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5-Aminovaleric Acid Suppresses the Development of Severe Seizures in the Methionine Sulfoximine Model of Mesial Temporal Lobe Epilepsy

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Abstract

Mesial temporal lobe epilepsy (MTLE) is one of the most common forms of drug-resistant, localization-related epilepsies in humans. One potential therapeutic target is the brain glutamine-glutamate-GABA metabolic pathway, which is perturbed in patients with MTLE. Loss of glutamine synthetase (GS) in astrocytes may be critically involved in this perturbation, which can be modeled by infusing the GS inhibitor methionine sulfoximine (MSO) into the entorhinal-hippocampal area in rats. Because 5-aminovaleric acid (5-AV) has been implicated in modulation of the glutamine-glutamate-GABA metabolic pathway, we hypothesized that 5-AV would alter the expression of seizures in the MSO model of MTLE.

Male Sprague Dawley rats (300-330 g) were implanted with an Alzet pump placed subcutaneously in the abdominal region to release either 5-AV (0.05 mg/mL, n = 6) or phosphate buffered saline (PBS, n = 6) at a rate of 2.5 μ L/hr over 28 days. Five to 7 days after surgery, all rats were implanted with an intracranial pump infusing MSO (2.5 mg/mL; 0.25 μ L/hr) unilaterally into the hippocampal formation. Following the second surgery, intracranial EEG was measured from the left and right hemispheres above the dorsal hippocampal formations for a continuous period of 21 days. The EEG was correlated with simultaneous video recordings to determine the stage of seizures according to a modified Racine scale.

Five-AV-treated rats experienced a 3.5 fold reduction in the number of seizures (6.7 ± 1.4 seizures/day) than PBS-treated rats (23.2 ± 6.3 seizures/day) during the first 2 days following

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MSO pump placement ($p < 0.005$). Both groups showed similar seizure frequency over days 3-21 (~ 1 seizure/day). However, the fraction of the most severe type of seizures (Racine stage 4 and 5) increased over time in the PBS treated group, but not in the 5-AV treated group. Notably, 5-AV treated rats experienced a 2.3 and 2.6 fold lower fraction of stage 4 and 5 seizures than PBS-treated rats during the 2nd and 3rd week of MSO treatment respectively ($p < 0.05$ and $p < 0.001$ respective to week).

Five-AV markedly reduces the number of seizures initially and suppresses the development of the most severe type of seizures in the MSO model of MTLE. These results may have implications for the therapeutic use of 5-AV in treating mesial temporal lobe seizures and for our understanding of the chemical pathology of epileptogenesis and MTLE.

Keywords

astrocytes; epileptogenesis; GABA; gliosis; glutamate; glutamine; glutamine synthetase; hippocampus

Mesial temporal lobe epilepsy (MTLE) is one of the most common forms of drug-resistant, localization-related epilepsies in humans (Hauser et al., 1991), and more efficacious treatments for this disease are therefore necessary. However, the discovery of better treatments has been lagging, partly due to a lack of in vivo models that recapitulate key features of the human condition. We recently developed a novel model of MTLE by chronic infusion of methionine sulfoximine (MSO) unilaterally into the hippocampal formation of rats (Wang et al., 2009). This paradigm results in inhibition of glutamine synthetase (GS) in astrocytes, and development of chronic temporal lobe seizures. Both of these features resemble human MTLE (Eid et al., 2004; van der Hel et al., 2005).

GS is the only enzyme in mammals known to effectively synthesize glutamine, which is critical for producing two of the most abundant excitatory and inhibitory neurotransmitters: glutamate and GABA (Petroff et al., 2002). GS is also important for clearance of glutamate released during excitatory synaptic transmission and for metabolism of brain ammonia (Albrecht and Jones, 1999; Martinez-Hernandez et al., 1977). A large proportion of extracellular glutamate is taken up by high affinity excitatory amino acid transporters present on the plasma membrane of astrocytes (Danbolt, 2001). Once in the astrocyte, glutamate binds to GS along with ammonia and ATP, for enzymatic conversion to glutamine and ADP. The known stoichiometry of glutamate transport across the astroglial plasma membrane suggests that rapid metabolism of intracellular glutamate via GS is a prerequisite for efficient glutamate clearance from the extracellular space (Otis and Jahr, 1998). A loss of GS in astrocytes is therefore likely to perturb the homeostasis of glutamine, glutamate, GABA, and ammonia in the brain. Studies using magnetic resonance spectroscopy have suggested that perturbations in the glutamine-glutamate-GABA pathway are present in human MTLE (Petroff et al., 2002), whereas alterations in the ammonia homeostasis are less well documented.

