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Remotely controlled proton generation for neuromodulation

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Abstract

Understanding and modulating proton-mediated biochemical processes in living organisms have been impeded by the lack of tools to control local pH. Here, we design nanotransducers capable of converting non-invasive alternating magnetic fields (AMFs) into protons in physiological environments by combining magnetic nanoparticles (MNPs) with polymeric scaffolds. When exposed to AMFs, the heat dissipated by MNPs triggered a hydrolytic degradation of surrounding polyanhydride or polyester, releasing protons into the extracellular space. pH changes induced by these nanotransducers can be tuned by changing the polymer chemistry or AMF stimulation parameters. Remote magnetic control of local protons was shown to trigger acid-sensing ion channels and evoke intracellular calcium influx in neurons. By offering a wireless modulation of local pH, our approach can accelerate the mechanistic investigation of the role of protons in biochemical signalling in the nervous system.

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Supporting Information Available

The Supporting Information is available free of charge on the ACS Publications website.

- Experimental and modeling methods, Figures S1–S13, and Table S1–S2 (PDF)

The authors declare no competing financial interest.

Graphical Abstract

Keywords

nanotransducers; wireless neuromodulation; protons; alternating magnetic fields; acid-sensing ion channels

The pH of biological fluids is one of the critical factors that determine cellular function and viability.^{1–4} In the nervous system, acidic environments affect neuronal function by triggering proton-activated ion channels, including acid-sensing ion channels (ASICs).^{5–7} ASICs play a role in multiple physiological processes, including synaptic plasticity, mechanosensation, learning and memory.^{7–9} Furthermore, acid-induced toxicity mediated by ASICs contributes to neurological diseases, such as ischemic stroke and epileptic seizures.⁷ Reduction in the extracellular pH also affects the function, morphology, and survival of non-neuronal glial cells.^{10,11} To understand proton-mediated neurobiological processes, multiple methods that can alter the pH in biological systems have been developed. While earlier studies evoked pH reduction with CO₂ inhalation, this method was often accompanied by off-target pH changes.^{12,13} More invasive techniques, such as microinjections of acidified medium through permanently implanted cannulas or control of protons with electrochemical devices, have been also applied to achieve localized pH modulation.^{13–16}

In search of a less invasive technique to locally modulate pH of a target region, we designed a wireless proton generating platform controlled by low-radiofrequency alternating magnetic fields (AMFs). The latter exhibit minimal coupling to biological matter due to its low conductivity and negligible magnetic permeability.¹⁷ Paired with hysteretic heating of magnetic nanoparticles (MNPs), AMFs have been applied to control neural activity, gene expression, and hormone release.^{18–22} In this study, we leveraged the MNP heating in AMFs to manipulate local pH by integrating MNPs with polyanhydrides (or polyesters), which generate carboxyl groups as their hydrolysis products (Figure 1a).^{23,24} MNP heating facilitated the hydrolytic degradation of the polymer, which in turn led to the rapid decrease in extracellular pH. We applied these chemomagnetic nanotransducers to convert an AMF cue into protons for the control of ASICs-mediated signalling processes in neurons (Figure 1b).

As the first step to develop MNP-polymer nanotransducers, we prepared monodisperse Fe₃O₄ MNPs with diameters of 23.8 ± 1.7 nm (Figure 2a), which possess hysteretic heat dissipation rate of ~ 500 W/g[Fe] in AMFs with amplitudes $H_0 \sim 15$ kA/m and a frequency ~ 500 kHz.²⁵ Similar particles have been previously shown to have negligible cytotoxic effects.¹⁸ Motivated by prior research showing significantly higher temperature inside the

sub-micrometer MNP-containing scaffolds as compared to the bulk ferrofluid during the MNP heating,²⁶ we then designed MNP-polyanhydride or MNP-polyester nanotransducers, where synthesized MNPs were embedded inside the nanoscale polyanhydride or polyester scaffolds, respectively (Figure 1b). We hypothesized that significant temperature increase within these nanotransducers would accelerate the hydrolytic degradation of polymers²⁷ without comparable changes in temperature of the surrounding solution in AMFs. We employed poly(sebacic acid) (PSA) and poly(lactic-co-glycolic acid) (PLGA) as test-beds for demonstrating a proof-of-concept of our chemomagnetic approach to control local pH. PSA and PLGA are widely investigated polyanhydride or polyester biomaterials, respectively, due to their biocompatibility, tunable degradation rates, and well-established fabrication and surface modification methods.^{23,24,28}

