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2 # Overexpression of transmembrane protein 168 in the mouse nucleus accumbens induces anxiety and

3 sensorimotor gating deficit

4

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18

19 # ABSTRACT

20 Transmembrane protein 168 (TMEM168) comprises 697 amino acid residues, including some
21 putative transmembrane domains. It is reported that TMEM168 controls methamphetamine (METH)
22 dependence in the nucleus accumbens (NAc) of mice. Moreover, a strong link between METH
23 dependence-induced adaptive changes in the brain and mood disorders has been evaluated. In the
24 present study, we investigated the effects of accumbal TMEM168 in a battery of behavioral
25 paradigms. The adeno-associated virus (AAV) *Tmem168* vector was injected into the NAc of
26 C57BL/6J mice (NAc-TMEM mice). Subsequently, the accumbal TMEM168 mRNA was increased
27 approximately by seven-fold when compared with the NAc-Mock mice (controls). The NAc-TMEM
28 mice reported no change in the locomotor activity, cognitive ability, social interaction, and
29 depression-like behaviors; however, TMEM168 overexpression enhanced anxiety in the
30 elevated-plus maze and light/dark box test. The increased anxiety was reversed by pretreatment with
31 the antianxiety drug diazepam (0.3 mg/kg i.p.). Moreover, the NAc-TMEM mice exhibited decreased
32 prepulse inhibition (PPI) in the startle response test, and the induced schizophrenia-like behavior was
33 reversed by pretreatment with the antipsychotic drug risperidone (0.01 mg/kg i.p.). Furthermore,
34 accumbal TMEM168 overexpression decreased the basal levels of extracellular GABA in the NAc
35 and the high K^+ (100 mM)-stimulated GABA elevation; however, the total contents of GABA in the
36 NAc remained unaffected. These results suggest that the TMEM168-regulated GABAergic neuronal
37 system in the NAc might become a novel target while studying the etiology of anxiety and

38 sensorimotor gating deficits.

39 # INTRODUCTION

40 Accumulating evidence indicates a link between the mood disorders and drug addiction within the
41 brain's rewarding circuitry [1, 2]. Mice chronically administered with methamphetamine (METH),
42 are generally used as a model to learn about the psychiatric disorders. The model is described by
43 related behavioral alterations, which suggests long-lasting influences on the gene and protein
44 expression within specific brain subregions, including the nucleus accumbens (NAc), striatum,
45 prefrontal cortex, and hippocampus [3-5]. Recently, several studies have attempted to elucidate the
46 link between the METH-induced maladaptive molecular changes in the brain and behavioral
47 alterations [4, 6, 7]. These studies may be crucial in discovering the mechanisms involved in the
48 regulation of psychiatric phenomena and may also suggest novel targets for pharmacotherapy.
49 Considering using animal models of addiction to study mood disorders, we focused on the NAc,
50 which plays an important role in both reward circuitry and mood regulation [1, 2]. Several
51 psychostimulant adaptive molecules in the NAc are known to be involved in psychiatric disorders,
52 including the cAMP response element-binding protein (CREB) [8, 9], brain-derived neurotrophic
53 factor (BDNF) [10, 11], orexin [12], and Shati/Nat8l [5, 13]; however, key signaling pathways and
54 novel molecular cascades related to behavioral regulation still remain to be identified. In a recent
55 study, we administered METH (2 mg/kg) in mice for 6 days, and then performed a polymerase
56 chain reaction-selected cDNA subtraction in the NAc of mice [3]. We found that a novel molecule
57 transmembrane protein 168 (TMEM168; GenBank accession number NM_028990) was increased

58 in the brain, especially in the NAc and hippocampus [14]. The accumbal overexpressed TMEM168
59 plays a crucial role in controlling the METH-induced pharmacological actions [14]; however,
60 whether TMEM168 in the NAc is associated to the other behavioral changes in vivo still needs to
61 be evaluated.

62 In the present study, the adeno-associated virus (AAV) comprising *tmem168* cDNA was
63 microinjected into the NAc of mice to overexpress TMEM168 mRNA. A series of behavioral tests
64 were performed to explore the behavioral changes following the interruption of the injections of
65 TMEM168. Furthermore, the in vivo microdialysis analysis was conducted to elucidate the
66 functional role of TMEM168 in the NAc. We identified TMEM168 in the NAc as a novel target to
67 induce anxiety and schizophrenia-like symptoms, by inhibiting the GABAergic system in the NAc.

68 # MATERIALS AND METHODS

69

70 ## Animals

71 Male C57BL/6J mice (8-week old; Nihon SLC, Inc. Hamamatsu, Japan) were housed in plastic cages
72 with a 12 h light/dark cycle (8 am–8 pm). The health and welfare of the animals was
73 monitored by staff at least once a day. All procedures were in accordance with the National
74 Institute of Health Guideline for the Care and Use of Laboratory Animals and were approved by the
75 Animal Experiments Committee of the University of Toyama (Permit Number A2015pha-21).

76

77 ## Drugs

78 Diazepam (045-18901; Wako Pure Chemical Industries, Japan) was dissolved in saline and 1%
79 Tween80. Risperidone (R3030; Sigma-Aldrich, Japan) was dissolved in saline. The behavioral
80 experiments were performed 30 min after the drug administration. The mice administered with
81 diazepam or risperidone were not used for other behavioral experiments.

82

83 ## AAV microinjection

84 The AAV vector was produced according to previously described methods [15] by encoding cDNA
85 *tmem168* (GenBank accession number NM_028990). Mice were anesthetized with a combination
86 anesthetic (medetomidine (0.3 mg/kg), midazolam (4.0 mg/kg), and butorphanol (5.0 mg/kg)), and

87 were fixed in a stereotactic frame (SR-5M, Narishige, Tokyo, Japan). AAV-TMEM168 vector or
88 AAV-Mock vector (0.7 μl/side) was injected bilaterally into the NAc (1.5 mm anterior and 0.8 mm
89 lateral from bregma, 3.9 mm below the skull surface [16]; NAc-TMEM mice or NAc-Mock mice at a
90 speed of 0.05 μL/min. Mice were used for the experiments 3 weeks later.
91 All procedures were in accordance with the Guideline for Recombinant DNA Experiment from the
92 Ministry of Education Culture, Sports, Science, and Technology, Japan and were approved by the
93 Gene Recombination Experiment Safety Committee of the University of Toyama (Permit Number
94 G2015pha-21).
95

