**Figure 3**

Working memory deficit in sdy mice. The percent of correct choices in T-maze forced alternation task was presented. Data were analyzed by two-way repeated measures ANOVA. Improvements of performance over training were analyzed one-way repeated measures ANOVA. (B) Apparatus of the T-maze forced alternation task.

35]. Wild type mice improved their performance over training as measured by an increase in the number of correct choices made ($F(14, 238) = 3.067$, $p = 0.0002$; session effect), while sdy mice did not ($F(14, 210) = 0.952$, $p = 0.5041$; session effect). There was a significant session by genotype interaction effect on the percent of correct choices ($F(14, 448) = 1.760$, $p = 0.0421$). After acquisition trials, sdy mice demonstrated significantly less correct choices made than wild type mice ($F(1, 32) = 8.031$, $p = 0.0079$; from 11th to 15th session) (Figure 3A).

To increase the difficulty of the task, a delay period (10, 30, 60 sec) was applied. Under these conditions, there was no significant difference in the percentage of correct choices made between sdy and wild type mice (10 sec delay, $F(1, 32) = 0.041$, $p = 0.8412$; 30 sec delay, $F(1, 32) = 1.096$, $p = 0.3030$; 60 sec delay, $F(1, 32) = 0.479$, $p = 0.4939$). However, DBA/2J, the background strain of sdy mice, has been reported to demonstrate relatively lower performance of working memory in radial maze compared to other inbred strains such as C57BL/6J and BALB/cJ [36]. Therefore, increasing the difficulty of the task with a delay period may have masked the effect of genotype.

Discussion

The lack of *Dtnbp1* did not lead to obvious abnormalities in overall health, appearance, sensory-motor reflex or muscular strength. However, sdy mice demonstrated abnormal behaviors some of which are reminiscent of schizophrenia. Firstly, sdy mice showed hypolocomotor activity, which is consistent with our previous report on the behavior of sdy mice [9]. In most animal models of schizophrenia, hyperlocomotor activity is considered of potential relevance to the positive symptoms of schizophrenia. Speculatively, the decreased activity, shown by sdy mice, could be related to negative symptoms of schizophrenia.

In addition, we found poor motor learning in the rotarod test. It is known that motor skill learning assessed by rotary pursuit is impaired in patients with schizophrenia [37,38]. One possibility is that deficit in motor learning could be part of a pattern of generalized neuropsychological impairment in schizophrenic patients. Such an explanation could also apply to the sdy mice since they demonstrated cognitive deficits in the present study as well as learning deficits. Another possible explanation for the motor learning deficits in sdy mice is that they reflect

poor motor coordination, likely to have affected the *sdyl* mice since they also exhibited swimming deficits in the Porsolt forced swim test. Neuromotor dysfunction is consistently found in schizophrenia [39-41]. Dysbindin-1, which binds to dystrobrevin, is expressed in neuromuscular junctions and cerebellar tissues [42]. Similar to our results of motor coordination deficits in *sdyl* mice, dystrobrevin knockout mice also show poor motor coordination [42].

In addition to abnormal motor related behaviors, *sdyl* mice also displayed cognitive impairments including impaired memory retention and working memory. Memory function is one of the representative neurobiological traits of schizophrenia and is deeply disturbed in patients with the disorder [43,44]. Although previous study reported enhanced anxiety-like behavior in *sdyl* mice [9], *sdyl* mice did not show altered anxiety-like behavior in the present study. For example, in the previous study, apparatus with a black walk way was used in the elevated plus maze test, while we used apparatus with white walk way. The illumination level and time behavior was observed for was also different between studies. Hattori et al. examined behavior for 20 minutes, while we examined for only 10 minutes. Consistent with our results, however, anxiety-like behavior was unaltered in *sdyl* mice within the first 10 minutes of the Hattori et al study, suggesting we may have observed anxiety-related changes had we observed the mice for longer. On the other hand, *sdyl* mice showed decreased locomotor activity in open field test (Additional figure 1) and light/dark transition test (Additional figure 2). In light/dark transition test, the time course of distance traveled in the dark box was different between genotypes (Additional figure 2B), suggesting abnormal habituation to a novel environment in *sdyl* mice. This reduction of locomotor activity in *sdyl* mice caused by novel and stressful environment may affect anxiety-like behavior in the previous study.

Overall therefore, the data presented show deficits in exploratory activity and memory which could be consistent with the negative and cognitive symptoms of schizophrenia and with neuropsychological and neuromotor deficits seen in the disorder. However, the schizophrenia phenotype is unlikely to be simulated in animal models through the disruption of a single gene, and it should be pointed out that similar behavioral effects can be seen following the disruption of genes thought to have little to do with schizophrenia. Notwithstanding, the phenotypes in the *sdyl* mice may be useful for understanding the neurobiological role of dysbindin and may be relevant to particular endophenotypes of schizophrenia.

Cognitive deficits of *sdyl* mice as an animal model of cognitive dysfunctions of schizophrenia

As dysbindin-1 is a susceptibility gene for schizophrenia, *sdyl* mice could be a good animal model of the disorder. In the present study, we demonstrated that *sdyl* mice displayed cognitive deficits including impaired long-term memory retention in the Barnes maze test and impaired working memory in the T-maze forced alternation task. Cognitive dysfunctions, including impaired attention, decreased working memory and decreased long term memory retention, are described as core symptoms of schizophrenia [45]. Cognitive deficits have also commonly been observed in other pharmacological, neurodevelopmental, and genetic animal models for schizophrenia [46,47]. Recently, mice with mutations in several schizophrenia susceptibility genes have been shown to have cognitive deficits. For example, mice with mutations in Neuregulin-1, Disrupted in schizophrenia 1 (DISC1) and calcineurin, all identified as risk genes for schizophrenia [3,48,49], show impaired working memory. [25,50-52]. YWHAE, a binding partner of DISC1, was also identified as a susceptibility gene for schizophrenia and heterozygous knockout mice of this gene also causes working memory deficits [24]. Mutant mice relevant to both the dopamine and glutamate hypotheses of schizophrenia similarly show working memory deficits, including mice lacking D2 and D3 receptors [53], mice with transient striatal overexpression of D2 [54], GluR1 knockout mice [55] and mice with dentate gyrus specific NMDA receptor knockout [56]. In addition, we have demonstrated working memory deficits in knockout mice for the alpha-isoform of calcium/calmodulin dependent kinase (alpha-CaMKII) [28], a major signaling molecule downstream of NMDARs associated with both schizophrenia and working memory in humans [57]. Thus several diverse animal models show behavioral abnormalities in memory function in common with the *sdyl* mouse. This suggests that the *sdyl* mouse may have not only construct validity but also reasonable face validity, as a model of the cognitive dysfunctions of schizophrenia.

Why does Dysbindin-1 deficiency cause impairments in working memory and long term memory retention?

Several lines of evidence indicate that the dentate gyrus and mossy fiber terminus play important roles in working memory and long term memory retention [58-61]. Dentate gyrus is activated by spatial working memory tasks [62,63] and others have reported a highly positive correlation between spatial working memory performance and the size of the mossy fiber terminals [64,65]. Likewise, Ramirez-Amaya and colleagues show mossy fiber synaptogenesis correlates with performance of spatial long-term memory retention in a water maze test [66]. We recently reported that continuous neurogenesis in dentate gyrus is essential for long-term memory retention [67]. In addition,

tion, mossy fiber – CA3 synapses exhibit long-term plasticity phenomena, such as long term potentiation [68], which may contribute to hippocampal memory processing.

In reference to deficits of the dentate gyrus and spatial working memory, mutant mice of the DISC1 gene and the alpha-CaMKII gene should be noted. Mice lacking a C-terminal portion of DISC1 show morphological abnormalities in the dentate gyrus and deficits of spatial working memory [69]. Heterozygous knockout mice of alpha-CaMKII, which displayed a severe working memory deficit, had remarkable abnormalities in their dentate gyrus that is referred as immature dentate gyrus [28]. In the dentate gyrus of alpha-CaMKII heteroknockout mice, increased transmission and reduced frequency facilitation at the synapses between mossy fibers and CA3 pyramidal cells [28]. These mice with deficits in working memory and long term memory retention showed abnormalities in dentate gyrus. Abnormality in dentate gyrus of sdy mouse has not been reported, however, dysbindin-1 could play a critical role in memory disturbance in schizophrenia via dentate gyrus. Indeed, dysbindin-1 is expressed at highly levels in the dentate gyrus and mossy fibers [6,70]. Moreover, dysbindin-1 mRNA in the hippocampal formation of patients with schizophrenia shows reduced expression in dentate granule and polymorph cells and in CA3, but not in CA1 [4]. Talbot and colleagues similarly reported that the reduction of dysbindin-1 was relatively restricted in dentate gyrus and mossy fiber terminus of patients with schizophrenia [6]. This presynaptic reduction of dysbindin-1 protein was inversely correlated with increased expression of vesicular glutamate transporter-1, indicating glutamatergic alterations within intrinsic hippocampal formation connections in schizophrenia. We previously reported that dysbindin-1 plays a role in the glutamate neurotransmission [13]. Overexpression of dysbindin-1 induced the expression of two pre-synaptic proteins, SNAP25 and synapsin I, and increased extracellular basal glutamate levels and release of glutamate evoked by high potassium in primary cortical neuronal culture. Conversely, knockdown of endogenous dysbindin-1 protein by small interfering RNA (siRNA) resulted in the reduction of pre-synaptic protein expression and glutamate release, suggesting that dysbindin-1 might influence exocytotic glutamate release via upregulation of the molecules in pre-synaptic machinery. Consistent with this role of dysbindin-1, altered regulation of exocytosis and vesicle biogenesis in neurons has been reported in sdy mice [71]. This included specific defects in neurosecretion and vesicular morphology in hippocampal synapses such as larger vesicle size, slower quantal vesicle release, lower release probability, and smaller total population of the readily releasable vesicle pool. Collectively therefore, these data suggest that defi-

ciency of dysbindin-1 could be linked to glutamatergic dysfunction in the dentate gyrus and mossy fibers, and this could possibly underpin cognitive deficits related to the dentate gyrus in both schizophrenia and sdy mice.

