Published in final edited form as:

Neuroscience. 2012 July 26; 215: 90–97. doi:10.1016/j.neuroscience.2012.04.011.

Rapamycin attenuates aggressive behavior in a rat model of pilocarpine-induced epilepsy

Xiaoxing Huang, John McMahon, and Yunfei Huang*

Center for Neuropharmacology and Neuroscience, Albany Medical College, NY, USA

Abstract

Psychiatric disorders are fairly common comorbidities of epilepsy in humans. Following pilocarpine-induced status epilepticus (SE), experimental animals not only developed spontaneous recurrent seizures, but also exhibited significantly elevated levels of aggressive behavior. The cellular and molecular mechanism triggering these behavioral alterations remains unclear. In the present study, we found that aggression is positively correlated with development of spontaneous seizures. Treatment with rapamycin, a potent mTOR pathway inhibitor, markedly diminished aggressive behavior. Therefore, the mTOR pathway may have significance in the underlying molecular mechanism leading to aggression associated with epilepsy.

Keywords

mTOR; epilepsy; psychiatric comorbidities; rapamycin; seizure

Introduction

Psychiatric disorders are fairly common comorbidities of epilepsy (Tellez-Zenteno et al., 2007, Garcia-Morales et al., 2008). The prevalence of psychiatric disorders is around 19–80% depending on the type and severity of epilepsy (Swinkels et al., 2005), being the highest in temporal lobe epilepsy (TLE) (Perini et al., 1996). Psychiatric disorders and epilepsy can reciprocally exacerbate each other. Psychiatric disturbances can occur around the same time as seizures. They are recognized as peri-ictal symptoms, by which the psychiatric behaviors can be initiated or exacerbated immediately before, during or following the seizures (Kanner and Balabanov, 2002, Mula and Monaco, 2011). Psychiatric disorders sometimes also independently precede the onset of spontaneous recurrent seizures, which suggests that the underlying mechanism(s) of psychiatric disorders may trigger seizures (Hesdorffer et al., 2000). Recent studies in animal models suggest that psychiatric symptoms may serve as an indication of development of spontaneous seizures and pharmacological resistance to antiepileptic drugs (Gastens et al., 2008). This reciprocal relationship may reflect that epilepsy and its comorbidities share related cellular neuropathological mechanisms. Indeed, initial neurological insults such as SE often induce alterations in several brain regions that are well known to mediate both psychiatric behaviors and seizures (Sheline et al., 2003, Trimble and Van Elst, 2003, Garcia-Morales et al., 2008).

© 2012 IBRO. Published by Elsevier Ltd. All rights reserved.

*To whom correspondence should be addressed: Yunfei Huang, MD, PhD., Center for Neuropharmacology and Neuroscience, Albany Medical College, Albany, NY 12208, USA, Phone: (518) 262-5873, Fax: (518) 262-5799, huangy@mail.amc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Aggression is one of several psychiatric disorders that have long been observed in epileptic patients including those with temporal lobe epilepsy (TLE) (Kligman and Goldberg, 1975, van Elst et al., 2000, Sumer et al., 2007), cortical dysplasia (Granieri and Fazio, 2011) and tuberous sclerosis complex (TSC) (Muzykewicz et al., 2007). This association has been reliably replicated in several animal models including those using pilocarpine (Liu et al., 1994, Rice et al., 1998, Desjardins et al., 2001) and domoic acid (Fuquay et al., 2012), in which aggression develops either in parallel to spontaneous seizures or precedes the development of recurrent seizures. The molecular mechanism underlying this comorbidity remains unclear. In animal models, aggressive behaviors tend to occur in animals with severe brain damage caused by initial status epilepticus (SE) (Desjardins and Persinger, 1995). Brain damages in hypothalamus and septum were also reported to be involved in the aggressive behavior (Toth et al., 2010, Comai et al., 2011).

