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Research report

Characterization of gene–environment interactions by behavioral profiling of selectively bred rats: The effect of NMDA receptor inhibition and social isolation

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HIGHLIGHTS

- ▶ Characterization of selectively bred rats in terms of schizophrenia-related alterations.
- ▶ Complex treatment with social isolation and ketamine injections.
- ▶ Both pain sensitivity and motor activity decreased in treated animals.
- ▶ Sensory gating deficit and memory dysfunctions could also be observed.
- ▶ Selective breeding and complex treatment are important in producing these changes.

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ABSTRACT

Gene–environment interactions have an important role in the development of psychiatric disorders. To generate and validate a new substrain of rats with signs related to schizophrenia, we used selective breeding after postweaning social isolation and chronic ketamine treatment through several generations of animals and compared the subsequent strain to naive rats that were not genetically manipulated. We further investigated whether social isolation and ketamine treatment augmented the appearance of schizophrenic-like signs in these rats. Four experimental groups were studied ($n = 6–15$ rats/group): naive rats without any treatment (NaNo); naive rats with postweaning social isolation and ketamine treatment (NaTr); 15th generation of selectively bred animals without any treatment (SelNo) or selectively bred rats with both isolation and ketamine treatment (SelTr). The startle reaction, tail-flick and novel object recognition tests were used to classify the animals into low- or high-risk for schizophrenia. Reduced pain sensitivity, higher degree of the startle reaction, disturbed prepulse inhibition, altered motor activity and decreased differentiation index in the memory test were observed in the 15th generation of the substrain, along with enhanced grooming behavior. Five functional indices (TF latency, startle reaction, prepulse inhibition, differentiation index, and grooming activity) were rated from 0 to 2, and the analysis of the summarized score revealed that the NaNo group had the lowest overall indication of schizophrenic-like signs, while the SelTr animals scored the highest, suggesting that both heritable and environmental factors were important in the generation of the behavioral alterations. We assume that further breeding after this complex treatment may lead to a valid and reliable animal model of schizophrenia.

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1. Introduction

Schizophrenia is a devastating psychiatric disorder that impairs mental and social function and affects approximately 1% of the population worldwide [1,2]. It is characterized by positive symptoms (hallucinations, delusions, and thought disorder), negative

symptoms (deficits in social interaction, emotional expression, and motivation), and cognitive dysfunction (impaired attention/information processing, problem-solving, processing speed, verbal and visual learning and memory). Schizophrenia is considered as a complex, multifactorial disease. It is clear that susceptibility is hereditary, in some cases; however, none of the identified risk genes are specific to schizophrenia but rather indicate a general vulnerability to mental health disorders [3]. The neurodevelopmental hypothesis of schizophrenia is also a major theory which suggests the significance of different stressors in

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the etiology of this disease [4,5]. Dopaminergic, serotonergic, glutamatergic and GABAergic deficits have been proposed as pathophysiological factors in schizophrenia. The underlying process of the disease occurs in the early stages of neurodevelopment and manifests only later, during the developmental restructuring of the central nervous system.

In order to understand the biological mechanisms underlying a complex disorder like schizophrenia, and in search of novel drug targets, valid animal models are necessary. There are four main groups of chronic animal models for schizophrenia: pharmacological-, lesion-, environmental- and genetic models, and a few studies applied certain combinations of these [6–9]. It is well-known that NMDA antagonists worsen symptoms in schizophrenia and can induce schizophrenia-like symptoms in normal individuals [10–12]. Animals treated with NMDA receptor antagonists exhibit a number of changes related to schizophrenia, including deficits in memory function, pain sensitivity, as well as hyper-responsiveness to stimulants such as amphetamine [6,13–17]. The NMDA receptor system has an effect on several transmitter systems in the cortico–limbic–striatal network, and also plays a crucial role in brain plasticity during early development [13,15,18]; therefore, the developing brain is more susceptible to a chronic, low-dose blockade of NMDA receptors: it causes synaptic weakening and elimination by over-pruning in several brain regions, including the prefrontal cortex and the hippocampus [19–25].

The profound neurobiological effects of stress are believed to be the basis of many neuropsychiatric changes. Since Hatch and colleagues first reported behavioral abnormalities in socially isolated rats [26], a large body of evidence has accumulated to suggest that postweaning social isolation has profound, long-term effects on rodent brain and behavior. Thus, postweaning social isolation is an alternative, non-pharmacological model that produces a number of behavioral consequences in adulthood that are similar to schizophrenia symptoms, including deficits in sensorimotor gating, pain sensitivity, motor activity and enhanced sensitivity to psychoactive drugs [27–32]. This intervention also causes specific deficits in the prefrontal cortex, significant neurotransmission abnormalities including enhanced dopamine and serotonin functions in the basal ganglia [8,33,34].

As the subchronic NMDA-receptor antagonist model and the postweaning social isolation model of schizophrenic symptoms produce somewhat complementary, but not always robust behavioral alterations, some authors applied a combined “double hit” model to investigate the hypothesis that these manipulations can enhance the reliability of the schizophrenia model [35,36]. This paradigm applied in adult or juvenile rats induced several behavioral abnormalities, such as hyperresponsiveness to different stress situations, drugs and altered pain sensitivity [6,15,32,37,38]. Importantly, combining the two manipulations did not produce detectable additive or synergistic effects on behavior or hippocampal plasticity, however, these animals showed more schizophrenia-like signs.

Animal models generated by artificial selection are tools that can be used to gain a better understanding of the genetic makeup behind the complex symptomatology of different syndromes including schizophrenia [39–41]. There are data to suggest that selective breeding approaches in rats may be a powerful strategy to unravel the genetic basis of schizophrenia [40,42,43].

We hypothesized that the combination of genetic and environmental factors could yield a reliable rat model of schizophrenia; thus, the aim of our study was to generate and validate a new substrain of rats by selective breeding after social isolation and chronic ketamine (which acts primarily as a noncompetitive NMDA receptor antagonist) treatment. Selective breeding started in March 2008, and the desired substrain was defined as exhibiting measurable alterations in acute heat-pain sensitivity, sensory gating and

memory functions. The behavioral profile (sensory gating, pain sensitivity, memory function) was characterized in the 15th generation of our substrain by using the tail-flick (TF), the prepulse inhibition (PPI) and novel object recognition (NOR) tests.