In addition to being implicated in glutamatergic neurotransmission, dysbindin is also highly expressed in dopaminergic nuclei [5,12]. A recent study found that *DTNBP1* siRNA transfection reduced dysbindin-1 protein, increased surface expression of dopamine D2 receptor and blocked dopamine-induced internalization of dopamine D2 receptor in SH-SY5Y cells [11]. Dysbindin-1, via its role in BLOC-1, may thus regulate recycling of dopamine D2 receptor in postsynaptic targets of dopaminergic synapses [11]. Another study reported that dopamine release was increased by siRNA-mediated silencing of dysbindin-1 in PC12 cells [12]. We found that sdy mice displayed lower levels of dopamine in the cerebral cortex, hippocampus, and hypothalamus compared to wild type mice [9,10], further demonstrating that dysbindin-1 plays a crucial role in the dopaminergic system. Altered dopaminergic transmission in sdy mice could be related to the deficits of working memory, since mice deficient for dopamine D2 and D3 receptors and mice selectively overexpressing striatal dopamine D2 receptors show working memory deficits [53,54]. The former mice exhibited abnormal dopamine D1 receptor activity in the frontal cortex, and the latter mice also displayed altered dopamine levels, rates of dopamine turnover, and activation of D1 receptors in the frontal cortex. In addition, D1 receptor blockade in hippocampus – prefrontal cortex circuits has been shown to disrupt working memory in the rat [72,73]. These data therefore suggest that the memory impairment observed in sdy mice might be related to the dopaminergic system and dopaminergic projections to the frontal cortex. Indeed, dopamine has a major role in regulating the excitability of the cortical neurons upon which the working memory function of the prefrontal cortex depends [74].

Conclusion

We have provided the first report of impaired long-term memory retention and working memory in sdy mutant mice, which lack the dysbindin-1 gene, a susceptibility gene for schizophrenia. The behavioral phenotype shows similarities with several other genetic animal models with mutations in putative schizophrenia susceptibility genes. Further studies to explore any shared mechanisms underpinning the cognitive deficits in the sdy mice and other genetic animal models of schizophrenia might provide novel insight into the pathophysiology of schizophrenia and new drug targets for the disorder.

Methods

Animals and experimental design

Sdy mice (dysbindin-1 mutant mice) were obtained from the Jackson Laboratory (Bar Harbor, Maine, USA). Mice were housed one per cage in a room with a 12-hr light/dark cycle (lights on at 7:00 a.m.) with access to food and water ad libitum. Behavioral testing was performed between 9:00 a.m. and 6:00 p.m. After the tests, the apparatus were cleaned with super hypochlorous water to prevent a bias due to olfactory cues. All behavioral tests were conducted in a manner similar to those described previously [75,76]. All behavioral testing procedures were approved by the Animal Care and Use Committee of Kyoto University Graduate School of Medicine.

Neurological screen

Neurological screen was performed with 10-wk-old male mice. The righting, whisker touch, and ear twitch reflexes were evaluated. A number of physical features, including the presence of whiskers or bald hair patches, were also recorded.

Neuromuscular strength

Neuromuscular strength was performed with 10-wk-old male mice, and tested with the grip strength test and wire hang test. A grip strength meter (O'Hara & Co., Tokyo, Japan) was used to assess forelimb grip strength. Mice were lifted and held by their tail so that their forepaws could grasp a wire grid. The mice were then gently pulled backward by the tail with their posture parallel to the surface of the table until they released the grid. The peak force applied by the forelimbs of the mouse was recorded in Newtons (N). Each mouse was tested three times, and the greatest value measured was used for statistical analysis. In the wire hang test, the mouse was placed on a wire mesh that was then inverted and waved gently, so that the mouse gripped the wire. Latency to fall (second: sec) was recorded, with a 60 s cut-off time.

Open field test

Locomotor activity was measured using an open field test. Open field test was performed with 11-wk-old male mice. Each mouse was placed in the center of the open field apparatus (40 × 40 × 30 cm; Accuscan Instruments, Columbus, OH). Total distance traveled (in cm), vertical activity (rearing measured by counting the number of photobeam interruptions), time spent in the center, the beam-break counts for stereotyped behaviors, and number of fecal boli were recorded. Data were collected for 120 min.

Light/dark transition test

Light/dark transition test was performed as previously described [77]. The apparatus used for the light/dark transition test consisted of a cage (21 × 42 × 25 cm) divided

into two sections of equal size by a partition containing a door (O'Hara & Co., Tokyo, Japan). One chamber was brightly illuminated (390 lux), whereas the other chamber was dark (2 lux). Mice were placed into the dark side and allowed to move freely between the two chambers with the door open for 10 min. The total number of transitions between chambers, time spent in each side, first latency to enter the light side and distance travelled were recorded automatically using Image LD software (see 'Image analysis').

Elevated plus maze test

Elevated plus-maze test was performed as previously described [28]. The elevated plus-maze (O'Hara & Co., Tokyo, Japan) consisted of two open arms (25 × 5 cm) and two enclosed arms of the same size, with 15-cm high transparent walls. The arms and central square were made of white plastic plates and were elevated to a height of 55 cm above the floor. To minimize the likelihood of animals falling from the apparatus, 3-mm high plastic ledges were provided for the open arms. Arms of the same type were arranged at opposite sides to each other. Each mouse was placed in the central square of the maze (5 × 5 cm), facing one of the closed arms. Mouse behaviour was recorded during a 10-min test period. The numbers of entries into, and the time spent in open and enclosed arms, were recorded. The illumination level was 100 lux at the center of the maze. For data analysis, we used the following four measures: the percentage of entries into the open arms, the time spent in the open arms (s), the number of total entries, and total distance travelled (cm). Data acquisition and analysis were performed automatically using Image EP software (see 'Image analysis').

Rotarod test

Motor coordination and balance were tested with a rotarod test. The rotarod test, using an accelerating rotarod (UGO Basile Accelerating Rotarod, Varese, Italy), was performed by placing 13-wk-old mice on rotating drums (3 cm diameter) and measuring the time each animal was able to maintain its balance on the rod. The speed of the rotarod accelerated from 4 to 40 rpm over a 5-min period.

Porsolt forced swim test

The apparatus for Porsolt forced swim test consisted of four plastic cylinders (20 cm height × 10 cm diameter). The cylinders were filled with water (23°C) up to a height of 7.5 cm. Mice were placed into the cylinders, and their behavior were recorded over a 10-min test period.

Barnes circular maze test

The Barnes task was conducted on "dry land," a white circular surface, 1.0 m in diameter, with 12 holes equally spaced around the perimeter (O'Hara & Co., Tokyo,

Japan). The circular open field was elevated 75 cm from the floor. A black Plexiglas escape box (17 × 13 × 7 cm), which had paper cage bedding on its bottom, was located under one of the holes. The hole above the escape box represented the target, analogous to the hidden platform in the Morris task. The location of the target was consistent for a given mouse, but was randomized across mice. The maze was rotated daily, with the spatial location of the target unchanged with respect to the visual room cues, to prevent a bias based on olfactory or proximal cues within the maze. The first training was started when wild type mice and sdy mice were 34 weeks old. Three trials per day were conducted for 9 successive days in the beginning (on days 5 and 6, no trial was undertaken). One day after the last training, a probe trial was conducted without the escape box, to confirm that this spatial task was acquired based on navigation using distal environment room cues. Time of latency to reach the target hole, number of errors, distance to reach the target hole, and time spent around each hole were recorded by video tracking software (Image BM, see 'Image analysis').