Recent studies suggest an important role of the mammalian target of rapamycin pathway (mTOR) in epilepsy. Hyperactivation of mTOR is responsible for genetically inherited epilepsies such as TSC and phosphatase and tensin homolog (PTEN) in both patients (Krueger et al., 2010) and animal models (Meikle et al., 2008, Zeng et al., 2008, Zhou et al., 2009). mTOR activity was found to be elevated in animal brains of acquired epilepsy (Buckmaster et al., 2009, Zeng et al., 2009, Huang et al., 2010, Raffo et al., 2011, Sunnen et al., 2011). Inhibition of mTOR by rapamycin has been reported to attenuate epileptogenesis, chronic seizures, and pathological changes in the hippocampus and even the improvement of cognitive functions in experimental animal models (Buckmaster et al., 2009, Zeng et al., 2009, Huang et al., 2010, Raffo et al., 2011, Sunnen et al., 2011), albeit not in a mouse model (Buckmaster and Lew, 2011). During our routine handling of rats for seizure monitoring we found that rats in the pilocarpine-treated group displayed overt struggling and exhibited aggressive behaviors such as biting (see Supplemental Videos 1 & 2). We also noticed that following rapamycin treatment, rats became calmer during handling (see Supplemental Video 3). These observations led us to further evaluate the effect of rapamycin on psychiatric behavior. We particularly focused on aggressive behavior as it is a prominent and easily detectable behavioral change in the rat model of pilocarpine-induced epilepsy.

Material and Methods

Animals

Sprague-Dawley rats were purchased from Taconic (Taconic, NY). Rats were housed in a room with *ad libitum* access to food and water. All experiments were performed in accordance with National Institutes of Health guidelines for the care and use of laboratory animals and were approved by our Institutional Animal Care and Use Committees.

Induction of status epilepticus by pilocarpine

The experiment started with a total of 150 age-matched Sprague-Dawley rats (50–75g). Of which, 113 rats were first injected i.p. with solution of LiCl (3 mEq/kg; 1 ml/kg) 16 h prior to i.p administration of pilocarpine hydrochloride (40 mg/kg; 1 ml/kg) as described (Hirsch et al., 1992). The remaining 37 rats were used as control and were handled and housed in the same manner as the pilocarpine-treated animals and received an equal volume of saline solution (0.9% NaCl). Rats were then put into individual clear plexiglass cages to allow optimal video recording under infrared light. Immediately following injection of pilocarpine seizure activity was continuously recorded by a DVR video surveillance system (*The Advanced Security Inc, Chino Hills, CA*) for 90 min. Seizure events were graded according to Racine's standard classification (Racine, 1972): Stage 1, behavioral arrest with mouth and facial movement; stage 2: head nodding; stage 3: forepaw clonus; stage 4: rearing and stage

5: rearing and falling. The majority (n=101) of pilocarpine-treated rats developed acute stage 5 seizures. Twelve rats did not initially develop stage 5 seizures, but did after receiving an additional dose of 10 mg/kg of pilocarpine. Seizures were terminated with sodium pentobarbital (25 mg/kg,i.p.) 90 min after administration of pilocarpine. The rats were allowed to spontaneously recover from pentobarbital treatment. Thirty four rats died within two weeks after pilocarpine treatment. All rats were kept in individual cages throughout the experiments.

Behavioral seizure monitoring

Thirty three pilocarpine-treated rats were monitored for spontaneous recurrent seizures for 12 hr/day by DVR video surveillance from weeks 3 to 5 after pilocarpine injection. Videos were then examined by the trained researchers and graded using the Racine scale (Racine, 1972). In the present study only stage 3–5 seizures were counted as they are most easily deciphered from typical rat behavior using video surveillance. After 3 weeks of monitoring, we found 17 (51.5%) of pilocarpine-treated rats developed spontaneous seizures. Pilocarpine-treated rats either with or without spontaneous seizures were then assigned to one of two groups. One group was further treated with rapamycin, the other group was treated with an equal volume of vehicle. EEGs were not performed due to concerns that surgical implantation of EEG electrodes could interfere with behavioral testing. We acknowledge that video recording could miss a small portion of animals that developed mild behavioral seizures or electrographic seizures without a behavioral manifestation. In such a case, those animals would have been assigned to the non-seizure pilocarpine-treated group.

Rapamycin treatment

Immediately before administration, rapamycin (*Tecoland*) was first dissolved in DMSO (*Sigma*) and diluted in a solution containing 5% tween-20 and 4% ethanol (defined as vehicle). To determine if rapamycin can suppress aggressive behavior, 10 control and 12 pilocarpine-treated rats were given rapamycin 5 mg/kg i.p. for 3 consecutive days, followed by every other day during a series of behavioral tests. In parallel, 10 additional control rats and 12 pilocarpine treated rats were given equal volume of vehicle. To determine if the effect of rapamycin is reversible, 12 pilocarpine treated rats were treated with rapamycin at 5 mg/kg daily for three days. Ten pilocarpine-treated and 5 control rats (untreated with pilocarpine) received an equal volume of vehicle daily for three days. Rapamycin treatment was administered starting 5–6 weeks after initial treatment with pilocarpine.

