



A laser-induced graphene neural probe for multiplexed multimodal readout via site-selective functionalization

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ABSTRACT

Simultaneous monitoring of multiple neurochemicals in the brain is critical for elucidating complex neuronal processes, yet it remains technically challenging because of limited spatial selectivity, device complexity, and signal cross-interference. Existing multiplexed neurochemical probes often depend on physically separated sensors or intricate microfluidic architectures, which constrain spatial resolution, scalability, and practical integration for in vivo applications. Here, we present a flexible neural probe based on laser-induced graphene (LIG) that enables multiplexed, multimodal readout within a compact, scalable platform. Using a site-selective, sequential enzyme functionalization strategy, we created independent sensing interfaces for glucose, lactate, and dopamine on closely spaced electrodes while minimizing cross-contamination and interference between adjacent channels. The fabricated probe exhibited selective, concentration-dependent electrochemical responses in vitro and successfully detected stimulus-dependent dopamine release in neuronal cell models. In vivo experiments further demonstrated stable sensing performance after implantation, enabling real-time, multiplexed monitoring of neurochemical dynamics in the mouse medial prefrontal cortex. In addition, the probe supported simultaneous recording of neurochemical signals and electrophysiological activity, highlighting its potential for multimodal neural interfacing in vivo. Collectively, this work establishes a practical and scalable approach to high-density multiplexed neurochemical sensing and provides a versatile platform for investigating dynamic neurochemical signaling and its relationship to brain function.

1. Introduction

The brain relies on tightly coordinated neurochemical signaling to regulate neuronal activity, energy metabolism, and circuit function [1–3]. Metabolic substrates such as glucose and lactate serve as essential energy sources for active neurons, whereas neurotransmitters such as dopamine modulate synaptic transmission, reward processing, and adaptive behavior [2–6]. Accordingly, the ability to simultaneously monitor these chemically distinct signals is critical for elucidating

dynamic brain function under both physiological and pathological conditions [7–10]. However, real-time, in vivo measurement of multiple neurochemicals with high spatiotemporal resolution remains a significant technical challenge [7–10].

Conventional approaches to multiplexed neurochemical monitoring have primarily relied on microdialysis coupled with analytical techniques such as liquid chromatography–mass spectrometry (LC–MS) [11–14]. Although these methods enable the detection of multiple analytes, they suffer from poor temporal resolution (on the order of

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minutes) and require bulky, invasive instrumentation [7,9,12,14]. Genetically encoded fluorescent sensors, including GRAB-based dopamine sensors and iGluSnFR, provide high spatiotemporal resolution and cell-type specificity [15–19]. However, their capacity for multiplexed detection is constrained by spectral overlap, and they do not readily provide absolute concentration measurements [15,16,20].

Electrochemical biosensors offer a compelling alternative for neurochemical monitoring due to their high sensitivity, rapid response, and compatibility with miniaturized, implantable devices [7,9,21–29]. Laser-induced graphene (LIG) is particularly attractive for electrochemical sensing because of its porous conductive structure and facile laser patternability [26–29]. Significant progress has been made in detecting individual analytes such as glucose, lactate, and dopamine. Nevertheless, most electrochemical systems remain limited to single-analyte detection or rely on physically separated sensing elements for multiplexing [7–9,30–32]. These designs increase implantation complexity and restrict the ability to perform localized measurements within small brain regions [31,33,34].

Implantable neural probes integrating multiple neurochemical sensors have been developed to enable concurrent *in vivo* monitoring (Table S1) [35–38]. Among these, a recent multimodal platform further demonstrated simultaneous detection of multiple analytes, such as glucose, lactate, glutamate, and choline, alongside electrophysiological recordings, representing an important step toward multimodal neural interfacing [35]. However, due to the difficulty of directly integrating multiple enzyme-functionalized sensing sites within a confined probe tip, this system positions biosensors along the probe body and integrates them to push–pull microfluidic mechanism. This design introduces architectural complexity, while the extraction and transport of extracellular fluid inherently delay signal acquisition, thereby limiting true real-time sensing.

Consequently, there remains a critical need for a simplified, scalable

neural probe capable of directly and simultaneously detecting multiple neurochemicals *in situ*, without reliance on complex microfluidic systems. Such a platform must support spatially localized sensing interfaces, robust and selective biochemical functionalization, and stable operation under physiological conditions [31,39–42].

Here, we present a flexible neural probe based on LIG that enables high-density multiplexed electrochemical sensing of glucose, lactate, and dopamine within a compact architecture (Fig. 1). Porous, conductive graphene electrodes are directly patterned onto polyimide (PI) substrates via laser processing, allowing scalable and reproducible fabrication of implantable microsensors. To achieve analyte-specific detection, individual sensing sites are sequentially exposed and selectively functionalized with glucose oxidase (GO_x), lactate oxidase (LO_x), and tyrosinase. This site-selective immobilization strategy establishes spatially confined biochemical recognition while minimizing cross-reactivity between adjacent channels. In addition, a temperature-controlled immobilization process is employed to enhance enzyme loading density and improve interfacial stability.

