HOKKAIDO UNIVERSITY

Title	Verification of quantitative trait loci located on chromosome 4 that affect mouse behaviors by use of congenic and sub-congenic strains generated from DBA/2 and C57BL/6 mice
Author(s)	Sato, Keisuke; Morimatsu, Masami; Agui, Takashi
Citation	Japanese Journal of Veterinary Research, 71(2), 48-55 https://doi.org/10.57494/jjvr.71.2_48
Issue Date	2023-11-27
Doc URL	https://hdl.handle.net/2115/90818
Type	departmental bulletin paper
File Information	JJVR71-2_48-55_KeisukeSato.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Japanese Journal of Veterinary Research 71(2): 48-55, 2023

JJVR

REGULAR PAPER Experimental Research

Verification of quantitative trait loci located on chromosome 4 that affect mouse behaviors by use of congenic and sub-congenic strains generated from DBA/2 and C57BL/6 mice

Keisuke Sato[1)], Masami Morimatsu[1)] and Takashi Agui[1,*)]

[1)] Laboratory of Laboratory Animal Science and Medicine, Faculty of Veterinary Medicine, Hokkaido University

Received for publication, March 20, 2023; accepted, June 19, 2023

Abstract

The open field test is a basic test used to assess exploratory behavior, anxiety, and locomotor activity in rodents. Some quantitative trait loci (QTLs) regarding mouse behavior in open field were identified so far. A QTL associated with grooming duration was identified in chromosome (Chr) 4 using recombinant inbred strain made from DBA/2 (D2) and C57BL/6 (B6) mice. We previously generated D2 congenic line, D2.B6-*Sen1*, in which most region of Chr 4 was replaced to B6 haplotype. In this paper, to confirm the position of this QTL and to investigate candidate genes, two sub-congenic lines, D2.B6-*Sen1.1* and D2.B6-*Sen1.2*, were generated. From results of open field test with a congenic and two sub-congenic strains, we found that D2.B6-*Sen1.2* increased grooming duration compared with D2, but D2.B6-*Sen1.1* demonstrated opposite result, suggesting two genes responsible for grooming duration located in Chr 4. We also found that only D2.B6-*Sen1* mice decreased the time in thigmotaxis zone, suggesting another gene responsible for this behavior located in B6-derived chromosomal region.

Key Words: Congenic mice, Mouse behavior, Mouse strain difference, Open field test, Quantitative trait loci

Introduction

Behavior analysis of mouse is one of the methods to investigate human psychological disorder and neurological analysis. In behavior analysis, mouse behaviors are measured in some tests such as open field test, elevated plus maze test, water maze test etc. In open field test we can observe exploratory behavior in novel environment, feeling of anxiety in open area, and locomotion activity. Grooming is one of the native behaviors in most animals including mammals[10)]. Further, grooming is an important factor in behavioral repertoire of rodents and accounts for the large portion of behaviors while awaking[14)]. In previous analysis in mice model for autism spectrum disorder (ASD) and obsessive-compulsive disorder (OCD), excessive or abnormal grooming has been observed[12,31)].

In behavioral analysis, many reports indicated difference in reactivity among mouse strains for locomotion activity, anxiety, susceptibility for anti-anxiety drug and so on[16,19,23)]. To investigate candidate genes to affect strain difference in

* Corresponding author: Takashi Agui
Laboratory of Laboratory Animal Science and Medicine, Faculty of Veterinary Medicine, Hokkaido University, Kita-8, Nishi-9, Kita-ku, Sapporo 060-0818, Japan
TEL & FAX: +81-11-743-1110
E-mail: agui@vetmed.hokudai.ac.jp
doi: 10.57494/jjvr.71.2_48

