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Heat induced temperature dysregulation and seizures in Dravet Syndrome/GEFS+ Gabrg2$^{+/Q390X}$ mice

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Abstract

It has been established that febrile seizures and its extended syndromes like generalized epilepsy with febrile seizures (FS) plus (GEFS+) and Dravet syndrome have been associated with mutations especially in *SCN1A* and *GABRG2* genes. In patients, the onset of FS is likely due to the combined effect of temperature and inflammation in genetically vulnerable individuals because fever is often associated with infection. Much effort has been spent to understand the mechanisms underlying fever induction of seizures. In addition to the role of cytokines in FS, previous studies in *Scn1a*$^{+/-}$ knockout mice, a model of Dravet syndrome, indicated that temperature elevation alone could result in seizure generation, and the effect of elevated temperature inducing seizures was age-dependent. Here, we report the thermal effect in a different mouse model of Dravet syndrome, the *Gabrg2*$^{+/Q390X}$ knockin mouse. We demonstrated age-dependent dysregulated temperature control and that temperature elevation produced myoclonic jerks, generalized tonic clonic seizures (GTCSs) and heightened anxietylike symptoms in *Gabrg2*$^{+/Q390X}$ mice. The study indicated that regardless of other inflammatory factors, brief heat alone increased brain excitability and induced multiple types of seizures in *Gabrg2*$^{+/Q390X}$ mice, suggesting that mutations like *GABRG2*(*Q390X*) may alter brain thermal regulation and precipitate seizures during temperature elevations.

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Disclosures

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this study is consistent with those guidelines.

Keywords

temperature; electroencephalography; febrile seizures; myoclonic jerks; generalized tonic clonic seizures; *GABRG2(Q390X*) mutation

Introduction

Fever is the most common seizure trigger for children between five months and six years of age [1], but the underlying mechanisms by which fevers precipitate seizures are not clear. In humans fever often occurs with infection that could cause release of inflammatory cytokines such as interleukin-1β, tumor necrosis factor (TNF)-α and other cellular mediators. These cytokines could increase brain excitability as seizures are commonly observed in meningitis or encephalitis [2]. However, external heat such as a hot bath has also been reported to induce seizures, especially in Dravet syndrome, which is the most severe epileptic encephalopathy with intractable seizures, cognitive impairment and sometimes unexpected death. This suggests that heat alone without infection also increases brain excitability and can lead to seizures in patients with certain genetic predispositions. In previous studies, the peak of the core body temperature has been proposed to be a determining factor for seizure onset during fever [4]. In patients, the condition of febrile seizures (FS) is likely due to a combination of temperature and inflammation. In experimental animal models of FS, nearly all animals have a seizure if heated sufficiently, suggesting that the duration of heating correlates with brain excitability [3].

Recent studies indicate that certain genetic factors increase susceptibility to FS. Among all the epilepsy genes, mutations in *SCN1A* and *GABRG2* have been strongly associated with FS and extended epilepsy syndromes like generalized epilepsy with febrile plus (GEFS+) and Dravet syndrome [5–7]. In sodium channels, computer simulation has shown that changes in inhibitory ion channels are unable to offset the increased excitability in the brain during a temperature increase [8]. Thus, the overall net effect of increased temperature would tilt the brain to a more excitable state, thus making it more prone to seizures. In $GABA_A$ receptor channels, it has been demonstrated that mutant γ2 subunit trafficking was impaired during increased temperature *in vitro* [9]. The γ2 subunit is essential for $GABA_A$ receptor clustering at synapses [10]. It has been demonstrated that the cytokine, TNF-α, caused endocytosis of $GABA_A$ receptors through phosphatase-1, thus resulting in decreased inhibitory strength [11]. The effect of temperature has also been studied in mouse models carrying mutations in sodium channel *Scn1a* and *Gabrg2* genes. A study of $Scn1a^{+/-}$ mice suggested that age plays a role in temperature-induced seizure susceptibility, and adult $Scn1a^{+/-}$ mice were more prone to thermogenic seizures than younger mice [12]. In $Gabrg2^{+/R82Q}$ (also named $Gabrg2^{+/R43Q}$) mice, the *GABRG2*(*R82Q*) mutation increased the susceptibility to thermogenic seizures, but FS could not be induced in the $Gabrg2^{+/-}$ knockout mice[13], which represents a condition of simple *Gabrg2* haploinsufficiency.

In the current study, we characterized the thermal effect in our recently constructed knockin mouse $Gabrg2^{+/Q390X}$, which is associated with Dravet syndrome [14]. The *GABRG2*(*R82Q*) mutation is associated with FS and childhood absence epilepsy [15], while the

GABRG2(*Q390X*) mutation [16] is associated with a more severe epilepsy, Dravet syndrome. It is unknown if temperature elevation has a similar effect on epilepsy syndromes with different severities. Additionally, it is unknown if brief temperature elevation has any effect on epilepsy although it has been reported that prolonged heating could cause seizures in nearly all the experimental animals [17]. We hypothesize that the increased age and core body temperature will increase thermogenic seizures and have tested the effect of brief temperature rise in the *Gabrg2*$^{+/Q390X}$ mice. Because pentylenetetrazol (PTZ) is a known $GABA_A$ receptor antagonist that induces seizures by blocking GABAergic neurotransmission, we thus administered PTZ as a control for seizure severity. We compared the severity of seizures induced by temperature elevation with the severity of seizures induced by PTZ in age- and strain-matched mice.

Methods

Mice

The *Gabrg2*$^{+/Q390X}$ knockin mouse model has been characterized in our previous study [14]. All mice were bred for at least 8–10 generations into a C57BL/6J or DBA/2J background, which is a seizure-resistant or seizure-prone mouse background, respectively [18]. All procedures were performed in accordance with policies and guidelines set forth by the Vanderbilt University Institutional Animal Care and Use Committee.

ELISA

A standard sandwich ELISA was performed to measure tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) levels in mouse plasma. The ELISA kits were purchased from ThermoFisher and all the cytokine standards were included in the kits. The blood was drawn from mouse tail and plasma was separated. Fifty μl of undiluted plasma was used for reaction. The optical density (OD) of each well was read at 450 nm with an absorbance-based microplate reader. The final concentration was calculated by converting the OD readings against a standard curve.