T-maze forced alternation task

The forced alternation task was conducted using an automatic T-maze that we devised (Figure. 3B, O'Hara & Co., Tokyo, Japan). It was constructed of white plastics runways with walls 25-cm high. The maze was partitioned off into 6 areas by sliding doors that can be opened downward. The stem of T was composed of area S2 (13 × 24 cm) and the arms of T were composed of area A1 and A2 (11.5 × 20.5 cm). Area P1 and P2 were the connecting passage way from the arm (area A1 or A2) to the start compartment (area S1). The end of each arm was equipped with a pellet dispenser that could provide food reward. The pellet sensors were able to record automatically pellet intake by the mice. One week before the pre-training, mice were deprived of food until their body weight was reduced to 80–85% of the initial level. Mice were kept on a maintenance diet throughout the course of all the T-maze experiments. Before the first trial, mice were subjected to three 10-min adaptation sessions, during which they were allowed to freely explore the T-maze with all doors open and both arms baited with food. On the day after the adaptation session, mice were subjected to a forced alternation protocol for 16 days (one session consisting of 10 trials per day; cutoff time, 50 min). The first training was started when wild type mice and sdy mice were 29 weeks old. Mice were given 10 pairs of training trials per day. On the first (sample) trial of each pair, the mouse was forced to choose one of the arms of the T (area A1 or A2), and received the reward at the end of the arm. Choosing the incorrect arm resulted in no reward and confinement to the arm for 10 sec. After the mouse consumed the pellet or the mouse stayed more than 10 sec without consuming the pellet, door that separated the arm (area A1 or A2) and

connecting passage way (area P1 or P2) would be opened and the mouse could return to the starting compartment (area S1), via connecting passage way, by itself. In this way, the potential stress could be reduced compared to the traditional forced alternation paradigm in which human experimenter brings back the mouse to the start box by hand. The mouse was then given 3 sec delay there and a free choice between both T arms and rewarded for choosing the other arm that was not chosen on the first trial of the pair. The location of the sample arm (left or right) was varied pseudo-randomly across trials using Gellermann schedule so that mice received equal numbers of left and right presentations. A variety of fixed extra-maze clues surrounded the apparatus. On the 16–21th day, delay (10, 30 or 60 sec) was applied after the sample trial. Data acquisition, control of sliding doors, and data analysis were performed by Image TM software (see 'Image analysis').

Image analysis

The applications used for the behavioral studies (Image LD, Image EP, Image BM, and Image TM) were based on the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>) and ImageJ program <http://rsb.info.nih.gov/ij/>, which were modified for each test by Miyakawa (available through O'Hara & Co., Tokyo, Japan).

Statistical analysis

Statistical analysis was conducted using Stat View (SAS institute). Data were analyzed by two-way ANOVA, or two-way repeated measures ANOVA, unless noted otherwise. In Porsolt forced swim test, mortality of each genotype was analyzed by Fisher's exact test. Values in text and graphs were expressed as mean ± SEM. All p-values reported are two tailed. Statistical significance was defined as $p < 0.05$.

List of abbreviations

alpha-CaMKII: Alpha-isoform of calcium/calmodulin dependent kinase; ANOVA: analysis of variance; DISC1: Disrupted-In-Schizophrenia 1; dysbindin-1 (DTNBP1): dystrobrevin binding protein 1; siRNA: small interfering RNA.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KTa carried out the behavioral studies of mice, performed the statistical analysis and wrote the manuscript. KTo, KN, SH and HT carried out the behavioral studies of mice. MT and TM participated in the design and coordination of the study and helped to draft the manuscript. RH supervised

the entire project, wrote the manuscript, was critically involved in the design, analysis and interpretation of the data and was responsible for performing the literature review. All authors read and approved the final manuscript.

Additional material

Additional file 1

Reduced locomotor activity in sdy mice in an open field test. (A) Total locomotor distance. (B) Count of vertical activity. (C) Time spent on the centre of the field. (D) Count of stereotypic behavior. Data were analyzed by two-way repeated measures ANOVA.

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Additional file 2

Reduced locomotor activity in sdy mice in a light/dark transition test. (A) Distance traveled in the light and dark boxes. (B) Time course of the distance traveled in the dark box. (C) Time spent in the light box. (D) Number of transitions between the light and dark boxes. (E) Latency of first entry into light box. Data were analyzed by two-way ANOVA and two-way repeated measures ANOVA.

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Additional file 3

Normal anxiety-like behavior in sdy mice in elevated plus maze test. (A) Total number of arm entries. (B) Percentage entries into open arms. (C) Distance traveled. (D) Percentage entries into open. Data were analyzed by two-way ANOVA and two-way repeated measures ANOVA.

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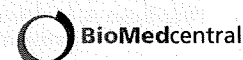
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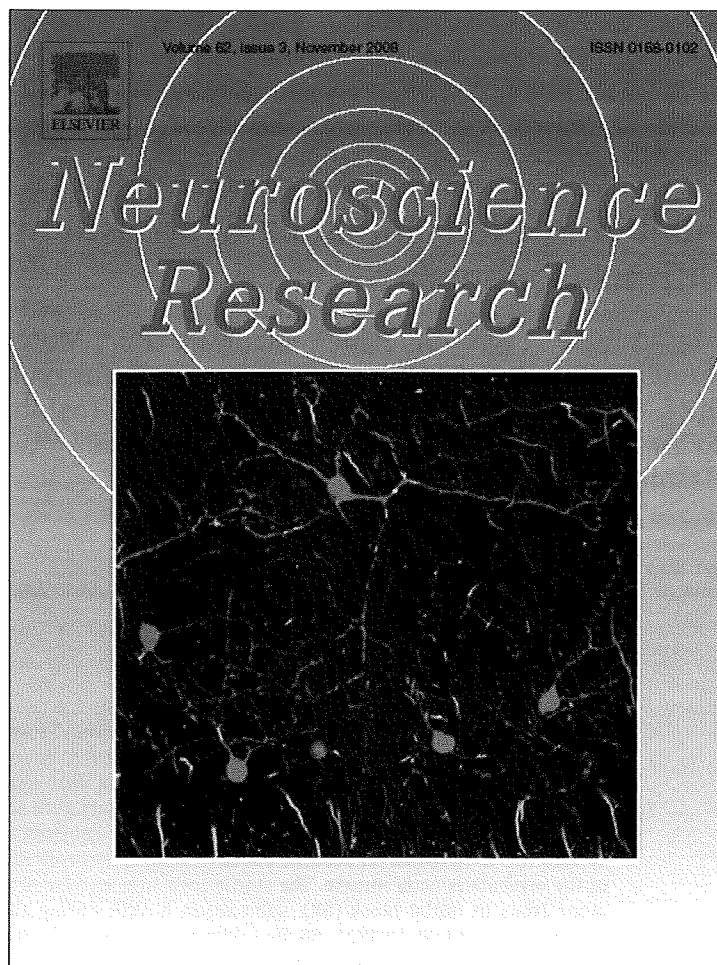
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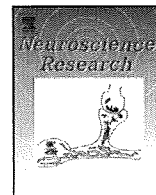


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Impaired prepulse inhibition and habituation of acoustic startle response in Japanese patients with schizophrenia

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ABSTRACT

Prepulse inhibition (PPI) and habituation of the acoustic startle reflex (ASR) are considered to be candidate endophenotypes of schizophrenia. However, to our knowledge, only one group has investigated these startle measures in Asian patients with schizophrenia. In the present study, we evaluated these startle measures in 51 Japanese patients with schizophrenia and compared them with those of 55 healthy age- and sex-matched Japanese controls. A human startle response monitoring system was used to deliver acoustic startle stimuli, and record and score the electromyographic activity of the orbicularis oculi muscle. The startle measures examined were mean magnitude of ASR to pulse alone trials in initial block (SR), habituation of ASR during the session (HAB), and PPI at prepulse intensities of 82 dB (PPI82), 86 dB (PPI86), and 90 dB (PPI90) sound pressure level. SR was not significantly different between the patients and controls. Patients displayed significantly reduced HAB and PPI for all prepulse intensities compared to controls. The greatest statistical difference in PPI between patients and controls was found with PPI86. This did not correlate with any clinical variable in each group. Our results indicate that PPI and habituation of ASR are impaired in Asian patients with schizophrenia.

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1. Introduction

Impaired sensorimotor gating is considered to be a common psychophysiological feature of schizophrenia that could theoretically lead to a variety of severe dysfunctions in perception, attention, and thinking (McGhie and Chapman, 1961). Prepulse inhibition (PPI) and habituation of the acoustic startle reflex (ASR)

are the most common psychophysiological indexes of sensorimotor gating.

