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In vivo glutamate sensing inside the mouse brain with perovskite nickelate-nafion heterostructures

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Abstract

Glutamate, one of the main neurotransmitters in the brain, plays a critical role in communication between neurons, neuronal development, and various neurological disorders. Extracellular measurement of neurotransmitters such as glutamate in the brain is important for understanding these processes and developing a new generation of brain-machine interfaces. Here, we demonstrate the use of a perovskite nickelate-nafion heterostructure as a promising glutamate sensor with a low detection limit of 16 nM and response time of 1.2 seconds via amperometric sensing. We have designed and successfully tested novel perovskite nickelate-nafion electrodes for recording of glutamate release *ex vivo* in electrically stimulated brain slices and *in vivo* from the primary visual cortex (V1) of awake mice exposed to visual stimuli. These results demonstrate the potential of perovskite nickelates as sensing media for brain-machine interfaces.

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Introduction

Perovskite oxides (formula of ABO_3) are an important materials family with a diverse range of physical properties and functionalities of interest to multiple disciplines in science and engineering. Among this class, rare-earth nickelates ($RNiO_3$ (RNO)), where R represents rare-earth lanthanide elements, have attracted significant interest in the fields of electronics, catalysis, and energy[1-3]. Perovskite nickelates are strongly correlated systems with electronic properties highly sensitive to the microstructure, strain, and defects[4-5]. The ground state at room or body temperature can be insulating or metallic depending on the steric effect due to the A-site cation, for instance, $NdNiO_3$ is a correlated metal at room temperature[6-8]. The highly tunable electronic properties of nickelates have served as motivation to exploit them as electrocatalysts in energy technologies[9] and bio-sensors[10]. There exists a great need for bio-sensing inside brain tissue in living animals for *in vivo* measurements of neurotransmitters. Advancing *in vivo* techniques to monitor neurotransmitter release in the brain is of great interest and significance to neuroscience, disease therapy, and bio-engineering fields, because these neurotransmitters play an essential role in critical brain functions such as information transmission, learning and memory [11-14]. Furthermore, neurotransmission is known to be impaired in neurodegenerative disorders such as Parkinson's and Alzheimer's Disease[15]. However, the precise measurements of neurotransmitters in the studies of these disorders are often lacking. Current technologies are being used to measure glutamate such as high-performance liquid chromatography, gas chromatography, mass spectrometry, and microdialysis. While these techniques result in a high level of accuracy, they require external analysis, and longer measurement time[16-18]. On the other hand, genetically encoded glutamate sensors require genetic modification of the target cells. Thus, electrochemical sensors are well-suited for *in vivo* measurements, in which direct and continuous measurements in deep brain tissue can be carried out without the need for artificial labels or gene therapy [19-22]. Indeed, benchtop experiments exploring oxide electrodes for sensing of bio-molecules have been reported. [23-25]. However, multiple disciplines across natural sciences and engineering have to be brought together and numerous hurdles crossed to go from material-level sensing experiments to their use in implanted electrode devices for *in vivo* brain recording from live animals.

A particular neurotransmitter of interest is glutamate, which is among the most abundant and the main excitatory neurotransmitter in the central nervous system. It is involved in many brain functions, including sensory perception, motor control, learning, memory formation, higher-level cognition, and behavior. As a result, changes in glutamate signaling or processing are involved in the pathophysiology of various neurological and neurodegenerative disorders[26]. Glutamate is highly excitatory and is rapidly removed from the synaptic cleft by transporters to prevent excitotoxic effects and excessive spillover to neighboring synapses[27]. Therefore, it is significant to understand how glutamatergic activity is regulated not only because much of the brain energy is spent on sustaining synaptic activity at the glutamatergic synapse, but also due to glutamate's critical role in brain function at normal and disease-related levels[28].

In a typical glutamate sensor fabrication process, glutamate oxidase ($GluO_x$) enzyme is immobilized on the surface of sensing medium to trigger enzymatic generation of hydrogen peroxide (H_2O_2), which can be detected amperometrically[29-30]. To avoid the interference of other chemicals such as ascorbate, which can be directly oxidized on the electrode surface without an enzyme, a Nafion polymer layer is incorporated between enzyme and sensing electrode. Nafion can electrostatically repel interference anions, but selectively allows the penetration of H_2O_2. Such nafion coated structure has been reported to provide enhanced selectivity for accurate measurements[31-32].

In recent years, several candidates for amperometric glutamate biosensors have been developed such as noble metals (e.g., Pt, Au, Pd[33-34]), glassy carbon, carbon fiber or carbon nanotubes (CNTs[35-36]), polymers[37-38], binary metal oxides (e.g., TiO_2 and CeO_2[39-40]) and perovskite oxides (e.g., titanates)[23, 41]. Additionally, several studies have been presented for implantation of glutamate sensors into brain matter for real-time recording[21-22, 42-43]. However, improving both response time scale and detection limit simultaneously motivates the discovery of new platforms for sensing. We report such a biosensor using a cross-linking immobilizing method with nafion-coated perovskite nickelate thin films. Glutamate oxidase ($GluO_x$), an enzyme that metabolizes glutamate and releases H_2O_2, was immobilized on nafion-coated NNO film. The as-generated H_2O_2 molecules penetrate through the nafion and released protons and electrons catalyzed by NNO film. The released charge carriers were monitored using a three-electrode setup amperometrically. Here, for

the first time, we present experimental demonstration of fast detection of low concentration (nM range) of the glutamate in phosphate buffer saline (PBS) using benchtop measurements, followed by *ex vivo* in mouse brain slices and *in vivo* in awake head-fixed mice using a correlated material system of perovskite nickelate (i.e., $NdNiO_3$ (NNO)) heterostructured with nafion, a polymeric ion-permeable membrane.

Results

Figure 1a shows the schematic pathway for the glutamate sensing mechanism using NNO as a biosensor device. The electrical stimulus leads to an increased level of glutamate (path ① in Figure 1b), which gets transported to the device interface. The glutamate oxidase ($GluO_x$) enzyme is immobilized on the film surface, which consumes the glutamate with oxygen and water to produce H_2O_2. The GluOx biosensor is O_2-dependent, as suggested in Eq. (1). However, it operates well in the brain under normal conditions without being limited by oxygen concentration [44]:

$$\text{Glutamate} + O_2 + H_2O \rightarrow \alpha\text{−ketoglutarate} + NH_3 + H_2O_2 \quad (1)$$

(Path ② in Figure 1b),

The H_2O_2 selectively penetrates through the nafion film (path ③ in Figure 1b). H_2O_2 is then catalytically oxidized at the working electrode of NNO according to Eq. (2) at an appropriate polarization potential and monitored by the electrochemical station.

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (2)$$

(Path ④ in Figure 1b).

The protons intercalate into the nickelate lattice in which the proton is weakly bonded with oxygen anions and occupies interstitial sites in NiO_6 octahedra, and the extra electron is filled into the ligand hole in Ni_{3d}-O_{2p} hybridized orbital[45-46]. After the intercalation of proton and electron, the electron-electron repulsion in Ni site orbital leads to the localization of electrons and an increase of resistivity, as a feedback mechanism.

