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Genetic Backgrounds Have Unique Seizure Response Profiles and Behavioral Outcomes Following Convulsant Administration

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Abstract

Three highly utilized strains of mice, common for preclinical genetic studies, were evaluated for seizure susceptibility and behavioral outcomes common to the clinical phenotypes of numerous psychiatric disorders following repeated low dose treatment with either a gamma-aminobutyric acid (GABA) receptor antagonist (pentylenetetrazole; PTZ) or a glutamate agonist (kainic acid; KA). Effects of strain and treatment were evaluated with classic seizure scoring and a tailored behavior battery focused on behavioral domains common in neuropsychiatric research: learning and memory, social behavior, and motor abilities, as well as seizure susceptibility and/or resistance. Seizure response was induced by a single daily treatment of either PTZ (30 mg/kg, i.p.) or KA (5 mg/kg, i.p.) for 10 days. PTZ-treated FVB/NJ and C57BL/6NJ strains of mice showed strong, clear seizure responses. This also resulted in cognitive and social deficits, and increased susceptibility to a high-dose of PTZ. KA-treated FVB/NJ and C57BL/6NJ strains of mice had a robust seizure response which resulted in hyperactivity. PTZ-treated C57BL/6J mice demonstrated mild hyperactivity while KA-treated C57BL/6J displayed cognitive deficits and resistance to a high-dose of KA but no social deficits. Overall, a uniquely different seizure response profile was detected in the C57BL/6J strain with few observable instances of seizure response despite repeated convulsant administration by two mechanisms. This work illustrated that differing background genetic strains have unique seizure susceptibility profiles and distinct social and cognitive behavior following PTZ and/or KA treatment and that it is therefore necessary to consider strain differences before attributing behavioral phenotypes to gene(s) of interest during preclinical evaluations of genetic mouse models, especially when outcome measures are focused on cognitive and/or social behaviors common to the clinical features of numerous neurological disorders.

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Author contributions:

Conceived and designed the experiments: NAC and JLS; Performed the experiments: NAC, AA, SP; Analyzed and interpreted the data: NAC, SP, JLS; Contributed reagents/materials/analysis tools: JLS; Drafted the article: NAC, JLS; Revised the article: All authors.

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Introduction:

Advances in genetic technologies allow mice to be manipulated in order to model virtually any human condition with a known genetic causal factor. In addition to genetics, the practical lifespan of a mouse allows for the opportunity to investigate the results of genetic insults relevant from neurodevelopment to neurodegeneration [1]. Due to the benefits of using mice in disease modeling, various inbred strains are used to ensure phenotypic consistency and long term stability [2]. Individual strains are unique and exhibit particular phenotypic characteristics that are important for addressing specific research questions (i.e., immunology, genetics, toxicology). Inbred strains represent fixed, renewable genotypes that are ideally suited for behavioral pharmacology, genetics, and toxicology systems approaches. However, an unintended byproduct may occur when new genetic models are created. False positives may attribute phenotypic trait(s) to be associated with a gene mutation without considering the background strain used. Often, positive phenotypic traits are attributed to a gene and lead to generalizations that the same phenotype will be seen across more than one inbred strain, when, in fact, it may not [3]. Or, in the broader scenario, that these phenotypes will generalize to the genetically heterogenous clinical population widening the gap from preclinical research to translational successes [4].

This is not surprising, given that phenotypic diversity of inbred mouse strains has been well described on numerous standardized behavior assays [5–10], and experiments restricted to a single strain will not necessarily reflect the heterogeneous nature of the human population [3]. A large body of literature suggests that background strain strongly influences phenotypes and survival rates related to seizures and seizure disorders [11–15], we desired to expand this research by assessing neurobehavioral outcomes following a chemoconvulsant regimen.

To begin this investigation, we selected three commonly utilized strains in genetic models of neurological disorders, FVB/NJ and C57BL/6. We chose FVB/NJ mice as they are a popular strain amongst geneticists due to their high pup yield and compatibility to embryonic cell manipulations, however, their blindness, hyperactivity, aggressive and anxiety-like phenotypes can act as confounding variables for behavior [16–20]. We also carefully evaluated the two most utilized B6 substrains (N and J). While C57BL6/J is probably the most well characterized B6 substrain, known for its reliability in behavioral assays [6, 21–23]; large trans-NIH initiatives, such as the Knockout Mouse Project (KOMP) and International Mouse Phenotyping Consortium, [24, 25], are in the process of characterizing mice that harbor null mutations for every protein-coding gene in the mouse genome on the C57BL/6N background, thus we included both substrains.

Our first goal was to determine if the three background strains FVB/NJ, C57BL/6J, and C57BL/6NJ had similar seizure response profiles and susceptibility characteristics, using a modified kindling protocol focused on administering an identical regimen of chemo-induction [26, 27]. This process consisted of sequential low dose administrations of convulsant, wherein one dose would cause mild seizure responses including immobility and small, generalized spasms while continued insult induced more extreme profiles such as forelimb and full body clonus. Our next objective was to determine if responses in the

strains differed between the glutamate or GABA driven mechanisms of action. We used two common chemoconvulsants that addressed glutamate and gamma-aminobutyric acid (GABA) homeostatic balance and are common in preclinical research [28–31]. Pentylenetetrazole (PTZ) is a non-competitive $GABA_A$ antagonist [32, 33] and kainic acid (KA) is a glutamate analog to the kainate glutamate channel receptor. Both lead to hyperexcitability [34, 35]. Finally, we sought to evaluate if behavioral impairments were detectable following the convulsant treatment. We designed a tailored battery to address motor, cognitive, and social behavioral domains to capture a broad scope of phenotypes common in neuropsychiatric disease.

Methods:

Subjects

Male FVB/NJ (Stock #001800), C57BL/6J (Stock #000664), and C57BL/6NJ (Stock #005304) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age and were socially housed in groups of 2–4 per cage. All mice were housed in Techniplast cages (Techniplast, West Chester, PA, USA). Cages were housed in ventilated racks in a temperature (68–72°F) and humidity (~25%) controlled colony room on a 12:12 light/dark cycle with lights on at 07:00, off at 19:00-h. Standard rodent chow and tap water were available *ad libitum*. In addition to standard bedding, a Nestlet square, shredded brown paper, and a cardboard tube (Jonesville Corporation, Jonesville, MI, USA) were provided in each cage. All subjects were tested between 2–3 months of age and all experimental procedures were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees (IACUC) of the University of California, Davis.

Design

A total of 116 mice were used in this experiment (38 FVB/NJ, 39 C57BL/6J, and 39 C57BL/6NJ). Of the 38 FVB/NJ mice, 15 were administered vehicle (sterile water in 0.9% sodium chloride), 11 PTZ, and 12 KA. Of the 39 C57BL/6J and 39 C57BL/6NJ mice, 15 were administered vehicle, 12 PTZ, and 12 KA of each group. Subjects received injections for 10 days then, on Day 11, began the behavioral battery. All behavioral tests were performed between 09:00 and 17:00-h during the light phase of the 12:12 light/dark cycle. Mice were brought to an empty holding room adjacent to the testing area at least 1-h prior to the start of behavioral testing. Mice were tested every other day in the follow order: open field, novel object recognition, 3-chambered social approach, male-female reciprocal social interaction, and high-dose induced seizures (Figure 1). All animals were littermates and housed mixed treatment to avoid group housing effects.

Low-Dose Convulsant Treatment

Subjects were weighed daily, then administered either pentylenetetrazole (30 mg/kg, PTZ), kainic acid (5 mg/kg, KA), or vehicle (sterile water in 0.9% sodium chloride) injected intraperitoneally (i.p.) for 10 days prior to behavioral testing. Both PTZ (SKU: P6500) and KA (SKU: K0250) were purchased from Sigma-Aldrich (Sigma Aldrich, St. Louis, MO, USA). Dosing was conducted in the afternoon (04:00–06:00) in a dim (~30 lux) empty

holding room. Directly after administration of specified treatments, subjects were placed in a clean, empty cage and subsequent seizure stages were live-scored for 20-min. Seizure stages were scored using a modified Racine scale where 0 = normal exploratory behavior, 1 = immobility, 2 = generalized spasm, 3 = Straub's tail, 4 = forelimb clonus, 5 = generalized clonus, 6 = clonic-tonic seizure, and 7 = full tonic extension/death. No subjects died (score of 7) in response to PTZ or KA, nor had a continued non-response (score of 0) to either convulsant.