Electroencephalography (EEG) electrode implant surgery, recording, and analysis

The EEG electrode implant surgery and synchronized video-EEG monitoring have been described before [19;20]. Briefly, synchronized video-EEGs were recorded from mice one week after electrode implantation and recorded with a synchronized video-EEG monitoring system from Pinnacle Technology. Mice were anesthetized with 1–3% isoflurane and four epidural electrodes (stainless steel screws affixed to one head mount) were placed on the brain surface and secured in place with dental cement and surgical stiches. EMG leads were inserted into the trapezius muscle. Video-EEG monitoring lasted for 24–48 hrs. Mice were freely moving during EEG recordings, but the headmount was connected to a cable box. At least 24 hours of baseline video-EEG recordings were obtained and analyzed for each mouse.

Seizure induction by temperature elevation

The heating procedure was adapted from a previous study [12]. Mice were placed in a Plexiglas cylinder with an infrared heat lamp (250 watt, HL-1, Physitemp Instruments Inc.)

kept in a fixed position above. A rectal temperature probe (RET-4, Physitemp Instruments Inc.) was carefully inserted and taped to the tail of the mouse. The temperature probe was connected to a temperature controller (TCAT-2DF, Physitemp Instruments Inc.) to monitor the core body temperature of the animal. Each mouse was allotted a minimum of 10 min for habituation in the Plexiglas cylinder without the heat lamp on. The mouse was then recorded for 30 min to 2 hrs under baseline activity. After baseline recordings, the heat lamp was turned on and the synchronized video-EEG/EMG recording began. The core body temperatures were then elevated approximately 0.5°C every 2 min until 42.5°C was achieved. The rate of temperature rise in the mice was adjusted by the height of the heating lamp. The heating lamp would stop once the mouse core temperature reached 42.5 °C. If the mouse had a GTCS, the heating process would be stopped immediately.

Pentylenetetrazol treatment

Adult mice were injected with a single dose of PTZ (Sigma-Aldrich, St. Louis, MO) 50 mg/kg i.p. to induce seizures and monitored during the first 30 min after administration [20]. PTZ has been used for inducing absence seizures at low doses [21] and GTCSs at high doses [22] in rodents because of its $GABA_A$ receptor-antagonizing effect.

Statistical analysis

Data were analyzed with GraphPad Prism 5 or the statistical package for the social sciences (SPSS) 23.0 software. Independent-samples t tests or a chi-square (χ^2) test were used for the comparisons between genotypes. All analyses used an alpha level of 0.05 to determine statistical significance. Data were presented as Mean ± SEM.

Results

Gabrg2$^{+/Q390X}$ mice had spontaneous GTCSs and myoclonic seizures during baseline activity

The *Gabrg2*$^{+/Q390X}$ mice had spontaneous GTCSs in the most seizure-resistant C57BL/6J background (Supplementary video 1). We first characterized the baseline EEGs of *Gabrg2*$^{+/Q390X}$ knockin mice. We observed spontaneous GTCSs starting from postnatal 16 days in the knockin mice during our routine handling. We could not do EEG recordings on the knockin mice under one month of age because the headmount could not be properly attached to the smaller skulls of the mice. We thus only recorded from mice over 2 months old. We observed sporadic, multiple forms of ictal discharges associated with behavioral seizures. The abnormal EEG activities included 4–7 Hz spike-wave-discharges (SWDs) associated with brief behavioral arrest or without any behavioral changes (Figure 1A and B), prolonged epileptiform discharges associated with GTCSs (Figure 1C), and high amplitude spikes associated with myoclonic jerks (Figure 1D). We also observed behavioral seizures without associated EEG abnormalities of irregular, less rhythmic SWDs. However, the convulsive seizures were rare during baseline activity. The average occurrence was 1.67 ± 0.27 for of GTCSs and 5.4 ± 1.45 for myoclonic jerks over a 24-hr period while no seizures were detected in their wild-type littermates (Figure 1E).

We then measured the levels of cytokines in peripheral blood to determine if there was inflammation involved in seizure generation in the *Gabrg2*$^{+/Q390X}$ mice. We measured TNF-α (Figure 1F) and Il-1β (Figure 1G) prior to and after heat exposure at 42.5°C for 30 min. The baseline level of both cytokines were very low and there was no difference of either cytokines between wild-type and *Gabrg2*$^{+/Q390X}$ mice before or after heat exposure (TNF-α: 1.89 ± 0.21 for wt vs 1.8 ± 0.14 for het before heat; 1.92 ± 0.14 for wt vs 1.72 ± 0.23 for het after heat; IL-1β: 35.13 ± 2.4 for wt vs 35.08 ± 3.6 for het before heat; 35.28 ± 3.6 for wt vs 35.22 ± 4.2 for het after heat). This thus suggested that there was no systematic inflammation involved in seizure generation in *Gabrg2*$^{+/Q390X}$ mice.

A brief, rapid temperature rise induced myoclonic jerks and SWDs in Gabrg2$^{+/Q390X}$ mice that were less frequent than seizures induced by PTZ injection

Because sustained high temperature exposure may cause cellular injury, and even cell death, we determined the effects of short temperature elevations on *Gabrg2*$^{+/Q390X}$ mice using a temperature-controlled heating lamp. The temperature induction protocol was modified based on a previously described study [12] using *Scn1a*$^{+/-}$ knockout mice (Figure 2A). Heating stopped once the mouse's core temperature reached 42.5 °C. We first recorded for 2 hrs for baseline EEGs before temperature elevation and 30 minutes after heating for recovery (Figure 2B upper panel). Because PTZ could induce seizures by blocking GABAergic neurotransmission, we administered PTZ as a control for seizure severity (Figure 2B, lower panel). Temperature elevation evoked myoclonic jerks and many SWDs, which tended to be irregular or less rhythmic but were associated with behavioral seizures (Figure 2C). This was thus quantified. With administration of PTZ (50 mg/kg), there were also many myoclonic jerks, which occurred alone or proceeding GTCSs. There were also more rhythmic SWDs in PTZ-induced seizures than heat-induced seizures (Figure 2D). We compared the severity of seizures induced by high temperature and PTZ administration because PTZ is a known $GABA_A$ receptor antagonist and our preliminary data indicated that administration of a moderate dose of PTZ (50 mg/kg) could induce GTCS with high mortality in *Gabrg2*$^{+/Q390X}$ mice. The comparison potentially provides insights into the mechanisms underlying temperature increases and compromised GABAergic inhibition.