Behavioral Tests

Excitability and sensory responsiveness of pilocarpine-treated and control rats were evaluated by three behavioral tests which include approach-response, touch response, and pick up as described (Rice et al., 1998, Gastens et al., 2008). The behavioral test was administered 5–6 weeks after pilocarpine treatment or 16 hr after rapamycin treatment. Each rat was tested every other day between 9:00 to 12:00 pm for a total of five times. With experiments to determine the time course of rapamycin's effect, tests were carried out daily between 1:00 to 5:00 pm. Before each test, individual rats were gently transferred to a designated testing area and habituated for 15 min. All tests were video recorded. The responsiveness to the test was graded using the Racine scale (Racine, 1972). Videos for behavior analysis were encoded so as to prevent the grader from knowing whether individual animals were in the test or control groups.

1. Approach-response test—A pen held vertically is moved slowly toward the face of the animal. Responses were scored as 1, no reaction; 2, the rat sniffs at the object; 3, the rat

moves away from the object; 4, the rat freezes; 5, the rat jumps away from the object; and 6, the rat jumps at or attacks the object.

2. Touch-response test—The animal is gently prodded in the rump with the blunt end of a pen. Responses were scored as 1, no reaction; 2, the rat turns toward the object; 3, the rat moves away from the object; 4, the rat freezes; 5, the rat jerks around toward the touch; 6, the rat turns away from the touch; and 7, the rat jumps with or without vocalizations.

3. Pick-up test—The animal is picked up by grasping around the body. Responses were scored as 1, very easy; 2, easy with vocalizations; 3, some difficulty, the rat rears and faces the hand; 4, the rat freezes (with or without vocalization); 5, difficult, the rat avoids the hand by moving away; and 6, very difficult, the rat behaves defensively, and may attack the hand.

4. Resident-Intruder Test—The resident-intruder test has been widely used to investigate offensive behavioral such as aggression (Koolhaas et al., 1980, Frye et al., 2002). Intruder rats are temporarily made anosmic (loss of ability to smell) to reduce the likelihood of retaliation towards resident rats. One week before the test, intruder rats (SD, 150 g) received daily intranasal zinc applications for four days as described (Frye et al., 2002). Resident rats were habituated in the observation cages for 7 days to facilitate territorial behavior. Before each test, individual rats were gently transferred to a designated testing area and habituated for 15 min. All tests were performed between 9:00 to 12:00 pm. After habituation on the testing day, the intruder was introduced into the resident's cage for 5 min and the test terminated if the intruder receives a maximum of 20 attacks (Frye et al., 2002). An attack was defined as overt aggressive behavior resulting in direct dental contact between rats. Each test session was video recorded and the number of attacks by resident rats (test subjects) was determined.

Statistical analysis

Statistic analysis was performed using GraphPad Prism. T-tests were used to determine significant differences between two groups. When the number of experimental groups exceeded two, significant differences between groups were determined based on one way ANOVA along with post hoc tests with $P < 0.05$ taken as significant. Results are presented as group means (with N = number of animals/group) $\pm$ standard error of the mean (SEM).

Results

Hyperexcitability in pilocarpine-treated rats in response to stimuli

To determine aggressive behavior in pilocarpine-treated rats, we employed the battery of four behavioral tests described in this report (Rice et al., 1998, Gastens et al., 2008). We found that pilocarpine-treated rats exhibited significantly elevated levels of aggression in response to external stimuli in all behavioral tests. With the approach-response test, the average score in control rats was 0.87 ± 0.09 versus 1.82 ± 0.14 in pilocarpine-treated rat (fig 1a). With the touch-response test, the average score for control rats was 0.92 ± 0.11 versus 1.57 ± 0.12 in the pilocarpine-treated rats (fig 1b). With the pick-up test, the average score for control rats was 1.20 ± 0.06 whereas in pilocarpine-treated rats the average score was 3.07 ± 0.27 (fig 1c). In the resident-intruder test, we found that 44 % ($n=33$) of pilocarpine-treated rats attacked their intruders at least once during a 5 min period (fig. 1d) with 1.62 average attacks per test session (fig. 1e). Only 1 ($n=12$) rats in the control group exhibited attacking behavior. These results confirm our observations that pilocarpine-treated rats displayed significantly increased levels of aggressive behavior over non-treated control rats.

