Role of abca7 in mouse behaviours relevant to neurodegenerative diseases

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Role of abca7 in mouse behaviours relevant to neurodegenerative diseases

Abstract
ATP-binding cassette transporters of the subfamily A (ABCA) are responsible for the translocation of lipids including cholesterol, which is crucial for neurological function. Recent studies suggest that the ABC transporter ABCA7 may play a role in the development of brain disorders such as schizophrenia and Alzheimer's disease. However, Abca7's role in cognition and other behaviours has not been investigated. Therefore, we characterised homozygous Abca7 knockout mice in a battery of tests for baseline behaviours (i.e. physical exam, baseline locomotion and anxiety) and behaviours relevant to schizophrenia (i.e. prepulse inhibition and locomotor response to psychotropic drugs) and Alzheimer's disease (i.e. cognitive domains). Knockout mice had normal motor functions and sensory abilities and performed the same as wild type-like animals in anxiety tasks. Short-term spatial memory and fear-associated learning was also intact in Abca7 knockout mice. However, male knockout mice exhibited significantly impaired novel object recognition memory. Task acquisition was unaffected in the cheeseboard task. Female mice exhibited impaired spatial reference memory. This phenomenon was more pronounced in female Abca7 null mice. Acoustic startle response, sensorimotor gating and baseline locomotion was unaltered in Abca7 knockout mice. Female knockouts showed a moderately increased motor response to MK-801 than control mice. In conclusion, Abca7 appears to play only a minor role in behavioural domains with a subtle sex-specific impact on particular cognitive domains. © 2012 Logge et al.

Keywords
relevant, mouse, behaviours, role, diseases, neurodegenerative, abca7

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Role of Abca7 in Mouse Behaviours Relevant to Neurodegenerative Diseases

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Abstract

ATP-binding cassette transporters of the subfamily A (ABCA) are responsible for the translocation of lipids including cholesterol, which is crucial for neurological function. Recent studies suggest that the ABC transporter ABCA7 may play a role in the development of brain disorders such as schizophrenia and Alzheimer’s disease. However, Abca7’s role in cognition and other behaviours has not been investigated. Therefore, we characterised homozygous Abca7 knockout mice in a battery of tests for baseline behaviours (i.e. physical exam, baseline locomotion and anxiety) and behaviours relevant to schizophrenia (i.e. prepulse inhibition and locomotor response to psychotropic drugs) and Alzheimer’s disease (i.e. cognitive domains). Knockout mice had normal motor functions and sensory abilities and performed the same as wild type-like animals in anxiety tasks. Short-term spatial memory and fear-associated learning was also intact in Abca7 knockout mice. However, male knockout mice exhibited significantly impaired novel object recognition memory. Task acquisition was unaffected in the cheeseboard task. Female mice exhibited impaired spatial reference memory. This phenomenon was more pronounced in female Abca7 null mice. Acoustic startle response, sensorimotor gating and baseline locomotion was unaltered in Abca7 knockout mice. Female knockouts showed a moderately increased motor response to MK-801 than control mice. In conclusion, Abca7 appears to play only a minor role in behavioural domains with a subtle sex-specific impact on particular cognitive domains.

Introduction

ATP-binding cassette (ABC) transporters are classified into seven subfamilies according to their structure and sequence homology. The subfamily A (ABCA) is responsible for the translocation of a variety of lipids including cholesterol across membranes. There is also evidence to suggest that they might contribute to regulation of neurogenesis, phagocytosis and host defense [1–3]. Although the brain is extremely enriched in lipids little is known about the role that ABCA transporters play in cognitive function. Emerging evidence indicates that tight regulation of brain cholesterol homeostasis is crucial for neurological function and that abnormal cholesterol homeostasis can contribute to neurodegeneration [4]. Thus, modulation of endogenous ABCA transporters could potentially have a significant impact on brain function and susceptibility to neurodegenerative diseases.