Gabrg2$^{+/Q390X}$ mice had a few myoclonic jerks (1.18 ± 0.26) while the wild-type mice had no myoclonic jerks during the baseline period. The number of myoclonic jerks was greater for *Gabrg2*$^{+/Q390X}$ mice following temperature elevation (4.93 ± 0.73) than for wild-type mice, which had almost no myoclonic jerks (0.18 ± 0.12, $p < 0.0001$) following temperature elevation, and *Gabrg2*$^{+/Q390X}$ mice during a baseline period (1.18 ± 0.26, $p = 0.0003$; Figure 2E). Similarly, the number of myoclonic jerks was greater for *Gabrg2*$^{+/Q390X}$ mice following PTZ treatment (19.91 ± 2.60) than for wild-type mice (0.27 ± 0.14, $p<0.0001$) and a *Gabrg2*$^{+/Q390X}$ baseline period (1.18 ± 0.26, $p < 0.0001$; Figure 2E).

There was also a greater incidence of SWDs for *Gabrg2*$^{+/Q390X}$ mice following seizure induction by temperature elevation (15.71 ± 2.46) compared to the wild-type mice (0.67 ± 0.33, $p < 0.0001$) and a *Gabrg2*$^{+/Q390X}$ baseline period (4.83 ± 0.42, $p < 0.002$). Moreover, there was a greater occurrence of SWDs for *Gabrg2*$^{+/Q390X}$ mice following PTZ treatment (17.88 ± 2.81) compared to the wild-type mice (0.71 ± 0.36, $p < 0.0001$) and the

Gabrg2$^{+/Q390X}$ baseline performance (4.83 ± 0.42 p=0.0019). However, there was not a significant difference in SWDs between *Gabrg2*$^{+/Q390X}$ mice that were either exposed to temperature elevation (15.71 ± 2.46) or PTZ (17.88 ± 2.81, p = 0.579; Figure 2F).

Seizures induced by brief high temperature were less severe than seizures induced by PTZ in Gabrg2$^{+/Q390X}$ mice

The seizures were scored based on the modified Racine Scale (Figure 3A) [23]. The latency to reach stage 2/3 of the modified Racine scale (i.e., head nodding or oro-facial seizure) during seizure induction by heat was significantly reduced for *Gabrg2*$^{+/Q390X}$ mice in both C57BL/6J (26.0 ± 5.36 sec for *Gabrg2*$^{+/Q390X}$ vs 125.5 ± 27.70 sec for wild-type, p = 0.028) and DBA/2J (28.1± 6.1 sec for *Gabrg2*$^{+/Q390X}$ vs 135± 31 sec for wild-type, p <0.0001) (Figure 3B–C). The latency to reach stage 2/3 was also significantly reduced following PTZ administration for *Gabrg2*$^{+/Q390X}$ mice in both C57BL/6J (39.33 ± 2.65 sec for *Gabrg2*$^{+/Q390X}$ vs 218.2 ± 53.71 sec for wild-type, p = 0.008; Figure 3B–C) and DBA/2J (21.1 ± 4.0 sec for *Gabrg2*$^{+/Q390X}$ vs 138 ± 16 sec for wild-type, p = 0.003) backgrounds. All of the *Gabrg2*$^{+/Q390X}$, but none of the wild-type, mice reached stage 3 (data not shown), but only 40% of *Gabrg2*$^{+/Q390X}$ mice in C57BL/6J and 16% in DBA/2J reached stage 5 with temperature induction (Figure 3D–E). However, all of *Gabrg2*$^{+/Q390X}$ mice in C57Bl/6J and 75% in DBA/2J backgrounds and none of the wild-type mice in either background reached stage 5 with PTZ injection (Figure 3D–E). Three out of 32 *Gabrg2*$^{+/Q390X}$ mice died after seizure induction by temperature elevation, 22 out of 35 *Gabrg2*$^{+/Q390X}$ mice died after PTZ induction (Figure 3F), but none of wild-type mice died after seizure induction by either method. The increased mortality of *Gabrg2*$^{+/Q390X}$ mice during seizure inductions was consistent with our previous report that the *Gabrg2*$^{+/Q390X}$ mice had spontaneous death with evidence favoring cardiorespiratory failure, which is a putative cause of sudden unexpected death in epilepsy (SUDEP)[14;24]. However, to our surprise, none of *Gabrg2*$^{+/Q390X}$ mice in DBA/2J background died during seizure induction by temperature elevation and only 1 out 10 mice died during PTZ seizure induction (Figure 3G), suggesting that some unknown mechanisms modify the phenotype of *Gabrg2*$^{+/Q390X}$ mice in the two different genetic backgrounds.

Adult Gabrg2$^{+/Q390X}$ mice had altered temperature regulation

We noticed that the core body temperature in adult *Gabrg2*$^{+/Q390X}$ mice rose at a faster rate than wild-type mice during temperature induction under the same heating conditions. We thus compared the rate of temperature elevation using the same heating conditions. We measured the percentage of mice that reached 42.5°C against the heating time (Figure 4A). The rate of body temperature rise was measured by how many minutes it took for a mouse to reach a core body temperature of 42.5°C. We measured core body temperature every 2 min because we used a previous study{Oakley, 2009 235 /id} as reference. In the study, the rate of temperature increase for wild-type mice was set at 0.5 °C per 2 min. For adolescent mice, there was no difference in the rate of body temperature rise for *Gabrg2*$^{+/Q390X}$ mice (0.46 ± 0.03 °C) and wild-type mice (0.50 ± 0.02 °C, p = 0.290) per 2 minutes, but the heating process raised the heterozygous mouse body temperature at a significantly faster rate (0.78 ± 0.05 °C) than their wild-type littermates (0.43 ± 0.03 °C; p < 0.001; Figure 4B) for adult mice. Regardless of age, all mice had a similar baseline temperature of approximately

36.9°C. However, all adult *Gabrg2*$^{+/Q390X}$ mice reached 42.5°C within 24 min while only 80% wild-type adult mice, 78% wild-type adolescent mice and 64 % of *Gabrg2*$^{+/Q390X}$ adolescent mice reached 42.5°C by 24 min (Figure 4B). It is unknown why temperature regulation was altered for *Gabrg2*$^{+/Q390X}$ mice. It is possible that the specific cells of the hypothalamus involved in temperature regulation may express γ2 subunits and were dysfunctional in the mutant mice, but this needs further study.