The top-view and cross-section morphology of NNO/LAO ($NdNiO_3$ thin film deposited on $LaAlO_3$ substrate) film were analyzed by transmission electron microscopy (TEM). The interface between NNO film and LAO substrate is sharp without any secondary phases (Figure 1c-d). The NNO film is epitaxially oriented along [001] direction, parallel to the c-axis of LAO (001) substrate. The zone axis of the specimen is [100] for imaging and can be seen by the symmetry in the FFT image shown in the inset of Figure 1c. STEM-EDX analysis shown in Figure 1e illustrates the uniform distribution of Nd and Ni elements across film thickness. The NNO film was coated with nafion (a widely used

ion permeating membrane) and the $GluO_x$ enzyme. Atomic force microscopy (AFM) measurements were then performed to verify the surface decoration (Figure 1f and Figure S3). The pristine NNO surface has a surface roughness of ~ 0.76 nm. In comparison, the first layer of nafion coating leads to the increase of surface roughness to 8.54 nm (Table S1). Further $GluO_x$ coating shows up as a bright spot with diameter of 5 μm.

Glutamate Biosensor Performance

The chronoamperometric method for the determination of glutamate utilizes the oxidation response of H_2O_2, a byproduct of enzymatic oxidation. From CV scan results (representative result shown in Figure S4), 0.6 V vs. Ag/AgCl is chosen as an optimal potential for amperometric detection of glutamate via oxidation of H_2O_2, which is also typically used in literature[20]. Prior to sensing measurements, the NNO film was confirmed to have sufficient conductivity for reliable electrocatalytic activity (Figure S5). In the presence of oxygen, $GluO_x$ catalyzes successive reaction of glutamate to form H_2O_2, which can be oxidized at the electrode. As shown in Figure 2a, the perovskite nickelate film coated with Nafion-$GluO_x$ exhibits prominent electrocatalytic activity toward increasing levels of glutamate. The successive addition of glutamate (50 μM each dosage) leads to a significant jump up of current density of ~ 18 nA mm^{-2} per dosage. In comparison, the nickelate without Nafion-$GluO_x$ demonstrates no current density variation upon addition of glutamate at 0.6 V vs. Ag/AgCl, suggesting that there is no occurrence of Faradaic reaction ($H_2O_2 \rightarrow O_2 + H^+ + 2e^-$) without the enzyme. A calibration plot is presented in Figure 1b, which presents a linear kinetic reaction with a sensitivity of 0.327 ± 0.07 nA μM^{-1} mm^{-2} (n=3) toward glutamate. For sensor selectivity, a Nafion layer which electrostatically repels anions was deposited onto the perovskite nickelate film before the immobilizing of $GluO_x$ enzyme layer. Figure S6a shows the amperometric response of our glutamate biosensor against AA, UA, AC. Figure S6b presents the current ratio between glutamate and AA, UA and AC. There is very slight response from the sensor to UA and AC. The current ratio of AA/Glutamate is 28% with negligible ratio of UA/glutamate and AC/glutamate of 4% and 7%, respectively. The results suggested that the Nafion membrane can only prevent about 80% interference signal from AA. Therefore, in future studies, another type of permselective layer such as m-Phenylenediamine dihydrochloride can be

applied to better block interferent signal from AA and other species such as Dopamine and 5-hydroxytryptamine[47-51]. One can also include a final layer of ascorbate oxidase enzyme (200 U/ml) to further prevent the interference of AA[47].

A comparison of the parameters for response time and limits of detection of various glutamate biosensors is shown in Figure 2c[31, 47, 52-58]. The light pink regime in Figure 2c represents ambient extracellular glutamate ranging from 20 nM to 20 μM[21, 59]. The NNO film demonstrates a fast response time of ~ 1.2s and low detection limit of 16 nM with a linear range between 1 to 700 μM (Figure S7). These metrics are comparable to state-of-the-art glutamate sensors suggesting their potential as *in-vivo* sensors as described further. Additionally, it is important for our glutamate sensor to maintain the balance between sensitivity, selectivity and temporal resolution. Thus, the existence of the permselective membrane is also significant to keep our sensor selective, which increases the response time as comparing to ultra-fast glutamate sensor[21]. Furthermore, as described in more detail later, the sensor is implanted at the layer 4 of the visual cortex next to the direct thalamocortical projection, where small concentration of glutamate is released by different types of visual stimulation. In this case, the response time and low detection limit of our sensor are suitable to detect the glutamate release. However, even faster response times, sensitivity and better resolution for transient glutamate detection can be achieved by optimizing the Nafion layer dimensions in future studies as shown theoretically by Clay et al[60].

Phase evolution in nickelate sensors after exposure to glutamate dosages

The hydrogen peroxide oxidation reaction at the nickelate electrode can be monitored via the electrochemical station. Separately, the nickelate thin film can be studied post-reaction by ex-situ methods using high energy resolution synchrotron radiation. The resistance evolution of the films (i-v curves) after treatment with different dosages of glutamate in 0.01 M PBS (pH 7.4) solution (Figure 2d) is shown in Figure 2e. The pristine metallic NNO film has a low resistivity of ~ 0.18 mΩ cm. The 1× dosage of glutamate leads to a slight increase to around 0.25 mΩ cm. The 2× and 3× dosage treatment further results in substantial change in resistivity by ~ 5X and ~ 2 orders of magnitude. Such PBS-mediated conductivity reduction due to electron filling is non-volatile at ambient conditions.

To investigate the mechanism of sensor response to glutamate, microstructural and electronic structure studies were performed on representative samples. Synchrotron X-ray diffraction curves taken from films after different dosage treatments are shown in Figure 2f. The pristine NNO was epitaxially grown on single crystalline LAO substrate, which shows a shoulder (220) diffraction peak (orthorhombic notation) at $Q_z \approx 3.27$ Å^{-1}. The substrate LAO (002) diffraction peak (in pseudocubic notation) is located at 3.31 Å^{-1}, indicating the NNO films' larger out-of-plane lattice parameter. Upon 1× dosage glutamate treatment, the diffraction peak of NNO becomes broader and shows a shift toward lower Q_z position, which arises from the proton/electron doping induced lattice expansion and distortion. The XRD pattern of NNO after further 2× dosage and 3× dosage treatments demonstrates no apparent diffraction peak which indicates a decrease in film crystallinity due to the greater concentration of protons that are intercalated through the film. Synchrotron X-ray absorption near-edge spectroscopy (XANES) measurements near the O K-edge of NNO films after different dosages are shown in Figure 2g. The gradual suppression of the O K-edge absorption peak at 529 eV occurs upon different dosage treatment, suggesting a decrease in oxygen-projected density of unoccupied states owing to the electron injection into O_{2p}-Ni_{3d} hybridized orbitals. Synchrotron XANES measurements near the Ni K-edge of NNO films after different dosages measured at different incidence angles are shown in Figure 2h and i, respectively. The choice of incidence angles allows us to determine if there are any significant changes occurring only near the surface as opposed to a significant fraction of the film thickness. The pre-edge region (shown in inset of Figure 2h and i) of XANES can be regarded as the fingerprint of the covalence status between O_{2p}-Ni_{3d} hybridized orbital. The intensity of white line peak depends on the occupancy of the bound final states. Both spectra at incidence angles of 0.3 ^{o} and 5.2 ° show similar evolution trend that the white line peak amplitude significantly gets weaker and the effective integrated area underneath the pre-edge peak apparently suppressed after the glutamate treatment. Both reductions indicate electron filling into hybridized d-orbital due to the neurotransmitter. The reduction in integrated pre-edge peak area of glutamate treated NNO is normalized by the area of pristine NNO. For an incidence angle of 0.3 °, the pre-edge area decreases by 48% for 3× dosage treatment (Table S2). In comparison, such change is estimated to be 28% for incidence angle of 5.2 ^{o} (Table S3).