2. Materials and methods

2.1. Selective breeding process

All procedures were ethically approved by the institutional animal care committee. Both male and female Wistar rats were used. Starting from a population of outbred Wistar rats (‘parental generation’: 10 males and 10 females), a breeding line was established by selective breeding according to the rats sensitivity to acute heat pain after social isolation and ketamine treatment. In further generations, the parental generation consisted of between 13 and 16 animals of each sex. The paradigm for selective breeding through several generations was as follows: rats, after weaning at 3 weeks of age (21–23 days), were tested with the TF test and then housed individually for 28 days (between 4 and 7 weeks of age) in cages of 42 × 15 × 12 cm (1 × w × h) with wood shavings as bedding and nesting material. The animal housing rooms, as well as the experimental rooms, were kept under standard laboratory conditions (light–dark cycle: 12:12 h; light on at 06:00 h; temperature 22 ± 1 °C; relative humidity: 55 ± 10%). Commercial rat diet and bottled tap water were available ad libitum. The cages were placed in shared racks so that auditory and olfactory contacts were maintained. The animals were treated with ketamine (CALYPSOL, Richter Gedeon Nyrt., Budapest, Hungary; 30 mg/kg intraperitoneally, 4 ml/1000 g body weight, daily, 5 times/week, 15 injections in total) from 5 to 7 weeks of age. Duration of ketamine treatment and isolation parameters were adapted from earlier studies [6,15,44]. At the end of the treatment, animals were re-housed in a group setting (4–5 rats per cage) and 1 week of recovery, with no treatment, followed. Behavioral assessment started at the age of 9 weeks with the TF test. Five rats of both sexes, that showed the highest pain threshold, as indicated by the TF test, were selected for the next breeding generation. Their offspring (1st generation) and the subsequent 2nd generation were also tested only in the TF test, and again 5 rats of each sex with the highest pain thresholds were chosen to parent the next generation of the breeding line. From the 3rd generation we also used the PPI test to investigate sensory gating (at the age of 10 weeks), and the animals showing a high pain threshold, along with a low PPI were selected for a further breeding line. From the 6th generation, the NOR test was also applied (at the age of 11 weeks) to investigate memory functions and motor activity. Thus, animals with impaired pain sensitivity, PPI and NOR were selected for the further breeding lines. From the second generation, 5–7 animals of both sexes were selected for breeding. Sibling mating was avoided by paying close attention to the litter of origin, and the litter size was reduced to a maximum of 6–8 pups (the number of males and females was approximately equal), ensuring that each family contributed equally to the next generation. We found no signs of inbreeding depression (i.e. reduction of fertility, deformed offspring, small litters, poor mothering ability) in the selected line. Male rats of the 15th generation were involved in the present experiment.

2.2. Experimental paradigm

Four experimental groups of male rats were compared ($n=6-15$ rats/group): naive socialized rats without any treatment (NaNo), or with isolation and ketamine treatment (NaTr) and 15th generation selectively bred animals without any treatment (SelNo), or with isolation and ketamine treatment (SelTr). Groups were matched according to body weight (50 ± 1.7 g) and their TF values at the age of 3 weeks. The testing schedule is presented in Fig. 1. The body weights of rats in all experimental groups were measured throughout the investigation period.

2.3. Nociceptive testing

Acute nociceptive threshold was assessed by the TF test. The reaction time was determined by immersing the distal 5 cm portion of the tail in hot water (48 °C) until a tail-withdrawal response was observed (cut-off time: 20 s or 40 s at the age of 3 or 9 weeks, respectively). TF latencies were obtained four times at 0, 30, 60, and 90 min and, since they did not differ significantly, were averaged to establish the pain threshold for each group.

2.4. Prepulse inhibition test

PPI of the acoustic startle response was measured in four startle chambers as described previously [45]. The Plexiglas startle chamber was in a sound-attenuated room and was divided into four identical compartments (12 × 17 × 15.3 cm each). Noise bursts were applied through a speaker mounted close to the backside of the chamber. Under the cage, a piezoelectric accelerometer (i.e. force transducer) sensitive to rat startle-like movements produced an electrical signal that was amplified by a signal conditioner and visualized on a computer screen. Rats were allowed to habituate to the background noise (70 dB) for 10 min, immediately thereafter they were exposed to three different trial types: a PULSE ALONE (PA) in which a 40 ms 95 dB white noise burst was presented; PREPULSE ALONE (PPA), 20 ms 76 dB; and the

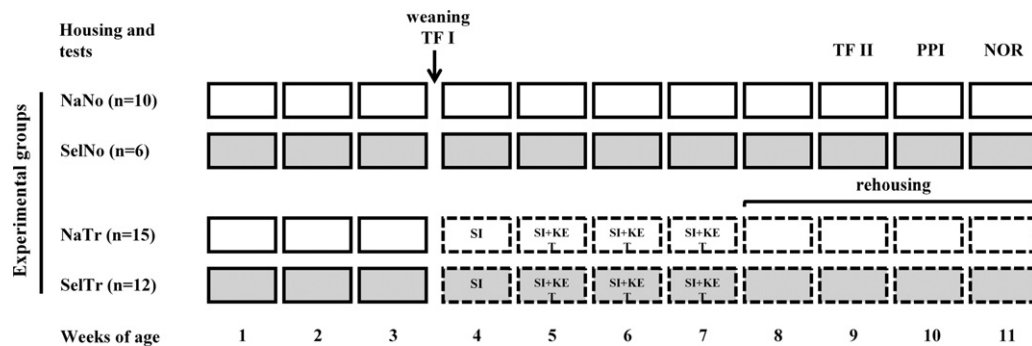


Fig. 1. Experimental paradigm. TF, tail-flick test; PPI, prepulse inhibition test; NOR, novel object recognition test; NaNo, naive animals without treatments; NaTr, naive animals with social isolation and ketamine treatment; SelNo, selectively bred rats without treatments; SelTr, selectively bred rats with social isolation and ketamine treatment; SI, social isolation; KET, ketamine treatment.

PREPULSE-PULSE PAIR (PP) in which prepulse stimuli were followed by the acoustic startle stimulus with a latency of 150 ms. All types were presented 10 times. The interstimulus intervals ranged from 7 s to 13 s, and there was a 10 min' resting period between each trial. The %PPI values were calculated as percentages using the following formula: $\%PPI = [1 - (\text{startle response for PREPULSE} + \text{PULSE trial}) / (\text{startle response for PULSE ALONE trial})] \times 100$. Since the startle reaction increases significantly with body weight, we normalized the reaction to body weight, accordingly:

$$\text{Relative startle reaction} = \frac{\text{startle reaction} \times 100}{\text{body weight (g)}}$$

2.5. Novel object recognition test

Rats were habituated to the testing room (with dim light) for 60 min prior to the start of the experiments. The NOR test was conducted in a Plexiglas box (arena, 60 × 34 × 33 cm) without bedding, with black walls to obscure the testing room from the animals. Toy brick towers (Lego Group, Billund, Denmark) of similar size but not exactly the same shape (~8 × 2 × 3 cm) were used as test objects. They were affixed to the floor of the box to prevent them from being displaced during testing. The objects were placed 13 cm from the opposing corners of the arena, and the rat was consistently placed in the middle of the arena to be equidistant from both objects. Between the testing of different animals, the arena and the objects were cleaned with 70% ethanol.