Hyperthermia induced more myoclonic jerks in younger Gabrg2$^{+/Q390X}$ mice, but GTCSs were more frequent in older Gabrg2$^{+/Q390X}$ mice

Previous studies of *Scn1a*$^{+/-}$ mice indicated that older mice (2 months or older) were more susceptible to temperature-induced seizures than younger mice (1 month or younger)[12]. We thus compared seizure activity in P18–20 day old (adolescent) and 2–4 month old (adult) mice. For adolescent mice, there was a significant increase in the number of myoclonic jerks for heterozygous *Gabrg2*$^{+/Q390X}$ mice (5.91 ± 0.96) compared with their wild-type littermates (1.46 ± 1.06, p = 0.040) during heating. For adult mice, there was also a significant increase in the number of myoclonic jerks for the *Gabrg2*$^{+/Q390X}$ mice (2.10 ± 0.89) compared to their wild-type littermates (0.10 ± 0.10, p = 0.038; Figure 4C). However, the myoclonic jerks were more frequent in the adolescent mice than in the adult mice, regardless of genotype, suggesting a possible role of age in the onset of myoclonic jerks. It has been noticed in epilepsy mouse models carrying *Gabra1* mutations that myoclonic seizures are more prominent in older mice [25]. Regardless, there were more myoclonic jerks in the *Gabrg2*$^{+/Q390X}$ mice in both adolescent and adult groups. This indicates the mutation increased myoclonic jerks regardless of age. For heat induced GTCSs in adult mice, there was a significant increase for the *Gabrg2*$^{+/Q390X}$ mice (0.40 ± 0.16) compared to their wild-type littermates (0.00 ± 0.00, p = 0.025). Four out of 10 adult *Gabrg2*$^{+/Q390X}$ *mice* had a GTCS (χ^2 = 5.00, p = 0.025; Supplementary video 2), while only 1 out of 11 adolescent *Gabrg2*$^{+/Q390X}$ mice had a GTCS (χ^2 = 1.33, p = 0.250; Figure 4D).

Adult, but not juvenile, Gabrg2$^{+/Q390X}$ mice had heightened anxiety-like symptoms

Severe epilepsies like Dravet syndrome or temporal lobe epilepsy often have high neuropsychiatric comorbidities-including anxiety, hyperactivity, impaired social ability and cognition[26]. We noticed frequent jumping bouts for all mice during the heating process. Based on a previous study, escape strategies, such as jumping, are closely linked with the psychopathological conditions related to anxiety [27]. We thus measured jumping bouts as a behavioral anxiety marker in addition to seizure-related activity. In the evaluation of jumping bouts (i.e., when the mouse was leaping in a vertical direction with all four paws off the ground; Supplementary video 3), adolescent *Gabrg2*$^{+/Q390X}$ mice (57.45 ± 5.87 bouts) did not jump at a greater rate in comparison to their wild-type littermates (56.43 ± 6.36 bouts, p = 0.909). However, adult *Gabrg2*$^{+/Q390X}$ mice (99.40 ± 8.75 bouts) jumped at a greater rate than wild-type mice (63.80 ± 5.30 bouts, p = 0.003; Figure 4E). Regardless of genotype, all mice began jumping consistently at a core body temperature of 39 ± 1°C (data not shown). It is important to note, however, that we compared the behavioral characteristics of jumping bouts with GTCSs (Supplementary video 2 for GTCSs and video 3 for jumping bouts). Based on our observations, there was no clear evidence for convulsive seizures or behavioral arrest associated with the jumping bouts.

Discussion

Some seizures in epilepsy patients may be precipitated occasionally by either internal or external stimuli. A rapidly rising and high fever is one of the most measurable parameters among these precipitants. Therefore, the effect of temperature elevation has been investigated in both wild-type and genetically modified mouse epilepsy models [12;13;28]. In humans, prolonged and complex FS promote later development of temporal lobe epilepsy associated with hippocampal sclerosis, suggesting prolonged complex FS could cause cellular injury. However, the effect of brief exposure to elevated temperature has never been addressed.

We determined the types of seizures evoked by brief heating in wild-type and in mutant *Gabrg2*$^{+/Q390X}$ knockin mice associated with GEFS+/Dravet syndrome [14;16]. Our data suggest that the *GABRG2*(*Q390X*) mutation increases susceptibility to thermogenic seizures, and that a brief temperature elevation alone could induce multiple forms of seizures in *Gabrg2*$^{+/Q390X}$ mice. Our findings do not necessarily come as a surprise given that multiple mutations in *GABRG2* in humans have been linked to FS including R136X, IVS6 + 2T→G [29], K328M [30], Q390X [16], R177G [31], and R82Q [15;32] mutations. Furthermore, the γ2 subunit has been suggested to have temperature-dependent effects due to its importance for receptor trafficking [33], clustering [34], and synaptic maintenance [35]. An alteration of temperature could change protein folding and trafficking due to their established temperature-dependent characteristics [9;36].

Our data indicate that the susceptibility to thermogenic seizures is age-dependent as 2–4 month old *Gabrg2*$^{+/Q390X}$ mice had a more severe seizure phenotype than 18–20 day old mice. A genetic mutation like *GABRG2*(*Q390X*) would increase brain excitability and the risk for developing seizures. This is consistent with the previous findings in *Scn1a*$^{+/-}$ mice[12]. In *Gabrg2*$^{+/Q390X}$ *mice*, hyperthermia induced more GTCSs in adult mice, but more myoclonic jerks in adolescent mice. Our results support a previous study in *Gabrg2*$^{+/R82Q}$ mice that mutant $GABA_A$ receptor γ2(R82Q) subunits increased susceptibility to FS [13]. However, the age-dependent seizure phenotypes induced by high temperatures have never been reported. This is the first report that high temperature evokes more myoclonic jerks in adolescent mice, but more GTCSs in adult mice, although both seizure types are seen at either age.

