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An animal model of genetic predisposition to develop acquired epileptogenesis: The FAST and SLOW rats

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ABSTRACT

Epidemiological data and gene association studies suggest a genetic predisposition to developing epilepsy after an acquired brain insult, such as traumatic brain injury. An improved understanding of genetic determinants of vulnerability is imperative for early disease diagnosis and prognosis prediction, with flow-on benefits for the development of targeted anti-epileptogenic treatments as well as optimal clinical trial design. In the laboratory, one approach to investigate why some individuals are more vulnerable to acquired epilepsy than others is to examine unique rodent models exhibiting either vulnerability or resistance to epileptogenesis. This review focuses on the most well-characterized of these models, the FAST (seizure-prone) and SLOW (seizure-resistant) rat strains, which were derived by selective breeding for differential amygdala electrical kindling rates. We describe how these strains differ in their seizure profiles, neuroanatomy, and neurobehavioral phenotypes, both at baseline and after a brain insult, with this knowledge proving fruitful to identify common pathological abnormalities associated with seizure susceptibility and psychiatric comorbidities. Importantly, accruing data on strain differences in multiple biological processes provide insight into why some individuals may be more vulnerable to epileptogenesis, although future studies are evidently needed to identify the precise molecular and genetic risk factors. Together, the FAST and SLOW rat strains, and other similar experimental models, are invaluable neurobiological tools to investigate the effect of genetic background on acquired epilepsy risk, as well as the poorly-understood relationship between epilepsy development and associated comorbidities.

Keywords: acquired epilepsy; animal models; FAST rats; SLOW rats; genetics; amygdala electrical kindling

Introduction

Acquired epilepsy develops following a brain insult, such as a stroke, infection, head trauma, tumor or neurodegenerative disease, and accounts for about one third of all epilepsies[1; 2]. Current understanding of the process by which epilepsy develops (i.e. 'epileptogenesis') identifies three different stages; the initial brain insult, a latent period of variable length (months to years), and a chronic period[3; 4]. During the latent period, progressive changes in

neuronal activity occur that ultimately result in an increased propensity to generate spontaneous recurrent seizures[5; 6].

Even after a severe brain insult, only a proportion of individuals go on to develop epilepsy, and this process may take many years to occur[7]. There is building evidence of a genetic predisposition to developing epilepsy following a brain insult[8-12]. Furthermore, genetic factors can shape how and when the disease manifests[8; 11; 13-15]. Research in this area remains in its infancy, and there is currently a pressing unmet need in the field to better understand how an individual's genetic background contributes to their risk of acquired epilepsy. The discovery of genetic factors associated with vulnerability (or resistance) to develop acquired epilepsies will advance the field by providing improved prediction, with flow-on benefits for the development of targeted anti-epileptogenic treatments as well as clinical trial design[12; 16].

Aiding this line of investigation, unique rodent models exhibiting vulnerability or resistance to epileptogenesis after an acquired insult have been established. Specifically, this review focuses on the FAST (seizure-prone) and SLOW (seizure-resistant) rat strains, first generated by Racine and colleagues[17]. These models have led to the identification of common pathological abnormalities associated with psychiatric comorbidities and seizure susceptibility. Here, we review the evidence demonstrating differences in seizure profiles, neuroanatomical and neurobehavioral phenotypes in FAST compared to SLOW rats, both at baseline as well as after a brain insult. We highlight how such comparisons have provided novel insights into biological mechanisms of why some individuals may be more vulnerable to epileptogenesis and identify the need for future studies to identify genetic risk variants. Together, this research aims to accelerate our understanding of genetic determinants of vulnerability to acquired epilepsies, with important implications for diagnosis and prognosis.

Genetic predisposition to acquired epilepsy in humans

While epilepsy can result from different environmental insults, clinical, epidemiological and molecular studies suggest a genetically-determined vulnerability to these 'acquired' epilepsies.

In an early study, first-degree relatives of children with hemiplegia who developed epileptic seizures had a higher prevalence of seizures and epileptiform EEG abnormalities than relatives of hemiplegics without seizures[18]. More recently, in a population-based Danish

study of >1.6 million people, a positive family history of epilepsy was associated with an approximately 10-fold increased risk of developing epilepsy following a severe brain injury[9].

Gene association studies have suggested common genetic variation raising the risk for post-traumatic epilepsy (PTE)[16]. For example, single-nucleotide polymorphisms (SNPs) in the adenosine A1 receptor (*A1AR*), methylenetetrahydrofolate reductase (*MTHFR*) enzyme, and glutamic acid decarboxylase 1 (*GAD1*) genes, increases the risk for developing PTE [19-21]. There is a high possibility that alterations of these 3 genes may be linked with epilepsy[16], with *GAD1* being important for catalyzing the production of gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter[19; 22]; the *A1AR* gene encoding receptors for adenosine, whereby binding causes seizure arrest[21; 23]; and the *MTHFR* gene being responsible for the metabolism of methionine, but when impaired increases the production of the by-product homocysteine, which lowers seizure threshold[20]. Lastly, Diamond et al. (2015) reported that a SNP in the interleukin-1β (*IL-1β*) gene is associated with increased risk of PTE, lower serum IL-1β levels and higher IL-1β CSF/serum ratios[15]. These data coincide with emerging evidence of a central role for IL-1β signaling in ictogenesis and epileptogenesis[24; 25].

