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Vascular endothelial growth factor (VEGF) attenuates status epilepticus-induced behavioral impairments in rats

Jamee N. Nicoletti¹, Janice Lenzer¹, Elisa A. Salerni¹, Sachin K. Shah², Ahmed Elkady², Sidra Khalid², Dean Quinteros², Francis Rotella², David Betancourth², and Susan D. Croll^{1,2,3,4,*}

¹ Neuropsychology Doctoral Subprogram of the Graduate Center of the City University of New York

² Department of Psychology, Queens College of the City University of New York

³ Neuroscience Doctoral Subprogram of the Graduate Center of the City University of New York

⁴ Regeneron Pharmaceuticals, Tarrytown, New York

Abstract

Vascular endothelial growth factor (VEGF) is a vascular growth factor more recently recognized as a neurotrophic factor (for review see ¹). We previously reported that endogenous VEGF protein is dramatically upregulated after pilocarpine-induced status epilepticus in the rat, and that intra-hippocampal infusions of recombinant human VEGF significantly protected against the loss of hippocampal CA1 neurons in this model². We hypothesized that we would see a preservation of cognitive and emotional functioning with VEGF treatment accompanying the neuroprotection previously observed in this paradigm. Using the Morris water maze to evaluate learning and memory, and the light-dark task to assess anxiety, we found a selective profile of preservation. Specifically, VEGF completely preserved normal anxiety functioning and partially but significantly protected learning and memory after status epilepticus. To determine whether VEGF's ability to attenuate behavioral deficits was accompanied by sustained preservation of hippocampal neurons, we stereologically estimated CA1 pyramidal neuron densities at four weeks after status epilepticus. At this time point, we found no significant difference in neuronal densities between VEGF- and control-treated status epilepticus animals, suggesting that VEGF could have protected hippocampal functioning independent of its neuroprotective effect.

1. Introduction

Vascular endothelial growth factor (VEGF) is a protein growth factor originally named for its potent trophic effects on endothelial cells, but more recently recognized as a neurotrophic factor (for review see ¹). We have previously reported that endogenous VEGF protein is dramatically upregulated after pilocarpine-induced status epilepticus in rat, and that intra-hippocampal infusions of recombinant human VEGF significantly protected against the loss of hippocampal CA1 neurons in the same model².

*Correspondence to: Susan D. Croll, Queens College of the City University of New York, 65-30 Kissena Boulevard, Flushing, NY 11367, USA. Tel: +1-718-997-3551; fax: +1-718-997-3257. susan.croll@qc.cuny.edu.

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While status epilepticus results in loss of hippocampal neurons in rat models, findings regarding hippocampal damage in humans with temporal lobe epilepsy (TLE) have been inconsistent. Many studies with human patients have demonstrated that acute status epilepticus can cause extensive loss of hippocampal neurons^{3,4}, and that damage progression occurs in patients who continually experience seizures^{5,6}. In contrast, Thom and colleagues⁷ demonstrated the absence of hippocampal neuronal loss in a post-mortem stereological analysis of patients with poorly controlled seizures.

In addition to the underlying neuropathology, individuals with epilepsy often experience functional impairments such as deficits in intellectual functioning, learning and memory, processing speed, attention and concentration, and executive functioning^{8,9}. These impairments can lead to substantial decreases in quality of life. In fact, individuals with epilepsy are more likely to be unemployed, resulting in lower socioeconomic status, and are also less likely to engage in marriage, ultimately leading to social isolation⁹. It would therefore be important to determine whether treatment interventions that protect the epileptic brain from damage would also preserve functional integrity.