The ABCA transporter ABCA7 is expressed in the mouse brain with particularly high expression in hippocampal and cortical neurons as well as microglia [4–6]. A potential role of ABCA7 in the development of brain disorders has recently been suggested. A single nucleotide polymorphism (SNP) of ABCA7 has been found to be associated with schizophrenia [7]. Furthermore, deregulation of apoptosis in cortical regions of schizophrenia patients has been reported [8]. Apoptosis is a key process in shaping neuronal circuits and functions. In this context, ABCA7’s role in phagocytosis [9] might be important to maintain tissue homeostasis and prevent the release of potentially cytotoxic or antigenic molecules from dying cells during apoptosis. In Alzheimer’s disease (AD), common variants in ABCA7 have been linked to AD and a recent meta-analysis provided compelling evidence that the SNP rs5764650 represents a new AD susceptibility locus [10]. Furthermore, modulating pathways that regulate cholesterol homeostasis seem to affect amyloid precursor protein (APP) processing and the production of β-amyloid peptides, which are neurotoxic and pro-inflammatory, impair memory and represent a major constituent of cerebral amyloid plaques associated with AD [4,11].
Kim et al. developed a viable homozygous Abca7 knockout mouse (i.e. Abca7−/−) with no remarkable phenotype except a reduction of white adipose tissue mass in knockout mice and lower total serum and high-density lipoprotein cholesterol levels in female Abca7−/− mice [5]. Importantly, the behavioural phenotype of this mouse model has not been determined to date. Therefore, we employed a battery of tests for baseline behaviours (i.e. physical exam, locomotion in the open field and anxiety in the elevated plus maze) as suggested by Crawley and others [12,13] and also considered behaviours relevant to schizophrenia (i.e. prepulse inhibition and locomotor response to psychotropic drugs in the open field; [14]) and Alzheimer’s disease (i.e. cognitive domains using the novel object recognition task, the Y maze, the fear conditioning paradigm, and the cheeseboard; [15]) in Abca7−/− mice to explore whether Abca7 influences a broad range of behavioural domains. This strategy will provide information about the potential involvement of Abca7 in a variety of behavioural domains with a particular focus on those relevant to neurodegenerative diseases.

Results

Male Abca7−/− mice exhibited wild type-like sensory abilities and neurological reflexes and showed no differences in motor abilities or motor function in the accelerated (i.e. latency to fall of the rotating rod), the pole test (i.e. latency to climb down the pole to reach platform), the wire hang test (i.e. latency to fall) and the beam walking test (i.e. latency to cross squared beams) (all p-values >.05; Table 1). The body weight was similar across genotypes (p>.05; Table 1).

Anxiety

In the EPM, locomotive activity (i.e. closed arm distance) and the occurrence of anxiety-related behaviours (i.e. time in open arm, open arm entry ratio, and frequency of stretch attend postures) was similar in WT and Abca7−/− mice (p-values >.05 for all parameters investigated), indicating that Abca7 deficiency had no impact on EPM locomotion or anxiety (Table 2). Female mice showed a somewhat lower level of risk assessment (i.e. frequency of stretch attend postures) than male mice [two-way ANOVA for ‘sex’: F(1,36)= 9.6, p = .004; Table 2].

Cognition

Y-maze. Test animals of both genotypes distinguished among the three arms (‘arm type’: familiar arm 1, familiar arm 2, and novel arm) in the test trial (F(2,80)= 26.7, p<.0001; no interaction] and developed a preference for the novel arm over the familiar arms [simple contrasts for arm distance: novel arm versus familiar arm 1: F(1,40) = 24.0, p<.001– novel arm versus familiar arm 2: F(1,40) = 49.1, p<.001; Fig. 1]. Short-term memory was not affected by Abca7 deficiency as novel time [F(1,40) = 3.0, p>.05] and novel distance [F(1,40) = 0.9, p>.05] were not different between genotypes (Table 3).

Table 1. Motor function/coordination in the physical exam and body weight.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td></td>
<td>WT</td>
<td>Abca7−/−</td>
</tr>
<tr>
<td>Accelerated: Latency to fall of rod [s]</td>
<td>149.8 ± 15.5</td>
<td>146.5 ± 14.0</td>
</tr>
<tr>
<td>Pole test: Latency to reach platform [s]</td>
<td>26.8 ± 4.5</td>
<td>22.6 ± 3.9</td>
</tr>
<tr>
<td>Wire hang test: Latency to fall [s]</td>
<td>18.5 ± 2.0</td>
<td>20.3 ± 3.6</td>
</tr>
<tr>
<td>Beam walking test: Latency to cross squared 10 mm² beam [s]</td>
<td>15.5 ± 1.2</td>
<td>16.0 ± 1.6</td>
</tr>
<tr>
<td>Beam walking test: Latency to cross squared 5 mm² beam [s]</td>
<td>13.1 ± 1.6</td>
<td>12.8 ± 1.3</td>
</tr>
<tr>
<td>Body weight [g]</td>
<td>31.8 ± 0.5</td>
<td>31.5 ± 0.8</td>
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</table>

Table 2. Locomotion and anxiety-related behaviours in the elevated plus maze.

