-95. They also link to the actin cytoskeleton, providing a multivalent scaffold and build the postsynaptic density.

There are three members in the shank family of proteins. Among three members of Shank family, shank2 proteins present in the cortex and hippocampus. Shank2 is almost absent in the thalamus and striatum, while Shank3 seems to be dominantly expressed in those regions. In the cerebellum, Shank2 is restricted to Purkinje cells, while Shank3 is restricted to granule cells (Sheng and Kim, 2000).

Shank3 was the first to be associated with autism. At the beginning of the investigation, a link between Shank3 and 22q13.3 deletion syndrome (Phelan-McDermid Syndrome; PMS) was established. PMS is a form of mental retardation often presenting together with autistic phenotypes (Bonaglia et al., 2001; Wilson, 2003). More analysis of ASD patients was carried and significant number of Shank3 mutations were found, suggesting a specific role in autistic pathology (Durand et al., 2007). The creation of genetic constructs resembling different mutations for the Shank proteins, and their use in live mice and in cultured neurons, has been key to enhancing our knowledge of the role of Shank within the synapse, and its contribution to autistic pathology. After the discovery of Shank3, within a few years, Shank2 was also identified as a risk gene (Berkel et al., 2010; Pinto et al., 2010).

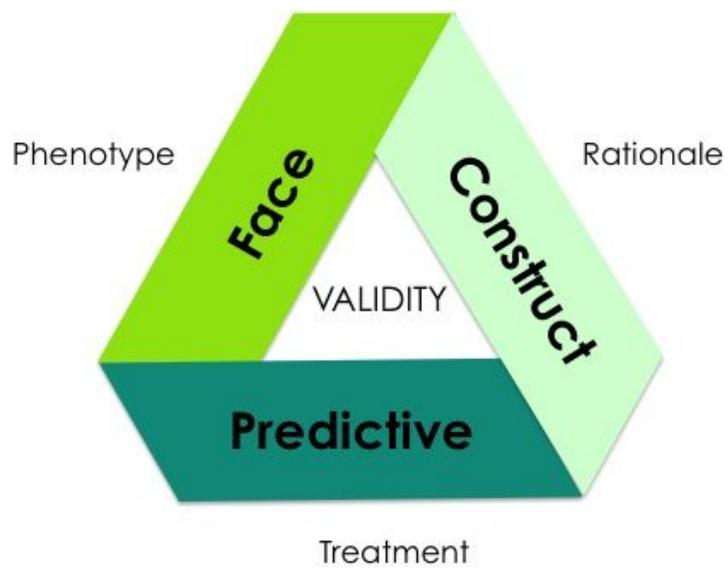
These genetic findings about shank proteins were soon used to generate mouse models.

### ***Validity of animal models and behavioral test in disease research***

Animal model is crucial for studying neuropsychiatric disease when the etiology is not clear, when specific biomarker is not found. In order to say that an animal model represent a certain disease, it should be agreed on three categories: face validity, predictive validity, construct validity (Blanchard et al., 2013; Willner, 1984) (**Figure 1**). Face validity describes behavioral, symptomatological similarities between human and model animal. Predictive validity is similarity in treatment effectiveness. It means the animal model should help predict the outcome of chemical or behavioral treatment to diseases. Lastly, construct validity is the model's theoretical background rationale. For example, anxiety disorder model is produced using threatening or giving painful experience and such events elicit behaviors related to anxiety. The rationale of this procedure is that anxiety disorders often follow after traumatic or painful experience. Of course, when genetic component of the disease is known it is a lot easier to make an animal model: deleting, knocking-down or overexpressing the gene in the animal.

Not only the animal model but also objective and reliable measurement of the behavior evaluation is crucial. Behavioral abnormality that human and animal model show can be correctly evaluated and interpreted only by proper behavioral assay protocols (Crawley, 2007b). When dealing with ASD, behavioral experiments are especially crucial because, as today, diagnosis is made by solely behavioral symptoms. However, there are no

standardized behavioral assay protocols for ASD model. Even though several animal models (knock-out mice) of ASD have been laid on the table for quite a while, each laboratory uses its own protocols to assess ASD model animals. So, it is difficult to compare behavioral results among studies and each result can be interpreted as conflicting in some cases.



**Figure 1.** Validity criteria for animal model of diseases

### ***Animal models of ASDs***

CD38 is a trans-membrane glycoprotein with ADP-ribosyl cyclase activity, which is recruited in calcium signaling in the cell. It is originally identified and studied for its role in immune responses in lymphocytes. Recently, CD38 is newly spotlighted as a regulating factor of oxytocin, a posterior pituitary hormone that modulates social and sexual behaviors (Dölen et al., 2013). CD38 knock out mice showed disturbance in oxytocin secretion and impaired social behavior (Jin et al., 2007). Moreover, CD38 genetic variants were discovered in autism spectrum disorder patients suggesting mutated CD38 as a possible causing factor of ASD (Higashida et al., 2012; Munesue et al., 2010).