2.5.1. Procedures

Habituation phase: Each rat was placed in the center of the chamber and allowed to explore the open field without any objects for a single 10-min session. **Sample phase:** One minute following the habituation session, the sample phase began. Two objects with the same size and shape (S1 and S2) were mounted in the open-field. Rats were put into the center of the open field again, and allowed to explore the two identical objects for 5 min. **Test phase:** At the end of the sample phase, each rat was returned to their home cage for a one-hour retention interval, while one of the objects was replaced with another, visually non-identical one (N: novel). The other object (F: familiar) was the same as in the sample phase. Afterwards, a 5-min test phase followed.

Behavior in all trials was recorded with an infrared video device (WCM-21VF, CNB, China). The scoring of the different behaviors was carried out by investigators blind to the applied treatment. The following parameters were scored in each phase: duration of stereotypic behaviors, such as rearing, self-grooming, the time of exploratory activity and walking. Object exploration was defined as animals' licking, sniffing or touching the object with the forepaws, but not leaning against, turning around, standing on or sitting on the object. Since the habituation phase lasted twice as long as the other phases, behavioral activity was divided into and scored in sub-phases (0–5 and 5–10 min) for analysis.

The discrimination index (DI) was calculated for both the sample and test phases as follows: $DI = (\text{time spent exploring N vs S1 object} - \text{time spent exploring F vs S2 object}) / (\text{total time spent exploring both the objects [S1 + S2] vs [N + F]})$. If the animals did not explore the objects during the sample or test phases, they were considered as non-responders and data from these animals were not included in the final analysis (altogether one animal from the SelTr group was excluded on such grounds).

2.6. Statistical analyses

Data are expressed as means ± SEM. The median split method was used for transforming continuous variables into categorical ones. A quartile-based scoring method was used. The values in the first (lower) quartile received 0 points, values in the third (upper) quartile received a score of 2, and the values between them received 1 point. Five aspects (TF latency at the age of 9 weeks, relative startle reaction, %PPI, DI, and grooming activity) were rated from 0 (lowest risk) to 2 (highest risk), and

summarized to generate the total schizophrenia score, which ranged from 0 to 10. Using this score, it was possible to classify animals as either low- or high-risk for schizophrenia using quartiles of the total schizophrenia score.

Effects of treatment (social isolation + ketamine) and strain (naive or selectively bred), and interactions were assessed using two-way ANOVA. Subsequent analysis was performed using the Fisher-LSD test. Level of significance was set at $p < 0.05$. For the analyses, STATISTICA for Windows 7.1 (Statsoft Inc., Tulsa, OK) was used.

3. Results

The body weight measured on the different testing days (Fig. 2) showed a significant effect of time ($F_{3,117} = 3077.81, p < 0.0001$) and strain ($F_{1,39} = 24.78, p < 0.001$), and the interaction between time and strain ($F_{3,117} = 7.13, p < 0.001$) was also significant. That is, the substrain started to exhibit a lower body weight from age of 9 weeks. The social isolation together with ketamine treatment did not result in further weight loss.

3.1. Tail-flick test

ANOVA revealed a significant effect of strain ($F_{1,39} = 4.23, p < 0.05$), time ($F_{1,39} = 328.15; p < 0.001$) and a significant interaction between time and strain ($F_{1,39} = 4.94; p < 0.05$) on the TF latencies measured at 3 and 9 weeks of age; thus, the TF latency significantly increased in all groups with time. Post hoc comparison did not reveal differences between the groups at the age of 3 weeks, but a tendency toward TF latency increase was observed in the new substrain (naive: 4.1 ± 0.21 s, 15th generation: 4.6 ± 0.27 s).

Significant differences were observed at the age of 9 weeks between NaNo and both of the substrain groups (SelNo and SelTr), with these groups having the lowest pain sensitivity (Fig. 3). The latency in the NaTr group did not differ from any other group.

3.2. Prepulse inhibition test

ANOVA revealed a significant effect of prepulse stimulation ($F_{1,39} = 72.47, p < 0.0001$), and strain ($F_{1,39} = 6.50, p < 0.05$) (Fig. 4A) on the magnitude of the startle reaction. The response significantly decreased in the case of prepulse stimulation in all groups, except the SelNo group. The post hoc comparison revealed significant differences between the NaNo and SelTr groups with the PA, while both of the selectively bred groups showed a significantly higher degree of relative startle reaction compared to both of the naive groups with the PP. Regarding %PPI, the effect of strain was significant ($F_{3,39} = 5.59; p < 0.005$); thus, both groups of the substrain (SelNo and SelTr) had lower PPI compared to the naive groups (NaNo and NaTr) (Fig. 4B).

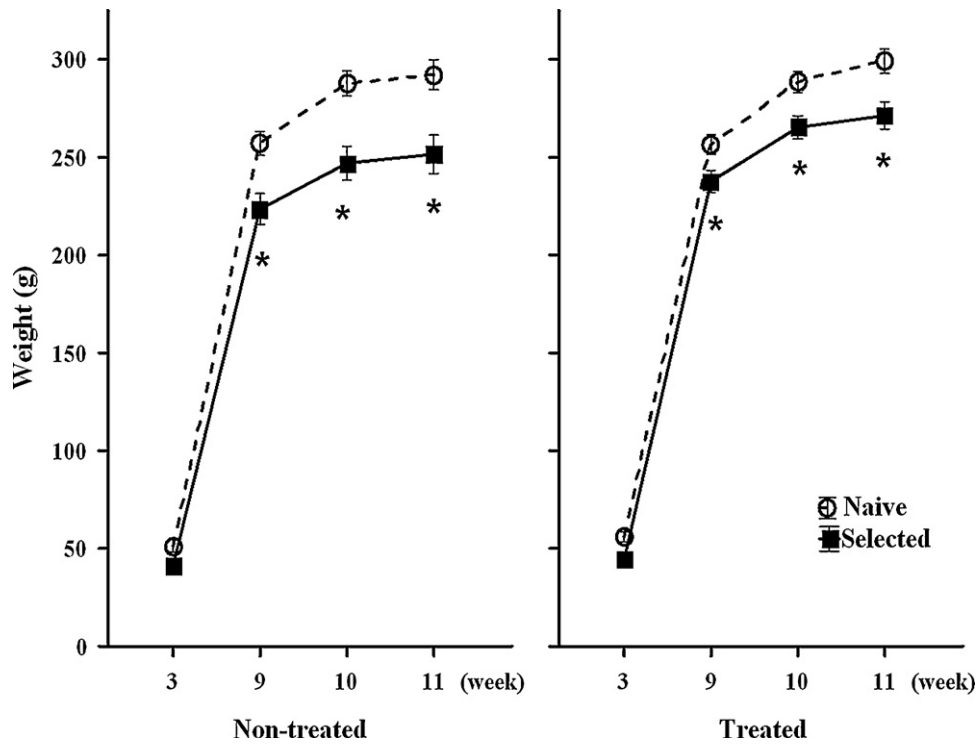


Fig. 2. The body weight measured on the day of different behavioral tests. * indicates significant differences as compared to the naive non-treated (NaNo) and naive treated (NaTr) groups. Data are expressed as means ± SEM.