Behavioral alteration correlates with development of spontaneous seizures

Behavioral changes typically evolve along with the development of spontaneous seizures following SE. A recent study revealed that rats with behavioral changes tend to be resistant to antiepileptic treatment (Gastens et al., 2008). Accordingly, we investigated the association of spontaneous seizures with aggressive behavior. Video records of each rat taken over a three week period revealed that among 33 pilocarpine-treated rats, 17 developed spontaneous seizures. The behavioral data were then sorted and analyzed according to rats with or without spontaneous seizures. Pilocarpine-treated rats in both groups (with or without spontaneous seizures) showed significantly higher scores for aggression than control rats in the approach-response test, the touch-response test, and the pick-up test (fig 2a–2c). Pilocarpine-treated rats with spontaneous seizures had significantly higher scores for aggression in the pick-up test than pilocarpine-treated rats without detectable seizures (3.69 ± 0.37 versus 2.40 ± 0.35) (fig 2c). Similarly, we noted that in the resident-intruder test, about 25% of pilocarpine-treated rats without spontaneous seizures exhibited attacking behavior with an average of 1.10 attacks per rat, while 63.54% of rats with spontaneous seizures exhibited attacking behavior with an average of 2.12 attacks per rat (fig 2d & 2e). Only 1 (n=12) rat in the control displayed attacking behavior. These data suggest a positive correlation the development of spontaneous seizures and aggressive behavior.

Rapamycin suppresses behavioral changes

Previous studies have reported that rapamycin attenuates epileptogenesis, chronic seizures, and improves cognitive functions (Meikle et al., 2008, Zeng et al., 2009, Zhou et al., 2009, Huang et al., 2010, Raffo et al., 2011, Sunnen et al., 2011). As behavioral alterations often develop in parallel with spontaneous seizures, we attempted to determine if rapamycin would have a direct and measurable effect on the aggressive behavior. Pilocarpine-treated rats were treated with rapamycin daily for three days and then continuously received treatment every other day during the 5 behavioral tests that were started 16 hr after the first injection of rapamycin and then performed every other day for 10 days. Rapamycin markedly reduced aggressive behavior in all behavioral tests including approach-response, touch-response, pick-up, and resident-intruder tests (2.53 ± 0.17 versus 1.17 ± 0.17 (fig 3a), 2.18 ± 0.19 versus 1.21 ± 0.10 (fig 3b), 4.24 ± 0.39 versus 1.83 ± 0.30 (fig 3c), 48.00 ± 6.63 versus 28.00 ± 4.90 (fig 3d), 2.02 ± 0.65 versus 0.69 ± 0.29 (fig 3e) in the group treated with pilocarpine versus in the group treated with both pilocarpine and rapamycin) (see Supplemental video 1–3).

The behavioral improvement by rapamycin is transient

We previously reported that rapamycin-mediated suppression of seizures is temporary (Huang et al., 2010), with seizures recurring shortly after cessation of rapamycin treatment. Considering the linkage of seizures with behavioral changes in epilepsy, we wished to determine the duration of behavioral improvement by rapamycin. We employed the pick-up test in this experiment because we have found that the response of rats is consistent and robust. Pilocarpine-treated rats and untreated control rats were given either rapamycin or vehicle on three consecutive days, beginning on day 7. The pick-up test was performed daily over a period of 32 days (fig. 4). We found that rapamycin treatment improved aggressive behavioral within 16 hr after the first dose (fig 4a). These lowered levels of aggressive behavior were maintained during the three-day treatment period. However, one week after the cessation of rapamycin treatment aggressive behavioral returned to the pre-treatment level. The aggressive behavior in the second group of pilocarpine-treated rats that were treated with the vehicle only remained largely unchanged over the 32 day course of testing, though a trend of slight elevation (fig 4b) was noted. Control rats that were not treated with pilocarpine or rapamycin and were only given the vehicle maintained a very low behavioral score over the course of testing (fig 4c).