It is intriguing that adult *Gabrg2*$^{+/Q390X}$ mice had an accelerated temperature increase despite the same heating parameters for all mice. This may be related to age-dependent temperature sensitivity, but needs further elucidation. Because the *GABRG2* is ubiquitously expressed in the brain including the brainstem, it is possible that the neurons in the brainstem nuclei responsible for temperature control are dysfunctional. It is also unknown why the temperature regulation was only altered for adult *Gabrg2*$^{+/Q390X}$ mice. We have previous demonstrated that the mutant γ2(Q390X) subunits accumulate intracellularly over time [14]. It is possible that the specific cells in the hypothalamus involving temperature regulation which express *GABRG2* gene are only dysfunctional in the older mutant mice, and relatively normal in the young mice, but this needs further elucidation. Nevertheless, elevating the core body temperature for *Gabrg2*$^{+/Q390X}$ mice revealed the emergence of ictal

discharges (unrelated to jumping) leading to a significant increase in myoclonic jerks and GTCSs, as well as evident progressions through the different stages of seizures based on the modified Racine scale [23]. This suggests that a brief elevated temperature alone can increase seizure activity in a susceptible host regardless of cellular inflammation.

Seizures induced by high temperature exposure were less severe than seizures produced by PTZ. There were more myoclonic jerks and GTCSs in PTZ-treated *Gabrg2*$^{+/Q390X}$ mice than the mice exposed to temperature induction. In comparison of the two stimuli (i.e., hyperthermia and PTZ) used to trigger seizures, both were effective in producing SWDs characteristic of epilepsy, but the effect was more robust with PTZ administration, suggesting that there may be some similarities in the mechanistic functions of the stimuli to increase brain excitability.

We first report here that an elevated core body temperature increased the level of anxietylike behavior in *Gabrg2*$^{+/Q390X}$ mice. It is clear that γ2 subunits are ubiquitously expressed in brain regions associated with anxiety including forebrain, hippocampus and amygdala [37]. A disruption in the GABAergic system, particularly genetic mutations, has been known to trigger anxiety [13,37]. In addition, anxiety has been viewed as a sign of impaired GABAergic neurotransmission [38;39]. Aside from genetic predispositions or mutations, antagonism of GABAergic function resulted in heightened anxiety, whereas enhancing the same system, via benzodiazepines, greatly diminished anxiety [40]. *Gabrg2*$^{+/-}$ knockout mice displayed increased anxiety[37]. The increased rate of jumping noted for *Gabrg2*$^{+/Q390X}$ mice could be viewed as an indirect measure of anxiety given that the greater efforts to escape (via jumping) an aversive stimulus has been suggested as a form of intense, panic-like anxiety [27]. The anxiety phenotype expressed in *Gabrg2*$^{+/Q390X}$ mice supports the notion that a $GABA_A$ receptor deficiency is a predisposition for anxiety disorders at the clinical level.

Overall, our findings indicated that thermogenic seizures occur in *Gabrg2*$^{+/Q390X}$ mice and were more severe compared to wild-type mice. Hyperthermia alone was more likely to induce myoclonic jerks in adolescent *Gabrg2*$^{+/Q390X}$ mice and GTCSs in adult *Gabrg2*$^{+/Q390X}$ mice. Our data also revealed a more rapid increase in body temperature and signs of anxiety during temperature elevation in *Gabrg2*$^{+/Q390X}$ mice. Our results demonstrated that the age of the mice played a key role with the aforementioned phenotypes showing an increased severity for the older mice. The thermogenic seizures noted for the *Gabrg2*$^{+/Q390X}$ mice did display similarities (albeit to a much lesser degree) to that of using PTZ to induce seizures, which could suggest a similar mechanistic pathway or pathways that at least converge for the resulting seizures. Based results from our *Gabrg2*$^{+/Q390X}$ mice, we suggests that, regardless of any infection related inflammatory mediators, a brief high temperature alone could induce multiple types of seizures and related comorbidities in a subset of the population with a lowered seizure threshold by a mutation like *GABRG2*(*Q390X*).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- A brief temperature rise evoked multiple types of seizures in *Gabrg2*$^{+/Q390X}$ mice.
- A brief temperature rise evoked more myoclonic jerks in adolescent *Gabrg2*$^{+/Q390X}$ mice and more GTCSs in adult *Gabrg2*$^{+/Q390X}$ *mice*.
- *Gabrg2*$^{+/Q390X}$ mice had altered thermoregulation as evidenced by more rapidly increased core temperature in adult than in adolescent *Gabrg2*$^{+/Q390X}$ mice.
- *Gabrg2*$^{+/Q390X}$ mice had heightened anxiety with temperature elevation.
- Seizure severity following temperature elevation was less than seizure severity following PTZ administration.