3.3. Novel object recognition test

ANOVA revealed a significant effect of strain ($F_{1,39} = 6.75, p < 0.05$), of phase ($F_{3,117} = 27.35, p < 0.001$), and significant

interaction between phase and strain ($F_{3,117} = 3.69, p < 0.05$) (Fig. 5A) on the rearing activity during the different phases of the NOR test. Rearing activity decreased with time (phase) in all groups, and the 15th generation showed lower rearing activity in the

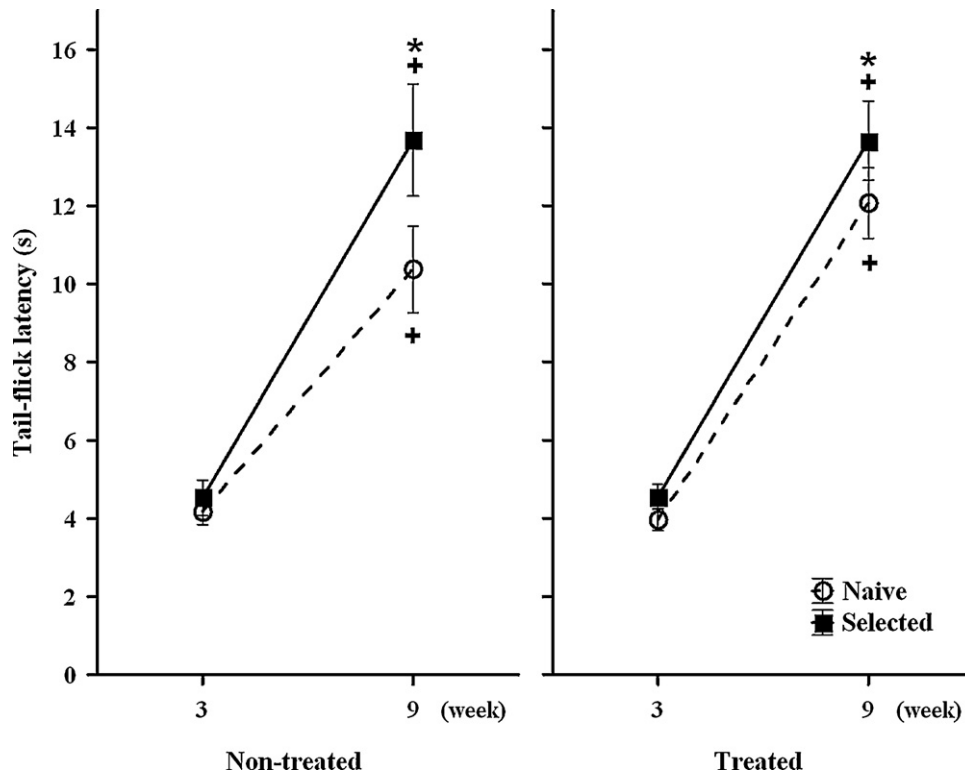


Fig. 3. Tail-flick latency at the age of 3 and 9 weeks. + indicates significant differences between the two time points. * indicates significant difference compared to the naive non-treated (NaNo) group. Data are expressed as means ± SEM.