Shank2 is a member of synaptic scaffolding protein family, Shank (also known as ProSAP, cortBP, SSTRIP et al.). Shank proteins indirectly bind to mGluR and NMDAR via Homer, GKAP and PSD-95 (Sheng and Kim, 2000). Human genetic studies showed that variation in Shank *de novo* mutation could be the cause of ASD (Berkel et al., 2010). As center of the synaptic scaffolding structure, Shank is being highlighted as an essential component in ASD. Previous studies using mouse models deleting exon 6-7, exon 7 respectively (Schmeisser et al., 2012; Won et al., 2012), showed autism-like behaviors. Mice had deficits in social behavior, difference in ultrasound vocalization compared to wild type which are interpreted as impaired social behavior, and showed stereotypy behavior such as jumping, increased grooming which represents repetitive behavior.

### ***Three-chamber test and pup retrieval test***

Mice are basically social species. In wild life, they live in packs composed of one male and one or two females. Pups share nest with their parents and get protection from predators, from coldness. When pups grow old enough, they leave the pack and make their own social group in new territory. Rodent social interactions include approaching, sniffing, following, climbing onto and allogrooming (Crawley, 2007a). These social characteristics remain the same in lab mice. Two unfamiliar mice placed together in a novel environment explore the new cage and investigate each other. Three-chamber test exploits these innate social characteristics of mice and pup retrieval test uses instinctual maternal care behavior of mice.

### ***Purpose of the study***

In this study, we set up rodent social behavior assay system including three-chamber test and pup retrieval test according to previous studies (Jin et al., 2007; Moy et al., 2007; Nadler et al., 2004; S S Moy et al., 2004; Won et al., 2012) and assessed validity assays as tool for screening autism spectrum model mice. To assess validity, we used previously established ASD model mouse lines, CD38 knockout and Shank2 knockout mice.

## Experimental Procedure

### *Animals*

All the animals were group caged in number of two to four and located in temperature, humidity controlled clean rack (Temperature: 22-23°C, humidity: 50-60%). Food and water were accessible to animals *ad libitum*. Light/Dark cycle was kept 12/12 (9a.m. to 9p.m.).

Only male mice were used for three-chamber test and only female mice were used for pup retrieval test.

2-, 3-months-old animals were used as subject for three-chamber test and pup retrieval test. Only P1 to P3 C57BL/6NCrljOri pups were used for pup retrieval assay since older ones could crawl around on their own without test mouse moving them.

Both mutant mice (CD38KO, Shank2KO) were maintained by crossing their hetero genotypes respectively. Genetic backgrounds of both mouse lines are C57BL/6N.

### *Three-chamber test*

Three-chamber social behavior test was devised to exploit innate social characteristics of mice. Naturally, they explore around the neighborhood and make social contact with other conspecific animals. The assay measures social preference and ability of social recognition. Social

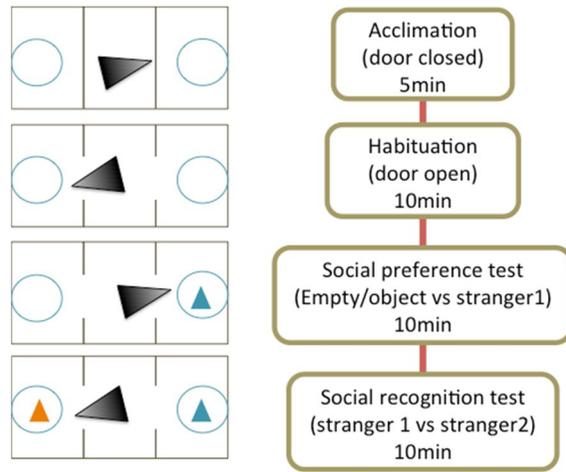
preference indicates animal's genuine tendency or preference toward moving animal over non-moving object.

Rectangular opaque Plexiglas arena (w 41.5cm x l 60.4cm x h 22cm) was divided into three chambers (w 40.5 cm x l 20cm x h 22cm each) by the same Plexiglas walls, with sliding gates. The size of the door way (4.5cm x 3.5cm) was big enough for mice to pass through. In two side chambers, wire cages were placed right by the wall (S S Moy et al., 2004) (**Figure 2B**).