Previous work using $SmNiO_3$ thin films for spontaneous transfer of hydrogen from glucose (with no electrical stimuli) showed only near-surface doping to a few unit cells[10]. In this work, we can see that electron filling from a neurotransmitter could be a bulk effect across the film thickness under the electrochemical bias used in the amperometric sensing methodology, confirmed by both synchrotron XRD and XANES measurements as shown above. To verify reproducibility, we annealed the sensor devices to remove the doped hydrogen, started the fabrication process from scratch for subsequent re-use (Figure S8).

***Ex vivo* studies of neurotransmitter release from mouse visual cortex**

Prior to demonstrating the use of these biosensors for real-time monitoring of neurotransmitter glutamate released from neuronal tissue of living animals, we first measured glutamate release in acute mouse brain slices *ex vivo*. Brain slices were prepared from the primary visual cortex (V1) of mice. In each experiment, a glutamate NNO sensor was placed underneath a brain slice and secured by a slice holder for glutamate measurement. To stimulate glutamate release from the presynaptic terminals within the slice, a bipolar stimulus electrode was placed on the surface of the layer 4 of the cortical slice. Electrical pulses were applied as described in Methods (Figure 3a-b). Stimulation of layer 4, the main recipient of the thalamic input, was justified by the anatomical organization of the V1 microcircuit and the extensive previous studies of the long-term synaptic plasticity at the layer 4 to layer 2/3 synapse[61]. Furthermore, this layer 4 to layer 2/3 synapse represents one of the strongest feedforward inputs in the cortical column. To achieve full contact of brain tissue with the biosensor, we made sure that the sensor was about twice as large as the brain slice (0.46×0.76 cm^2) (Figure 3a). When the glutamate reached the glutamate oxidase layer of the biosensor, the redox current was captured by the perovskite nickelate biosensor. The sampling rate for the redox current recording was 10 Hz and all the current density shown in Figure 3c, f is normalized to square millimeter in order to compare the data obtained with sensors of different surface areas. About 8s following the onset of electrical stimulation (500 μA 0.1ms width pulse, 40Hz for 10s), the peak current density per mm^2 is up to 20 nA (baseline subtracted), indicated the highest glutamate concentration recorded is about 60 μM (Figure 3f). The baseline is the average current density value when no prominent current peak is detected in consecutive 20s of a

recording trial. The results suggest that NNO can be successfully used for sensing glutamate signal ex vivo.

The large surface area of the sensor increased the sensitivity of glutamate detection, but its size limited the spatial resolution. Furthermore, the size of the sensor was prohibitively large for *in vivo* recordings from the live mouse. To develop a more compact sensory design that could be inserted into the brain of a live mouse, we developed a needle-like prototype of the biosensor with the 250×300 μm^2 dimensions. Although it is larger than the latest state-of-the-art silicon probe for electrophysiological recordings, this design represented a major improvement compared to the benchtop version and could be successfully used for the recordings. Before using the new design *in vivo*, we first tested it in brain slices. We had placed the new needle-shape sensor on top of layer 2/3 and successfully recorded glutamate release when applying the same high-frequency stimulation (500 μA 0.1s width pulse, 40Hz for 5s) of layer 4 in V1 as described earlier (Figure 3d-f). The current density peak was detected about 2s after the stimulation began, the peak is up to 7 nA/mm^2, which corresponds to >21 μM of glutamate during this experiment(Figure 3f). The faster and more transient response detected by the needle-shape sensor compared to the large baseplate sensor could be explained by the closer proximity of the needle-shape sensor to the presynaptic terminals in layer 2/3 releasing glutamate directly compared to the slow excessive glutamate spillover required to reach the baseplate sensor. The results indicate that the needle-like sensor is more precise and sensitive and is promising for glutamate monitoring in living animals.

***In vivo* studies of neurotransmitter release from mouse brain**

In order to demonstrate the potential for real-time biological applications, the nickelate-nafion sensors were used to record glutamate release *in vivo* in awake, head-fixed mice. A needle-shaped version of the sensor was inserted into the binocular region of the visual cortex to record the release of glutamate in response to visual stimulation (Figure 4). The experiment setup and mouse brain atlas (from Allen Brain Institute[62]) are shown in Figure 4a and b, respectively. Figure 4d shows the measured response to the Figure 4d shows the measured response to the drifting sinusoidal grating (spatial frequency = 0.03 (cpd), temporal frequency = 3 Hz, speed = 100 deg/s) presented for 10 s. The sensor with the

coating shows a stimulus-induced peak suggesting it is detecting extracellular glutamate. Recordings made in response to other visual stimuli patterns are shown in Figure S9. Some differences in the amount of glutamate detected may be potentially explained by the differences in the ability of different visual stimulation protocols to activate presynaptic terminals in the visual cortex and release glutamate. To compare *in vivo* glutamate release in response to visual stimulation to the standard electrophysiological recordings performed in mice, we performed extracellular recordings of visually evoked potentials (VEPs) and neuronal unit responses as described previously (Figure S10)[63].

There was a temporal delay between stimulation and glutamate detection both *ex vivo* and *in vivo*. This delay was higher than the response latency in extracellular electrophysiological recordings of local field potentials and neuronal spikes (Figure S10). The distinct behavior may be potentially explained by the action of the glutamate transporters in the presynaptic terminal and astrocytes, which can swiftly remove glutamate from the synaptic cleft and extracellular space following visual stimulation. Consequently, we may be able to detect only excess glutamate released following extensive, prolonged electrical stimulation in brain slices or visual stimulation in vivo. The slow diffusion of this excess glutamate may also explain the latency between the stimulation and the glutamate signal detection by the biosensor compared to electrophysiological methods. Future studies can include further miniaturization of electrode devices for multiplexed long-term in vivo brain research and bio-compatibility analysis.

Conclusions

We have presented the demonstration of an amperometric biosensor using a correlated metallic perovskite nickelate (i.e.,$NdNiO_3$ (NNO)) for neurotransmitter sensing in both *ex vivo* brain slices and *in vivo* inside the brain of awake mice. The biosensor consists of a NNO/nafion/$GluO_x$ hetero-structure with high selectivity towards glutamate, fast response time (1.2 s) and low detection limit (16 nM). Correlated metallic systems interfaced with polymers can therefore contribute to design of components for neurotransmitter sensing and brain-machine interfaces.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at

Photograph of a NNO based glutamate biosensor; Photograph of a needle shape NNO based glutamate biosensor; AFM image of Nafion/NNO film; Cyclic voltametry (CV) scan of NNO based glutamate biosensor in different solutions; Control C-V measurement with $NdNiO_3$ electrode;Amperometric sensing and interference (selectivity) studies of nickelate-nafion heterostructure; Sensor performance measurements; Recovery and re-use of the treated NNO biosensor; *In vivo* glutamate sensing in awake mice exposed to various visual stimuli; Electrophysiology experiments in awake mice with silicon probes (control); Summary of film thickness; Summary of normalized pre-edge area of Ni K-edge XANES at incidence angle of 0.3 and 5.2 ^{o}.