<table>
<thead>
<tr>
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<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td></td>
<td>WT</td>
<td>Abca7−/−</td>
</tr>
<tr>
<td>Closed arm distance [cm]</td>
<td>911.3 ± 37.6</td>
<td>849.7 ± 48.3</td>
</tr>
<tr>
<td>Stretch attend postures [n]</td>
<td>11.0 ± 1.2</td>
<td>13.5 ± 1.9</td>
</tr>
<tr>
<td>Open time [s]</td>
<td>19.6 ± 3.2</td>
<td>18.6 ± 1.9</td>
</tr>
<tr>
<td>Open entry ratio [%]</td>
<td>26.0 ± 3.1</td>
<td>25.3 ± 1.7</td>
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</table>

Distance travelled in enclosed arms, frequency of stretch attend postures, time spent on open arms (open time) and number of open arm entries as a percentage of total arm entries (open entry ratio) are shown. Significant one-way ANOVA split by ‘genotype’ effects of females versus males of the corresponding genotype are indicated by ‘*’ (p<.01).

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Figure 1. Recognition of familiarity in the Y-maze. Overall arm distance [cm], in familiar (familiar 1 and familiar 2) and novel arms are shown.

doi:10.1371/journal.pone.0045959.g001

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**Table 3. Cognitive performance in the Y-maze (YM) and novel object recognition task (NORT).**

<table>
<thead>
<tr>
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<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td></td>
<td>WT</td>
<td>Abca7−/−</td>
</tr>
<tr>
<td>Novel time [%]</td>
<td>34.7±2.5</td>
<td>36.0±2.2</td>
</tr>
<tr>
<td>Novel distance [%]</td>
<td>40.3±2.5</td>
<td>42.9±2.7</td>
</tr>
<tr>
<td>NORT: Exploration of familiar object [s]</td>
<td>14.9±2.6</td>
<td>20.9±3.8</td>
</tr>
<tr>
<td>NORT: Exploration of novel object [s]</td>
<td>20.6±2.7</td>
<td>16.9±2.0</td>
</tr>
</tbody>
</table>

YM: Time spent in the novel arm (novel time) and distance travelled in the novel arm (novel distance) as a percentage of the total measure [%] are shown as measures for short-term memory. NORT: The duration [s] of exploring (time spent nosing + rearing) the familiar and the novel object are shown as a measure of working memory.

doi:10.1371/journal.pone.0045959.t003

**Novel object recognition task.** Animals distinguished between the novel and the familiar object presented during the test trial as indicated by a significant effect of ‘object’ (i.e. novel versus familiar) on exploration time [F(1,33) = 4.8, p = .04; Table 3]. Importantly, the ability to recognise the novel object was genotype-dependent [interaction of ‘object’ with ‘genotype’: F(1,33) = 6.5, p = .02] as only WT mice showed a clear preference for the novel object [WT: F(1,17) = 14.9, p<.001; Abca7−/−: F(1,16) = 0.1, p = not significant]. Impaired short-term novel object recognition memory of Abca7 null mice was confirmed when analysing the % time mice spent exploring the novel object. Abca7−/− mice exhibited a reduced level of % novel exploration [F(1,33) = 7.8, p = .009] with males failing to develop a significant preference for the novel object (Fig. 2).

**Fear conditioning.** All animals responded to the electric foot shocks delivered during the conditioning phase (i.e. vocalization of all mice). The level of baseline freezing during conditioning (i.e. in the first 2 min before US presentation) was similar in all mice regardless of genotype and sex (all p-values >.05; Table 4). As expected, freezing during the first 2 min of the context test was significantly increased compared to the first 2 min of freezing in the conditioning phase regardless of genotype and sex [2 min: F(1,36) = 39.5, p<.001; no interactions; Table 4]. The ability of Abca7 knockout mice to associate the context with the US exposure during conditioning was WT-like, as the total time spent freezing during the context test was not significantly different across test groups (all p-values >.05; Table 4). In the cue test, test animals learned the association between US and CS. Mice responded to the CS presentation with increased levels of freezing after cue onset as confirmed by a significant main effect of ‘1 min block’ for time spent freezing one minute before and one minute post cue presentation [F(1,36) = 29.8, p<.001; Fig. 3], indicating associative learning of WT and Abca7−/− mice.