The designed MNP-PSA or MNP-PLGA nanotransducers were synthesized by using an oil-in-water, emulsion-solvent evaporation method.²⁹ Briefly, the mixtures of MNPs and PSA or PLGA dispersed in chloroform were emulsified with a high-speed homogenizer in the presence of the surfactant polyvinyl alcohol dissolved in deionized water. After evaporation of the chloroform, MNP-PSA or MNP-PLGA nanotransducers were collected and re-dispersed in Tyrode's solution. The percentage of MNPs in a nanotransducer was defined by the initial MNP-polymer ratio in the chloroform (Figure 2b, Figure S1). Transmission electron microscopy (TEM) images of representative MNP-PSA and MNP-PLGA nanotransducers with a 1:2 MNP:polymer weight ratio revealed high-density MNPs embedded within the spherical polymer scaffolds (Figure 2c–d and Figure S2). The diameters of the synthesized MNP-PSA and MNP-PLGA nanotransducers were measured as 432 ± 87 nm and 203 ± 32 nm, respectively (Figure 2e). Fourier transform infrared (FT-IR) spectrum of MNP-PLGA nanotransducers showed absorption bands near 1750, 1200, and 1100 cm^{-1} ,³⁰ which could be attributed to the C=O and two C-O stretching vibrations in the ester, confirming the presence of PLGA in the nanotransducer. Similarly, the existence of PSA in the MNP-PSA nanotransducer was confirmed by C=O, C-O, and -CH₂- bands in the FT-IR spectra (Figure S3).

We then evaluated the feasibility of applying the synthesized nanotransducers as wireless proton generators by recording the pH changes of a nanotransducer solution following exposure to an AMF. Physiologically safe AMFs with amplitudes $H_0 \sim 50$ kA/m and a frequency ~ 150 kHz were generated by a custom-designed resonant coil^{18,31,32} and applied to Tyrode's solutions of nanotransducers (1:2 MNP:polymer weight ratio) with concentrations of ~ 15 mg/mL. Following AMF exposure, the pH of the collected solution was recorded using a ratiometric pH-indicating fluorescent dye seminaphtharhodafleur (Figure 2f).³³ After 60 s of exposure to AMFs, pH of Tyrode's solution containing MNP-PSA nanotransducers was reduced from 7.40 to 6.87 ± 0.17 . Further exposure to the AMF led to the gradual decrease in pH to 6.52 ± 0.11 and 6.38 ± 0.22 after 300 s and 600 s, respectively. In the absence of AMF stimuli, only a slight decrease in solution pH to 7.18 ± 0.07 was observed even after 1 h of spontaneous hydrolytic degradation at room temperature (Figure 2g).

Exposure to the AMF of the MNP-PLGA nanotransducers did not result in significant pH changes compared to the MNP-PSA nanotransducers. The pH of a solution loaded with

MNP-PLGA nanotransducers was only slightly reduced from 7.40 to 7.30 ± 0.05 and 7.24 ± 0.03 after 300 s and 600 s of exposure to AMFs, respectively. Even in the modified Tyrode's solution with buffer capacity reduced 10 fold, MNP-PLGA nanotransducers lowered the solution pH to 6.88 ± 0.06 and 6.60 ± 0.07 after 300 s and 600 s, respectively, which were smaller changes than those induced by MNP-PSA nanotransducers in unmodified Tyrode's solution (Figure 2g). The difference between these two groups can likely be attributed to an intrinsically slower hydrolysis rate of ester group in PLGA compared to that of anhydride group in PSA.³⁴ This observation was further confirmed in a follow up experiment where the PSA and PLGA nanospheres were hydrolytically degraded in the absence of AMF stimuli (Figure S4). Together, these data suggest that MNP-PSA nanotransducers can remotely lower the solution pH in response to AMFs. Furthermore, the proton release kinetics of the nanotransducers are dependent on polymer chemistry. This dependency provides an opportunity to tune the release kinetics of the nanotransducers, minimize their spontaneous degradations before exposure to AMFs, and optimize their application *in vitro* or *in vivo* while reducing thermal effects.

