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Minocycline increases quality and longevity of chronic neural recordings

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Abstract

Brain/machine interfaces could potentially be used in the treatment of a host of neurological disorders ranging from paralysis to sensory deficits. Insertion of chronic micro-electrode arrays into neural tissue initiates a host of immunological responses, which typically leads to the formation of a cellular sheath around the implant, resulting in the loss of useful signals. Minocycline has been shown to have neuroprotective and neurorestorative effects in certain neural injury and neurodegenerative disease models. This study examined the effects of minocycline administration on the quality and longevity of chronic multi-channel microwire neural implants 1 week and 1 month post-implantation in auditory cortex. The mean signal-to-noise ratio for the minocycline group stabilized at the end of week 1 and remained above 4.6 throughout the following 3 weeks. The control group signal-to-noise ratio dropped throughout the duration of the study and at the end of 4 weeks was 2.6. Furthermore, 68% of electrodes from the minocycline group showed significant stimulus-driven activity at week 4 compared to 12.5% of electrodes in the control group. There was a significant reduction in the number of activated astrocytes around the implant in minocycline subjects, as well as a reduction in total area occupied by activated astrocytes at 1 and 4 weeks.

Introduction

A fundamental requirement of next-generation brain/machine interfaces is to provide a long-term, stable and reliable interface with the nervous system. Of the many technological and biological impediments confronting neural engineers, perhaps most challenging is improving the biocompatibility of electrode arrays. Studies have shown that the insertion of micro-electrode arrays for chronic recording/stimulation of brain cells initiates a host of immunological responses [1,2]. The immune response leads to the formation of a cellular sheath around the implants consisting predominantly of astrocytes and microglia [3–8]. The cellular encapsulation is a highly capacitive and resistive layer that ranges from 50 to 400 μm in diameter [3,8] and contracts tightly around the implant by week 12 [6]. While neural density is normal immediately outside the sheath ~60 μm [8], the encapsulation typically results in signal degradation and can ultimately render the neural interface nonfunctional. If neural interfaces are to become a viable solution for treating neurological conditions long-term, methods must be developed to prevent or minimize encapsulation.

Minocycline, a second generation antibiotic belonging to the tetracycline family, has been shown to have neuroprotective and neurorestorative effects in certain ischemic injury/stroke [9,10] and neurodegenerative disease models such as Huntington disease [11], amyotrophic lateral sclerosis [12], Alzheimer's [13], Parkinson disease [14] and spinal cord injuries [15].

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The precise target(s) and complete mechanism underlying its actions are still under investigation, but beneficial effects on inflammation, microglial activation, matrix metalloproteinases (MMP), nitric oxide production and apoptotic cell death are believed to impart minocycline its neuroprotective properties [10,16,17].

The purpose of this study was to determine if minocycline enhances the quality and longevity of chronic multi-channel neural recordings. Twenty rats were implanted with eight-channel microwire electrodes in primary auditory cortex; one group was given orally-administered minocycline and the control group was given water. The effects were evaluated with neural recording properties as well as histology at 1 week and 4 weeks for both groups. The effects were quantified using measures of the signal-to-noise ratio, number of driven channels, as well as the number, size and area of glial fibrillary acidic protein (GFAP)—positive cells.

Methods

Subjects were 20 male Long Evans rats (200–300 g) obtained from Charles River Labs (Dallas, TX). All the rats were individually housed in a temperature- and humidity-controlled environment and were exposed to a 12:12 h light-to-dark cycle. The rats were divided into four groups: 1 week controls and minocycline; 4 week controls and minocycline (five each). The minocycline group was given 100 mg L^{-1} minocycline–HCl (Watson Inc., CA) dissolved in water and administered *ad libitum* 2 days prior and for 5 days following surgical implantation. Prior to surgery, it was estimated that the subjects consumed 40 mL of water per day, which is equivalent to ~4 mg of minocycline per day. Two subjects from the 1 week control group were removed from the study due to unrelated health factors.