Genetic variation has also been associated with the risk of epilepsy following ischemic stroke, including a functional polymorphism in the transient receptor potential cation channel subfamily M member 6 (*TRPM6*) gene, associated with reduced serum levels of Mg^{2+}[26]. Further, a polymorphism of mitochondrial aldehyde dehydrogenase 2 (*ALDH2*), is reportedly associated with post-stroke epilepsy susceptibility and levels of oxidative stress[27].

These gene association studies have implicated common genetic variation in the vulnerability to human acquired epilepsies. However, such studies are time consuming and expensive, and to date have only examined a small number of genes, typically in a hypothesis-driven approach, and none have yet been validated in more than one population. An important complementary approach is the utilization of genetic animal models with differing seizure susceptibility, which can be compared in order to more rapidly investigate novel genetic risk factors for acquired epilepsies. The most well-characterized rodent model of great utility for this purpose is the FAST (seizure-prone) and SLOW (seizure-resistant) rats[17].

FAST and SLOW rats – generation and seizure profile

To investigate genetically-determined neurobiological factors that may predispose towards epileptogenesis, selective breeding based on differing susceptibility resulted in two rat strains: the seizure 'prone' FAST and seizure 'resistant' SLOW rats[17]. In the electrical kindling model, animals receive an electric stimulation through an electrode implanted in a limbic structure, such as the hippocampus or amygdala[28]. Repetitive stimulations elicit increasingly more intense and prolonged electroencephalographic seizure responses which progress through the various stages of severity involving other brain regions; resembling focal seizures that generalize in humans[29]. The various stages of behavioral manifestations of the seizures induced by kindling are traditionally classified using the Racine Class Scale; Class I is defined by immobility and facial automatisms; Class II by head nodding; Class III by contralateral forelimb clonus; Class IV by bilateral forelimb clonus and rearing; and Class V by rearing and falling with or without secondarily generalized seizures[30].

A cross between Wistar and Long-Evans rats served as the parent population from which the FAST and SLOW rats were selectively bred[17; 31]. The rationale to use these parental strains was based on previous experiments that showed an interesting variability of the number of amygdala electrical stimulations required to evoke a class V seizure (range from 8 to 28)[32] (Figure 1).

----- *Figure 1 near here* -----

From the parent generation, the rats with the fastest rates to develop the first class V seizure (mean kindling rate of 8.8 stimulations) were selected as breeders for the seizure-prone (FAST) strain. Conversely, the rats that required the largest amount of stimulations to reach the first class V seizure (mean of 15.0 stimulations) were selected as breeders for the seizure-resistant (SLOW) strain[17; 33]. Similar selection procedures continued through F11, although there was little or no overlap in the distribution of kindling rates for the FAST and SLOW strains by F6. At F11, the FAST rats achieved a class V seizure with daily amygdala kindling within the first 7 days, while a SLOW rat required 3-4 weeks to display the same seizure class[17]. The progression of the behavioral seizures was also significantly different between the two strains. Between generations F7 to F10, 71.0% of the FAST rats progress directly from a class II seizure to a class V, whereas only 23.1% of SLOW rats showed this accelerated progression[17]. Moreover, FAST rats exhibited seizures of longer duration[33].

Similar results were seen with electrical kindling of other structures. As in other rat strains, the fastest kindling rates in the FAST and SLOW rats were observed in the perirhinal cortex which was followed by the piriform cortex, amygdala and hippocampus[33].

FAST and SLOW rats – behavioral phenotype

Neurodevelopmental trajectory

Developmental delay, a common indicator of abnormal brain development, can manifest as delays in the acquisition of speech and language, motor control (gross and fine), auditory processing and social communication in humans[34-36]. Exploratory neurodevelopmental studies in FAST and SLOW strains have revealed developmental delay in FAST rats, observed as delayed physical development (eye opening), neuromuscular development (decreased locomotor activity), neuromotor and sensory system development (increased latencies to respond to righting reflex, cliff avoidance and negative geotaxis tests)[37]. These delays were evident within the first two postnatal weeks (PND5-15), a time period comparable to late third trimester through to eight months of postnatal age in humans. Of note, these behavioral phenotypes correlate with the below-mentioned neuroanatomical findings observed in FAST rats, including delayed myelination and enlarged ventricles, which have also been reported in children with neurodevelopmental disorders such as autism spectrum disorder (ASD) and attention deficiency hyperactivity disorder (ADHD)[38-41].

Fear, anxiety, and general activity

Differences in fear and anxiety behaviors are probably the best characterized behavioral phenotype between the FAST and SLOW rat lines, with various studies demonstrating that SLOW rats exhibit heightened fear and anxiety responses in different testing paradigms compared to FAST rats. For example, in an elevated plus maze, where 'normal' animals will typically explore both the open and enclosed maze arms, an anxious animal will preferentially spend more time in the enclosed arms. FAST rats were observed to spent twice the amount of time in the open arms compared to the SLOW rats, which rarely explored outside of the enclosed arms[42]. This anxiety-like behavior is further amplified in the SLOW rats when they have previously been exposed to a shock stimulus or adverse conditions, as they exhibit reduced exploratory activity and freezing in an inhibitory avoidance task[42].