Methods

Fabrication of $NdNiO_3$ (NNO)/Nafion/Enzyme heterostructures

$NdNiO_3$ film deposition： Perovskite nickelate $NdNiO_3$ (NNO) thin films were grown on single crystal (001) $LaAlO_3$ (LAO) substrates (MTI corp) using a AJA UHV magnetron sputtering at room temperature. All substrates were rinsed by toluene, acetone, and isopropanol, and dried with high purity N_2 before deposition. The optimized growth condition was calibrated using Phenom SEM equipped with energy-dispersive X-ray spectroscopy (EDS). The deposition gas atmosphere is mixture of 40/10 sccm Ar/O_2 at total deposition background pressure of 5 mTorr. Two metallic Ni (DC, 66W) and Nd (RF, 145W) targets were used for deposition. The film growth rate is ~ 2.5 nm per minute. After deposition, the samples were treated by post-annealing in air at 500 °C for 24 h in a tube furnace with ramping and cooling rate of 1.5 ^{o}C min^{-1}. Films with thickness of ~ 50 nm were used in this work.

Nafion coating synthesis: Perovskite nickelate $NdNiO_3$ (NNO) thin films were used as a working electrode for neurotransmitter detection (image shown in Figure S1). The films were connected using magnetic wire (34 AWG, Digi-Key Corp, MN) by silver paste. The contact was insulated using polydimethylsiloxane (PDMS) leaving only the working area open for electrochemical activity. The thin-film electrode was then coated with a thin layer

of Nafion as permselective membrane to improve selectivity for glutamate over other interferences such as ascorbic acid (AA), acetaminophen (AC) and uric acid (UA). Prior to coating, thin-film electrodes were baked at 175 °C for 4 minutes to remove any moisture. They were then removed from oven and lowered into the amber vial containing Nafion solution, such that the recording sites were submerged in the solution. The films were rotated in a circular motion 5 times (~ 1s per rotation). The films were removed from the Nafion solution and baked at 175 °C for 4 minutes. They were removed from the oven and cooled down for at least 10 min at room temperature before coating with $GluO_x$ enzyme for glutamate sensing.

Enzyme immobilization: After Nafion coating, the films were functioned with glutamate oxidase ($GluO_x$), bovine serum albumin (BSA) and glutaraldehyde protein matrix. BSA (2.5%) and glutaraldehyde (0.4%) were added to the microcentrifuge tube containing glutamate oxidase (500 U/ml). The solution was mixed by centrifuging for 30 seconds. The resulting solution was 1% BSA, 0.15% glutaraldehyde and 100 U/ml GluOx. GluOx was crosslinked with BSA and glutaraldehyde to be immobilized on the surface of Nafion coated film. The protein matrix solution was used immediately. A 0.1-10 µL micropipette was used to coat the NNO sensor. 2 µL of glutamate matrix was drawn up. A small droplet of the solution was formed at the pipet tip without completely releasing the droplet. The solution droplet was then lowered to briefly contact the film surface and is raised straight up and off the NNO sensor surface. This was repeated 4 times with at least 1min wait in between. The coating film were cured at room temperature from 48-72 h and then stored at 4 °C before first measurement [19,65].

Chemical agent procurement: Glutamate oxidase ($GluO_x$) from Streptomyces, with a rated activity of 25 units per mg protein was obtained from Cosmo Bio USA (Carlsbad, CA). Nafion perfluorinated resin solution (5 wt.% in water and alcohol), glutaraldehyde (25% in water) were obtained from Sigma Aldrich (St. Louis, MO). L-Glutamic acid, 0.1 M phosphate buffer saline (PBS, pH 7.4) were obtained from Fisher Scientific (Waltham, MA). Ascorbic acid (AA), uric acid (UA), acetaminophen (AC) were purchased from Alfa Aesar (Fisher Scientific, Waltham, MA). Elastomeric polydimethylsiloxane (PDMS, Sylgard 184) was purchased from Dow Corning (Midland, MI). Water was purified by

Milli-Q (Millipore, Bedford, MA). Ag/AgCl/NaCl (3.5 M) reference electrode was acquired from (Bio-logic USA, LLC, Knoxville, TN, USA) for three-electrode measurements.

Microscopy: Cross-sectional area images of NNO/LAO film were obtained using a Cs and Cc aberration-double corrected FEI Titan transmission electron microscopy (TEM) at 200 keV. The TEM specimen was prepared by focused ion beam (FIB) lift-out and subsequently milled in a Gatan PIPS at 200 eV to remove any excess damage layers introduced by the FIB. The scanning TEM energy dispersive X-ray spectroscopy (STEM-EDS) data was collected using an FEI Talos equipped with a Super X EDS at 200keV.

Electrical conduction measurements: After each glutamate treatment, the NNO film (without nafion coating) was rinsed with DI water and dried with high-purity N_2 gas. Two Pd pads with the thickness of 100 nm were deposited onto film as contact using magnetron sputtering from Pd target. The current-voltage (I-V) curves were measurement between two Pd contact by using a Keithley 2635A Source Measure Unit. For measurements of in-plane conductivity of the film, the cyclic voltammetry measurement was performed using a three-electrode setup on a Solatron 1260 potentiostat. A silver paste was scratched into a corner of the NNO film (5mm ×10mm), and a stainless steel wire was in contact with Ag paste and baked at 50 ^{o}C until the paste become solid. Thereafter, the back and sides and Ag paste area were sealed with inert epoxy (Locite 9460) leaving NNO film (~ 0.3 cm^2) exposed to the electrolyte only. In our measurement, the NNO film served as working electrode, and the Pt wire and Ag/AgCl in 3.5 M KCl served as counter electrode and reference electrode, respectively. 5×10^{-3} M each of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6 \cdot 3H_2O$ (Sigma, >99%) were added to the 0.1 M KCl electrolyte. Before measurement, the electrolyte was bubbled with ultra-high purity N_2 for 30 min.

Synchrotron X-ray measurements: The X-ray measurements were carried out on beamline 33-ID-D at Advanced Photon Source, Argonne National Laboratory. A 6-circle Newport Kappa diffractometer was used for the X-ray diffraction measurement near the substrate (002) Bragg peak. The nominal incident X-ray photon energy of 8.333 keV was used for the XRD measurements, which is lower than the measured Ni absorption edge of ~ 8.345 keV. XRD and XANES measurements were done on the same spot of the sample.

For XAS measurement, a single element Vortex detector was used to collect the fluorescence signal from the sample as the incident X-ray energy was scanned through the Ni-K absorption edge (8.31 - 8.56 keV). Two different incident angles of X-rays were chosen for the XAS measurements: one at 5.2 degree to survey whole depth of the film; another at 0.3 degree, i.e., below the critical angle, where the X-ray extinction depth is reduced to less than ten nanometers, to probe the Ni cation valence state of the surface layer. O K-edge X-ray absorption spectroscopy was conducted at beamline 29-ID IEX at the Advanced Photon Source, Argonne National Laboratory. Data were collected in a pressure better that 1×10^{-8} Torr in total fluorescence yield (TFY) using a micro-channel plate with 7° angular acceptance located at $2\theta = 20°$. We used circular polarization with an overall energy resolution better than 100 meV. The incidence angle was set to theta = 5°, as to limited the penetration depth to 10 nm at the O K- edge. The total fluorescence yield was normalized by the incident x-ray intensity (I_0) using the drain current from a gold mesh upstream of the sample.