The hippocampus plays a fundamental role in learning, memory, and emotional functioning, and it is therefore reasonable to predict that loss of neurons after status epilepticus could be associated with functional deficits in these behaviors. Indeed, animal models of epilepsy have revealed seizure-associated impairments in hippocampally-mediated memory tasks as well as a blunting of normal anxiety responses in anxiety tasks^{10,11,12}. Based on our findings that VEGF decreased status epilepticus-induced cell loss in the hippocampus, we hypothesized that VEGF would also attenuate behavioral impairments. In order to investigate functional preservation, animals were continuously treated with VEGF and were given pilocarpine-induced status epilepticus. Behavioral testing was then conducted to evaluate learning, memory, and emotional functioning during the period between status epilepticus and the eventual development of chronic seizure behavior.

2. Methods

2.1. Subjects

All subjects were adult male Sprague-Dawley rats (Charles River Laboratories, Kingston, NY) weighing 250–350g. Animals were housed 2 to 3 per cage within a temperature-stabilized animal facility with food (Rat LabDiet 5001, Purina Mills, LLC, St. Louis, MO) and water available *ad libitum*. Animals were maintained on a 12:12 light:dark cycle (lights on 07:00) and acclimated to their colony room for at least one week prior to any manipulations.

2.2. Proteins

The VEGF used for protein infusions was recombinant human VEGFA₁₆₅ (generously provided by Regeneron Pharmaceuticals). VEGF was stored frozen until use and then diluted in sterile phosphate buffered saline (PBS) (Sigma-Aldrich, St. Louis, MO) to deliver 60ng/day VEGF in a 12μl volume at a rate of .5μl/hour via osmotic minipump. PBS was purchased in powder form, mixed with distilled water, sterilized, and used as a vehicle control. In addition, some control animals received inactivated VEGF as a control instead of PBS to control for protein load. VEGF was inactivated by repeated freeze-thaw cycles, which has previously been shown to eliminate VEGF's bioactivity (unpublished data), rather than by heat, which results in a precipitate.

2.3. Pump implantation and protein infusion

Animals were anesthetized with 65mg/kg sodium pentobarbital administered intra-peritoneally (Henry Schein, Melville, NY). Animals were placed in a stereotaxic apparatus, the skull was exposed, and three burr holes were drilled to insert anchor screws (Plastics One, Roanoke, VA). A sterile 4mm cannula (Plastics One), with an attached heat-sealed polyvinyl catheter (Plastics One) containing sterile PBS, was implanted bilaterally into the hilus of the dentate gyrus of the dorsal hippocampus (3.8mm posterior and 2.7mm lateral as measured from bregma). Dental acrylic was then applied to secure the cannula and anchor screws and polyamid nylon suture thread (Henry Schein) was used to close the incision.

One week following cannula implantations, animals were briefly re-anesthetized under 2.5% isoflurane anesthesia and an incision was made at the nape of the neck. The heat-sealed tip of the catheter was snipped and an Alzet osmotic minipump (0.5 μ l per hour, Durect Corporation, Palo Alto, CA), containing rhVEGF₁₆₅, sterile PBS, or inactivated VEGF, was attached to the catheter or catheters and glued. The pump was inserted into the subcutaneous space at the nape of the neck and the incision was closed with nylon sutures. Pumps continuously administered VEGF protein into the hippocampus for two weeks.

One group of animals, the “control” group, received no surgical manipulations or protein infusions.

2.4. Acute seizure induction

Five days following pump implantations for protein infusions, animals were pre-treated with 1mg/kg atropine methylbromide (Sigma-Aldrich) injected subcutaneously 30 minutes prior to receiving either 350mg/kg pilocarpine hydrochloride (Sigma-Aldrich) or an equivalent volume of saline intraperitoneally (the “control” group). Seizures were scored from stages 1–8 based on Racine’s scale¹³ modified as previously described¹⁴. Status epilepticus was defined as seizures with no intervening return to normal behavior for greater than five minutes. Status epilepticus was truncated with 10mg/kg diazepam (Henry Schein) after 60 minutes. Animals not achieving status epilepticus received diazepam 90 minutes after pilocarpine injection, and were removed from further analyses. All pilocarpine animals were hydrated immediately following diazepam with 3cc of isotonic saline and were given fresh apple slices for further hydration. Seized animals received hydration injections and fresh apple slices daily for one week.

