DOI 10.1007/s11055-018-0590-8
Neuroscience and Behavioral Physiology, Vol. 48, No. 4, May, 2018

Characteristics of the Formation of Memories Relating to Fear in Mice with Depression- and Schizophrenia-Like Phenotypes: Effects of Gender and Age

N. I. Dubrovina,[1] M. V. Khrapova,[1,2] and T. V. Lipina[1,2]

Translated from Rossiiskii Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 103, No. 1, pp. 10–21, January, 2017. Original article submitted June 20, 2016.

We report here a comparative analysis of the acquisition of a conditioned passive avoidance reaction in mice of the mutant strains DISC1-Q31L and DISC1-L100P and mice of the control strain C57Bl/6NCr1 and detection of the effects of gender and age on learning. DISC1-L100P mice showed impairments to associative learning to avoid the dangerous dark sector of the apparatus regardless of gender and age. In DISC1-Q31L mice, the fear memory trace formed only in young males. A deficit of aversive learning was demonstrated in old mice of all the strains tested, with identically lower levels of learning ability in female DISC1-Q31L and C57Bl/6NCr1 mice than males. These characteristics of learning avoidance provide an additional argument for regarding DISC1-L100P mice as a genetic model of a schizophrenia-like state and DISC1-Q31L mice as a model of a depression-like state.

Keywords: fear memory, conditioned passive avoidance reaction, age, gender-related differences, DISC1-Q31L, DISC1-L100P and C57Bl/6NCr1 mice.

The ability to identify threats in the surrounding world and mount targeted responses to them are critical for adaptation of the body. Fear is a fundamental adaptive manifestation of behavior supporting survival [15]. This adaptive function is linked with the potential for rapid and consistent acquisition of associations between neutral stimuli, including context, and a negative event. A typical example of such associative learning is the conditioned fear reaction. Paradigms forming fear memories (classical conditioned fear reaction, fear-potentiated twitch reactions, conditioned passive avoidance reactions) are very widely used for identifying the neuronal and molecular mechanisms of memory in studying cognitive impairments in psychopathological states using experimental models.

As the genetic component determines the risk of developing psychopathology [17], many investigators display considerable interest in studying fear memory in mice of inbred and genetically modified strains [1, 2, 21, 29, 35, 39]. The genetic factor has been shown to have a significant influence on the neuronal processes of fear memories, including learning, consolidation, retention, and extinction of the memory trace.

Finding an experimental model in rodents with the full set of pathological signs of any disease is very difficult. In the cases of schizophrenia and depression, this is made more complex by the heterogenous nature of the symptoms and difficulties in establishing a reliable pathological etiology [34]. Among a multitude of experimental models of these diseases, the greatest interest in recent years has been in genetic models with impairment of the *DISC1* gene (Disrupted in Schizophrenia 1), which has a very strong link with the development of schizophrenia and depression [16, 27, 31, 36]. Two mouse strains were created carrying point mutations in exon 2 of the *DISC1* gene – *DISC1-L100P* (substitution of a leucine by proline at position 100), which is regarded as a genetic model of a schizophrenia-like state, and *DISC1-Q31L* (replacement of glutamine by leucine at

[1] Research Institute of Physiology and Basic Medicine, Novosibirsk, Russia; e-mail: dubrov@physiol.ru.
[2] Novosibirsk National Research State University, Novosibirsk, Russia.

position 31), which is regarded as a genetic model of a depression-like state [8, 27]. The behavioral profiles of DISC1-L100P and DISC1-Q31L mice were shown by these investigators to differ from the behavioral profile of mice of the control strain C57Bl/6NCr1 [8, 25, 26]. DISC1-L100P mice displayed a high level of movement activity in the open field test, with no alteration in anxiety in the elevated plus maze test or in social behavior, or in measures of depression-like behavior in the forced swimming test. DISC1-L100P mice showed a clear deficit of prestimulus inhibition of an acoustic startle reaction, reflecting impairment to sensorimotor coupling and information filtration. As regards memory, experimental studies have demonstrated impairments to latent inhibition and significant weakening of working memory, but retention of spatial learning and memory in the Morris test. DISC1-Q31L mice did not differ from control mice in terms of movement activity or exploratory and anxiety behavior, though they differed from DISC1-L100P mice in terms of the main measure of depressivity in the forced swimming test – they showed high levels of immobility. They also displayed a deficit of social behavior and anhedonia behavior typical of the depression-like state, i.e., a decrease in interest in pleasures detected by testing preference reactions for sucrose solutions. Weakening of working memory and latent inhibition was demonstrated, though spatial learning and memory were not altered. At the same time, other data have been reported on the properties of the behavioral phenotypes in DISC1-L100P and DISC1-Q31L mice [36]. However, the literature lacks data on the formation of a conditioned fear reaction in mice of these strains, though fear memory is highly susceptible to psychopathological impairments.