Atomic Force Microscopy (AFM): The topographic AFM mapping of the NNO films upon different glutamate treatments were performed using an Asylum MFP3D stand-alone atomic force microscope using Asylum ASYELEC-01 conductive tips (Si coated with Ti/Ir).

***Ex vivo* glutamate sensing experiments on brain slices**

Needle shape NNO/LAO electrode fabrication: The needle shape NNO/LAO electrodes used in the Ex vivo experiments were fabricated by using a wafer saw to cut the NNO/LAO wafer into 1x10 mm needle like structures. The electrodes were then mechanically polished with 3 μm polishing paper such that the width was approximately the same as the original wafer thickness (250 μm) resulting in a 0.25×0.25×10 mm final structure.

Animals and acute brain slices preparation: All animal procedures were approved by Purdue University Animal Care and Use Committee. Brain slices were prepared as described [64]. P28-P35 female C57BL/6 mice were anesthetized with the mix of 90 mg/kg ketamine and 10 mg/kg xylazine delivered by intraperitoneal injection. After confirmation of deep anesthesia, trans-cardiac perfusion was performed with oxygenated (carbogen from

Airgas: 95% O_2, 5% CO_2) high sucrose dissection buffer (HSDB, composition in mM: 1.25 NaH_2PO_4, 25 $NaHCO_3$, 212.7 sucrose, 10 dextrose, 2.5 KCl, 0.5 $CaCl_2$, 7 $MgCl_2$, 1.3 ascorbic acid). After the perfusion has completed, the brain was quickly isolated by dissection. The brain was trimmed to desirable shape and fixed in the cutting chamber of the vibratome (Leica VT1000) filled with the HSDB while constantly oxygenated with carbogen flow. The coronal brain slices containing the striatum and primary visual cortex (V1) were cut into 300 μm of thick sections. The brain slices were transferred immediately to a 32 °C incubation chamber containing oxygenated artificial cerebrospinal fluid (ACSF, composition in mM: 124 NaCl, 2.5 KCl, 2 $CaCl_2$, 0.8 $MgCl_2$, 1.23 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose) and incubated there for 30 min. The slices were then kept at room temperature (about 25 °C) for a least 1 hour before use.

Electrical stimulation of brain slices: The electric current was generated by a stimulation isolator (WPI A365) and output to a bipolar electrode (FHC CE2C55). The thin tip of the bipolar electrode was placed on the surface of the target brain areas. To induce glutamate release in V1, 5 sec or 10 sec constant 40 Hz stimulations were applied, and the width of one pulse was 0.1 ms, the amplitude was 500 μA.

***Ex vivo* experiment setup:** For ex vivo experiments shown in Figure 3a, a piece of NNO/LAO was fixed on the experiment platform, on which the brain slide was placed. Pt wire and Ag/AgCl were served as a counter electrode and a reference electrode, respectively. During the experiment, the recording chamber was continuously perfused with an oxygenated ASCF solution. A slide hold-down was placed in order to avoid slice movement during measurements. The ex vivo experiment shown in Figure 3d shared the same setup. Alternatively, a needle shape NNO/LAO connected via magnetic wire (30 AWG, Digi-Key Corp, MN) by solder served as a working electrode. PDMS was used as an insulation layer over the solder connection to prevent any contact with the solution. The device was then attached to a stainless tube for insertion.

***Ex vivo* electrochemical evaluation:** Electrochemical sensing experiments were carried out using SP-200 potentiostat (Bio-logic USA, LLC, Knoxville, TN, USA). Investigation of glutamate detection was done through the chronoamperometry i-t curve technique. All chronoamperometry data were collected after 20 min of settling time unless stated otherwise. A conventional three-electrode cell was used for chronoamperometry

measurements with Ag/AgCl/NaCl (3.5 M) as a reference electrode and graphite rod as a counter electrode for all evaluations. Parameters for chronoamperometry were at 0.6 V vs. Ag/AgCl with 0.1 s sampling interval. All chronoamperometry was performed in a stirring solution of 0.01 PBS (pH 7.4) as supporting electrolyte and rotation rate of 180 rpm.

In vivo glutamate sensing experiments on awake mice

Fabrication of *in vivo* sensors: A complete three electrode chemical sensor system was prepared for *in vivo* brain implantation experiments (image shown in Figure S2). The shank was prepared by spin-coating SU-8 2050 resin (Microchem, Newton, MA) on a 4-inch silicon wafer substrate with 3500 rpm of spin-speed to obtain 50 μm of thickness. The sample was soft baked for 3 min in 65 ℃, and 6 min in 95 ℃. UV light (dose: 160 mJ/cm^2) was exposed using a mask aligner (Suss MA6, Suss Microtech, Garching, Germany). Post-exposure bake was done for 1 min in 65 ℃, and 6 min in 95 ℃. The sample was developed in the SU-8 developer (Microchem, Newton, MA) for 5 min, and rinsed with isopropyl alcohol. Hard bake was done at 200 ℃ for 10 min. Shanks were released from the silicon wafer by etching the natural oxide with buffered oxide etch followed by rinsing in DI water for 5 times.

After fabricating the desired SU-8 shank structure, platinum nanoparticle nanocomposite was printed on the backside of the shank as CE, and as a conductive trace for RE on the front side.[65] Ag/AgCl ink was used to print RE on top of the conductive trace in the front of the shank. Silver ink was printed as contact pads, and PDMS was printed as an insulated layer exposing only the electrodes and contact pads. A 3-axis microfluid dispensing robot (Pro-EV 3, Nordson EFD, East Providence, RI) was used for the printing process. The needle shape NNO/LAO was then attached right below the reference electrode and connected via magnetic wire (34 AWG, Digi-Key Corp, MN) by silver ink. The whole system was placed on a bared LAO substrate (1 cm×1 cm) before attached to a stainless-steel tubing, which helps guide the insertion (Figure 4a).

Mice and Surgical Procedures

Surgical procedures were performed as described previously[66]. C57BL/6 mice were housed on a 12 hr light/dark cycle. P55 old mice were anesthetized with 5% inhaled isoflurane (in oxygen) and maintained at 1.5% during surgery. Once deep anesthesia was confirmed the

mice were affixed with ear bars to a Neurostar™ stereotaxic surgery frame and ophthalmic ointment was applied to the eyes. The scalp was shaved and sterilized with Dynarex™ ethanol wipes before a midline incision was made and expanded to uncover the lambda and bregma skull sutures. The skull was sterilized using 3% H_2O_2, and the periosteum was removed. Once the skull was dry, coordinates for the binocular visual cortex (from lambda: AP 0.8 mm, ML +/- 3.2 mm) were marked using Neurostar™ stereodrive software. A 9.5 mm long head post was glued in place with cyanoacrylate to a point on the midline of the skull 3.5 mm anterior to bregma. A reference pin made from a 1.5 mm tungsten wire soldered to the end of a 0.79 mm diameter gold plated pin was also glued into place with cyanoacrylate after insertion through the skull at a point on the midline 0.2 mm anterior of bregma. Metabond™ bone cement was used to seal the skull under a head cap.