96 ## Quantitative real time RT-PCR analysis

97 After 3 weeks of AAV microinjection, the NAc-Mock mice and NAc-TMEM mice were decapitated
98 by animal guillotine without feeling any suffering and the brains were quickly removed, since the
99 fresh brain tissues were needed for the isolation of mRNA or brain slices. This procedure
100 were done without anesthesia to avoid the effect of anesthetic drugs. All procedures
101 followed the National Institute of Health Guideline for the Care and Use of Laboratory
102 Animals (NIH publication No. 85–23, revised in 1996) and were approved by the
103 committee for Animal Experiments of the University of Toyama (Permit Number
104 A2015-PHA21). The NAc tissues were dissected according to the atlas of mouse brain [16] and
105 were preserved at −80 °C until further use. The analysis of real time RT-PCR was described as a

106 previous method [14]. The total RNA (1 μg) from each tissue was extracted (RNeasy Plus Mini Kit
107 protocol; QIAGEN, Tokyo, Japan) and was converted into cDNA using the Prime Script RT reagent
108 kit (Takara, Shiga, Japan), following the manufacturer's instructions. Quantitative real time RT-PCR
109 was performed in a Thermal Cycler Dice Real Time System (Takara) using Power SYBR Green PCR
110 Master Mix (Applied Biosystems, Foster, CA) with cDNA and primers (1 μM), according to the
111 manufacturer's recommendation. The primers of TMEM168 used for real time RT-PCR were as
112 follows: 5'-GACAGAATCATGGCATCCAAAGG-3', and
113 5'-ATGGACTCCAGCGGCAAGACAA-3'. The 36B4 transcript amount was quantified using
114 primers 5'-ACCCTGAAGTGCTCGACATC-3', and 5'-AGGAAGGCCTTGACCTTTTC-3'.
115

116 ## Schedule of the behavioral tests

117 We performed the behavioral tests in the following order: locomotor activity test, Y-maze test, novel
118 object recognition test, social interaction test, elevated plus maze test, light/dark box test, tail
119 suspension test, forced swim test, and prepulse inhibition test. The time interval between each test
120 was 2–3 days.
121

122 Locomotor activity test

123 The locomotor activity test was performed, as previously reported [17]. Mice were placed into a
124 Plexiglas box with a frosting Plexiglas floor (40 × 40 × 30 cm), and the test was performed for 60
125 min using digital counters with infrared sensors (Scanet MV-40; MELQEST, Toyama, Japan).
126

127 Y-maze test

128 Y-maze test was performed, according to a previously described method [18]. The three-arm maze
129 (each arm measuring 40 cm × 3 cm × 12 cm) was used for the test. Mice were placed at the end of
130 one arm and were allowed to move freely through the maze for 10 min. During this time, the arm
131 entries were enumerated. Alternation was defined as successive entries into the three arms on the
132 overlapping triplet sets. The percentage alternation was calculated using the following formula:
133 (number of alternations)/(total number of arm entries-2) × 100.
134

135 Novel object recognition test

136 Novel object recognition test was performed, according to a previously described method [18]. After
137 habituation for 3 days, the NAc-Mock or NAc-TMEM mice were allowed to explore two familiar
138 floor-fixed objects (A and B) in a Plexiglas box (30 cm × 30 cm × 35 cm) for 10 min (familiar
139 process). The familiar object A and a novel object C were set in the Plexiglas box 24 h after the trail
140 and the mice were allowed to explore the novel object process for 10 min (novel process). The

141 exploratory preference percentage was calculated using the following formula: (approach time of
142 object B or C)/(total approach time of the two objects in each process) × 100.
143

144 Social interaction test

145 Social interaction test was performed according to a previously described method [19]. The
146 apparatus for this test was designed as a Plexiglas box (60 cm × 40 cm × 22 cm) comprising three
147 connected chambers. After habituation for 2 days, both the NAc-Mock and NAc-TMEM mice were
148 randomly assigned to a partner male mouse, which was confined to one side of the chamber. The test
149 mice were placed in the apparatus for 10 min and the total duration they spent interacting with the
150 partner mouse was recorded.
151

152 Elevated plus-maze test

153 Elevated plus-maze test was performed according to a previously described method [20]. The
154 apparatus comprised four black plastic arms (25 cm × 5 cm). Two opposite arms were enclosed by
155 walls (15 cm in height) and the other two “open” arms had only a small rim (0.2 cm) around the
156 edges. The apparatus was elevated to a height of 70 cm above the floor level. For testing, mice were
157 placed in the center region facing an open arm, and were allowed to freely explore the maze for 10
158 min. The time spent on open arms and the number of entries into the open arms was evaluated.
159

160 ## Light–dark box test

161 Light–dark test was performed according to a previously described method [20]. The apparatus
162 comprised two plastic chambers. The dark chamber (black plastic) measured 15 cm × 15 cm × 20 cm
163 (l × w × h) and was covered by a lid. The light chamber, 15 cm × 15 cm × 20 cm (l × w × h), made of
164 transparent plastic, was brightly illuminated from above with tubular fluorescent lamps (1000 lux).
165 Mice were placed into the dark chamber and their behaviors were monitored by Scanet MV-40 LD
166 (MELQUEST) for 10 min. The time spent in the light box was measured.
167

168 ## Tail suspension test

169 Tail suspension test was performed according to a previously described method [19]. The mice were
170 suspended by their tails, i.e., the body dangled in the air, with the head pointing downward. The
171 duration of immobility from 2 min to 6 min within the 10 min test was recorded visually.
172

173 ## Forced swim test

174 Forced swim test was performed according to a previously described method [19]. Mice were placed
175 in a transparent Plexiglas cylinder (diameter: 14.5 cm; height: 19 cm), filled with water (depth: 15
176 cm; temperature: 25 °C). The immobility time was monitored by Scanet MV-40 AQ (MELQUEST)
177 from 2 min to 6 min within the 10 min test.
178

179 ## Prepulse inhibition test

180 Prepulse inhibition (PPI) test was performed according to a previously described method [21]. The
181 test was evaluated using the SR-LAB apparatus (San Diego Instruments, CA, USA). Briefly, the test
182 was performed by exposing the animals to a 70 dB background noise. After a 5 min acclimatization
183 period, 5 pulses (120 dB each lasting 40 ms) were presented. Subsequently, the randomly prepulse
184 plus pulse trials were administered as a 20 ms prepulse of 74, 78, 82 or 86 dB, followed by a 100 ms
185 delay and a startle pulse (120 dB each lasting 40 ms). Eventually, 5 pulses (120 dB each lasting 40
186 ms) were presented once again. The PPI was calculated as (1 − [startle amplitude on prepulse + pulse
187 trial/mean startle amplitude on pulse alone trials]) × 100.