2.5. Behavioral Analyses

Animals underwent the behavioral tests described below two to four weeks after status epilepticus to evaluate learning, memory, and emotional functioning. Before each behavioral test, animals were acclimated to the testing room for at least one hour. All behavioral testing was conducted by experimenters blind to the treatment condition of the animals.

2.5.1. Morris Water Maze—Each animal was placed in a 130cm diameter water maze, made opaque with white, non-toxic paint, back-end first to avoid stress. Each animal was placed in a pseudo-randomly selected start location in the pool for three trials per day with an inter-trial interval of one minute. Each trial ended when the animal escaped onto a submerged, hidden goal platform or when the animal had been in the maze for two minutes. Any animal that had not located the platform within two minutes was guided to the platform by hand. Each animal was tested daily until acquisition of the memory was achieved. Acquisition was defined as non-seized animals (which had no surgical manipulations, protein treatment, or convulsant injection) in each cohort locating the platform in less than 10 seconds. This typically occurred by the fourth day (twelfth trial) although acquisition trials in some cohorts were extended to the fifth day. Following the acquisition trials, the

goal platform was removed for a spatial probe trial in which each animal was placed in the maze for 30 seconds, and the proportion of time spent in the goal and other quadrants was recorded. Additionally, mean quadrant crossing time was calculated as a control for swim speed.

2.5.2. Grid Locomotor Activity—Each animal was placed in the center of an 86cm square open field divided by laboratory tape into nine 29cm squares. Animals were observed for six minutes to measure the number of grid crossings as a measure of exploratory locomotor activity.

2.5.3. Light-Dark Exploration—Each animal was placed into the open side of a 43cm × 86cm light-dark box in which one side was covered and painted black and the other side was open and painted white. The amount of time spent in the black chamber versus the white chamber was recorded for a total of five minutes as a measure of anxiety-like behaviors, with more time spent hidden in the closed side indicative of greater levels of anxiety.

2.6. Histology

After receiving an overdose of Euthasol euthanasia solution (Henry Schein, Inc., Melville, NY), animals were transcardially exsanguinated with heparinized isotonic (0.9%) saline, and then perfusion-fixed with 4% paraformaldehyde in acetate and then borate buffer, as previously described¹⁵. The brains were removed and placed in 30% sucrose borate buffer at 4°C until sectioned.

After 3–7 days in buffered sucrose solution, brains were sectioned coronally at 40µm using a sliding microtome (American Optical Company, Buffalo, New York). Sections were stored in 24-well plates in an ethylene glycol-based cryoprotectant solution¹⁶ at -20 degrees Celsius until stained.

Some sections were stained with cresyl violet for subjective evaluation of hippocampal damage. Additional sections were stained with methylene blue for stereological evaluation of neuronal loss. In both cases, sections were hydrated through graded ethanols before being stained with a 1.6%/1% methylene blue/azure II solution or 5% cresyl violet.

2.7. Quantification of neuronal density and hippocampal volume

Estimates of status epilepticus-related neuronal loss were made using the optical fractionator method¹⁷ as previously described². Briefly, the total number of neurons was determined within a region of interest in area CA1 that was within the diffusion range of the cannula tip (i.e., 1.5mm radius). The region of interest was defined as the area of the pyramidal cell layer between area CA2 and the subiculum in the medial-lateral axis, and from the initial appearance of the CA1 pyramidal cell layer to the portion of the hippocampus where the dorsal and ventral portions of area CA3 united in the rostral-caudal axis.