Finally, to test our hypothesis that the local heating near the MNPs surfaces rather than the bulk solution heating (Figure 2h), predominately accelerated the hydrolysis of PSA upon exposure to an AMF, we compared pH and temperature changes of Tyrode's solutions containing MNP-PSA nanotransducers and those of the concentration-matched physical mixtures of MNPs and PSA-only nanospheres after exposure to AMFs. We anticipated that hydrolysis of PSA nanospheres in physical mixtures with MNPs would be mainly attributed to the increase in the bulk solution temperature during ferrofluid heating in the AMF. We found that in contrast to the solutions of MNP-PSA nanotransducers, the exposure to the AMF stimuli did not evoke noticeable pH reductions in physical mixtures of MNPs and PSA nanospheres. The pH of solutions containing physically mixed MNPs and PSA nanospheres was only slightly reduced from 7.4 to 7.06 ± 0.09 and 7.03 ± 0.03 after 300 s and 600 s of exposure to AMF, respectively. However, no statistically significant difference in bulk temperature changes was found between the two groups (Figure S5). These findings suggest that the hydrolytic degradation of PSA in MNP-PSA nanotransducers is largely driven by the local heat dissipation from MNPs.

To investigate the ability of the nanotransducers to modulate proton-mediated signaling processes, we applied them to trigger ASICs, which are endogenously expressed Na^+ -selective cation channels in the central nervous system.⁷ Activation of ASICs induces entry of Na^+ ions into the cells, causing membrane depolarization and activation of voltage-gated Ca^{2+} channels.^{5,7} We chose to focus on hippocampal neurons because of their well-characterized ASIC expression and culture methods.^{9,35} We recorded the intracellular Ca^{2+} changes using the green fluorescent Ca^{2+} indicator fluo-4 as a proxy for membrane depolarization (Figure 3a).³⁶ We first confirmed the functionality of endogenously expressed ASICs in the cultured hippocampal neurons. Consistent with prior studies,^{6,37} we observed that a rapid extracellular pH reduction induced Ca^{2+} influxes into the neurons. When exposed to an acidified medium with pH of 6.8, the normalized fluo-4 fluorescence (F/F_0) in the neurons increased by $24 \pm 7\%$. Ca^{2+} influx into the neurons at identical conditions was greatly diminished by the addition of 500 μM of the non-specific ASIC blocker

amiloride,⁵ indicating that Ca^{2+} influxes observed in the hippocampal neurons in response to pH decrease are predominately mediated by ASICs (Figure 3b).

Acid-evoked Ca^{2+} responses in the hippocampal neurons were further enhanced following viral transgene delivery and over-expression of ASIC1a, the predominant ASIC subunit in the central nervous system.⁵ To enhance the expression levels of ASIC1a, neurons were transduced with lentivirus carrying ASIC1a transgene along with a fluorescent protein mCherry separated by the post-transcriptional cleavage linker p2A under the excitatory neuronal promoter calmodulin kinase II α -subunit (*Lenti-CaMKII α ::ASIC1a-p2A-mCherry*). The expression of mCherry-tagged ASIC1a was confirmed via fluorescent imaging in the transduced neurons (Figure 3c). The ASIC1a-overexpressing (ASIC1a⁺) neurons showed a robust fluo-4 fluorescence increase ($F/F_0 \sim 137 \pm 12\%$) following the injection of an acidified medium with pH of 6.8 (Figure 3d). At identical extracellular pH conditions, ASIC1a⁺ neurons exhibited significantly higher Ca^{2+} influxes as compared to genetically intact neurons (Figure 3e). Addition of the amiloride has similarly led to a decrease in proton-mediated Ca^{2+} influxes in ASIC1a⁺ neurons, akin to our observation in the genetically intact cultures (Figure 3b).