Interestingly, FAST rats in general show a higher level of activity compared to SLOW rats,

as typically detected in the open field test[42-44]. When restrained, FAST rats are observed to struggle more than SLOW rats[43-46], and generate more vocalizations[44; 47]. This hyperactivity in FAST rats is correlated with enlarged cerebrum and reduced myelin integrity of corpus callosum[44]. It has also been proposed that FAST rats are more impulsive compared to SLOW rats or the parental strains, exhibiting abnormal sexual assertiveness to both estrous and non-estrous female rats when paired for breeding purposes[48; 49]. Of note, hyperactivity and impulsivity may confound the interpretation of anxiety-like phenotypes noted above. For example, hyperactivity and impulsivity may cause increased exploratory behavior in an elevated plus maze task, which would mask anxiety-like behavior in FAST rats.

However, to date these findings point towards FAST rats exhibiting a more active behavioral phenotype, while SLOW rats are more vulnerable to environmental stressors. As such, it has been suggested that FAST rats are a useful model of the neurobehavioral profile of patients diagnosed with ASD or ADHD[44; 50], in addition to their utility for acquired epileptogenesis studies; and indeed, reflects common neurobehavioral comorbidities seen in patients with acquired epilepsies.

Cognitive dysfunction

Several studies have demonstrated cognitive impairments in FAST rats compared to SLOW rats or their parental strain lines. Using various maze paradigms to assay cognitive performance, FAST rats typically exhibit slower learning, impaired working and reference memory, and are more readily distracted from the task at hand compared to SLOW rats[50-52]. Performance of FAST rats in the delayed alternation T-maze test was found to decay further when the rats had developed class V amygdala-kindled seizures prior to testing, whereas no changes were observed in the SLOW rats, suggesting that memory function in FAST rats may be particularly vulnerable to CNS challenges[51]. Impulsivity might also be a factor of their poor performance, as it is noted that FAST rats tend to race to whichever direction that they are oriented at the choice point of a T-maze alternation test, instead of pausing to strategize[51].

Poor performance of FAST rats in the Morris Water Maze has primarily been attributed to their failure to adopt appropriate searching strategies, as FAST rats tend to display aberrant thigmotaxis, swimming repeatedly around the pool edges. Instead, the SLOW rats were more flexible and tend to develop methods which increased pool crossings[50]. Of note, however,

performance of FAST rats in the water-maze spatial memory task was greatly enhanced when animals were assisted during the learning acquisition phase of the test (i.e. by elevating the escape platform to ensure its visibility). This observation raises the question of whether FAST rats, which seemed to be unable to form the concept of facile escape, actually have impairments in concept formation[50].

Social behavior

FAST and SLOW rats also show differences in social behaviors, which have been likened to an ASD/ADHD-like phenotype. It is reported that FAST rats displayed a more juvenile-like play fighting behavior, initiating more playful attacks, and being more likely to defend against attacks compared to the SLOW rats[53]. It is also noted that the defensive behavior of FAST and SLOW rats differed, with FAST rats trying to avoid contacts for defense, while SLOW rats try to block the contact[53]. Moreover, the observations of stereotypic behaviors such as circling, excessive grooming and repetitively moving their pups, seen only in FAST rats, indicates abnormal social behaviors in FAST rats[54]. However, others groups have failed to detect deficits in social interactions in FAST versus SLOW rats[55].

Polydipsia

Polydipsia, or excessive thirst and water intake[43; 44; 54; 56], has been observed in FAST rats as compared to SLOW rats. Of note, polydipsia is another disorder observed in individuals with ADHD, ASD, and psychiatric illnesses[57; 58]. Further, excessive water intake due to polydipsia can cause hyponatremia (low serum sodium levels), which has been identified as a risk factor for seizure disposition in epilepsy patients[59]. Such findings emphasize the commonality between genetic predisposition, neurodevelopmental disorders, and epilepsy vulnerability.

Sensorimotor Gating

Pre-pulse inhibition (PPI) is a well-established measure of sensorimotor gating, and is frequently aberrant in psychiatric disorders such as schizophrenia[60]. Changes in PPI and hippocampal auditory-evoked potentials have been reported in FAST rats after seizures induced by hippocampal CA1 kindling, suggesting that this strain is more vulnerable to psychosis-related symptoms[61]. However, these deficits in sensorimotor gating were alleviated by administration of haloperidol, a D2 and D1 dopaminergic antagonist, and CGP7930, a positive allosteric modulator of $GABA_B$ receptors[61]. This finding suggests that dysfunction of dopamine and $GABA_B$ receptors may be an important mechanism which underlies the

sensory and sensorimotor gating differences in FAST and SLOW rats[61]. Unexpectedly, the same study also revealed that partially kindled SLOW rats showed enhanced methamphetamine-induced hyperlocomotion compared to FAST rats, a result suggestive of particular vulnerability to psychiatric disturbances. Although the mechanism behind this is not clear, the authors propose that higher α5 $GABA_A$ receptor expression in SLOW rats may contribute to this behavioral comorbidity[61].

In summary, FAST rats have been reported to display a phenotype of hyperactivity, impulsivity, cognitive deficits, social abnormalities, and neurodevelopmental delays[62], whereas SLOW rats exhibit heightened fear and anxiety but otherwise a largely 'normal' behavioral phenotype (Table 1). It is worth highlighting that most of these ASD/ADHD-like phenotypes reported in FAST and SLOW rats, such as anxiety, cognitive dysfunction, diminished attention, impulsivity and social impairment, are also observed in various chronic epilepsy rat models, such as pilocarpine-induced status epilepticus[63-65]. However, the relationship between the above noted behaviours and risk of epilepsy remains unclear and requires further investigation.