All the surgical procedures were carried out in accordance with the University of Oklahoma's Laboratory Animal Resources and Institutional Animal Care and Use Committee regulations. Surgical procedures have been reported elsewhere [18]. Briefly, rats were anesthetized using ketamine, xylazine and acepromazine (targeted dosage 50, 20, 5 mg kg^{-1} respectively). A midline incision was made in the scalp and the connective tissue was dissected from the skull. The temporal bone and lateral suture were exposed by partial dissection of the right temporalis muscle. Six bone screws were implanted in the skull for electrode fixation. A 2 mm × 3 mm portion of the temporal bone was removed adjacent to the lateral suture exposing primary auditory cortex. An incision was made in the dura exposing the pia. Custom-made multi-channel microwire electrodes consisted of 50 μm diameter micro wires with 500 μm center-to-center spacing in a 4 × 2 matrix [18,19]. A bone screw was used as a reference. Using a custom-fabricated mechanical inserter mounted on the microdrive, the array was implanted to a depth of 550 μm in layer IV/V of the primary auditory cortex at a rate of 1.49 m s^{-1} [19]. The craniotomy was covered by a layer of silicone elastomer (Kwik-Cast; World Precision Instruments, Inc., FL) and another layer of acrylic was added to seal the craniotomy and secure the implant to the bone screws. After the acrylic dried, the electrode array was carefully separated from the insertion device and sutures were sewn covering the incision. All implantations were performed by the same surgeon to minimize technique variability.

Neural data were obtained starting 2 days post-implantation and every other day during the first week. During weeks 2, 3 and 4 recordings were made two to three times per week. During recording sessions, animals were suspended in a custom restraint harness beneath a calibrated free-field speaker inside a double-walled acoustic chamber. Stimuli sets consisted of ten repetitions of 140 pure tones ranging in frequency from 2 to 32 kHz and in intensity from 0 to 60 dB SPL. Neural responses to these auditory stimuli were digitized at a rate of 25 kHz and band-passed filtered from 500 Hz to 5000 Hz, 6 dB per octave. Most active channels recorded multi-unit activity from neuron clusters.

Auditory stimulus-driven activity from the multi-unit clusters was plotted as peri-stimulus time histograms (PSTHs) for each channel with bin sizes of 5 ms. Spontaneous rates were measured 30 ms prior to stimulus presentation and averaged over the stimulus set. Channels were defined as significantly driven if the onset response rate (from 10 to 30 ms post-stimulus-onset) was significantly different from spontaneous rate (*t*-test, $\alpha = 0.01$). Signal-to-noise ratio (SNR) was defined as the peak-to-peak magnitude of isolated action potentials divided by the RMS value of the signal. Channels that did not exhibit any unit activity were assigned a SNR of 1.

At the completion of the experimental period of 1 and 4 weeks subjects were euthanized and perfused. Subjects were administered 3–6 mL of anesthetic urethane (concentration 0.25 g mL^{-1}) intraperitoneally, and then transcardially perfused with 100 mL of phosphate buffered saline (PBS) and 100–200 mL 4% paraformaldehyde. The brain was removed and stored (4 ° C) for 48 h immersed in a solution of 30% sucrose and 4% paraformaldehyde by volume. The brain was cut into 40 μm thick coronal sections which were collected free-floating in PBS. Sections had endogenous peroxidase activity blocked by a 5 min rinse in 3% hydrogen peroxide, and were then rinsed in 3% normal goat serum. Slices were incubated overnight in rabbit anti-GFAP polyclonal antibody (DAKO; diluted 1:1000) in a buffer containing 3% normal goat serum and 0.3% Triton X-100. The following day the sections were rinsed in PBS and incubated in a goat, anti-rabbit secondary antibody (Vector). The slices were reacted according to the Vectastain kit instructions and final staining visualized with Vector SG chromogen. Sections were mounted on gelatin subbed slides, dehydrated and cover-slipped with Histomount.