We next examined whether our nanotransducers could similarly trigger ASICs. Before applying AMF stimulation, we confirmed that both MNP-PSA and MNP-PLGA nanotransducers did not evoke significant cytotoxic responses *in vitro* (Figure S6). To elicit robustly measurable Ca^{2+} responses, we used ASIC1a⁺ neurons and MNP-PSA nanotransducers. ASIC1a⁺ neurons immersed in Tyrode's solution containing MNP-PSA nanotransducers (15 mg/mL, 1:2 MNP:PSA weight ratio) were exposed to AMFs for 60 s with the parameters previously shown to evoke the reduction of extracellular pH from 7.40 to 6.87 (50 kA/m at 150 kHz). Here, the MNP-PSA nanotransducer concentration and MNP-PSA ratio were optimized to induce a significant pH reduction required for ASIC activation (pH for half-maximum responses $\text{pH}_{50} \sim 6.8$),⁷ while reducing non-specific thermal effects (Figure S7 and S8).³⁸ Note that the resulting solution temperature following AMF application was 36 °C, which is below the activation threshold for thermosensitive ion channels.^{18,38} After ~ 32 s of exposure to AMFs, noticeable Ca^{2+} influxes were found in the ASIC1a⁺ neurons (Figure 3f). Significantly greater proportion of ASIC1a⁺ neurons exhibited Ca^{2+} influxes (as identified by $F/F_0 \geq 10\%$) in response to the AMF stimulation in the presence of MNP-PSA nanotransducers as compared to control groups, which included neurons immersed in MNP-PSA nanotransducer solutions and not subjected to the AMF stimulation, neurons subjected to the AMF stimulation in the presence of PSA-only nanospheres, and neurons immersed in bulk MNP-only solutions without PSA and subjected to the AMF (Figure 3f–j). These findings indicate that proton release from the nanotransducers during AMF stimulation dominates over other potential mechanisms leading to Ca^{2+} influxes, including spontaneous hydrolytic degradation of PSA, which might result in the reduction in the local pH, or the increase in the bulk solution temperature mediated by the MNPs, which could potentially cause depolarization and action potentials in neurons.³⁸ Indeed, a small fraction of neurons exhibited Ca^{2+} influx in response to the AMF-driven temperature increase in MNP solutions without PSA, however the extent of the response was significantly lower than that observed in the presence of MNP-PSA nanotransducers (Figure 3j, Table S1). Moreover, Ca^{2+} influx into ASIC1a⁺ neurons in the

presence of the nanotransducers and AMF stimulation was greatly blocked by the addition of amiloride (Figure S9), further supporting that Ca^{2+} responses in the ASIC1a⁺ neurons are mainly attributable to proton generation by the nanotransducers.

To explore the potential feasibility of future applications of the nanotransducers *in vivo*, we employed a finite element model (FEM) to approximate local pH and temperature changes induced by the MNP-PSA nanotransducers in the buffering system of the brain (Figure S10, Table S2). In this model, 1 μL of MNP-PSA nanotransducer solution (15 mg/mL, 1:2 MNP:PSA weight ratio) used for *in vitro* studies was injected into the brain. Based on experimentally observed pH (Figure 2g) and temperature (Figure 2h) changes induced by identical experimental conditions *in vitro*, we calculated the theoretical proton and heat flux of the nanotransducer solutions in AMFs. A mean-field approximation was made to estimate a bulk homogeneous reaction rate for the simulation derived from the rate of heterogeneous surface reactions occurring on individual nanotransducers. After 60 s of exposure to AMFs, the MNP-PSA nanotransducer solution caused a noticeable pH reduction (< 6.8) inside the injected fluid volume (Figure 4a, c). The pH gradually recovered over time following turning off the AMF (Figure 4b, d). At the same AMF conditions, no significant temperature increase ($< 1\text{ }^{\circ}\text{C}$) was found between the injection site and the surrounding environment (Figure 4e, g). The small temperature gradients disappeared in 120 s in the absence of AMF stimulation (Figure 4f, h), owing to the higher diffusivity of heat compared to mass. It should be noted that MNP concentration (and iron content, 3.6 mg_[Fe]/mL) in our system is significantly smaller than that of ferrofluids (50–100 mg/mL) commonly used for thermal stimulation of electroactive cells *in vivo*.^{18,22} A finer meshing scheme yielded identical results (Figure S11), and turning off the heat sink into brain recovered the *in vitro* findings (Figure S12). Along with our experimental observations during *in vitro* Ca^{2+} imaging, these numerical results suggest that our approach could in the future be applied to target ASIC enriched neurons, such as those in the amygdala³⁹ (Figure S13), while avoiding non-specific thermal effects.