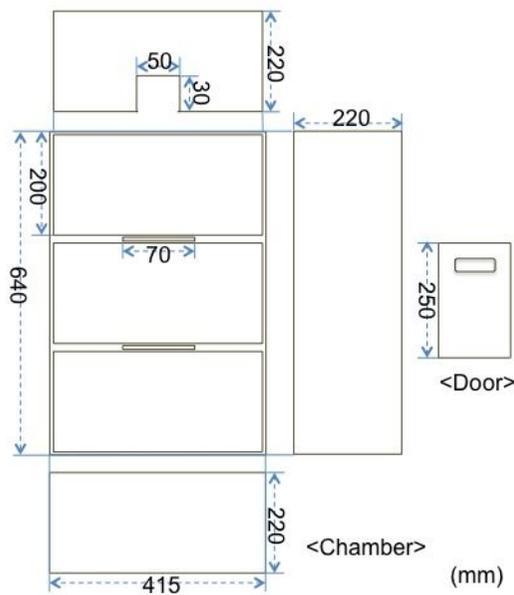
Three-chamber assay constituted three phases (**Figure 2A**). First phase was acclimation and habituation period. In acclimation period, doors were closed and test animal was introduced into the middle chamber and freely explore the chamber for five minutes so that it can adjust to the experimental area. After five minutes, doors were opened and the test mouse was allowed to explore the whole three compartments for another ten minutes so it can be accustomed to the wire cage in each side chamber (habituation period). The second phase measured sociability. After habituation, the test animal was gently guided into the middle chamber then doors were closed. New conspecific animal (stranger 1, S1) was introduced into the wire cage of one side chamber and the wire cage of the other side was left empty (empty, E). Doors were opened, the test animal moved around freely to investigate either S1 or E chamber for ten minutes. This period measured sociability of the animal. Before last phase, the test animal was guided into the middle chamber again and doors were closed. In third phase, social recognition ability of the animal was tested. The same mouse used in social preference test phase (S1) was placed in a wire cage, and in the other cage, a new conspecific animal (stranger 2, S2) was put in. The doors were opened and test animal explored freely in the arena for another 10min.

During tests, video was recorded via CCD camera and later analyzed with Noldus Ethovision XT 9.

A



B



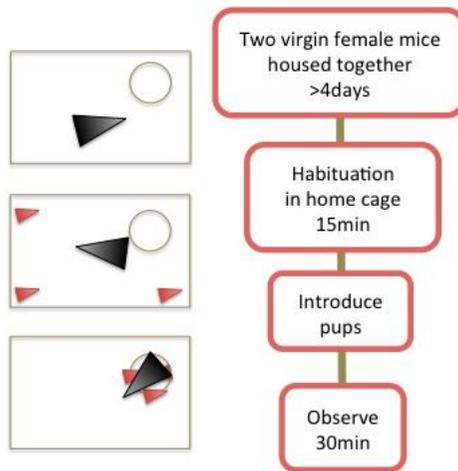
**Figure 2. Schematics of three-chamber test** (A) Experimental procedure for three-chamber test. Black triangle represents the subject mouse, blue and orange triangle represent social partner. (B) Dimensional plan of three-chamber apparatus

### ***Pup retrieval test***

Pup retrieval test procedure was designed by modifying previous studies (Jin et al., 2007; Won et al., 2012). Before carrying out pup retrieval assay, two virgin female animals were housed together for at least a week to prevent possible anxiety of single caging. Postnatal day 1 to 3 (P1-P3) C57BL/6 mice pups were used for the experiment. Because pups older than 3 days can crawl around on their own will and can gather together without test mouse retrieving them. The home cage was held on the shelf in the experiment area for 30 min so the animals can adjust to the environment (e.g. scent, white noise). Test mouse was left in home cage and her cage mate was moved to temporary cage. The test mouse in the home cage was habituated in the experiment room under the recording camera for 15min. Three pups were placed at three different corners apart from the nest. Test animal was allowed to retrieve pups up to 30 min (**Figure 3**). Video was recorded by CCD camera and latency to retrieve each pup (the 1<sup>st</sup> pup, 2<sup>nd</sup> pup, 3<sup>rd</sup> pup) was counted manually.

### ***Data analysis***

Statistical analyses were performed using GraphPad Prism 5.01. All data are presented as mean  $\pm$  SEM (standard error of mean).



**Figure 3. Schematics of pup retrieval test** Black triangle represents the subject mouse and small red triangles represent pups.

## Results

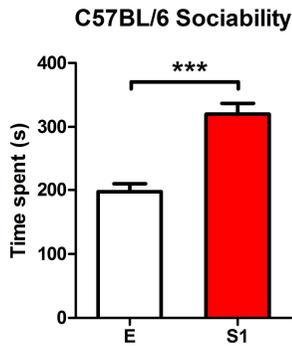
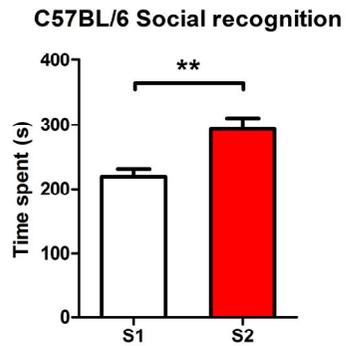
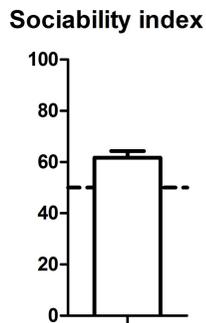
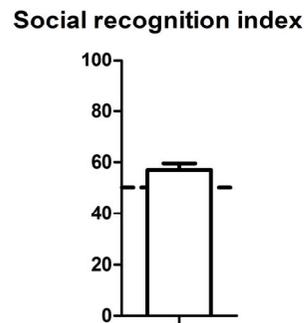
### *C57BL/6 mice showed preference for social interaction partner to empty cage.*