Open Field

General exploratory locomotion in a novel open field arena was evaluated as previously described [36–39]. Briefly, each subject was tested in a VersaMax Animal Activity Monitoring System (Accuscan, Columbus, OH, USA) for 30-min in a ~30 lux testing room. Total distance traversed, horizontal activity, vertical activity, and time spent in the center were automatically measured to assess gross motor abilities in mice.

Novel Object Recognition

The novel object recognition test was conducted as previously described [40–42] in opaque matte white (P95 White, Tap Plastics, Sacramento, CA, USA) arenas (41 cm *l* × 41 cm *w* × 30 cm *h*). The assay consisted of four sessions: a 30-min habituation session, a second 10-min habituation phase, a 10-min familiarization session, and a 5-min recognition test. On day 1, each subject was habituated to a clean empty arena for 30-min. 24-h later, each subject was returned to the empty arena for an additional 10-min habituation session. The mouse was then removed from testing arena and was placed in a clean temporary holding cage while two identical objects were placed in the arena. Subjects were returned to the testing arena and given a 10-min of familiarization period in which they had time to investigate the two identical objects. After the familiarization phase subjects were returned to their holding cages for a 1-h interval period. One familiar object and one novel object were placed in the arena, where the two identical objects had been located during the familiarization phase. After the 1-h interval, each subject was returned to the arena for a 5-min recognition test. The familiarization session and the recognition test were recorded using Ethovision XT video tracking software (version 9.0, Noldus Information Technologies, Leesburg, VA, USA). Sniffing was defined as head facing the object with the nose point within 2 cm from the object. Time spent sniffing each object was scored by an investigator blind to both genotype and treatment. Recognition memory was evaluated by time spent sniffing the novel object versus the familiar object and innate side bias was accounted for by comparing sniff time of the two identical objects during familiarization.

3-Chambered Social Approach

For social behavior, a three-chambered social approach assay was used as described previously [38, 43, 44]. Each rectangular 3-chambered apparatus (40 cm *w* × 60 cm *l* × 23 cm *h*) was made of non-reflective matte white finished acrylic (P95 White, Tap Plastics, Sacramento, CA, USA). Opaque retractable doors (12 cm *w* × 33 cm *h* with 5 cm × 10 cm doorways) separated the apparatus and allowed entries across chambers. The assay consisted of three sessions: a 5-min habituation session, a 10-min familiarization session, and a 10-min sociability test. All sessions were run in their entirety and no subjects were removed due

to overt aggression. Subjects were placed into the middle chamber of the 3-chambered arena with the retractable doors closed and allowed to habituate for 5-min. After the 5-min habituation period, the doors of the apparatus were lifted and the animals were given 10-min to investigate all three chambers of the testing arena during a familiarization phase. At the end of this familiarization period, subjects were placed back into the middle chamber with both doors closed. While the mice were kept in the middle chamber a wire cup (inverted wire cup, Galaxy Cup, Kitchen Plus, http://www.kitchen-plus.com) was placed in both the top and bottom chamber. One wire cup was empty (serving as the novel object) while the other wire cup had an age- and sex-matched stimulus mouse (novel mouse). Sniffing was defined as head facing the cup enclosure with the nose point within 2 cm from the enclosure. Time spent in each chamber and time spent sniffing each cup were scored by an investigator blind to both genotype and treatment. Sociability was evaluated by time spent in the chamber and sniffing the novel mouse compared to the novel object and innate side bias was accounted for by top and bottom chamber time during familiarization.

Male-Female Reciprocal Social Interaction

The male-female reciprocal social interaction assay was conducted as previously described [45, 46]. Male mice were paired with an unfamiliar female in estrous for 5-min in a novel, square arena with clean bedding covering the floor (30 cm *l* × 30 cm *w*). Female stimulus mice were introduced to male urine and bedding for 3 days prior to the interaction task to induce estrous. On the day of testing females were visually scored by an investigator to determine the phase of their reproductive cycle. Females were scored on a 0–3 scale where 0: diestrous (vaginal opening is small with no swelling), 1: metestrous (vaginal opening is slightly more open with no swelling), 2: proestrous (vaginal opening is pink, swollen, and moist), and 3: estrous (vaginal opening is large with less swollen and moist characteristics). Only females that had a score of 3 were used as stimulus animals. Interaction video was captured using a recording camera and ultrasonic vocalizations were gathered using an ultrasonic microphone (Avisoft UltraSoundGate condenser microphone capsule CM15; Avisoft Bioacoustics, Berlin, Germany) placed 20 cm above the apparatus. Sampling frequency for the microphone was 250 kHz. The entire arena was contained in a sound-attenuating environmental chamber (Lafayette Instruments, Lafayette, IN, USA) under red light illumination (~10 lux). Duration of nose-to-nose sniffing, nose-to-anogenital sniffing, grooming and frequency of ultrasonic vocalizations were scored by an investigator blind to both genotype and treatment.

Seizure Susceptibility by High-Dose Convulsant

Subjects were weighed then administered either pentylenetetrazole (80 mg/kg) or kainic acid (30 mg/kg) intraperitoneally. Mice that were previously treated with PTZ were administered a high dose of PTZ while mice that were previously treated with KA were administered a high dose of KA. Vehicle-treated animals were split evenly by background strain into high-PTZ or - KA drug groups. Dosing was conducted in the afternoon (04:00–06:00) in a dim (~30 lux) empty holding room. Directly after administration of the convulsant, subjects were placed in a clean, empty cage and subsequent seizure stages were live-scored for 2-hr. Seizure stages were scored using latencies to (1) first jerk/Straub's tail, (2) loss of righting,

(3) generalized clonic-tonic seizure, and (4) death. Time to each stage was taken in seconds and compared by treatment and background strain.

Statistical analysis

Data were analyzed using GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA) with significance level defined at $p < 0.05$. All results are presented as mean ± SEM, using statistical tests previously described [43, 45, 47]. Data for treatment response and behavioral assays all fit Gaussian distribution by Shapiro-Wilk normality tests.

Low-Dose Convulsant Treatment analysis: Linear regression analyses were used to determine change in Racine score over the 10-d treatment time for PTZ- and KA-treated background strain groups. R^2, *F*, degrees of freedom, and *p*-values are reported. Two-way ANOVA was used to analyze Racine score response over treatment days between strains. *F*, degrees of freedom, and *p*-values are reported.

Open Field analysis: Repeated-measures ANOVA was used to detect differences in horizontal, vertical, total, and center time activity obtained during the open field assay. Treatment groups were tested within background strain across time bins. Multiple comparisons were corrected for using Sidak post hoc methods and *F*, degrees of freedom, and *p*-values are reported.

Novel Object Recognition analysis: Within genotype repeated-measures ANOVA (~paired t-test) was used to analyze novel object recognition using novel versus familiar objects as comparison. *F*, degrees of freedom, and *p*-values are reported. Since there are only two variables, posthoc analysis for multiple comparison is not required. These methods have been established as laboratory standard [42] as well as disseminated via the National Institute of Child Health and Human Development (NICHD)'s network of Intellectual Developmental Disabilities Resource Center(IDDRC) Behavioral Cores [48].

3-Chambered Social Approach analysis: Within genotype repeated-measures ANOVA (~paired *t*-test) was used to analyze time spent in novel mouse chamber and time spent sniffing novel mouse compared to the novel object. Time spent in the middle chamber was included for illustrative purposes, but was not included in statistical analyses. *F*, degrees of freedom, and *p*-values are reported. These methods have been established as laboratory standard in collaboration with the Crawley laboratory, inventor of the behavioral assay [38, 44, 49].