188

189 ## Tissue extraction

190 From each brain, the NAc tissue was bilaterally extracted and homogenized in a homogenizing
191 buffer, containing 200 mM perchloric acid and 100 μM ethylenediaminetetraacetic acid (EDTA). The
192 homogenates were kept in ice for 30 min and were then centrifuged at 20,000 × g for 15 min at 0 °C.
193 Supernatant was collected and was adjusted to pH 3.0 by adding 1 M sodium acetate. After filtration
194 (0.45 μm Membrane Filter, MF-Millipore, Japan), the extraction samples was preserved at −80 °C
195 until the measurement by high-performance liquid chromatography (HPLC).

196

197 ## In vivo microdialysis

198 In vivo microdialysis was performed according to a previously described method [17]. The guide
199 cannula (AG-4, Eicom, Kyoto, Japan) was implanted into the NAc (+1.5 mm anterolateral, +0.7 mm
200 mediolateral from bregma, and +3.25 mm dorsoventral from dura mater). On the following day, a
201 dialysis probe (A-I-4-01, Eicom) was inserted into the guide cannula, and a ringer's solution (147
202 mM NaCl, 4 mM KCl, and 2.3 mM $CaCl_2$) was continuously perfused through the probe into the left
203 side of the NAc.
204 In the case of GABA dialysis, the dialysate was collected every 30 min at a rate of 1.0 μL/min by a
205 fraction collector (EF-80; Eicom), placed in biotubes and preserved at −80 °C until it was subjected
206 to HPLC. High K^+-stimulation (100 mM) was applied for 15 min, 4.5 h after the probe insertion. The
207 baseline of extracellular GABA levels was the mean of the averages amount of the last three samples
208 before high K^+-stimulation. The 100 mM K^+ solution means an identical amount of sodium is
209 replaced in the ringer's solution with potassium.
210 In case of dopamine and serotonin dialysis, the dialysate was collected in 15 min fractions at a rate of
211 0.5 μL/min and was simultaneously subjected to HPLC.
212

213 ## HPLC Detection

214 Using sampling injector (M-500; Eicom), 7 μL of o-phthalaldehyde solution (4 mmol/L) and 0.04%
215 mercaptoethanol in carbonate buffer (pH 9.5) were added to a 21 μL of dialysate sample or extraction

216 sample. Subsequently, 21 μL of the mixture was injected into the HPLC system (HTEC-50; Eicom).
217 GABA was separated on the SA-50DS column (Eicom), which was maintained at 25 °C, using a
218 phosphate buffer (pH 3.5) containing EDTA (0.5 μg/L) and 50% methanol as the mobile phase with a
219 flow rate of 500 μL/min. An electrochemical detector that used a glassy carbon and a working
220 electrode (set at +600 mV) against a silver–silver chloride reference electrode (WE-GC; Eicom) was
221 used to quantify the compounds. Chromatograms were controlled by an integrator (PowerChrom:
222 AD Instruments, NSW, Australia) connected to a personal computer.
223 In the case of dopamine and serotonin detection, the dialysate was injected into the HPLC system
224 (HTEC-50; Eicom) directly by an auto injector (Eicom). Dopamine and serotonin were separated on
225 a PP-ODS column (Eicom), which was maintained at 25 °C, using a phosphate buffer (pH 6.0)
226 containing decane sulfonic acid (0.5 g/L), EDTA (50 μg/L), and 1% methanol as the mobile phase at
227 a flow rate of 500 μL/min. An electrochemical detector that used a glassy carbon working electrode
228 (set at + 400 mV) against a silver–silver chloride reference electrode (WE-3G; Eicom) was used to
229 quantify the compounds. Four hours after the probe was inserted, the baseline of dopamine and
230 serotonin levels was measured as the average of the last three samples. Chromatograms were
231 controlled by an integrator (PowerChrom: AD Instruments, NSW, Australia) connected to a personal
232 computer.
233

234 ## Statistical Analyses

235 All data were expressed as the mean ± standard error of mean (S.E.M.). Statistical differences
236 between the two groups were determined using a Student's *t*-test. Statistical differences among
237 values for individual groups were determined by one-way analysis of variance (ANOVA), followed
238 by the Bonferroni's post hoc tests when F ratios were significant ($p < 0.05$). The influences of drug
239 administration on individual groups were determined by two-way ANOVA, followed by the
240 Bonferroni's post hoc tests when F ratios were significant ($p < 0.05$). To analyze the GABA
241 development in the microdialysis experiment, statistical differences were evaluated by ANOVA with
242 repeated measurement, followed by Bonferroni's post hoc tests (Prism version 5).

243 # RESULTS

244

245 ## Microinjection of AAV-TMEM168 vector increased the TMEM168
246 ## mRNA expression in the NAc

247 The mRNA expression level was measured by using real time RT-PCR experiment and was presented
248 as the value relative to 36B4 mRNA level. The average of the TMEM168 mRNA levels in the
249 NAc-TMEM mice was 0.214 ± 0.05 and the average of TMEM168 mRNA levels in the NAc-Mock
250 mice (controls) was 0.0282 ± 0.003. TMEM168 mRNA levels in the NAc of the NAc-TMEM mice
251 were increased significantly when compared with the levels in the NAc-Mock mice ($N = 6$, $p < 0.01$, t
252 $= 3.979$; Student-*t* test).

253

254 ## Overexpression of TMEM168 did not change the locomotion,
255 ## spontaneous alternation, cognitive ability, social interaction, and
256 ## depression-like behaviors in mice

257 A series of behavioral tests were performed to detect the changes in the emotional behavior induced by
258 TMEM168 overexpression. The NAc-TMEM mice reported no changes in the locomotor activity test
259 (Fig 1A, $t = 1.167$) or the Y-maze test (Fig 1B, $t = 0.9495$), novel object recognition test (Fig 1C, $F_{(3,}$
260 $_{32)} = 20.98$), three chamber social interaction test (Fig 1D, $F_{(3, 32)} = 15.7$), tail suspension test (Fig 1E, t
261 $= 0.2432$), and forced swimming test (Fig 1F, $t = 0.7084$) when compared with the NAc-Mock mice.

262

263 **Figure 1 Similar preferences in locomotor activity, Y-maze, novel object recognition, three**
264 **chambers, tail suspension, and forced swimming tasks in the NAc-TMEM mice compared with**
265 **the NAc-Mock mice** (A) The locomotor activity in the NAc-Mock and NAc-TMEM mice were
266 measured for 60 min (No significant difference; Student-*t* test). (B) Working memory was assessed in
267 the Y-maze spontaneous alternation task in the NAc-Mock and NAc-TMEM mice (No significant
268 difference; Student-*t* test). (C) Cognitive function was assessed in the novel object recognition task.
269 Percentage of total exploratory time on the novel object was expressed as exploratory preference (%)
270 (No significant difference; ANOVA followed by the Bonferroni's post hoc tests). (D) Social interaction
271 was assessed in the three chambers task. Average time (10 min per phase) spent in the chamber with
272 an object or a stranger mouse was detected (No significant difference; ANOVA followed by the
273 Bonferroni's post hoc tests). (E) Immobility time of the NAc--Mock and NAc-TMEM mice in the
274 tail-suspension task was measured for 5 min (No significant difference; Student-*t* test). (F) Immobility
275 time of the NAc-Mock and NAc-TMEM mice in the forced swimming task was measured for 5 min
276 (No significant difference; Student-*t* test). Values are presented as mean ± S.E.M. N = 9.