Methylene blue-stained sections were viewed with an Olympus BX-51 microscope and Optronics video camera. Using a stereological software package (Stereo Investigator, Microbrightfield Inc.), a pre-determined counting frame (25µm²) was systematically moved along a randomly placed grid (125µm²), and the number of cell nucleoli that came into focus within a portion of the section (excluding 4µm upper and lower guard zones) were counted. Only cells that had a darkly stained nucleolus surrounded by a lightly stained nucleus and cytoplasm were counted. In the event that two nucleoli could not be distinguished as belonging to two separate neurons, only one neuron was counted. Neurons that were pyknotic were not considered viable neurons and were excluded from the analysis. The total number of neurons within the region of interest were estimated with the formula: $N = \text{sum}$

$Q- \times 1/tsf \times 1/asf \times 1/ssf$, where the number of neurons counted (sum Q-) were multiplied by the reciprocal value of the sampling probabilities based on the proportion of section thickness (tsf), cell layer area (asf), and total number of sections (ssf).

Sections were taken in a 1:6 series and utilized for evaluation of hippocampal volume with the NeuroLucida system (MicroBrightField, Inc). Immunostained areas were quantified using the contrast threshold measurement in NIH Image.

Nissl stained sections representing a 1:6 series were used for evaluation of dorsal hippocampal volume. Mean volumes of dorsal hippocampus were obtained using the NeuroLucida (7.003) image analysis software (Microbrightfield, Inc). Serial coronal areas were traced from the first section displaying pyramidal neurons in rostral hippocampus to the disappearance of dorsal hippocampus.

2.8. Data Analysis

To determine if there were any statistical differences between groups, quantitative data were analyzed with a one-way analysis of variance (ANOVA), or a factorial ANOVA, depending on the nature of the data. If statistical significance was attained in ANOVAs, Tukey LSD post-hoc tests were performed, when appropriate, to determine which groups were significantly different from each other. All statistical analyses were conducted using SPSS software (version 11.5) with alpha set at .05.

All behavioral tasks were assessed for outliers, which were defined as scores greater than two standard deviations from the mean. Outliers were subsequently removed and data re-analyzed. For the Morris water maze, if an animal's data on two or more days was an outlying score, the animal was considered an overall outlier and was completely removed from the analysis for that task. If an animal's datum was an outlier on only one day of the maze, the animal's datum was interpolated by transforming all scores for that animal on the specific task to z-scores, obtaining the mean z-score, and then transforming the z-score for the outlying day back to a raw score.

3. Results

3.1. Behavioral Functioning

The experiment was conducted in three independent cohorts to assure replicability of results. For each behavioral test, a preliminary Factorial analysis was conducted using cohort as a factor to determine if there was a significant difference between the experimental cohorts. Results revealed an overall significant cohort effect for most tests; however, the variations in baseline behavioral measurements occurred for all treatment groups in the experiment and never interacted with the effect of treatment. Therefore, data from all three cohorts were collapsed into one data set. In addition, preliminary statistical tests were used to compare results for seized animals treated with the PBS vehicle control versus those treated with the inactivated VEGF control. These groups were statistically identical, and were therefore collapsed into a single "SE" group. All animals in this "SE" group received pilocarpine and a control intra-hippocampal infusion of either inactivated VEGF or PBS vehicle.

3.1.1. Learning and Memory—Learning and memory were evaluated in the Morris water maze. Data were analyzed using a mixed 3(treatment) $\times$ 4(trial block) Factorial ANOVA, where treatment groups were untreated controls (controls) vs. pilocarpine-treated animals receiving vehicle or inactivated protein infusions (SE) vs. pilocarpine-treated animals receiving active VEGF (SE+VEGF). Results revealed an overall significant difference in learning between groups ($F(2,19)=5.369$, $p<.05$, see Figure 1A). Post hoc

analyses revealed that SE only animals learned significantly more slowly than control animals ($p < .001$), consistent with previous literature showing a negative impact of SE on learning. However, VEGF-treated SE animals also learned significantly better than SE animals receiving vehicle or inactivated VEGF ($p < .05$). These differences were not likely to be accounted for by differences in swim speed, as analysis of mean quadrant crossing time revealed no significant difference between groups on this measure ($p > .05$, data not shown).