Male-Female Reciprocal Social Interaction analysis: One-way ANOVA was used to analyze time spent nose-anogenital sniffing, nose-nose sniffing, grooming, and produced ultrasonic vocalizations. *F*, degrees of freedom, and *p*-values are reported, as previously illustrated [38, 45, 47, 50]

Seizure Susceptibility by High-Dose Convulsant analysis: One-way ANOVA was used to analyze latencies to first jerk, loss of righting, generalized clonic-tonic seizure, and death. *F*, degrees of freedom, and *p*-values are reported.

Results:

Strain differences in behavioral seizure response to PTZ or KA: increased severity of responses in FVB/NJ and C57BL/6NJ and minimal responses in C57BL/6J mice.

Seizure susceptibility to PTZ or KA was evaluated daily during a sequential, low-dose treatment (30 mg/kg of PTZ or 5 mg/kg KA) of chemoconvulsants in an adapted kindling paradigm. No seizures were observed in the vehicle-treated group. PTZ-treated FVB/NJ mice displayed a significant increase in Racine score across treatment days, indicating heightened seizure susceptibility (Figure 2A: $R^2 = 0.381$, $F_{(1, 108)} = 66.43$, $p < 0.0001$). KA-treated FVB/NJ mice also displayed a significant increase in Racine score across treatment days (Figure 2B: $R^2 = 0.284$, $F_{(1, 118)} = 46.69$, $p < 0.0001$). KA-treated C57BL/6J mice displayed a significant, smaller increase in Racine score across treatment days compared to FVB/NJ and C57BL/6NJ (Figure 2D: $R^2 = 0.041$, $F_{(1, 118)} = 5.012$, $p = 0.027$). PTZ-treated C57BL/6J mice displayed no change in Racine score across the 10-d treatment time (Figure 2C: ns, $p > 0.05$). PTZ- and KA-treated C57BL/6NJ mice displayed a significant increase in Racine score across treatment days, indicating a strong susceptibility response (Figure 2E–F: $R^2 = 0.453$, $F_{(1, 118)} = 97.55$, $p < 0.0001$, $R^2 = 0.260$, $F_{(1, 118)} = 41.40$, $p < 0.0001$).

PTZ-treated FVB/NJ and C57BL/6NJ mice had higher Racine scores compared to C57BL/6J ($F_{(1, 21)} = 200.2$, $p < 0.0001$, $F_{(1, 22)} = 95.26$, $p < 0.0001$). KA-treated FVB/NJ mice also had a significantly larger increase in Racine scores compared to C57BL/6J, while C57BL/6NJ mice exhibited the largest increase in score compared to both FVB/NJ and C57BL/6J (*, $p < 0.05$). Together, these findings highlighted that the frequently utilized C57BL/6J substrain is resistant to induced seizure response by never achieving seizure progression beyond myoclonic jerks and never reaching generalized clonus, clonic-tonic seizure, or tonic extension by either a PTZ or KA-mediating convulsant, extending a wide breadth of literature [51–55].

Deficits in novel object recognition learning and memory were observed in PTZ-treated FVB/NJ and C57BL/6NJ and KA-treated C57BL/6J and C57BL/6NJ mice.

In novel object recognition, all vehicle-treated FVB/NJ, C57BL/6J, and C57BL/6NJ showed typical, significant preference towards the novel object (Figure 3A, 3B, and 3C: $F_{(1, 14)} = 8.505$, $p = 0.011$, $F_{(1, 14)} = 19.386$, $p = 0.001$, $F_{(1, 14)} = 32.081$, $p = 0.0001$). Deficits in cognitive behavior, defined as a lack of preference for the novel object over the familiar object, were observed in PTZ-treated FVB/NJ subjects (Figure 3A: $F_{(1, 10)} = 0.263$, $p = 0.619$), and KA-treated C57BL/6J and C57BL/6NJ subjects (Figure 3B, 3C: $F_{(1, 11)} = 0.170$, $p = 0.688$, $F_{(1, 11)} = 1.530$, $p = 0.242$), while KA-treated FVB/NJ and PTZ-treated C57BL/6J and C57BL/6NJ subjects showed typical preference (Figure 3A, 3B, 3C: $F_{(1, 11)} = 8.892$, $p = 0.013$, $F_{(1, 11)} = 17.829$, $p = 0.001$, $F_{(1, 11)} = 11.360$, $p = 0.006$). To test for innate side preference, subject investigation of both the right and left object during familiarization phase was analyzed. No side preference was detected in either background or treatment-grouped subjects (Figure 3D, 3E, 3F: ns, $p > 0.05$). These data show a consistent adverse effect of increased seizure susceptibility on learning and memory, regardless of the mechanism and

strain, consistent with a large body of literature on epilepsy and cognitive impairments [56–58].

Impairments in social behavior were detected in PTZ-treated FVB/NJ and C57BL/6NJ mice while KA-treated C57BL/6NJ showed elevated repetitive behavior.

Social behavior was assessed using male-female reciprocal social interactions to provide a nuanced, detailed evaluation of sensitive social and repetitive behavior while 3-chambered social approach was used as a yes/no qualitative social parameter [45, 59]. Social behavioral parameters were measured as time spent nose-anogenital sniffing, nose-nose sniffing, and sum ultrasonic vocalizations emitted by the male during the 5-min interaction. Time spent self-grooming during the interaction captured repetitive-like behavior. Social deficits were observed in PTZ-treated FVB/NJ mice as reductions in nose-anogenital sniffing and sum ultrasonic vocalizations when compared to vehicle-treated controls (Figure 4A(i), 4A(ii): $F_{(1, 24)} = 6.807$, $p = 0.015$, $F_{(1, 23)} = 6.529$, $p = 0.018$). No effect was detected in PTZ-treated FVB/NJ mice on duration of time spent nose-to-nose sniffing (Figure 4A(iii): $F_{(1, 24)} = 0.494$, $p = 0.489$) or self-grooming, a repetitive behavior seen in various mouse models of neurodevelopmental disorders [45] (Figure 4A(iv): $F_{(1, 24)} = 1.680$, $p = 0.207$). No significant treatment effect was observed in KA-treated FVB/NJ animals on any social or repetitive readout, (Figure 4A(i–iv): ns, $p > 0.05$). No significant treatment effect was observed in PTZ or KA-treated C57BL/6J animals on any social or repetitive readout, (Figure 4A(i–iv): ns, $p > 0.05$). Partial social deficits were observed by reduced bouts of nose-nose sniffing in PTZ-treated C57BL/6NJ mice when compared to vehicle-treated controls (Figure 4C(iii): $F_{(1, 25)} = 6.839$, $p = 0.015$). No PTZ-treatment effect was detected on duration of time spent nose-to-anogenital sniffing, sum vocalizations, or self-grooming (Figure 4C (i–ii, iv): ns, $p > 0.05$). KA-treated C57BL/6NJ mice showed an increase in self-grooming compared to vehicle-treated animals (Figure 4C(iv): $F_{(1, 25)} = 5.210$, $p = 0.045$), but had no significant difference in nose-anogenital sniffing, sum ultrasonic vocalizations, or nose-to-nose sniffing (Figure 4C(i–iii): ns, $p > 0.05$). There was a significantly higher amount of time spent nose-anogenital and nose-nose sniffing in vehicle-treated FVB/NJ animals compared to vehicle-treated C57BL/6J and C57BL/6NJ while vehicle-treated C57BL/6J mice made more ultrasonic vocalizations during the interaction period (Data not shown: *, $p < 0.05$), extending earlier data on strain differences in nuanced social behavior [5, 8, 60]. The 3-chambered social approach task defines sociability in mice as preference for the chamber with a novel mouse over the chamber with a novel object and more time spent sniffing the novel mouse over the novel object. PTZ and KA-treated FVB/NJ, C57BL/6J, and C57BL/6NJ showed typical, significant sociability by chamber time (Figure 4D(i), 4E(i), 4F(i): *, $p < 0.05$). Additionally, all groups exhibited significantly more time social sniffing, which was defined as time spent within 2-cm of the wire cup, with the head facing the wire cup containing the stimulus mouse, as compared to the time spent sniffing the novel object (Figure 4D(ii), 4E(ii), 4F(ii): *, $p < 0.05$). For all other treatment groups, number of entries and time spent in the top or bottom chamber was not affected by treatment (Data not shown, $p > 0.05$). Total entries were similar for all groups indicating that chemoconvulsant treatment had no effect on general exploratory activity throughout the 3-chambered apparatus during the social approach assay. These data show an adverse effect of increased seizure susceptibility on sociability during the sensitive male-female dyadic

interaction but not the three chambered assay, as seen by others with genetic perturbations [35]. Moreover, these data are consistent with a large body of literature on epilepsy and social impairments in the autism spectrum disorder population [45, 61, 62].