The second factor, along with the genetic characteristics of behavior, with significant influences on the acquisition of the conditioned fear reaction, is gender [9, 12, 19, 32]. Marked gender-related differences in learning of aversive stimuli in rodents have been noted mainly in tests with powerful emotional contexts. Studies of gender-related differences in aversive learning have recently become important because of the need for individual approaches to the treatment of stress-induced psychopathological disorders [11]. Furthermore, epidemiological investigations have identified significant differences between men and women in the manifestation of psychopathological impairments [4, 13]. In particular, men had a greater predisposition to diseases such as autism and schizophrenia, while women are more susceptible to post-traumatic disorders, depression, and pathological anxiety. Another important factor on which the intensity of fear memory trace acquisition depends is age [14, 18, 20, 28, 37]. Older animals generally show decreased formation and retention of emotional memories. It should be noted that no studies of the influences of gender and age on behavioral and cognitive status in DISC1-L100P and DISC1-Q31L mice have been reported.

On the basis of these data, and considering the lack of data on fear memory formation in DISC1-L100P and DISC1-Q31L mice, there is a need for comparative analysis of the acquisition of a conditioned passive avoidance reaction in these mice and C57Bl/6NCr1 control mice in relation to gender and age.

Methods. Experiments used homozygous DISC1-Q31L and DISC1-L100P males, as well as C57Bl/6NCr1 mice in the control group. Experiments were performed using 128 mice of these strains, making up 12 groups: group 1 consisted of male C57Bl/6NCr1 mice aged 3–5 months (n = 10); group 2 were female C57Bl/6NCr1 mice aged 3–5 months (n = 10); group 3 was male C57Bl/6NCr1 mice aged 9–11 months (n = 8); group 4 consisted of female C57Bl/6NCr1 mice aged 9–11 months (n = 10); group 5 was male DISC1-Q31L mice aged 3–5 months (n = 25); group 6 was female DISC1-Q31L mice aged 3–5 months (n = 10); group 7 consisted of male DISC1-Q31L mice aged 9–11 months (n = 10); group 8 consisted of female DISC1-Q31L mice aged 9–11 months (n = 11); group 9 was male DISC1-L100P mice aged 3–5 months (n = 8); group 10 consisted of female DISC1-L100P mice aged 3–5 months (n = 8); group 11 was male DISC1-L100P mice aged 9–11 months (n = 12); group 12 was female DISC1-L100P mice aged 9–11 months (n = 6). Animals were kept in groups of four individuals per cage with free access to food and water.

All experiments were performed in compliance with international rules [European Communities Council Directive of November 24, 1986 (86/609/EEC)].

Training to the conditioned passive avoidance reaction was by a standard single-session method in an experimental chamber with dark and light sectors and an automated Gemini Avoidance System apparatus (San Diego Instruments). On the training day, mice were placed in the light sector with the tail towards the open door. On transfer to the dark sector, the door was closed and the mouse received a painful electric shock (0.5 mA, 2 sec). After 10–20 sec, the animal was transferred to the home cage. On testing 24 h after acquisition of the conditioned reflex, the mouse was again placed in the light sector with the door openth. The Gemini program was used for automatic recording of the latent period of transfer to the dark sector. The latent period of the transfer on the training day before pain stimulation reflects the initial duration of preference for the dark chamber, while on testing it is a measure of the acquisition of the conditioned passive avoidance reaction. The maximum duration of observation of the animal was 180 sec. Potential variation in the learning measure (latent period of transfer) due to the animal keeping conditions and the environment were excluded, allowing more accurate assignment of differences in learning to the genotype, gender, and age factors.

Results were processed statistically by analysis of variance (ANOVA).

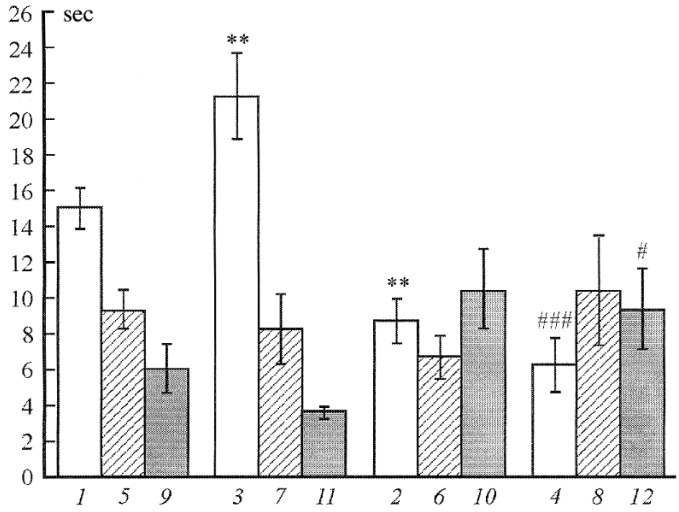

Fig. 1. Comparison of mean latent periods of transfer into the dark sector in C57Bl/6NCr1, DISC1-Q31L, and DISC1-L100P mice on the day of training to the conditioned passive avoidance reaction. Light columns show C57Bl/6NCr1 mice, shaded columns show DISC1-Q31L mice, and dark columns show DISC1-L100P mice. The ordinate shows latent periods of transfers to the dark sector; the abscissa shows groups of mice: *1*, *5*, *9*) male C57Bl/6NCr1, DISC1-Q31L, and DISC1-L100P mice aged 3–5 months; *2*, *6*, *10*) female C57Bl/6NCr1, DISC1-Q31L, and DISC1-L100P mice aged 3–5 months; *3*, *7*, *11*) male C57Bl/6NCr1, DISC1-Q31L, and DISC1-L100P mice aged 9–11 months; *4*, *8*, *12*) female C57Bl/6NCr1, DISC1-Q31L, and DISC1-L100P mice aged 9–11 months. $^{**}p < 0.01$ compared with young males; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ compared with old males.