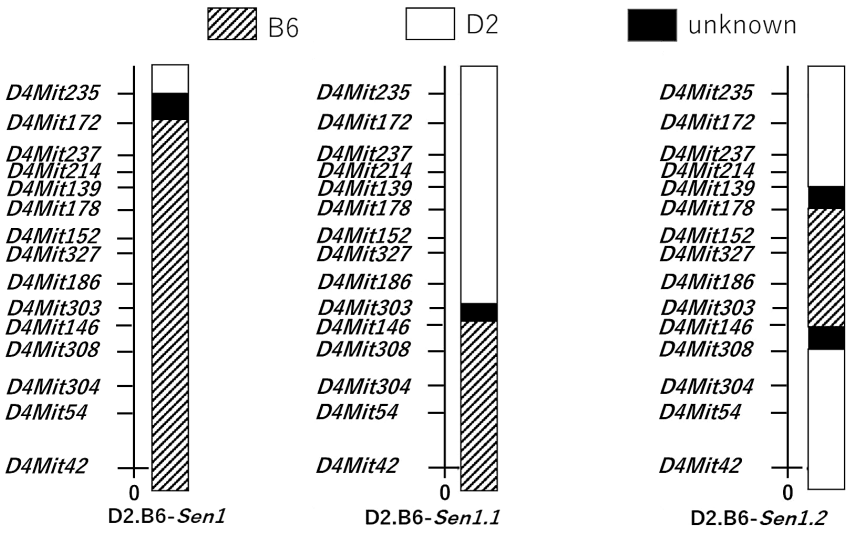

Fig. 1. Chromosomal map of congenic strain and 2 sub-congenic strains generated in this study. Shaded, open, and black columns indicate B6-derived, D2-derived, and unknown chromosomal portions, respectively

these quantitative traits, quantitative trait loci (QTL) analysis in two strains or collaborative cross of eight strains was conducted and provided information for genetic variation[18,27]. Many genetic analyses concerning behaviors in F2 generation from DBA/2 (D2) and C57BL/6 (B6), one of the major inbred mouse strains, have been conducted so far[8,23,24].

In the previous study, QTL analysis with recombinant inbred strain made from D2 and B6 mice indicated a significant QTL for grooming behavior in chromosome (Chr) 4[7]. To verify this QTL, the generation of congenic mice, in which a region locating this QTL is introduced each other between D2 and B6 mice, is necessary. We previously established a congenic strain possessing the D2-genetic background, D2.B6-*Sen1*, in which most region of Chr 4 were replaced with B6 haplotype, in order to investigate a QTL for the susceptibility to Sendai virus infection[1]. In this study, we used this congenic strain to verify a QTL affecting grooming behavior. Further, to identify the position of QTL for grooming behavior in Chr 4, we established new sub-congenic lines from D2.B6-*Sen1* and conducted open field test using these congenic and sub-congenic strains.

Materials and Methods

Mice

Establishment of congenic strain, D2.B6-*Sen1*, is described in the previous paper[1]. Two sub-congenic lines were developed from this congenic mouse as follows. D2.B6-*Sen1* was mated with D2 female mice to generate heterozygous F1 and then F1 generations were backcrossed to D2 females to establish homozygous sub-congenic lines. Introgression of chromosomal regions was confirmed by genotyping of microsatellite markers as shown in Fig. 1. Congenic and sub-congenic lines were named according to the international nomenclature guidelines and abbreviated as in parenthesis; D2.B6-(*D4Mit235-D4Mit42*)/NSlcHkv (D2.B6-*Sen1*), D2.B6-(*D4Mit146-D4Mit42*)/NSlcHkv (D2.B6-*Sen1.1*), and D2.B6-(*D4Mit178-D4Mit146*)/NSlcHkv (D2.B6-*Sen1.2*). Specific pathogen-free inbred mice, C57BL/6NSlc (B6) and DBA/2CrSlc (D2) were purchased from Japan SLC (Shizuoka, Japan) to generate congenic and sub-congenic lines. For generation of congenic and sub-congenic lines and behavioral tests with these mice, we were adhered to the AAALAC International-accredited program and the Regulation for the Care and Use of Laboratory Animals in Hokkaido University. Animal use

protocols (16-0158, 18-0160) were approved by the President of Hokkaido University after the review by the Institutional Animal Care and Use Committee.