277

278 Overexpression of TMEM168 in the NAc induced the increased 279 anxiety and decreased sensorimotor gating in mice

280 TMEM168 overexpression in the NAc increased anxiety in mice, such as entries (Fig 2A, $p < 0.05$, $t =$

281 2.844) and time (Fig 2B, $p < 0.05$, $t = 2.2.253$) on open arms in the elevated plus-maze) as well as
282 time in the light box in light/dark box tasks (Fig 2C, $p < 0.05$, $t = 2.964$). Although the startle
283 responses were not affected (Fig. 2D, $F_{(11, 96)} = 58.07$), decreased sensorimotor gating in the
284 NAc-TMEM mice was observed in the prepulse intensity of 74 dB and 82 dB in the auditory PPI test
285 (Fig. 2E, $p < 0.05$, $F_{(7, 64)} = 16.61$). It is suggested that overexpression of TMEM168 in the NAc
286 induced sensorimotor gating deficit in mice.
287

288 **Figure 2 Increased anxiety and decreased PPI in the NAc-TMEM mice compared with the**
289 **NAc-Mock mice** (A) Number of entries spent on open arms were measured for 10 min in the elevated
290 plus-maze task; $N = 9$; Values are presented as mean ± S.E.M. *$p < 0.05$ vs. NAc-Mock (Student-*t*
291 test). (B) Time spent on open arms was measured for 10 min in the elevated plus-maze task; $N = 9$;
292 Values are presented as mean ± S.E.M. *$p < 0.05$ vs. NAc-Mock (Student-*t* test). (C) Time in the light
293 box was measured for 10 min in the light/dark box task; N = 9; Values are presented as mean ± S.E.M.
294 *$p < 0.05$ vs. NAc-Mock (Student-*t* test). (D) Startle responses were measured at 70, 80, 90, 100, 110,
295 and 120 dB, respectively (background noise: 70 dB). $N = 9$; Values are presented as mean ± S.E.M. No
296 significant difference between NAc-TMEM and NAc-Mock mice (ANOVA followed by the
297 Bonferroni's post hoc tests). (E) PPI was measured for 74, 78, 82, and 86 dB, respectively, of the
298 prepulse intensity (background noise: 70 dB). Values are presented as mean ± S.E.M. $N = 9$. *$p < 0.05$
299 vs NAc-Mock (ANOVA followed by the Bonferroni's post hoc tests).

300

301 # Anxiety-like behaviors induced by TMEM168 overexpression in
302 the NAc were reversed by the administration of diazepam

303 Diazepam is an (Food and Drug Administration in USA (FDA)-approved benzodiazepine known to
304 alleviate anxiety, by activating the inotropic $GABA_A$ receptors [22]. To investigate whether the
305 anxiety-like behaviors detected in the NAc-TMEM mice could be reduced by the administration of
306 anxiolitic drugs, mice were administered with diazepam (0.3 mg/kg i.p.) or vehicle, 30 min before a
307 performance in the elevated plus-maze and the light/dark box tasks. The dose of diazepam for mice
308 administration was referred to the previous study [23], which would not affect anxious behaviors in
309 mice as a criticality. In the elevated plus-maze tasks, the decreased number of open arm entries in the
310 TMEM mice was reversed (Fig 3A, $F_{(1, 20)} = 1.169$, $p < 0.05$) and the decreased time spent in open
311 arms tend to be normalized in the NAc-TMEM mice (Fig 3B, $F_{(1, 20)} = 5.2$), following the
312 administration of diazepam. Similarly, in the light/dark box task, the decreased time spent in the light
313 box in the NAc-TMEM mice was also reversed after the administration of diazepam (Fig 3C, $F_{(1, 28)} =$
314 1.628, $p < 0.05$).

315

316 **Fig 3. Reversal of anxiety behaviors in the elevated plus-maze and light/dark box task following**
317 **the administration of diazepam in the NAc-TMEM mice** (A) and (B) Diazepam (0.3 mg/kg i.p.) or
318 vehicle was administered 30 min before performance in the elevated plus-maze task. Number of

319 entries and time on open arms were measured for 10 min in the elevated plus-maze task, $N = 6$; Values
320 are presented as mean ± S.E.M. *$p < 0.05$ vs. NAc-TMEM (VEH) (two-way ANOVA followed by the
321 Bonferroni's post hoc tests). (C) Diazepam (0.3 mg/kg i.p.) or vehicle was administered 30 min before
322 the light/dark box test. Time in the light box was measured for 10 min in the light/dark box test, $N = 8$;
323 values are presented as mean ± S.E.M. *$p < 0.05$ vs. NAc-TMEM (VEH) (two-way ANOVA followed
324 by the Bonferroni's post hoc tests); VEH: vehicle administration group, DZP: diazepam administration
325 group.
326

327 Decreased PPI induced by TMEM168 overexpression in the NAc 328 was reversed following the administration of risperidone

329 Sensorimotor gating deficit, which is detected by auditory PPI test, is assumed to be a distinctive
330 phenomenon of schizophrenia [24]. Previous studies reported that antipsychotic drugs, such as
331 risperidone, significantly reverse the low levels of sensorimotor gating [25]. Mice were injected with
332 risperidone (0.1 mg/kg i.p.) or saline, 30 min before performing the auditory PPI task. The
333 concentration of risperidone administration was referred to the previous studies [26, 27], which would
334 not affect locomotor activity and startle response in mice. No between-group difference was observed
335 in the startle response to any pulse intensity between the NAc-Mock mice and NAc-TMEM mice,
336 when these were administrated with saline or risperidone (Fig 4A, $F_{(11, 162)} = 0.6238$). However, the
337 decreased PPI in the NAc-TMEM mice was reversed following the administration of risperdone at a

338 prepulse of 74 dB ($p < 0.01$) and 78 dB ($p < 0.05$) (Fig 4B, $F_{(7, 108)} = 2.293$).

