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Inhibition of glutamine synthetase in the central nucleus of the amygdala induces anhedonic behavior and recurrent seizures in a rat model of mesial temporal lobe epilepsy

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Abstract

The prevalence of depression and suicide is increased in patients with mesial temporal lobe epilepsy (MTLE); however, the underlying mechanism remains unknown. Anhedonia, a core symptom of depression that is predictive of suicide, is common in patients with MTLE. Glutamine synthetase, an astrocytic enzyme that metabolizes glutamate and ammonia to glutamine, is reduced in the amygdala in patients with epilepsy and depression and in suicide victims. Here, we sought to develop a novel model of anhedonia in MTLE by testing the hypothesis that deficiency in glutamine synthetase in the central nucleus of the amygdala (CeA) leads to epilepsy and comorbid anhedonia. Nineteen male Sprague–Dawley rats were implanted with an osmotic pump infusing either the glutamine synthetase inhibitor methionine sulfoximine [MSO (n = 12)] or phosphate buffered saline [PBS (n = 7)] into the right CeA. Seizure activity was monitored by video-intracranial electroencephalogram (EEG) recordings for 21 days after the onset of MSO infusion. Sucrose preference, a measure of anhedonia, was assessed after 21 days. Methionine sulfoximine-infused rats exhibited recurrent seizures during the monitoring period and showed decreased sucrose preference over days when compared with PBS-infused rats ($p < 0.01$). Water consumption did not differ between the PBS-treated group and the MSO-treated group. Neurons were lost in the CeA, but not the medial amygdala, lateral amygdala, basolateral amygdala, or the hilus of the dentate gyrus, in the MSO-treated rats. The results suggest that decreased glutamine synthetase activity in the CeA is a possible common cause of anhedonia and seizures in TLE. We propose that the MSO CeA model can be used for mechanistic studies that will lead to the development and testing of novel drugs to prevent seizures, depression, and suicide in patients with TLE.

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Conflict of interest

The authors have no conflicts of interest to declare.

Keywords

Anhedonia; Epilepsy; Glutamine synthetase; Central amygdala; Sucrose preference

1. Introduction

Epilepsy is a common and often lifelong neurological disorder with a prevalence of approximately 1% in the general population [1]. Patients with epilepsy have a 5- to 10-fold increased risk of depression [2,3] and an 11-fold increased risk of suicide [2,4] compared with the general population. In patients with mesial temporal lobe epilepsy (MTLE), the rate of suicide is 25 times higher than in the general population [5,6]. The current treatments for MTLE, which include either the use of antiepileptic drugs or the surgical removal of the temporal lobe, can themselves increase depression and the risk of self-harm and suicide [4,7–10]. To provide more effective treatments for seizures, depression, and suicide prevention in patients with MTLE, we must achieve development of an effective animal model of MTLE with depressive comorbidity.

Commonly used animal models of MTLE include acute systemic injections of pilocarpine [11] or kainic acid [12] and electrical stimulation of the hippocampus or amygdala [13]. The depression-related behaviors that have been tested in these models have included the forced swim test and the sucrose or the saccharine preference test. Mixed results have been obtained in all these models with these tests. For the kainic acid model, studies have shown that sucrose preference is decreased and immobility in the forced swim test is increased in response to systemic administration of kainic acid in rats [14,15], indicative of depressive-like behavior; however, other studies that used the kainic acid model failed to demonstrate depressant effects [16]. Similarly, some studies using the pilocarpine model have shown a decrease in saccharin preference and an increase in immobility in the forced swim test [17–19]. Other studies, however, did not show this effect [20–22]. With respect to the stimulation models, one study showed that kindling of the ventral hippocampus produces depressant effects in the forced swim test and saccharine preference test [23]. Other studies have shown no such effect with amygdala or hippocampal stimulation [24,25].