Gross motor skills varied widely between background strains and convulsant treatment.

To assess broad motor ability, subjects were tested in an open field assay. KA-treated FVB/NJ animals exhibited significantly higher vertical and total activity (Figure 5A(ii, iii): $F_{(2, 25)} = 5.247$, $p = 0.031$, $F_{(2, 25)} = 5.775$, $p = 0.026$) and no activity difference in horizontal and center time measures when compared to vehicle-treated controls (Figure 5A(i, iv): $F_{(2, 25)} = 1.401$, $p = 0.248$, $F_{(2, 25)} = 0.133$, $p = 0.719$). PTZ-treated FVB/NJ mice showed no significant difference in any activity readout (Figure 5A(i–iv): ns, $p > 0.05$). PTZ-treated C57BL/6J animals exhibited significantly higher total activity (Figure 5B(iii): $F_{(2, 25)} = 13.40$, $p = 0.001$) and while there were trends towards increased horizontal activity (Figure 5B(i): $F_{(2, 25)} = 3.709$, $p = 0.066$), no difference was detected in vertical or center time measures when compared to vehicle-treated controls (Figure 5B(ii, iv): $F_{(2, 25)} = 1.365$, $p = 0.254$, $F_{(2, 25)} = 2.126$, $p = 0.157$). KA-treated C57BL/6J mice showed no significant difference in any activity readout (Figure 5B(i–iv): ns, $p > 0.05$). KA-treated C57BL/6NJ animals exhibited significantly higher horizontal, vertical and total activity (Figure 5C(i–iii): $F_{(2, 25)} = 5.902$, $p = 0.023$, $F_{(2, 25)} = 10.28$, $p = 0.004$, $F_{(2, 25)} = 4.73$, $p = 0.039$) and no activity difference in center time when compared to vehicle-treated controls (Figure 5C(iv): $F_{(2, 25)} = 0.147$, $p = 0.705$). PTZ-treated C57BL/6NJ mice showed significantly lower center time when compared to vehicle-treated controls (Figure 5C(iv): $F_{(2, 25)} = 12.68$, $p = 0.002$) and no deficit in any other motor parameter (Figure 5B(i–iv): ns, $p > 0.05$). While varied in response to chemoconvulsant, all vehicle-treated subjects had significantly different motor phenotypes by background strain. FVB/NJ mice were significantly more active by horizontal, vertical, and total activity measurements compared to C57BL/6NJ and C57BL/6J (Data not shown: *, $p < 0.05$). C57BL/6NJ subjects were also significantly more active by horizontal, vertical, and total activity when compared to C57BL/6J, indicating that the C57BL/6J background strain is the least active of the three strains (Data not shown: *, $p < 0.05$). Finally, C57BL/6NJ exhibited more center time activity compared to FVB/NJ, with C57BL/6J showing the lowest center time activity of the various strains (Data not shown: *, $p < 0.05$).

FVB/NJ and C57BL/6NJ showed increased seizure susceptibility to a high-dose of PTZ while C57BL/6J exhibited increased resistance to seizures following a high-dose of KA.

To better understand seizure susceptibility and resistance after continued convulsant insult, high-doses of PTZ or KA were administered. Previously PTZ-treated subjects were congruently administered a high-dose of PTZ (80 mg/kg), previously KA-treated mice were administered a high-dose of KA (30 mg/kg), and previously vehicle-treated, convulsant naïve mice were evenly split between high-PTZ and -KA drug groups. After administration of either PTZ or KA, animal's latencies to (1) first jerk (Straub's tail), (2) loss of righting, (3) generalized clonic-tonic seizure, and (4) death were measured. A reduction in latency, or shorter time to respond, indicated susceptibility, while an increase in latency, or longer time to respond, indicated resistance when compared to latencies of convulsant naïve animals. PTZ-treated FVB/NJ mice exhibited seizure susceptibility via reduced latency in loss of

righting and trends towards reduced latency in generalized clonic-tonic stages (Figure 6A(ii–iii): $F_{(1, 16)} = 5.797$, $p = 0.029$, $F_{(1, 16)} = 3.055$, $p = 0.099$) when compared to the naïve, non-treated control group. No differences were detected in latency to first jerk or death (Figure 6A (i, iv): $F_{(1, 14)} = 0.386$, $p = 0.643$, $F_{(1, 17)} = 0.222$, $p = 0.643$). While there were trends towards reduced latency to generalized clonic-tonic seizure in PTZ-treated C57BL/6J mice (Figure 6B(iii): $F_{(1, 18)} = 3.084$, $p = 0.096$), they had no significant difference on latency to first jerk, loss of righting, or death when compared to the naïve control group (Figure 6B(i–ii, iv): $F_{(1, 18)} = 0.010$, $p = 0.922$, $F_{(1, 18)} = 0.002$, $p = 0.643$, $F_{(1, 18)} = 2.778$, $p = 0.113$). A decrease of latency time in PTZ-treated C57BL/6NJ mice indicate high seizure susceptibility in loss of righting, generalized clonic-tonic, and death (Figure 6C(ii–iv): $F_{(1, 18)} = 4.258$, $p = 0.054$, $F_{(1, 18)} = 20.349$, $p = 0.0003$, $F_{(1, 18)} = 7.753$, $p = 0.012$), but is not significantly different from the naïve, non-treated group in first jerk (Figure 6C(i): $F_{(1, 18)} = 0.335$, $p = 0.570$). While not significant, KA-treated FVB/NJ and C57BL/6NJ mice showed an opposite pattern to their PTZ-treated counterparts with increased latency times at all seizure stages when compared to the naïve, non-treated control groups (Figure 6D, 6F: Latencies of PTZ-treated FVB/NJ compared to latencies of KA-treated FVB/NJ on all seizure stages, *, $p < 0.05$; Latencies of PTZ-treated C57BL/6NJ compared to latencies of KA-treated C57BL/6NJ on first jerk, loss of righting, and clonic tonic seizure, *, $p < 0.05$). KA-treated C57BL/6J mice exhibited seizure resistance via increased latencies in loss of righting, generalized clonic-tonic, and death (Figure 6E(ii–iv): $F_{(1, 17)} = 5.484$, $p = 0.032$, $F_{(1, 17)} = 9.993$, $p = 0.006$, $F_{(1, 17)} = 5.143$, $p = 0.037$) when compared to the naïve, non-treated control group. No difference was detected in latency to first jerk in KA-treated C57BL/6J (Figure 6E(i): $F_{(1, 15)} = 0.026$, $p = 0.874$). PTZ-treated FVB/NJ mice had the shortest latencies to first jerk, loss of righting, and tonic-clonic seizures compared to both C57BL/6J and C57BL/6NJ and a shorter latency to death compared to C57BL/6J (*, $p < 0.05$) indicating that FVB/NJ subjects were the most susceptible to PTZ-induced seizures. C57BL/6NJ mice had a faster onset to generalized clonic-tonic seizure compared to C57BL/6J (*, $p < 0.05$). KA-treated C57BL/6NJ mice displayed a faster onset to first jerk compared to FVB/NJ ($F_{(1, 22)} = 2.149$, $p = 0.043$) and both KA-treated C57BL/6J and C57BL/6NJ subjects demonstrated a faster onset to loss of righting compared to FVB/NJ (*, $p < 0.05$). KA-treated C57BL/6J had a faster onset to clonic-tonic seizures compared to C57BL/6NJ (*, $p < 0.05$). Interestingly, while FVB/NJ mice seem to have the fastest onset to various seizure stages when induced with PTZ, they have the longest onset stages with KA. Similarly, C57BL/6NJ subjects are more susceptible to PTZ-induction compared to C57BL/6J but have longer onset to clonic-tonic seizures compared to C57BL/6J.