Memory was assessed using the mean proportion of time spent swimming in the goal quadrant during a spatial probe trial. Results revealed an overall significant difference between groups ($F(2,19) = 7.205$, $p < .01$, see Figure 1B). Post hoc analyses revealed that animals which did not receive pilocarpine (controls) showed significantly better memory of the goal platform location than either the SE animals that received no VEGF ($p < .001$) or the VEGF-treated SE animals ($p < .05$). However, in spite of their impairment relative to the controls, SE+VEGF animals showed significantly better memory for the goal platform location relative to SE animals not receiving VEGF ($p < .05$). That is, VEGF-treated SE animals spent a significantly longer time swimming in the goal quadrant than SE animals treated with vehicle or inactivated VEGF.

3.1.2. Anxiety—The light-dark exploration task, a well-validated test of anxiety, was used to assess anxiety-like behaviors in the animals. Results revealed an overall significant difference in the amount of time spent in the dark compartment between groups ($F(2,17) = 5.720$, $p < .05$, see Figure 2). Post hoc analyses revealed a significant impairment in the expression of normal anxiety in the SE animals that did not receive VEGF relative to the control group ($p < .05$). VEGF prevented this impairment, as VEGF-treated SE animals showed significantly more anxiety-like behavior than the SE animals not receiving VEGF ($p < .01$), and were statistically indistinguishable from control animals ($p = .506$).

3.1.3. Grid Locomotion—Grid crossings on the open field revealed no significant difference in the amount of exploratory locomotion between control, SE, and SE+VEGF animals ($F(2,21) = 1.770$, $p = .195$, data not shown). While there were no statistically significant differences between groups in locomotion on the open field, SE animals did tend to cross more grids (mean of 40.8 for SE animals not given VEGF and 32.7 for VEGF-treated SE animals) than control animals (mean of 25.2), further refuting the idea that decrements in general behavioral drive or locomotor drive were likely to contribute to the longer time taken for SE animals to find the goal platform in the water maze.

3.2. CA1 Neuronal Loss

Because treatment with VEGF prior to pilocarpine-induced status epilepticus was previously found to preserve neurons 24 hours after SE², we investigated whether this neuronal preservation persisted at one month after status by conducting stereological CA1 neuron counts on tissue collected from a subset of the animals behaviorally tested in this experiment. Neuronal density estimates, quantified stereologically, revealed a significant difference in neuronal densities between groups ($F(2,6) = 6.834$, $p < .05$, see Figure 3). Specifically, control animals, which had not received pilocarpine, had significantly more neurons than either SE ($p < .05$) or SE+VEGF ($p < .05$) animals 30 days after SE, verifying that SE resulted in hippocampal neuronal loss as has been reported previously. This effect could not be accounted for by shrinkage of the hippocampus as we found no significant difference in hippocampal volumes between the SE and SE +VEGF groups ($t(7) = -.350$, $p = .737$). In contrast to our previously reported result of VEGF-induced neuroprotection measured 24 hours after SE, we observed no significant difference between neuronal densities in SE animals treated with VEGF versus SE animals receiving vehicle or

inactivated VEGF, suggesting that the neuronal preservation observed 24 hours after status epilepticus was not sustained for the entire first month after status.

4. Discussion

In our previous work, we observed significant VEGF-mediated neuronal preservation 24 hours after status epilepticus² which led us to hypothesize that we would see preservation of cognitive and emotional functioning with VEGF treatment in this paradigm. When we investigated behavioral functioning, we found a selective profile of preservation. That is, VEGF completely preserved normal anxiety functioning and partially protected learning and memory as assessed during the latent period between acute status epilepticus (SE) and the development of chronic overt seizure behavior in the rat pilocarpine model of SE.

To determine whether VEGF's ability to attenuate behavioral deficits was accompanied by sustained preservation of hippocampal neuron density, we assessed CA1 pyramidal neuron densities at four weeks post-SE. At this time point, we found no significant difference in neuronal densities between VEGF-treated SE animals and SE animals not given VEGF, showing that both seized groups suffered significant CA1 neuron loss. Although behavioral testing was conducted up until the fourth week after status, we cannot be certain about neuronal densities during the exact time point that each behavior was tested. However, the order of behavioral testing was counterbalanced so that the differences in the degree of behavioral preservation between tasks could not be attributed to a gradual loss of protected neurons during the two-week testing period.