We present a recently developed model of MTLE with comorbid anhedonia. While depression is a complex disorder with multiple symptoms, we have chosen to focus on the anhedonic symptom of depression because it is a key symptom of depression that is highly predictive of suicidal thoughts and behaviors [26–30] and is common in patients with MTLE [31,32]. The model we are introducing is produced by inhibiting glutamine synthetase, an astrocytic enzyme that is critical for the metabolism of glutamate and ammonia to glutamine, in the central nucleus of the amygdala (CeA). Unlike the classically used models, our approach recapitulates a possible causative mechanism of seizures and concurrent depression in humans with MTLE. This is because glutamine synthetase activity has been shown to be reduced in the amygdala in patients with MTLE [33], and glutamine synthetase levels have been shown to be significantly decreased in patients with major depressive disorder [34], in suicide victims with major depression [35], and in suicide victims with no major depression [35].

Our working hypothesis when developing the new model was that inhibition of glutamine synthetase with methionine sulfoximine (MSO) in the CeA would induce both recurrent seizures and a lack of preference for a sucrose solution in a limited access two-bottle-choice procedure. Such a model may be used to effectively investigate the anatomical and chemical mechanisms that underlie MTLE and comorbid anhedonia, thereby potentially leading to more effective ways to prevent seizures, depressive symptoms, and suicide in patients with TLE.

2. Material and methods

2.1. 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). Rats were housed (2 per 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 acclimated for at least 1 week 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.

2.2. Surgery

Rats were anesthetized with 0.25–3% isoflurane (Baxter, Deerfield, Ill.) in O₂ and placed in a stereotaxic frame (David Kopf Instruments, Tujunga, Calif.). A 30-gauge stainless steel cannula, with a length of 8.1 mm, attached to a plastic pedestal (Plastics One, Roanoke, Va.) was stereotactically lowered into the CeA using 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 = –2.6 mm, ML = +4.6 mm, DV = –8.1 mm.

The cannula was lowered into the brain until the pedestal touched the skull. The pedestal was then glued to the skull with medical grade cyanoacrylate (Vetbond Tissue Adhesive, Butler Animal Health Company, Chicago, IL). The cannula and pedestal were connected via plastic tubing to a subcutaneously implanted Alzet osmotic pump (Model 2004, Durect Corp., Cupertino, Calif.) which delivers a continuous flow of 0.25 µL/h for ~28 days. Treatment pumps were filled with MSO (2.5 mg/mL; dissolved in Dulbecco's phosphate buffered saline (PBS)) to achieve a delivery of 0.625 µg of MSO per hour. Control pumps were filled with PBS. Following placement of the cannula and pedestal, four stainless steel epidural screw electrodes (Plastics One, Roanoke, Va.) were implanted to record cortical EEG activity. Two electrodes (one in each hemisphere) were positioned in the epidural space over the cortex. On the left side (contralateral to the injection), the recording screw electrode was placed in the skull above the dorsal anterior hippocampal formation (AP = –2.0 mm, ML = –2.5 mm). On the right side, the recording screw electrode was placed in the skull above the temporal lobe (AP = –6.25 mm, ML = 5.4 mm). One screw electrode was positioned in the epidural space (AP = –8.5 mm, ML = –2.2 mm) to serve as the reference. A fourth electrode, which was positioned above the cerebellum (AP = –10.0 mm, ML = 1.5 mm), served as the ground. Three additional stainless steel mounting screws

(Plastics One) were inserted into the skull to reduce the risk of headcap detachment. Two screws were positioned in AP = 2.5 mm, ML = ± 2.5 mm, and one was positioned in AP = -4.5 mm, ML = 4.0 mm.

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

2.3. Video-intracranial EEG monitoring and seizure quantitation

Video-EEG recording was conducted over the first 21 days following MSO pump placement. Sucrose preference testing was performed after the completion of the EEG recordings. We chose to carry out EEG recording for the first 21 days because in other models we have developed, for example, where MSO is infused into the molecular subiculum, there is an increase in the percent severity of seizures over time [36]. We wanted to test if this was also the case with MSO infusion into the CeA. The experimental setup for recording video-EEG was adapted from Bertram et al. [37]. 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., San Carlos, Calif.). Digital cameras with infrared light detection capability were used to record animal behavior (two cages per camera). The digital video signal was encoded and synchronized with the digital EEG signals. Seizures were identified by visual inspection of the EEG record. As detailed in Avoli and Gloor [38], seizures were defined by EEG characteristics and not by the duration of the discharge. Specifically, seizures displayed distinct signal changes from background (interictal) activity. Such signal changes included sustained rhythmic or spiking EEG patterns and a clear evolution of signal characteristics from onset to termination. Sub-clinical seizures were distinguished from clinical seizures by examination of the video record. The 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 a modification of Racine's criteria [39] as follows: 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; and stage 5 — rearing, falling, and generalized convulsions.