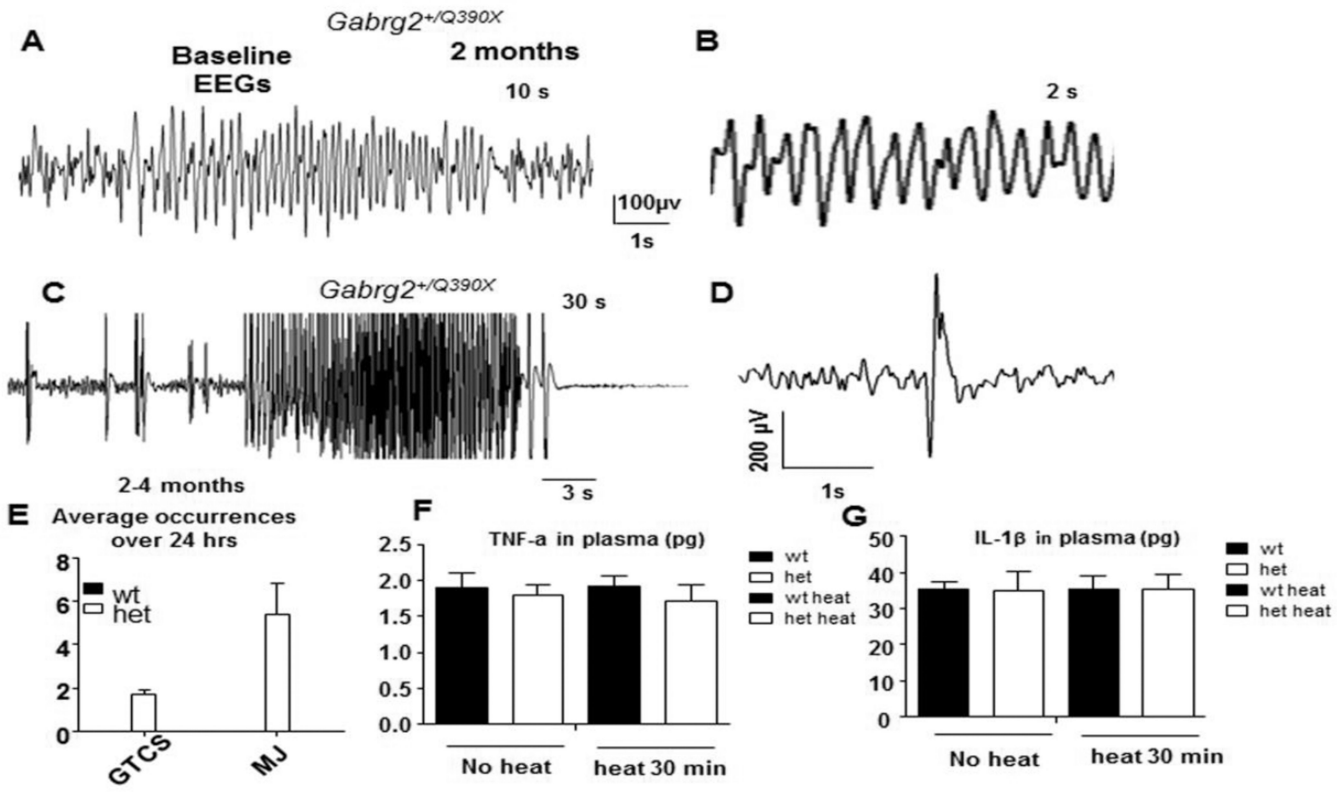

Figure 1. Interictal and ictal EEG s in Gabrg2$^{+/Q390X}$ knockin mice

(A) Representative EEG recordings show that a 2 month old *Gabrg2*$^{+/Q390X}$ knockin mouse had spike-wave-discharges (SWDs). **(B)** The frequency of SWDs in *Gabrg2*$^{+/Q390X}$ mice was 4–7 Hz. **(C)** Spontaneous generalized tonic clonic seizures (GTCSs) were associated with epileptiform activity on EEG **(D)**. Spontaneous myoclonic jerks were associated with spike discharges on EEG. **(E)** Average occurrences of GTCSs and myoclonic jerks (MJ) with noticeable behavioral seizures over 24 hours EEG recordings were measured in 2–4 month old mice (n = 31 for each genotype). (**F,G**) The pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α) (**F**) or interleukin-1beta (IL-1β) (**G**) in 50 µl plasma from peripheral blood of mice untreated or treated at 42.5^{C} for 30 min were measured with Elisa.