Sections were digitally imaged with an Olympus BH-2 microscope on a 10× objective and an Olympus DP-70 digital camera. Acquired images centered around individual electrode tracks and comparable regions in the contralateral, unimplanted hemisphere. Images were analyzed with ImageJ software (NIH), which allowed for automated cell counting and volume measurements (see figure 3). Total GFAP-labeled cell number, average cell size (including processes) and total area occupied by GFAP-labeled cells within the viewing area were determined using ImageJ software. The outermost cortical surface was not included in the analysis and a constant threshold was used for all automated imaging. Although quantification was automated, these analyses were performed by an experimenter blind to the group assignment of the sections. A total of 85 (28 controls and 57 minocycline) images were analyzed from the 1 week subjects and 109 images (57 controls and 52 minocycline) from 4 week subjects.

Results

There were no significant differences in signal-to-noise ratio or percentage of driven channels during the first 6 days post-implantation. Both groups exhibited significant (ANOVA, $\alpha = 0.05$), parallel drops in SNR during the first week, but after day 6 the minocycline group SNR stabilized while the control group SNR continued to decrease (figure 1). From day 7 onward, the minocycline group SNR was significantly greater than the control group SNR (ANOVA, $\alpha = 0.01$).

To provide a measure of multi-channel functionality, the percentage of channels with significant driven activity was counted for each recording session. Again, significant differences between the groups emerged after day 6 (figure 2). Note that the large variability shown in figure 2 is due to the fact that there is only one measure of channel count per subject. Despite the variability, minocycline significantly increased the percentage of driven channels compared to controls. In the control subjects, only one to two electrodes on average exhibited driven activity after day 9, whereas four to five of the eight channels were driven in minocycline subjects through the duration of the study.

GFAP-immunoreactivity is a standard measure for assessing reactive gliosis [3,4]. Figure 3 displays representative histological images and corresponding identified/counted cells for both minocycline and control groups. Cursory examination of 1 week subjects (figures 3(A)–(D)) suggests that the minocycline group has fewer activated astrocytes (figures 3(A) and (B)) than the control image (figures 3(C) and (D)). The astrocytes also appear to be larger in minocycline images than in controls. Images from 4 week subjects (figures 3(E)–(H)) are more similar in appearance between the two groups than those from 1 week subjects. In 4 week controls, activated astrocytes appear to be more localized around the tip of the electrode track, with broad activation near the surface of the cortex in all cases.

Quantitative analysis of the histological images was performed by an automated software package. Differences in automated histology results were significant at week 1 (figure 4). For 1 week subjects, those in the minocycline group showed significantly fewer activated astrocytes (*t*-test, $p < 0.0001$) covering less cortical area than controls ($p < 0.0001$). However, astrocytes seen in the minocycline group had a significantly larger area per cell than controls ($p = 0.007$). Control group subjects at 4 weeks had significantly more activated astrocytes (figure 4(B)) than minocycline subjects ($p = 0.016$), taking up significantly more area ($p = 0.021$), but there was no significant difference in area per cell ($p = 0.108$).

Discussion and conclusion

The results presented here demonstrate that minocycline improves the quality (SNR) and longevity (% active channels) of neural recordings obtained from a chronic neural interface. The mean SNR for the minocycline group stabilized at the end of week 1 and remained above 4.6 for the duration of the study, which is a considerable improvement compared to the final control SNR of 2.6. The improvement due to minocycline is likely a function of several factors, including decreased inflammation [10], increased neuronal survival near the electrode tip [16] and decreased microglia [17] and astrocyte activity in the minocycline-treated subjects. Neuronal injury, such as that caused by electrode implantation, typically initiates a host of responses which eventually result in a cellular sheath of astrocytes and laminin around the offended region [1–5]. The data presented here show that treatment with minocycline results in a significant reduction in the total number of activated astrocytes and the total amount of area occupied by these cells. This reduced activity may account in part for the stability of the recording properties in the minocycline subjects past week 1. Longer-term studies could be performed to determine how long this improved recording quality persists.

Similar to minocycline, the administration of dexamethasone for 6 days post-implantation has been shown to reduce astrocyte activity, but it increases microglial and vascular responses [6]. The combination of minocycline and dexamethasone may provide an optimal treatment to reduce both microglial [10,17] and astrocyte activation [6,7]. Future studies should examine this treatment regimen.