2.4. Sucrose preference testing

After the completion of video-EEG monitoring, the animals proceeded to sucrose preference testing. For the sucrose preference test, rats were disconnected from the EEG recording equipment, taken out of the Plexiglas EEG recording cages, and housed individually in standard rat cages. Following placement, rats were given one day to acclimate to their new cage. During this time, they were provided with food and water ad libitum. Following this

24-hour period, the water bottle was removed and replaced with a bottle containing a 1% sucrose solution, which was the only source of fluid for 48 h. Sucrose preference testing occurred following the 48-hour period of acclimation to the sucrose solution. On the first day of testing, 4 h prior to the beginning of the dark cycle, the sucrose solution was removed, and the rats were fluid-deprived for 4 h. The sucrose preference test began at the beginning of the dark cycle, when rats were given one-hour exposure to two bottles, one filled with 1% sucrose solution and the other with water. Following the sucrose preference test, the sucrose bottle was removed, leaving only a water bottle for fluid. On each of the six remaining days of the sucrose preference test, the water bottle was removed 4 h before the beginning of the dark cycle. The sucrose preference test then began at the beginning of the dark cycle.

2.5. Histology

Rats were anesthetized with 0.25–3% isoflurane and perfused transcardially with 0.9% NaCl followed by 4% freshly dissolved paraformaldehyde in phosphate buffer (PB; 0.1 M, pH 7.4). The brains were removed and left in the same fixative at 4 °C for 24 h and then transferred to PB. The brains were stored at 4 °C until being sectioned on a vibratome horizontally at 50- μ m thickness. Every fifth section was mounted on gelatin-coated slides and stained with cresyl violet. For NeuN staining, the primary antibody used was (MAB377 Millipore Corp., Bellerica, MA; 1:000 dilution), and the secondary antibody was biotinylated goat antimouse secondary antibody (BA-2000, Vector Laboratories, Burlington, CA). Staining was visualized using 3–3'-diaminobenzidine tetrahydrochloride (DAB) (Polysciences Inc., Warrington, PA). The slides were covered and examined under a light microscope. Neuronal loss was assessed through quantification of NeuN-positive neurons.

2.6. Statistical methods

The temporal distribution and total number of seizures in all animals were determined by reviewing the entire video-EEG record. All seizures were staged according to the modified Racine scale [39]. Repeated measures analysis of variance (ANOVA) was used to compare the total frequency of nonsevere (stages 1–2) and severe (stages 3–5) seizures over 21 days in 3-day bins (1–3, 4–6, 7–9, 10–12, 13–15, 16–18, and 19–21). Analysis of variance was followed by a post hoc Fisher least significant difference (LSD) test. Significance was defined as $p < 0.05$ for all tests performed.

One-way ANOVA was used to compare the body weight of the MSO-treated group with that of the PBS control group at two separate time points (day 7 and day 21 following MSO surgery). A one-way ANOVA was used to compare water consumption levels averaged over a one-week period between these two groups. A separate one-way ANOVA was used to compare sucrose consumption levels averaged over the same time period. Percent sucrose preference, which was defined as the ratio of the volume of sucrose divided by the total volume of fluid consumed multiplied by 100, was analyzed over one week with repeated measures ANOVA. Analysis of variance was followed by a post hoc Fisher LSD test.

Neurons were counted in the following brain regions, both ipsilateral and contralateral to the infusion site: the CeA, the hilus of the dentate gyrus, the medial amygdala, the lateral

amygdala, and the basolateral amygdala. A one-way ANOVA comparing the MSO-treated group with the PBS-treated group was carried out separately for each of these brain regions.