The performance of the platform is validated through *in vitro* studies demonstrating concentration-dependent responses, high selectivity, and minimal crosstalk between adjacent sensing channels. Its *in vivo* utility is further established in the mouse medial prefrontal cortex (mPFC), where glucose, lactate, and dopamine are simultaneously monitored in real time. Notably, cocaine administration induces a pronounced increase in dopamine levels, accompanied by a modest elevation in glucose and minimal change in lactate, as well as enhanced neuronal electrical activity, highlighting the capability of the system to resolve distinct neurochemical and electrophysiological dynamics. Collectively, we introduce a practical and scalable strategy for multiplexed neurochemical sensing directly at the probe interface. By eliminating the need for microfluidic coupling and enabling closely spaced, independently functionalized sensing sites, this platform advances the capability for

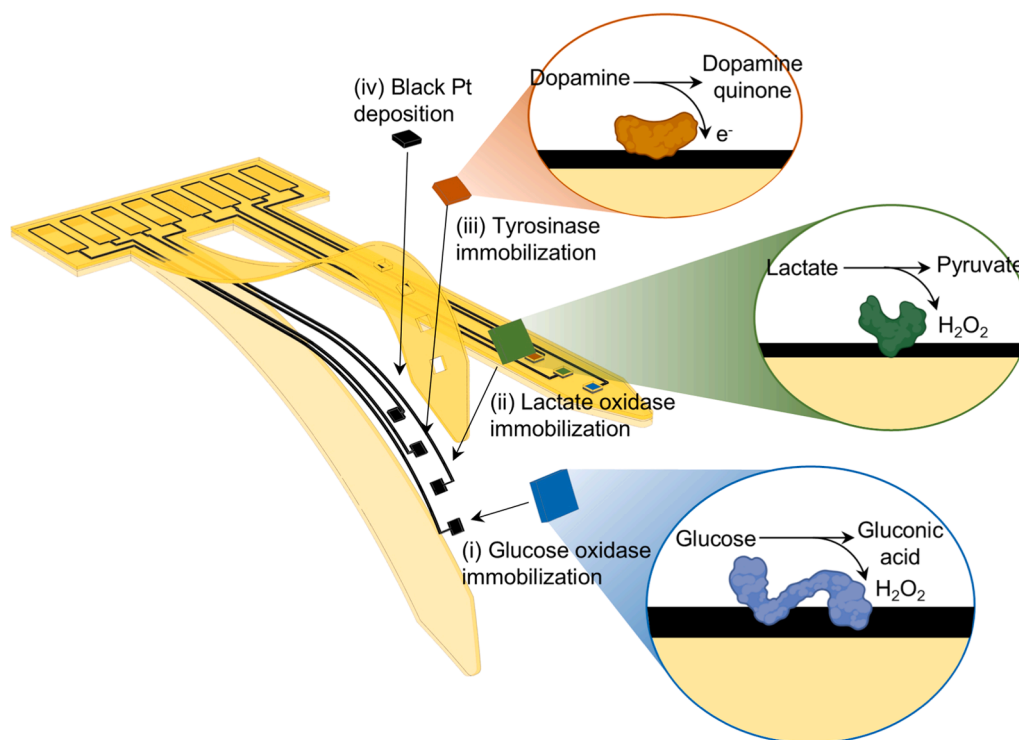


Fig. 1. Design and site-selective functionalization of the multiplexed LIG-based neural probe. Schematic illustration of the flexible laser-induced graphene (LIG)-based neural probe and the sequential, site-selective enzyme functionalization strategy. Individual sensing sites are sequentially exposed and functionalized with (i) GO_x, (ii) LO_x, and (iii) tyrosinase to enable selective detection of glucose, lactate, and dopamine, respectively. (iv) Additional electrode sites are modified with black platinum (Pt) for electrophysiological recording. Insets illustrate the corresponding electrochemical sensing mechanisms for each analyte, including enzymatic reactions that generate H₂O₂ for glucose and lactate detection and dopamine oxidation to dopamine quinone with electron transfer.

high-resolution, multimodal investigation of neurochemical signaling, neurometabolic coupling, and pharmacological responses in the living brain.

2. Materials and methods

2.1. Fabrication of the multichannel LIG probe for multiplexed chemical sensing

Multichannel neural probes for multiplexed neurochemical sensing were fabricated based on our previously reported LIG platform [41,43], with key modifications to enable high-density, site-selective functionalization (Fig. S1). A 25 μm -thick PI film (Kapton) was laminated onto a glass substrate and patterned using a CO_2 laser system (ML-3040, bgm tech Inc., Korea). Laser irradiation was employed to define conductive interconnects, sensing electrode sites, connector pads, and the overall probe geometry. The resulting LIG conductive traces exhibited a width of approximately 90 μm and a thickness of ~ 13 μm .

To electrically insulate the probe surface while preserving access to sensing regions, a PI insulating layer was deposited via spin coating. A PI adhesion promoter (PICO 300) was first applied, followed by coating of a PI precursor to achieve an insulation thickness of approximately 20 μm . The coated substrate was prebaked at 110 $^\circ\text{C}$ for 5 min and subsequently thermally cured at 220 $^\circ\text{C}$ for 12 h to ensure mechanical robustness and dielectric stability.

Following insulation, individual sensing sites were sequentially exposed using additional CO_2 laser irradiation to enable site-selective biochemical functionalization. Each opened electrode site had a diameter of approximately 180 μm . In the first step, two electrode sites were exposed and converted into LIG electrodes for glucose sensing. These electrodes were electrochemically activated and functionalized with GO_x . In the second step, an additional two electrode sites were opened and processed in the same manner, followed by immobilization of LO_x for lactate sensing. In the final step, two electrode sites were exposed for dopamine sensing and subsequently functionalized with tyrosinase (Fig. S1).