Genotyping

Genomic DNA was prepared from ear snips of mice. Samples were incubated at 54°C for 3 h in 500 µl of lysis buffer [10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10 mM thylenediaminetetraacetic acid (EDTA) and 1% sodium dodecyl sulfate (SDS)] containing 20 mg/ml proteinase K and 10 mg/ml RNase. Genomic DNA was extracted by the standard phenol chloroform extraction method, purified by ethanol precipitation in the presence of 0.3 M sodium acetate, and finally resolved in TE buffer [10 mM Tris-HCl and 1 mM EDTA (pH 8.0)]. Informative microsatellite markers that show difference between B6 and D2 were used for genotyping. The genetic map positions (cM) of markers were obtained from the Mouse Microsatellite Database of Japan (MMDBJ).

The touchdown PCR was performed with Taq DNA polymerase (Ampliqon A/S, Odense Denmark) as follows; denaturing at 95°C for 1 min, followed by 10 cycles of denaturing at 95°C for 30 sec, primer annealing at 65°C for 30 sec (-2°C in 2 cycles), and extension at 72°C for 30 sec, 35 cycles of denaturing at 95°C for 30 sec, primer annealing at 55°C for 30 sec, and extension at 72°C for 30 sec, and final extension at 72°C for 1 min. The amplified samples were electrophoresed in 12% polyacrylamide gels in TBE solution (89 mM Tris, 89 mM boric acid and 2 mM EDTA), and then, stained with ethidium bromide. The stained samples were visualized under ultraviolet lamp and photographed.

Open field test

The open field was a square cage (50 × 50 × 40 cm) with vinyl chloride resin panel that was placed in a brightly lit room. The light intensity was 300 lx. At 30 min prior to the test, mice were transferred to the experimental room from the housing room (lighting on from 7 AM to 7 PM). All tests took place between 10:00 and 14:30. Animals were placed in the center and the test started after 5 sec and continued continuously for 20 min. Overall locomotor activity and time spent in thigmotaxis zone (5 cm of the edge of open field) were automatically recorded using ImageJ with ImageOF plugin (Ver.12011123, https://cbsn.neuroinf.jp/database/item/id/ImageOF). Rearing (standing upright on the hind legs, while forepaws are free), leaning (standing upright on the hind legs, one or two forepaws leaned on the wall), grooming frequency (number of grooming episodes), and grooming duration were manually scored. Grooming included all self-cleaning activities such as tail and ventral licking, facial wiping, etc. The numbers of each mouse strain used in this study were 6, 6, 7, 11, and 10 for B6, D2, D2.B6-*Sen1*, D2.B6-*Sen1.1*, and D2.B6-*Sen1.2*, respectively. Only males were used in this test.

Statistical analysis

The groups were compared with the student's t-test and data were showed as means ± standard errors of mean. Alpha level of $P = 0.05$ was considered as the statistical limit of significance of comparisons.

Results

Congenic and sub-congenic lines

Previous study demonstrated that grooming behavior was regulated by a QTL located in Chr 4[7]. To confirm the position of this QTL, we generated sub-congenic strains from D2.B6-*Sen1* strain, which we previously generated as a congenic line as showing that most region of Chr 4 was replaced with B6-derived chromosomal region. Fig. 1 shows haplotypes of replaced Chr 4 in sub-congenic mice. Other chromosomes except for Chr 4 were confirmed to be recipient genetic background by genotyping microsatellite markers located in other chromosomes as shown in the previous study[1]. Replaced region was confirmed to include highly significant region of the QTL peak detected as behavior-controlling locus in the previous QTL analysis[7]. D2.B6-*Sen1* and D2.B6-*Sen1.2* possessed B6-derived chromosomal region between *D4Mit146* and *D4Mit42* (51.4 cM and