----- *Table 1 near here* -----

FAST and SLOW rats - neuroanatomical differences

Studying brain morphology facilitates the identification of neuroanatomical biomarkers for diagnosis and treatment of neurological disorders. In general, this is achieved by comparing a healthy versus diseased population. Differences in brain structure may contribute to why some patients develop epilepsy after a brain insult, while others do not. However, identifying neuroanatomical changes that contribute to the risk of developing epilepsy has been a challenge in clinical populations, as an individual's brain has usually already been exposed to the insult and recurrent seizures by the time they present. FAST and SLOW rat strains provide a platform to understand neuroanatomical alterations related to inherited susceptibility versus resistance towards epileptogenesis, in the absence of factors associated with a brain insult.

Using *ex-vivo* magnetic resonance imaging (MRI), absolute volumetric comparison of brains

from FAST and SLOW rats detected a larger third ventricle, larger posterior inferior cerebellum, cerebral hemispheres (left and right) and larger corpus callosum (major white matter cerebral region), and smaller anterior cerebellar vermis in FAST rats[44] (refer to Table 2). Alongside these volume increases, differences in regional tissue length were also identified in the cerebrum and corpus callosum, with both showing elongation along the rostral-caudal axis in FAST rats[44]. Furthermore, 3D volume rendering showed localized shape differences in the ventral corpus callosum, with SLOW rats having more prominent curvature extending medially towards the amygdala (Figure 2)[44]. In addition to MRI volumes, differences were observed within cerebellar regions, with histological analysis revealing a smaller molecular layer (decreased number of Purkinje cells) and larger white matter layer in the cerebellum of FAST rats[44]. The above-described neuroanatomical differences observed between naïve adult male FAST and SLOW rats are similar to what have been reported in patients (both adults and children) with new onset epilepsy, suggesting that these changes might be related to factors implicated in epilepsy susceptibility rather than being a secondary effect of seizure damage[44; 56; 66; 67].

----- *Figure 2 near here* -----

The corpus callosum, being the major cerebral white matter tract, plays a critical role in interhemispheric brain connectivity and secondary generalization of epileptic seizures[68]. Based on volumetric and shape differences in the corpus callosum between the strains, another study using diffusion tensor imaging (DTI) demonstrated a region-specific reduction in fractional anisotropy in the corpus callosum of FAST rats, indicating lower myelin fiber integrity compared to SLOW rats[56]. At a cellular level, electron microscopy to study the microstructure of the corpus callosum revealed larger myelinated axons and reduced g-ratio in FAST rats, indicating increased myelin sheath thickness compared to SLOW rats[56] (Figure 2).

Given that adult FAST and SLOW rats have epileptogenesis vulnerability divergence, but do not develop epilepsy *per se* until an acquired brain insult is sustained, these white matter microstructural alterations suggest underlying neurodevelopmental pathology that is associated with their inherent predisposition to acquired epileptogenesis. Indeed, FAST rats exhibit delayed myelination and lower levels of mRNA expression for myelin-specific genes at PND 5 and 11[37] (Figure 2), a developmental time period that is comparable to myelination

processes occurring between late trimester gestation and eight months of age after birth in humans[69]. Interestingly, a study of patients with mesial temporal lobe epilepsy revealed that late myelinating white matter tracts were more vulnerable to seizure-associated damage than early myelinating white matter tracts[70], indicating a critical period of myelination during neurodevelopment that may be associated with vulnerability towards developing seizure disorders later in life. Indeed, animal models of cortical malformation have suggested that white matter development could be an underlying pathology for enhanced seizure vulnerability. For example, promoting myelination with quetiapine, an atypical antipsychotic known to enhance oligodendrocyte regeneration and myelin repair[71], has been found to reduce seizure susceptibility and severity[72]. Therefore, the FAST/SLOW rat model serves as a useful tool to facilitate investigations into neurodevelopmental contributions to seizure vulnerability.

The most common neuropathological change associated with acquired limbic epilepsy in animal models and humans is mossy fiber sprouting, a form of hippocampal synaptic reorganization associated with network hyperexcitability[73]. Pilocarpine-induced status epilepticus in FAST and SLOW rats results in differences in the degree of mossy fiber sprouting. Compared to SLOW rats, FAST rats displayed more mossy fiber granules in the 'naïve' brain state, but were in fact more resistant to seizure-induced mossy fiber sprouting in this model[74]. This paradox in FAST rats, of increased seizure susceptibility but resistance towards morphological changes after a brain insult, is observed in both juvenile and adult animals[74]. Alongside previous evidence that reduced mossy fiber sprouting is observed in younger rats post-seizure compared to adult rats[75-77], it is suggested that FAST rats may have genetic variants that result in brain maturation arrest, preventing them from developing as a seizure-resistant strain[74]. This theory is supported by the observed enlarged corpus callosum in adult FAST rats, which also suggests a failure in brain maturation[44].

----- Table 2 near here -----

Biological mechanisms underlying genetic-based differences in susceptibility to acquired epilepsy and comorbidities

The above-described differences in phenotype between FAST and SLOW rats provide strong rationale for common pathogenic mechanisms underlying vulnerability to epilepsy and

comorbid psychiatric disorders, and future studies to identify particular genetic variations between the two strains promise to reveal insights into such mechanisms. To date, several biological processes have been identified as distinct between the strains (in addition to neuroanatomical differences), including GABAergic signaling, stress responses, metabolism, synaptic plasticity, and neuroinflammation.