Habituation of the mice to the head-fixation apparatus began after a day of recovery from the initial surgery. Habituation lasted for a minimum of three days for 90 min/day. When attached to the head-fixation apparatus via the implanted head post, the mice stood on a vertical treadmill facing the center of a 47.63 cm x 26.99 cm monitor screen placed 16.51 cm in front of them. After the last day of habituation, the following day, a craniotomy was performed at one of the marked coordinates. The same method of anesthesia was used for this surgery, as for the initial head post-implantation. Once affixed to the head-fixation apparatus, the biosensor was inserted normal to the surface of the now exposed binocular area of the primary visual cortex. Once inserted, the sensor was allowed to settle, and the mouse allowed to fully awaken from anesthesia over 30 minutes before recording. For the electrophysiology data, the process was the same as with the biosensor, but instead, a 64 channel silicon electrode was inserted. After a recording session, the craniotomy was re-sealed with Kwik-Cast™ Silicone Elastomer and Ortho-Jet™ orthodontic acrylic resin.

Visual Stimulation: To generate and present the visual stimuli, the open-source psychology software PsychoPy was used. During the habituation of the mice, they were shown a control screen made with the color space “gray” on a monitor with a mean luminance of 73 cd/m^2. The visual stimuli provided to promote a neural response were single 10 s sinusoidal drifting gratings (spatial frequency (SF) = 0.03 cycles per degree of visual angle, temporal frequency (TF) = 3 Hz, speed = 100 deg/s, oriented and drifting at

an angle of 150 degrees) with an inter-trial interval of 8 s, a drifting checkerboard pattern (temporal frequency (TF) = 3 Hz, speed = 100 deg/s, oriented and drifting at an angle of 150 degrees) displayed for 10 s with an inter-trial interval of 8s as well as a 10s display time with an inter-trial interval of 30 s, a full contrast modulation following a 2.5 Hz square wave displayed for 10 s with an inter-trial interval of 60s.

Perfusions and Histology: Before starting the perfusion, the mice were anesthetized with intraperitoneal injections of a 90 mg/kg ketamine and 10 mg/kg xylazine solution. The inner cavity of the peritoneum was exposed with an incision under the rib cage before cutting the lateral sides of the rib cage and removing the diaphragm. The heart was then exposed by peeling back the rib cage and any other connective tissue. 1X PBS was gravity-fed through a 25-gauge needle inserted into the left ventricle to force blood out of an incision made in the right atrium. Perfusion of 4% PFA was then used to fix the tissue. To remove the brain, the animal was decapitated, and the head cap sealing the skull was removed. Then cuts were made up the midline and along the lambda and bregma sutures in order to remove the skull and expose the brain enough to remove it from the skull. Once extracted the brain was placed in 4% PFA for 24 h before slicing it into 100 μm thick coronal sections. The slices were carefully attached on slides and mounted by NPG-Glycerol. The sensor track was then visualized by light microscopy.

Analysis of electrophysiological data: Raw traces were digitized at 30 kHz and acquired with OpenEphys acquisition hardware and software. For LFP analysis, the raw signal traces were filtered (1–300 Hz) and downsampled to 1 kHz, manually inspected for artifacts, and filtered with a notch filter removing 60 Hz noise. In order to compare LFPs between mice, the first and strongest trial-averaged visually evoked potential (VEP) from a visual stimulus (putative layer 4 VEPs) from each column of a silicon probe was used. For spike analysis, raw traces were bandpass-filtered (300-6000 Hz). Spikes were detected and sorted with the use of Kilosort, a template-based clustering algorithm implemented in Matlab[67]. The default Kilosort parameters were used except for a 6 SD threshold for spike detection and initializing the templates from data. Manual inspection of the resulting clusters for unit quality was performed using the Phy template GUI, based on criteria [68] that have been previously described[66].

Single unit analysis: To analyze single unit activity, they were put into peristimulus time histograms (PSTHs) with a 10ms bin size and were smoothed with a Gaussian smoothing kernel (width = 100ms). Z-score heatmaps were made by normalizing to the mean firing rate (FR) across time, so z = (FR – mean FR)/(SD FR), and the z-scores of the population time course line plots were made by normalizing FR to the baseline period preceding stimuli presentation, so z = (FR – mean baseline FR)/(SD baseline FR).

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Author contributions

Y.S. T.N. J.L A.A X.C and Z.Z. contributed to fabricating the samples and conducted measurement and data analysis of all experiments. T.G and I.A conducted the TEM characterization and needle electrode preparation. Z.Z H.Z and F.R conducted the X-ray diffraction and X-ray absorption measurments. S.R. H.L. and A.A.C conceived and designed the project. All authors contributed to writing the paper.

Competing financial interests. The authors declare no competing financial interests