While minocycline did stabilize neural recording quality during weeks 2–4, it did not prevent the initial decrease in SNR during the first week. Szarowski *et al* proposed that the strength of early tissue response is proportional to the device size and hence the amount of initial tissue damage [4]. Reducing the amount of cortical tissue deformation by using small electrodes with a reduced surface area relative to the cortical surface during implantation [8] could reduce this initial response. In a previous study, we have also demonstrated that rapid insertion techniques, which have been shown to result in lower mean effective strain on cortical tissue [20], improve the quality and longevity of neural recordings compared to slow insertion [19].

While it has been shown that the use of drug interventions to minimize reactivity following surgery will improve the quality of recordings short term (4 weeks), it is likely that other factors

will contribute to failure of these devices over longer time periods. Continuous administration of minocycline or any other antibiotic is not a viable solution for long-term treatment. It is apparent that other strategies will need to be employed to maximize the long-term viability of neural interfaces. The development of bioactive coatings to induce neural growth toward the implant, the development of specialized surface coatings and a reduction of those factors that result in chronic tissue injury all need to be addressed.

Micromotion has been identified as one of the most likely contributors to continued tissue injury. Micromotion, relative motion between the implant and cortical tissue, has been shown to be on the order of 10's of μm [21] in anesthetized subjects. This motion is likely to be even greater in behaving subjects. The optimal solution is to develop a small wireless neural implant that can be fully embedded in cortical tissue. This will allow the neural interface to move with the tissue as it is deformed.

Chronic intracortical recordings have the potential to improve the lives of people with neurological disorders. Efforts to make these devices acceptable for chronic human use will require the development of treatment strategies, improved device designs and surgical methods to ensure the chronic viability of the neural interface for decades. The results of this study demonstrate that minocycline improves the functionality of neural interfaces over the course of 4 weeks. As a result, future studies on the efficacy of minocycline are warranted.

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References

1. Polikov VS, Tresco PA, Reichert WM. Response of brain tissue to chronically implanted neural electrodes. J Neurosci Methods 2005;148:1–18. [PubMed: 16198003]
2. Landis DM. The early reactions of non-neuronal cells to brain injury. Annu Rev Neurosci 1994;17:133–51. [PubMed: 8210172]
3. Turner JN, et al. Cerebral astrocyte response to micromachined silicon implants. Exp Neurol 1999;156:33–49. [PubMed: 10192775]
4. Szarowski DH, et al. Brain responses to micro-machined silicon devices. Brain Res 2003;983:23–35. [PubMed: 12914963]
5. Schultz RL, Willey TJ. The ultrastructure of the sheath around chronically implanted electrodes in brain. J Neurocytol 1976;5:621–42. [PubMed: 1003257]
6. Spataro L, et al. Dexamethasone treatment reduces astroglia responses to inserted neuroprosthetic devices in rat neocortex. Exp Neurol 2005;194:289–300. [PubMed: 16022859]
7. Shain W, et al. Controlling cellular reactive responses around neural prosthetic devices using peripheral and local intervention strategies. IEEE Trans Neural Syst Rehabil Eng 2003;11:186–8. [PubMed: 12899270]
8. Edell DJ, Toi VV, McNeil VM, Clark LD. Factors influencing the biocompatibility of insertable silicon microshafts in cerebral cortex. IEEE Trans Biomed Eng 1992;39:635–43. [PubMed: 1601445]
9. Hua R, Walz W. Minocycline treatment prevents cavitation in rats after a cortical devascularizing lesion. Brain Res 2006;1090:172–81. [PubMed: 16647693]
10. Yrjanheikki J, Tikka T, Keinanen R, Goldsteins G, Chan PH, Koistinaho J. A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. Proc Natl Acad Sci USA 1999;96:13496–500. [PubMed: 10557349]
11. Bonelli RM, Hodl AK, Hofmann P, Kapfhammer HP. Neuroprotection in Huntington's disease: a 2-year study on minocycline. Int Clin Psychopharmacol 2004;19:337–42. [PubMed: 15486519]
12. Zhu S, et al. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. Nature 2002;417:74–8. [PubMed: 11986668]

13. Ryu JK, Franciosi S, Sattayaprasert P, Kim SU, McLarnon JG. Minocycline inhibits neuronal death and glial activation induced by beta-amyloid peptide in rat hippocampus. Glia 2004;48:85–90. [PubMed: 15326618]