339

340 **Fig 4. Reversal of sensorimotor gating deficit in the PPI task following the administration of**
341 **risperidone in the NAc-TMEM mice** (A) Risperidone (0.01 mg/kg i.p.) or saline was administrated
342 30 min before the task performance. Startle responses was measured at 70, 80, 90, 100, 110, and 120
343 dB, respectively (background noise: 70 dB), $N = 8$; values are presented as mean ± S.E.M. No
344 significant difference between NAc-TMEM and NAc-Mock mice (two-way ANOVA followed by the
345 Bonferroni's post hoc tests). (B) Risperidone (0.01 mg/kg i.p.) or saline was administered 30 min
346 before the task performance. PPI was measured for 74, 78, 82, and 86 dB respectively, of prepulse
347 intensity (background noise: 70 dB), $N = 8$; values are presented as mean ± S.E.M. **$p < 0.01$, *$p <$
348 0.05 vs. NAc-TMEM (VEH) (two-way ANOVA followed by the Bonferroni's post hoc tests). VEH:
349 saline administration group, RIS: risperidone administration group.

350

351 Overexpression of TMEM168 in the NAc did not change the total 352 contents of glutamate and GABA, but decreased the basal levels of 353 accumbal extracellular GABA and high K^+ -stimulated GABA 354 release from the NAc

355 The contents of GABA and glutamate in the NAc were analyzed by HPLC. No difference was
356 observed between the NAc-TMEM and NAc-Mock mice (Fig 5A, $F_{(1, 40)} = 0.5878$). The TMEM168

357 overexpression inhibited the basal extracellular GABA levels (Fig 5B, $p < 0.05$, $t = 2.281$). Moreover,
358 GABA release following the potassium stimulation was decreased in the NAc-TMEM mice when
359 compared to the NAc-Mock animals (Fig 5C, $p < 0.01$, $F_{(6, 60)} = 7.683$). These results suggest that
360 TMEM168 baseline overexpression attenuated GABA neurotransmission in the NAc.

361

362 **Fig 5. Inhibitory effects of TMEM168 on GABA neurotransmission in the NAc** (A) Glutamate
363 (GLU) and GABA concentrations in the NAc tissue were measured by HPLC. The NAc-Mock mice
364 and NAc-TMEM mice were sacrificed and then the NAc tissue was extracted immediately, $N = 11$;
365 values are presented as mean ± S.E.M. No significant difference between NAc-TMEM and
366 NAc-Mock mice (two-way ANOVA followed by the Bonferroni's post hoc tests). (B) Basal levels of
367 extracellular GABA in the NAc were detected by the in vivo microdialysis task, $N = 6$; values are
368 presented as mean ± S.E.M. *$p < 0.05$ vs. NAc-Mock (Student-*t* test). (C) Dynamic changes in the
369 extracellular GABA levels in the NAc after high K^+-stimulation was analyzed in the NAc by the in
370 vivo microdialysis task, $N = 6$; values are given as mean ± S.E.M. **$p < 0.01$ vs. NAc-Mock.
371 (ANOVA with repeated measures followed by the Bonferroni's post hoc test) (D) Basal levels of
372 extracellular dopamine in the NAc were detected by the in vivo microdialysis task, $N = 4$; values are
373 presented as mean ± S.E.M. No significant difference between NAc-TMEM and NAc-Mock mice
374 (Student-*t* test). (E) Basal levels of extracellular serotonin in the NAc were detected by the in vivo
375 microdialysis task, $N = 3–4$; values are given as mean ± S.E.M. No significant difference between

376 NAc-TMEM and NAc-Mock mice (Student-*t* test).

377

378 Overexpression of TMEM168 did not change the basal amount of 379 extracellular dopamine and serotonin in the NAc

380 The basal levels of accumbal extracellular dopamine (Fig 5D, $t = 0.5635$) and serotonin (Fig 5E, $t =$ 381 0.09495) in the NAc-Mock and NAc-TMEM mice were analyzed using the in vivo microdialysis 382 method. No significant between-group difference was observed.

383 # DISCUSSION

384 Both sensorimotor gating deficit and increased anxiety are often found in patients with addiction
385 disorder [4, 28]. Repeated METH administration in rodents is usually used as a model to mimic the
386 decreased PPI and anxiety in schizophrenia [21, 29, 30]; however, a clear link between METH
387 addiction and emotional properties or sensorimotor gating function still needs to be assessed. In the
388 present study, we found that the increased METH related molecule TMEM168 in the nucleus
389 accumbens, induced anxiety in the elevated plus-maze and light/dark box tasks, and resulted in
390 sensorimotor gating deficit in the auditory PPI task. These findings suggest that TMEM168 in the NAc
391 is crucial for the modulation of anxiety and schizophrenia-like behaviors in mice.

392 GABA is a primary inhibitory neurotransmitter associated with emotion regulation anomalies,
393 including anxiety and panic disorders [31]. Specifically, the reduced concentration of GABA is
394 thought to be associated with increased anxiety levels [31]. As the injected AAV-TMEM168 vector can
395 transduce into local neurons preferentially [32, 33], approximately 99% of the affected neuronal
396 populations in the NAc of NAc-TMEM mice should be GABA neurons [2, 34]. In vivo microdialysis
397 analysis revealed that the basal levels of extracellular GABA were reduced in the NAc, and GABA
398 release was also reduced after K^+ stimulation in the NAc-TMEM mice when compared with the
399 control mice. Furthermore, the pharmacological action of anxiety reducing drug, diazepam, which is
400 known to facilitate GABAergic transmission by binding $GABA_A$ receptors [22], reversed the
401 TMEM168 overexpression-induced anxiety as measured in both the elevated plus-maze and light/dark

402 box tasks. These results suggest that a reduction in GABAergic neurotransmission could be linked to
403 TMEM168-induced anxious behaviors.
404 The trigger of anxiety is a complex process in the brain, which is related to the activity in multiple
405 neural circuits. Briefly, the amygdala, bed nucleus of the stria terminalis, and prefrontal cortex (PFC)
406 are usually identified as the key regions controlling anxiety. As a central relay structure between the
407 amygdala, basal ganglia, ventral tegmental area (VTA), and PFC, the NAc seems to play a modulatory
408 role in the anxious signal transmission from the amygdaloid complex to the latter areas [35]. In the
409 present study, we found that GABA release was inhibited following a TMEM168 transfection in the
410 NAc neurons locally, including 95% GABAergic medium spiny neurons (MSN) projecting to other
411 brain regions [2, 34]. As the direct projected targets of the accumbal MSN, the VTA and pallidum are
412 demonstrated to be relevant to anxiety symptoms via GABAergic dysfunction [36, 37]. Thus, the
413 interrupted GABAergic projection from the NAc might underlie the mechanism of the increased
414 anxiety in the NAc-TMEM mice.
415 The NAc-TMEM mice also showed reduced PPI when compared with the NAc-Mock mice in the
416 present auditory startle response test. Increased dopaminergic and serotoninergic neurotransmission in
417 the brain is presumed to reduce PPI in rodents [38, 39]. Risperidone is an antagonist of dopamine
418 receptor D2, and serotonin receptor 2A in multiple brain regions [40]. In the present study, risperidone
419 reversed the sensorimotor gating deficit associated with the overexpression of TMEM168 in the NAc.
420 This might indicate that the overexpression of TMEM168 in the NAc could mediate sensorimotor