The completed probe consisted of two shanks containing a total of eight electrodes. Among them, six electrodes were assigned to neurochemical sensing (glucose, lactate, and dopamine), while the remaining two electrodes were reserved for electrophysiological recording.

For stable electrical interfacing, the connector region of the fabricated probe was mechanically reinforced with adhesive tape and interfaced with an 8-pin flexible printed circuit (FPC) connector (Molex, USA) mounted on a custom-designed printed circuit board (PCB, Han Saem Digtec Inc., Korea). The assembled device was connected to an external potentiostat system for electrochemical measurements.

2.2. Site-selective enzyme immobilization on LIG electrodes

To achieve multiplexed sensing within a compact probe architecture, we developed a site-selective enzyme functionalization strategy that enables spatially localized and independent biochemical recognition at each sensing site. Specifically, three sensing electrodes were sequentially opened and functionalized with GO_x for glucose sensing, LO_x for lactate sensing, and tyrosinase for dopamine sensing. Individual sensing sites were defined through stepwise opening of the PI insulation layer, followed by enzyme immobilization on each exposed electrode. This sequential approach enables selective immobilization of different enzymes on adjacent electrodes while minimizing cross-contamination.

Prior to opening the first electrode and subsequent glucose enzyme immobilization, electrochemical activation was selectively performed on the LIG electrode designated for glucose sensing to enhance the density of surface functional groups and improve interfacial stability. This selective treatment ensures robust enzyme attachment capable of withstanding subsequent functionalization steps.

The fabricated LIG probes were first rinsed with deionized (DI) water

to remove surface contaminants. Electrochemical activation was then carried out in 1 M sulfuric acid (H_2SO_4) using cyclic voltammetry (CV) with a potentiostat system. The potential was swept from -0.2 V to $+1.2$ V (vs. Ag/AgCl) at a scan rate of $100 \text{ mV}\cdot\text{s}^{-1}$ for 10 cycles. This process was applied exclusively to the glucose sensing electrode to introduce oxygen-containing functional groups, thereby improving the stability of enzyme immobilization during repeated processing.

The predefined glucose sensing site was then incubated in an activation solution containing 0.2 M EDC and 0.05 M NHS dissolved in 0.1 M MES buffer (pH 5.5) for 30 min at room temperature to activate surface carboxyl groups, followed by rinsing with MES buffer to remove excess reagents. The electrode was subsequently immersed in a GO_x solution (1 mg ML^{-1} in PBS, 0.1 M, pH 7.4). To enhance enzyme loading and ensure stable covalent attachment, a two-step incubation process was employed: incubation at 4 $^\circ\text{C}$ for 6 h to facilitate controlled adsorption and minimize premature denaturation, followed by an additional 6 h at room temperature to promote covalent bonding via amide formation.

Subsequently, additional sensing sites were sequentially opened and processed using the same procedure. The lactate sensing site was exposed, followed by EDC/NHS activation and immobilization of LO_x under identical conditions. The dopamine sensing site was then opened and functionalized with tyrosinase using the same activation and immobilization protocol.

After each immobilization step, the electrodes were thoroughly rinsed with PBS to remove loosely bound enzymes before proceeding to the next step. Upon completion of all functionalization steps, the devices were stored at 4 $^\circ\text{C}$ until further use.

2.3. Black Pt electroplating for neural recording electrodes

Following enzyme immobilization, the remaining two electrodes were opened and designated as neural recording sites. To enhance electrochemical performance for electrophysiological measurements, black Pt was electrochemically deposited onto the exposed LIG electrode surfaces.

The plating solution consisted of 8% (w/v) chloroplatinic acid (H_2PtCl_6) and 0.025% (w/v) lead(II) acetate dissolved in DI water. The LIG electrode served as the working electrode (WE), with a Pt wire as the counter electrode (CE) and an Ag/AgCl electrode as the reference electrode (RE). Electrochemical deposition was performed by applying a constant potential of -0.2 V for 120 s using a potentiostat system. This black Pt coating increases the effective surface area and reduces electrode impedance, thereby improving sensitivity and signal quality for neural recording.

2.4. Electrochemical measurements and calculation

Electrochemical measurements were performed using a PalmSens4 potentiostat (PalmSens, Netherlands). The fabricated LIG probe served as the WE, while an Ag/AgCl electrode and a platinum wire were used as the reference and CEs, respectively, for *in vitro* characterization. For *in vivo* measurements, a stainless-steel screw implanted in the skull or a reference wire electrode was used as the reference/ground electrode.

All measurements were conducted in phosphate-buffered saline (PBS, 0.1 M, pH 7.4) at room temperature unless otherwise specified. Chronoamperometric measurements were performed under gentle stirring to ensure homogeneous analyte distribution. The applied potentials were set to $+0.6$ V for glucose sensing, $+0.6$ V for lactate sensing, and $+0.2$ V for dopamine sensing (vs. Ag/AgCl).

For calibration, analyte solutions were sequentially added to achieve final concentrations of 0.1–5.0 mM for glucose, 0.1–5.0 mM for lactate, and 0.05–1.0 μM for dopamine. The current response was continuously recorded, and the steady-state current after each addition was used for quantitative analysis. Calibration curves were constructed by plotting current change versus analyte concentration. The sensitivity of each

sensing channel was determined from the slope of the calibration curve normalized to the geometric electrode area.