TABLE 1. Classification of Mice in Terms of the Level of Learning the Conditioned Passive Avoidance Reaction, %

Mice		Time, sec			
		5–30	31–60	61–120	121–180
C57BL/6NCrl					
♂	Young	0	0	30	70
	Old	25	35	25	15
♀	Young	30	10	0	60
	Old	60	10	20	10
DISC1-Q31L					
♂	Young	28	20	12	40
	Old	60	40	0	0
♀	Young	40	20	30	10
	Old	90	10	0	0
DISC1-L100P					
♂	Young	62.5	12.5	25	0
	Old	75	8	17	0
♀	Young	62.5	25	0	12.5
	Old	66	34	0	0

Results. As males and females are known to differ in terms of movement activity [9, 19, 30], there is a need to analyze initial latent periods of transfers recorded before application of painful stimuli on the day of training to the conditioned passive avoidance reaction (Fig. 1). Three-factor analysis of variance (factors: genotype, age, gender) identified a statistically significant genotype influence ($F_{2,123} = 7.7936$, $p < 0.001$) on the latent period of transfer, though the influences of gender and age were not statistically significant. Post hoc analysis (Fisher LSD) demonstrated statistically significant differences between the C57Bl/6NCr1 mice of the control group and DISC1-Q31L ($p < 0.05$) and DISC1-L100P ($p < 0.001$) mutant mice. Figure 1 shows that male but not female DISC1-Q31L and DISC1-L100P mice had shorter latent periods than C57Bl/6NCr1 mice.

Analysis by groups (with genotype as the grouping feature) showed that gender affected the initial latent period of transfer in C57Bl/6NCr1 mice ($F_{1,34} = 47.1387$, $p < 0.001$) and DISC1-L100P mice ($F_{1,30} = 10.67242$, $p < 0.01$) but had no statistically significant effect in DISC1-Q31L mice ($F_{1,52} = 0.01560$, $p > 0.05$). Post hoc analysis (Fisher's LSD) showed the following: no statistically significant differences were seen between groups of DISC1-Q31L mice; statistically significant differences in DISC1-L100P mice were found between old male and old females ($p < 0.05$); young males in groups of C57Bl/6NCr1 control mice entered the dark sector more quickly than old mice ($p < 0.01$) but more slowly than young ($p < 0.01$) and old ($p < 0.01$) females, while old males showed significantly more delayed transfer to the dark sector than old females ($p < 0.001$). This is evidence that gender and age make contributions to motor and motivational components of the reaction to contextual novelty only in C57Bl/6NCr1 mice, but not in mice of the mutant strains DISC1-Q31L and DISC1-L100P.

Training to the conditioned passive avoidance reaction was manifest as an increase in the latent period of transfer to the dark sector on testing at 24 h as compared with the training day. Qualitative analysis in terms of the learning abilities of DISC1-L100P and DISC1-Q31L mice compared with the control strain showed, first, that DISC1-L100P mice had a gender-independent inability to form an association between the context of the experimental apparatus and the danger of the dark sector where they had received pain punishment (see Table 1). Second, among young male and female DISC1-Q31L mice, there were significantly fewer showing good learning (latent periods of transfer 121–180 sec) than C57Bl/6NCr1 mice (40 and 10%, compared with 70 and 60%, respectively). Third, there was a clear deficit in the reproduction of the conditioned passive avoidance reaction in older male and female C57Bl/6NCr1 mice but only in old male DISC1-Q31L mice. Fourth, lower levels of learning were seen in young female DISC1-Q31L mice than young males, which was not the case in mice of the control group.

We will consider the quantitative results of the acquisition of the conditioned passive avoidance reaction in