PPI is usually defined as a reduction of the startle reflex due to weak sensory prestimulation (Braff et al., 1978). PPI is thought to regulate sensory input by filtering out irrelevant or distracting stimuli, to prevent sensory information overflow, and to allow for selective and efficient processing of relevant information (Braff et al., 1978, 1992; Geyer and Braff, 1987). Reductions in PPI have been consistently demonstrated in schizophrenia (reviewed by Braff et al., 2001a,b). PPI is not only reduced in schizophrenia patients but also in unaffected relatives (Cadenhead et al., 2000; Kumari et al., 2005b), and it has showed substantial heritability (Anokhin et al., 2003). Recently, PPI of ASR has been considered a candidate endophenotype of schizophrenia (Braff and Light, 2005).

Habituation is defined as the decrement in behavioral responses to repeated presentations of an identical, initially novel

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stimulus that is not due to sensory adaption or effector fatigue (Geyer and Braff, 1982). Reduced habituation of ASR is also thought to reflect impaired gating of repeatedly presented simple stimuli that might result in cognitive disruption by sensory overload. However, the impairment of habituation in patients with schizophrenia is still a controversial issue. Several studies demonstrated reduced habituation in patients with schizophrenia (Geyer and Braff, 1982; Braff et al., 1992; Parwani et al., 2000; Ludewig et al., 2003a) while others could not replicate these findings (Cadenhead et al., 2000; Braff et al., 2001a,b; Leumann et al., 2002; Ludewig et al., 2002; Mackeprang et al., 2002; Oranje et al., 2002; Kumari et al., 2002, 2004, 2005a,b; Kunugi et al., 2007a,b).

A recent study (Swerdlow et al., 2005) has suggested there may be an ethnic difference in PPI and the magnitude of the startle response between normal Caucasian and Asian subjects. Asians showed decreased ASR amplitude and increased PPI compared with Caucasians. However, the difference in PPI disappeared when the subjects were matched for ASR amplitude.

Findings from a study by Swerdlow et al. (2005) suggest that the profile of startle measures, which includes startle amplitude, habituation and PPI, might be different in Asian patients with schizophrenia compared with Caucasian patients. However, to our knowledge, only one group (Kunugi et al., 2007a,b) has reported on the difference in startle measures between patients with schizophrenia and healthy controls in Asians (Japanese). Japanese patients with schizophrenia showed decreased amplitude of startle response and decreased PPI compared with healthy Japanese controls, and there was no significant difference between two groups in habituation. In this study, we evaluated startle measures in Japanese patients with schizophrenia and compared them with those of healthy Japanese subjects matched for age, sex, years of education and estimated premorbid IQ. The number of subjects in each group was more than 50 and sufficiently large. In

addition, we investigated the relationship between startle measures and clinical variables.

2. Methods

2.1. Subjects

Fifty-one Japanese patients with schizophrenia and fifty-five Japanese healthy control subjects participated in this study. Patients were recruited at Osaka University Hospital. Controls were recruited by local advertisements in Osaka.

Consensus diagnosis was made for each patient by at least two trained psychiatrists, according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV; American Psychiatric Association, 1994) criteria, based on clinical interview and other available information including medical records and other research assessments. No patient was diagnosed by medical records alone. Controls were psychiatrically, medically, and neurologically healthy volunteers who were not receiving psychiatric medication and had no first- or second-degree relatives with psychosis. Controls were screened for psychiatric disease with the non-patient edition of the modified structured clinical interview for the Diagnostic and Statistical Manual-Fourth Edition Axis I disorders (SCID-I/NP) (First et al., 1997). Premorbid IQ was estimated using the Japanese Adult Reading Test (Matsuoka et al., 2006). Symptoms of schizophrenia were assessed with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), and also by the five-factor model of schizophrenia presented by Lindenmayer et al. (1994). Drug-related extrapyramidal symptoms in schizophrenia patients were assessed using the Drug-Induced Extrapyramidal Symptoms Scale (Yagi and Inada, 1996). None of the subjects had any hearing impairments. Pregnant or lactating women were not included. Clinical and demographic characteristics of all subjects are presented in Table 1. Patients and controls

Table 1
Demographic data

	Schizophrenia (N = 51)		Control (N = 55)		χ^2	d.f.	p		
Male:female	29:22		28:27		0.38	1	0.56		
Current nonsmoker:smoker	37:14		38:17		0.15	1	0.83		
	Schizophrenia (N = 51)			Control (N = 55)			t	d.f.	p
	Mean	S.D.	Range	Mean	S.D.	Range			
Age (year)	38.1	10.2	20–59	40.4	10.1	21–60	1.14	104	0.26
Education (year)	14.1	2.2	9–18	14.7	1.9	12–18	1.64	104	0.10
Estimated premorbid IQ	100.0	10.4	77.8–120.2	103.1	6.9	87.5–119.3	1.80	85.84	0.08
Age of onset (year)	23.7	8.1	10–44						
Duration of illness (year)	14.4	10.7	0–35						
Duration of medicated period (year)	11.6	10.3	0–34						
Duration of unmedicated period (year)	1.7	4.0	0–26						
Dose of antipsychotic medication (chlorpromazine-equivalent mg per day)	701.7	518.3	100–2366						
Patients with antipsychotics, typical:atypical (both)	26:47 (22)								
Overall severity of Drug-Induced Extrapyramidal Symptoms Scale	0.5	0.7	0–2						
Positive and Negative Syndrome Scale									
Total score	76.0	21.3	11–124						
Positive syndrome	18.2	7.0	1–36						
Negative syndrome	19.4	7.1	2–36						
General psychopathology	38.4	10.6	8–64						
Five-factor model									
Negative	17.2	6.7	0–30						
Positive	14.7	6.0	2–30						
Excitement	7.3	3.3	0–18						
Cognitive	12.1	4.2	4–23						
Depression/anxiety	9.5	3.4	2–18						

were matched for age, sex distribution, years of education and estimated premorbid IQ. All patients were treated with antipsychotics, and had few drug-related extrapyramidal side effects.

The study procedure was conducted according to the Helsinki declaration and approved by the Research Ethical Committee of Osaka University. All subjects gave written informed consent after the study procedures had been fully explained to them.

2.2. Startle response measurement

2.2.1. Apparatus and stimuli

A commercial computerized human startle response monitoring system (Startle Eyeblink Reflex Analysis System Map1155SYS, NIHONSANTEKU Co., Osaka, Japan) was used to deliver acoustic startle stimuli, and record and score the corresponding electromyographic activity. Stimulus presentation and data acquisition were controlled through a laptop computer with Windows XP operating system installed on it. Sound pressure levels (SPL) were calibrated with precision every 1 dB using Artificial Ears (type 4153, Brüel & Kjaer Sound and Vibration Measurement A/S, Denmark) and a sound level meter NL-20 (RION Co., Kokubunji, Japan). All the auditory stimuli and the background noise were produced by a custom built tone and noise generator and delivered binaurally to the subjects through stereophonic headphones (type DR-531, Elega Acous. Co. Ltd., Tokyo, Japan) with hard plastic bells that were connected to an audio headphone amplifier (type AT-HA20, Audio-Technica Co., Machida, Japan). A 24-bit PCMCIA soundcard (Audigy 2 ZS Notebook, Creative Technologies Ltd., Creative Resource, Singapore) was directly plugged into the stimulus computer.

Startle eyeblink electromyographic responses were recorded from the left orbicularis oculi muscle with two Ag/AgCl disposable electrodes (sensor area 15 mm²) filled with wet gel. The first electrode (Blue Sensor N-00-S, Ambu, Ballerup, Denmark) was positioned approximately 1 cm directly below the pupil of the left eye and low enough to not touch the lower eyelid, while the second electrode (Blue Sensor M-00-S, Ambu, Ballerup, Denmark) was placed laterally and slightly superior to the first one, with the centers of the electrodes separated by approximately 2 cm. The impedance between the two electrodes was measured and deemed acceptable if below 10 k Ω . The impedance was measured with an electrode impedance meter (MaP811, NIHONSANTEKU Co., Osaka, Japan) at a measurement frequency = 30 Hz. The ground electrode (Blue Sensor M-00-S) was placed on the left angle of the mandible. The skin area at the electrode site was cleaned with a cotton swab saturated with rubbing alcohol, then prepared by gently rubbing a small amount of NUPREP EEG & ECG Skin Prepping Gel (Bio-Medical Instruments Inc., Warren, USA), and cleaned with a cotton swab saturated with rubbing alcohol again.

Electromyography (EMG) data were measured with an EMG Telemeter (PolyTele EMG, NIHONSANTEKU Co., Osaka, Japan). The measurement condition was adjusted as follows: the time constant was 0.03 s which was equivalent to the low frequency filter of 5 Hz; the high frequency filter was 300 Hz. The sensitivity of the amplifier was 1000 times. The amplification gain control for the EMG signal was kept constant for all subjects. EMG data were digitized with a 12-bit A/D converter (MaP222, NIHONSANTEKU Co., Osaka, Japan) and collected on the PC. The sampling frequency was 1 kHz. Sampling on each trial began 1000 ms prior to the onset of the startle eliciting stimulus and continued for 1000 ms after the onset of the startle eliciting stimulus. The resulting data were baseline corrected with a moving average. The eyeblink amplitude of every startle response was defined as the voltage of the peak activity of the EMG within a latency window of 20–85 ms following startle eliciting stimulus onset. The data were stored and exported for analyses in microvolt values.