For electrochemical characterization of the electrode interface, CV and electrochemical impedance spectroscopy (EIS) were performed using the same potentiostat system. CV measurements were conducted in PBS (0.1 M, pH 7.4) using a standard three-electrode configuration, with the LIG probe as the WE, an Ag/AgCl electrode as the RE, and a platinum wire as the CE. The potential was scanned from -0.9 V to $+0.9$ V versus Ag/AgCl at scan rates ranging from 10 to 1000 $\text{mV}\cdot\text{s}^{-1}$ to evaluate electrochemical activity, effective surface area, and changes in interfacial properties during surface activation and enzyme immobilization. In addition, EIS was performed to characterize the impedance of the neural recording electrodes after black Pt electroplating, confirming their suitability for stable electrophysiological signal acquisition.

Calibration curves were obtained from in vitro chronoamperometric measurements by plotting the steady-state current (I) after each analyte addition against the corresponding analyte concentration. Linear regression was performed within the concentration ranges exhibiting proportional current responses (glucose and lactate: 0.1–5.0 mM; dopamine: 0.05–1.0 μM). The resulting calibration equations were used to determine the sensor sensitivity from the slope of the fitted line.

For the cell and in vivo experiments, representative current values at selected measurement points were converted into estimated analyte concentrations using the corresponding calibration equation:

$$C = \frac{I - b}{S}$$

where I is the measured current, S is the slope (sensor sensitivity), and b is the intercept of the corresponding calibration curve. The calculated values represent estimated analyte concentrations based on the in vitro calibration. Because electrochemical responses measured in biological environments may be affected by factors such as the local tissue environment, mass transport, and biofouling, the converted values should be interpreted as estimated analyte concentrations rather than absolute extracellular concentrations.

The limit of detection (LOD) was calculated according to the conventional analytical expression:

$$\text{LOD} = \frac{3\sigma}{S}$$

where σ is the standard deviation of the baseline current measured in the absence of the target analyte and S is the slope of the corresponding calibration curve.

2.5. SH-SY5Y cell culture and dopamine induction

SH-SY5Y cell experiments were performed to evaluate whether the fabricated dopamine sensor could detect dopamine release at the cellular level. Human neuroblastoma SH-SY5Y cells were obtained from ATCC (CRL–2266) and cultured in complete medium consisting of a 1:1 mixture of Eagle's Minimum Essential Medium (MEM) and Ham's F–12 nutrient mixture (MEM/F–12), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cells were maintained at 37 °C in a humidified incubator with 5% CO_2 .

For differentiation, SH-SY5Y cells were seeded into 12-well plates at a density of 50,000 cells per well and allowed to attach overnight. The culture medium was then replaced with differentiation medium composed of MEM/F–12 supplemented with 20 μM retinoic acid, 2% FBS, and 1% penicillin–streptomycin. The medium was refreshed every 2–3 days, and cells were maintained under these conditions for 7 days to induce neuronal differentiation.

Following differentiation, the cells were pretreated for 3 h with differentiation medium without retinoic acid (MEM/F–12 supplemented with 2% FBS and 1% penicillin–streptomycin) containing 50 μM

L-DOPA to promote intracellular dopamine accumulation. To induce dopamine release via exocytosis, the cells were subsequently exposed to a depolarization buffer consisting of 10 mM HEPES, 170 mM KCl, 2 mM CaCl_2 , and 1 mM MgCl_2 .

For electrochemical measurements, the collected medium was centrifuged at 3000 rpm for 5 min to remove cell debris, and the supernatant was immediately used for dopamine sensing or stored at -20 °C until further use.

2.6. Fiber photometry recording

Fiber photometry was performed to validate dopamine dynamics in the mPFC. Mice were anesthetized with isoflurane (1.5–2%) and placed in a stereotaxic frame. An adeno-associated virus encoding a genetically encoded dopamine sensor (AAV-hSyn-GRAB_DA2m, Addgene #140553) was used for in vivo expression.

The viral suspension (1 μL) was injected into the mPFC at coordinates AP + 1.8 mm, ML + 0.4 mm, and DV -2.5 mm relative to bregma at a rate of 100 $\text{nL}\cdot\text{min}^{-1}$ using a syringe pump. The injection needle was maintained in place for 10 min after delivery to allow diffusion before being slowly withdrawn. Animals were allowed to recover for 2 weeks to ensure sufficient expression of the dopamine sensor.

An optical fiber cannula was then implanted above the injection site and secured with dental cement. After an additional 1 week of recovery, the animals were habituated for 3 days prior to recording.

Photometry recordings were performed using a fiber photometry system. Excitation light (470 nm) was delivered through the optical fiber, and fluorescence signals were continuously recorded.

To induce dopamine release, cocaine (15 $\text{mg}\cdot\text{kg}^{-1}$, intraperitoneal) was administered, and fluorescence signals were recorded before and after injection. The recorded signals were expressed as $\Delta F/F$ (%) relative to baseline.

2.7. In vivo probe implantation and neurochemical and electrophysiological recording

In vivo experiments were conducted to evaluate the capability of the proposed probe for simultaneous multiplexed neurochemical sensing and electrophysiological recording. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Korea Brain Research Institute (KBRI) (Approval No. IACUC–25–00039).