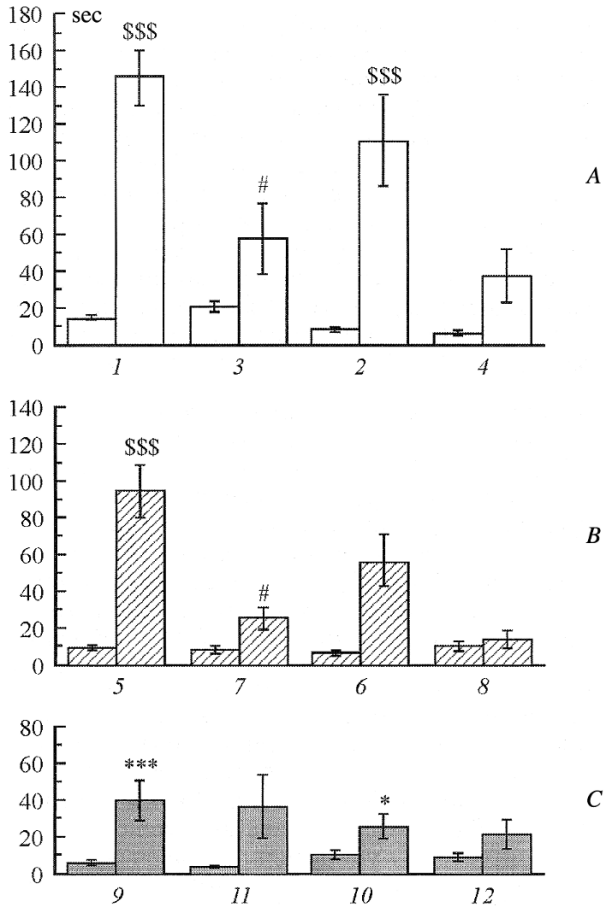

Fig. 2. Comparison of the acquisition of the conditioned passive avoidance reaction in C57Bl/6NCr1, DISC1-Q31L, and DISC1-L100P mice. *A*) C57Bl/ 6NCr1 mice; *B*) DISC1-Q31L mice; *C*) DISC1-L100P mice. The ordinate shows the latent periods of transfers into the dark sector, sec; the abscissa shows mouse group identification numbers as in Fig. 1. The first column in each pair shows the latent period of transfers on the day of training to the reflex and the second shows the latent period on testing 24 h after acquisition. $^{\$\$\$}p < 0.001$ compared with latent periods before training (day 0); $^{*}p < 0.005$, $^{***}p < 0.001$ compared with young C57Bl/6NCr1 females and males; $^{\#}p < 0.05$ compared with young C57Bl/6NCr1 and DISC1-Q31L males.

DISC1-Q31L and DISC1-L100P mice as compared with C57Bl/6NCr1 mice (Fig. 2). Analysis of the repeat-measures variance of the latent period of transfer identified statistically significant factors – genotype ($F_{2,116} = 14.36$, $p < 0.001$), age ($F_{1,116} = 26.63$, $p < 0.001$), and gender ($F_{1,116} = 4.57$, $p < 0.05$). A genotype × gender interaction was found ($F_{2,116} = 3.20$, $p < 0.05$), though there was no genotype × age and no gender × age interaction. There was also no interaction between the three factors. At the same time, repeat measures analysis of the latent period of transfer on testing showed a statistically significant difference from the value recorded on the day of acquisition of the conditioned passive avoidance reaction ($F_{1,116} = 91.58$, $p < 0.001$). The repeat measures factor interacted with the genotype factor ($F_{2,116} = 10.17$, $p < 0.001$) and age ($F_{1,116} = 29.02$,

$p < 0.001$). A repeat measure factor × genotype × age interaction was found ($F_{2,116} = 4.21$, $p < 0.05$). Other complex interactions (repeat measure × genotype × gender; repeat measure × gender × age; repeat measure × genotype × gender × age) were not statistically significant.

Comparative analysis of differences in the acquisition of the conditioned reflex between mice of the mutant strains and control animals showed that only young male DISC1-Q31L mice showed formation of a memory trace; learning did not occur in DISC1-L100P mice; young male and female C57Bl/6NCr1 mice demonstrated good learning (Fig. 2). This was evident as a statistically significant increase in the latent period of transfer on testing (as compared with initial values) in young male and female C57Bl/6NCr1 mice ($p < 0.001$ and $p < 0.001$, respectively) and young male DISC1-Q31L mice ($p < 0.001$). We would like to draw attention to the marked tendency of young female DISC1-Q31L mice to learn, as 60% of these mice showed an intermediate level of reproduction of the conditioned reaction (see Table 1). The same type of tendency was characteristic of old male and female C57Bl/6NCr1 mice, but not for DISC1-Q31L or DISC1-L100P mice. Although Fig. 2 shows a reduction in learning in young male and female DISC1-Q31L mice from the level seen in the control strain, statistically significant differences in the latent period of transfers on testing were seen only for young male and female C57Bl/6NCr1 and DISC1-L100P mice ($p < 0.001$ and $p < 0.05$, respectively).

As regards the influence of age, there was a statistically significant decrease in learning in males of both the DISC1-Q31L ($p = 0.03$) and C57Bl/6NCr1 ($p = 0.02$) strains aged 9–12 months (Fig. 2). A decrease in the reproduction of the conditioned passive avoidance reflex with age was also recorded in females of both strains, though differences in the latent period of transfers on testing did not reach statistical significance because of the high level of individual variation in this parameter. However, the decrease in the acquisition of the conditioned reflex with age in DISC1-Q31L mice was less marked than in normal animals. This could be followed in terms of the relative decrease in the latent period of transfers in old individuals (Fig. 2) (by 27% in male DISC1-Q31L mice, 40% in C57Bl/6NCr1 males, 25% in DISC1-Q31L females; 33% in C57Bl/6NCr1 mice) and a decrease in the proportion of animals displaying good learning (see Table 1) (from 40% to 0% in in DISC1-Q31L males, from 70% to 15% in C57Bl/6NCr1 males, from 10% to 0% in DISC1-Q31L females, form 10% to 0% in DISC1-Q31L females, and from 60% to 10% in C57Bl/6NCr1 females).