421 deficits through an increase of dopaminergic or serotoninergic activity. However, no significant
422 difference in accumbal extracellular dopamine or serotonin between the NAc-TMEM and NAc-Mock
423 mice was observed. Numerous animal and human studies have indicated that sensorimotor gating
424 function is regulated by the cortico-striatal-pallido circuit [24, 41]. Hence, the interruption in the NAc
425 might not be a solitary part of the integral neural pathways. There is a possibility that the
426 dopaminergic and serotoninergic functions in other accumbal relevant regions such as the PFC,
427 striatum, and pallidum are indirectly affected by the GABAergic suppression in the NAc, and their
428 dysfunctions are subsequently normalized by the administration of risperidone in the NAc-TMEM
429 mice. Although the neurotransmissions in these accumbal relevant regions of the NAc-TMEM mice
430 are needed to be analyzed in the next study, the functional roles of accumbal TMEM168 in the
431 METH-induced schizophrenia-like behaviors were demonstrated firstly in the present experiment. As
432 TMEM168 is an adaptive molecule responding to METH exposure, the study of the increased
433 TMEM168 in the NAc might open a branch to elucidate the mechanism of the METH-induced
434 psychotic complications, of which one characteristic symptom is sensorimotor gating deficit.
435 The downstream signaling pathways of TMEM168 in influencing GABAergic activity or behavioral
436 events still remain unclear. Repeated administration of METH does not influence the extracellular
437 GABA levels in the NAc, but the overexpression of TMEM168 via the AAV vector transfection
438 inhibits the accumbal GABA release. It is suggested that TMEM168 may play some functional roles in
439 GABAergic regulation independent on the pharmacological effect of METH. The Crk-like protein

440 (CrkL), for example, has been found to interact with TMEM168 in a yeast two-hybrid screening study
441 [42]. CrkL, collectively with Crk, participates in the reelin signaling cascade downstream of DAB1
442 [43, 44]. The reduced expression of reelin can weaken the GABAergic neurotransmission in
443 transgenic mice and also schizophrenia or bipolar patients [45-48]. Thus, it could be suggested that the
444 activation of the TMEM168-CrkL-reelin pathway might induce behavioral changes in the
445 NAc-TMEM mice altering the GABAergic neurotransmission. Furthermore, in a previous study, we
446 found that extracellular osteopontin (OPN) was increased in the NAc-TMEM mice [14]. Activation of
447 integrin receptors is usually determined as the downstream signaling pathway of the secreted OPN
448 [49]. Mutations of β1- and β3-containing integrins in mice have been linked to anxiety disorders [50].
449 Thus, the TMEM168-OPN-integrin receptor could also be implicated in the mechanisms underpinning
450 TMEM168-effects on behavior.
451 In summary, TMEM168 overexpression in the NAc neurons could induce a decrease in the
452 extracellular GABA levels in the NAc, with effects on both anxiety levels and sensorimotor gating
453 ability. Future research should further explore the role of TMEM168 in emotional properties or
454 sensorimotor gating function.
455

456 # ACKNOWLEDGMENTS

457 We thank Naomi Takino and Mika Ito for technical assistance in producing the AAV-TMEM168
458 vectors.

459 # Authorship Contributions

460 KF, YM and AN were responsible for the study concept and design. KF YM KS ES SM and
461 KU and contributed to the acquisition of all data. KF YM KS ES and KU assisted with data
462 analysis and interpretation of findings. KF drafted the manuscript and final version of the
463 manuscript was confirmed by YM, and AN. All authors critically reviewed content and
464 approved final version for publication.

465 # REFERENCES

466 1. Nestler EJ, Carlezon WA Jr. The mesolimbic dopamine reward circuit in depression. Biol
467 Psychiatry. 2006; 59:1151-1159. https://doi.org/10.1016/j.biopsych.2005.09.018 PMID: 16566899
468 2. Russo SJ, Nestler EJ. The brain reward circuitry in mood disorders. Nat Rev Neurosci. 2013;
469 14:609-625. https://doi.org/10.1038/nrn3381 PMID: 23942470
470 3. Niwa M, Nitta A, Mizoguchi H, Ito Y, Noda Y, Nagai T, et al. A novel molecule "Shati" is involved
471 in methamphetamine-induced hyperlocomotion, sensitization, and conditioned place preference. J
472 Neurosci. 2007; 27:7604-7615. https://doi.org/10.1523/JNEUROSCI.1575-07.2007 PMID: 17626222
473 4. Grant KM, LeVan TD, Wells SM, Li M, Stoltenberg SF, Gendelman HE, et al.
474 Methamphetamine-associated psychosis. J Neuroimmune Pharmacol. 2012; 7:113-139.
475 https://doi.org/10.1007/s11481-011-9288-1 PMID: 21728034
476 5. Miyamoto Y, Ishikawa Y, Iegaki N, Sumi K, Fu K, Sato K, et al. Overexpression of Shati/Nat8l, an
477 N-acetyltransferase, in the nucleus accumbens attenuates the response to methamphetamine via
478 activation of group II mGluRs in mice. Int J Neuropsychopharmacol. 2014; 17:1283-1294.
479 https://doi.org/10.1017/S146114571400011X PMID: 24559655
480 6. Homer BD, Solomon TM, Moeller RW, Mascia A, DeRaleau L, Halkitis PN. Methamphetamine
481 abuse and impairment of social functioning: a review of the underlying neurophysiological causes and
482 behavioral implications. Psychol Bull. 2008; 134:301-10.
483 https://doi.org/10.1037/0033-2909.134.2.301 PMID: 18298273