In sociability test phase in three-chamber assay, C57BL/6 mice spent significantly more time in the chamber where novel social interaction partner (S1) ( $320.0 \pm 6.90s$ ) was than empty wire cage side (E) ( $197.0 \pm 12.67s$ ) (Student's t-test, two-tailed,  $p < 0.001$ ) (**Figure 4A**). During acclimation period, when animal was allowed to explore only the center chamber, distance moved was measured in order to see the animal's anxiety and locomotor activity. ( $1476 \pm 62.65$  cm) (**Figure 5A**). During habituation period, when side gates were opened and animal explored all three compartments freely, the animal didn't show place preference for either side of the chamber (Left : center : right = 40.04 % : 15.56 % : 42.21 %) (**Figure 5B**). Sociability index score ( $61.71 \pm 2.568$ ) and social recognition index score ( $57.04 \pm 2.495$ ) were calculated respectively (**Figure 4C, D**).

### *C57BL/6 mice showed preference for novel social interaction partner to familiar one.*

In social recognition test phase, C57BL/6 spent significantly more time in the chamber where novel social interaction partner (S2) was ( $293.5 \pm 16.87s$ ) than where familiar one (S1) was ( $218.8 \pm 11.68s$ ) (**Figure 4B**). The

result showed that C57BL/6 mice had preference for novel social stimulus rather than for familiar stimulus.

**A****B****C****D**

**Figure 4. Social Behavior of C57BL/6 in three-chamber test.** C57BL/6 mice showed preference (A) for social partner to empty cage in sociability test (Student's t-test,  $***p < 0.001$ ,  $n = 12$ ) and (B) for novel social partner than familiar one. (Student's t-test,  $**p = 0.0014$ ,  $n = 12$ ) (C) C57BL/6 sociability

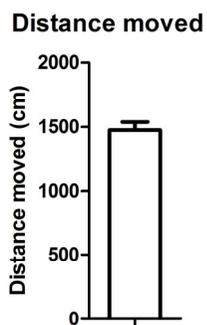
index =  $61.71 \pm 2.568$  (Sociability index =  $\frac{\text{Duration in S1}}{(\text{Duration in S1} + \text{Duration in E})} \times$

100). (D) C57BL/6 social preference index =  $57.04 \pm 2.495$

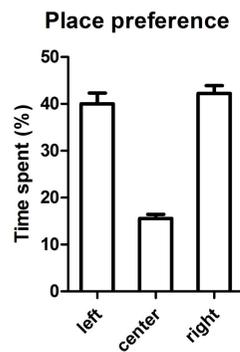
(Social recognition index =  $\frac{\text{Duration in S2}}{(\text{Duration in S1} + \text{Duration in S2})} \times 100$ ).

Error bars represent SEM.

A



B

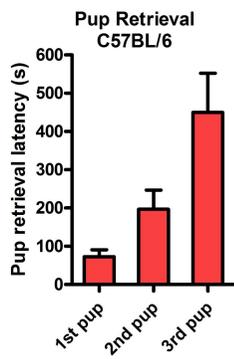


**Figure 5. Locomotor activity and place preference of C57BL/6 mice in habituation period of three-chamber test.** (A) The distance C57BL/6 mice moved in the center chamber during habituation 1 period was measured (1476 ± 62.65 cm). (B) The duration C57BL/6 mice spent in either side of the chamber had no significant difference. Left : center : right = 40.04 % : 15.56 % : 42.21 %. (n=12) Error bars represent SEM.

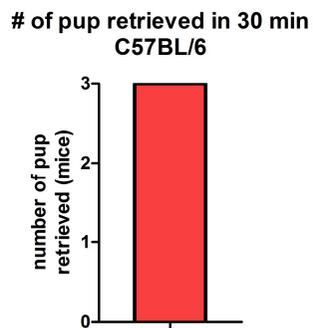
***C57BL/6 female mice retrieved all three pups within 30min***

In pup retrieval test, virgin female C57BL/6 mice retrieved all three pups within 30min-limit ( $449.9 \pm 102.3s$ ) even though they had no experience of giving birth before and had no experiences of nurturing pups (**Figure 6A, B**).