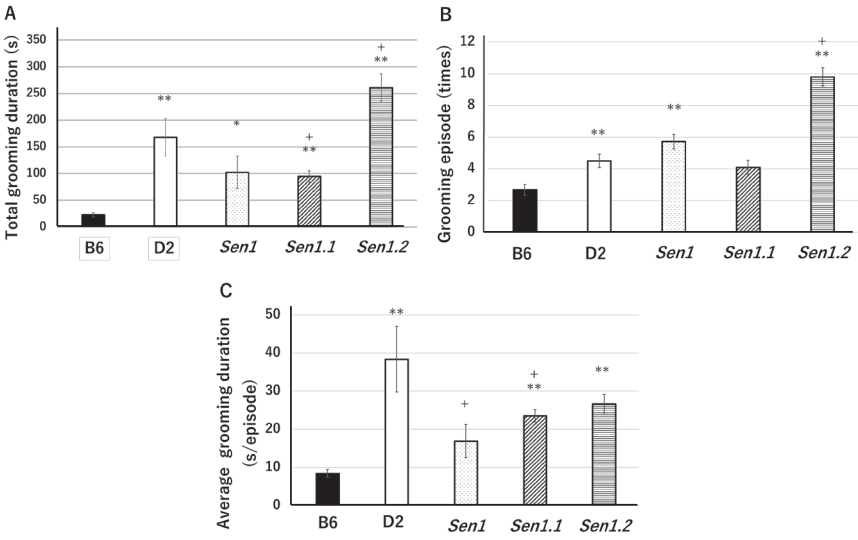

Fig. 2. Grooming behaviors in open field. Data represent the mean ± SEM. * and ** indicate $P < 0.05$ and $P < 0.01$ compared with C57BL/6, respectively. + and ++ indicate $P < 0.05$ and $P < 0.01$ compared with DBA/2, respectively. (A); total grooming duration, (B); grooming episode, and (C); grooming duration per episode

82.6 cM) and *D4Mit178* and *D4Mit146* (34.9 cM and 51.4 cM), respectively. We used these congenic and sub-congenic strains for open field test.

Grooming behavior

We measured some mouse behaviors in open field by using software. In the total duration of grooming, all D2-genetic background mice spent more time for grooming than B6 mice (Fig. 2A). D2.B6-*Sen1.1* and D2.B6-*Sen1.2* decreased and increased the total time for grooming compered to D2 mice, respectively. There was no difference between D2 and D2.B6-*Sen1*, in which most of region in Chr 4 were replaced with B6 haplotype. Grooming frequency of all D2-genetic background strains except for D2.B6-*Sen1.1* was higher than that of B6 mice. Of note, the frequency of D2.B6-*Sen1.2* was much higher than that of D2 mice. The average times per grooming episode of D2, D2.B6-*Sen1.1*, and D2.B6-*Sen1.2* were much higher than that of B6 mice, whereas those of D2.B6-*Sen1* and D2.B6-*Sen1.1* were lower than that of D2 mice (Fig. 3C).

Locomotion activity

In all locomotion activities, all D2-genetic background mice showed significant difference from donor strain, C57BL/6 (Figs. 3A, 3B, 3C, 3D, and 3E). In only D2.B6-*Sen1*, total locomotion duration was higher than that of background strain, DBA/2 (Fig. 3B). Furthermore, moving distance was higher than D2, although it was not significant (Fig. 3A). Generally, D2.B6-*Sen1* showed more moving activity than background strain. We measured the time of spending in thigmotaxis zone, the edge of open field (5 cm), which showed tendency of mouse anxiety and exploration activity. The time spending in thigmotaxis zone in only D2.B6-*Sen1* was significantly lower than that of D2 mice (Fig. 3C). Standing behavior such as rearing and leaning was comparable among all D2-genetic background strains (Figs. 3D and 3E).