**Schizophrenia-relevant Behaviours**

**Startle response and prepulse inhibition.** There were no effects of Abca7 on the startle response of mice [p>.05] (Table 5). Female mice exhibited lower acoustic startle responses to a 120dB tone than male mice [F(1,39) = 7.0, p = .01]. Startle habituation over trials occurred in all mice ['ASR block': F(2,78) = 8.1, p = .001] and was similar across genotypes and sex (Fig. 5A). Prepulse intensities had a significant effect on % PPI ['prepulse': F(2,78) = 137.7, p<.001; no interactions; Table 4]. The ability of Abca7 mice to associate the context with the US exposure during conditioning was WT-like, as the total time spent freezing during the context test was not significantly different across test groups (all p-values >.05; Table 4). In the cue test, test animals learned the association between US and CS. Mice responded to the CS presentation with increased levels of freezing after cue onset as confirmed by a significant main effect of ‘1 min block’ for time spent freezing one minute before and one minute post cue presentation [F(1,36) = 29.8, p<.001; Fig. 3], indicating associative learning of WT and Abca7−/− mice.

**Figure 2. Novel object recognition.** Percentage exploration [%] (i.e. duration of nosing and rearing) of the novel object (% novel exploration) is shown for males and females of both genotypes. Exploration of the novel object according to chance (= 50%) is marked with a dotted line. Significant one-way ANOVA effects of Abca7−/− versus WT of the corresponding sex are indicated by * (p<.05).
doi:10.1371/journal.pone.0045959.g002

**Figure 3. Fear conditioning in cue test.** Time spent freezing [s] for 1 min before (block min: 2) and 1 min post CS onset (block min: 3) during the cue test is shown.
doi:10.1371/journal.pone.0045959.g003
30 min of testing ['5 min block': F(5,190) = 35.0, p < .001; Fig. 6], although habituation was influenced by 'genotype' and 'sex' interaction of '5 min block' with 'genotype' and 'sex': F(5,190) = 3.1, p = .01. However, this was first of all due to increased baseline locomotion of WT males in the first 5 min block compared to Abca72/2 males and all females (Fig. 6).

As expected, MK-801 stimulated locomotion of all test animals ['5 min block' for 90 min OF test session: F(17,646) = 17.5, p < .001; Fig. 6]. An interaction of '5 min block' with 'genotype' [F(17,646) = 1.9, p = .02] was based on increased locomotion levels of female Abca7 knockout mice in the later test stages ['5 min block' by 'genotype' interaction: females: F(17,340) = 17.4, p < .05–males: not significant].

**Discussion**

Abca7 null mice have normal sensory abilities, neurological reflexes and motor functions and show wild type-like anxiety behaviour and sensorimotor gating. Short-term memory, fear-associated conditioning and spatial learning were also unaltered in Abca72/2 mice. However, deficiency in Abca7 disrupted novel object recognition in male mice. Furthermore, female mice exhibited disrupted spatial reference memory. This phenomenon was somewhat more pronounced in Abca72/2 females. Female Abca72/2 mice also exhibited a moderate longer-lasting response to the locomotor-stimulating effects of MK-801 treatment than control females.

Our study expands on the initial finding that Abca7 deficiency does not result in a remarkable physiological phenotype (i.e.
unaltered food consumption, body weight development and weight of major organs; [5]), as our baseline tests revealed normal neurophysiological functioning of Abca7 null mice and no gross behavioural abnormalities in locomotion, exploration and anxiety.

We investigated the cognitive phenotype of Abca7 null mice as human research has linked common variants in ABCA7 to AD [10]. The test battery incorporated tests for short-term memory of context familiarity (Y-maze), object recognition, amygdalar and

**Figure 5. A+B: Habituation of startle response and sensorimotor gating:** A) Habituation of the acoustic startle response to a tone stimulus of 120dB [millivolts] and B) % PPI for different prepulse intensities [%].

doi:10.1371/journal.pone.0045959.g005

**Table 5.** Acoustic startle response to a 120 dB startle stimulus during PPI testing.

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<th>Female</th>
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<tr>
<td>PPI</td>
<td>WT</td>
<td>Abca7&lt;sup&gt;−/−&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acoustic startle response</td>
<td>110.2 ± 18.3</td>
<td>114.7 ± 11.4</td>
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Acoustic startle response [millivolts] is shown.
doi:10.1371/journal.pone.0045959.t005

**Figure 6. Baseline and MK-801 induced locomotion in the open field.** Overall distance travelled [cm] (in 5 min blocks) in the open field in a 90 min session where animals received an i.p. injection of MK-801 (0.25 mg/kg body weight) after 30 min of baseline testing (indicated by black arrow). There was an interaction of ‘5 min block’ with ‘genotype’ in females (p<.05).