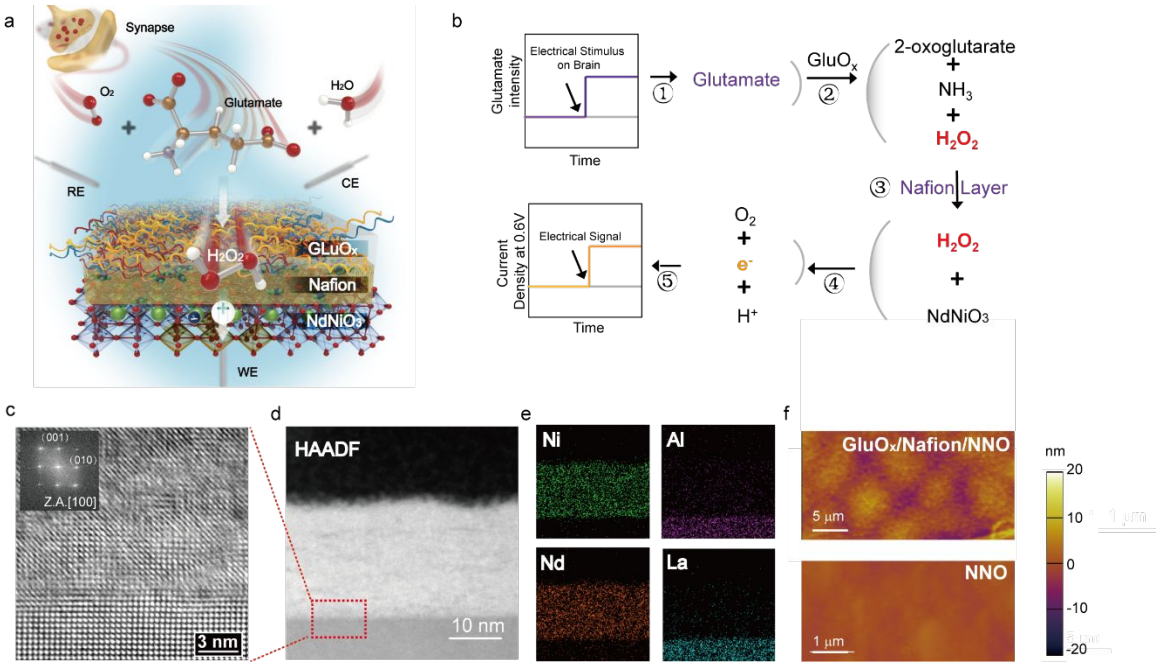

Figure 1. **$NdNiO_3$/Nafion heterostructure as a glutamate biosensor. a.** Schematic of glutamate sensing with nickelate-nafion sensor. The $NdNiO_3$ (NNO) thin film was coated with nafion followed by $GluO_x$ enzyme. The nafion serves as a ion selective permeable membrane while $GluO_x$ enzyme is immobilized on top of nafion. **b.** Bio-sensing reaction mechanism of glutamate by NNO. Electrical stimulus application led to the release of glutamate. The $GluO_x$ enzyme coated on NNO catalyzes the enzymatic reaction to form α−ketoglutarate, NH_3 and H_2O_2. The H_2O_2 diffuses through the nafion to reach surface of NNO. Under applied bias (i.e., 0.6 V vs. Ag/AgCl), the H_2O_2 oxidation is catalyzed by NNO, which is monitored by electrochemical station. **c.** High resolution TEM image of NNO/LAO cross-section. The Fast Fourier Transform (FFT) image of interface is shown in inset. **d.** HAADF-STEM, and **e.** STEM-EDX image of cross-section of NNO/LAO film. The selected area diffraction pattern is shown in inset of Figure 1c. The epitaxially grown NNO film on LAO substrate with uniformly dispersed Nd and Ni across film thickness could be observed. **f.** The surface morphology of $GluO_x$/Nafion/NNO and NNO characterized by atomic force microscopy (AFM). The surface of NNO is quite smooth. In comparison, the $GluO_x$ coating has a rough morphology of round-shaped particles with the diameter of ~5 μm which is distinguishable from bare NNO surface as well as Nafion/NNO surface.

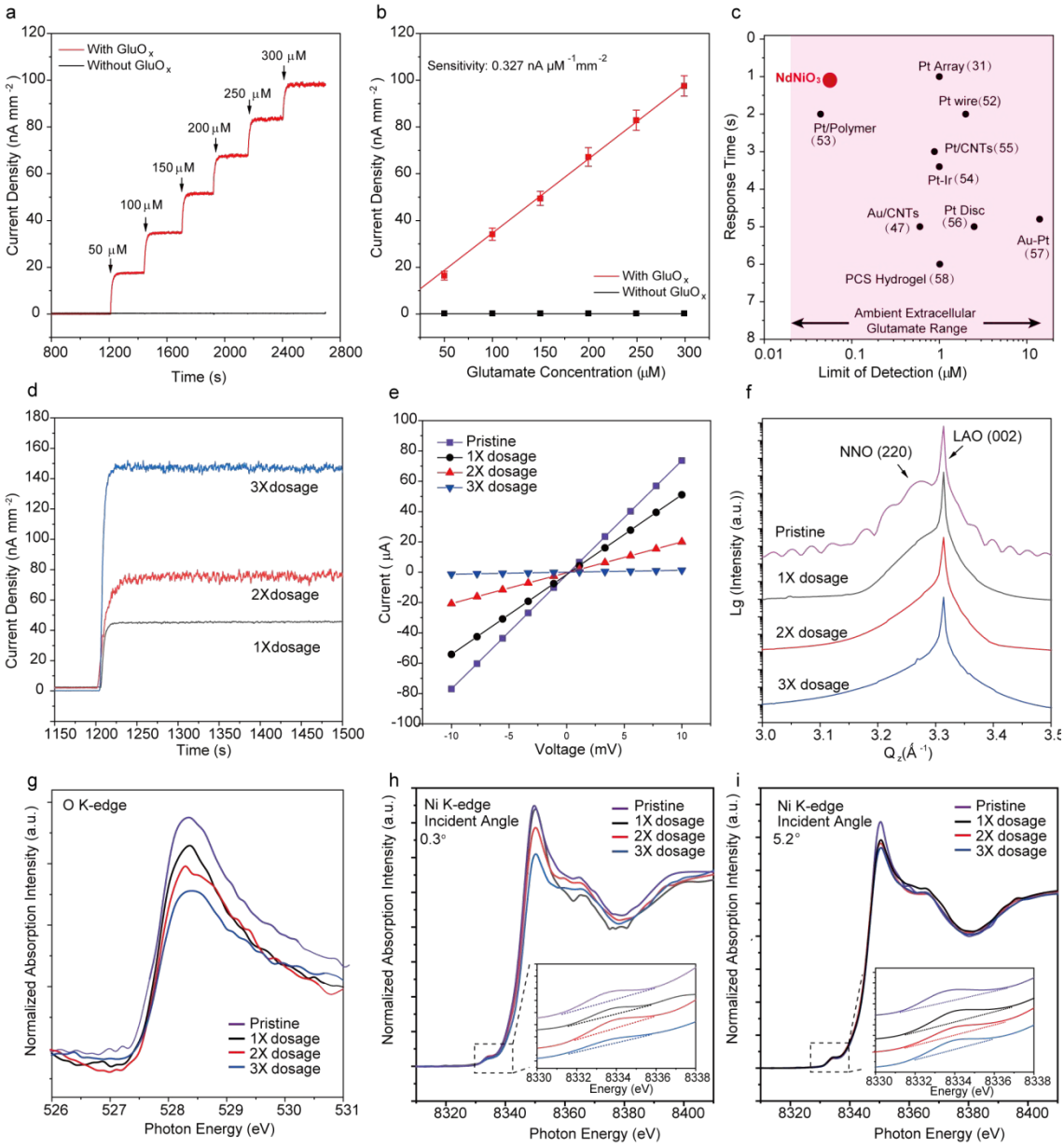

Figure 2. Benchtop experiments and nickelate characterization post-glutamate dosage. a. Representative amperometric curves for NNO as a glutamate biosensor at applied potential of 0.6 V vs. Ag/AgCl in 0.01 M PBS (pH 7.4) with and without the presence of $GluO_x$ enzyme (control)**. b.** The corresponding calibration curves and the sensitivity is shown. **c.** Comparison of the performance metrics in terms of response time and detection limit of various representative glutamate biosensors from literature. The light pink regime indicates the ambient extracellular glutamate level without any external stimulus. **d.** Representative amperometric curves for NNO as a glutamate biosensor for different dosages of glutamate (100 μM per dosage) at applied potential of 0.6 V vs. Ag/AgCl in 0.01 M PBS (pH 7.4). **e.** The i-V curves taken from NNO films after treatment with 0× (pristine), 1×, 2×, 3× dosages of glutamate. The evolution of resistivity of the films

is shown in inset. For instance, 3× dosage treatment of glutamate led to the increase of film resistivity by 2 orders of magnitude due to proton-electron incorporation. **f.** Synchrotron X-ray diffraction scans of identical NNO films upon the treatment of pristine film and films subjected to 1×, 2×, 3× dosage of glutamate. The scans are along Q_z direction around the (002) diffraction peak of $LaAlO_3$ (LAO) substrate (pseudocubic notation). **g.** X-ray absorption curves of the O K-edge of NNO films after treatment of 0× (pristine), 1×, 2×, 3× dosages of glutamate. **h-i.** Angle-dependent X-ray absorption spectra of the Ni K-edge of NNO films upon the treatment of 0× (pristine), 1×, 2×, 3× dosages of glutamate at the incident angle of (**h**) 0.3 ^{o} and (**i**) 5.2 ^{o}. The zoom-in feature of pre-edge area spectra are shown in inset. The glutamate treatment leads to the gradually decreased intensity of O K-edge absorption peak, pre-edge hump area and white line intensity of Ni K-edge XANES, due to the intercalation of proton and electron into NNO lattice from the hydrogen peroxide oxidation.