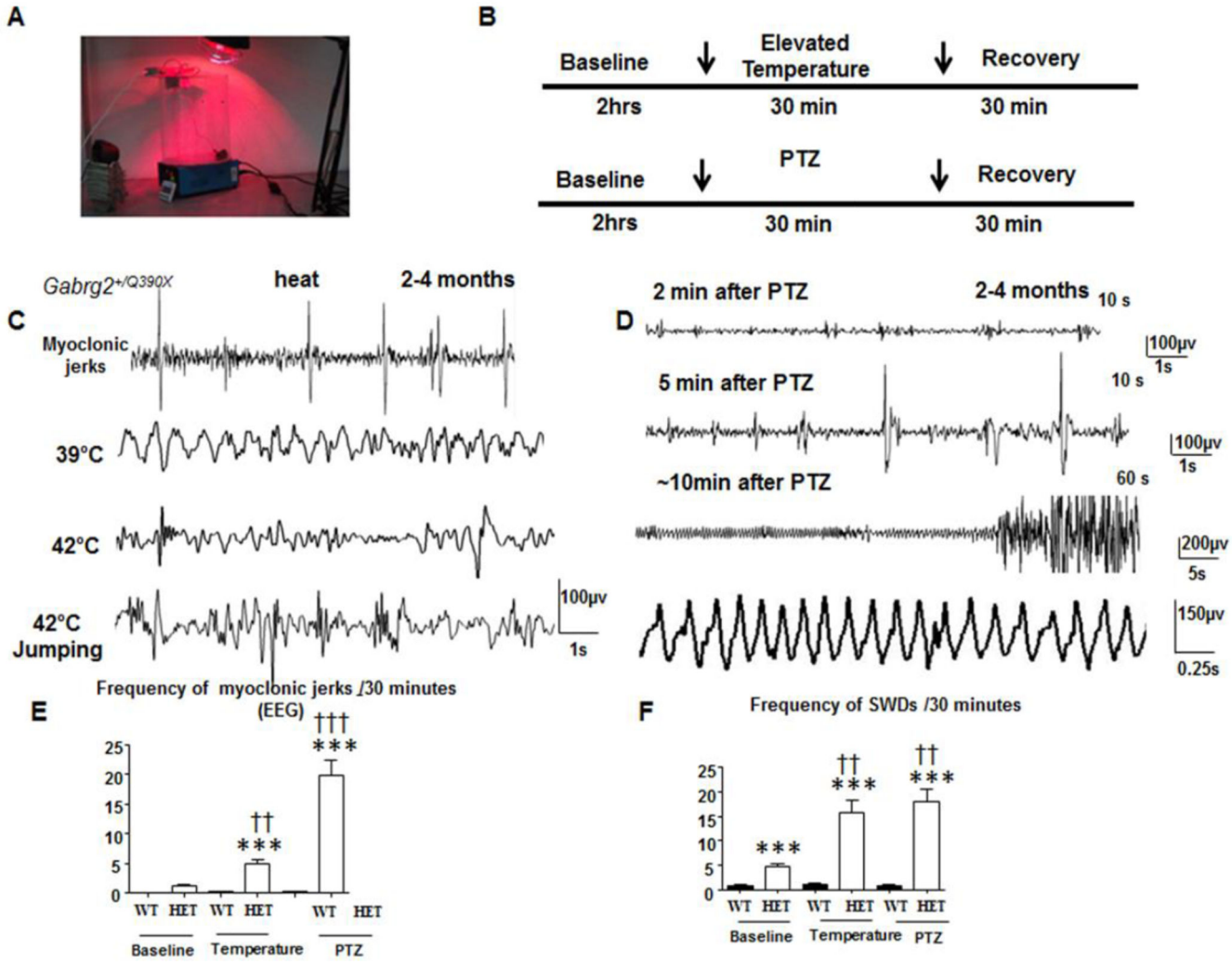

Figure 2. Temperature elevation and PTZ induced seizures and abnormal EEGs in Gabrg2$^{+/}$Q^{390X} knockin mice

(**A)** The temperature induction apparatus and heating setup are shown. **(B)** A diagram of the temperature induction and pentylenetetrazol (PTZ) seizure induction procedures are shown. **(C)** Sample EEGs traces are shown for 2–4 month old *Gabrg2*$^{+/(Q390X)}$ knockin mice during temperature elevation. **(D)** Sample EEGs traces are shown for 2–4 month old *Gabrg2*$^{+/(Q390X)}$ knockin mice during PTZ seizure induction. **(E)** Number of myoclonic jerks for 2–4 month old wild-type (wt) and *Gabrg2*$^{+/Q390X}$ knockin (het) mice are plotted for baseline and for temperature elevation and PTZ seizure induction segments. **(F)** Frequency of SWDs for 2–4 month old wild-type (wt) and *Gabrg2*$^{+/Q390X}$ knockin (het) mice are plotted for baseline, temperature induction, and PTZ segments. (Temperature seizure induction, n = 10 for wt and 10 for het; PTZ seizure induction, n = 6 for wt and 10 for het) (***p < 0.001 vs wt); (†† p < 0.01; ††† p < 0.001 vs baseline het).

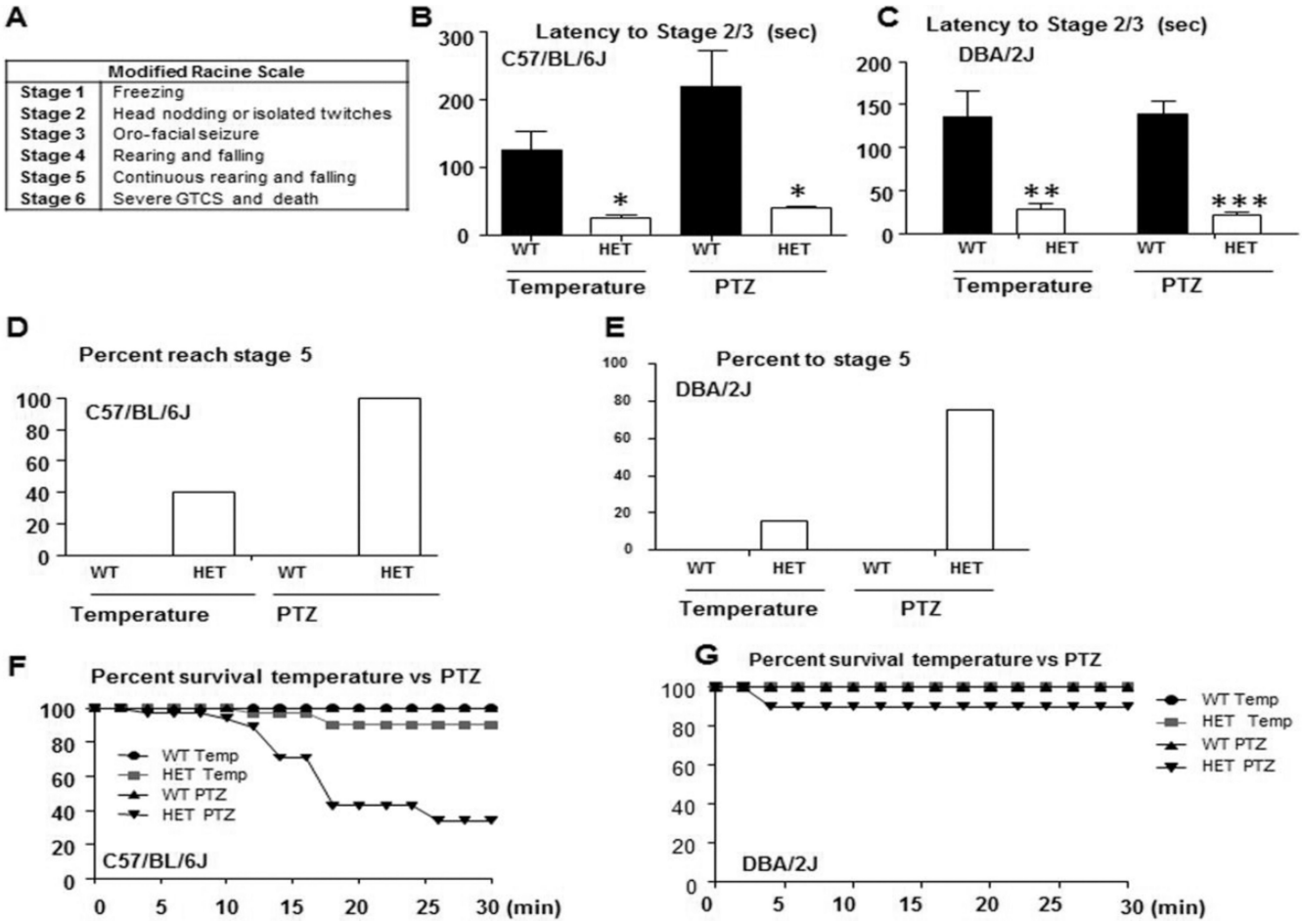

Figure 3. Temperature elevation and PTZ induced seizure latency, severity, and survival in *Gabrg2*$^{+/Q390X}$ knockin mice