Regardless of whether the neurons initially rescued by VEGF were still present during behavioral testing, research has demonstrated that there are many physiological changes which occur after seizures that could contribute to post-SE behavioral deficits, and therefore to VEGF's beneficial behavioral effects. That is, even if there were no longer any rescued neurons present when testing was performed, VEGF may have partially protected against abnormal circuit functioning. VEGF decreases synaptic activity and excitability¹⁸, which may ultimately function to preserve some behaviors (such as anxiety) more completely than others (such as learning and memory).

Because VEGF preserved anxiety-related behaviors to a greater extent than learning and memory in our model, it may be the case that memory is more sensitive to seizure-associated neuroanatomical and neurophysiological changes than anxiety. Anxiety, which can be considered an expression of fear, is a natural, adaptive reaction to the environment¹⁹. Animals typically experience fear and anxiety when faced with an acute life-threatening situation. Since this basic biological response evolved to ensure survival, it may be the case that alterations in this behavior are more resistant to impairment by damage after SE than memory would be.

At this point, we cannot be sure of the mechanisms responsible for the differential profile of functional preservation observed after SE in our model, in part because the relationships between behavior, cellular distribution, and circuit properties are complex and incompletely understood. There are many other mechanisms which could account for the incomplete preservation of learning and memory in our model. One potential explanation was proposed by Liu and colleagues²⁰, who found that rats which experienced SE, regardless of when the insult was experienced during their life spans, demonstrated impaired learning and memory on a spatially-mediated task. Concomitant with these impairments, they found defective place cells at the network level and abnormal connectivity at the structural level. Specifically, place cells recorded from rats which had experienced SE were less "coherent." That is, the local smoothness of their firing fields was lower, and their fields were less

stable. However, place cell discharge frequency was not significantly different between the groups, suggesting that abnormal electrical activity alone could not account for the observed memory deficits.

Brun et al.²¹ also investigated the place cell network within the hippocampus and related structures. By effectively isolating CA1, they demonstrated that direct connections from the entorhinal cortex into CA1 were sufficient for maintaining the fundamental properties of place cells in area CA1, ultimately preserving spatial recognition memory. They further demonstrated that an intact CA3-CA1 associate network was necessary for recall. In kindled rats, Leung and Shen²² observed changes in the medial perforant path-evoked dentate gyrus population spike and medial perforant path-evoked polysynaptic wave. They suggested that these changes in synaptic transmission underlie kindling-induced deficits in spatial performance. Given these findings, it is possible that both local place cell abnormalities and disrupted connectivity play a role in impaired spatial ability after SE, regardless of the degree of neuronal protection.

Biochemical changes related to neurotransmitters such as glutamate could also have contributed to differences in behavior. For example, during a seizure, extracellular glutamate levels increase, causing an influx of calcium through NMDA glutamate receptors which can lead to excitotoxic cell death^{23,24,25}. While it is likely that glutamate plays a role in the neuroanatomical changes after SE, it is also possible that it directly affects behavior after seizures. It is well-established that glutamate plays a role in modulating hippocampally-mediated cognitive processes such as memory and long-term potentiation^{26,27,28,29}.

Szyndler et al.³⁰ investigated the relationship between amino acid transmitters and behavior after kindling in rats. They found that learning and memory deficits were associated with a decrease in brain concentrations of glutamate-related amino acids including, glycine, alanine, and glutamate itself. Although neuronal loss is a likely explanation for decreased glutamate levels, this decrease may also be the result of an upregulation of glutamate transporters^{31,32} in response to initial increases in extracellular glutamate. In fact, Ghijsen and colleagues³³ found an increase in both glial and neuronal glutamate transporters after seizures. We have previously proposed the possibility that VEGF's enhancement of glial phenotype could have increased glutamate transporters further^{18,34}. Regardless of the cause, dysfunction of the glutamatergic system within the brain could contribute to the cognitive deficits observed after seizures.