Discussion:

Genetically engineered models are used to describe genotype-phenotype relationships broadly across biomedical research. The majority of behavioral studies of genetic mouse models are performed using the C57BL/6 mouse [6, 21–23]. There are a number of C57BL/6 substrains that differ in key phenotypes ranging from immunologic profiles, microbiome, to performance on most standardized behavioral assays [6, 21–23]. The work presented herein illustrated that FVB/NJ, C57BL/6J, and C57BL/6NJ, background strains used in the preclinical genetic biomedical research field, exhibited markedly different

seizure and behavioral responses. These differences were observed following low-dose chemoconvulsant treatment that used a set number of administration days and post-treatment intervals following treatment with compounds that act via two distinct pharmacological mechanisms: a $GABA_A$ antagonist (pentylenetetrazole; PTZ) and an ionotropic glutamate receptor agonist (kainic acid; KA). These strain-dependent seizure response profiles resulted in observable deficits on several behavioral assays commonly used in preclinical evaluation of neurological disorders, when compared to vehicle treated controls. The results may be of interest to the broad neuropsychiatric community because, while seizure induction using convulsants is a standard tool used in animal models of epilepsy [28, 29, 63], there has been little work from a preclinical perspective, focused on neuropsychiatric disorders despite the fact that seizures are the most common medical comorbidity of many neurodevelopmental disorders, such as autism spectrum disorders (ASD) and syndromic forms of intellectual disability.

We observed that FVB/NJ and C57BL/6NJ mice exhibited more pronounced seizure responses throughout treatment by either PTZ or KA-mediating mechanisms. We hypothesized these findings based on standard kindling literature [26, 28]. Specifically, both PTZ and KA treated FVB/NJ and C57BL/6NJ spent less time in low/mild level seizure stages and more time in the moderate and high stages with the only distinction between the two treatments being that no Racine high level stage scores were observed with KA treatment in either FVB/NJ and C57BL/6NJ. This data lead us to conclude the two chemoconvulsants lowered susceptibility to behavioral seizure, as expected, with similar severity and within a similar window of time (10 days). 5 mg/kg/day of KA was mildly less potent than 30 mg/kg/day of PTZ. We also discovered the highly utilized C57BL/6J were resistant to convulsants by not achieving progressed seizures defined by generalized clonus, clonic-tonic seizure, nor tonic extension by either PTZ or KA-mediating treatment. This study adds to previous reports of protective or seizure resistant effects observed in the C57BL/6J strain [51, 52, 54, 55, 64]. Interestingly, this lack of seizure responsivity did not preclude the observation of behavioral alterations as a result of the chemoconvulsant treatments. C57BL/6J mice had increased total activity in the open field following PTZ and deficits in novel objection recognition following KA-treatment.

There is a rich history describing the positive correlation between seizure susceptibility following repeated stimulation using many inbred mouse strains [65]. Information from kindling in rodents models has informed on the effects of repeated seizures in the brain, the neural correlates that regulate seizure duration and spread, the complexities and subtypes of epilepsies and the heterogeneity regarding the genetics of seizure susceptibility [63, 66]. While others have observed the effects of genetic strain on susceptibility following various seizure inducing paradigms such as audiogenic threshold, electroconvulsive shock and a variety of glutamatergic, cholinergic and/or GABAergic chemoconvulsants agents including but not limited to PTZ, nicotine, caffeine, strychnine, physostigmine, thiosemicarbazide, pilocarpine, picrotoxin and KA [67–69], we found only four direct reports of the C57BL/6J and C57BL/6N substrains used herein and all four earlier reports used cholinergic agents [70–73]. These studies reported seizure susceptibility in some C57BL/6N mouse strains compared to C57BL/6J and observed increased motor activity and cognitive deficits supporting our findings, despite the different neurotransmitter systems in action. The

susceptibility and resistance of the N sub-strain of C57BL/6 was of interest given two key pieces of evidence: 1) the C57BL/6NJ was separated from C57BL/6J in 1951 and has different variants of several key genes including the retinal degeneration gene, a spontaneous mutation in the cytoplasmic FMR1 interacting protein 2 implicated in neuropsychiatric disorders (Cyfip2M1N), 34 Single Nucleotide Polymorphisms (SNPs) and variable phenotypes in open field activity, metabolism, cardiac and other biological systems [74], and 2) the influence of the C57BL/6N substrain background is critical for any new lines created via the trans-NIH initiative, knockout mouse phenotyping project (KOMP) [75].

Chemoconvulsant treatment in FVB/NJ and C57BL/6NJ resulted in significant behavioral deficits in the cognitive and social behavioral domains. In fact, the repeated low dose treatment in every strain induced learning and memory deficits in the novel object recognition task. PTZ-treated FVB/NJ and KA-treated C57BL/6NJ and C57BL/6J did not exhibit typical novel object preference. This finding was prominent and utilized strict manual and automated scoring [42], as well as standardized methods [48]. Our data suggest that when learning and memory deficits are only detectable by this singular assay and are attributed to single gene nulls, that the underlying seizure susceptibility may be contributing to the phenotype in B6 background strains. Emerging literature has considered this inbred strain confound [76, 77] but it is far from standard in discussion. Similarly, when interpreting data from FVB/NJ based genetic mouse models, it is possible that impaired novel object recognition and social interaction deficit phenotypes could be the result of elevated seizure propensity from the gene mutation or its interaction with FVB/NJ background strain [78, 79] and interpretations of single gene effects using one background strain may benefit from more cautious interpretation over causal statements impacting disease research. In fact, conclusions on seizure susceptibility in ASD models have been asserted by laboratories despite their using models of broadly differing genetic backgrounds [79, 80], without considering background influence. This led us to our reductionist study of assessing behavioral effects by strain alone following mild convulsant treatment.

Our data showed that PTZ-induced susceptibility alone produced social deficits in FVB/NJ and C57BL6/N as well as elevated grooming times in C57BL6/N. Our data also delineated heightened seizure susceptibility in both FVB/NJ and C57BL/6NJ mice compared to C57BL/6J and subsequent motor, cognitive, and sociability deficits. If this was a study looking at a genetically modified mouse model, behavioral deficits could be attributed to the genetic manipulation without proper consideration of the effect of the background strain genetics. Wildtype littermates can control for strain effects and allow for interpretation of results, but different background strains may highlight or preclude detection of disease-relevant phenotypes and should be considered upon interpretation of a novel preclinical model. This notion is highly supported by Sittig et al, (2016) that discovered that use of single strains in preclinical models is a big barrier to robust characterization of genotype-phenotype relationships, with clear analyses to prove that genetic background is a stronger modulator than sex and should be a considerable variant for interpretations and limits generalizability [3].

These data also have strong relevance to multiple clinical populations, as there is high co-morbidities between epileptic disorders and ASD [81]. As previously reviewed by Mazarti et

al., (2017) ~30% of patients with ASD develop epilepsy at some point of their lives, and at the same time ~30% of patients with epilepsy as a primary diagnosis fit the criteria for ASD diagnosis [82–85]. Seizures are the most common medical co-morbidity in ASD, including associations with various types of seizures, such as complex, partial, and primary generalized [86, 87]. The multifactorial nature of both ASD and epilepsy implies that an ASD-epilepsy connection should be addressed on an etiology-specific basis to develop effective therapeutic strategies with broad implications and stratify patient populations for improved success in clinical trial design [88–91].

Emerging literature demonstrates failures to reproduce and validate results in disease modeling across research teams [92]. While some lack of reproducibility is inevitable via procedural variables, given this report, we postulate that conclusions may also be confounded by the use of a single background strain for phenotypic analysis to identify and rescue or reverse certain traits. In 2016, the NIH mandated the inclusion of sex as a biological variable, yet attention to strain effects have not been as highly publicized. Given this knowledge, and the data presented herein, consideration should be employed when discussing interpretations of a genetic mouse model's behavior when performed using a single background strain.