We created a tool to locally lower the pH of targeted regions on-demand in response to a non-invasive AMF cue. Rationally designed nanotransducers based on MNPs and PSA composites converted a magnetic stimulus into proton mass transfer via thermally triggered hydrolysis of anhydride group. This enabled remotely controlled activation of ASICs in primary neurons. As AMFs permit remote targeting of structures deep within the body, nanotransducers may be applied to modulate activity of ASICs enriched neurons in the brain, advancing the understanding of proton-mediated processes in the nervous system. Furthermore, our approach potentially provides a platform to investigate diverse proton-mediated biochemical and physiological processes, such as enzymatic reactions,³ protein folding,⁴⁰ and immune response.⁴¹

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- (1). Chesler M Regulation and Modulation of pH in the Brain. *Physiological Reviews* 2003, 83, 1183–1221. [PubMed: 14506304]
- (2). Tannock IF; Rotin D Acid pH in Tumors and Its Potential for Therapeutic Exploitation. *Cancer Research* 1989, 49, 4373–4384. [PubMed: 2545340]
- (3). Kohse S; Neubauer A; Pazidis A; Lochbrunner S; Kragl U Photoswitching of Enzyme Activity by Laser-Induced pH-Jump. *Journal of the American Chemical Society* 2013, 135, 9407–9411. [PubMed: 23688056]
- (4). Saftig P; Klumperman J Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. *Nature Reviews Molecular Cell Biology* 2009, 10, 623. [PubMed: 19672277]
- (5). Xiong Z-G; Pignataro G; Li M; Chang S.-y.; Simon RP Acid-sensing ion channels (ASICs) as pharmacological targets for neurodegenerative diseases. *Current Opinion in Pharmacology* 2008, 8, 25–32. [PubMed: 17945532]
- (6). Yermolaieva O; Leonard AS; Schnizler MK; Abboud FM; Welsh MJ Extracellular acidosis increases neuronal cell calcium by activating acid-sensing ion channel 1a. *Proceedings of the National Academy of Sciences of the United States of America* 2004, 101, 6752–6757. [PubMed: 15082829]
- (7). Wemmie JA; Taugher RJ; Kreple CJ Acid-sensing ion channels in pain and disease. *Nature Reviews Neuroscience* 2013, 14, 461. [PubMed: 23783197]
- (8). Jasti J; Furukawa H; Gonzales EB; Gouaux E Structure of acid-sensing ion channel 1 at 1.9 Å resolution and low pH. *Nature* 2007, 449, 316. [PubMed: 17882215]
- (9). Wemmie JA; Chen J; Askwith CC; Hruska-Hageman AM; Price MP; Nolan BC; Yoder PG; Lamani E; Hoshi T; Freeman JH; Welsh MJ The Acid-Activated Ion Channel ASIC Contributes to Synaptic Plasticity, Learning, and Memory. *Neuron* 2002, 34, 463–477. [PubMed: 11988176]
- (10). Chen Y; Swanson RA Astrocytes and Brain Injury. *Journal of Cerebral Blood Flow & Metabolism* 2003, 23, 137–149. [PubMed: 12571445]
- (11). Kempfski O; Staub F; Jansen M; Schödel F; Baethmann A Glial swelling during extracellular acidosis in vitro. *Stroke* 1988, 19, 385–392. [PubMed: 3354026]
- (12). Ziemann AE; Schnizler MK; Albert GW; Severson MA; Howard MA III; Welsh MJ; Wemmie JA Seizure termination by acidosis depends on ASIC1a. *Nature Neuroscience* 2008, 11, 816–822. [PubMed: 18536711]
- (13). Ziemann AE; Allen JE; Dahdaleh NS; Drebot II; Coryell MW; Wunsch AM; Lynch CM; Faraci FM; Howard MA; Welsh MJ; Wemmie JA The Amygdala Is a Chemosensor that Detects Carbon Dioxide and Acidosis to Elicit Fear Behavior. *Cell* 2009, 139, 1012–1021. [PubMed: 19945383]
- (14). Ugawa S; Ueda T; Ishida Y; Nishigaki M; Shibata Y; Shimada S Amiloride-blockable acid-sensing ion channels are leading acid sensors expressed in human nociceptors. *The Journal of Clinical Investigation* 2002, 110, 1185–1190. [PubMed: 12393854]
- (15). Deng Y; Miyake T; Keene S; Josberger EE; Rolandi M Proton mediated control of biochemical reactions with bioelectronic pH modulation. *Scientific Reports* 2016, 6, 24080. [PubMed: 27052724]
- (16). Josberger EE; Hassanzadeh P; Deng Y; Sohn J; Rego MJ; Amemiya CT; Rolandi M Proton conductivity in ampullae of Lorenzini jelly. *Science Advances* 2016, 2, e1600112. [PubMed: 27386543]
- (17). Chen R; Canales A; Anikeeva P Neural recording and modulation technologies. *Nature Reviews Materials* 2017, 2, 16093.