**A**



**B**



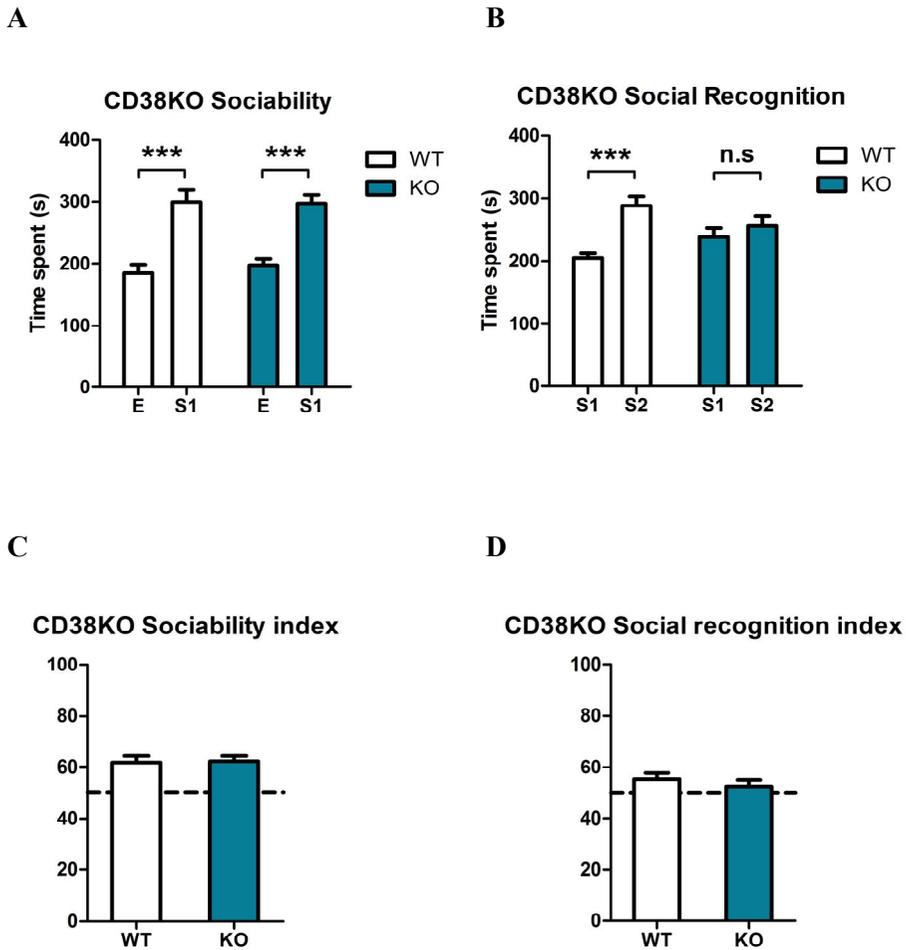
**Figure 6. C57BL/6 mice pup retrieval test.** (A) Pup retrieval latency of C57BL/6 mice. (B) No animal failed in retrieving all three pups within 30 min limit (n = 8). Error bars represent SEM.

***CD38KO mice showed preference for social interaction partner to empty cage.***

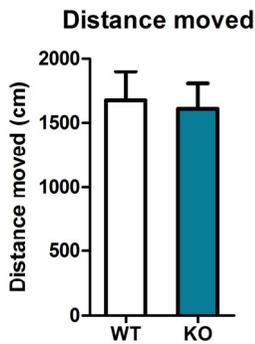
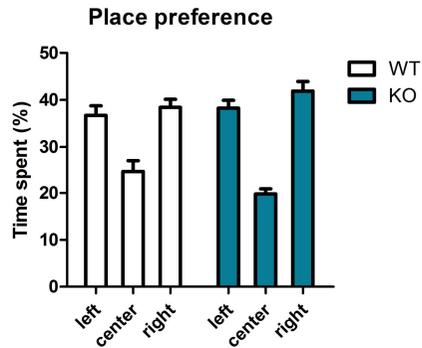
CD38 wild type mice spent significantly more time in the chamber where stranger mouse was thus showed preference for social interaction partner rather than for empty cage (WT; E =  $184.5 \pm 13.63s$ , S1 =  $299.3 \pm 19.86s$ ). CD38 knockout mice exhibited preference for social partner as well (KO; E =  $197.4 \pm 10.51s$ , S1 =  $296.8 \pm 14.25s$ ) (**Figure 7A**). There was no difference in sociability index score between genotypes (WT;  $61.69 \pm 2.703$ , KO;  $62.21 \pm 2.129$ ) (**Figure 7C**). Both genotypes showed no difference in locomotor activity (WT;  $1680 \pm 216.2cm$  KO;  $1616 \pm 189.6cm$ ) and didn't have place preference for either side of the chamber (Left : center : right =  $37.60\% : 23.17\% : 39.21\%$  (WT),  $38.28\% : 19.82\% : 41.90\%$  (KO)) (**Figure 8B**).