3. Results

3.1. Location of MSO infusion site

Fig. 1 illustrates the infusion sites for MSO- and PBS-treated rats. Column A represents the injection site for a representative animal at 4× magnification. Column B illustrates the injection site for the MSO-treated rats (n = 12). Column C illustrates the injection site for the PBS-treated rats (n = 7). The injection site for each rat is illustrated with a red circle. Data are presented at 4 different dorsoventral (DV) levels, with each row on the figure representing a different level. The sections at DV = −8.4 mm demonstrate one injection site for the MSO-treated rat and one injection site for the PBS-treated rat, both located in the area between the central and medial portions of the posterior regions of the CeA. The sections at DV = −8.1 mm demonstrate 8 injection sites for the MSO-treated rats and 6 injection sites in the PBS-treated rats in the medial and central portions of the mid to posterior regions of the CeA. The sections at DV = −7.8 mm demonstrate two injection sites for the MSO-treated rats in the medial and central portions of the CeA. The sections at DV = −7.6 mm demonstrate one injection site in the MSO-treated rat in the anterior portion of the CeA.

3.2. Total number of nonsevere (stages 1–2) and severe (stages 3–5) seizures over 21 days

We first quantified the total number of seizures in the MSO- and PBS-treated rats by analysis of video-intracranial EEG records continuously collected over a period of 21 days after the onset of intracranial infusion of MSO. All of the rats treated with MSO in the CeA and none of the PBS-infused rats developed recurrent seizures, which were defined as 2 seizures occurring at least 1 h apart. The total number of seizures was documented for each MSO-infused rat and is illustrated in Table 1. Seizures for rat 2 were only recorded for ten days because the EEG pedestal became disconnected from the electrodes at this time and the EEG could no longer be recorded from this animal. The total number of seizures for rat 2 has not been reported. The effect of MSO infusion on the daily number of nonsevere (stages 1–2) and severe (stages 3–5) seizures was evaluated (Fig. 2). Repeated measures ANOVA over days indicated no significant effect of seizure severity, a significant effect of days [$F(1, 60) = 3.46, p < 0.005$], and no seizure severity by day interaction. Post hoc Fisher LSD indicated that both nonsevere and severe seizures were highest during the first three days when compared with all other days ($p < 0.05$). While the frequency of both severe and nonsevere seizures was highest over the first three days, the frequency of nonsevere versus severe seizures did not differ from each other at any time point.

3.3. Body weight, water consumption, and sucrose consumption

Body weight at 7 days postsurgery (postpump implantation) did not significantly differ between the MSO-treated group and the PBS-treated group ($538.1 \text{ g} \pm 6.4$ and $546 \text{ g} \pm 9.4$, respectively). There was also no significant weight difference between the MSO-treated group and the PBS-treated group at day 21 postsurgery ($579.7 \text{ g} \pm 7.9$ and $582.8 \text{ g} \pm 7.1$, respectively).

Fig. 3 illustrates averaged limited access water (left) and sucrose (right) consumption over 7 days in MSO- and PBS-treated rats. One-way ANOVA demonstrated no significant effect of treatment on water consumption ($2.1 \text{ mL} \pm 0.6$ and $1.9 \text{ mL} \pm 0.4$, respectively), but a significant effect of treatment on sucrose consumption [$F(1, 17) = 11.1$, $p < 0.005$], with the PBS-treated group showing a higher level of consumption compared with the MSO-treated group ($10.4 \text{ mL} \pm 1.7$ and $4.9 \text{ mL} \pm 0.8$, respectively).

3.4. Sucrose preference

Fig. 4 illustrates that rats treated with MSO in the CeA showed a decreased sucrose preference over days when compared with PBS-treated controls. Repeated measures ANOVA indicated a significant effect of group [$F(1, 17) = 9.52$, $p < 0.01$], but no effect of day, and no interaction between groups. Post hoc Fisher LSD demonstrated that sucrose preference on day 1 of the MSO-treated group was significantly lower than days 1 and 3–7 of the PBS control group ($p < 0.001$). Furthermore, sucrose preference on days 5 and 6 of the MSO-treated group was significantly lower than days 4–7 of the PBS control group ($p < 0.01$), and day 7 of the MSO treatment group was significantly lower than days 4–6 of the PBS control group ($p < 0.05$). Overall, the results show that the MSO-treated rats had a lower level of sucrose preference over days than the PBS-treated rats.