- (18). Chen R; Romero G; Christiansen MG; Mohr A; Anikeeva P Wireless magnetothermal deep brain stimulation. *Science* 2015, 1261821.
- (19). Munshi R; Qadri SM; Zhang Q; Castellanos Rubio I; del Pino P; Pralle A Magnetothermal genetic deep brain stimulation of motor behaviors in awake, freely moving mice. *eLife* 2017, 6, e27069. [PubMed: 28826470]
- (20). Rao S; Chen R; LaRocca AA; Christiansen MG; Senko AW; Shi CH; Chiang P-H; Varnavides G; Xue J; Zhou Y; Park S; Ding R; Moon J; Feng G; Anikeeva P Remotely controlled chemomagnetic modulation of targeted neural circuits. *Nature Nanotechnology* 2019, 14, 967–973.
- (21). Stanley SA; Gagner JE; Damanpour S; Yoshida M; Dordick JS; Friedman JM Radio-Wave Heating of Iron Oxide Nanoparticles Can Regulate Plasma Glucose in Mice. *Science* 2012, 336, 604–608. [PubMed: 22556257]
- (22). Rosenfeld D; Senko AW; Moon J; Yick I; Varnavides G; Gregure D; Koehler F; Chiang P-H; Christiansen MG; Maeng LY; Widge AS; Anikeeva P Transgene-free remote magnetothermal regulation of adrenal hormones. *Science Advances* 2020, 6, eaaz3734. [PubMed: 32300655]
- (23). Kumar N; Langer RS; Domb AJ Polyanhydrides: an overview. *Advanced Drug Delivery Reviews* 2002, 54, 889–910. [PubMed: 12384314]
- (24). Makadia HK; Siegel SJ Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. *Polymers* 2011, 3, 1377–1397. [PubMed: 22577513]
- (25). Chen R; Christiansen MG; Anikeeva P Maximizing Hysteretic Losses in Magnetic Ferrite Nanoparticles via Model-Driven Synthesis and Materials Optimization. *ACS Nano* 2013, 7, 8990–9000. [PubMed: 24016039]
- (26). Dong J; Zink JI Taking the Temperature of the Interiors of Magnetically Heated Nanoparticles. *ACS Nano* 2014, 8, 5199–5207. [PubMed: 24779552]
- (27). Agrawal CM; Huang D; Schmitz JP; Athanasiou KA Elevated Temperature Degradation of a 50:50 Copolymer of PLA-PGA. *Tissue Engineering* 1997, 3, 345–352.
- (28). Domb AJ; Nudelman R In vivo and in vitro elimination of aliphatic polyanhydrides. *Biomaterials* 1995, 16, 319–323. [PubMed: 7772672]
- (29). Kim J; Lee J; Lee S; Yu J; Lee J; Park T; Hyeon T Designed Fabrication of a Multifunctional Polymer Nanomedical Platform for Simultaneous Cancer- Targeted Imaging and Magnetically Guided Drug Delivery. *Advanced Materials* 2008, 20, 478–483.
- (30). Meng ZX; Wang YS; Ma C; Zheng W; Li L; Zheng YF Electrospinning of PLGA/gelatin randomly-oriented and aligned nanofibers as potential scaffold in tissue engineering. *Materials Science and Engineering: C* 2010, 30, 1204–1210.
- (31). Hergt R; Dutz S Magnetic particle hyperthermia—biophysical limitations of a visionary tumour therapy. *Journal of Magnetism and Magnetic Materials* 2007, 311, 187–192.
- (32). Romero G; Christiansen MG; Stocche Barbosa L; Garcia F; Anikeeva P Localized Excitation of Neural Activity via Rapid Magnetothermal Drug Release. *Advanced Functional Materials* 2016, 26, 6471–6478.
- (33). Buckler KJ; Vaughan-Jones RD Application of a new pH-sensitive fluoroprobe (carboxy-SNARF-1) for intracellular pH measurement in small, isolated cells. *Pflügers Archiv* 1990, 417, 234–239. [PubMed: 2084617]
- (34). Burkersroda F. v.; Schedl L; Göpferich A Why degradable polymers undergo surface erosion or bulk erosion. *Biomaterials* 2002, 23, 4221–4231. [PubMed: 12194525]
- (35). Baron A; Waldmann R; Lazdunski M ASIC-like, proton-activated currents in rat hippocampal neurons. *The Journal of Physiology* 2002, 539, 485–494. [PubMed: 11882680]
- (36). Gee KR; Brown KA; Chen W-N; Bishop-Stewart J; Gray D; Johnson I Chemical and physiological characterization of fluo-4 Ca²⁺-indicator dyes. *Cell Calcium* 2000, 27, 97–106. [PubMed: 10756976]
- (37). Xiong Z-G; Zhu X-M; Chu X-P; Minami M; Hey J; Wei W-L; MacDonald JF; Wemmie JA; Price MP; Welsh MJ; Simon RP Neuroprotection in Ischemia: Blocking Calcium-Permeable Acid-Sensing Ion Channels. *Cell* 2004, 118, 687–698. [PubMed: 15369669]