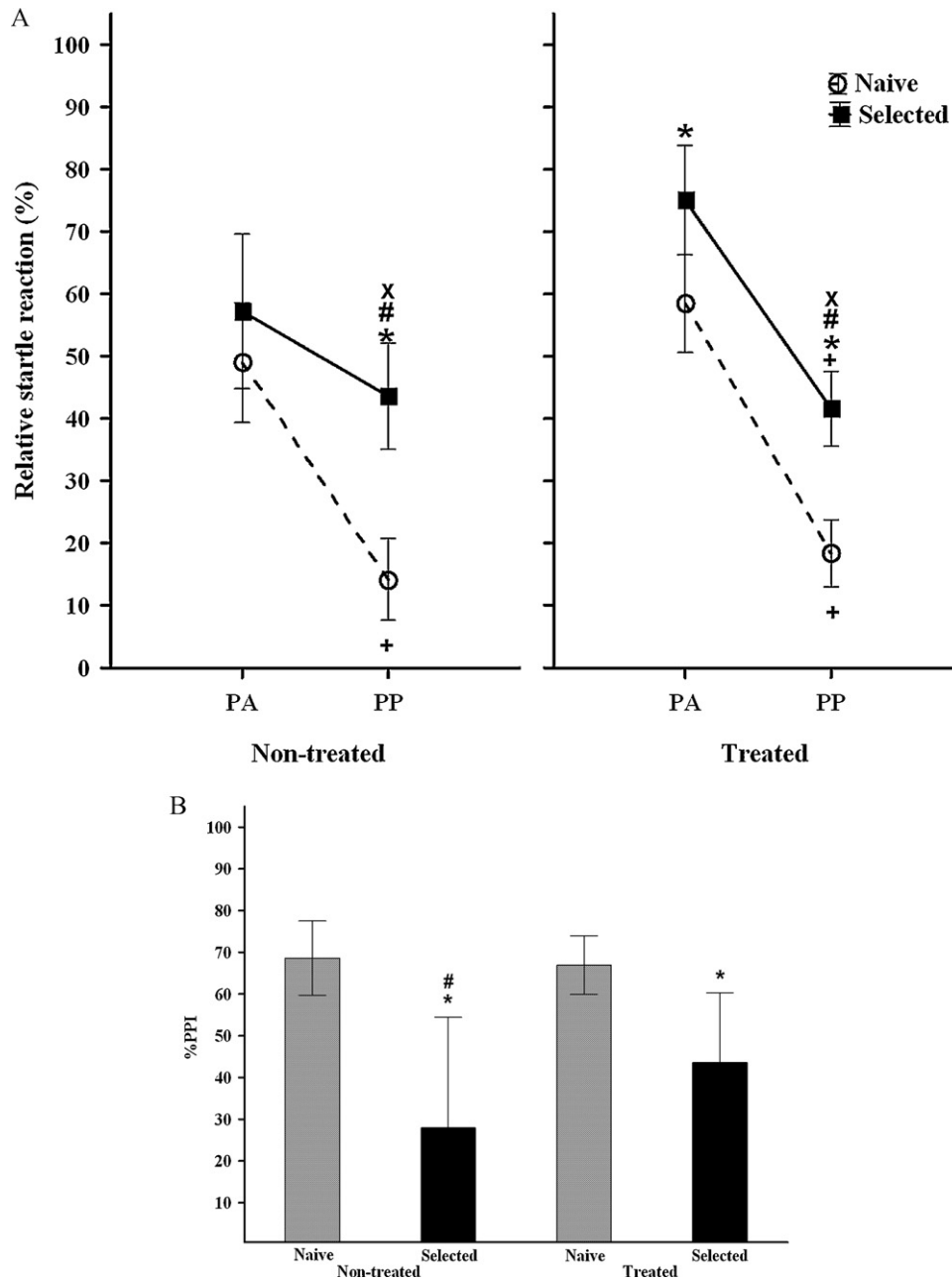


Fig. 4. (A) Degree of relative startle reaction without (PA) and with prepulse (PP) stimulus. (B) %PPI values in the different groups. + indicates significant difference between PA and PP, * and # indicate significant difference compared to the naive non-treated (NaNo) and treated (NaTr) groups, respectively. Data are expressed as means \pm SEM.

sample and/or testing phases. The NaTr group showed enhanced rearing activity in the test phase compared to all the other groups.

Strain differences were also found in the grooming behavior, i.e. the substrain showed increased grooming activity during the second part (5–10 min) of the habituation phase ($F_{1,39} = 4.18, p < 0.05$; Fig. 5B).

Analysis of walking duration revealed a significant effect of strain ($F_{1,39} = 8.88, p < 0.01$), phase ($F_{3,117} = 56.61, p < 0.001$) and a phase-treatment interaction ($F_{3,117} = 3.39, p < 0.05$); thus, walking activity decreased with time (phase), and was lower in the new substrain, while the NaTr group showed enhanced activity in the sample and test phases (Fig. 5C). Both NaTr and SelTr groups showed an increased exploring time of the objects (Fig. 5D). As for the DI, ANOVA revealed a significant effect of phase ($F_{1,38} = 5.70, p < 0.05$). The post hoc comparison revealed that in the NaNo group

DI was significantly enhanced in the presence of the new object, while this enhancement could not be observed in any other groups (Fig. 5E).

3.4. Categorization

Since the quality of motor activity significantly differed between the NaTr and SelTr groups, these parameters were not used for categorization. ANOVA revealed significant differences between the four groups ($F_{3,39} = 9.47, p < 0.001$) in the summarized score, i.e. the NaNo group had the lowest score, while the SelTr group scored the highest (Fig. 6A). The histogram of the summarized score shows that all NaNo animals scored lower than 6 points, while in all of the other groups there were some animals that scored higher, and the highest ratio of these was observed in the SelTr group (Fig. 6B).

4. Discussion

Schizophrenia poses a challenging degree of complexity with respect to genetic and environmental factors; nonetheless, only a few studies have addressed possible gene–environment

interactions in the context of schizophrenia models [5,46,47]. We combined selective breeding, postweaning social isolation and subchronic NMDA antagonist treatment to determine the effects of these interventions on responses to acoustic stimulation, memory function, pain sensitivity and motor activity; the parameters

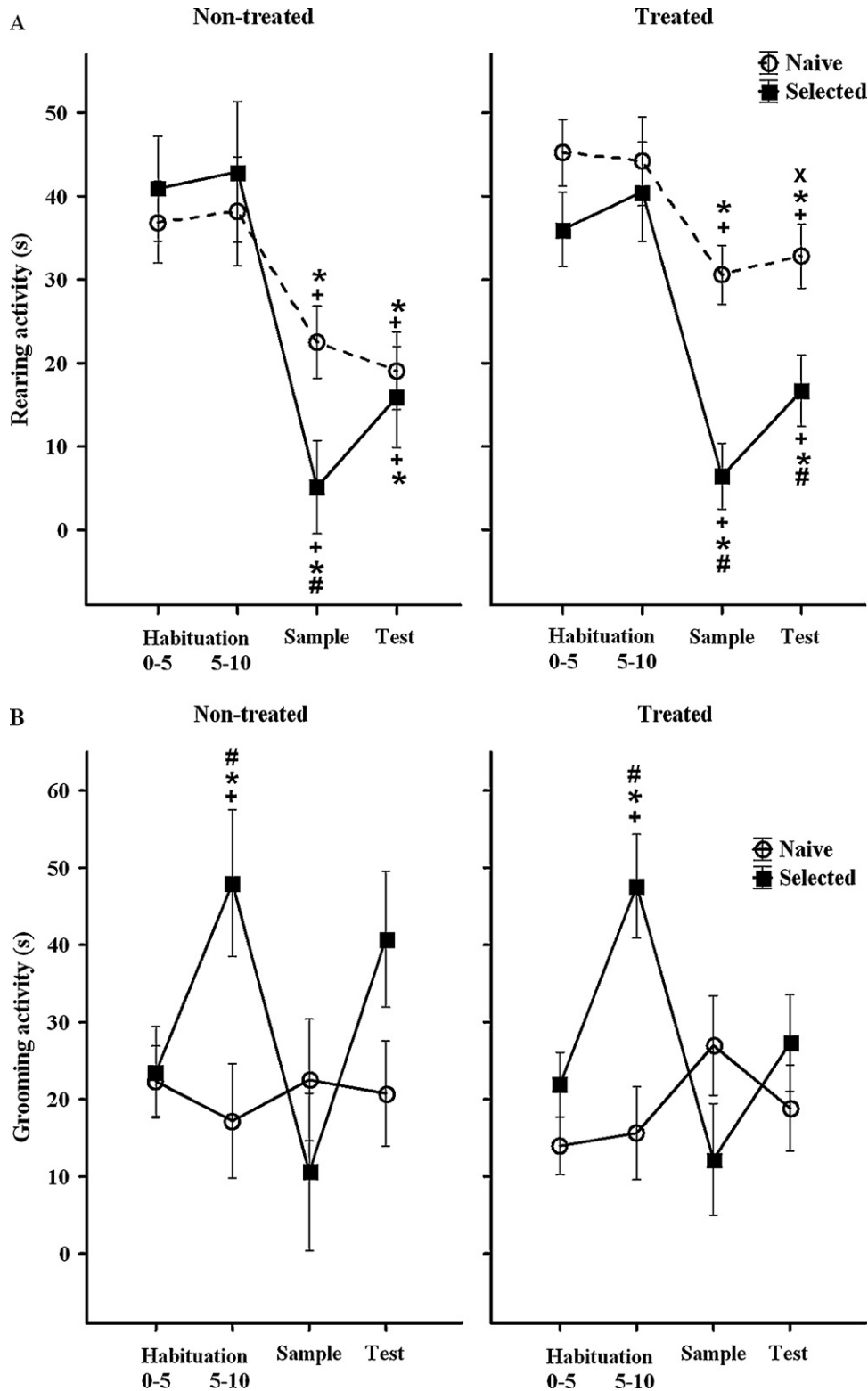


Fig. 5. Rearing (A), grooming (B), walking (C) and exploratory (D) activities in the different phases of the NOR test. + and * indicate significant difference from the first (0–5 min) and second (5–10 min) habituation period; × and # sign significant difference with treatment and strain, while the symbol o indicates significant difference between the naive and selected groups. (E) Differentiation index in the sample and test phases. \$ indicates significant difference between the phases. Data are expressed as means ± SEM.