Discussion

Aggressive behavior, a comorbidity of epilepsy, has long been observed in pilocarpine-treated rats, however, the molecular and cellular mechanism of this aggression is unclear. In this study we found that administration of rapamycin to pilocarpine-treated rats led to a significant improvement of aggression within 16 hours. However, this positive effect was gradually reversed within a week after cessation of rapamycin treatment. Similarly, in a previous study with the same model, we observed a significant reduction in seizure events within days of rapamycin treatment (Huang et al., 2010). Therefore, the improvement of aggressive behavior by rapamycin clearly parallels the reduction in frequency of spontaneous seizures. As rapamycin is a potent mTOR inhibitor, these findings raise the possibility that hyperactivation of mTOR may be a common pathogenic mechanism in both epilepsy and its associated aggression. It is currently unclear if the effects of rapamycin on seizures and its comorbidity aggression are independent or linked. As seizures and aggression can exacerbate each other, conceivably rapamycin could independently attenuate either spontaneous seizures or aggression, which, in turn, would then influence the other. To further understanding the mechanism of action of rapamycin, one possible approach would be to use either anti-epileptic or anti-psychiatric drugs as probes to selectively target each of them individually. However, there is an overt limitation in this method because many standard anti-epileptic drugs such as valproic acid and phenytoin not only suppress seizures, but also possess anti-psychiatric effect (Comai et al., 2012a, Comai et al., 2012b). Similarly, psychiatric drugs were also reported to have various effects on epilepsy (Ruffmann et al., 2006). Therefore it will be difficult to get any conclusive result. We acknowledge that further study should be conducted when adequate tools are available. Results from our previous and present studies indicate that rapamycin has dual anti-epileptic/antiaggression properties. Again, some anti-epileptic drugs (AEDs) such as valproic acid also possess dual activities (Comai et al., 2012a, Comai et al., 2012b). It will be interesting to see if other AEDs have a similar effect in this model in the future.

We observed that pilocarpine-treated rats gradually became aggressive, which replicates the previous findings (Liu et al., 1994, Rice et al., 1998, Desjardins et al., 2001). We also found that rats with overt aggressive behavior tend to develop early onset spontaneous seizures. This is in agreement with previous studies that report that rats with psychiatric behaviors are also likely to have refractory seizures (Gastens et al., 2008). The mechanistic role of mTOR in aggression remains to be elucidated. However, the therapeutic effect of rapamycin appears to be temporary, as we observed the return of aggression within one week of cessation of administration. This observation is consistent with recent reports that chronic seizures and mossy fiber sprouting reoccur even after a prolonged rapamycin treatment ranging from 3 to 8 weeks (Huang et al., 2010, Lew and Buckmaster, 2011). This seems to indicate that rapamycin can only temporally attenuate, but not completely negate the underlying mechanism resulting in spontaneous seizures and aggression. It remains to be determined if rapamycin has any effect on depression, a psychiatric disorder frequently associated with epilepsy (Mazarati et al., 2008). In the present study, we used rapamycin at 5 mg/kg rather than testing other doses because this dose has ready been demonstrated to effectively inhibit mTOR activity (Huang et al., 2010). More importantly, this dose has been shown to attenuate chronic spontaneous seizures in the same model (Huang et al., 2010).

The precise molecular mechanism of mTOR in regulating aggression in this model remains to be elucidated. At the molecular level, the mTOR pathway has been implicated in various neuronal processes including regulating axonal growth, synaptogenesis, and expression of receptor/channels (Jaworski and Sheng, 2006). All these processes play an important role in regulating neuronal plasticity and excitability (Hoeffer and Klann, 2010) and are likely altered in response to elevated mTOR activity in the brains in the pilocarpine model (Huang

et al., 2010). It is conceivable that rapamycin could act through attenuating these processes. At a cellular level, pilocarpine induces wide spread damages to many regions (Honchar et al., 1983) of the brain including these known to regulate aggressive behavior, such as amgdala, hypothalamus and septum (Malick, 1970, Eclancher and Schmitt, 1972, Toth et al., 2010, Comai et al., 2011). Future study may need to address if rapamycin's effect on aggression involves these structures.