2.2.2. The stimulus sequence

Subjects were tested in a startle paradigm which consisted of three blocks with a continuously presented 70 dB SPL background white noise. Pulse stimuli consisted of broadband white noises with an instantaneous rise/fall time lasting for 40 ms and presented at 115 dB SPL. Prepulse stimuli were also broadband white noises with an instantaneous rise/fall time lasting for 20 ms and presented at three different intensities (82 dB, 86 dB, and 90 dB SPL).

The lead interval (LI) (from prepulse onset to pulse onset) in our study was set at 120 ms. In the literature, PPI at LI of 60 ms (Braff et al., 1978; Kumari et al., 2002; Ludewig et al., 2002, 2003a,b; Leumann et al., 2002; Swerdlow et al., 2006) or 120 ms (Braff et al., 1992, 2001a,b, 2005; Kumari et al., 2000; Mackeprang et al., 2002; Oranje et al., 2002; Parwani et al., 2000; Perry et al., 2002; Quednow et al., 2006; Weike et al., 1999) often enabled discrimination between patients with schizophrenia and controls. However, it has been demonstrated that Asian patients with schizophrenia have significant lower PPI at LI of 120 ms in three prepulse intensities (82 dB, 86 dB and 90 dB) than controls, while there is no difference between patients and controls in PPI at LI of 60 ms in any of these three prepulse intensities (Kunugi et al., 2007a). Based on this finding we decided to employ LI of 120 ms in the present study of Asian subjects.

In block 1, the startle response for pulse alone trial (PA trial) was recorded six times. Block 2 consisted of PA trials or trials of pulse with prepulse at three intensities (PP trials), performed eight times for each condition. Block 3 was the same as block 1 to see the habituation phenomenon. All trials were presented in a fixed pseudorandom order, separated by inter-trial intervals of 15–25 s (20 s on average). The startle paradigm consisted of a total of 44 trials. Together with 5 min acclimation to the background noise, the session lasted approximately 20 min.

2.2.3. Procedure

The experiment took place in a dimly lit electrically and acoustically shielded chamber where the temperature was kept comfortable (approximately 20 °C). The EMG recordings were obtained with the subjects sitting comfortably in a chair in a moderately reclined position.

Upon arriving at the laboratory, each subject read and signed an informed consent form and completed a brief medical history questionnaire including demographic data. The subjects were informed about the general purpose of the study, about the stimuli and procedure, and that they could withdraw from the study at any time. Subjects were told that the experiment aimed to measure their reactivity to a number of noise bursts. There was no restriction on smoking intake but we took care to avoid testing smokers within 30 min of smoking a cigarette, as this could potentially increase PPI (Kumari et al., 2001).

Subjects were then seated in the testing room. During the task, the subjects were instructed to keep their eyes open and to maintain their gaze on a fixed point 100 cm away. Thereafter, the skin area at the electrode site was cleaned and the electrodes were attached. The door to the experimental chamber was closed.

2.2.4. Response scoring and data reduction

The following startle response measures were examined: (i) SR, average eyeblink amplitude of startle response to PA trials in block 1; (ii) HAB, habituation of the startle response during the session, computed as the percentage of amplitude reduction between block 1 and block 3 by the formula [(1 – average eyeblink amplitude of startle response in block 3/average eyeblink amplitude of startle response in block 1) \times 100]; (iii) PPI82, PPI86, PPI90: prepulse inhibition at prepulse intensities of 82 dB, 86 dB, and 90 dB SPL,

respectively. PPI for each prepulse intensity was computed as the percentage of amplitude reduction between PA and PP trials in block 2 by the formula $[(1 - \text{average eyeblink amplitude of startle response to PP trials in block 2} / \text{average eyeblink amplitude of startle response to PA trials in block 2}) \times 100]$.

Prior to data analyses, exclusion criteria were established for both trials and subject data. To minimize the potential impact of voluntary and spontaneous blink activity on startle measures, trials were discarded if the voltage of their peak activity of the EMG within a latency window of 0–20 ms following startle eliciting stimulus onset was more than 30 μV . Subjects were excluded from further analyses as nonresponders, if their magnitudes of startle response were less than 30 μV in more than half of the trials in block 1. The SR was not evaluated if more than half of the trials in block 1 were discarded. Analyses for HAB were not conducted for participants who had more than half of their trials in block 1 or block 3 discarded. Analyses of PPI for each prepulse intensity were not conducted if more than half of the PP trials at the corresponding prepulse intensity or PA trials in block 2 were discarded. Two patients and one control could not complete the session because they could not stand the startle stimuli, and were excluded from the analyses. There were 12 nonresponders in the patient group and 8 in the control group. There was no significant difference in the distribution of nonresponders ($\chi^2 = 1.82$, d.f. = 1, $p = 0.22$) between controls and patients. Nonresponders and responders did not differ significantly in demographic characteristics.

2.2.5. Statistical analysis

Individual t -test and χ^2 -tests (Fisher's exact test when appropriate) were used to compare means and categorical proportions, respectively. In addition, the differences in startle measures between patients and controls were analyzed by analysis of covariance (ANCOVA) with sex and smoker status as covariates, since these variables are considered to have an effect on startle measures (Swerdlow et al., 1993, 1997; Kumari et al., 1996, 2001). PPI measures were examined with analysis of variance (ANOVA) with repeated measures of prepulse intensities. Probability levels

were adjusted using the Greenhouse-Geisser correction. Pearson r correlations were performed to assess the possible correlation between startle measures and clinical characteristics. All p -values reported were two-tailed. Statistical significance was considered when p -value was <0.05 . All statistical analyses were performed using SPSS ver12 (SPSS Japan, Tokyo, Japan).

3. Results

3.1. Difference in startle measures between patients with schizophrenia and healthy controls

Fig. 1 presents the difference in startle measures between patients with schizophrenia and healthy controls. There was no significant difference between patients and controls in SR (Fig. 1a: $t = 1.56$, d.f. = 99, $p = 0.12$). Patients with schizophrenia exhibited significantly reduced habituation compared with controls (Fig. 1b: $t = 3.12$, d.f. = 71, $p = 0.0026$). This group difference for HAB did not disappear after the data were analyzed by ANCOVA (sex and smoker status as a covariate: $F = 9.57$, d.f. = 1, 70, $p = 0.0029$).

Patients had significantly decreased PPI for all prepulse intensities (Fig. 1c, PPI82: $t = 2.08$, d.f. = 77, $p = 0.041$; PPI86: $t = 2.44$, d.f. = 41.96, $p = 0.019$; PPI90: $t = 2.28$, d.f. = 76, $p = 0.025$). These group differences for PPI remained after analysis of the data using ANCOVA (sex and smoker status as a covariate, PPI82: $F = 4.83$, d.f. = 1, 76, $p = 0.031$; PPI86: $F = 6.98$, d.f. = 1, 75, $p = 0.010$; PPI90: $F = 5.35$, d.f. = 1, 75, $p = 0.023$).

We examined the possible effects of prepulse intensity (within-subjects factors), sex, smoker status, and case-control status (between-subjects factors) on PPI by using ANOVA with repeated measures on prepulse intensities. There was a significant main effect of case-control status on PPI ($F = 6.04$, d.f. = 1, 70, $p = 0.016$); however, we could not detect a significant effect of sex, smoker status or prepulse intensity on PPI. There was no significant interaction among sex, smoker status, case-control status and prepulse intensity.