(**A**) The modified Racine scale used for seizure scoring is shown. **(B, C)** Latency to reach stages 2 and 3 of the modified Racine scale for 2–4 month old *Gabrg2*$^{+/Q390X}$ knockin mice in C57BL/6J (**B**) or DBA/2J (**C**) was plotted for temperature elevation and PTZ seizure inductions. (**D,E**) Percentage to reach stage 5 of the modified Racine scale was plotted for 2–4 month old *Gabrg2*$^{+/Q390X}$ knockin mice in C57BL/6J (**D**) or DBA/2J (**E**) during temperature elevation and PTZ seizure inductions. **(F,G)** Survival percentage following temperature and PTZ induction was plotted for 2–4 month old *Gabrg2*$^{+/(Q390X)}$ knockin mice in C57BL/6J (**F**) or DBA/2J (**G**) (In **F**, temperature seizure induction, n = 10 for wt and 10 for het; PTZ seizure induction, n = 6 for wt and 6 for het; in G, n = 6 for each genotype for temperature induction and n = 10 for each genotype for PTZ seizure induction).

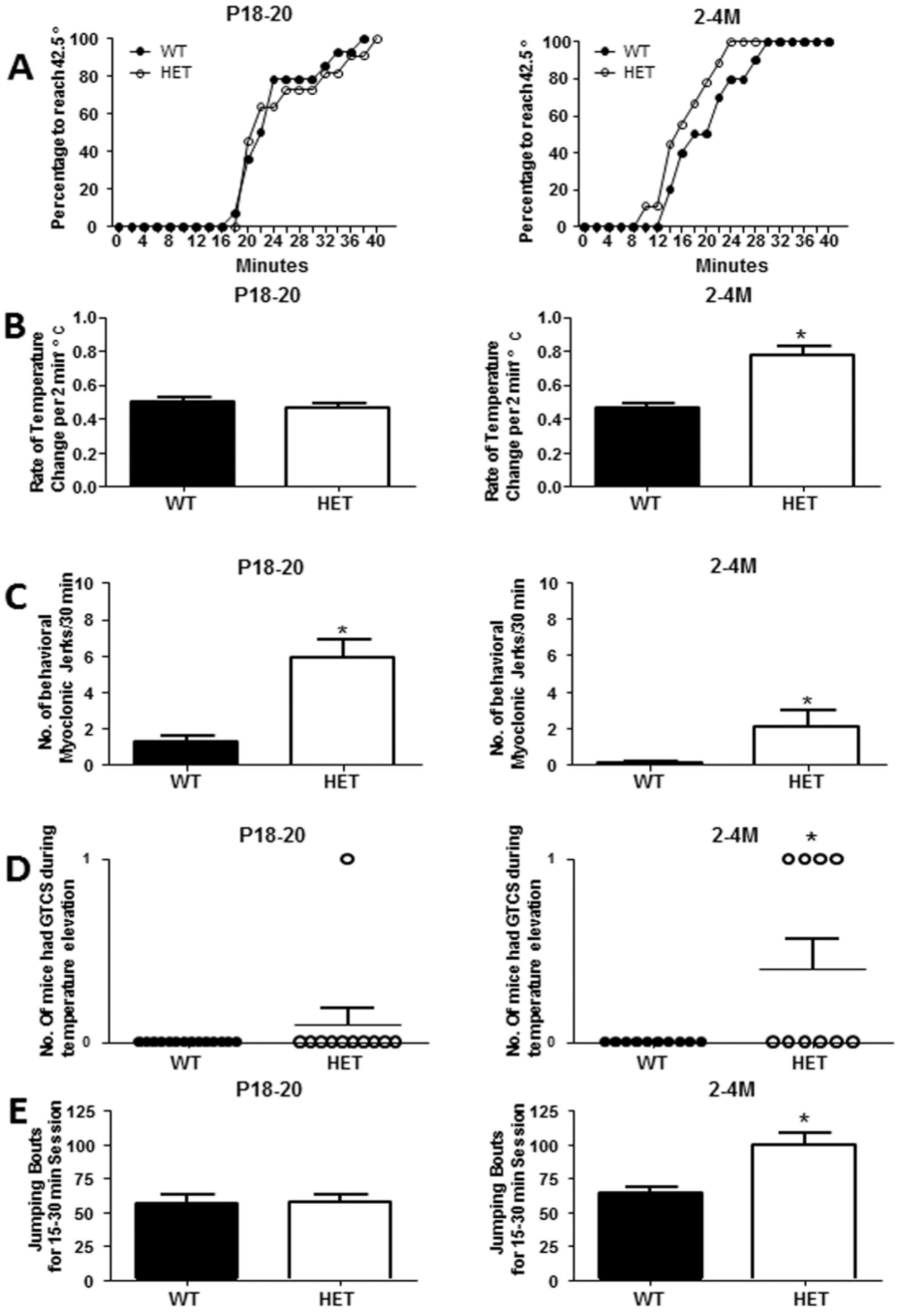

Figure 4. Heat induced body temperature changes as well as seizures and anxiety-like phenotypes were differentially expressed in Gabrg2$^{+/Q390X}$ mice at different age

(A) The percentage of mice to reach 42.5 °C during temperature induction was plotted for P18–20 and 2–4 month old wild-type (wt) and *Gabrg2*$^{+/Q390X}$ knockin (het) mice. **(B)** The rate of core body temperature change assessed over 2-minute intervals during temperature induction was plotted for P18–20 and 2–4 month old wild-type (wt) and *Gabrg2*$^{+/Q390X}$ knockin (het) mice. (**C)** The number of myoclonic jerks during temperature induction was plotted for P18–20 and 2–4 month old wild-type (wt) and *Gabrg2*$^{+/Q390X}$ knockin (het)

mice. **(D)** The number of GTCS (GTCSs) during temperature induction was plotted for P18–20 and 2–4 month old wild-type (wt) and *Gabrg2*$^{+/Q390X}$ (het) mice. **(E)** Frequency of jumping bouts during temperature induction was plotted for P18–20 and 2–4 month old wild-type (wt) and *Gabrg2*$^{+/Q390X}$ knockin (het) mice (n = 10 for wt and het for 2–4 month old mice; n = 11 for wt and 14 for het for P18–20); (*$p < 0.05$ vs wt).