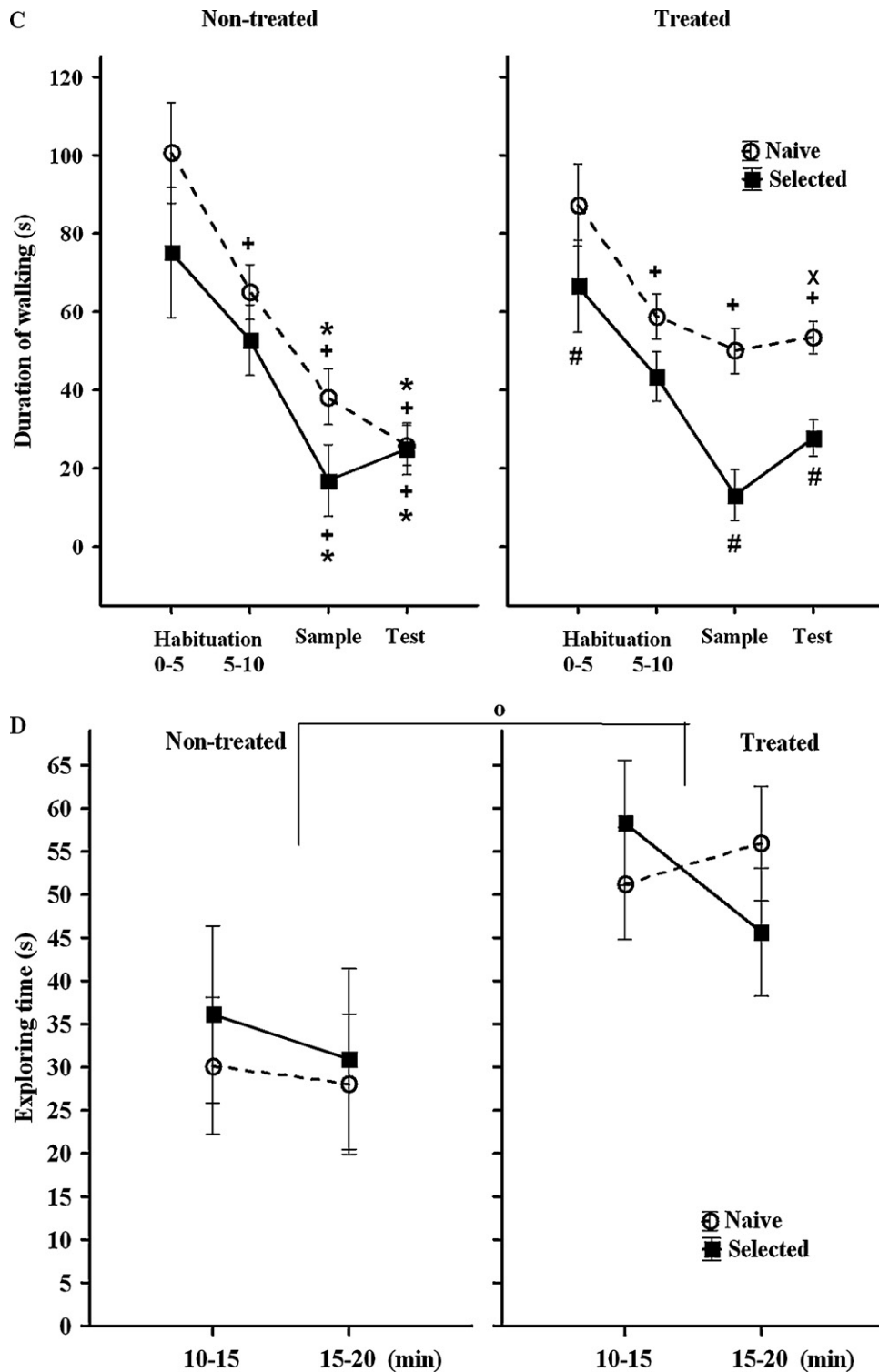


Fig. 5. (Continued)

that are impaired in several neuropsychiatric disorders, like schizophrenia. Our data suggest that this complex paradigm can lead to an improved model of schizophrenia; although, further breeding is required to enhance its reliability.

4.1. Sensory gating

Deficits in sensory gating have been described as a prominent area of information processing dysfunction in individuals with

psychosis, and it may contribute to the characteristic thought disorder and cognitive fragmentation seen in schizophrenia [11,48–51]. It is important to mention that PPI deficits are not unique to schizophrenia, as patients with other neuro-psychiatric diseases (e.g. Huntingtons’s disease, Tourette’s syndrome, autism, bipolar or panic disorder) also show this disturbance, and a lack of this phenomenon in schizophrenia has also been reported [50,52].

Previous studies revealed that repeated NMDA-antagonist treatment of neonatal or adult rats led to the disruption of PPI