14. Du Y, et al. Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. Proc Natl Acad Sci USA 2001;98:14669–74. [PubMed: 11724929]

15. Wells JE, Hurlbert RJ, Fehlings MG, Yong VW. Neuroprotection by minocycline facilitates significant recovery from spinal cord injury in mice. Brain 2003;126:1628–37. [PubMed: 12805103]

16. Lee SM, et al. Minocycline inhibits apoptotic cell death via attenuation of TNF-alpha expression following iNOS/NO induction by lipopolysaccharide in neuron/glia co-cultures. J Neurochem 2004;91:568–78. [PubMed: 15485488]

17. Tikka T, Fiebich BL, Goldsteins G, Keinanen R, Koistinaho J. Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. J Neurosci 2001;21:2580–8. [PubMed: 11306611]

18. Rennaker RL, Ruyle AM, Street SE, Sloan AM. An economical multi-channel cortical electrode array for extended periods of recording during behavior. J Neurosci Methods 2005;142:97–105. [PubMed: 15652622]

19. Rennaker RL, Street S, Ruyle AM, Sloan AM. A comparison of chronic multi-channel cortical implantation techniques: manual versus mechanical insertion. J Neurosci Methods 2005;142:169–76. [PubMed: 15698656]

20. Bjornsson CS, et al. Effects of insertion conditions on tissue strain and vascular damage during neuroprosthetic device insertion. J Neural Eng 2006;3:196–207. [PubMed: 16921203]

21. Gilletti A, Muthuswamy J. Brain micromotion around implants in the rodent somatosensory cortex. J Neural Eng 2006;3:189–95. [PubMed: 16921202]

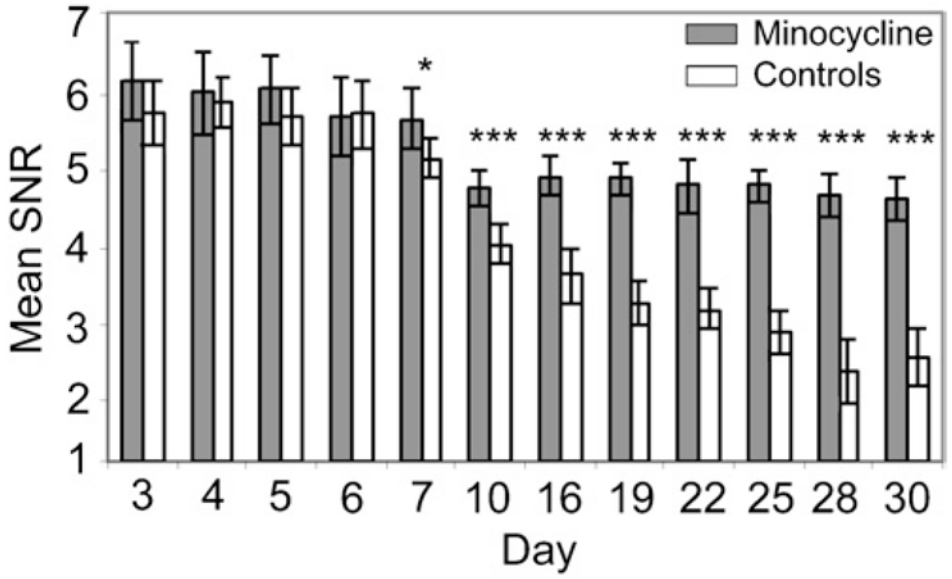

Figure 1.
SNR data. There were no significant differences between the groups during the first 6 days. On day 7, the control group SNR decreased significantly, while the minocycline group did not change. Error bars are the 95% confidence interval. (*$p < 0.05$, ***$p < 0.001$)