The current study highlighted the differences of seizure susceptibility in three common genetic mouse background strains and the various behavioral alterations that resulted from repeated subthreshold chemoconvulsant treatment. We demonstrated a heightened acute behavioral seizure response in FVB/NJ and C57BL/6NJ compared to C57BL/6J, deficits in sociability and cognitive domains and hyperactivity specific to treatment and background strain, and increased susceptibility to a high-dose of PTZ in PTZ-treated FVB/NJ and C57BL/6NJ but resistance to a high-dose of KA in KA-treated C57BL/6J mice. This study was limited in that it did not look at varying doses and timepoints of convulsants to try to elicit a similar intensity of seizure response in C57BL/6J as seen in FVB/NJ and C57BL/6NJ mice. Differences observed in C57BL/6J may be representative of their lessened seizure response and future studies are needed to determine an optimal kindling paradigm for this strain. Another major limitation was the need to address sex differences. This is planned for future follow up studies but could not be included here given the number of variables for feasibility. Finally, in the future, it will be interesting to examine F1 hybrids of B6J and B6N as many novel conditional or inducible mouse models are made using N while the genetic driver line is on J (or vice versa).

In summary, a direct comparison of seizure susceptibility in typical background strains used when modeling neurodevelopmental disorders and their relevant behavioral characteristics has not been described. Our modified kindling paradigm and subsequent behavioral analyses not only demonstrate clear background strain differences in response to PTZ and KA, but distinct behavioral responses within and between strains. Our study, therefore, adds to a growing body of literature recognizing the value of strain effect on seizure response profiles and behavior and the importance of its interpretation when behaviorally phenotyping a genetic model of neurological disorder.

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- Mouse strains used in biomedical research exhibited markedly different seizure responses following chemoconvulsant administration.
- Susceptibility responses led to unique seizure-relevant profiles that illustrated resistance in C57BL6/J.
- Behavioral assays used in preclinical evaluation of neuropsychiatric disorders were altered by seizure induction.
- Strain is an important consideration for behavioral interpretations in animal models of epilepsy and comorbid disorders.

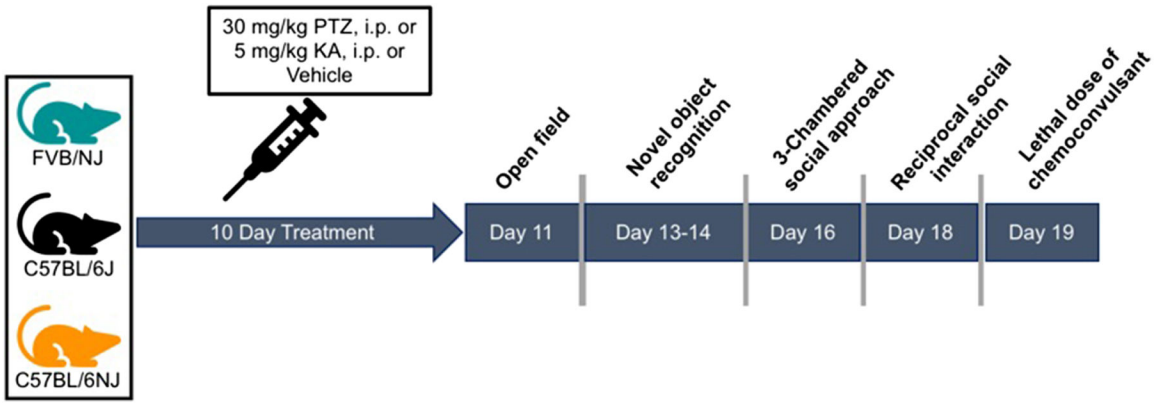

Figure 1: Schematic of study design.

Timeline for modified kindling paradigm and subsequent behavioral battery of motor, cognitive, and social domains.

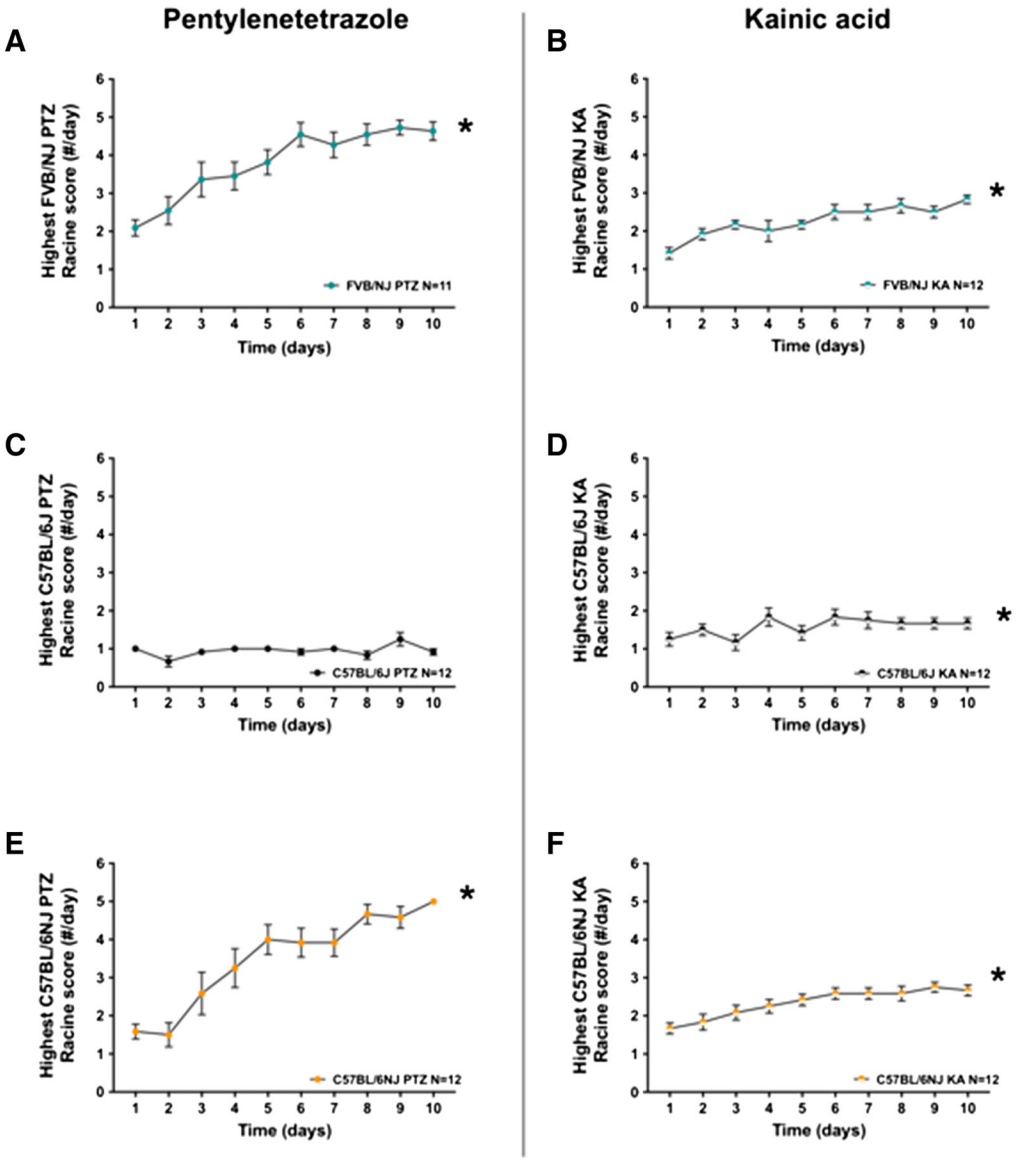

Figure 2: Inbred strains exhibit different Racine responses across a 10-day convulsant treatment time.