In summary, psychiatric comorbidities, including aggression, have been recognized as a part of epilepsy. Previous studies with experimental animals reported that rapamycin reduced seizures. Our data suggest rapamycin treatment also temporarily attenuates aggressive behavior in the rat pilocarpine model for epilepsy, suggesting that hyperactivation of mTOR may be a common mechanism underlying both the development of aggression and seizures. Our findings suggest that rapamycin or other mTOR pathway inhibitors could have significance in the clinical management of epilepsy and its associated psychiatric comorbidities which include aggressive behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Drs. Lauren Jacobson and Cheryl Frye for assistance on plasma corticosterone assay and resident-intruder test respectively. This work was supported by grants from the National Institute of Neurological Disorders and Strokes NS062068 (to YH) and NARSAD (Young Investigator award)(to YH).

References

- Buckmaster PS, Ingram EA, Wen X. Inhibition of the mammalian target of rapamycin signaling pathway suppresses dentate granule cell axon sprouting in a rodent model of temporal lobe epilepsy. *J Neurosci.* 2009; 29:8259–8269. [PubMed: 19553465]
- Buckmaster PS, Lew FH. Rapamycin suppresses mossy fiber sprouting but not seizure frequency in a mouse model of temporal lobe epilepsy. *J Neurosci.* 2011; 31:2337–2347. [PubMed: 21307269]
- Comai S, Tau M, Gobbi G. The Psychopharmacology of Aggressive Behavior: A Translational Approach: Part 1: Neurobiology. *J Clin Psychopharmacol.* 2011
- Comai S, Tau M, Gobbi G. The psychopharmacology of aggressive behavior: a translational approach: part 1: neurobiology. *Journal of clinical psychopharmacology.* 2012a; 32:83–94. [PubMed: 22198449]
- Comai S, Tau M, Pavlovic Z, Gobbi G. The psychopharmacology of aggressive behavior: a translational approach: part 2: clinical studies using atypical antipsychotics, anticonvulsants, and lithium. *Journal of clinical psychopharmacology.* 2012b; 32:237–260. [PubMed: 22367663]
- Desjardins D, Parker G, Cook LL, Persinger MA. Agonistic behavior in groups of limbic epileptic male rats: pattern of brain damage and moderating effects from normal rats. *Brain Res.* 2001; 905:26–33. [PubMed: 11423076]
- Desjardins D, Persinger MA. Association between intermale social aggression and cellular density within the central amygdaloid nucleus in rats with lithium/pilocarpine-induced seizures. *Percept Mot Skills.* 1995; 81:635–641. [PubMed: 8570370]
- Eclancher F, Schmitt P. Effect of early lesions of the amygdala and median hypothalamus on the development of interspecific aggression behavior in rats. *J Physiol (Paris).* 1972; 65(Suppl):231A.
- Frye CA, Rhodes ME, Walf A, Harney JP. Testosterone enhances aggression of wild-type mice but not those deficient in type I 5alpha-reductase. *Brain Res.* 2002; 948:165–170. [PubMed: 12383970]
- Fuquay JM, Muha N, Pennington PL, Ramsdell JS. Domoic acid induced status epilepticus promotes aggressive behavior in rats. *Physiol Behav.* 2012; 105:315–320. [PubMed: 21875611]