Male C57BL/6 J mice (8 weeks old, 20–25 g) were anesthetized with isoflurane (1.5–2%) and positioned in a stereotaxic frame. A craniotomy was performed above the mPFC, and the LIG-based multichannel probe was implanted at coordinates AP + 1.8 mm, ML + 0.4 mm, and DV -2.5 mm relative to bregma using a micromanipulator. A stainless-steel screw fixed to the skull served as the reference and ground electrode.

Electrochemical recordings were performed using a PalmSens potentiostat in chronoamperometric mode. The LIG probe served as the WE, and analyte-specific bias potentials were applied ($+0.6$ V for glucose and lactate, and $+0.2$ V for dopamine vs. Ag/AgCl). After a stabilization period (~ 10 min), glucose (3 $\text{g}\cdot\text{kg}^{-1}$, tail vein) or cocaine (15 $\text{mg}\cdot\text{kg}^{-1}$, intraperitoneal) was administered, and current responses were continuously recorded for 10–30 min.

Simultaneously, electrophysiological signals were acquired using an RHX2000 system (Intan Technologies) at a sampling rate of 20 $\text{kS}\cdot\text{s}^{-1}$ per channel with a 300–6000 Hz band-pass filter. Spike events were detected using a threshold criterion ($\sim 2 \times$ baseline noise) and further analyzed using MATLAB-based clustering.

Electrochemical signals were recorded at 10 Hz and analyzed based on steady-state current changes. At the end of the experiments, animals were euthanized according to institutional guidelines.

3. Results and discussion

3.1. Development of the LIG neural probe for multiplexed neurochemical sensing

An LIG-based neural probe was developed to enable multiplexed neurochemical sensing within a compact, scalable, and implantable architecture (Fig. 1). The device consists of multiple closely spaced electrode sites patterned on a flexible PI substrate, with each electrode individually functionalized for the selective detection of a specific neurochemical. The use of LIG as the electrode material provides a porous and conductive graphene structure that offers a high surface area for enzyme immobilization and supports efficient electrochemical signal transduction (Fig. 1).

The LIG electrodes were directly patterned onto the PI substrate by laser processing, eliminating the need for complex lithographic procedures and enabling rapid, scalable fabrication. Following laser patterning, a PI insulation layer was applied to define individual sensing sites. Importantly, the sensing electrodes were not exposed simultaneously; instead, a stepwise opening process was used to selectively define each sensing interface.

To achieve multiplexed sensing within a single probe, a site-selective enzyme functionalization strategy was implemented. The PI layer was sequentially opened to expose one electrode at a time, followed by localized enzyme immobilization on the exposed site. The first electrode was opened and functionalized with GO_x, followed by sequential opening and functionalization of the second and third electrodes with LO_x for lactate detection and tyrosinase for dopamine detection, respectively. Finally, an additional electrode was opened to serve as a signal-recording site. This stepwise process enables selective immobilization of different enzymes on adjacent electrodes while minimizing cross-contamination during fabrication (Fig. 1 and Fig. S1).

A key consideration in this process is the stability of the initially immobilized enzyme during subsequent processing steps. To address this issue, electrochemical activation was selectively applied to the glucose-sensing electrode prior to enzyme immobilization. This treatment introduces oxygen-containing functional groups onto the LIG surface, thereby improving covalent coupling efficiency and enhancing the mechanical and chemical stability of the enzyme layer. As a result, the glucose-sensing interface remains stable throughout the sequential functionalization of neighboring electrodes.

Enzyme immobilization at each sensing site was carried out using EDC/NHS coupling chemistry, enabling covalent attachment of the enzymes to the LIG surface. A two-step incubation protocol was employed to enhance enzyme loading while preserving enzymatic activity. The electrodes were first incubated at low temperature to promote controlled adsorption, followed by incubation at room temperature to facilitate stable amide bond formation. This approach produced uniform and stable enzyme layers across all sensing sites (Fig. 1).

Through the combination of LIG-based electrode fabrication and sequential, site-selective functionalization, the proposed neural probe enables multiplexed integration of multiple biochemical sensing interfaces within a single miniaturized device. In contrast to conventional approaches that rely on physically separated sensors or complex microfluidic systems, this strategy offers a simpler and more scalable route to multi-analyte sensing while preserving spatial selectivity and minimizing cross-interference. These features highlight the potential of the platform as a practical tool for high-resolution, multiplexed neurochemical monitoring in vivo.

3.2. Selective and multiplexed sensing performance of the integrated multichannel LIG probe

Building on our previous fabrication approach [41,43], a flexible LIG-based neural probe was constructed on a PI substrate. Laser patterning conditions were first optimized to achieve reproducible

electrode dimensions. Among the tested laser scanning speeds, 120 mm·s⁻¹ produced an electrode radius closest to the target value (90 μm) with the smallest variation (Fig. S2) and was therefore selected for subsequent fabrication. Under the optimized condition, LIG conductive traces with a width of approximately 90 μm and a thickness of approximately 13 μm were formed. Four sensing electrodes with a diameter of 180 μm and an edge-to-edge spacing of 100 μm were then defined by laser patterning. Following parylene insulation, the first sensing site was selectively exposed by opening the insulating layer, enabling site-selective functionalization (Fig. 2a-c). Subsequent sensing sites were sequentially opened and functionalized using the same procedure to construct the multiplexed neurochemical sensing array.

Specifically, the first electrode was opened. The porous structure formed by laser-induced graphene (LIG) was confirmed, providing an enlarged surface area for enzyme immobilization (Fig. S3). The electrode was then electrochemically activated, followed by immobilization of glucose oxidase (GO_x). Successful enzyme immobilization was confirmed by cyclic voltammetry (CV), which showed distinct changes in electrochemical behavior after enzyme coating (Fig. 2d).