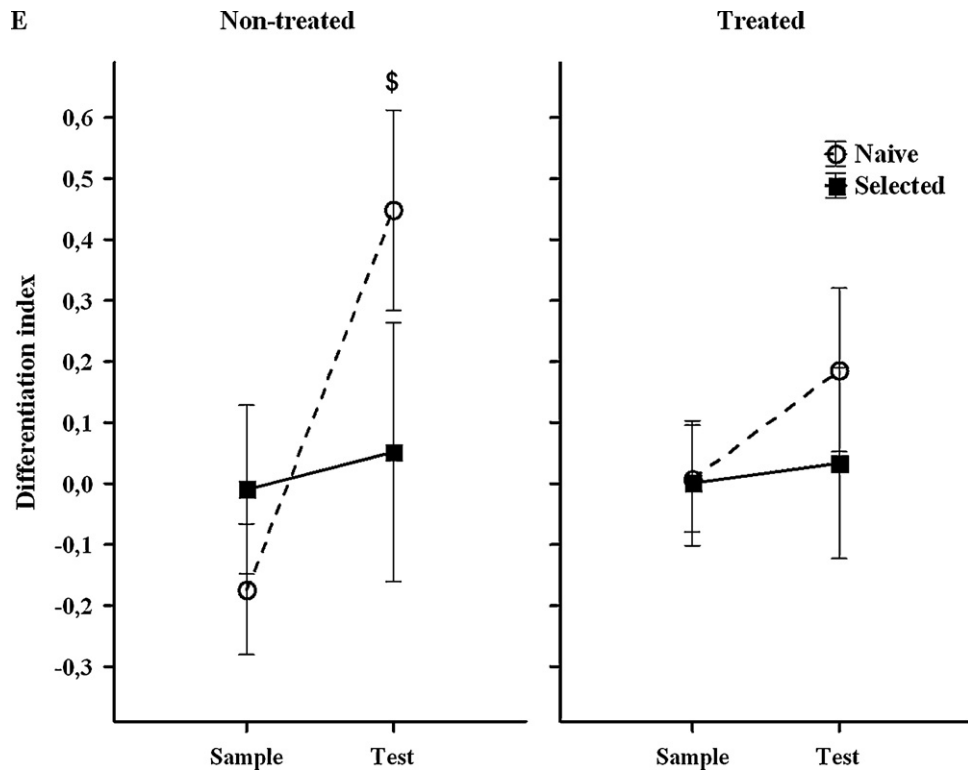


Fig. 5. (Continued).

in some but not all the animals [53–56], and the cessation of NMDA-antagonist treatment resulted in remission; which, suggests that this treatment by itself is not sufficient to produce a long-term simulation of the PPI disturbance [17,57–59]. Plenty of evidence suggests that long-term postweaning social isolation may also cause impaired PPI in rodents, but resocialization may lead to recovery in these changes; however, the data are somewhat inconsistent [11,60–69]. It is assumed that changes in the prefrontal cortex after social isolation and/or imbalances between neural connections within the cortico–striato–limbic circuitry lead to the observed PPI disturbances [8]. Altered sensitivity to novelty and disturbed PPI as a result of selective breeding was proven to be heritable, and this can be used to develop an animal model for schizophrenia [43,70–73]. The rats with impaired PPI also exhibited deteriorated social behavior, impaired reward responses, and abnormalities in information processing; which indicates that rats with low PPI show other schizophrenia-like disturbances, as well. Regarding the combination of genetic manipulation with environmental factors, it has been shown that postweaning social isolation for 12 weeks did not impair PPI in Nurr1 wild type mice, but it was disturbed in heterozygotic animals [74].

We did not find a striking effect of social isolation and ketamine treatment on PPI after two weeks of treatment cessation in naive animals. Selective breeding was effective, but the combination of these interventions did not lead to further impairment; which suggests that genetic factors played the major role in the development of PPI disturbance.

4.2. Memory

Cognition, including memory, is impaired in schizophrenia, and both social deprivation and repeated treatment with NMDA antagonists of juvenile animals can disrupt memory functions which are related primarily to the prefrontal cortex [13,75–80]; however, several studies failed to induce impairments in tests of

memory with these treatments, or only modest learning disturbances were observed [13,17,20,21,52,55,56,78–82]. Ashby et al. investigated the effects of subchronic NMDA-antagonist, MK-801, and postweaning social isolation (for 5 weeks) on hippocampal long term potentiation (LTP) after a 7 days' washout period [36]. While subchronic MK-801 treatment enhanced hippocampal LTP, postweaning social isolation did not influence it, and the combination of the two manipulations did not result in detectable additive or synergistic effects on hippocampal plasticity.

The NOR task is based on the spontaneous novel object preference of rodents. A reduction in novel object recognition might be interpreted as a memory deficit, and the underlying process is a possible analog of declarative memory in humans [83–85]. Anatomically, this task is assumed to depend on the hippocampus, the nigrostriatal dopaminergic pathway and rhinal cortex [86,87]. Both postweaning isolation and NMDA antagonist treatment can lead to impairment in the NOR test, but the results are controversial in this respect, too [19,66,79,88–92].

We have found that all the groups, except for NaNo, showed impairment in the NOR test, i.e. the ability to discriminate between novel and familiar objects was disturbed; thus, we assume that both genetic and environmental factors play a role in the memory deficit. However, it would be useful to apply further memory tests (e.g. T-maze or holeboard) to characterize this deficit in detail.

4.3. Motor activity

Altered motor activity has been reported in schizophrenia. Depending on the disease subtype, psychopathology and medication, excessive motor agitation, reduced motor activity, even akinetic episodes, are observed, and these motor disturbances have been related to basal ganglia dysfunction [93–98]. Postweaning social isolation increases activity in novel environments, but data are controversial and the effect depends on the strain of rodents [62–64,66,89,90,99–103]. Most studies investigated motor