FVB/NJ and C57BL/6NJ subject mice (A, B and E, F) displayed increased seizure susceptibility to both PTZ and KA while C57BL/6J were only mildly affected by KA. Subjects received an intraperitoneal injection of PTZ, KA, or vehicle and highest Racine score achieved each day was recorded. The Racine score was evaluated for 10-d during a 20-min observation period. Racine scores of 0 indicated no response, 1 immobility, 2 generalized spasm and forelimb clonus, 3 Straub's tail, 4 loss of righting, 5 running seizure, 6 generalized clonic-tonic seizure, and 7 death. (A-B) PTZ and KA-treated FVB/NJ mice displayed a significant increase in seizure severity score over the 10-day injection period. In subjects that received PTZ, severe Racine score (5–6) were observed while those that were treated with KA did not display scores in the 5–6 level range. (C) PTZ-treated C57BL/6J mice had no significant change in seizure severity score over the 10-d treatment time. (D) KA-treated C57BL/6J mice displayed a significant increase in seizure severity score over the

10-day injection period. No 5–6 Racine score stages were observed in either PTZ or KA-treated group. (E-F) PTZ and KA-treated C57BL/6NJ mice displayed a significant increase in seizure severity score over the 10-day injection period. In subjects that received PTZ severe Racine score (5–6) were observed while those that were treated with KA did not display scores in the 5–6 level range. Both PTZ and KA-treated FVB/NJ and C57BL/6NJ mice spent more time in moderate and severe Racine seizure stages compared to C57BL/6J. *, $p < 0.05$, linear regression of highest Racine score over convulsant injection days within strain and treatment.

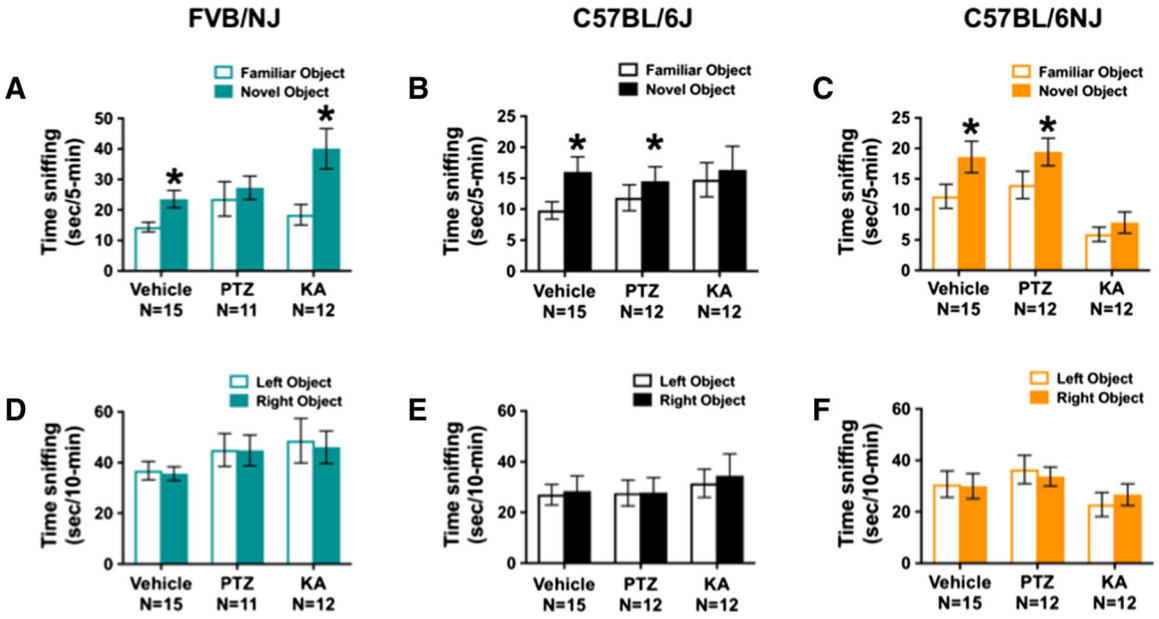

Figure 3: PTZ and KA treatment resulted in learning and memory impairments in the novel object recognition assay.

Recognition memory was assessed using a novel object recognition assay. Subjects were habituated to a novel arena, then were given a 10-min familiarization session with two identical objects. After familiarization, subjects were removed from the testing arena and, after a 1-h inter-trial interval, were placed back into the arena with one familiar object and one novel object. Time spent investigating both objects was recorded. (A) PTZ-treated FVB/NJ mice did not spend more time sniffing the novel object over the familiar object. Both vehicle and KA-treated subjects preferred novel object investigation compared to the familiar object. (B) KA-treated C57BL/6J mice did not spend more time sniffing the novel object over the familiar object. Both vehicle and PTZ-treated subjects preferred the novel object compared to the familiar object. (C) KA-treated C57BL/6NJ mice did not spend more time sniffing the novel object over the familiar object. Both vehicle and PTZ-treated subjects did prefer the novel object compared to the familiar object. These data illustrate both convulsant insults can cause disruption in object recognition learning and memory circuitry, however, the cognitive deficits are strain and drug dependent. (D-F) All vehicle, PTZ, and KA-treated subjects of FVB/NJ, C57BL/6J, and C57BL/6N strains showed no preference for either the left or right object during the familiarization phase indicating no innate side bias confounds in the novel object recognition trials. *, $p < 0.05$, repeated-measures ANOVA within strain and treatment using the familiar versus novel object for comparison.

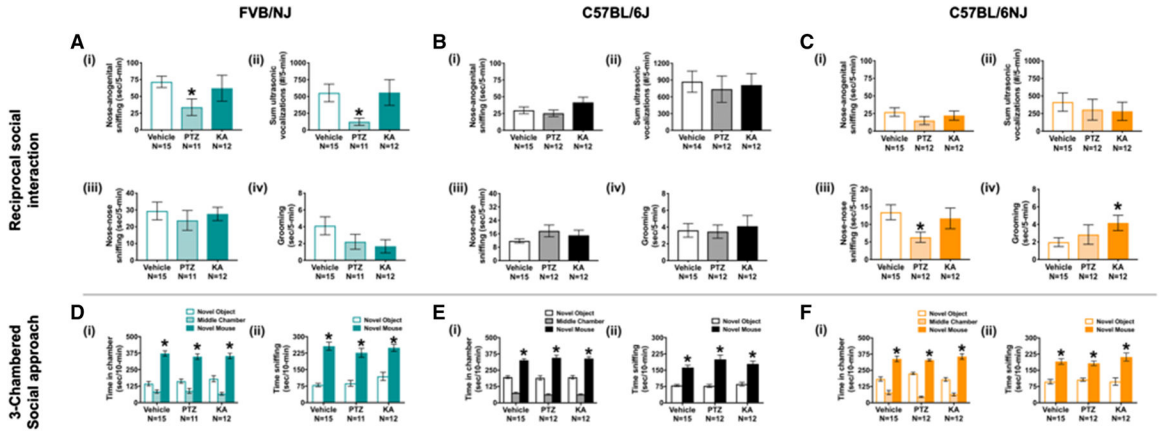

Figure 4: PTZ-treated FVB/NJ and C57BL/6NJ illustrated deficits on sociability parameters while KA-treated C57BL/6NJ showed elevated repetitive behavior during reciprocal social interaction.