Subsequently, the second electrode was then selectively opened and functionalized with LO_x for lactate sensing. The corresponding CV response showed clear post-functionalization changes, confirming successful enzyme immobilization (Fig. 2d). Importantly, the CV response of the previously GO_x-functionalized electrode remained unchanged, indicating that no unintended enzyme deposition occurred (Fig. 2e-f). This result highlights effective spatial isolation during the sequential functionalization process.

The third electrode was opened and functionalized with tyrosinase for dopamine sensing (Fig. 2d). Consistent with the previous functionalization steps, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) confirmed successful enzyme immobilization on each sensing site (Fig. 2e-f and Fig. S4). In particular, enzyme-functionalized electrodes exhibited increased impedance compared with bare LIG electrodes, indicating the formation of enzyme-containing interfacial layers on the porous LIG surface (Fig. S4). Collectively, these results demonstrate that the sequential, site-selective functionalization strategy enables independent biochemical modification of closely spaced electrodes without cross-contamination.

Following enzyme immobilization, the electrochemical performance of the integrated multichannel probe was evaluated toward glucose, lactate, and dopamine (Fig. 3a). Each sensing channel exhibited a pronounced response to its target analyte, whereas non-target analytes produced minimal signal changes (Fig. 3b-d).

In the GO_x-functionalized channel, successive glucose additions from 0.1 to 5.0 mM produced clear staircase-like current increases with stable plateaus, reaching approximately 6.5 μA at 5.0 mM glucose (Fig. 3b). The LO_x-functionalized channel also showed a target-specific stepwise response to lactate over the same concentration range, reaching approximately 5.0 μA at 5.0 mM lactate (Fig. 3c). In the tyrosinase-functionalized channel, dopamine additions from 0.05 to 1.0 μM generated distinct concentration-dependent current increases, reaching approximately 0.8 μA at 1.0 μM dopamine (Fig. 3d). In contrast, non-target analytes produced only minimal signal changes in each channel, indicating selective detection of glucose, lactate, and dopamine. These tested concentration ranges are consistent with calibration ranges reported for implantable neurochemical biosensors and cover the expected extracellular concentration ranges for glucose and lactate [35, 37], as well as cocaine- or stimulation-induced dopamine changes reported in previous in vivo studies [44,45]. To further quantify the concentration-dependent sensing performance, calibration curves were constructed from the steady-state current responses obtained from each target-specific channel (Fig. 3e-g). The sensors exhibited linear responses over the tested concentration ranges, with sensitivities of 1.1862 μA·mM⁻¹, 0.9093 μA·mM⁻¹, and 0.6691 μA·μM⁻¹ for glucose, lactate, and dopamine, respectively. The corresponding limits of detection (LODs) were 0.068 mM, 0.105 mM, and 0.098 μM,