Due to the alterations in the glutamine-glutamate-GABA homeostasis in MTLE, we hypothesize that compounds with actions directed towards these metabolites, may represent

a novel and more effective therapeutic approach for this disorder. Here we test the hypothesis that one such compound – 5-aminovaleric acid (5-AV) – reduces the frequency, duration, and clinical severity of seizures in our model of MTLE. Five-AV is a GABA analogue (Fritsche et al., 1999; Nanavati and Silverman, 1989), which has been reported to inhibit GABA uptake (Tasaka et al., 1989), inactivate GABA aminotransferase (Nanavati and Silverman, 1989), and to both agonize and antagonize GABA_b receptors. A particularly interesting feature of 5-AV is its ability to increase brain glutamine concentrations, thereby possibly compensating for the reduced concentration of brain glutamine caused by the loss of GS in MTLE (Samuels et al., 1983).

Materials and Methods

Chemicals and animals

All chemicals were obtained from Sigma Chemical Co. (St. Louis, Mo.) unless otherwise noted. Male Sprague Dawley rats were obtained from Charles River Laboratories (Wilmington, Mass.) at a mass of 300 to 330 g. Upon arrival, rats were group housed (2-3/cage) and maintained in a temperature-controlled colony room (21°C–23°C) on a 12 h light–dark cycle. Rats were allowed free access to food and water, and were given at least 1 week of acclimation prior to surgery. All procedures were approved by the Institutional Animal Care and Use Committee at Yale University and were conducted in accordance with current guidelines.

Surgery

Rats were anesthetized with 1-2% Isoflurane (Baxter, Deerfield, Ill.) in O₂ and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, Calif.). The animals were implanted with an Alzet osmotic pump (Model 2ML4, Durect Corp., Cupertino, Calif.) in the subcutaneous abdominal region. The pump, which delivers a continuous flow of 2.5 µL/hr for approximately 28 days, released either 5-AV [0.05 mg/mL, dissolved in phosphate buffered saline (PBS)] or PBS.

Five to 7 days after implantation of the abdominal pump, a second surgery, using the same anesthesia procedure and stereotaxic frame as the first surgery, was performed to implant an Alzet osmotic pump (Model 2004) that delivers a continuous flow of MSO (2.5 mg/mL) at a rate of 0.25 µL/hr for approximately 28 days into the molecular layer of the right subiculum or the polymorphic layer of the right dentate gyrus. The pump was connected via plastic tubing to a 30-gauge, 6.5 mm long, stainless steel cannula, which was fitted to a plastic pedestal (Osmotic pump connector cannula; Plastics One, Roanoke, Va.). The cannula was lowered into a burr hole in the skull until the pedestal touched the surface at the following coordinates with bregma marking zero for the mediolateral (ML) and anteroposterior (AP) directions, and the top of the skull marking zero for the dorsoventral (DV) direction: AP = 6.25 mm, ML = 5.4 mm, DV = –6.5 mm. The pedestal was then glued to the skull with cyanoacrylate. The pump reservoir was placed subcutaneously in the interscapular region of the rat. Following placement of the cannula, four stainless steel epidural screw electrodes (Plastics One, Roanoke, Va.) were implanted to record EEG activity. Two electrodes (one over each hemisphere) were positioned in the epidural space above the neocortex overlying

the left and right dorsal hippocampus ($AP = -2.0$, $ML = \pm 2.5$). One electrode was positioned in the epidural space at $AP = -8.5$, $ML = -2.2$ to serve as the reference, and a fourth electrode was positioned in the skull, without touching the dura, at $AP = -8.5$, $ML = 1.5$ to serve as the ground. Three additional stainless steel mounting screws (Plastics One, Roanoke, Va.) were used as anchors to reduce the risk of headcap detachment. Two of these mounting screws were positioned at $AP = 2.5$, $ML = \pm 2.5$, and the third was positioned at $AP = -4.5$, $ML = 4.0$.