GABAergic responsiveness

One of the most widely studied mechanisms thought to underpin differential behavioral comorbidities as well as seizure susceptibility in FAST versus SLOW rats is the GABAergic system[33]. Differential responses were observed in response to administered GABAergic antagonists, with FAST rats being more responsive to lower doses of pentylenetetrazol, bicuculline and picrotoxin[78]. On the other hand, SLOW rats are more vulnerable to pentobarbital and the neurosteroid tetrahydrodeoxycorticosterone (THDOC), and can be anesthetized with doses that only modestly affect FAST rats[33; 79]. THDOC injection also seemed to deteriorate cognition abilities of the SLOW rats, greatly reducing their performance in the Morris Water Maze, while marginally enhancing these behaviors in FAST rats[79]. It is suggested that the differences in GABAergic responsiveness may be due to different profiles of spontaneous GABAergic miniature inhibitory postsynaptic currents (mIPSCs) observed in the perirhinal cortex and amygdala, where SLOW rats showed fast and large amplitude mIPSCs and FAST rats showed decaying small amplitudes[80].

Further exploration of $GABA_A$ receptor subtypes revealed strain differences in the expression of α1, α2, α3, and α5 subunits in the amygdala, perirhinal cortex and adjacent paleocortex, with FAST rats overexpressing the α2, α3, and α5 subunits (as commonly found in the embryonic state), and under-expressing the α1 subunit, below the levels typically found in the adult brain[81]. In contrast, SLOW rats had a higher expression of α1 subunit expression, with lower expression of α2, α3, and α5 subunits[81]. This might explain why FAST rats exhibit a more juvenile-like behavioral profile, with higher seizure susceptibility similar to what is reported in children with epilepsy[82; 83]. Similar observations are made in the mossy fiber system, where FAST rats showed more mossy fiber granules compared to the SLOW rats, consistent with what is observed in juvenile animals[84].

$GABA_B$ receptors are also thought to be related to sensory and sensorimotor gating functions, as CGP7930, a positive allosteric modulator of $GABA_B$ receptors, alleviated the deficits in sensory and sensorimotor gating of FAST rats[61].

Synaptic plasticity

Another mechanism which might explain why FAST and SLOW rats exhibit differing behavioral profiles and seizure susceptibility, is a difference in stimulation-induced neuroplastic changes. After stimulating the corpus callosum to induce long-term polysynaptic potentiation of the transcallosal pathway to the sensorimotor neocortex, cortex mapping using intracortical microstimulation found a doubling in the mean size of the caudal forelimb area in FAST rats but not in the SLOW rats[85]. Staining of the frontal neocortical layer III pyramidal neurons following the stimulation revealed an increase in apical dendritic length and branching in SLOW rats, while FAST rats displayed reduced dendritic length and decrease branching[85]. Spine densities of basilar and apical dendrites were also significantly increased in post-stimulated FAST rats, while no changes were observed in that of SLOW rats[85]. With evidence that seizures correlate with changes in caudal forelimb area movement representations[86] and motor map topography[87], it is possible that this differential stimulation-induced synaptic plasticity may also modulate seizure susceptibility and epileptogenesis[85].

Similarly, another study also showed that adult FAST rats in which febrile seizures were induced by intracortical microstimulation at PND14 had larger motor maps, with lower stimulation thresholds compared to those without febrile seizures, which were not observed in their parental control strain (Long Evans) under the same conditions[88]. The study further reported several changes in neurotransmitter and transporter levels, including increased $GABA_A$ α2 subunit, a higher NKCC1/KCC2 ratio, and significantly lower levels of the $GABA_A$ α1 subunit, GluR2 and NR2A receptor subunits in the sensorimotor cortex of PND14 FAST rats compared to age-matched Long Evans rats. These findings suggest that the increased excitability observed in the neocortex of FAST rats may also be related to their immature-like GABAergic system[88].

Seizure-induced neurotrophin expression

Growth factors including brain derived neurotrophic factor (BDNF) have a broad range of functions in the CNS, including well-characterized roles in synaptic plasticity and cognition[89], as well as an emerging role in epilepsy[90]. Although basal levels of BDNF mRNA are

comparable between FAST and SLOW rats, following an induced seizure, widespread increases of BDNF mRNA was observed in FAST rats, most remarkably in the dentate gyrus, piriform and amygdala, as well as fronto-parietal cortex[91]. In contrast, SLOW rats showed an increase mainly in the amygdala and piriform, and no change in the fronto-parietal cortex[91]. FAST rats also showed increase nerve growth factor (NGF) and decreased neurotrophin-3 (NT-3) mRNA levels accompanied by the increase in TrkB and TrkC receptor expression in dentate granule cells, which was not seen in the SLOW rats[91]. As neurotrophins are thought to be responsible for the regulation of synaptic plasticity and efficacy, these observations may account for differing seizure susceptibility in SLOW and FAST rats[91].

Stress-induced hormone response

Several studies have identified differential stress responses in FAST and SLOW rats. For example, in response to the presence of a predator (ferret), one study reported that SLOW rats exhibited a more pronounced increase in adrenocorticotropic hormone (ACTH) and corticosterone levels[45]. In contrast, while under restraint, FAST rats showed a remarkably higher expression of ACTH[45]. These differences in adrenal hormonal responses parallel with the anxiety-like behavior of SLOW rats observed in the open field test, and the struggling of FAST rats in the restraint paradigm, respectively[31; 46].