- (38). Kim J; Connors B High temperatures alter physiological properties of pyramidal cells and inhibitory interneurons in hippocampus. *Frontiers in Cellular Neuroscience* 2012, 6, 27. [PubMed: 22783167]
- (39). Wemmie JA; Askwith CC; Lamani E; Cassell MD; Freeman JH; Welsh MJ Acid-Sensing Ion Channel 1 Is Localized in Brain Regions with High Synaptic Density and Contributes to Fear Conditioning. *The Journal of Neuroscience* 2003, 23, 5496–5502. [PubMed: 12843249]
- (40). Donten ML; Hassan S; Popp A; Halter J; Hauser K; Hamm P pH-Jump Induced Leucine Zipper Folding beyond the Diffusion Limit. *The Journal of Physical Chemistry B* 2015, 119, 1425–1432. [PubMed: 25536860]
- (41). Lardner A The effects of extracellular pH on immune function. *Journal of Leukocyte Biology* 2001, 69, 522–530. [PubMed: 11310837]

a Polyanhydride **Polyester** **Figure 1:**

(a) Hydrolytic degradation of polyanhydride and polyester. Water-labile linkages in polyanhydride and polyester are converted into carboxyl groups following hydrolysis. (b) An illustration of the wireless proton generating system. MNP heating elevates temperature inside the nanotransducers and accelerates the hydrolysis of polyanhydride and polyester. The carboxyl groups generated during the hydrolysis reduce the extracellular pH and activate neurons by triggering ASICs.

Figure 2:

(a) A transmission electron microscope (TEM) image of synthesized Fe_3O_4 MNPs. Scale bar: 50 nm. (b) An illustration of the synthesis of MNP-PLGA and MNP-PSA nanotransducers via the emulsion-solvent evaporation method. (c-d) Representative TEM images of a MNP-PLGA (c) or a MNP-PSA (d) nanotransducer (1:2 MNP:polymer weight ratio), respectively. Scale bar: 100 nm. (e) Dynamic light scattering spectra of the MNP-PLGA (blue) and MNP-PSA (red), illustrating their size distributions. (f) Experimental scheme for the analysis of proton release kinetics from nanotransducers in AMFs. AMFs were applied to Tyrode's solutions containing nanotransducers placed into the gap of an electromagnet of a toroid ferrite core. After exposure to an AMF, the solution was mixed with ratiometric pH indicator (seminaphtharhodafleur) solution. The ratio of fluorescence emission intensity at 580 nm (acidic form) versus the emission intensity at 640 nm (basic form) was used for measuring the solution pH. Standard curve was obtained by using