Sociability was measured using a male-female reciprocal dyad social interaction assay where parameters such as nose-anogenital sniffing, ultrasonic vocalizations, nose-nose sniffing, and groom time were evaluated. Male subjects were placed in a novel arena with an age-matched, wildtype female in estrous and their interaction was observed for 5-min. (A) PTZ-treated FVB/NJ mice displayed reductions in (i) nose-anogenital sniffing (ii) and ultrasonic vocalizations, compared to vehicle-treated controls. (iii, iv) No differences were observed in nose-nose sniffing and grooming parameters. (i-iv) KA-treated FVB/NJ mice did not differ from the vehicle group on any measured parameter. (B) (i-iv) PTZ and KA-treated C57BL/6J groups did not significantly differ from the vehicle group on any parameter during the reciprocal social interaction assay. (C) (iii) PTZ-treated C57BL/6NJ male subjects exhibited reduced nose-nose sniffing, while KA-treated C57BL/6NJ exhibited increased repetitive behavior, seen as increased grooming. No other significant differences were observed in either PTZ or KA-treated C57BL/6NJ subjects (i-iv). Using the less sensitive but standard measure of 3-chambered social approach, all strains and all treatments groups met the criterion of sociability. (D) Time spent in the novel mouse chamber versus the object chamber (i), as well as, time spent sniffing the novel mouse versus object were statistically significant for both PTZ and KA-treated FVB/NJ. (E, F) C57BL/6J and C57BL/6NJ also spent more time in the chamber with the novel mouse (i) and more time sniffing the novel mouse (ii) compared to the object. No significant difference was identified between vehicle and PTZ, KA-treated FVB/NJ, C57BL/6J, and C57BL/6NJ on the number of transitions between chambers, confirming no locomotor confound (data not shown). *, $p < 0.05$, Reciprocal social interaction assay: one-way ANOVA comparing vehicle-treated group to either convulsant group by strain factor. 3-Chambered social approach: repeated-measures ANOVA within strain and treatment using the factor of chamber side (novel mouse side vs. novel object side).

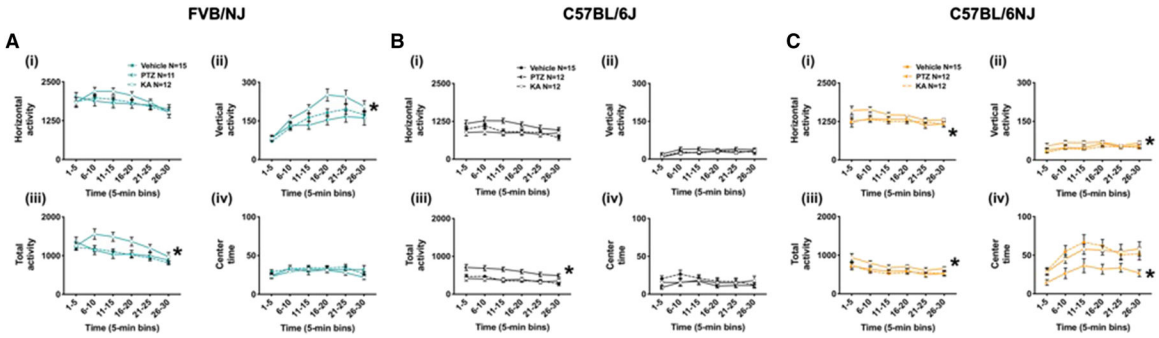

Figure 5: PTZ and KA treatment elevated motor activity in a novel open field arena on various parameters specific to strain and treatment.

Gross motor abilities were assessed using activity in a novel open field arena by total, horizontal, and vertical activity and time spent in the center across a 30-min session. Data is shown in 5-min bins. (A) Open field parameters for vehicle, PTZ, and KA-treated FVB/NJ mice. KA-treated FVB/NJ animals exhibited (iii) higher total activity and an elevated number of (ii) vertical movements, compared to the PTZ-treated and vehicle controls. No differences were observed by (i) horizontal activity nor (iv) time spent in the center of the arena. (B) Open field parameters for vehicle, PTZ, and KA-treated C57BL/6J mice. (iii) Robust increased total activity was observed in PTZ-treated C57BL/6J mice, but not KA-treated, compared to the vehicle group. (i-ii, iv) No differences detected on horizontal activity, vertical activity, or center time between the PTZ and KA-treated mice and the vehicle treated controls. (C) Open field parameters for vehicle, PTZ, and KA-treated C57BL/6N mice. (iv) Reduced center time was detected in PTZ-treated, but not KA-treated, C57BL/6N mice compared to the vehicle control. Increased activity measured by (i) horizontal, (ii) vertical, and (iii) total activity was detected in KA-treated C57BL/6NJ mice, compared to the PTZ-treated and vehicle controls. *, $p < 0.05$, repeated-measures ANOVA within strain and treatment using the factor of time across 5-min bins.

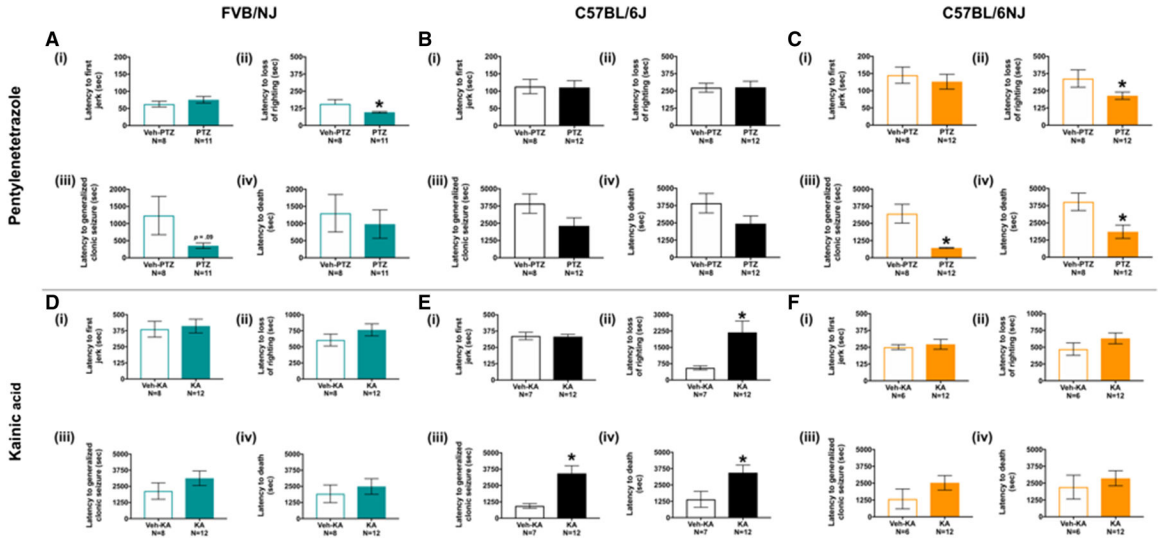

Figure 6: PTZ-treated FVB/NJ and C57BL/6NJ showed increased susceptibility to a high-dose of PTZ. In contrast, KA-treated C57BL/6J showed seizure resistance to a high-dose of KA.

Response to a high-dose of PTZ (80 mg/kg, i.p.) or KA (30 mg/kg, i.p.) was evaluated in previously treated PTZ (30 mg/kg, i.p.) and KA (5 mg/kg, i.p.) subjects as well as their naïve, vehicle control littermates. Subjects received a high-dose of PTZ or KA and then placed in an empty cage. Latencies to first jerk, loss of righting reflex, clonic-tonic seizure, and death were collected. (A) High-dose administration of PTZ in FVB/NJ mice. (ii-iii) Faster onsets to loss of righting and a trend ($p = 0.09$) towards faster onset to clonic-tonic seizures were observed in PTZ-treated mice compared to control littermates. (i, iv) No change in latency to onset of first jerk or death after a high-dose treatment of PTZ was observed in PTZ-treated FVB/NJ mice compared to control littermates. (B) High-dose administration of PTZ in C57BL/6J mice. (i-iv) No change in any behavioral seizure metrics following PTZ were observed in PTZ-treated subjects compared to control littermates. (C) High-dose administration of PTZ in C57BL/6NJ mice. (ii-iv) Faster onsets to loss of righting, clonic-tonic seizure, and death were observed in PTZ-treated mice compared to control littermates. (D) High-dose administration of KA in FVB/NJ subjects. (i-iv) No change in any behavioral seizure metrics following KA were observed in KA-treated subjects compared to control littermates. (E) High-dose administration of KA in C57BL/6J subject mice. (ii-iv) Increased latency to loss of righting, clonic-tonic seizure, and death were observed in KA-treated compared to control littermates. (F) High-dose administration of KA in C57BL/6NJ subjects. (i-iv) No change in any behavioral seizure metrics following KA were observed in KA-treated subjects compared to control littermates. *, $p < 0.05$, one-way ANOVA comparing vehicle-treated group to either convulsant group by strain factor.