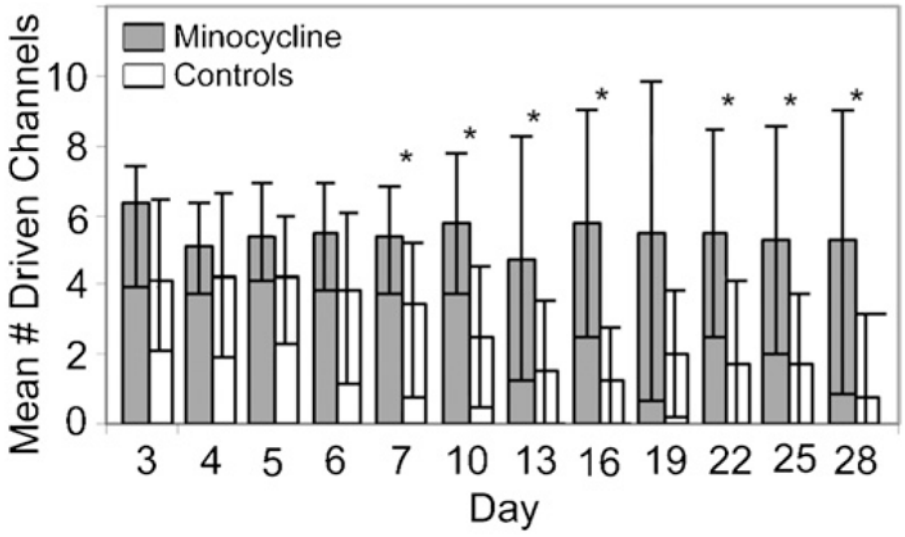

Figure 2.

Per cent of driven channels. The per cent of channels with driven activity was measured for each subject. The control group has a steady decline over the 4 week period while the minocycline group stays relatively stable across 4 weeks. Error bars are 95% confidence interval. (*$p < 0.05$)