The female socket contacts on the ends of each electrode were inserted into a plastic pedestal (Plastics One, Roanoke, Va.), and the entire implantation was secured by UV light cured acrylated urethane adhesive (Loctite 3106 Light Cure Adhesive, Henkel Corp., Rocky Hill, Conn.) to form a headcap.

Video-intracranial EEG monitoring, seizure quantitation and statistics

The experimental setup for recording video-EEG was adapted from Bertram et al. (Bertram et al., 1997). The rats were placed individually in custom-made Plexiglas cages. A spring-covered, 6-channel cable was connected to the electrode pedestal on one end and to a commutator (Plastics One) on the other. A second cable connected the commutator to the digital EEG recording unit (CEEGraph Vision LTM, Natus/Bio-Logic Systems Corp., Mundelein, Ill.). Digital cameras with infrared light detection capacity were used to record animal behavior (two cages per camera). The digital video signal was encoded and synchronized to the digital EEG signals. Seizures were identified by visual inspection of the EEG record. As detailed in Avoli and Gloor (Avoli and Gloor, 1994) seizures were defined by EEG characteristics and not by the duration of the discharge (Nagao et al., 1994). Specifically, seizures displayed distinct signal changes from background and interictal activity. Such signal changes included sustained rhythmic or spiking EEG patterns and a clear evolution of signal amplitude and frequency characteristics from onset to termination. Subclinical seizures were distinguished from clinical seizures by examination of the video record. The electrographic start and stop points of seizures were identified by the following commonly used method. By visual inspection of the EEG, we determined a point that was unequivocally within the seizure. Next, we moved backward in time to determine the seizure start time as the first point where the EEG was different from background activity and forward in time to establish the seizure end time. The video record was examined to stage the seizures, using modified Racine criteria (Racine et al., 1973): subclinical, no remarkable behavior; stage 1, immobilization, eye blinking, twitching of vibrissae and mouth movements; stage 2, head nodding, often accompanied by facial clonus; stage 3, forelimb clonus; stage 4, rearing; stage 5, rearing, falling and generalized convulsions. According to Gastaut (Gastaut, 1970), stage 1 and 2 seizures are partial seizures, stage 3 seizures are partial or secondary generalized, and stage 4 and 5 are secondary generalized. Proper classification and staging of experimental seizures are further reviewed by Sutula and Ockuly (Sutula and Ockuly, 2006).

Statistics

The temporal distribution and total number of seizures as well as the duration and percentage of total seizures spent in each stage were determined by reviewing the entire

EEG record. All seizures were staged according to the modified Racine scale. Repeated Measures ANOVA, followed by post hoc Tukey's HSD test, was used to compare the percent sum of seizures at each seizure stage. Significance was defined as $p < 0.05$.

Histology

Rats were anesthetized with Isoflurane and perfused transcardially with saline followed by 4% paraformaldehyde in phosphate buffer (PB; 0.1 M, pH 7.4). The brains were removed from the skull and left in the same fixative at 4°C for 24h and then transferred to PB. The brains were stored at 4°C until being sectioned on a Vibratome at 50- μ m thickness. Slices were captured into four consecutive groups. Every fifth section was mounted on gelatin-coated slides and stained with cresyl violet. One of the four groups of slices was stained with NeuN to quantify neuronal loss. The primary antibody used was (MAB377 Millipore Corp., Bellerica, MA; 1:1000 dilution), and the secondary antibody was biotinylated goat anti-mouse secondary antibody (BA-2000, Vector Laboratories, Burlington, CA). The slides were covered and examined under a light microscope. A quantitative assessment of neuronal loss in the entorhinal-hippocampal area was performed by an experienced observer who was blinded to the identity of the experimental groups. Neurons were counted in the polymorphic layer of the dentate gyrus, CA3, CA2, CA1, and the lateral and medial portions of entorhinal cortex (layer 3 and deep layers (5&6) of entorhinal cortex). A one way ANOVA was carried out separately for each of the brain regions studied. Neurons were counted at the dorso-ventral level of the injection site (6.5 mm ventral to bregma) as the area surrounding the drug delivery was hypothesized to exhibit the greatest neuronal loss. The area in which the microinjector was located was not counted due to mechanical damage to this brain region.