Similarly, Merali and colleagues (2001) showed increase immunoreactivity of the corticotropin-releasing hormone and bombesin (BH) in several brain regions of SLOW rats compared to FAST rats exposed to the same stressors[46]. BH is a peptide which is associated with anxiety, and shown to impact various biological and behavioral responses, such as the suppression of feeding, increased locomotor activity, and enhanced grooming[46]. The difference in these adrenal hormonal responses may serve as the basis as to explain why anxiety responses of the SLOW and FAST rats differ.

Neuroinflammation

Given the known association between brain immunophenotype and psychiatric disorders as well as epilepsy[92-94], it is feasible that FAST and SLOW rats also differ in their immune systems and responses. While this has been minimally investigated to date, immunofluorescence for glial fibrillary acidic protein revealed astrogliosis in the cortex of FAST rats, suggesting that basal immunological differences may also affect behavioral phenotype and other comorbidities[55].

Fundamental metabolic differences

Recent studies incorporating dietary manipulations have identified fundamental metabolic differences between FAST and SLOW rats. Due to the fact that lipid-handling strategies of the FAST and SLOW rats seemed to differ (see '**Genetic variations underlying differing susceptibility to epileptogenesis'** section below), suggesting metabolic differences between the strains, the effect of caloric restriction was examined in the two rat lines. Upon feeding the rats with calorically-restricted diets to 80% of their body weight, while no effect was observed in SLOW rats, FAST rats displayed a decrease in their activity levels in the open field test with reduced vocalizations in the restraint paradigm[47]. Moreover caloric restriction also appeared to prolonged the kindling rate of FAST rats, while shortening seizure duration of class V kindled SLOW rats[47].

The effects of omega-3 supplementation on FAST and SLOW rats were also very different. While omega-3 supplementation in FAST rats was found to abolish impulsivity, in SLOW rats it actually increased their seizure sensitivity, with kindling rates being nearly halved compared to the naïve SLOW rats[95]. The supplementation also lowered the initially higher plasma non-esterified fatty acids (NEFA) level of SLOW rats to a similar level observed in FAST strains, which raises the question as to whether plasma NEFA levels are related to seizure susceptibility[95]. Moreover, the supplementation also increased food and water consumption of SLOW rats, inducing them to consume water up to a comparable amount to FAST rats, which displays polydipsia[95]. This suggests that seizure predisposition and ASD/ADHD-like behaviors may also be influenced by differences in diet and nutrition.

Homeostatic regulation of synaptic zinc

FAST and SLOW rats reportedly show innate differences in the homeostatic regulation of synaptic zinc[96]. It was found that SLOW rats had 30% lower levels of synaptic zinc throughout the telencephalon compared to FAST rats, with the ventrolateral cortical regions exhibiting the greatest difference between the two rat lines[96]. This is consistent with a study by Xu et al. (2004), whom found slightly higher levels of zinc in the dentate gyrus in FAST rats compared to the SLOW rats, associated with reduced resistance to seizure-induced mossy fiber sprouting[74]. However, as zinc may have both convulsive and anti-convulsive effects, it is unclear how alterations in zinc regulation may contribute to epileptogenesis in these two rat lines[96].

In summary, various biological processes have been identified as distinct between the FAST and SLOW rat strains (Table 3). However, a causal relationship between these biological mechanisms and the observed differences in seizure susceptibility of the two strains has not been established. Further investigation into whether these biological mechanisms play a role in seizure susceptibility is needed.

----- Table 3 near here -----

Genetic variations underlying differing susceptibility to epileptogenesis in FAST and SLOW rats

Due to the fact that various clinical studies have suggested genetic variations that affect the risk for PTE in humans (as described above), there is clear rationale for gene expression analysis in FAST and SLOW rats to assess whether distinct genomic and transcriptomic profiles underpin the between-strain phenotypic differences. Surprisingly, this has been little studied to date. Gilby and colleagues (2007) examined the embryonic expression of 112 genes that were believed to play a role in neurodevelopment of FAST and SLOW rats[97]. Their study revealed 2 genes, Apolipoprotein (*APOE*) and β2 subunit of the voltage-gated sodium channel (*SCN2β*), that were significantly under-expressed in the brain of FAST rats[97]. The *APOE* gene is responsible for coding an intracellular cholesterol and fatty acid transport protein, which is particularly important for lipoprotein metabolism and neurodevelopment[97]. This may account for the differential metabolic profiles of these two strains, as described above. Further, studies employing mice genetically deficient in *APOE* genes/receptors have shown cognitive impairments and abnormalities in fear behavior, which are also comorbidities in FAST rats[98; 99].

The *SCN2β* gene expression surprisingly showed a six-fold reduction in FAST rats compared to SLOW rats[97]. As alterations to voltage-gated sodium channels have long been hypothesized to relate to epilepsy pathology[100], it is possible that differences in the expression of this gene influence the differing seizure susceptibility of FAST and SLOW rat lines[97]. However, more integration between clinical and animal models is needed to confirm the role in which *APOE* and *SCN2β* genes play in epileptogenesis.