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Abca7’s Impact on Mouse Behaviour

hippocampal associative learning and spatial reference memory. Abca7−/− mice exhibited a task- and moderate sex-specific impairment of cognitive abilities. Male Abca7 null mice showed disrupted object recognition (a better performance of females in a visual memory task has also been shown in other studies and has been related to sex hormones [16,17]). Female knockout mice on the other side exhibited impaired spatial reference memory. It has been found that male rats perform better in reference memory, when it has been differentiated from the working memory aspects of a paradigm [18] and mice studies have discussed reduced reference memory of females in relation to estradiol levels [19,20]. The cognitive impairments of Abca7 deficient mice are in line with the expression profile of ABCA7, which is abundant in hippocampal and cortical neurons [5,6], suggesting a potential moderate role of the ABC transporter in cognitive domains. Importantly, the impact of Abca7 on cognition cannot be generalised across different ABC transporters: for example, the Abcg1 transporter is not involved in cognitive functioning as mice selectively overexpressing Abcg1 performed equivalently to their WT littermates in the spatial reference and working memory version of the Morris water maze [21], which is a paradigm similar to the cheeseboard test [22].

Interestingly, validated mouse models for familial AD have shown disrupted spatial reference memory and object recognition [23–25] and deficiency in Abca1, which is one of the main ABC transporters and essential for cholesterol homeostasis, exacerbated amyloidogenesis in amyloid mouse models of AD [26,27], whereas Abca1 overexpression ameliorated amyloid load [28]. Another study reported that agonists of liver X receptors reduce Ab levels and improve cognition in AD mice but only in the presence of ABCA1 [29]. As ABCA7 has the highest homology of all ABC transporters to ABCA1, it appears logical to investigate Abca7’s role in amyloid pathology as well. Indeed, our earlier in vitro work found that ABCA7 stimulates cholesterol efflux to apoE discs, regulates processing of amyloid precursor protein and inhibits the generation of neurotoxic Aβ peptides [11]. Future research should focus on the role that Abca7 may play in cognition in established mouse models for AD.

A SNP of ABCA7 has been found to be associated with schizophrenia [7] and ABCA7’s role in phagocytosis might impact on the deregulation of apoptosis in cortical regions of schizophrenia patients [8,9]. The defects in the apoptotic pathway of schizophrenia patients may be a factor contributing to dysfunctional autoimmune system and elevated inflammatory cytokines observed in these patients [7]. Thus, we tested Abca7 null mice in behavioural tasks relevant to schizophrenia: Abca7 deficient mice showed unaltered baseline locomotion and sensorimotor gating [39]. However, Cre-lox mice showed normal cognitive abilities in the Morris water maze. Thus, the behavioural phenotype of Abca7−/− mice appears quite different to what has been described for Abca1−/− mice. Importantly, in vivo work confirms that ABCA7 does not recapitulate ABCA1 function but rather has distinct activities on apolipoprotein-derived generation of high-density lipoprotein [40] and cellular phagocytic function [9]. Furthermore, Abca7 null mice have no defect in apolipoprotein-stimulated sterol or phosphatidycholine transport, suggesting that cholesterol and phosphatidycholine are not likely to be the primary physiological substrates of ABCA7 transporter activity [5].

In conclusion, this study is the first to provide a detailed analysis of Abca7 function in behavioural domains relevant to neurodegenerative diseases in vivo. Abca7 appears to impact on particular aspects of cognitive functioning and to have a subtle effect on the locomotor response to NMDA blockade. Further research will clarify, if therapeutic strategies targeting ABCA7 might be beneficial in improving cognitive functioning in AD.