Discussion

Strain difference

The result of this study must be considered within genetic background and behavioral trait of both B6 and D2 mouse strains. Previous studies showed that behavioral status of both strains was significantly different in some behavior tests[4,5]. In previous exploration test in open field, B6

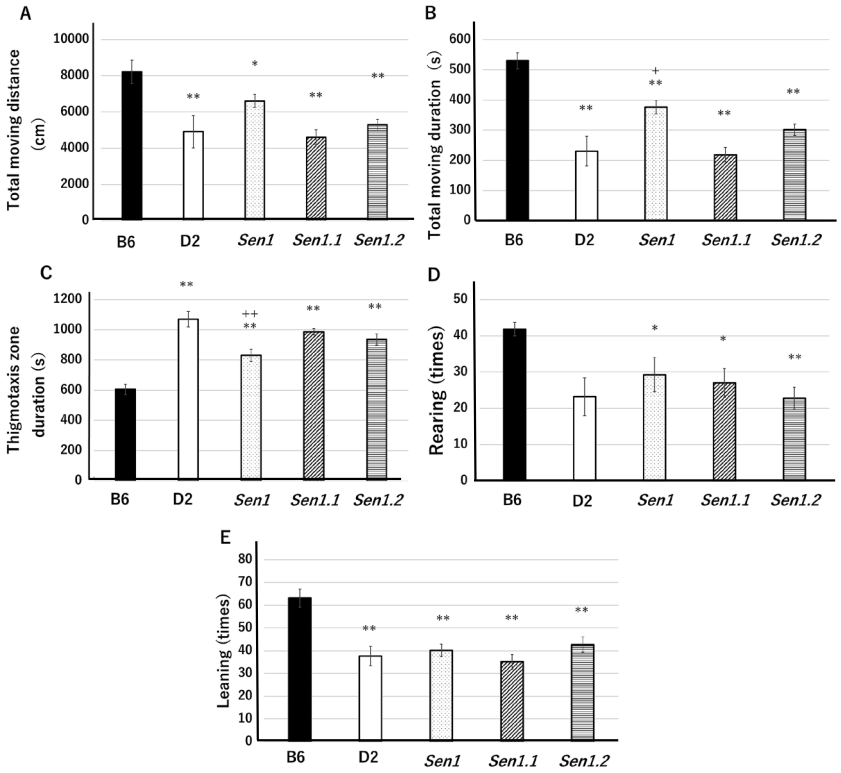

Fig. 3. Locomotion activities in open field. Data represent the mean ± SEM. * and ** indicate $P < 0.05$ and $P < 0.01$ compared with C57BL/6, respectively. + and ++ indicate $P < 0.05$ and $P < 0.01$ compared with DBA/2, respectively. (A); total moving distance, (B); total moving duration, (C); duration in thigmotaxis zone (5 cm of the edge of open field), (D); rearing, and (E); leaning behavior

mice were more exploratory and spent less time for grooming for the first thirty minutes than D2 mice, but there was no difference in time for spending grooming behavior between both strains after that[3]. Both strains showed different phenotypes in psychotropic drug administration in terms of scare, fight, and social behavior in their stress response[14,17,28]. Considering them, it is important to compare genetic factors of each strain that control behaviors.

Sex difference

In rodent behavior experiments, sexual difference has been discussed for long time. Female F_2 mice generated from B6 and 129 strains showed more locomotion activity than males in open field[11]. In addition, female mice showed higher activity in open field connected with home cage for suppressing anxiety[22]. Fluctuations in ovarian hormones have been considered to cause behavioral changes related to emotion or anxiety. Reductions of anxiety behavior in female rats have been reported during the phases of proestrus and estrus compared with phases of metaestrus and diestrus[2,20,32]. Moreover, ventral hippocampal modulation of anxiety in B6 mice revealed significant biological sex difference in several tests[30]. These data suggest that there is different pathway against anxiety in sex difference. Therefore, we used only male mice in this study.