484 7. Hellem TL. A Review of Methamphetamine Dependence and Withdrawal Treatment: A Focus on
485 Anxiety Outcomes. J Subst Abuse Treat. 2016; 71:16-22. https://doi.org/10.1016/j.jsat.2016.08.011
486 PMID: 27776672
487 8. Barrot M, Olivier JD, Perrotti LI, DiLeone RJ, Berton O, Eisch AJ, et al. CREB activity in the
488 nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. Proc Natl Acad
489 Sci U S A. 2002; 99:11435-40. https://doi.org/10.1073/pnas.172091899 PMID: 12165570
490 9. Barrot M, Wallace DL, Bolaños CA, Graham DL, Perrotti LI, Neve RL, et al. Regulation of anxiety
491 and initiation of sexual behavior by CREB in the nucleus accumbens. Proc Natl Acad Sci U S A.
492 2005;102:8357-62. https://doi.org/10.1073/pnas.0500587102 PMID: 15923261
493 10. Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, et al. Essential
494 role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science. 2006; 311:864-8.
495 https://doi.org/10.1126/science.1120972 PMID: 16469931
496 11. de la Tremblaye PB, Linares NN, Schock S, Plamondon H. Activation of CRHR1 receptors
497 regulates social and depressive-like behaviors and expression of BDNF and TrkB in
498 mesocorticolimbic regions following global cerebral ischemia. Exp Neurol. 2016; 284:84-97.
499 https://doi.org/10.1016/j.expneurol.2016.07.019 PMID: 27498336
500 12. Azogu I, Plamondon H. Inhibition of TrkB at the nucleus accumbens, using ANA-12, regulates
501 basal and stress-induced orexin A expression within the mesolimbic system and affects anxiety,
502 sociability and motivation. Neuropharmacology. 2017 pii: S0028-3908(17)30326-X.

503 https://doi.org/10.1016/j.neuropharm.2017.07.008 PMID: 28705440

504 13. Toriumi K, Mamiya T, Song Z, Honjo T, Watanabe H, Tanaka J, et al. Deletion of SHATI/NAT8L

505 decreases the N-acetylaspartate content in the brain and induces behavioral deficits, which can be

506 ameliorated by administering N-acetylaspartate. Eur Neuropsychopharmacol. 2015; 25:2108-17.

507 https://doi.org/10.1016/j.euroneuro.2015.08.003 PMID: 26361739

508 14. Fu K, Miyamoto Y, Otake K, Sumi K, Saika E, Matsumura S, Sato N, Ueno Y, Seo S, Uno K,

509 Muramatsu SI, Nitta A. Involvement of the accumbal osteopontin-interacting transmembrane protein

510 168 in methamphetamine-induced place preference and hyperlocomotion in mice. Sci Rep. 2017;

511 7:13084. https://doi.org/10.1038/s41598-017-13289-0 PMID: 29026117

512 15. Krzyzosiak A, Szyszka-Niagolov M, Wietrzych M, Gobaille S, Muramatsu S, Krezel W. Retinoid

513 x receptor gamma control of affective behaviors involves dopaminergic signaling in mice. Neuron.

514 2010; 66:908-920. https://doi.org/10.1016/j.neuron.2010.05.004 PMID: 20620876

515 16. Paxions G,Franklin KBJ.The mouse brainin stereotaxic coordinates, Compact,3rd ed. Elsevier,

516 Amsterdam;2008.

517 17. Fu K, Lin H, Miyamoto Y, Wu C, Yang J, Uno K, et al. Pseudoginsenoside-F11 inhibits

518 methamphetamine-induced behaviors by regulating dopaminergic and GABAergic neurons in the

519 nucleus accumbens. Psychopharmacology (Berl). 2016; 233:831-840.

520 https://doi.org/10.1007/s00213-015-4159-8 PMID: 26621348

521 18. Furukawa-Hibi Y, Nitta A, Fukumitsu H, Somiya H, Toriumi K, Furukawa S, et al. Absence of

522 SHATI/Nat8l reduces social interaction in mice. Neurosci Lett. 2012; 526:79-84.
523 https://doi.org/10.1016/j.neulet.2012.08.028 PMID: 22940080
524 19. Furukawa-Hibi Y, Nitta A, Fukumitsu H, Somiya H, Furukawa S, Nabeshima T, et al.
525 Overexpression of piccolo C2A domain induces depression-like behavior in mice. Neuroreport. 2010;
526 21:1177-81. https://doi.org/10.1097/WNR.0b013e3283411685 PMID: 20980930
527 20. Alkam T, Nitta A, Mizoguchi H, Itoh A, Nabeshima T. A natural scavenger of peroxynitrites,
528 rosmarinic acid, protects against impairment of memory induced by Abeta(25-35). Behav Brain Res.
529 2007; 180:139-145. https://doi.org/10.1016/j.bbr.2007.03.001 PMID: 17420060
530 21. Arai S, Takuma K, Mizoguchi H, Ibi D, Nagai T, Takahashi K, et al. Involvement of
531 pallidotegmental neurons in methamphetamine- and MK-801-induced impairment of prepulse
532 inhibition of the acoustic startle reflex in mice: reversal by GABAB receptor agonist baclofen.
533 Neuropsychopharmacology. 2008; 33:3164-75. https://doi.org/10.1038/npp.2008.41 PMID: 18354384
534 22. Riss J, Cloyd J, Gates J, Collins S. Benzodiazepines in epilepsy: pharmacology and
535 pharmacokinetics. Acta Neurol Scand. 2008; 118: 69–86.
536 https://doi.org/10.1111/j.1600-0404.2008.01004.x PMID: 18384456
537 23. Crestani F, Lorez M, Baer K, Essrich C, Benke D, Laurent JP, et al. Decreased GABAA-receptor
538 clustering results in enhanced anxiety and a bias for threat cues. Nat Neurosci. 1999; 2:833-9.
539 https://doi.org/10.1038/12207 PMID: 10461223
540 24. Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal

541 subjects, patient groups, and pharmacological studies Psychopharmacology (Berl). 2001; 156:234-258.

542 PMID: 11549226

543 25. Ouagazzal AM, Jenck F, Moreau JL. Drug-induced potentiation of prepulse inhibition of acoustic

544 startle reflex in mice: a model for detecting antipsychotic activity? Psychopharmacology (Berl) 2001;

545 156:273-283. PMID: 11549229

546 26. Miyamoto Y, Yamada K, Noda Y, Mori H, Mishina M, Nabeshima T. Hyperfunction of

547 dopaminergic and serotonergic neuronal systems in mice lacking the NMDA receptor epsilon1 subunit.

548 J Neurosci. 2001; 21:750-7. PMID: 11160454

549 27. Mierzejewski P, Kolaczkowski M, Marcinkowska M, Wesolowska A, Samochowiec J, Pawlowski

550 M, et al. Antipsychotic-like effects of zolpidem in Wistar rats. Eur J Pharmacol. 2016; 773:51-8.

551 https://doi.org/10.1016/j.ejphar.2016.01.009 PMID: 26825544

552 28. Merikangas KR, Mehta RL, Molnar BE, Walters EE, Swendsen JD, Aguilar-Gaziola S, et al.