Results

Location of MSO infusion site

We first assessed the location of the distal end of the MSO microinfusion cannula in Nissl stained brain sections (Fig. 1). In 5 rats in each of the two groups, the microinfusion location was in the molecular layer of the right subiculum, and in 1 rat in both groups, the location was in the hilus of the right dentate gyrus. Thus, the MSO infusion sites were similar for both the PBS-($n = 6$) and 5-AV-treated ($n = 6$) rats.

Pathology of the entorhinal-hippocampal area in PBS and 5-AV treated rats

All rats showed mechanical damage around the injection site as indicated in Fig. 1. Thus, neurons in the subiculum were not counted in these rats. Neurons in the polymorphic layer of the dentate gyrus were not counted in 2 of the 12 rats. One way ANOVA indicated no significant difference between PBS- and 5-AV treated rats in the number of neurons in any of the brain regions analyzed.

Temporal pattern, frequency, and duration of electrographic seizures over 21 days

We next visually reviewed the temporal distribution of electrographic seizures obtained from both cerebral hemispheres in each of the PBS- and 5-AV-treated rats (Fig. 2). All rats developed seizures that recurred for the duration of the video-EEG recordings which started on the day of MSO infusion and continued for 21 days. Total number of seizures over 21

days for the 5-AV treated rats was 43 ± 7.8 and for the PBS treated controls was 30 ± 3.45 . There was a striking increase in the number of seizures during the first 1-2 days of recordings in the PBS-treated vs. the 5-AV treated rats (Fig. 2 and 3). Repeated Measures ANOVA indicated no effect of group, a significant effect of days [$F(20,220) = 4.15$, $p < 0.0001$], and a significant group by days interaction [$F(20,220) = 1.97$, $p = 0.01$]. A post hoc Tukey's HSD test indicated that the effects were mainly driven by the PBS treated rats on days 1 and 2, which significantly differed from the 5-AV treated rats on all days ($p < 0.005$). Fig. 3B shows the average number of seizures over days 1-2, which were 23.2 ± 6.3 for the PBS treated group and 6.7 ± 1.4 for the 5-AV treated group. Average number of seizures over days 3-21 was 1.0 ± 0.2 for PBS treated rats and 1.2 ± 0.2 for 5-AV treated rats. According to the post hoc Tukey's HSD test, this difference between groups during days 3-21 was not significant. No differences were found between PBS and 5-AV treated rats in the average *duration* of each seizure at any day of recording.

As stated above, the MSO infusion in one of the rats in each group was in the polymorphic layer of the dentate gyrus, while in the other five rats in each group, the infusion was in the molecular subiculum. Removal of the rat with the infusion in the dentate gyrus from the data set did not eliminate the significance of the results. However, the significance level of the difference between the 5-AV group and the PBS group in seizure frequency on day 2 changed from $p < 0.005$ to $p < 0.05$.

Behavioral stage of seizures over 21 days

Finally, we assessed the effect of 5-AV on the behavioral (Racine) stage of seizures (Fig. 4) between post-MSO infusion day 1 and 28. Repeated measures ANOVA performed over 21 days, grouped into 4 time bins (Days 1-3, 4-7, 8-14, 15-21), which demonstrated no significant effect of group, a significant effect of days [$F(3,33) = 17.11$, $p < 0.0001$], and a significant group by days interaction [$F(3,33) = 3.64$, $p < 0.05$]. There was a striking decrease in the frequency of the milder stage seizures (subclinical, stage 1 and stage 2), over time, in the PBS treated rats (Fig. 4, left panel). A similar, but less pronounced decrease in milder stage seizures was evident in the 5-AV treated rats.