Grooming behavior

Rodent grooming behavior is suggested as an indicator of the limited repetitive behavior and anxiety[9,15,21]. It has been known that rodents' grooming activity increases in the relax situation in contrast to feeling anxiety. Previous study showed that relax or low stress condition induced coherent grooming from leg to tail, but high stress condition induced very short grooming[29].

In contrast to grooming induced by anxiety, it has been reported that mutations in some genes such as *Dab1*, *Hoxb8*, and *VDR* induce pathological grooming[13,15,26]. *Dab1* gene locates in Chr 4 and the grooming position and frequency were changed due to the disorder in cerebellum in *Dab1* gene mutant mice known as the scrambler mouse[26]. SNPs in *Dab1* have been implicated in influencing grooming associated with fear experiences in multiple mouse inbred strains[25] In *Hoxb8* mutation mice, the change of grooming was indicated under the influence of impairment in microglia development in the brain, but the details of the mechanism are still unclear[6]. In this study, we assume that stress- or nerve-related genes altered to B6 alleles from D2 alleles caused a decrease in both grooming and whole grooming durations in D2.B6-*Sen1.1*, compared with that of D2. In another sub-congenic line, D2.B6-*Sen1.2*, the grooming duration was expected to decrease as well as D2.B6-*Sen1*. However, we observed a little reduction of grooming duration and significant increase in both grooming frequency and whole grooming duration in D2.B6-*Sen1.2* than D2. In D2.B6-*Sen1* strain, in which most region of Chr 4 was altered to B6 allele, we found the decrease in grooming duration per episode only. From these results, we hypothesized that there were two genes affecting grooming behavior in Chr 4. One putative gene locating downstream of Chr 4, namely downstream of *Mit146*, upregulates grooming behavior in D2 mice, whereas another putative gene locating mid-region of Chr 4, namely between *Mit139* and *Mit308*, downregulates grooming behavior in D2 mice.

Spending time in thigmotaxis zone

Duration spending in thigmotaxis zone suggests a dilemma between anxiety behavior in open area and exploration of novel environments. The reduction of exploration is made by freezing behavior induced by strong anxiety. Previous study conducting QTL analysis with many mouse strains in open field test showed that B6 strain stayed for the shortest time in thigmotaxis zone and proposed the presence of a candidate gene in Chr 4[18]. In this study, D2.B6-*Sen1* spent significantly lower time than D2 in the area. This result suggests that gene suppressing anxiety may locate upstream of *Mit178* in Chr4, which is replaced from D2 allele to B6 allele in only D2.B6-*Sen1*. This prediction strongly supports the presence of candidate gene proposed in the previous report [8].

References

1) Abbas RMF, Torigoe D, Kameda Y, Tag-El-Din-Hassan HT, Sasaki N, Morimatsu M, Agui T. Verification of genetic loci responsible for the resistance/susceptibility to the Sendai virus infection using congenic mice. Infect Genet Evol 57, 75–81, 2018. doi: 10.1016/j.meegid.2017.11.008.
2) Bath KG, Chuang J, Spencer-Segal JL, Amso D, Altemus M, McEwen BS, Lee FS. Variant brain-derived neurotrophic factor (Valine66Methionine) polymorphism contributes to developmental and estrous stage-specific expression of anxiety-like behavior in female mice. Biol Psychiatry 72, 499–504, 2012. doi: 10.1016/j.biopsych.2012.03.032.
3) Cabib S, Algeri S, Perego C, Puglisi-Allegra S. Behavioral and biochemical changes monitored in two inbred strains of mice during exploration of an unfamiliar environment. Physiol Behav 47, 749–753, 1990. doi: 10.1016/0031-9384(90)90089-m.
4) Cabib S, Puglisi-Allegra S, Ventura R. The contribution of comparative studies in inbred strains of mice to the understanding of the hyperactive phenotype. Behav Brain Res 130, 103–109, 2002. doi: 10.1016/s0166-4328(01)00422-3.
5) Carola V, D'Olimpio F, Brunamonti E, Mangia F, Renzi P. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behav Brain Res 134, 49–57, 2002. doi: 10.1016/s0166-4328(01)00452-1
6) Chen SK, Tvrdik P, Peden E, Cho S, Wu S, Spangrude G, Capecchi MR. Hematopoietic