In the literature it has been reported sex difference in PPI in normal subjects, with women exhibiting less PPI than men (Abel

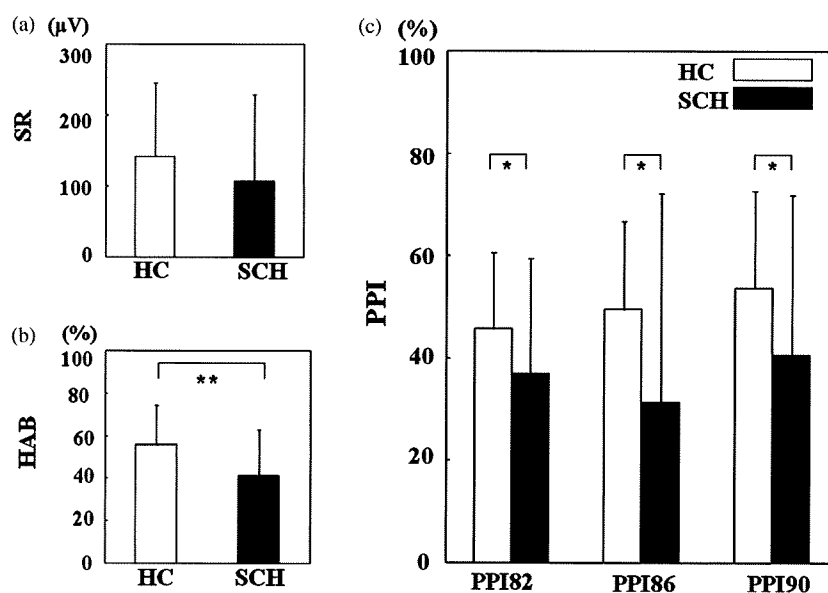


Fig. 1. Comparison of startle measure between patients with schizophrenia and healthy controls. (a) Comparison of SR between SCH and HC (SCH: $N = 47$; HC: $N = 54$). (b) Comparison of HAB between SCH and HC (SCH: $N = 33$; HC: $N = 40$). (c) Comparison of PPI82, PPI86, PPI90 between SCH and HC (PPI82: SCH, $N = 35$; HC, $N = 43$; PPI86: SCH, $N = 34$; HC, $N = 44$; PPI90: SCH, $N = 35$; HC, $N = 44$). SCH, patients with schizophrenia; HC, healthy controls; SR, mean magnitude of acoustic startle reflex (ASR) to pulse alone trials in block 1; HAB, Habituation of ASR in block 3 compared with block 1; PPI82, PPI86, PPI90: prepulse inhibition of ASR in prepulse of 82 dB, 86 dB, and 90 dB, respectively. The figures show the means, and the error bars show the S.D. Individual t -test: * $p < 0.05$, ** $p < 0.01$.

Table 2
Relationships of startle measures to clinical variables in controls and patients

	SR	HAB	PPI90	PPI86	PPI82
Controls					
N	54	40	43	44	44
Age	-0.158	0.176	-0.300	-0.163	-0.086
Education	0.127	0.177	0.277	0.176	0.126
Estimated premorbid IQ	-0.133	-0.131	0.226	0.270	0.260
Schizophrenia					
N	47	33	35	34	35
Age	-0.241	0.212	-0.486**	-0.258	-0.338*
Education	0.200	0.042	0.172	0.005	0.250
Estimated premorbid IQ	0.154	-0.504**	0.170	0.046	-0.008
Age of onset	-0.020	0.331	-0.347*	-0.151	-0.183
Duration of illness	-0.215	-0.064	-0.204	-0.145	-0.190
Duration of medicated period	-0.191	-0.291	-0.180	-0.186	-0.216
Duration of unmedicated period	0.007	0.185	-0.072	0.032	0.030
Dose of antipsychotic medication (chlorpromazine-equivalent mg per day)	-0.243	-0.321	-0.113	-0.050	-0.204
Positive and Negative Syndrome Scale					
Total score	0.084	-0.206	0.212	0.017	0.204
Positive syndrome	-0.007	-0.280	0.057	-0.250	0.107
Negative syndrome	0.079	-0.050	0.148	0.144	0.137
General psychopathology	0.121	-0.201	0.284	0.088	0.244
Five-factor model					
Negative	-0.054	-0.294	0.001	-0.319	0.037
Positive	0.048	-0.071	0.212	0.196	0.201
Excitement	0.156	-0.081	0.294	0.151	0.298
Cognitive	-0.019	-0.242	-0.075	-0.252	-0.051
Depression/anxiety	0.305*	-0.052	0.353*	0.121	0.308

Valuables are Pearson *r*. SR, mean magnitude of acoustic startle reflex (ASR) to pulse alone trials in block 1; HAB, habituation of ASR in block 3 compared with block 1; PPI82, PPI86, PPI90: prepulse inhibition of ASR in prepulse of 82 dB, 86 dB, and 90 dB, respectively. Pearson *r* correlation; **p* < 0.05; ***p* < 0.01.

et al., 1998; Swerdlow et al., 1997). Thus, we examined sex difference across groups and group difference for sex. There was no significant difference for the distribution of age, years of education and estimated premorbid IQ between controls and patients by sex and between male and female in each group. In the control group, males exhibited larger PPI than females at all prepulse intensities (PPI90: male 62.8 ± 15.8 , female 43.5 ± 17.4 , $t = 3.82$, d.f. = 41, $p < 0.001$; PPI86: male 55.2 ± 15.6 , female 42.8 ± 16.9 , $t = 2.53$, d.f. = 42, $p = 0.015$; PPI82: male 52.6 ± 13.7 , female 37.4 ± 12.2 , $t = 3.85$, d.f. = 42, $p < 0.001$). These sex differences for PPI remained after analysis of the data using ANCOVA (smoker status as a covariate, PPI90: d.f. = 1, 40, $F = 16.34$, $p < 0.001$; PPI86: d.f. = 1, 41, $F = 6.13$, $p = 0.018$; PPI82: d.f. = 1, 41, $F = 18.95$, $p < 0.001$). On the other hand, we could not detect significant sex difference in PPI at any prepulse intensity in the patient group. Male patients had lower PPI at all prepulse intensities compared with male controls (PPI90: 62.8 ± 15.8 , SCH 39.2 ± 34.4 , $t = 2.94$, d.f. = 40, $p = 0.005$; PPI86: HC 55.2 ± 15.6 , SCH 29.3 ± 49.1 , $t = 2.45$, d.f. = 41, $p = 0.019$; PPI82: HC 52.6 ± 13.7 , SCH 39.6 ± 22.8 , $t = 2.32$, d.f. = 41, $p = 0.025$). These group differences for PPI also remained after analysis of the data using ANCOVA (smoker status as a covariate, PPI90: d.f. = 1, 39, $F = 8.58$, $p = 0.006$; PPI86: d.f. = 1, 40, $F = 6.17$, $p = 0.017$; PPI82: d.f. = 1, 40, $F = 5.06$, $p = 0.030$). However, female patients did not show significant difference in PPI at any prepulse intensity compared to female controls.

3.2. Relationship of startle measures to clinical variables in controls and patients

The relationship of startle measures to clinical variables in controls and patients is shown in Table 2. In the control group, age, years of education and estimated premorbid IQ did not correlate significantly with any startle measures. Similarly, in the patient group, PPI86, which exhibited the greatest statistical difference in PPI between patients and controls, did not correlate with any

clinical variables. On the other hand, age showed negative correlation with PPI82 ($p = 0.047$) and PPI90 ($p = 0.0031$). Estimated premorbid IQ was correlated negatively with HAB ($p = 0.0028$). Age at onset of disease had a significant correlation with PPI90 ($p = 0.041$). Depression/anxiety symptoms scores on the five-factor model of schizophrenia (Lindenmayer et al., 1994) correlated with SR ($p = 0.037$) and PPI90 ($p = 0.038$). No other significant correlation was found between startle measures and clinical variables in the patient group.

4. Discussion

In this study, Japanese patients with schizophrenia demonstrated significantly decreased PPI for all prepulse intensities compared with Japanese healthy controls. Patients also exhibited significantly smaller habituation. We could not detect a group difference in SR. In the patient group, PPI86, which exhibited the greatest statistical difference in PPI between patients and controls, did not correlate with any clinical variables. On the other hand, other startle measures were associated with clinical variables, namely age, age at onset of disease, estimated premorbid IQ and depression/anxiety symptoms.

Kunugi et al. (2007a,b) are the first group reporting on PPI and HAB of ASR in Asian (Japanese) patients with schizophrenia. Our main finding that schizophrenia patients showed decreased PPI at prepulse intensities of 82, 86 and 90 dB SPL is consistent with that of Kunugi et al. studies (2007a,b). However, our results do not support the decrease in the magnitude of startle to pulse alone trial in Asian patients with schizophrenia reported by Kunugi et al. (2007a,b). Patients included in our study had a smaller magnitude of ASR compared with controls. However, this group difference did not reach statistical significance. Although some studies reported a significantly reduced startle magnitude in patients with schizophrenia (Meincke et al., 2004b; Quednow et al., 2006), the vast majority of previous studies did not find a difference in the

magnitude of ASR for PA trials (Braff et al., 1978, 1992, 1999, 2001a,b, 2005; Grillon et al., 1992; Ford et al., 1999; Weike et al., 1999; Cadenhead et al., 2000; Parwani et al., 2000; Kumari et al., 2002, 2004, 2005a; Leumann et al., 2002; Ludewig et al., 2002, 2003a; Mackeprang et al., 2002; Duncan et al., 2003). This is consistent with our findings.

In studies by Kunugi et al. (2007a,b), habituation did not differ between patients and controls. Conversely, in our study, habituation was significantly reduced in patients with schizophrenia compared with controls, and this group difference remained significant after the data were analyzed by ANCOVA. The existence of deficits of habituation in patients with schizophrenia remains controversial, even in non-Asian subjects. Some studies found reduced habituation in schizophrenia (Geyer and Braff, 1982; Braff et al., 1992; Parwani et al., 2000; Akdag et al., 2003; Ludewig et al., 2003a; Meincke et al., 2004a), while others have failed to find such deficits (Cadenhead et al., 2000; Braff et al., 2001a,b; Kumari et al., 2002, 2004, 2005a,b; Leumann et al., 2002; Ludewig et al., 2002; Mackeprang et al., 2002; Oranje et al., 2002). Possible explanations for this inconsistent result might be related to difference in measurement apparatus or clinical samples. Moreover, estimated premorbid IQ correlated negatively with HAB in our patients (Table 2); however, this variable was not shown in Kunugi et al. (2007a,b) studies. Thus, possible difference in patients' premorbid IQ between studies might account for the discrepancy in the findings. Further replication studies from other Asian independent groups will be necessary.