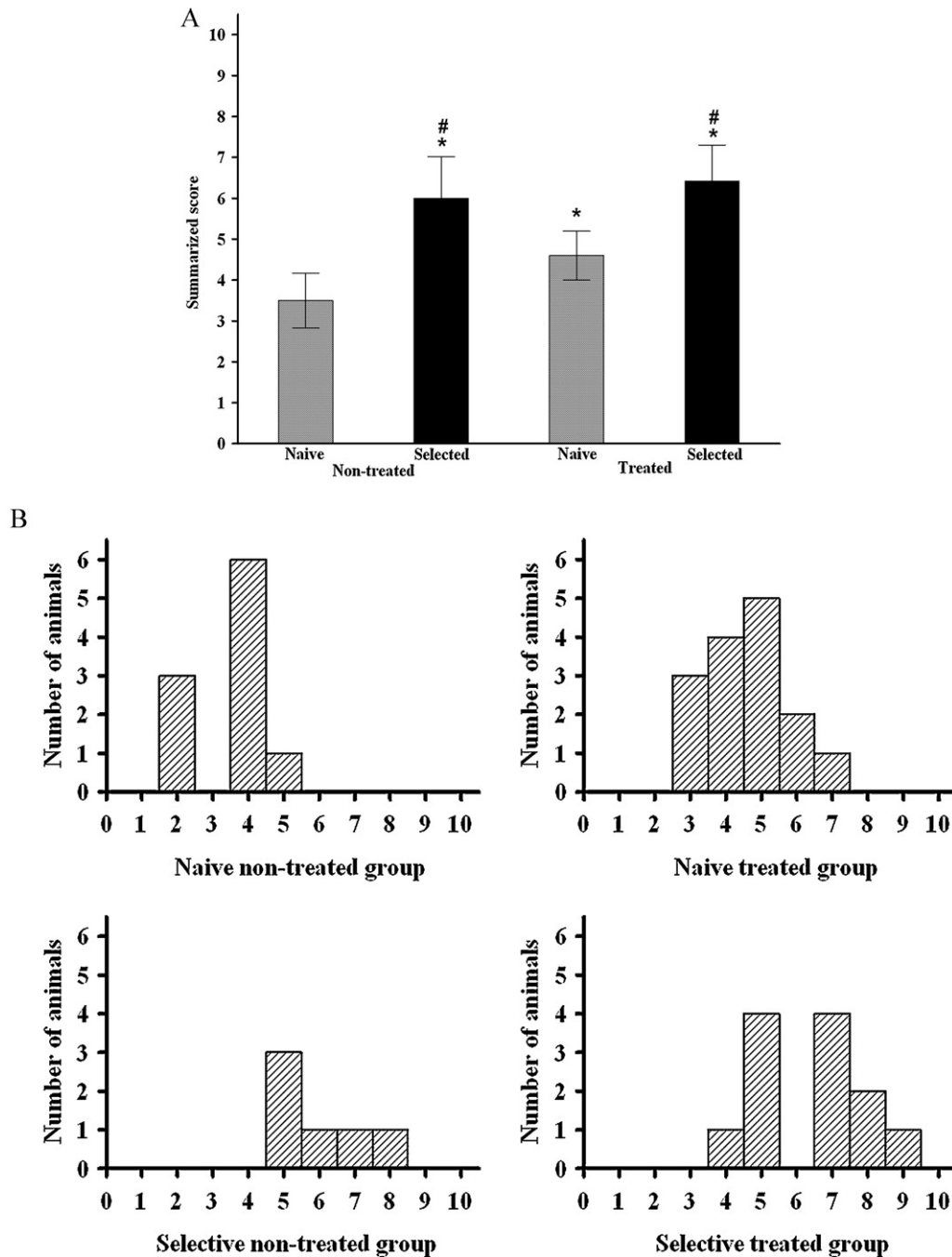


Fig. 6. Means \pm SEM (A) and the distribution (B) of the summarized score within each group. The symbols * and # indicate significant differences compared to the naive non-treated (NaNo) and treated (NaTr) groups, respectively.

activity during isolation, but social isolation by itself did not produce long-term changes in motor activity [60,104]. Some reports suggest motor disturbances after NMDA antagonist treatment, but the results are inconsistent, and the effect depends on the age of the animals [19,82,91]. Cessation of treatment in adult or juvenile rodents did not cause gross changes in motor activity, while early postnatal treatment was effective in this respect [19,21,56,105]. Beninger's laboratory investigated the effects of subchronic MK-801 and postweaning social isolation on motor activity [35,36,106]. Postweaning social isolation enhanced locomotor activity, while MK-801 treatment, alone, did not alter it, but blunted the amphetamine-induced hyperlocomotion. The combination of the two manipulations did not produce detectable additive or synergistic effects on behavior.

In the present study, the complex analysis of motor activity during the NOR test revealed that the selective breeding decreased overall motor activity but increased the grooming behavior. The ketamine treatment+social isolation induced increased exploratory activity in both naive and selected groups. Interestingly, the complex treatment in selectively bred animals resulted in an altered motor phenotype with decreased rearing and walking activity, accompanied by increased exploratory and grooming activities. The enhanced grooming behavior can indicate anxiety, and might present a useful strategy to investigate stress-related responses in animal models of neuropsychiatric disorders [107–109]. Hyper-exploratory activity was also observed in animals with hippocampal lesions, which is another model for schizophrenia [110]. To clarify these results regarding the different

aspects of motor behavior, further investigation of motor behavior is necessary in these animals.

4.4. Pain sensitivity

Clinical reports pointed out that many patients with schizophrenia are less sensitive to pain than other individuals, and this is likely associated with increased morbidity and mortality [111–113]. Data are available to suggest that juvenile isolation causes significant changes in pain sensitivity, which might be due, at least partially, to changes in the number and activity of opioid receptors; suggesting a high importance of housing conditions in schizophrenia models [6,15,29,38,104,114–117]. Subchronic ketamine treatment and subsequent social isolation in adult rats produces slight changes in pain sensitivity [38]. Our recent study demonstrated that juvenile isolation, but not ketamine treatment, attenuated responses evoked by acute heat stimuli; while the combination of the two manipulations did not result in a further increase in TF latency. In the same study we showed that social isolation, ketamine treatment and their combination enhanced the antinociceptive effect of morphine [6]. While selective breeding led to a significant increase in pain threshold, the complex treatment applied in the present study did not result in a further enhancement; suggesting that genetic factors played a larger role in this effect.

It should be noted that our study is not without limitations. Although we did observe deficits persisting for weeks after the last drug administration, further studies are needed to determine whether these deficits are maintained over a longer time period following treatment cessation. It is well-known that no neuropsychological test is a perfect indicator of psychiatric disorder and indication of disease must be present on a battery of tests in order to justify a diagnosis of schizophrenia [118]. In animals, it is impossible to determine most of these parameters; therefore, other appropriate parameters must be chosen to indicate schizophrenia in rodents. While none of the measured parameters are specific to schizophrenia, we hypothesize that categorization and the cluster sampling of the described parameters could improve the model, as the presence of many of the described indicators together can suggest psychotic-like disturbance.

We support that this new substrain is a promising model for schizophrenia, but acknowledge that further complex environmental and genetic manipulations should be investigated to improve the model. Social isolation together with pharmacological treatment can increase the prevalence of the animals with schizophrenic signs, and the reliability of the model. Our present results confirm that selective breeding is still one of the most fundamental and effective methods for the assessment of complex traits influenced by multiple genes. A classic example of such complexity is the demonstration of cognitive abilities that show a significant inheritance, as indicated by the experiments of Tryon (1929) in selectively bred rats [119,120]. In the largest published familial schizophrenia cohort, Touloupoulou et al. demonstrated that a major portion of phenotypic correlation between schizophrenia patients in certain cognitive measures could be explained by shared multiple genes [121]. In spite of these facts, selective breeding is a relatively rare method used in schizophrenia research. Although many of the measures we used did not indicate a straightforward gene–environment interaction, the summarized score based on categorization revealed that the selectively-bred and treated animals differed most markedly from the naive, non-treated rats. This suggests that genetically pre-disposed traits together with environmental risk factors resulted in the most prominent impairment relative to naive animals with no environmental perturbation. A further limitation of our assessment of gene–environment interaction was that we used a limited set of tests. A more comprehensive

battery of behavioral measures may lead to a better detection of such interactions.

In conclusion, selective breeding after juvenile isolation and ketamine treatment produces several signs which resemble those found in schizophrenia; however, further breeding is required to improve our animal model. Molecular biological studies are also required to reveal any changes of various neurotransmitter systems and genetic abnormalities. We suggest that the resulting rat line may serve as a potentially powerful model for the examination of the gene–environment interaction in the development of schizophrenia.

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