origin of pathological grooming in Hoxb8 mutant mice. Cell 141, 775–785, 2010. doi: 10.1016/j.cell.2010.03.055.
7) Delprato A, Algéo MP, Bonheur B, Bubier JA, Lu L, Williams RW, Chesler EJ, Crusio WE. QTL and systems genetics analysis of mouse grooming and behavioral responses to novelty in an open field. Genes Brain Behav 16, 790–799, 2017. doi: 10.1111/gbb.12392.
8) Demarest K, Koyner J, McCaughran J Jr, Cipp L, Hitzemann R. Further characterization and high-resolution mapping of quantitative trait loci for ethanol-induced locomotor activity. Behav Genet 31, 79–91, 2001. doi: 10.1023/a:1010261909853.
9) Estanislau C, Veloso AWN, Filgueiras GB, Maio TP, Dal-Cól MLC, Cunha DC, Klein R, Carmona LF, Fernández-Teruel A. Rat self-grooming and its relationships with anxiety, dearousal and perseveration: Evidence for a self-grooming trait. Physiol Behav 209, 112585, 2019. doi: 10.1016/j.physbeh.2019.112585.
10) Fentress JC. Expressive contexts, fine structure, and central mediation of rodent grooming. Ann N Y Acad Sci 525, 18–26, 1988. doi: 10.1111/j.1749-6632.1988.tb38592.x.
11) Fritz AK, Amrein I, Wolfer DP. Similar reliability and equivalent performance of female and male mice in the open field and water-maze place navigation task. Am J Med Genet C Semin Med Genet 175, 380–391, 2017. doi: 10.1002/ajmg.c.31565.
12) Graybiel AM. Habits, rituals, and the evaluative brain. Annu Rev Neurosci 31, 359–387, 2008. doi: 10.1146/annurev.neuro.29.051605.112851.
13) Greer JM, Capecchi MR. Hoxb8 is required for normal grooming behavior in mice. Neuron 33, 23–34, 2002. doi: 10.1016/s0896-6273(01)00564-5.
14) Griebel G, Belzung C, Perrault G, Sanger DJ. Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. Psychopharmacology (Berl) 148, 164–170, 2000. doi: 10.1007/s002130050038.
15) Kalueff AV, Tuohimaa P. Grooming analysis algorithm for neurobehavioural stress research. Brain Res Brain Res Protoc 13, 151–158, 2004. doi: 10.1016/j.brainresprot.2004.04.002.
16) Kalueff AV, Tuohimaa P. Contrasting grooming phenotypes in three mouse strains markedly different in anxiety and activity (129S1, BALB/c and NMRI). Behav Brain Res 160, 1–10, 2005. doi: 10.1016/j.bbr.2004.11.010.
17) Kempf E, Puglisi-Allegra S, Cabib S, Schleef C, Mandel P. Serotonin levels and turnover in different brain areas of isolated aggressive or non-aggressive strains of mice. Prog Neuropsychopharmacol Biol Psychiatry 8, 365–371, 1984. doi:10.1016/S0278-5846(84)80023-8.
18) Logan RW, Robledo RF, Recla JM, Philip VM, Bubier JA, Jay JJ, Harwood C, Wilcox T, Gatti DM, Bult CJ, Churchill GA, Chesler EJ. High-precision genetic mapping of behavioral traits in the diversity outbred mouse population. Genes Brain Behav 12, 424–437, 2013. doi: 10.1111/gbb.12029.
19) Meier SM, Trontti K, Purves KL, Als TD, Grove J, Laine M, Pedersen MG, Bybjerg-Grauholm J, Bækved-Hansen M, Sokolowska E, Mortensen PB, Hougaard DM, Werge T, Nordentoft M, Breen G, Børglum AD, Eley TC, Hovatta I, Mattheisen M, Mors O. Genetic variants associated with anxiety and stress-related disorders: A genome-wide association study and mouse-model study. JAMA Psychiatry 76, 924–932, 2009. doi: 10.1001/jamapsychiatry.2019.1119.
20) Mora S, Dussaubat N, Díaz-Véliz G. Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. Psychoneuroendocrinology 21, 609–620, 1996. doi: 10.1016/s0306-4530(96)00015-7.
21) Moyaho A, Valencia J. Grooming and yawning trace adjustment to unfamiliar environments in laboratory Sprague-Dawley rats (Rattus norvegicus). J Comp Psychol 116, 263–269, 2002. doi: 10.1037/0735-7036.116.3.263.
22) Palanza P. Animal models of anxiety and