3.5. Pathology of the CeA in PBS and MSO-treated rats

All rats showed mechanical damage around the injection site. Methionine sulfoximine-treated rats did not significantly differ from PBS-treated rats in the number of NeuN-positive neurons in the ipsilateral and contralateral medial amygdala, lateral amygdala, basolateral amygdala, and hilus of the dentate gyrus. The two groups also did not differ in the number of NeuN-positive neurons in the contralateral CeA. Methionine sulfoximine-treated rats showed significantly fewer NeuN-positive neurons in the ipsilateral CeA compared with the PBS-treated control rats (922.7 ± 681.8 versus 1981.8 ± 480.0), respectively [$F(1, 13) = 8.74$, $p = 0.01$].

4. Discussion

The results from this study support our hypothesis that inhibition of glutamine synthetase in the CeA induces recurrent seizures and comorbid anhedonic behavior, as indicated by the lack of preference for a sucrose solution over a water solution. In addition, the results demonstrate the occurrence of neuronal loss in the CeA as a result of GS inhibition. The decrease in sucrose consumption was not due to a general decrease in fluid consumption, as water consumption levels were not different between the glutamine synthetase-treated group and the nontreated controls. The decrease in sucrose consumption was also not due to a general decrease in overall consumption of food and water, as body weights did not differ between the glutamine synthetase-treated group and the nontreated control group. Furthermore, it is unlikely that the decreased consumption of sucrose solution was merely due to the recurrent seizures (i.e., false positive effect) because there were no observed differences in water consumption between treated rats and nontreated rats. Overall, we demonstrate that glutamine synthetase inhibition in the CeA induces recurrent seizures and

anhedonic behavior, demonstrating that this approach can be used to model MTLE with comorbid depressive features.

One of the key findings of our study is that the CeA is implicated in the causation of both recurrent seizures and depressive-like (i.e., anhedonic) behavior. The notion that the amygdala is also implicated in seizures and depression in human MTLE is supported by several observations. First, depth electrode EEG recordings have shown that the amygdala is involved in the onset of seizures in MTLE [40–42]. Second, studies have shown that some patients with MTLE who have received a temporal lobectomy, which often involves the removal of the amygdala [43], have demonstrated postsurgical depression. Specifically, one study found that temporal lobectomy patients with a smaller amygdalar volume due to greater amygdalar resection were more likely to develop postoperative depression compared with temporal lobectomy patients who had a larger postoperative amygdalar volume [44]. Finally, one functional magnetic resonance imaging (fMRI) study further supports the role of the amygdala in depression in patients with MTLE by finding that preoperative right amygdala activation correlates significantly with a postoperative change in anxiety and depression scores, with higher postoperative anxiety and depression corresponding with a higher preoperative activation of the right amygdala [45]. Future studies of the role of the amygdala in recurrent seizures and depressive-like behavior will help elucidate the underlying mechanism of seizures and depression in patients with MTLE.

We have shown that astrocytic glutamine synthetase inhibition in the CeA results in anhedonic behavior, as demonstrated by a lack of sucrose preference. Anhedonia is an important behavior to study because, in humans, the presence of anhedonic symptoms in a setting of depression is predictive of suicidal thoughts and behaviors [26–30]. Our amygdala-dependent anhedonia model is, therefore, consistent with human studies, in which numerous gene and protein expression studies have demonstrated a relationship between the physiologic state of the amygdala and the occurrence of a successful suicide. Analysis of postmortem tissue obtained from suicide victims demonstrates changes in cytoskeletal proteins associated with astrocytic function [46]. Other studies have found that glucocorticoid receptors [47,48] and GABA_A receptors [49] are downregulated in the amygdala of suicide victims. When examining the volume of the amygdala, studies have found that the right amygdala is larger in volume in suicide victims than in healthy controls [50]. Most relevant to our study, the activity of glutamine synthetase is reduced in the amygdala of patients with epilepsy [33] and suicide victims who do and do not have major depression [35]. By inhibiting glutamine synthetase in the amygdala, our current model recapitulates a possible causative mechanism of suicide in patients with TLE. A better understanding of the functional role of glutamine synthetase in the amygdala on depressive symptoms that are predictive of suicide, such as anhedonia, will facilitate the prevention of debilitating suicidal thoughts and behaviors, thereby leading to successful suicide prevention.