The most severe seizures (stage 4 and 5) increased significantly over time in the PBS treated rats, but not in the 5-AV treated animals (Fig. 4, right panel). Repeated measures ANOVA performed over 21 days, binned into 4 time groups (Days 1-3, 4-7, 8-14, 15-21), indicated a significant effect of group [$F(1,10) = 11.66$, $p < 0.01$], a significant effect of day [$F(3,33) = 25.67$, $p < 0.0001$], and a significant group by day interaction [$F(3,33) = 5.72$, $p < 0.01$]. Tukey's HSD test indicated that the 5-AV group showed a significantly lower level of stage 4 and 5 seizures than the PBS treatment group, both for week 2 ($p < 0.05$) and for week 3 ($p < 0.001$). Removal of the rats with the MSO infusion in the polymorphic layer of the dentate gyrus from the data analysis for both the stage 1 and 2 seizures and the stage 4 and 5 seizures had no effect on the significance level of the results.

Discussion

The temporal development of MTLE in humans is often associated with an initial brain insult (childhood febrile seizures, head trauma, infection), followed by a "latent" period

during which no clinical seizures are observed (Annegers et al., 1996). Studies have indicated that the initial brain insult triggers a sequence of cellular, molecular, and biochemical events that ultimately result in the manifestation of spontaneous recurrent, clinical seizures, months to years later (Dudek and Staley, 2012). The MSO model of MTLE replicates multiple key features of the human condition, such as: (1) an initial brain insult, as evidenced by several hours of subclinical or low-grade clinical seizures that starts within 12 hours of MSO-infusion; (2) a latent period of variable length during which no seizures or only subclinical/low-grade clinical seizures occur; (3) later manifestation of recurrent, more severe seizures; (4) a hippocampal pathology that in some cases resemble human MTLE (Wang et al., 2009); and (5) loss (inhibition) of GS in hippocampal astrocytes (Eid et al., 2004; van der Hel et al., 2005). We have shown here that systemic administration of 5-AV decreases the frequency of seizures during the first 2 days of MSO-infusion and reduces the behavioral severity of seizures later in the course of the model. These findings are important because they indicate that early treatment with 5-AV can modify the development of MTLE and lead to less severe seizures once the disorder is manifest. The findings that 5-AV attenuates the initial seizure insult and reduces the frequency of the most severe seizures later in the course of the MSO model, suggest that 5-AV interferes with key events in epileptogenesis, i.e. the pathological process that leads to MTLE.

Understanding how 5-AV affects the development of MTLE is likely to provide new insight into the mechanisms that underlie epileptogenesis. Most of the studies regarding the neurophysiological effects of 5-AV are related to GABA-mediated signaling and neurotransmission. Five-AV is a GABA analogue (Fritsche et al., 1999; Nanavati and Silverman, 1989) and GABA_b receptor agonist. It also inhibits glial uptake of extracellular GABA (Tasaka et al., 1989) and inactivates GABA aminotransferase (Nanavati and Silverman, 1989), thereby leading to increased extracellular GABA levels. Five-AV also replicates the dose dependent inhibition of baclofen (a GABA_b agonist) on potassium stimulated calcium channels in rat brain synaptosomes (Stirling et al., 1989). With regard to intracellular second messenger systems, 5-AV replicates the effect of baclofen to inhibit adenylyl cyclase in cultured cerebellar granule cells (Holopainen et al., 1992), and mimics the effect of baclofen to inhibit forskolin-stimulated cyclic AMP accumulation in rat cortical slices (Robinson et al., 1989). All these characteristics are consistent with a GABA-mediated, anticonvulsant effect of 5-AV.