5. Conclusions

Although VEGF preserved neurons 24 hours after SE in our earlier studies², we were unable to demonstrate here that VEGF protected neurons longer-term. In spite of this, VEGF significantly protected behavioral functioning after SE, although the extent of the functional preservation was contingent on which behavior was tested. Deficits in anxiety-like behaviors were completely prevented by VEGF while learning and memory impairments were only partially attenuated. Further studies will need to be conducted to elucidate the mechanisms of VEGF's ability to provide partial or full protection of behavior in this model.

Although exogenous VEGF protein confers some beneficial behavioral effects in this paradigm, it is unlikely that exogenous VEGF will be useful as a clinically therapeutic agent to protect neurons during severe seizures. As a large protein with multiple effects, issues of delivery and specificity of effect will be significant barricades to its use as a drug. However, if the receptor systems underlying these effects could be elucidated, small molecule reagents could be developed with specificity for the relevant receptors. Based on our current findings,

it appears possible that these reagents could confer some behavioral protection to patients suffering from the cognitive or emotional sequelae of severe or chronic seizures.

Research Highlights

VEGF protects neurons from status epilepticus-induced death 24 hours after the acute seizure event. To test whether this protection is accompanied by preservation of function, we infused VEGF during status epilepticus and then evaluated learning and memory in the Morris water maze and anxiety in the light-dark exploration apparatus. Animals treated with VEGF showed statistically significant preservation of behavioral function, suggesting a protective role for VEGF against seizure-induced behavioral impairments. Surprisingly, cell counts conducted one month after status epilepticus showed that the protective effects of VEGF against neuronal loss were transient, suggesting that other factors contributed to the behavioral preservation.

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Figure 1. Morris water maze performance after pilocarpine-induced status epilepticus (SE)

A. Control animals (Controls, n=15) learned significantly better than SE animals receiving vehicle or inactivated VEGF (SE, n=7) based on latency to find the goal platform. VEGF-treated SE animals (SE+VEGF, n=5) also learned significantly better than SE animals not receiving VEGF, suggesting partial functional preservation by VEGF.

B. Control animals spent significantly more time in the goal quadrant than either SE or SE +VEGF animals. However, VEGF-treated SE animals (SE+VEGF) spent significantly more time in the goal quadrant than SE animals receiving control infusions (SE), reflecting partial functional preservation by VEGF.

significantly different from control animals, * significantly different from SE animals receiving vehicle or inactivated VEGF infusions

Figure 2. Light-dark exploration task after pilocarpine-induced status epilepticus (SE)

Animals that experienced SE but no VEGF (SE, n=6) spent significantly more time in the light compartment compared to VEGF-treated animals that experienced SE (SE+VEGF, n=4) and control, non-seized animals (Controls, n=10). There was no significant difference between VEGF-treated SE animals and controls, suggesting that VEGF completely prevented anxiety impairments after SE.

significantly different from control animals, * significantly different from SE animals receiving vehicle or inactivated VEGF infusions

Figure 3. Neuronal density estimates after treatment with VEGF one month following status epilepticus (SE)

A. Photomicrograph of a Nissl-stained hippocampal section in a representative SE animal receiving an inactivated VEGF infusion.

B. Photomicrograph of a Nissl-stained hippocampal section in a representative VEGF-treated SE animal. Scale bar=100μm

C. Both SE+VEGF and SE animals not receiving VEGF had significantly fewer CA1 neuronal profiles 30 days after SE than control animals. However, CA1 neuronal densities were statistically similar for the two SE groups, VEGF infusions (SE+VEGF group) versus comparison infusions (SE group), suggesting no significant long-term preservation by VEGF.

significantly different from control animals