***CD38KO mice exhibited deficits in social recognition***

In social recognition phase where the animal was presented by familiar social partner (S1) and novel social partner (S2), wild type mice showed strong preference for S2 (WT; S1 =  $205.2 \pm 7.359s$ , S2 =  $287.7 \pm 15.21s$ ). On the other hand, knockout mice spent similar amount of time in each chamber (KO; S1 =  $238.0 \pm 146.0s$ , S2 =  $256.0 \pm 14.84s$ ). From this result, knockout mice seemed not being able to differentiate between familiar and novel social partner (**Figure 7B**). There was difference in the social recognition index score between genotypes (WT;  $55.31 \pm 2.380$ , KO;  $52.38 \pm 2.572$ ) but statistically not significant (**Figure 7D**).



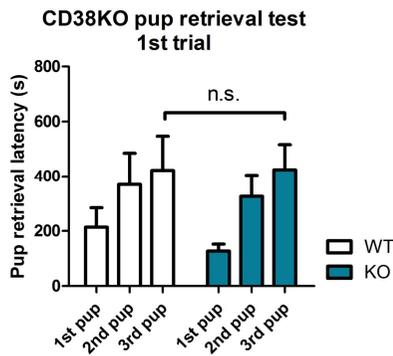
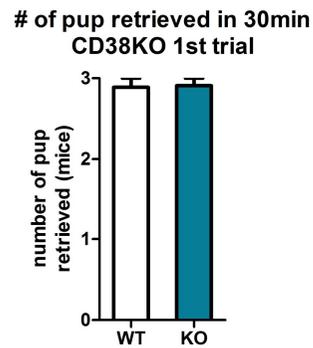
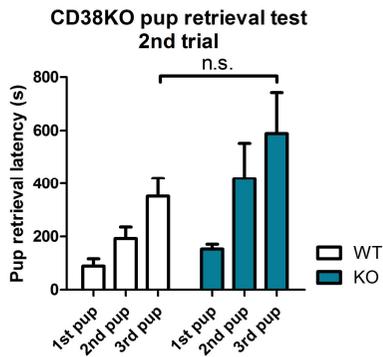
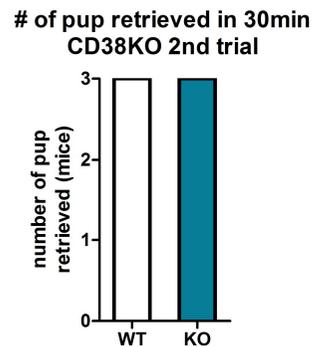
**Figure 7. Impaired social behavior of CD38KO mice in three-chamber test.** (A) Both WT and KO showed preference for social partner. (B) WT had preference for novel social partner. KO lacked this preference. (C), (D) WT and KO had no difference in social indices. (Student's t-test,  $***p < 0.001$ ,  $n.s = \text{not significant}$ . WT;  $n = 9$ , KO;  $n = 9$ ) Error bars represent SEM.

**A****B**

**Figure 8. CD38KO mice locomotor activity and place preference during habituation period in three-chamber test.** (A) WT and KO mice showed no difference in locomotor activity in acclimation period during three-chamber test. (WT;  $1680 \pm 216.2$  cm KO;  $1616 \pm 189.6$  cm) (B) WT and KO mice didn't have place preference for either side of the chamber as measured in habituation 2 period. Left : center : right = 37.60 % : 23.17 % : 39.21 % (WT), 38.28 % : 19.82 % : 41.90 % (KO) (WT; n = 9, KO; n = 9) Error bars represent SEM.

### ***CD38KO mice in pup retrieval test***

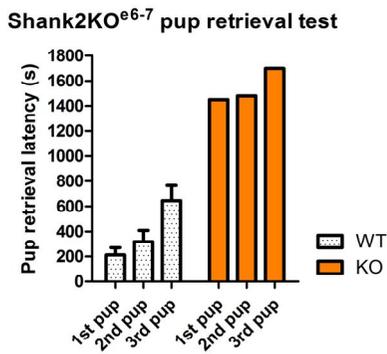
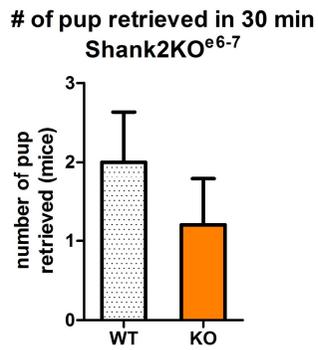
Pup retrieval test was done twice for each animal. In the first trial, wild type and knockout mice showed no difference in pup retrieval latency for all three pups (WT;  $422.4 \pm 124.8$ s, KO;  $424.1 \pm 92.33$ s) (**Figure 9A**). One mouse of each genotype failed to retrieve all three pups in 30min time limit (**Figure 9B**). In the second trial, wild type mice retrieved all pups with similar latency ( $351.3 \pm 69.61$ s) as the first trial. Knockout mice, however, took even longer time than the first trial ( $588.7 \pm 153.8$ s) (**Figure 9C**). Both genotypes succeeded in retrieving all three pups (**Figure 9D**).