In our study, patients with schizophrenia showed significantly reduced PPI for all prepulse intensities. This is consistent with findings from most previous studies including the study by Kunugi et al. (2007a,b). Moreover, many studies have provided evidence suggesting that PPI deficits in patients with schizophrenia are improved by antipsychotics. Especially atypical antipsychotics appear to have a close association with PPI improvement in schizophrenia (Braff et al., 2001b; Kumari et al., 2002; Leumann et al., 2002; Mackeprang et al., 2002; Oranje et al., 2002; Perry et al., 2002; Duncan et al., 2003; Carroll et al., 2004; Quednow et al., 2006; Swerdlow et al., 2006; Wynn et al., 2007). All patients included in our study were treated with antipsychotics, and most of them were on atypical antipsychotics. PPI impairment in the patient group may have been more apparent if they were not under medicated with atypical antipsychotics at the time of the examination.

Our result that significant group difference was only detected in male is in line with findings of Kumari et al. (2004) and Kunugi et al. (2007b) studies. However, Braff et al. (2005) reported prepulse inhibition deficits in female patients with schizophrenia. Sex dimorphism of PPI has been reported in normal subjects, with lower levels of PPI in females than in males (Abel et al., 1998; Swerdlow et al., 1997). Normal females had fluctuations in PPI across the menstrual cycle, with the lowest levels of PPI manifested in the mid-luteal phase (Swerdlow et al., 1997). Possible explanations for the lack of group difference between female patients and controls may be that some of the female patients with schizophrenia might have menstrual disorder as a side effect of their medication. Further studies concerning menstrual cycle are needed to clarify impairment of PPI in female patient with schizophrenia.

Although some relationships were found between startle measures and clinical variables, we found that PPI86, which showed the greatest statistical difference in PPI between patients and controls, did not correlate with any clinical variable. The effect of aging on PPI remains controversial. For instance, Ludewig et al. (2003b) reported no aging effect on PPI in healthy subjects, which is in line with our results. On the other hand, Ellwanger et al. (2003)

reported a U-shaped function between age and PPI (greatest PPI in intermediate ages). Although one study reported a negative correlation between age and PPI in patients with schizophrenia (Braff et al., 1999), the majority of previous studies found no significant correlation between these two variables (Kumari et al., 2000, 2008; Parwani et al., 2000; Ludewig and Vollenweider, 2002; Meincke et al., 2004b; Kunugi et al., 2007a). Most previous studies found no association between age at onset of disease and PPI at any intensity (Ludewig and Vollenweider, 2002; Perry et al., 2002; Kumari et al., 2005a; Kunugi et al., 2007a), except for one study (Kumari et al., 2000). Little is known about the relationship between startle measures and estimated premorbid IQ. Consistent with our findings, Kumari et al. (2008) reported no significant relationship between PPI and predicted IQ, as assessed using the National Adults Reading test. Thus the effects of age, age at onset of disease and estimated premorbid IQ on PPI appear to be rather small in patients with schizophrenia. Further studies are needed to confirm this argument.

Although PPI has been reported in association with positive symptoms (Braff et al., 1999; Weike et al., 1999) and negative symptoms (Braff et al., 1999; Ludewig and Vollenweider, 2002) of schizophrenia, most previous studies do not support a link between PPI and psychiatric symptoms (Perry et al., 2002; Swerdlow et al., 2006; Kunugi et al., 2007a; Kumari et al., 2008). This is consistent with our findings indicating no correlation between startle measures and positive or negative symptoms. To our knowledge, this is also the first study to investigate the relationship between PPI and psychiatric symptoms using the five-factor model of schizophrenia (Lindenmayer et al., 1994). Despite the fact that medication status may affect the relationship of psychiatric symptoms to PPI in schizophrenia patients (Duncan et al., 2006), a positive correlation of depression/anxiety symptoms with SR and PPI90 in our study was an unexpected finding which needs further investigation before any conclusion can be drawn.

With regard to the medication, the dose of antipsychotic medication (chlorpromazine-equivalent mg per day), as well as the duration of the medicated and unmedicated periods showed no significant correlation with any startle measure. Although findings from several studies suggest PPI deficits improvement in patients with schizophrenia by antipsychotic medication, especially atypical antipsychotics (Braff et al., 2001b; Kumari et al., 2002; Leumann et al., 2002; Mackeprang et al., 2002; Oranje et al., 2002; Perry et al., 2002; Duncan et al., 2003; Carroll et al., 2004; Quednow et al., 2006; Swerdlow et al., 2006; Wynn et al., 2007), earlier studies have reported no relationship between PPI and dose of antipsychotic medication (Ludewig and Vollenweider, 2002; Weike et al., 1999) or duration (Weike et al., 1999). Recently Vollenweider et al. (2006) suggested that clozapine enhances PPI in healthy humans with low but not with high PPI levels. On the other hand, haloperidol failed to increase PPI in subjects exhibiting low levels of PPI, although PPI was attenuated in those subjects with high sensorimotor gating levels (Csomor et al., 2008). Effect of antipsychotics on PPI might differ depending on medications or severity of PPI deficits. Longitudinal studies evaluating PPI before and after medication change will help elucidate the effect of antipsychotics on PPI in patients with schizophrenia.

5. Conclusions

Our results indicate that Asian patients with schizophrenia exhibit impaired PPI and habituation. Further investigation is needed to elucidate the relationship between sensorimotor gating deficits and clinical aspects of patients with schizophrenia.

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Letter to the Editor

Difficulty identifying spinocerebellar ataxia 17 from preceding psychiatric symptoms

SPINOCEREBELLAR ATAXIA (SCA) with preceding psychiatric symptoms has only ever been reported for SCA17.^{1,2} SCA17, a rare autosomal dominant disorder, is characterized by various neurological symptoms and/or psychiatric symptoms. SCA17 with psychiatric symptoms is often diagnosed as a variety of psychiatric disorders such as schizophrenia and bipolar disorder before the onset of neurological symptoms.¹ SCA17 is a polyglutamine disease caused by expanded CAG/CAA repeats in the TATA box-binding protein (*TBP*) gene.¹ Here, we report two rare cases of undefined hereditary SCA other than SCA17 with preceding psychiatric symptoms.

A 65-year-old man had presented with hypomanic and depressive episodes on more than 10 occasions since the age of 18. He was diagnosed with bipolar disorder at the age of 45. The patient noticed progressive unsteadiness of gait from 58 years of age, with double vision and difficulties in fastening a button.

A 54-year-old man had exhibited social withdrawal since he was in high school. He complained of cenesthopathy and persecutory delusion since his 30s, and was diagnosed with schizophrenia. He complained of feeling vertigo since 47 years of age, and subsequently reported further symptoms, such as double vision, gait disturbance, dysphagia and slurred speech. There were past histories of paralytic ileus and aspiration pneumonia at 53 years of age. He had autonomic symptoms, such as mild orthostatic hypotension and severe constipation.

Both patients had limb and gait ataxia, intention tremor, saccades during slow pursuit, gaze nystagmus and convergent disturbance, and had mild rigidity only in the former patient. They had no other neurological symptoms. They had several biological relatives with SCA. The latter had relatives with progressive muscular dystrophy and schizophrenia. Brain magnetic resonance imaging indicated atrophy of the cerebellum (hemisphere and vermis) in both cases; additionally lower pons in the latter case. They were diagnosed with SCA in their late 50s. Mutation analysis failed to identify any mutations in well-known causative genes reported in Japan for SCA1, SCA2, DRPLA, SCA3 and SCA6 in the former patient. No abnormally expanded triplet repeats were identified in the *TBP* gene (SCA17) in both patients. Their mental state is relatively stable with medication (patient 1, paroxetine 40 mg/day and taltirelin hydrate 10 mg/day; patient 2, aripiprazole 12 mg/day),

while their neurological symptoms and cerebral atrophy are gradually progressing. These patients met the diagnostic criteria for schizophrenia or bipolar disorder from DSM-IV and matched the criteria of SCA from the Japanese Ministry of Health, Labor and Welfare. Informed consent was obtained from both patients.