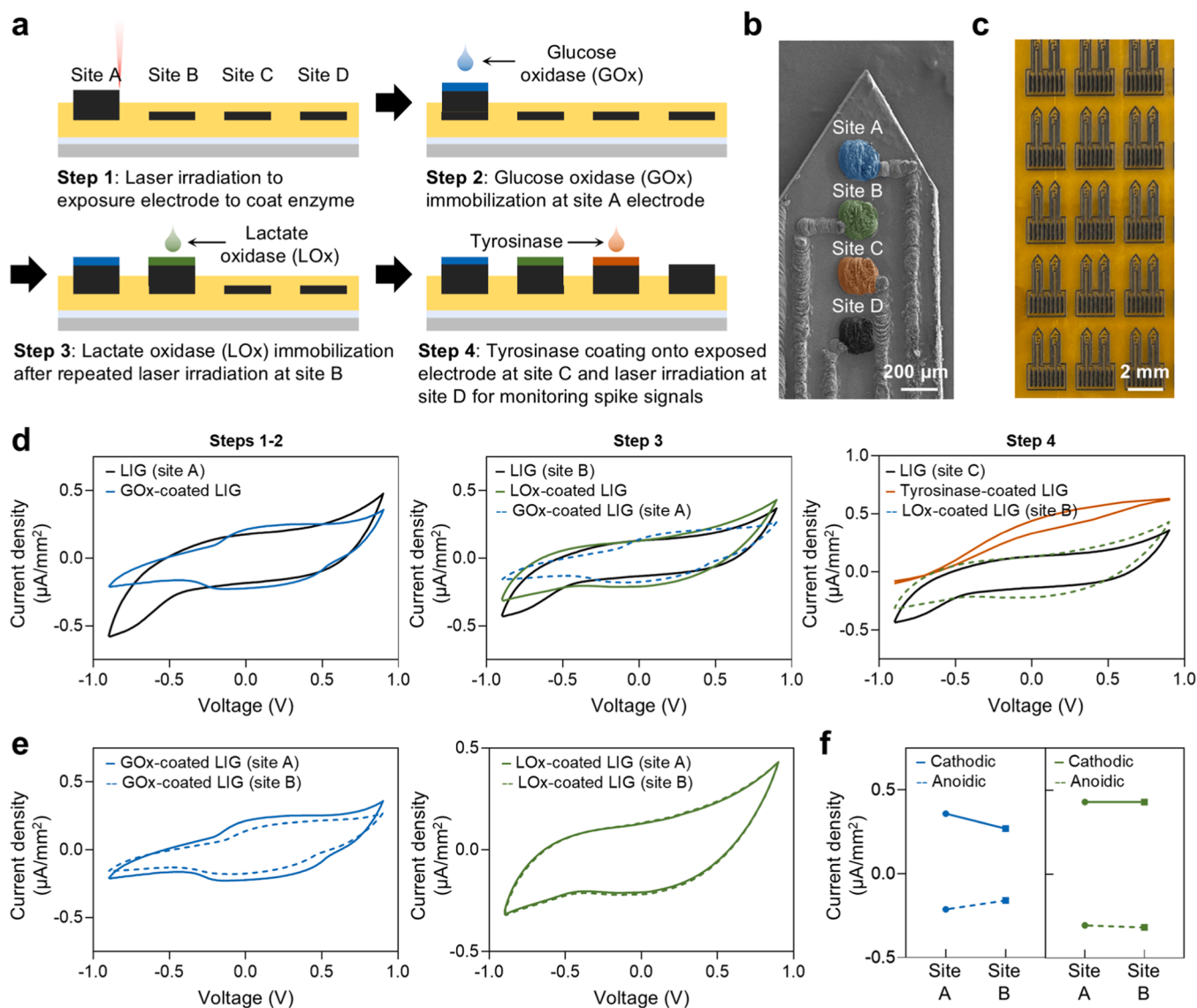


Fig. 2. Sequential site-selective enzyme functionalization and electrochemical characterization of the LIG probe. (a) Schematic illustration of the stepwise, site-selective functionalization process. Individual electrode sites are sequentially exposed by laser irradiation and functionalized with (Step 2) GO_x at site A, (Step 3) LO_x at site B, and (Step 4) tyrosinase at site C, while site D is reserved for electrophysiological recording. (b) SEM image of the LIG probe tip, showing spatially defined electrode sites (A–D). (c) Optical image of the fabricated LIG probe array. (d) CV responses measured after each functionalization step. (e) Comparison of CV responses at previously functionalized electrodes during sequential processing (GO_x-coated electrode: Step 2, blue solid; Step 3, blue dashed; LO_x-coated electrode: Step 3, green solid; Step 4, green dashed). (f) Quantitative comparison of anodic and cathodic current densities at previously functionalized electrodes during sequential processing (GO_x-coated electrode: Step 2, blue solid; Step 3, blue dashed; LO_x-coated electrode: Step 3, green solid; Step 4, green dashed).

demonstrating reliable quantitative detection within physiologically relevant concentration ranges. Notably, the enlarged chronoamperometric traces further confirmed negligible signal changes in adjacent non-target channels during each analyte addition (Fig. S5). This result indicates that the 100 μm edge-to-edge spacing between sensing sites, together with the site-selective enzyme functionalization strategy, effectively minimized H₂O₂-mediated crosstalk and cross-contamination between neighboring sensing channels.

We further examined whether the subsequent functionalization steps affected the activity of enzymes immobilized in earlier steps (Fig. S6). Target-response retention was calculated as the ratio of the target response after subsequent immobilization to the initial target response measured immediately after enzyme immobilization. The GO_x-functionalized channel retained 91.8% of its initial glucose response after subsequent LO_x and tyrosinase immobilization, while the LO_x-functionalized channel retained 94.9% of its initial lactate response

after subsequent tyrosinase immobilization. These results demonstrate that the sequential enzyme immobilization process minimally affected the catalytic activity of previously immobilized enzymes while preserving target-specific sensing performance throughout the fabrication process.

Overall, the integrated multichannel LIG probe exhibited stable, target-specific responses to glucose, lactate, and dopamine, with minimal responses in adjacent non-target channels. The preserved enzyme activity after sequential functionalization further confirms that the site-selective immobilization process enables independent biochemical modification of closely spaced electrodes without compromising sensing performance. These results demonstrate the suitability of the proposed LIG-based platform for compact and scalable multiplexed neurochemical sensing.