Finally, it is worth considering the potential role of epigenetics in the manifestation of epilepsy vulnerability. Various epigenetic mechanisms such as alterations of DNA methylation, post-translational histone modification, as well as changes in microRNA and long non-coding RNAs, have been observed to accompany the development of temporal lobe epilepsy both in animal models as well as in humans[101]. Due to the fact that epigenetics is involved in embryogenesis and early brain development, regulation of epigenetic mechanisms in the neurodevelopment stages may predispose the brain to varying susceptibility to epileptogenesis, and hence may underlying factors which impact the phenotypes of FAST and SLOW rats[102]. Hence, more thorough understanding of how changes in epigenetics may affect the phenotypes of FAST and SLOW rats is warranted.

Other models of genetic vulnerability to acquired epilepsy

FAST and SLOW kindling in Sprague Dawley rats

Less extensively characterized is the generation of seizure-prone and seizure-resistant strains on a different genetic background, the Sprague Dawley (SD) rat. Similar to the above-described model generation, outbred SD rats were selected for "fast" or "slow" rates of kindling development by selective inbreeding for ~ 15 generations[103]. The *seizure-susceptible* SD (fast kindling) and *seizure-resistant* (slow kindling) strains were originally selected in response to stimulation of the perforant path input to the hippocampus, with *seizure-susceptible* SD rats requiring fewer kindling stimulations to develop class V seizures. These SD strains also differ in specific behaviors that depend in part on circuitry activated during the kindling selection process. The *seizure-susceptible* SD strain demonstrates a significant increase in motor exploratory activity in the open field test, and impaired spatial learning in the Morris water maze, similar to the FAST strain described above. In contrast, *seizure-susceptible* SD rats show normal fear conditioning learning comparable to the *seizure-resistant* SD strain as well as outbred SD rats[103].

Conclusion

Rodent strains that are either vulnerable or resistant to developing epilepsy after an acquired brain insult are valuable neurobiological tools to characterize the effect of the genetic background on epilepsy risk, as well as the poorly-understood relationship between epilepsy

development and associated comorbidities. It is worth noting that several limitations exist in any of the reported studies in FAST and SLOW rats – for example, experiments may be underpowered, often lack reference to the parental control strains, or struggle with effective experimental blinding due to overt differences in behavior. Nonetheless, a growing body of literature clearly demonstrates striking differences between the two strains across a range of measures. Further research is needed to define the specific molecular underpinnings as well as epigenetic mechanisms underlying the susceptibility or resistance to the development of epilepsy in these strains. Moreover, it would be of great interest to examine differential susceptibility of these strains to clinically-relevant models or second-hit insults such as the post-status epilepticus, post-stroke epilepsy or posttraumatic epilepsy models[104]. Such investigations may increase our understanding of the role of genetics in the development of acquired epilepsy, aid to the development of biomarkers[105], and identify novel disease-modifying therapies in epilepsy[25].

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Conflicts of Interest

None of the authors has any conflict of interest to disclose relevant to this work.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Key Point Box:

- Increasing data suggests a genetic predisposition to the development of epilepsy after an acquired brain insult
- To investigate why some people are susceptible to acquired epilepsy, we examine unique rodent models with vulnerability or resistance to epileptogenesis

- FAST (seizure-prone) and SLOW (seizure-resistant) rat strains exhibit differential seizure profiles, neuroanatomy and behavioral phenotypes
- Strain differences provide insight into why some individuals may be more vulnerable to epileptogenesis
- Future studies should identify the precise molecular and genetic risk factors

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Figure Legends

Figure 1: Generation of FAST and SLOW rats by selective breeding based on amygdala kindling rates. Amygdala kindling rates (x-axis) in parent generation rats (*; lowest row), as well as FAST (▲, ●) and SLOW (△, ○) rats are depicted across each subsequent generation (F1-F10 and F20; y-axis). Rats were kindled at a fixed intensity of 400 μA (*, ▲, △) or just above the afterdischarge threshold level (●○). Some SLOW rats (△, ○) had not yet developed stage 5 seizures when kindling was ceased; in these cases, the number of afterdischarges to that point, while an underestimation, was used as the kindling rate. Kindling rates of FAST and SLOW rats were significantly different for all generations beyond F5, and by F20, there was no overlap between strains. Reprinted from Racine et al., Epilepsy Research 35 (1999) 183-195, with permission from Elsevier.

Figure 2: Neuroanatomical differences in the corpus callosum of FAST and SLOW rats. (A) From 3D-rendered volumetric reconstruction of magnetic resonance imaging, FAST rats exhibit an elongated corpus callosum mid-body (white line) and shorter curved ventral region (white circle) compared to SLOW rats. (B) No differences were found in the shape or length of the anterior commissure, either anteriorly (ant) or posteriorly (post). (C) and (D) illustrate electron microscopy images of the midline corpus callosum (scale bar = 1 μm). FAST rats exhibit larger myelinated axons (increased axon diameter) compared to SLOW rats, although the myelinated axon density did not differ between the strains. Panels A and B reprinted from Sharma et al., Epilepsy & Behavior 65 (2016) 42-48, with permission from Elsevier.