The effect of the gender factor consisted of a decrease in learning ability in females as compared with males, as evidenced by shorter latent periods of transfers (Fig. 2) and comparisons of the proportions of males and females with good learning ability – 40 vs. 10% in DISC1-Q31L mice and 70 vs. 60% in in C57Bl/6NCr1 mice (see Table 1).

Discussion. Thus, the data presented here provide evidence of differences in the acquisition of a conditioned passive avoidance reaction in DISC1-Q31L and DISC1-L100P mice as compared with mice of the C57Bl/6NCr1 control group. Among the characteristics of fear memory trace formation, we draw attention to: 1) unlike control mice and DISC1-Q31L mice, DISC1-L100P mice showed gender- and age-independent impairment to associative learning to avoid the dark sector of the apparatus which had become dangerous after pain punishment; 2) learning was seen only in young males among DISC1-Q31L mice, while memory traces formed in control animals in both young males and young females; 3) there were identical statistically significantly decreases in learning passive avoidance with age in male DISC1-Q31L mice and control C57Bl/6NCr1 mice, while females of these strains showed tendencies to learning deficits.

It is important to note that our results on learning deficit in DISC1-L100P mice are consistent with data reported from other investigations detecting learning deficit in a contextual conditioned fear reaction in DBA/2J mice, which are regarded as a genetic model of a schizophrenia-like state [7, 38, 40, 41], and rats in a pharmacological model of this behavior [6]. Along with data on the behavioral status of DISC1-L100P mice [8, 27], this is supported by the view of DISC1-L100P mice as a genetic model of a schizophrenia-like state and widens our understanding of the role of the fear memory formation.

The potential to learn passive avoidance in young male DISC1-Q31L mice, in contrast to that in DISC1-L100P mice, was consistent with results obtained in an experimental model of a depression-like state ("behavioral despair") [3]. This serves as a cognitive indicator additional to the behavioral characteristics of these mice [8, 25, 26], corresponding to the first criterion (face validity) of the view of DISC1-Q31L mice as a model of depression.

We will now consider the possible causes of the selectivity of learning the conditioned passive avoidance reaction seen in young DISC1-Q31L mice and the absence of acquisition of the fear memory trace in DISC1-L100P mice as compared with mice of the C57Bl/6NCr1 control group. These differences may be related to the genotypic features of behavior, as such a relationship has been noted by investigators using mice of other strains [21, 29, 35, 39]. Screening of behavioral characteristics revealed differences in movement activity between C57Bl/6NCr1, DISC1-Q31L, and DISC1-L100P mice (hyperactivity in DISC1-L100P mice) and depression-like manifestations (passivity, low social motivation, and anhedonia in DISC1-Q31L mice) [8, 24–27]. Our experiments noted that DISC1-L100P mice showed the shortest latent periods of transfers during familiarization with the experimental apparatus before training, at least in males. This is indirect evidence of hyperactivity in these mice, which can partially be explained in terms of the short latent periods of transfer on testing reflex acquisi-

tion. Linear differences in learning conditioned fear reactions have often been linked in previous studies with differences in the extents of anxiety behavior [5], though this link has not been regarded as important in recent years [21]. The studies of Lipina et al. [8, 24–26] showed that young male C57Bl/6NCr1, DISC1-Q31L, and DISC1-L100P mice had identical anxiety levels, i.e., basal anxiety cannot be taken as a prerequisite for the selectivity in the acquisition of conditioned passive avoidance reaction seen here.

Several possible explanations for role of the genotypic differences in forming the conditioned passive avoidance reaction in C57Bl/6NCr1, DISC1-Q31L, and DISC1-L100P mice can be suggested on the basis of individual-typological characteristics of behavior. In particular, defects in learning the conditioned passive avoidance reaction in DISC1-L100P mice may be due to use of a different behavioral strategy, i.e., attempts to escape the "dangerous" sector instead of avoiding it (freezing), which was observed visually in these mice but not in DISC1-Q31L or C57Bl/6NCr1 mice. This type of explanation of poor aversive learning in mice of some strains was suggested by Bothe et al. and Voikar et al. [7, 40]. A contribution from the different levels of sensitivity to stress-inducing stimuli (the danger of the dark sector after training) in DISC1-L100P mice as compared with controls cannot be excluded. For example, a relationship between a deficit in fear memory formation and low levels of unconditioned fear reactions has been demonstrated in terms of measures of tail beating and defecation in assessment of the aversiveness of the context on testing in DBA/2J mice as compared with C57Bl/6J mice [41].

However, data on differences in the behavioral profiles of the mice used in our studies – mutant strains DISC1-Q31L and DISC1-L100P and control C57Bl/6NCr1 mice – are insufficient to explain such different learning abilities in these mice.