However, some studies have suggested that 5-AV is an *antagonist* to GABA_b receptors, and therefore blocks the anticonvulsant properties of GABA_b agonists (Allan and Dickenson, 1986; Hwang and Wilcox, 1989; Muhyaddin et al., 1982; Santicoli et al., 1986; Sawynok, 1986). . For example, 5-AV antagonizes the anticonvulsant effects of baclofen in mice with seizures induced by maximal electroshock (MES) (Manocha et al., 2003a; Manocha et al., 2001; Manocha et al., 2003b; Manocha et al., 2005). The effect of 5-AV on GABA_b receptors and GABA metabolism are therefore complex and require further investigation, especially in models of epilepsy where 5-AV has been scarcely studied thus far.

In one of the few investigations analyzing the effect of 5-AV on seizures, 5-AV was administered subcutaneously to mice that received either a systemic injection of MSO, treatment with electroconvulsive shock, or picrotoxin to induce seizures (Samuels et al.,

1983). Five-AV had anticonvulsant effects in all 3 paradigms, and the authors discovered that the levels of glutamine and GABA were increased in the brains of 5-AV vs. saline treated mice. The authors report that 5-AV was taken up by the brain at a rate of 18.7 nmol/g/min. They hypothesized that intracerebral 5-AV is used as a substrate by gamma-glutamyl transpeptidase (gamma-GT), which normally converts glutamine to gamma-glutamyl glutamine. This reaction would spare glutamine from degradation and lead to increased GABA concentrations via the glutaminase reactions which convert glutamine to GABA. They postulated that the increased brain GABA caused by 5-AV would facilitate inhibition and lead to reduced seizure activity. However, such a scenario needs further validation because an increase in tissue (intracellular) GABA does not necessarily imply increased overall inhibition. Firstly, it is the synaptic (extracellular) GABA that mediates inhibitory neurotransmission. Secondly, increased GABA-ergic neurotransmission may sometimes lead to increased excitation, such as in complex neuronal networks where GABA-mediated inhibition of other inhibitory neurons may result in net excitation. Thirdly, the effects of GABA may be quite different in a pathological (“epileptic”) brain vs. a normal brain. During development, and possibly also in certain pathological conditions, GABA has excitatory effects on neurons (Cohen et al., 2002).

In the present study, we have demonstrated that 5-AV inhibits the development of clinically severe, recurrent seizures in the MSO model of MTLE. This observation is important for two reasons; first, it demonstrates that the MSO model is an operative model of epileptogenesis that can be used effectively to screen and test for therapeutic agents. Second, it suggests that 5-AV has antiepileptogenic properties, and that further investigations of 5-AV and 5-AV-analogues/metabolites for the treatment of MTLE are warranted. However, a few issues regarding the clinical utility and mechanism of 5-AV should be pointed out. First, we do not know if the MSO model responds to antiepileptic treatments in the same way as humans with medication refractory MTLE. I.e. is the MSO model refractory to the same drugs as patients with MTLE, and is 5-AV likely to be effective also in these patients? Second, because 5-AV was administered prior to the induction of epilepsy, i.e. MSO pump implantation, it is unclear if the drug is effective if given after the onset of seizures. Third, 5-AV was given systemically; therefore, it is possible that a peripheral metabolite of 5-AV is responsible for the antiepileptic effects. Intracerebral infusion of 5-AV and quantitation of brain tissue 5-AV and 5-AV metabolites would address this issue. In summary, future studies using the MSO model of MTLE are expected to resolve all of the issues above, thereby providing important new information regarding the utility of 5-AV and 5-AV analogues/metabolites for the treatment of medication refractory MTLE.

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Highlights

- 5-aminovalefic acid (5-AV) modifies seizure development (epileptogenesis) in rats
- The drug halts the development of the most severe type of seizures
- Further studies of 5-AV as a treatment of epilepsy are therefore warranted.

Figure 1.

Nissl-stained brain sections indicating the MSO microinfusion location (arrow) in all rats included in the study. Five of the 6 rats in each of the PBS-treated and 5-AV treated groups had the microinjector positioned within the molecular layer of the subiculum (Molec Subic; PBS Rat: #2, 3, 4, 11, 12; and MSO Rat: #6, 9, 10, 16, 18). One of the rats from each group had the microinjector placed within the hilus of the dentate gyrus (PBS Rat: #13; and MSO Rat: #8). Abbreviations: CA1 and CA3, Cornu Ammonis subfields 1 and 3 of the hippocampus; Dentate, dentate gyrus; Presubic, presubiculum.