553 Comorbidity of substance use disorders with mood and anxiety disorders: results of the International

554 Consortium in Psychiatric Epidemiology. Addict Behav. 1998; 23:893-907. PMID: 9801724

555 29. Dai H, Okuda H, Iwabuchi K, Sakurai E, Chen Z, Kato M, et al. Social isolation stress

556 significantly enhanced the disruption of prepulse inhibition in mice repeatedly treated with

557 methamphetamine. Ann N Y Acad Sci. 2004; 1025:257-66. https://doi.org/10.1196/annals.1316.032

558 PMID: 15542725

559 30. Schutová B, Hrubá L, Pometlová M, Rokyta R, Slamberová R. Responsiveness to

560 methamphetamine in adulthood is altered by prenatal exposure in rats. Physiol Behav. 2010; 99:381-7.

561 https://doi.org/10.1016/j.physbeh.2009.12.004 PMID: 20006633

562 31. Lydiard RB. The role of GABA in anxiety disorders. J Clin Psychiatry. 2003; 64 Suppl 3: 21-27.

563 PMID: 12662130

564 32. Davidson BL, Stein CS, Heth JA, Martins I, Kotin RM, Derksen TA, et al. Recombinant

565 adeno-associated virus type 2, 4, and 5 vector: transduction of variant cell types and regions in the

566 mammalian central nervous system. Proc Natl Acad Sci USA. 2000; 97:3428-3432.

567 https://doi.org/10.1073/pnas.050581197 PMID: 10688913

568 33. Tenenbaum L, Chtarto A, Lehtonen E, Velu T, Brotchi J, Levivier M. Recombinant AAV-mediated

569 gene delivery to the central nervous system. J Gene Med. 2004; 6:212-222.

570 https://doi.org/10.1002/jgm.506 PMID: 14978764

571 34. Warner-Schmidt JL, Schmidt EF, Marshall JJ, Rubin AJ, Arango-Lievano M, Kaplitt MG, et al.

572 Cholinergic interneurons in the nucleus accumbens regulate depression-like behavior. Proc Natl Acad

573 Sci USA. 2012; 109:11360-11365. https://doi.org/10.1073/pnas.1209293109 PMID: 22733786

574 35. Calhoon GG, Tye KM1. Resolving the neural circuits of anxiety. Nat Neurosci. 2015;

575 18:1394-1404. https://doi.org/10.1038/nn.4101 PMID: 26404714

576 36. Kim SY, Adhikari A, Lee SY, Marshel JH, Kim CK, Mallory CS, et al. Diverging neural pathways

577 assemble a behavioural state from separable features in anxiety. Nature. 2013; 496:219-223.

578 https://doi.org/10.1038/nature12018 PMID: 23515158

579 37. Talalaenko AN, Krivobok GK, Pankrat'ev DV, Goncharenko NV. Neurochemical mechanisms of
580 the dorsal pallidum in the antiaversive effects of anxiolytics in various models of anxiety. Neurosci
581 Behav Physiol. 2006; 36:749-754. https://doi.org/10.1007/s11055-006-0083-z PMID: 16841156
582 38. Mansbach RS, Geyer MA, Braff DL. Dopaminergic stimulation disrupts sensorimotor gating in
583 the rat. Psychopharmacology (Berl). 1988; 94: 507-514. PMID: 3131796
584 39. Fletcher PJ, Selhi ZF, Azampanah A, Sills TL. Reduced brain serotonin activity disrupts prepulse
585 inhibition of the acoustic startle reflex. Effects of 5,7-dihydroxytryptamine and p-chlorophenylalanine.
586 Neuropsychopharmacology. 2001; 24:399-409. https://doi.org/10.1016/S0893-133X(00)00215-3
587 PMID: 11182535
588 40. Lang DJ, Kopala LC, Vandorpe RA, Rui Q, Smith GN, Goghari VM, et al. An MRI study of basal
589 ganglia volumes in first-episode schizophrenia patients treated with risperidone. Am J Psychiatry.
590 2001; 158:625-31. https://doi.org/10.1176/appi.ajp.158.4.625 PMID:11282699
591 41. Swerdlow NR, Geyer MA, Braff DL. Neural circuit regulation of prepulse inhibition of startle in
592 the rat: current knowledge and future challenges. Psychopharmacology (Berl). 2001; 156:194-215.
593 PMID: 11549223
594 42. Grossmann A, Benlasfer N, Birth P, Hegele A, Wachsmuth F, Apelt L, et al. Phospho-tyrosine
595 dependent protein-protein interaction network. Mol Syst Biol. 2015; 11:794.
596 https://doi.org/10.15252/msb.20145968 PMID: 25814554
597 43. Matsuki T, Pramatarova A, Howell BW. Reduction of Crk and CrkL expression blocks

598 reelin-induced dendritogenesis. J Cell Sci. 2008; 121:1869-75. https://doi.org/10.1242/jcs.027334
599 PMID: 18477607
600 44. Park TJ, Curran T. Crk and Crk-like play essential overlapping roles downstream of disabled-1 in
601 the Reelin pathway. J Neurosci. 2008; 28:13551-62.
602 https://doi.org/10.1523/JNEUROSCI.4323-08.2008 PMID: 19074029
603 45. Fatemi SH, Earle JA, McMenomy T. Reduction in Reelin immunoreactivity in hippocampus of
604 subjects with schizophrenia, bipolar disorder and major depression. Mol Psychiatry. 2000; 5:654-63,
605 571. PMID: 11126396
606 46. Fatemi SH, Stary JM, Earle JA, Araghi-Niknam M, Eagan E. GABAergic dysfunction in
607 schizophrenia and mood disorders as reflected by decreased levels of glutamic acid decarboxylase 65
608 and 67 kDa and Reelin proteins in cerebellum. Schizophr Res. 2005; 72:109-22.
609 https://doi.org/10.1016/j.schres.2004.02.017 PMID: 15560956
610 47. Liu WS, Pesold C, Rodriguez MA, Carboni G, Auta J, Lacor P, et al. Down-regulation of dendritic
611 spine and glutamic acid decarboxylase 67 expressions in the reelin haploinsufficient heterozygous
612 reeler mouse. Proc Natl Acad Sci USA. 2001; 98:3477-82. https://doi.org/10.1073/pnas.051614698
613 PMID: 11248103
614 48. Lubbers BR, Smit AB, Spijker S, van den Oever MC. Neural ECM in addiction, schizophrenia,
615 and mood disorder. Prog Brain Res. 2014; 214:263-284.
616 https://doi.org/10.1016/B978-0-444-63486-3.00012-8 PMID: 25410362

617 49. Uede T. Osteopontin, intrinsic tissue regulator of intractable inflammatory diseases. Pathol Int.

618 2011; 61:265-280. https://doi.org/10.1111/j.1440-1827.2011.02649.x PMID: 21501293

619 50. Park YK, Goda Y. Integrins in synapse regulation. Nat Rev Neurosci. 2016; 17:745-756.

620 https://doi.org/10.1038/nrn.2016.138 PMID: 27811927