As learning a contextual conditioned fear reaction involves obligatory roles for neural networks in the hippocampus, amygdala, and prefrontal cortex [33, 35, 39], variation in learning in mice of the DISC1-L100P, DISC1-Q31L, and C57Bl/6NCr1 strains may be determined by the morphological properties of these structures. Currently there are only data showing a 6% decrease in brain volume in DISC1-Q31L mice and a 13% decrease in DISC1-L100P mice [8], with decreases in the numbers of cortical neurons, but no difference in dendrite branching and no changes in the morphology of hippocampal neurons [23] were seen in comparison with normal animals (control strain). Thus, it should be recognized that there is a lack of the data required for suggesting a link between differences in the ability to learn passive avoidance between DISC1-L100P and DISC1-Q31L mice, as well as between mutants and C57Bl/6NCr1 mice, with the anatomical phenotypes of these mice.

Synaptic pathology, particularly decreases in the density of dendritic spines in the brain, is regarded as one of the causes of cognitive deficits in psychopathologies with different origins, because the density of dendritic spines, as the postsynaptic targets of synaptic transmission, serves as a measure of the efficiency of connections in neural networks and functional plasticity. It can therefore be suggested that genotype-dependent modifications of learning the conditioned passive avoidance reaction in DISC1-L100P, DISC1-Q31L, and C57Bl/6NCr1 mice receive contributions from the different synaptic phenotypes of these individuals. However, despite the differences seen in learning in DISC1-L100P and DISC1-Q31L mice, these animals showed identical decreases in dendritic spine density in the prefrontal cortex and hippocampus from the level in mice of the control strain [23]. It should be recognized that the problem of analyzing behavioral, synaptic, and cognitive phenotypes of DISC1-L100P and DISC1-Q31L mice, as compared with normal animals, requires further multidisciplinary investigations for precise identification of the importance of the contribution of fear memory formation to the characteristics of genetic models of schizophrenia- and depression-like states. Analysis of the process of extinction of the fear memory trace in these mice is also important, as impairments to this process constitute the main cognitive symptom of a number of psychopathologies, particularly depression.

Comparative analysis of the relationship between fear memory formation and gender in the mice used in genotype studies showed that young female mice of both the DISC1-Q31L and C57Bl/6NCr1 strains learned worse than males. Decreased aversive learning in females is consistent with most data obtained in rodents [9, 32]. The main cause of gender-related differences in learning in response to pain stimuli is believed to consist of the selectivity of reactions to stressful and emotionally significant actions, as well as the level of anxiety behavior [10, 11, 19, 22]. Females are characterized by a mainly active style of responding to aversive stimuli, including contextual stimuli, while the behavioral repertoire in males is dominated by behavioral inhibition, i.e., passive reactions. The fact of different styles of behavior in response to threats is supported by data showing better learning in female rats in tests where active-type responses to pain stimulation are needed (conditioned active avoidance reactions) [9]. Different behavioral strategies in males and females on testing in "threatening" conditions may be reflected as decreases in the reproduction of the conditioned passive avoidance reaction in female DISC1-Q31L and C57Bl/6NCr1 mice, where freezing would be a more appropriate response. We should note that in our experiments, visual observation showed the active type of defensive behavior (attempts to exit the sector of the apparatus) as a means of responding to the aversive situation (the "dangerous" dark sector) on the day of testing acquisition of the reflex. In addition, gender-related differences in emotional memory in rodents may involve neuromorphological and neurochemical selectivity in males and females [11], though consideration of this possibility requires more experimental data on DISC1-Q31L and C57Bl/6NCr1 mice than as yet available.

Thus, we did not find selective influences of gender on learning between DISC1-Q31L mice and control animals.

As regards the contribution of age to the acquisition of the conditioned passive avoidance reaction, our experiment showed that DISC1-Q31L, DISC1-L100P, and C57Bl/6NCr1 mice aged 9–12 months, regardless of gender, showed no statistically significant increases in the latent period of transfer on testing from the baseline levels. This is evidence of impairment to aversive learning in old mice of the three strains used in these experiments. The appearance of deficit in fear memory formation with age in DISC1-Q31L and C57Bl/6NCr1 mice, on comparison of both the number of animals with different levels of learning (see Table 1) and the latent period of transfers on testing (Fig. 2) was quite clear. Previously, decreases in the formation of emotional memory were seen in old (8–19 months) C57BL/6 mice [14, 18, 37] and rats [28]. The authors of [18, 20] took the view that age-related learning deficit, at least in tests of contextual conditioned fear reactions and conditioned passive avoidance reactions, may be due to decreases in the functional activity of the hippocampus, which has been actively discussed in analysis of data on synaptic plasticity. The links between age-related changes in cognitive (fear memory) and behavioral (locomotor, anxiety, depressivity, etc.) phenotypes are difficult to assess because of significant conflicts in the data [14, 18, 20, 28, 37]. We believe that the age-related learning deficit in DISC1-Q31L and C57Bl/6NCr1 mice may be based on age-associated decreases in the ability to recognize the contexts of the dangerous and safe sectors of the apparatus. It should be noted that unconditioned reactions to pain stimuli did not change with age (squeaking and jumping in response to electric shocks in female and male mice of all strains were expressed identically), as has been observed in C57Bl/6J mice [37].