depression: how are females different? Neurosci Biobehav Rev 25, 219–233, 2001. doi: 10.1016/s0149-7634(01)00010-0.
23) Parker CC, Cheng R, Sokoloff G, Palmer AA. Genome-wide association for methamphetamine sensitivity in an advanced intercross mouse line. Genes Brain Behav 11, 52–61, 2012. doi: 10.1111/j.1601-183X.2011.00747.x.
24) Parker CC, Sokoloff G, Cheng R, Palmer AA. Genome-wide association for fear conditioning in an advanced intercross mouse line. Behav Genet 42, 437–448, 2012. doi: 10.1007/s10519-011-9524-8.
25) Seemiller LR, Mooney-Leber SM, Henry E, McGarvey A, Druffner A, Peltz G, Gould TJ. Genetic background determines behavioral responses during fear conditioning. Neurobiol Learn Mem 184, 107501, 2021. doi: 10.1016/j.nlm.2021.107501.
26) Strazielle C, Lefevre A, Jacquelin C, Lalonde R. Abnormal grooming activity in Dab1^{scm} (scrambler) mutant mice. Behav Brain Res 233, 24–28, 2012. doi: 10.1016/j.bbr.2012.04.038.
27) Talbot CJ, Nicod A, Cherny SS, Fulker DW, Collins AC, Flint J. High-resolution mapping of quantitative trait loci in outbred mice. Nat Genet 21, 305–308, 1999. doi: 10.1038/6825.
28) van der Veen R, Piazza PV, Deroche-Gamonet V. Gene-environment interactions in vulnerability to cocaine intravenous self-administration: a brief social experience affects intake in DBA/2J but not in C57BL/6J mice. Psychopharmacology (Berl) 193, 179–186, 2007. doi: 10.1007/s00213-007-0777-0.
29) van Erp AMM, Kruk MR, Meelis W, Willekens-Bramer DC. Effect of environmental stressors on time course, variability and form of self-grooming in the rat: handling, social contact, defeat, novelty, restraint and fur moistening. Behav Brain Res 65, 47–55, 1994. doi: 10.1016/0166-4328(94)90072-8.
30) Wang C, Zhang Y, Shao S, Cui S, Wan Y, Yi M. Ventral hippocampus modulates anxiety-like behavior in male but not female C57BL/6 J mice. Neuroscience 418, 50–58, 2019. doi: 10.1016/j.neuroscience.2019.08.032.
31) Wurzman R, Forcelli PA, Griffey CJ, Kromer LF. Repetitive grooming and sensorimotor abnormalities in an ephrin-A knockout model for Autism Spectrum Disorders. Behav Brain Res 278, 115–128, 2015. doi: 10.1016/j.bbr.2014.09.012.
32) Zimmerberg B, Farley MJ. Sex differences in anxiety behavior in rats: role of gonadal hormones. Physiol Behav 54, 1119–1124, 1993. doi: 10.1016/0031-9384(93)90335-d.