**A****B****C****D**

**Figure 9. CD38KO mice pup retrieval test.** (A) Pup retrieval latency of WT and KO showed no difference in the first trial. And (B) one of each genotype failed to retrieve all three pups in 30 min (WT; n=9, KO; n=11). (C) In the second trial, WT showed similar retrieval latency as the first trial, however, KO took longer time to retrieve (WT; n=6, KO; n=8). Error bars represent SEM.

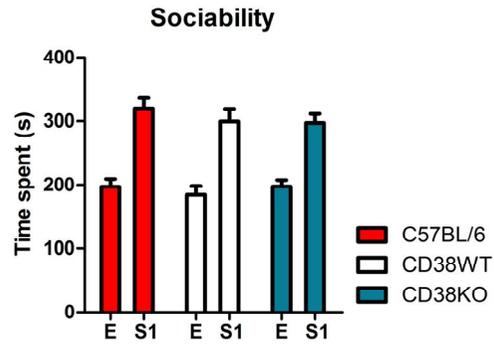
*Shank2KO<sup>e6-7</sup> mice seem to have disrupted maternal behavior in pup retrieval test*

Within given 30min time limit, only one of knockout mouse finished the task and the rest of the knockout animals failed to retrieve all three pups within 30min (WT; n = 6, KO; n = 5) (**Figure 10B**). And the only knockout mouse that finished the task showed longer pup retrieval latency (1698s) compared to average wild type mice ( $645.0 \pm 120.4s$ ) (**Figure 10A**).

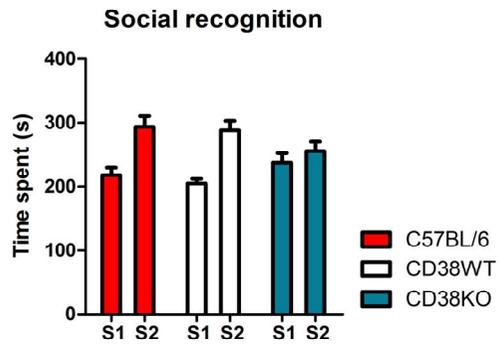
**A****B**

**Figure 10. Shank2KO<sup>e6-7</sup> mice pup retrieval test.** (A) Pup retrieval latency of Shank2 WT and KO mice. Only mice that retrieved all three pups were included in this data. (B) Number of pups retrieved in 30min. KO mice retrieved less pups than WT (WT; n = 6, KO; n = 5). Error bars represent SEM.

A



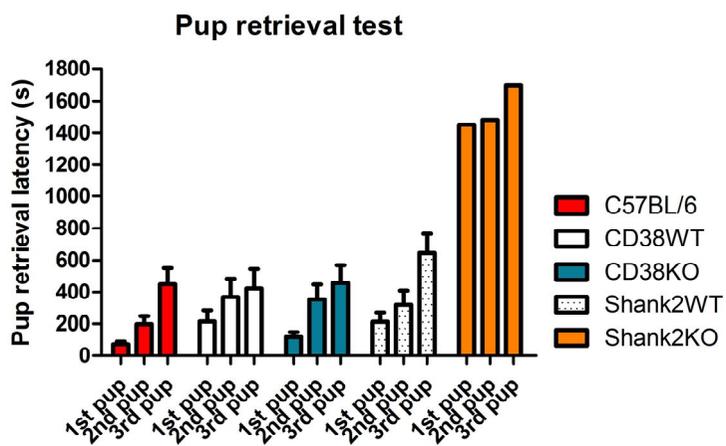
B



**Figure 11. Comparison of three-chamber test results between genotypes.**

(A) Sociability result comparison of C57BL/6, CD38WT and CD38KO mice.

(B) Social recognition result comparison of three genotypes.



**Figure 12. Comparison of pup retrieval test results between genotypes.** Pup retrieval latency data of five genotypes were presented together. Error bars represent SEM.

## Discussion

Translation of animal behavior into human behavior with psychiatric disease has long been studied since early as 1930s but development or shift through long period of time has not been much (Kalueff et al., 2007). This is because fundamental limitations of using different species as a model: We cannot say confidently that mouse behavior parallels human behavior especially when it comes to complicated cognitive or emotional process. Besides its translation problem, there are many reports about both inward factor (e.g. mouse genetics) and outward factor (e.g. experimental environment, diet and handling procedure) that affect the outcome of the result (Crabbe et al., 1999; Wahlsten et al., 2003). Practically, controlling every detailed factor such as experimental room environment, the vivarium condition is impossible. But at least, we need to control all what we can. Thus standardizing behavioral experimental protocol is necessary. By reproducing and comparing the result of previously introduced protocol, we can contribute validating behavioral paradigm that many research labs are supporting now.