Thus, the overall data demonstrated the characteristics of the acquisition of a conditioned passive avoidance reaction in mice of the mutant strains DISC1-Q31L and DISC1-L100P in comparison with the C57Bl/6NCr1 control strain. The process of fear memory trace formation was impaired in DISC1-L100P mice, while the only DISC1-Q31L mice able to learn passive avoidance were young males. There was similarity between the emotional cognitive measure (fear memory) and the schizophrenia-like phenotype in DISC1-L100P mice and the depression-like phenotype in DISC1-Q31L mice. A deficit of aversive learning was found in old mice of all three strains used in this study, along with identical decreases in learning ability in female DISC1-Q31L and C57Bl/6NCr1 mice compared with males.

REFERENCES

1. A. P. Bel'nik, R. U. Ostrovskaya, and I. I. Poletaeva, "Genotype-dependent behavioral characteristics in mice in cognitive tests. The effects of Noopept," *Zh. Vyssh. Nerv. Deyat.*, **57**, No. 6, 721–728 (2007).
2. N. I. Dubrovina, D. R. Zinov'ev, and D. V. Zinov'eva, "Extinction of a conditioned passive avoidance reaction in mice of different strains," *Zh. Vyssh. Nerv. Deyat.*, **60**, No. 6, 712–718 (2010).
3. N. I. Dubrovina and D. R. Zinov'ev, "The contribution of GABA receptors to extinction of a memory trace in health and a depression-like state," *Ros. Fiziol. Zh.*, **93**, No. 11, 1285–1291 (2007).
4. D. A. Bangasser and R. J. Valentino, "Sex differences in stress-related psychiatric disorders: neurobiological perspectives," *Front. Neuroendocrinol.*, **35**, No. 3, 303–319 (2014).
5. A. Beuzen and C. Belzung, "Link between emotional memory and anxiety states: A study by principal component analysis," *Physiol. Behav.*, **58**, 111–118 (1995).
6. M. M. Bolton, C. F. Heaney, J. J. Sabbagh, et al., "Deficits in emotional learning and memory in an animal model of schizophrenia," *Behav. Brain Res.*, **233**, No. 1, 35–44 (2012).
7. G. W. Bothe, V. J. Bolivar, M. J. Vedder, and J. G. Geistfeld, "Behavioral differences among fourteen inbred mouse strains commonly used as disease models," *Comp. Med.*, **55**, No. 4, 326–334 (2005).
8. S. J. Clapcote, T. V. Lipina, J. K. Millar, et al., "Behavioral phenotypes of Disc1 missense mutations in mice," *Neuron*, **54**, No. 3, 387–402 (2007).
9. C. Dalla and T. J. Shors, "Sex differences in learning processes of classical and operant conditioning," *Physiol. Behav.*, **97**, No. 2, 229–238 (2009).
10 N. C. Donner and C. A. Lowry, "Sex differences in anxiety and emotional behavior," *Pflügers Arch.*, **465**, No. 5, 601–626 (2013).
11. M. R. Farrell, D. R. Sengelaub, and C. L. Wellman, "Sex differences and chronic stress effects on the neural circuitry underlying fear conditioning and extinction," *Physiol. Behav.*, **122**, 208–215 (2013).
12. G. E. Fenton, A. K. Pollard, D. M. Halliday, et al., "Persistent prelimbic cortex activity contributes to enhanced learned fear in females," *Learn. Mem.*, **21**, No. 2, 55–60 (2014).
13. A. R. Gobinath, R. Mahmound, and L. A. Galea, "Influence of sex and stress exposure across the lifespan on endophenotypes of depression: focus on behavior, glucocorticoids, and hippocampus," *Front. Neurosci.*, **8**, 420 (2015).
14. T. J. Gould and O. R. Feiro, "Age-related deficits in the retention of memories for cued fear conditioning are reversed by galantamine treatment," *Behav. Brain Res.*, **165**, No. 2, 160–171 (2005).
15. C. A. Hartley and E. A. Phelps, "Changing fear: the neurocircuitry of emotion regulation," *Neuropsychopharmacology*, **35**, No. 1, 136–146 (2010).
16. T. Hikida, N. J. Gamo, and A. Sawa, "DISC1 as a therapeutic target for mental illnesses," *Expert Opin. Ther. Targets*, **16**, No. 12, 1151–1160 (2012).
17. L. H. Jacobson and J. F. Cryan, "Genetic approaches to modeling anxiety in animals," *Curr. Top. Behav. Neurosci.*, **2**, 161–201 (2010).
18. C. C. Kaczorowski and J. F. Disterholt, "Memory deficits are associated with impaired ability to modulate neuronal excitability in middle-aged mice," *Learn. Mem.*, **16**, No. 6, 362–366 (2009).
19. S. A. Karisson, K. Haziri, E. Hansson, et al., "Effects of sex and gonadectomy on social investigation and social recognition in mice," *BMC Neurosci.*, **16**, 83 (2015).
20. J. A. Kennard and D. S. Woodruff-Pak, "Age sensitivity of behavioral tests and brain substrates of normal aging in mice," *Front. Aging Neurosci.*, **3**, No. 9, 1–22 (2011).
21. S. Keum, J. Park, A. Kim, et al., "Variability in empathic fear response among 11 inbred strains of mice," *Genes Brain Behav.*, **15**, No. 2, 231–242 (2016).
22. N. Kokras and C. Dalla, "Sex differences in animal models of psychiatric disorders," *Br. J. Pharmacol.*, **171**, No. 20, 4595–4619 (2014).
23. F. H. Lee, M. P. Fadel, K. Preston-Maher, et al., "Discl point mutations in mice affect development of the cerebral cortex," *J. Neurosci.*, **31**, No. 9, 3197–3206 (2011).