Tyrode's solutions with different pH values. **(g)** pH changes (mean $\pm$ standard deviation) of MNP-PLGA containing Tyrode's solution (blue, n=3), MNP-PSA containing Tyrode's solution (red, n=4), and MNP-PLGA containing 0.1 $\times$ Tyrode's solution (green, n=4) as a function of AMF stimulation time. First point in each plot represents the pH changes 3600 s after reaction in the absence of AMF stimuli. **(h)** Calorimetry measurements (mean $\pm$ standard deviation) for MNP-PLGA (blue, n=3) and MNP-PSA (red, n=3) containing Tyrode's solutions, respectively, before and during the AMF stimulation.

Figure 3:

(a) A schematic illustrating proton-mediated Ca^{2+} responses in the hippocampal neurons. Acidic extracellular environment leads to the depolarization of the cell membrane by triggering cation ($\text{Na}^+/\text{Ca}^{2+}$) permeable ASICs, followed by the activation of voltage-gated Ca^{2+} channels (VGCCs). (b) Normalized fluo-4 fluorescence profiles averaged across 100 genetically intact neurons (red) and 100 ASIC1a-overexpressing (ASIC1a $^{+}$) neurons (blue) following the rapid infusion of acidified medium (pH=6.8) at 30 s. At the same experimental condition, the proton-mediated Ca^{2+} influxes were greatly diminished by the addition of ASICs blocker amiloride to 100 native neurons (black) and 100 ASIC1a $^{+}$ neurons (green). Solid lines and shaded areas represent the mean and standard error of the mean (s.e.m.), respectively. (c) A fluorescent image of ASIC1a $^{+}$ neurons. Scale bar: 50 μm . (d) Representative fluo-4 fluorescent images of ASIC1a $^{+}$ neurons before and after the infusion of acidified medium (pH 6.8), showing robust Ca^{2+} influx in ASIC1a $^{+}$

in respond to acidic environment. Scale bar: 50 μm . (e) Extracellular pH dependent maximum of normalized fluo-4 fluorescence increases (mean $\pm$ s.e.m.) averaged across 100 native neurons (red) and 100 ASIC1a⁺ neurons (blue) during 60 s of measurement. (f-i), Individual fluo-4 fluorescence traces for 100 ASIC1a⁺ neurons at different experimental conditions. AMF(–) in (g) indicates that AMF is not applied during the measurement. MNP(–) in (h) and PSA(–) in (i) indicate PSA-only control particles without MNP (h) and concentration-matched MNP-only solutions without PSA (i). AMFs were turned on at 30 s and applied for 60 s (dashed lines). (b), (e), and (f-i) F_0 was obtained by averaging the fluorescence intensity during the initial 10 s of measurement. Averaged F/F_0 and individual fluo-4 fluorescence traces were analyzed with 100 neurons randomly selected from three independently conducted experiments. (j) The percentages (mean $\pm$ s.e.m.) of the stimulated ASIC1a⁺ neurons (as identified by $F/F_0 \geq 10\%$) at different experimental conditions (one-way ANOVA, $n = 3$ plates, $F_{3,8} = 11.70$, $p = 0.0027$, ** $p < 0.01$). Post-hoc Tukey's honest significant difference method was used for pairwise comparison. All P-values are listed in Table S1.

Figure 4:

(a-b) Calculated pH profiles within and around a 1 μL injection of MNP-PSA nanotransducer solution in the buffering system of cerebral spinal fluid (CSF) at 0 s and 60 s of AMF stimulation (a), and 60 s or 1800 s after the AMF stimulation is turned off (b). Black circle indicates the CSF/injection interface. Scale bar: 1 mm. (c-d) The corresponding values for the pH at the center of the injection and at the interface. (e-f) Calculated temperature profiles within and around the same injection at 0 s and 60 s of the identical AMF stimulation (e), and 60 s or 1800 s after the AMF stimulation is turned off (f). Black circle indicates the CSF/injection interface. Scale bar: 1 mm. (g-h) The corresponding values for the temperature at the center of the injection and at the interface.