The finding here that the CeA is implicated in the causation of anhedonia is consistent with previously published studies. For example, injection of the GABA_A receptor agonist muscimol into the CeA of rats decreases self-administration of amphetamine and sucrose [51]. In C57BL/6J mice, electrolytic lesion of the CeA decreases preference for ethanol in a

limited access two-bottle-choice procedure [52]. Similarly, in rats, ibotenic acid lesion of the CeA decreases ethanol preference in a free access two-bottle-choice procedure [53]. Finally, ibotenic acid lesion of the CeA decreases saccharin preference and increases aversion to quinine [54]. All of these observations suggest that the CeA plays a key role in reward and, hence, the closely associated condition of anhedonia.

Despite a continuous infusion of MSO into the CeA, we observed a dramatic reduction in seizure frequency following the first three days of MSO infusion. While the mechanism of the seizure decline is poorly understood, several explanations are plausible. The decrease in seizure frequency might be due to increased GABA-mediated inhibition. While there are many seizure-induced changes observed in the GABAergic system that are believed to induce seizures, such as loss of hippocampal interneurons with decreased GABAergic inhibition [55], and decreased expression of GABA_A receptors [56] and neuronal K-Cl cotransporter (KCC2) [57], some changes are thought to facilitate inhibition, such as an increase in spontaneous inhibitory postsynaptic currents after memantine-induced seizures [58] and an increase in extracellular GABA levels after pilocarpine-induced seizures [59]. The decrease in seizure frequency may also be explained by a depletion of astrocytic glutamine that results from GS inhibition, resulting in glutamate reduction in axon terminals and reduced excitatory transmission [60,61]. However, because glutamate is a precursor for GABA, depletion of glutamine may also lead to decreased GABAergic neurotransmission. Interestingly, GS is upregulated in hippocampal astrocytes early in the kainic acid and amygdala-kindling models of MTLE [62,63]. Thus, the consequences of GS inhibition are multifaceted, and its effects on seizure activity are likely determined by the magnitude and timing of inhibition.

We have previously demonstrated that MSO infusion in the hippocampus at the doses used in the present study inhibits GS activity in vivo without affecting glutathione levels [64]. In addition, we have shown that MSO treatment in the hippocampus increases astrocytic glutamate levels [61]. Future studies, thus, may involve similar experiments in the intra-CeA MSO model to determine GS activity and levels of intracellular glutamine and glutamate in CeA neurons and astrocytes. Furthermore, through the use of microdialysis, extracellular levels of glutamine and glutamate may be quantified and correlated with the occurrence of simultaneously recorded seizures and anhedonic behavior. Simultaneously recording EEG and sucrose preference could help determine if there is a correlation between these two measures. Lastly, future studies could use an MSO dose–response curve to determine the threshold of MSO activity necessary to produce an effect on seizure frequency and severity, anhedonic behavior, and GS activity in the CeA.

The key finding of our study is that astroglial glutamine synthetase inhibition in the CeA is involved in seizures and comorbid anhedonic behavior. Although the extent of MSO diffusion from the infusion site is unknown, we do have anatomical controls in nearby brain regions, where the MSO infusion cannula was placed, but sucrose preference was unaffected. The nearby brain regions tested were the medial amygdala (n = 2), the lateral amygdala (n = 1), and the amygdalostratial transition region (n = 1). While there were no anatomical controls in the basolateral amygdala, the distance from the lateral amygdala and the amygdalostratial transition area to the basolateral amygdala were similar in distance as

the distance of the CeA to the basolateral amygdala. These observations indicate that nearby brain regions are not responsible for the behavioral effects seen in our study.