Because the presence of pre-existing psychiatric symptoms was consistent with SCA17,¹ we suspected that these symptoms were referable to SCA17, but SCA17 was excluded in both cases on genetic analysis. SCA is a heterogeneous syndrome with similar clinical phenotypes, such as ataxia and cerebellar atrophy, and is caused by different genetic abnormalities at different chromosomal loci. Some characteristic symptoms are considered to be useful to distinguish several subtypes of SCA. This approach failed to identify the subtype of SCA in the present patients. We suggest that it is difficult to identify SCA17 on the basis of preceding psychiatric symptoms.

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Brief Research Communication

No Association Between Tagging SNPs of SNARE Complex Genes (STX1A, VAMP2 and SNAP25) and Schizophrenia in a Japanese Population

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Abnormalities in neural connections and the neurotransmitter system appear to be involved in the pathophysiology of schizophrenia. The soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, which consists of Syntaxin1A, vesicle-associated membrane protein 2 (VAMP2) and synaptosomal-associated protein 25 kDa (SNAP25), plays an important role in the neurotransmitter system, and is therefore an attractive place to search for candidate genes for schizophrenia. We conducted a two-stage genetic association analysis of Syntaxin1A (*STX1A*), VAMP2 and SNAP25 genes with schizophrenia (first-set screening samples: 377 cases and 377 controls, second-set confirmation samples: 657 cases and 527 controls). Based on the linkage disequilibrium, 40 SNPs (*STX1A*, 8 SNPs; VAMP2, 3 SNPs; SNAP25, 29 SNPs) were selected as 'tagging SNPs'. Only nominally significant associations of an SNP (rs12626080) and haplotype (rs363014 and rs12626080) in *SNAP25* were detected in the first-set screening scan. To validate this significance, we carried out a replication analysis of these SNP and haplotype associations in second-set samples with a denser set of markers (including five additional SNPs). However, these associations could not be confirmed in the second-set analysis. These results suggest that the SNARE complex-related genes do not play a major role in susceptibility to schizophrenia in the Japanese population.

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This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at <http://www.interscience.wiley.com/jpages/1552-4841/suppmat/index.html>.

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There is growing evidence that the presynapse is involved with the pathophysiology of schizophrenia. Within the presynaptic area, neurotransmitters are released by synaptic vesicle exocytosis, and the regulation of this release is critical for neural function. The machinery for this release consists of several groups of proteins that work together as a functional unit, the soluble *N*-ethylmaleimide sensitive factor attachment protein receptor (SNARE) complex [Montecucco et al., 2005].

The SNARE complex consists of Syntaxin1A, vesicle-associated membrane protein 2 (VAMP2) and synaptosomal-associated protein 25 kDa (SNAP25) [Marz and Hanson, 2002], and it has been reported that alterations in the components in the SNARE complex may underlie the pathophysiology of schizophrenia. First, postmortem studies measuring the level of SNARE complex protein or its mRNA revealed specific brain region alternations in schizophrenia [Gabriel et al., 1997; Thompson et al., 1998; Young et al., 1998; Karson et al., 1999; Sokolov et al., 2000; Hemby et al., 2002; Honer et al., 2002; Halim et al., 2003; Thompson et al., 2003]. Second, genetic association studies showed a significant association between SNPs in the Syntaxin1A gene (*STX1A*) and schizophrenic patients from Portugal and Toronto [Wong et al., 2004]. In addition, a very recent report showed that SNPs in *SNAP25* were associated with schizophrenia in Irish high-density families [Fanous et al., 2007].

In this study, we investigated whether genetic polymorphisms within *STX1A* (7p11.23: OMIM *186590), VAMP2 (17p13.1: OMIM *185881) and *SNAP25* (20p12-p11.2: OMIM *600322) were associated with schizophrenia in a Japanese population.

A first-set screening analysis was conducted with 377 schizophrenic patients (196 males and 181 females; mean age ± standard deviation (SD) 42.4 ± 14.8 years) and 377 healthy controls (212 males and 172 females; 35.9 ± 14.7 years). In a

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confirmation analysis a different panel of samples was used, consisting of 657 patients with schizophrenia (350 male and 307 female; 50.1 ± 14.4 years) and 527 controls (303 male and 224 female; 40.8 ± 15.3 years).

The patients were diagnosed according to DSM-IV criteria with the consensus of at least two experienced psychiatrists on the basis of unstructured interviews and a review of medical records. All healthy control subjects were also psychiatrically screened based on unstructured interviews. None of the subjects was known to be related to each other, and all were ethnically Japanese.

Written informed consent was obtained from each subject. This study was approved by the ethics committees at Fujita Health University, Nagoya University Graduate School of Medicine, Osaka University Graduate School of Medicine and Teikyo University School of Medicine.

After consulting the HapMap database (release#16.c.1, June 2005, www.hapmap.org, population: Japanese Tokyo: minor allele frequencies (MAFs) of more than 0.05 for *STX1A* and *VAMP2*, and 0.1 for *SNAP25*), 39 SNPs (*STX1A*, 7 SNPs; *VAMP2*, 3 SNPs; *SNAP25*, 29 SNPs) were selected as 'tagging SNPs' based on the criterion of an r^2 threshold greater than 0.8 in 'pair-wise tagging only' mode using the 'Tagger' program (Paul de Bakker, <http://www/broad.mit.edu/mpg/tagger>). For *STX1A*, since a previous report showed the positive association of an SNP in intron 7 [Wong et al., 2004], we included this SNP with the aforementioned 'tagging SNPs' for the association analysis. Overall, 40 SNPs were examined in this study (Supplementary Figures 1–3).

For denser mapping in the confirmation analysis, we added five SNPs around nominally significant SNPs or haplotypes detected in the first-set screening scan (rs610457, rs363013, rs363015, rs6039792 and rs363050).

For genotyping of these SNPs, a TaqMan assay (Applied Biosystems, CA), PCR-RFLP assay, and direct sequencing techniques were used. Detailed information is available in Supplementary Table 1. Genotype deviation from the Hardy-Weinberg equilibrium (HWE) was evaluated by chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc., Tokyo, Japan). Marker-trait association was evaluated by a likelihood ratio test (allele-wise and haplotype-wise analyses) and χ^2 -test (genotype-wise analysis). For exhaustive screening, we tested all one-marker (by conventional allele-wise analysis), two-marker, and three-marker haplotypes (and seven-marker haplotypes for second-set confirmation analysis) using the COCAPHASE 2.403 program [Dudbridge, 2003].

The power and sample size calculations were performed with a statistical program (<http://biostat.mc.vanderbilt.edu/twiki/bin/view/main/powersamplesize>). This significance threshold for all statistical tests was 0.05.

All genotype frequencies of each group were in HWE (data not shown). The LD structures examined in our control samples were almost the same as the one shown in HapMap database (Supplementary Figure 1–3).

The SNP (rs12626080: *SNAP25*-M8: $P = 0.0236$, uncorrected) in *SNAP25* and the haplotype constructed by M7 (rs363014) and M8 in *SNAP25* showed a nominally significant association with schizophrenia in the first-set screening samples (global $P = 0.0215$, uncorrected), although no association was detected with any tagging SNP in *STX1A* and *VAMP2*, including the SNP reported to be associated with schizophrenia in Caucasian samples [Wong et al., 2003] (Tables I and II and Supplementary Table 2).

To validate this nominal significance, we carried out a replication analysis using an independent set of samples. In this analysis, five additional SNPs were further included for denser mapping around M7 and M8 (rs610457, rs363013, rs363015, rs6039792 and rs363050: Supplementary Figure 4). However, this second-set confirmation analysis showed no

TABLE I. First-Set Association Analysis of Tagging SNPs in *STX1A* and *VAMP2*

Genes	Marker IDs	Distance to next SNP (bp)	N ^a		MAF ^b		P-Values			
			SCZ	CON	SCZ	CON	Genotype	(1) Window ^c	(2) Window	(3) Window
<i>STX1A</i> (minus strand)	SNP1	rs867500	375	375	0.204	0.217	0.754	0.527	0.947	0.946
	SNP2	Intron7 SNP	373	372	0.260	0.261	0.767	0.978	0.859	0.896
	SNP3	rs4363087	164	370	0.326	0.311	0.424	0.537	0.592	0.775
	SNP4	rs3793243	3151	375	0.419	0.386	0.304	0.196	0.553	0.698
	SNP5	rs875342	5751	375	0.228	0.238	0.211	0.653	0.781	0.924
	SNP6	rs6951030	6143	373	0.0563	0.0565	0.826	0.594	0.593	0.872
	SNP7	rs9654749	7145	376	0.483	0.469	0.460	0.602	0.869	
	SNP8	rs2030921	785	376	0.249	0.240	0.728	0.696		
<i>VAMP2</i> (minus strand)	m1	rs2278637	372	369	0.430	0.436	0.348	0.724	0.732	0.802
	m2	rs1061032	377	377	0.399	0.403	0.750	0.975	0.694	
	m3	rs8067606	375	371	0.425	0.431	0.490	0.765		

^aN, number; SCZ, schizophrenia; CON, control.

^bMAF, minor allele frequency.

^cIdentical as conventional allele-wise analysis.