24. T. V. Lipina, O. Kaidanovich-Beilin, S. Patel, et al., "Genetic and pharmacological evidence for schizophrenia-related Discl interaction with GSK-3," *Synapse*, **65**, No. 3, 234–248 (2011).
25. T. V. Lipina, M. Wang, F. Liu, and J. C. Roder, "Synergistic interactions between PDE4B and GSK-3: DISC1 mutant mice," *Neuropharmacology*, **62**, No. 3, 1252–1262 (2012).
26. T. V. Lipina, P. J. Fletcher, F. H. Lee, et al., "Disrupted-in-schizophrenia-1/Gln3lLeu polymorphism results in social anhedonia associated with monoaminergic imbalance and reduction of CREB and P-arrestin-1,2 in the nucleus accumbens in a mouse model of depression," *Neuropsychopharmacology*, **38**, No. 3, 423–436 (2013).
27. T. V. Lipina and J. C. Roder, "Disrupted-in-schizophrenia-1 (DISC1) interactome and mental disorders: impact of mouse models," *Neurosci. Biobehav. Rev.*, **45**, 271–294 (2014).
28. Y. Luo, J. Zhou, M. X. Li, et al., "Reversal of aging-related emotional memory deficits by norepinephrine via regulating the stability of surface AMPA receptors," *Aging Cell*, **14**, No. 2, 170–179 (2015).
29. A. March, D. Borchelt, T. Golde, and C. Janus, "Differences in memory development among C57BL/6NCrl, 129S2/SvPasCrl, and FVB/NCrl mice after delay and trace fear conditioning," *Comp. Med.*, **64**, No. 1, 4–12 (2014).
30. T. P. O'Leary, R. K. Gunn, and R. E. Brown, "What are we measuring when we test strain differences in anxiety in mice?" *Behav. Genet.*, **43**, No. 1, 34–50 (2013).
31. D. J. Porteous, J. K. Millar, N. J. Brandon, and A. Sawa, "DISC1 at 10: connecting psychiatric genetics and neuroscience," *Trends Mol. Med.*, **17**, No. 12, 699–706 (2011).
32. A. M. Ribeiro, F. F. Barbosa, M. R. Godinho, et al., "Sex differences in aversive memory in rats: possible role of extinction and reactive emotional factors," *Brain Cogn.*, **74**, No. 2, 145–151 (2010).
33. R. R. Rozeske, S. Valerio, F. Chaudun, and C. Herry, "Prefrontal neuronal circuits of contextual fear conditioning," *Genes Brain Behav.*, **14**, No. 1, 22–36 (2015).
34. J. N. Samsom and A. H. Wong, "Schizophrenia and depression co-morbidity: What we have learned from animal models," *Front. Psychiatry*, **6**, 13 (2015).
35. L. A. Schimanski and P. V. Nguyen, "Multidisciplinary approaches for investigating the mechanisms of hippocampus-dependent memory: a focus on inbred mouse strains," *Neurosci. Biobehav. Rev.*, **28**, 463–483 (2004).
36. H. Shoji, K. Toyama, Y. Takamiya, et al., "Comprehensive behavioral analysis of ENU-induced Disc1-Q31L and Disc1-L100P mutant mice," *BMC Res. Notes*, **5**, 108 (2012).
37. H. Shoji, K. Takao, S. Hattori, and T. Miyakawa, "Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age," *Mol. Brain*, **9**, No. 1, 11 (2016).
38. P. Singer, J. Feldon, and B. K. Yee, "Are DBA/2 mice associated with schizophrenia-like endophenotypes? A behavioural contrast with C57BL/6 mice," *Psychopharmacology (Berl.)*, **206**, No. 4, 677–698 (2009).
39. M. E. Tipps, J. D. Raybuck, K. J. Buck, and K. M. Lattal, "Delay and trace fear conditioning in C57BL/6 and DBA/2 mice: issues of measurement and performance," *Learn. Mem.*, **21**, No. 8, 380–393 (2014).
40. V. Voikar, A. Polus, E. Vasar, and H. Rauvala, "Long-term individual housing in C57BL/6J and DBA/2 mice: assessment of behavioral consequences," *Genes Brain Behav.*, **4**, No. 4, 240–252 (2005).
41. Y. M. Wilson, T. C. Brodnicki, A. J. Lawrence, and M. Murphy, "Congenic mouse strains enable discrimination of genetic determinants contributing to fear and fear memory," *Behav. Genet.*, **41**, No. 2, 278–287 (2011).