5. Conclusion

While other studies have shown that stimulation of the CeA induces seizures and reinforcing behaviors, such as intracranial self-stimulation [65], to our knowledge, this is the first demonstration of a model exhibiting recurrent seizures and comorbid depressive-like behavior (i.e., anhedonia) that recapitulates the human condition. Future studies with this model should be carried out to evaluate other depressive-like and anxiety-like behaviors that are dependent on the CeA. In summary, this model can be used for a variety of studies aimed at understanding the underlying neurochemical, cellular, and neural circuit mechanisms of epileptic seizures, comorbid depression, and suicidality in patients with MTLE.

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Fig. 1.

Representative photomicrographs and infusion sites for MSO- and PBS-treated rats. Column A represents the injection site for a representative animal at 4× magnification. Column B illustrates the injection site for the MSO-treated rats (n = 12). Column C illustrates the injection site for the PBS-treated rats (n = 7). The injection site for each rat is illustrated with a red circle. Data are presented at 4 different dorsoventral (D/V) levels relative to bregma. The sections at DV = −8.4 mm demonstrate one injection site for the MSO-treated rats and one injection site for the PBS-treated rats, both located in the area between the

central and medial portions of the posterior regions of the CeA. The sections at DV = -8.1 mm demonstrate 8 injection sites for the MSO-treated rats and 6 injection sites for the PBS-treated rats in the medial and central portions of the mid to posterior regions of the CeA. The sections at DV = -7.8 mm demonstrate two injection sites for the MSO-treated rats in the medial and central portions of the CeA. The sections at DV = -7.6 mm demonstrate one injection site for the MSO-treated rats in the anterior portion of the CeA. Abbreviations: CeA, central nucleus of the amygdala; BLA, basolateral amygdala; LA, lateral amygdala; MeA, medial amygdala; ast, amygdalostratial transition region.

Fig. 2.

Effect of MSO infusion on the number of nonsevere (stages 1–2) and severe (stages 3–5) seizures over days. Days are binned, 1–3, 4–6, 7–9, 10–12, 13–15, 16–18, and 19–21.

Nonsevere (stages 1–2) seizures are shown in white, and severe (stages 3–5) seizures are shown in black. Values are given as mean ± SEM * $p < 0.05$ when compared with days 4–21 of all stage 1–5 seizures.

Fig. 3.

Averaged limited access to water and sucrose consumption over 7 days in MSO- and PBS-treated rats. The MSO-treated rats are depicted in black, and the PBS control rats are depicted in white. Values are given as mean $\pm$ SEM *** $p < 0.005$ versus the MSO-treated group.

Fig. 4.

Sucrose preference over 7 days in MSO- and PBS-treated rats. Rats receiving a chronic infusion of MSO into the CeA are indicated with filled markers, and those receiving PBS are displayed with unfilled markers. Values are given as mean $\pm$ SEM * $p < 0.05$ versus days 4–6 of the PBS control group; ** $p < 0.01$ versus days 4–7 of the PBS control group; *** $p < 0.001$ versus days 1 and 3–7 of the PBS control group.

Table 1

Overview of the temporal distribution and frequency of seizures in rats infused with MSO into the unilateral CeA. Electrographic seizure counts are given per day over 21 days. The day in which seizures occurred and the number of seizures that occurred are highlighted in color; 1–14 seizures per day are highlighted in yellow, 15–30 seizures per day are highlighted in blue, 31–50 seizures per day are highlighted in red, and 51 and greater seizures per day are highlighted in black. All animals were recorded with continuous video-EEG and synchronous intra-cranial EEG.

Rat #	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	0	37	59	26	0	0	0	0	0	0												
4	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	1	0	0	1	
6	18	64	66	9	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	3	0	
10	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1	1	0	1	0	1	0	
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	
16	0	0	16	0	0	0	0	0	0	1	5	0	0	0	0	0	1	0	0	0	0	
27	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	
29	0	10	1	0	0	0	0	0	0	1	2	3	0	0	1	0	0	0	0	1	1	
33	0	0	0	2	2	0	1	0	1	0	0	1	1	3	1	0	0	0	1	1	0	
37	0	6	4	0	0	0	0	0	1	1	0	0	2	0	1	0	2	0	0	0	1	
39	0	0	0	0	0	0	0	2	0	0	0	0	0	1	1	2	0	1	0	0	0	
41	8	12	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	