Materials and Methods

Animals

The generation of Abca7 null (Abca7−/−) mice, which were generously provided by Prof. Mason W. Freeman (Centre for Computational and Integrative Biology, Massachusetts General Hospital, Harvard Medical School), has been described elsewhere [5]. Test mice were adult (males: 19 ± 3 weeks; females: 20 ± 4 weeks) cohorts of male and female Abca7−/− mice and wild type-like control littermates (WT), which had been backcrossed for 15 generations onto a C57BL6/J background (males: Abca7−/− = 11 and WT = 10; females: Abca7−/− = 8 and WT = 15). Mice were bred and housed in independently ventilated cages (Airlaw, Smithfield, Australia) at Animal BioResources (Moss Vale, Australia). Two weeks before testing mice were transported to Neuroscience Research Australia (NeuRA), where they were pair-
housed in Polysulfone cages (1144B: Tecniplast, Rydalmere, Australia), which were enriched with certified polycarbonate mouse igloo (Bioserv, Frenchtown, USA), tissues for nesting material and a steel ring in the cage lid. Mice were kept pair-housed under a 12:12 h light:dark schedule [light phase: white light (illumination: 124 lx) – dark phase: red light (illumination: <2 lx)]. Food and water were available ad libitum.

Ethics statement. Research and animal care procedures were approved by the University of New South Wales Animal Care and Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Behavioural Phenotyping

Animals were tested in a battery of behavioural tasks relevant to schizophrenia and cognition, which are well-established at Neuroscience Research Australia (the least aversive/disruptive tasks were carried out first) [15,41,42]: elevated plus maze, Y maze, novel object recognition task, fear conditioning, prepulse inhibition, open field [baseline and after treatment with non-competitive N-methyl-D-aspartate (NMDA) antagonist MK-801] and cheeseboard using an inter-test interval of at least six days. All devices (and objects) were cleaned thoroughly with 70% ethanol in between trials and sessions. All testing occurred during the light phase (within 1–5 h of light onset) with the exemption of the elevated plus maze, which was run during the dark phase (2 h prior to light onset). An additional male cohort (N: Abca7+/− = 6 and WT = 6) was tested for motor functions and coordination as well as sensory abilities to avoid that impairments in those domains result in false positive or negative phenotyping outcomes [12,43].

Elevated plus maze (EPM). Mice were allowed to explore the apparatus freely for 5 min (as described previously [43]). Arm entries (when the mouse entered an arm with all four paws), distance travelled, time spent in arms as well as the frequency of rearing, and head dipping were scored for the different arms. Anxiety-related behaviour was measured by time spent on open arms (open time) and open arm entries as a percentage of the total measures (open entry ratio), and the frequency of stretch attend postures.

Y-maze (YM). The Y-maze assessed short-term memory of context familiarity [44]. Arms were equipped with different internal visual cues. Bedding covered the apparatus floor and was changed in between sessions. The Y-maze test consisted of two trials with a 30 min inter-trial interval (ITI). The trial duration for training and test was 10 and 5 min respectively. During training, one arm was blocked off (novel arm). In the test trial, all arms were accessible, and mice were allowed to explore the apparatus freely. Time spent and distance travelled was recorded for each arm using Any-Maze™ video tracking software (Stoelting Co., Wood Dale, USA). The percentage of time spent in the novel arm (novel time) was calculated using [(novel time/total time) × 100]. The corresponding calculation was performed for distance travelled in the novel arm (novel distance).

Novel object recognition task (NORT). The distinction between familiar and unfamiliar objects is an index of recognition memory, and its measurement is aided by the innate preference of rodents for novel over familiar objects [45]. The NORT was conducted over 3 days; two trials (10 min per trial) were conducted per day with a 1 h ITI (for details see [15,41,42]). On the test day (following two days of habituation to the arena and the test procedure), mice were exposed to two identical objects in trial 1 (sample trial), and then one familiar and one novel object in trial 2 (test trial). Objects and their location were counterbalanced across genotypes. The frequency and duration of nosing and rearing the familiar and novel objects were recorded offline by a trained experimenter blind to the sex and genotype of the test animals using Any-Maze™ tracking software. The percentage exploration time (time spent nosing + rearing objects) for the novel object (% novel exploration) was calculated using [(novel object exploration time/novel + familiar object exploration time) × 100] and served as an indicator for short-term object recognition memory.