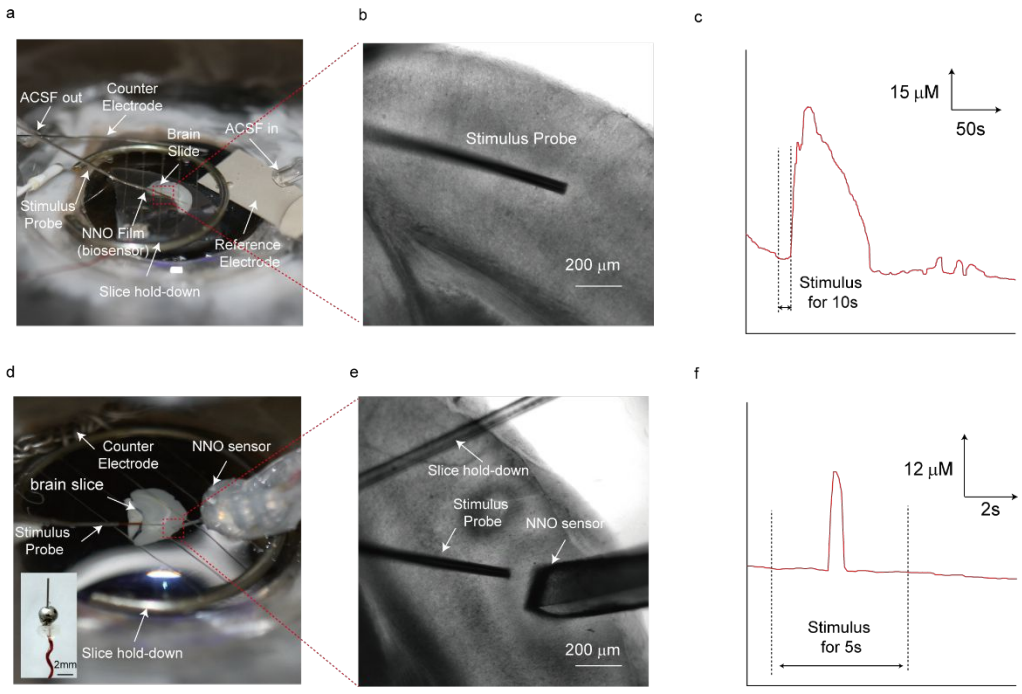

Figure 3. ***Ex vivo*** **studies of glutamate release in stimulated brain slices. a.** *Ex vivo* stimulated glutamate sensing setup using a visual cortex brain slice of a mouse. The size of the visual cortex brain slice is ~ 0.46 cm×0.76 cm. **b.** Zoom-in image of brain slice with the electrical stimulus probe is attached. **c.** Representative *ex vivo* stimulated glutamate sensing curve is shown. The glutamate sensing experiment was performed using a three-electrode setup at 0.6 V vs. Ag/AgCl. A sharp current peak was observed ~ 8s after the electrical stimulus (500 μA 0.1ms width pulse 40Hz for 10s) was applied to the brain slice. **d.** The same setup shown in **d** is identical to **a**. Here, the glutamate sensor was machined into a needle-shape probe and was inserted into the brain slice. **e**. The zoom-in image of the brain slice with the stimulus probe and the glutamate sensor. **f.** Representative *ex vivo* stimulated glutamate sensing curve recorded by the needle-shape sensor. The recording and stimulation protocol is the same as in **a-c**, but the stimulation time is 5s. The peak was detected ~ 2s after the electrical stimulus was turned on.

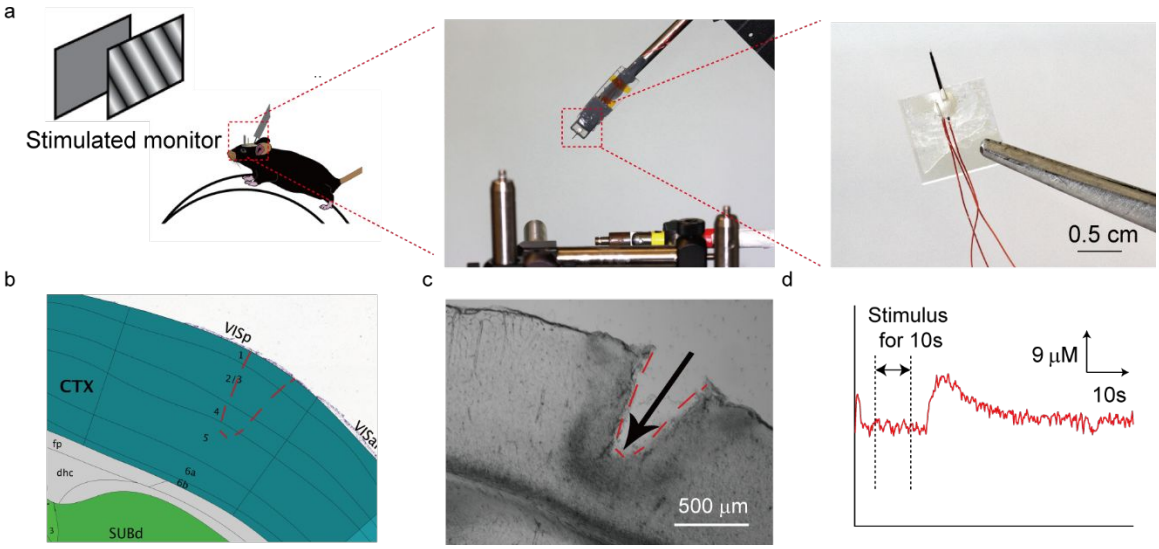

Figure 4. ***In vivo*** **glutamate release studies on awake mice. a.** Setup for the *in vivo* glutamate sensing from awake, head-fixed mouse and close-up images of the sensor used. The visual stimulus presented was drifting sinusoidal grating (Spatial frequency = 0.03 (cpd), temporal frequency = 3 Hz, speed = 100 deg/s) presented for 10s with an inter-trial delay of 8s where a gray screen was displayed. **b.** Mouse brain atlas (from Allen Brain Institute) showing the sensor was inserted into the binocular V1 region of the primary visual cortex (V1) outlined in red dashed line. **c.** Slice histology showing the insertion path of the sensor in V1. **d.** Representative *in vivo* glutamate recording is shown during visual stimulus made by the glutamate sensor (red).

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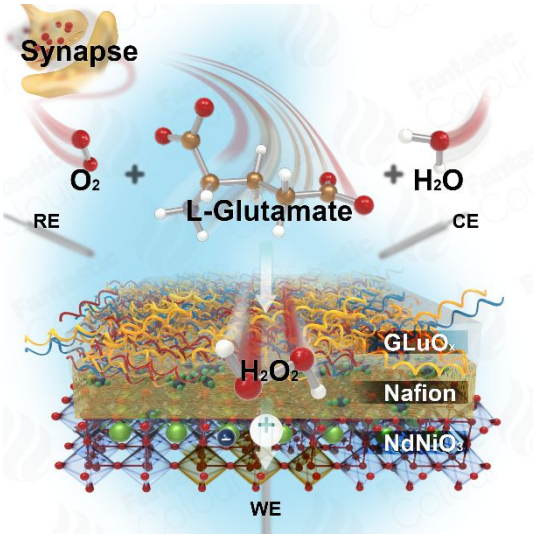

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