Rat #	Treatment	Injection Site	Days																					Total Seizures
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
2	PBS	Subiculum/Moleculare	25	8	3	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	3	0	0	42
3	PBS	Subiculum/Moleculare	11	7	0	0	2	0	5	4	3	0	2	8	0	0	0	0	1	2	0	0	0	45
4	PBS	Subiculum/Moleculare	8	12	1	0	0	6	2	0	0	2	1	1	0	1	1	1	2	0	1	2	41	
11	PBS	Subiculum/Moleculare	9	0	0	1	1	1	1	0	1	1	3	0	0	0	1	3	1	0	0	0	2	25
12	PBS	Subiculum/Moleculare	4	6	2	3	0	0	1	2	0	0	1	1	1	0	1	0	0	1	0	1	3	27
13	PBS	Hilus of Dentate Gyrus	0	49	5	0	0	0	0	0	0	0	3	4	1	0	0	3	3	1	6	2	1	78
6	5-AV	Subiculum/Moleculare	1	2	0	0	0	7	6	1	0	3	0	0	0	0	3	1	0	1	1	1	0	29
9	5-AV	Subiculum/Moleculare	3	0	0	0	2	5	5	1	1	1	0	1	1	1	0	7	1	1	2	0	0	39
10	5-AV	Subiculum/Moleculare	4	3	0	0	0	3	1	0	2	0	0	3	0	1	1	1	0	1	2	0	0	22
16	5-AV	Subiculum/Moleculare	0	7	7	1	3	1	0	0	1	1	1	0	0	0	0	2	2	0	0	1	1	28
18	5-AV	Subiculum/Moleculare	7	5	2	0	2	3	3	1	0	2	2	0	0	1	1	1	0	2	3	4	3	42
8	5-AV	Hilus of Dentate Gyrus	3	5	1	0	0	1	3	1	1	1	1	1	0	0	2	1	0	0	0	1	0	22

Figure 2.

Daily number of electrographic seizures encountered in all PBS (n = 6) and 5-AV (n = 6) treated rats, over a continuous 21-day-period starting at the day of intrahippocampal MSO infusion. The days with seizures are highlighted in yellow. An initial cluster of very frequent seizures is present during the first 2 days in the PBS-treated, but not in the 5-AV-treated rats. Less frequent seizures occur between days 3 and 21 in both groups.

Figure 3.

A: Average daily frequency of seizures in PBS (n = 6) and 5-AV (n = 6) treated rats, starting at the day of intrahippocampal MSO infusion and ending at day 21. The PBS and 5-AV treated groups did not differ in seizure frequency following the 3rd day post-MSO infusion. B: Average daily frequency of seizures in PBS and 5-AV treated rats over days 1-2 post-MSO infusion. The 5-AV treated rats showed a significantly lower frequency of seizures than PBS control (*p<0.005). All values are expressed as the mean $\pm$ SEM.

Figure 4.

Effect of 5-AV on the behavioral manifestation of seizures according to a modified Racine scale. The left panel shows the fraction (% of total) of subclinical, stage 1, and stage 2 seizures over 21 days, while the right panel shows the fraction (%) of stage 4 and stage 5 seizures over the same period. A: Rats in the PBS treated group showed a more drastic decrease over time in subclinical and low grade (stage 1-2) seizures than the 5-AV treatment group ($p < 0.05$) although the difference between groups was not significant. B: Rats in the PBS control group showed a significant increase in the fraction of severe (stage 4-5) seizures over time, while rats in the 5-AV treatment group did not show such increase. Notably, rats in the 5-AV treatment group showed a significantly lower fraction of stage 4 and 5 seizures than the PBS treated group during weeks 2 and 3 post-MSO infusion. All values are the mean $\pm$ SEM, and * $p < 0.05$, *** $p < 0.001$.