Fear conditioning (FC). Fear conditioning (FC) is a form of associative learning that occurs when a previously neutral stimulus (e.g. tone) elicits a fear response after it has been paired with an aversive stimulus [46]. On conditioning day, animals were placed in the test chamber (Model H10-11R-TC, Coulbourn Instruments, Whitehall, USA) for 120 s. A 80 dB conditioned stimulus (CS) was presented for 30 s with a co-terminating 0.4 mA 2 s foot shock (unconditioned stimulus; US) twice with an inter-pairing interval of 120 s. The test concluded 120 s later. On day 2 (context test), the animals were returned to the apparatus for 7 min. On day 3 (cue test), animals were placed in an altered context for 9 min. After 120 s (pre-CS/baseline), the CS was presented continuously for 5 min. The test concluded after another 120 s without the CS. Time spent freezing was measured using Any-Maze™ software [15,41,42].

Prepulse inhibition (PPI). PPI is an operational measure of sensorimotor gating, which is impaired in schizophrenia patients [47,48]. Startle reactivity was measured in millivolts using SR-LAB startle chambers (San Diego Instruments, San Diego, USA). The PPI test consisted of 5 min acclimatisation to 70 dB background noise, followed by 121 trials in a pseudorandom order: 5×70 dB trials (background); 5×90 dB trials; 15×120 dB trials (startle) and 96 trials comprising a prepulse of either 74, 82 or 96 dB presented 32, 64, 128, or 256 ms (variable interstimulus interval; ISI) prior to a startling pulse of 120 dB (PPI response) [for further details see [49]]. The blocks of startle responses at the beginning, middle and end of the PPI protocol (averaged across 5 trials each) were used for ASR habituation. Percentage PPI (% PPI) was calculated as [(mean startle response – PPI response)/mean startle response] × 100. % PPI was averaged across ISIs to produce a mean % PPI for each prepulse intensity.

Open field (OF). General motor activity was evaluated by placing the mouse into an infrared photobeam controlled open field activity chambers (41 cm × 41 cm; Tri-Scan Photo Beam Activity System: Coulbourn Instruments). Animals were tested for 30 min (baseline) before MK-801 (0.25 mg/kg body weight; dissolved in saline) was administered i.p. (injection volume: 10 ml/kg body weight). Following the injection, animals were put back into the OF chambers for another 60 min (MK treatment). Distance travelled and rearing were measured using Any-Maze™ software [15,41,42].

Cheeseboard (CB). The cheeseboard (CB) paradigm was employed as a dry-land equivalent of the Morris water maze [13,22,50]. Mice were trained to find a food reward over a number of days; reference memory was indexed by a decreased latency to find the reward over days. During habituation (three days to the blank side of the CB) two 2 min trials were conducted each day with a 10 min ITI. Mice were food-restricted (starting 24 h before the first habituation session) and kept at 85–90% of their pre-test body weight throughout testing (mice were fed for 1–2 h per day).

Task Acquisition/Spatial Reference Memory Training: Mice were trained over 13 days (three trials per day with a 10 min ITI) to locate the food reward. The location of the target well (controlled across genotypes) was kept constant for each mouse between trials and across days. If the target well was not located within 2 min, mice were placed next to the target well and allowed to consume the food reward. Spatial Reference Memory: A probe trial was conducted on day 9 (males and females) and day 16 (males only),
where no wells were bailed and mice were given 2 min to explore the board freely (probe trial 16 results are shown for males as no clear target preference was detected on day 9). For this, the board was divided into 8 zones corresponding to each line of 4 caps as well as a centre zone (40 cm diameter = start zone). The time spent in each zone was measured using Any-Maze\textsuperscript{TM}. Data are presented for ‘% target zone time’, which calculates the time spent in the target zone as a percentage of time spent in all zones (minus the time spent in the start zone).

### Statistical Analysis

Results were analysed using two-way analysis of variance (ANOVA: between factor: ‘genotype’ and ‘sex’) followed by one-way ANOVA split by corresponding factor(s) where appropriate as published previously \[51,52\]. Three-way repeated measures (RM) ANOVAs were used to control for successful learning (Y-maze: ‘arm type’, NORT: ‘object’), for effects across trial types (FC: ‘first min block’, OF: ‘5 min block’, FC: ‘1 min block’), which were followed by simple contrasts where appropriate. For the cheese-over time (OF: ‘5 min block’, FC: ‘1 min block’), which were presented for ‘% target zone time’, which calculates the time spent in the cheese target zone as a percentage of time spent in all zones (minus the time spent in the start zone).

### References