In this study, three-chamber test and pup retrieval test was used to measure behavior of C57BL/6 mice and two ASD model mice (CD38KO and Shank2KO). C57BL/6 mouse line, which is normally used for cognitive experiment in laboratory, exhibited preference for social partner to non-social empty cage in social preference test. Also, they showed preference for novel social partner to familiar social partner in social recognition test. In pup

retrieval test, C57BL/6 virgin female mice retrieved all three pups within 30min limit.

CD38KO mice, an ASD model, showed normal sociability as they preferred social partner to non-social empty cage and this behavior was not different from wild type. Wild type (WT) mice showed preference for novel social partner to familiar partner; however, CD38KO mice had no preference for either partner. In first trial of pup retrieval test, CD38KO and wild type mice both finished the task within 30min time limit. CD38KO seemed to have longer pup retrieval latency than WT but the difference was not statistically different. The same mice did pup retrieval test again. In second trial, WT exhibited better performance and finished task a little more quickly; in contrast, CD38KO mice took longer time than the first trial. WT seemed to have learnt nurturing behavior but CD38KO had not. This may be able to be interpreted as another deficit in social behavior.

Comparing three-chamber test data of 3 tested genotypes (C57BL/6, CD38WT, CD38KO) (**Figure 11**), CD38WT and CD38KO mice both showed similar level of sociability to C57BL/6 mice. However, in social recognition test, CD38WT exhibited comparable level of ability, however, CD38KO tend not to differentiate novel to familiar social partner. When comparing pup retrieval test data of 5 tested genotypes, all had similar pup retrieval latency except Shank2KO.

Counting how long had the animal spent in each chamber could be deemed as a vague indicator for social interaction because subject mouse can just wander around the chamber not making direct contact with interaction partner (stranger 1). This can be considered as a shortcoming we have to bear in order to have easy criteria for the assessment. Indeed in recent paper, when

another member of shank protein, Shank3 was deleted, the knockout mice showed reduced duration in social partner chamber but also showed overall avoidance to novel environment (Han et al., 2013). So it cannot be explicitly said that seemingly reduced sociability in three-chamber test is entirely due to deficit in social behavior. Like in this instance, if more precise measurement of social behavior is wanted, manual counting and other complementary experiment can be the only solution, which takes away long time and effort from experimenters.

Despite the shortcomings mentioned above, three-chamber test and pup retrieval test still are valuable strategies for screening autism-like symptoms and help discover etiology of the disease and develop therapy, since they detect disruption in social or maternal behavior easily, and those limitations can be complemented by other behavioral tests.

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국문초록

# 자폐 스펙트럼 장애 쥐 모델의 사회적 행동 연구

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자폐 스펙트럼 장애 (autism spectrum disorder, ASD)는 복합적인 장애로 그 원인이 아직까지 확실히 밝혀져 있지 않다. ASD는 생체표지나 영상표지가 규명되어 있지 않기 때문에 겉으로 보이는 행동적 특성을 관찰하는 것에 따라서만의 학적 진단이 이루어지고 있다. 따라서 동물모델을 이용한 ASD 연구 또한 많은 부분을 행동학적 실험에 의존해야하는 상황이다.

신경정신병 모델 쥐들은 병의 분자학적 기작을 밝히거나 치료약을 개발하는 데에 사용된다. 한편, 여러 실험실에서 같은 종류의 쥐와 실험 방법을 써도 서로 다른 결과를 얻게 되는 경우가 다반사이기 때문에 모델 쥐를 만드는 것과는 또 다른 측면에서 적절한 행동학적 시험법을 개발하는 것 또한 중요하다. 특히 ASD와 같은 생체 표지가 없는 질병의 경우에는 약이나 치료법의 효과를 확인하는데 행동학적 결과가 가장 중요한 관문이 된다.

본 연구에서 우리는 three-chamber apparatus와 pup retrieval test를 사용하여 C57BL/6 쥐를 먼저 시험하고 ASD 모델 쥐인 CD38KO과 Shank2KO 쥐를 시험하였다. 여러 실험실에서 널리 쓰이고 있는 C57BL/6 쥐는 사회성이 높고 유전자가 여러 유전자변형 쥐의 바탕 유전자로 사용되기 때문에 대조군으로 적당하다. CD38KO 쥐는 사회적 행동을 측정하는 three-chamber test에서 wild type(WT)과 비교했을 때 익숙한 쥐와 새로운 쥐를 구별하지 못하는 경향을 보였고, pup retrieval test에서 WT보다 새끼를 한 곳에 모으는데 더 오랜시간이 걸렸다. Shank2KO 쥐는 pup retrieval test에서 WT에 비해 새끼를 모으는데 시간이 더 오래 걸리는 경향을 보였다.

**핵심어 :** *사회적행동, 자폐 스펙트럼 장애, ASD, three-chamber test, pup retrieval test*

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