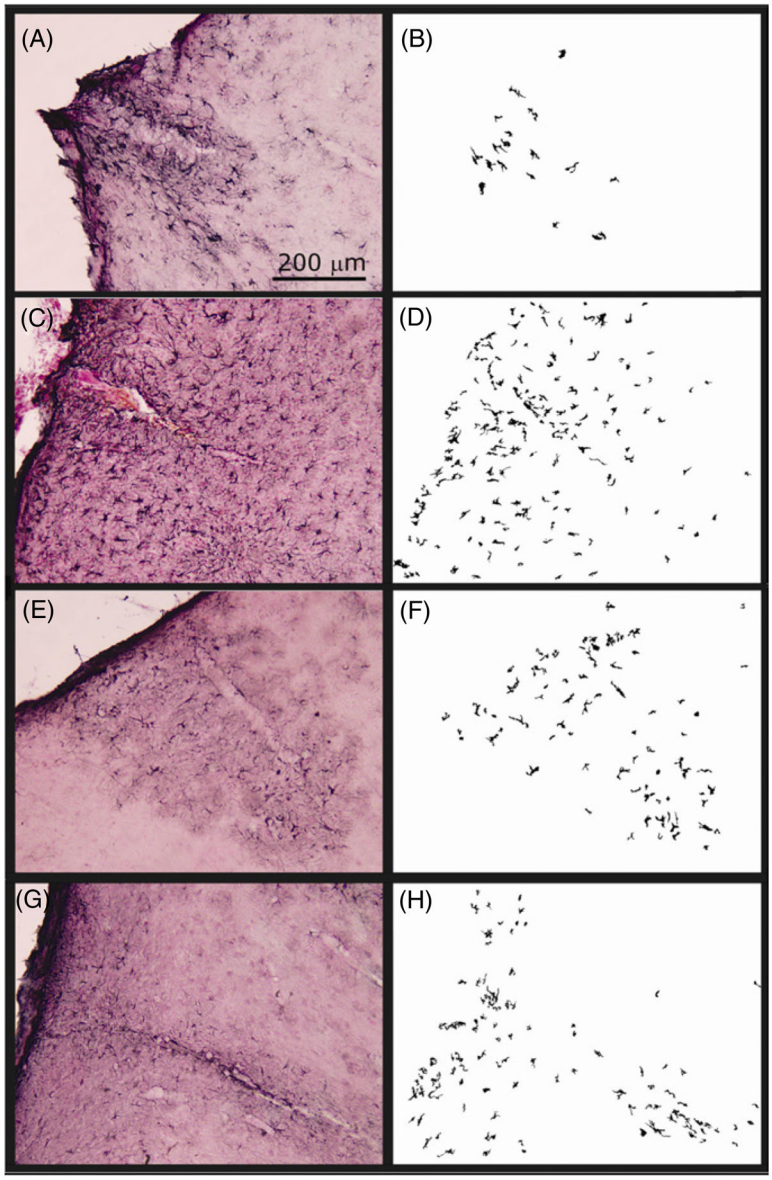

Figure 3.
Histology images. Panels (A)–(D) display representative examples of histology at 1 week. The minocycline group ((A) and (B)) displayed fewer activated cells and covered a smaller area than the controls ((C) and (D)). The 4 week data are presented in (E)–(H). At 4 weeks the two groups look very similar. The figures in the right column are the threshold figures used to count the number and size of cells.
(This figure is in colour only in the electronic version)

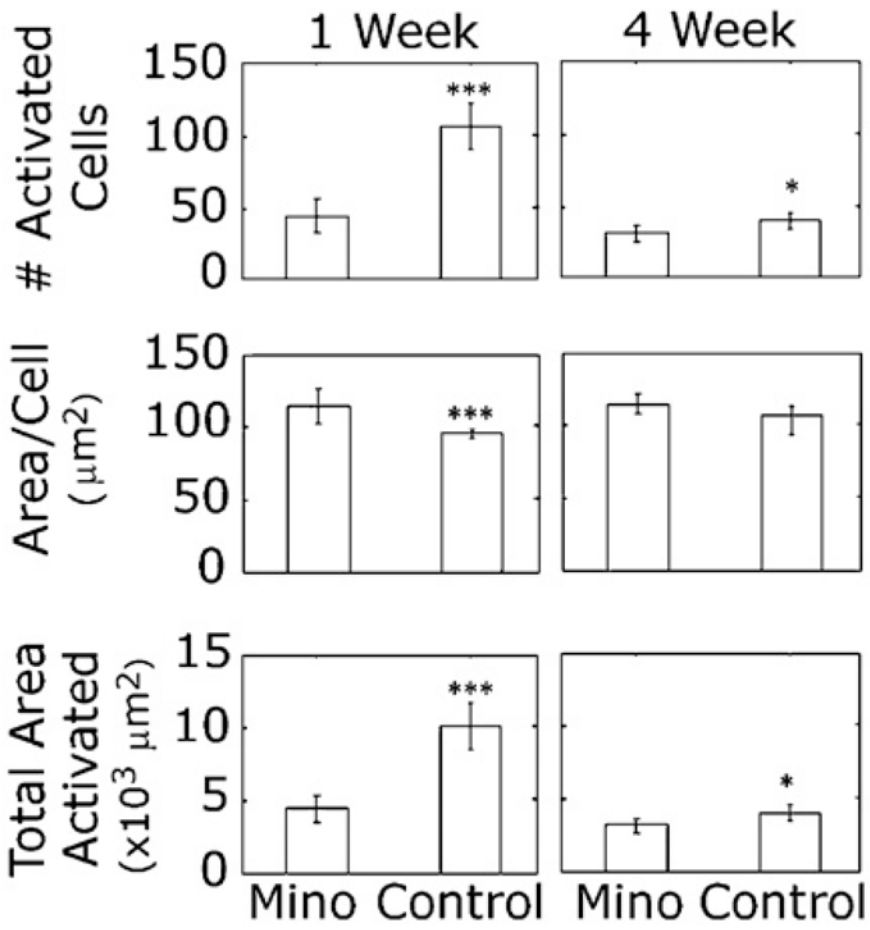

Figure 4.

Quantitative histology. Using automated image analysis software (ImageJ), the area occupied by each cell, the total number of activated cells and the total area occupied by activated astrocytes were measured. The 1 week data are presented in the left column and the 4 week data are in the right column. At both 1 and 4 weeks, the control group exhibited an increase in the total number of activated astrocytes and the total area occupied by these cells. However, the activated astrocyte size was larger in the minocycline group at week 1 only. Error bars are 95% confidence interval. (*$p < 0.05$; ***$p < 0.001$).