- Garcia-Morales I, de la Pena Mayor P, Kanner AM. Psychiatric comorbidities in epilepsy: identification and treatment. *Neurologist*. 2008; 14:S15–25. [PubMed: 19225366]
- Gastens AM, Brandt C, Bankstahl JP, Loscher W. Predictors of pharmacoresistant epilepsy: pharmacoresistant rats differ from pharmacoresponsive rats in behavioral and cognitive abnormalities associated with experimentally induced epilepsy. *Epilepsia*. 2008; 49:1759–1776. [PubMed: 18494789]
- Granieri E, Fazio P. The Lombrosian prejudice in medicine. The case of Epilepsy. *Epileptic psychosis. Epilepsy and aggressiveness*. *Neurol Sci*. 2011
- Hesdorffer DC, Hauser WA, Annegers JF, Cascino G. Major depression is a risk factor for seizures in older adults. *Ann Neurol*. 2000; 47:246–249. [PubMed: 10665498]
- Hirsch E, Baram TZ, Snead OC 3rd. Ontogenic study of lithium-pilocarpine-induced status epilepticus in rats. *Brain Res*. 1992; 583:120–126. [PubMed: 1324090]
- Hoeffler CA, Klann E. mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends Neurosci*. 2010; 33:67–75. [PubMed: 19963289]
- Honchar MP, Olney JW, Sherman WR. Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats. *Science*. 1983; 220:323–325. [PubMed: 6301005]
- Huang X, Zhang H, Yang J, Wu J, McMahon J, Lin Y, Cao Z, Gruenthal M, Huang Y. Pharmacological inhibition of the mammalian target of rapamycin pathway suppresses acquired epilepsy. *Neurobiol Dis*. 2010; 40:193–199. [PubMed: 20566381]
- Jaworski J, Sheng M. The growing role of mTOR in neuronal development and plasticity. *Mol Neurobiol*. 2006; 34:205–219. [PubMed: 17308353]
- Kanner AM, Balabanov A. Depression and epilepsy: how closely related are they? *Neurology*. 2002; 58:S27–39. [PubMed: 11971130]
- Kligman D, Goldberg DA. Temporal lobe epilepsy and aggression. *J Nerv Ment Dis*. 1975; 160:324–341. [PubMed: 1151352]
- Koolhaas JM, Schuurman T, Wiepkema PR. The organization of intraspecific agonistic behaviour in the rat. *Prog Neurobiol*. 1980; 15:247–268. [PubMed: 7005965]
- Krueger DA, Care MM, Holland K, Agricola K, Tudor C, Mangeshkar P, Wilson KA, Byars A, Sahmoud T, Franz DN. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med*. 2010; 363:1801–1811. [PubMed: 21047224]
- Lew FH, Buckmaster PS. Is there a critical period for mossy fiber sprouting in a mouse model of temporal lobe epilepsy? *Epilepsia*. 2011; 52:2326–2332. [PubMed: 22092282]
- Liu Z, Gatt A, Werner SJ, Mikati MA, Holmes GL. Long-term behavioral deficits following pilocarpine seizures in immature rats. *Epilepsy Res*. 1994; 19:191–204. [PubMed: 7698095]
- Malick JB. A behavioral comparison of three lesion-induced models of aggression in the rat. *Physiology & behavior*. 1970; 5:679–681. [PubMed: 5535529]
- Mazarati A, Siddarth P, Baldwin RA, Shin D, Caplan R, Sankar R. Depression after status epilepticus: behavioural and biochemical deficits and effects of fluoxetine. *Brain*. 2008; 131:2071–2083. [PubMed: 18559371]
- Meikle L, Pollizzi K, Egnor A, Kramvis I, Lane H, Sahin M, Kwiatkowski DJ. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *J Neurosci*. 2008; 28:5422–5432. [PubMed: 18495876]
- Mula M, Monaco F. Ictal and peri-ictal psychopathology. *Behav Neurol*. 2011; 24:21–25. [PubMed: 21447894]
- Muzykewicz DA, Newberry P, Danforth N, Halpern EF, Thiele EA. Psychiatric comorbid conditions in a clinic population of 241 patients with tuberous sclerosis complex. *Epilepsy Behav*. 2007; 11:506–513. [PubMed: 17936687]
- Perini GI, Tosin C, Carraro C, Bernasconi G, Canevini MP, Canger R, Pellegrini A, Testa G. Interictal mood and personality disorders in temporal lobe epilepsy and juvenile myoclonic epilepsy. *J Neurol Neurosurg Psychiatry*. 1996; 61:601–605. [PubMed: 8971108]
- Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol*. 1972; 32:281–294. [PubMed: 4110397]