Table 1. Summary of behavioral differences in FAST and SLOW rats

Behaviors	FAST rats	SLOW rats	References
Neuro-developmental trajectory	Physical, neuromuscular, neuromotor and sensory	-	[37]

	developmental delays		
Fear, anxiety and activity	Increased activity in EPM, open field, and restraint paradigms	Increased fear, anxiety in EPM	42-46
Impulsive behavior	Abnormal sexual assertiveness of FAST rats; rapid latency to mounting	Delayed latency to mount potential partner compared to parental strains	48-50
Cognitive dysfunction	Impaired concept formation, working and reference memory, and easy distractibility in T-maze and MWM	-	50-52
Social behaviors	Abnormal repetitive behaviors; more juvenile-like play fighting behavior	More adult-like play fighting behavior	53; 54
Water consumption	Polydipsia	-	43; 44; 54
Sensorimotor gating	Changes in PPI and hippocampal auditory-evoked potentials after seizures	Enhanced methamphetamine-induced hyperlocomotion	61

Table abbreviations: EPM = Elevated plus maze; MWM = Morris Water Maze; PPI = Pre-pulse inhibition.

Table 2. Summary of neuroanatomical differences between FAST and SLOW rats

Brain regions	**FAST rats**	**SLOW rats**	**References**
Third Ventricle	Larger volume	Smaller volume	44
Cerebellum	Larger posterior inferior cerebellum. Elongation along the rostral caudal axis. Smaller molecular layer (fewer Purkinje cells) and larger white matter	Smaller posterior inferior cerebellum	44

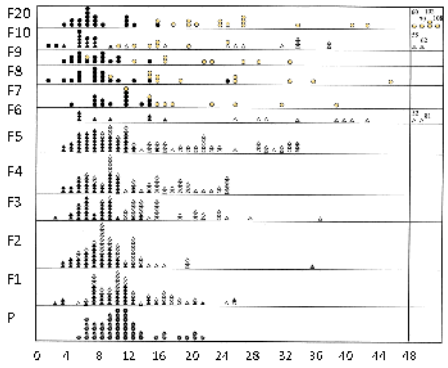
	layer		
Cerebral hemisphere	Larger volume	Smaller volume	[44]
Corpus Callosum	Larger volume. Elongation along the rostral caudal axis. Lower myelin fiber integrity but increased myelin sheath thickness. Delayed myelination	Smaller volume. Greater curvature of ventral corpus callosum has more curvature	[37; 44; 56]
Anterior cerebellar vermis	Smaller volume	Larger volume	[44]
Hippocampus	More mossy fiber granules, but more resistant to seizure-induced mossy fiber sprouting	Less mossy fiber granules, but less resistant to seizure-induced mossy fiber sprouting	[74]

Table 3. Summary of the biological mechanisms underlying differences in susceptibility to acquired epilepsy and comorbidities in FAST and SLOW rats

Biological mechanisms	**FAST rats**	**SLOW rats**	**References**
GABAergic responsiveness	Increased response to pentylenetetrazol, bicuculline and picrotoxin	Lower response threshold to pentobarbital; deterioration of cognition abilities with THDOC	[78; 79]
$GABA_A$ receptor expression	Overexpression of α2, α3, and α5 embryonic subunits and under-expression of α1 subunit.	Overexpression of α1 subunit, with lower expression of α2, α3, and α5 subunits	[81; 88]
Mossy fibers	Increased mossy fiber granules	Reduced mossy fiber granules	[84]
Synaptic plasticity (post-stimulation)	Reduced dendritic length and branching with increased spine densities of basilar and apical dendrites. Expanded	Increased apical dendritic length and branching	[85; 88]

	cortical mapping with lower stimulation thresholds		
Neurotrophin expression	Widespread increased brain BDNF mRNA expression post-kindling Increased NGF mRNA, TrkB and TrkC receptor expression, and decreased NT-3 mRNA expression in dentate granule cells	Increase in BDNF mRNA expression mainly in the amygdala and piriform post-seizure.	91
Stress-induced hormone response	Higher protein expression of plasma ACTH under restraint	Pronounced increase in circulating ACTH, BH, CRH and corticosterone protein levels in the presence of a predator	45; 46
Neuroinflammation	Cortical astrogliosis	-	55
Metabolic differences	Differential behavioral responses to caloric restriction (reduced locomotor activity) and omega-3 supplementation (abolished impulsivity)	Differential behavioral responses to caloric restriction (reduced seizure duration) and omega-3 supplementation (increased seizure sensitivity)	47; 95
Homeostatic regulation of synaptic zinc	Higher levels of zinc in the dentate gyrus	Lower levels of synaptic zinc in the telencephalon	74; 96

Table Abbreviations: adrenocorticotropic hormone (ACTH); brain-derived neurotrophic factor (BDNF); bombesin (BH); corticotropin-releasing hormone (CRH); *nerve growth factor (NGF); neurotrophin-3 (NT-3);* tetrahydrodeoxycorticosterone *(THDOC); tropomyosin receptor kinase (TrkB/C).*



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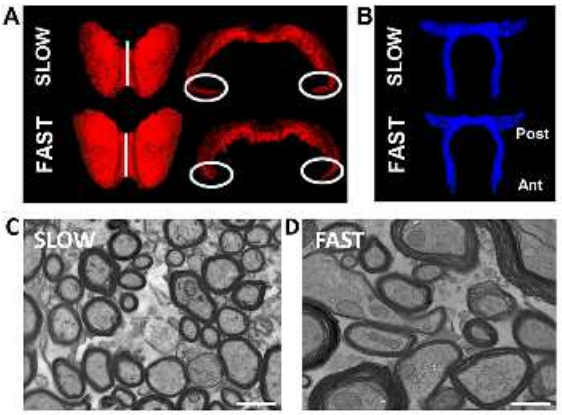

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