- Raffo E, Coppola A, Ono T, Briggs SW, Galanopoulou AS. A pulse rapamycin therapy for infantile spasms and associated cognitive decline. *Neurobiol Dis.* 2011; 43:322–329. [PubMed: 21504792]
- Rice AC, Floyd CL, Lyeth BG, Hamm RJ, DeLorenzo RJ. Status epilepticus causes long-term NMDA receptor-dependent behavioral changes and cognitive deficits. *Epilepsia.* 1998; 39:1148–1157. [PubMed: 9821978]
- Ruffmann C, Bogliun G, Beghi E. Epileptogenic drugs: a systematic review. *Expert Rev Neurother.* 2006; 6:575–589. [PubMed: 16623656]
- Sheline YI, Gado MH, Kraemer HC. Untreated depression and hippocampal volume loss. *Am J Psychiatry.* 2003; 160:1516–1518. [PubMed: 12900317]
- Sumer MM, Atik L, Unal A, Emre U, Atasoy HT. Frontal lobe epilepsy presented as ictal aggression. *Neurol Sci.* 2007; 28:48–51. [PubMed: 17385097]
- Sunnen CN, Brewster AL, Lugo JN, Vanegas F, Turcios E, Mukhi S, Parghi D, D’Arcangelo G, Anderson AE. Inhibition of the mammalian target of rapamycin blocks epilepsy progression in NS-Pten conditional knockout mice. *Epilepsia.* 2011; 52:2065–2075. [PubMed: 21973019]
- Swinkels WA, Kuyk J, van Dyck R, Spinhoven P. Psychiatric comorbidity in epilepsy. *Epilepsy Behav.* 2005; 7:37–50. [PubMed: 15975853]
- Tellez-Zenteno JF, Patten SB, Jette N, Williams J, Wiebe S. Psychiatric comorbidity in epilepsy: a population-based analysis. *Epilepsia.* 2007; 48:2336–2344. [PubMed: 17662062]
- Toth M, Fuzesi T, Halasz J, Tulogdi A, Haller J. Neural inputs of the hypothalamic “aggression area” in the rat. *Behav Brain Res.* 2010; 215:7–20. [PubMed: 20685366]
- Trimble MR, Van Elst LT. The amygdala and psychopathology studies in epilepsy. *Ann N Y Acad Sci.* 2003; 985:461–468. [PubMed: 12724177]
- van Elst LT, Woermann FG, Lemieux L, Thompson PJ, Trimble MR. Affective aggression in patients with temporal lobe epilepsy: a quantitative MRI study of the amygdala. *Brain.* 2000; 123 (Pt 2): 234–243. [PubMed: 10648432]
- Zeng LH, Rensing NR, Wong M. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. *J Neurosci.* 2009; 29:6964–6972. [PubMed: 19474323]
- Zeng LH, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol.* 2008; 63:444–453. [PubMed: 18389497]
- Zhou J, Blundell J, Ogawa S, Kwon CH, Zhang W, Sinton C, Powell CM, Parada LF. Pharmacological inhibition of mTORC1 suppresses anatomical, cellular, and behavioral abnormalities in neural-specific Pten knock-out mice. *J Neurosci.* 2009; 29:1773–1783. [PubMed: 19211884]

A positive correlation between incidence of aggression and development of seizures.
Rapamycin suppresses aggressive behavior.
Aggression relapses after the cessation of rapamycin treatment.

Figure 1.

Pilocarpine-treated rats exhibited aggressive behaviors. Pilocarpine-treated rats (Pilo) showed hyperexcitatory reactions in approach-response (A), touch-response (B) and pick-up (C) tests. In the resident-intruder test, significantly more rats in the pilocarpine-treated group (Pilo) displayed attacking behavior to intruders (D) along with increased average number of attacks per animal (E). N=12 for the control and 33 for the pilocarpine-treated rats. Data are presented as mean $\pm$ SE. ** indicates $P < 0.01$ with T-test.

Figure 2.

Comparison of aggressive behaviors between the groups of rats with spontaneous recurrent seizures (SRS (+)) and without seizures (SRS (-)). The symbols ** or * indicate significant difference between the control rats (N=12) and the pilocarpine-treated rats either with (N=17) or without behavioral seizures (N=16) with $P < 0.01$ or 0.05 respectively by ANOVA in all four tests. The symbol ## indicates significant difference between the groups of rats with seizures and without seizures with $P < 0.01$ by one way ANOVA. Data are presented as mean $\pm$ SE.

Figure 3.

Rapamycin suppresses aggressive behaviors. The average scores in the pilocarpine-treated group (N=12) are significantly lower than that in the other three groups including control (N=10), control plus rapamycin (Rapa) (N=10), and Pilo plus Rapa (N=12) (a–e). The symbols ** or * indicate $P < 0.01$ or 0.05 respectively. The # indicates a statistical difference between the Pilo plus Rapa group and the control or control plus Rapa groups with $P < 0.05$ (d). The data are presented as mean $\pm$ SE and analyzed using one way ANOVA.

Figure 4.

Transient effect of rapamycin on aggressive behavior. Pilocarpine-treated rats were treated with either rapamycin at 5 mg/kg (a) (N=12) or vehicle (b) (N=10) daily from day 7 to 9. Similarly, control rats (N=5) were treated with vehicle (c). The data are presented as mean $\pm$ SE and analyzed using one way ANOVA. The * indicates a statistic difference of the average scores as compared with that on day 7 (a).