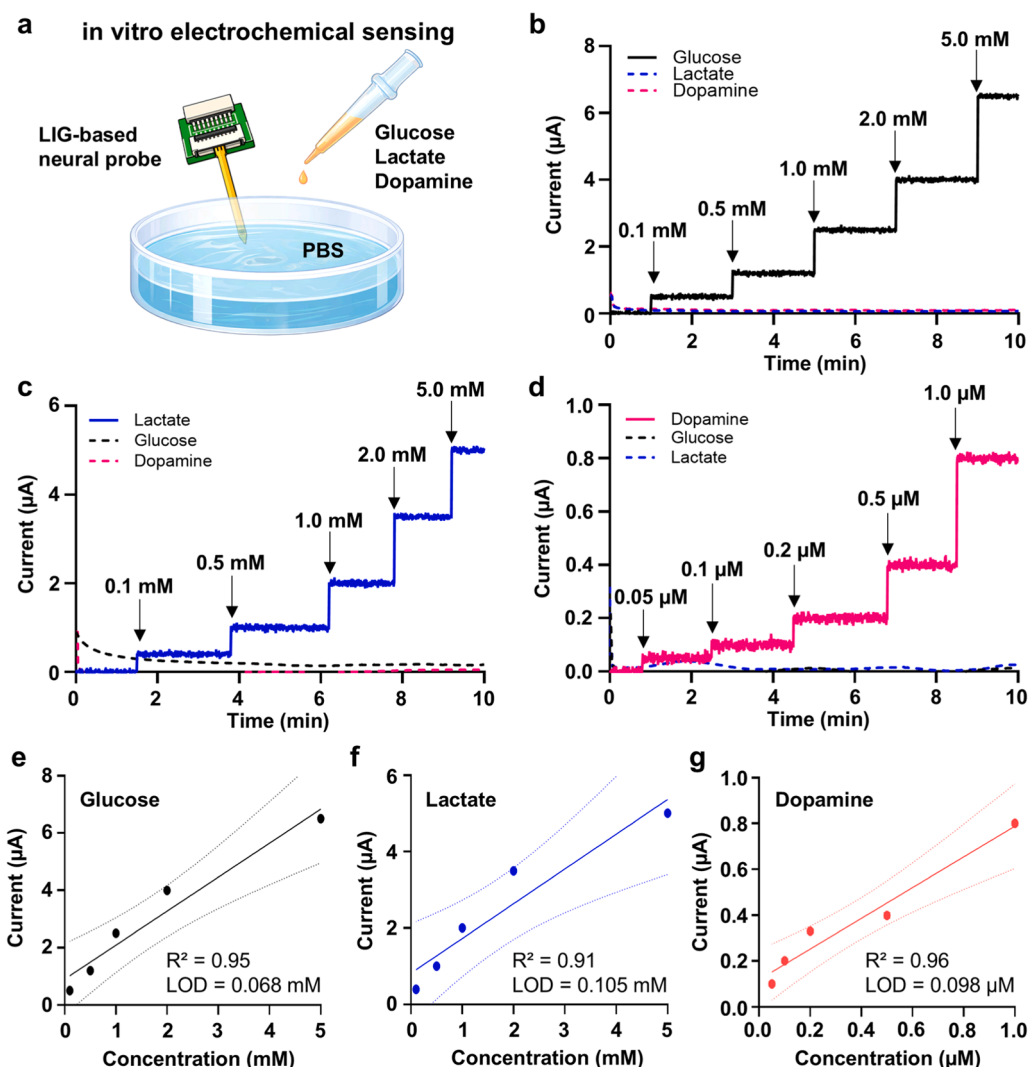


Fig. 3. In vitro electrochemical performance of the multiplexed LIG-based neural probe. (a) Schematic illustration of the in vitro electrochemical sensing setup, in which the LIG-based neural probe is immersed in PBS and analytes (glucose, lactate, and dopamine) are sequentially introduced. (b) Amperometric response of the glucose-sensing electrode to stepwise additions of glucose, with corresponding responses to lactate and dopamine. (c) Amperometric response of the lactate-sensing electrode to stepwise additions of lactate, with corresponding responses to glucose and dopamine. (d) Amperometric response of the dopamine-sensing electrode to increasing dopamine concentrations, with corresponding responses to glucose and lactate. (e–g) Calibration curves obtained from the steady-state current responses of the (e) glucose-, (f) lactate-, and (g) dopamine-sensing electrodes, respectively. Linear fitting yielded calibration equations of $I = 1.1862 C + 0.8988$ for glucose, $I = 0.9093 C + 0.8166$ for lactate, and $I = 0.6691 C + 0.1187$ for dopamine, where I is the current response in μA and C is the analyte concentration in mM for glucose/lactate and μM for dopamine. Corresponding LOD values were 0.068 mM, 0.105 mM, and 0.098 μM , respectively.

3.3. Electrochemical detection of stimulus-dependent dopamine release from SH-SY5Y cells

Dopamine is present in the brain at relatively low concentrations, typically in the nanomolar to low micromolar range under physiological conditions. To evaluate whether the fabricated LIG-based dopamine sensor can reliably detect such low-level signals in a biologically relevant environment, its performance was assessed using SH-SY5Y neuronal cells under different stimulation conditions.

Conditioned media collected from SH-SY5Y cells subjected to different stimulation protocols were analyzed by chronoamperometry to quantify dopamine release (Fig. 4a). The untreated group exhibited a minimal current response of approximately 0.02 μA . Following L-DOPA treatment, which enhances intracellular dopamine synthesis, the current increased to 0.10 μA . A higher response of 0.18 μA was observed in the depolarization group, reflecting stimulation-induced dopamine release. Notably, the combined L-DOPA and depolarization treatment produced the highest current response of 0.32 μA (Fig. 4a). These results indicate

that dopamine release is enhanced by precursor loading and further amplified by depolarization-induced exocytosis.

The concentration dependence of the measured signals was further examined using serially diluted supernatants (Fig. 4b). In the untreated group, the current increased from 0.008 μA (1/8 dilution) to 0.020 μA (1/2 dilution). The L-DOPA-treated group exhibited responses of 0.035, 0.060, and 0.100 μA at 1/8, 1/4, and 1/2 dilutions, respectively. The depolarization group showed an increase from 0.065 μA (1/8 dilution) to 0.180 μA (1/2 dilution), while the combined treatment group exhibited the highest responses of 0.110, 0.190, and 0.320 μA at the corresponding dilution levels. The stepwise increase in current with decreasing dilution confirms the concentration-dependent electrochemical detection of dopamine (Fig. 4b).

Across all dilution conditions, the combined treatment group consistently exhibited the highest response, followed by the depolarization group, the L-DOPA-treated group, and the untreated control. These results demonstrate that the LIG-based dopamine sensor is capable of detecting physiologically relevant dopamine levels and

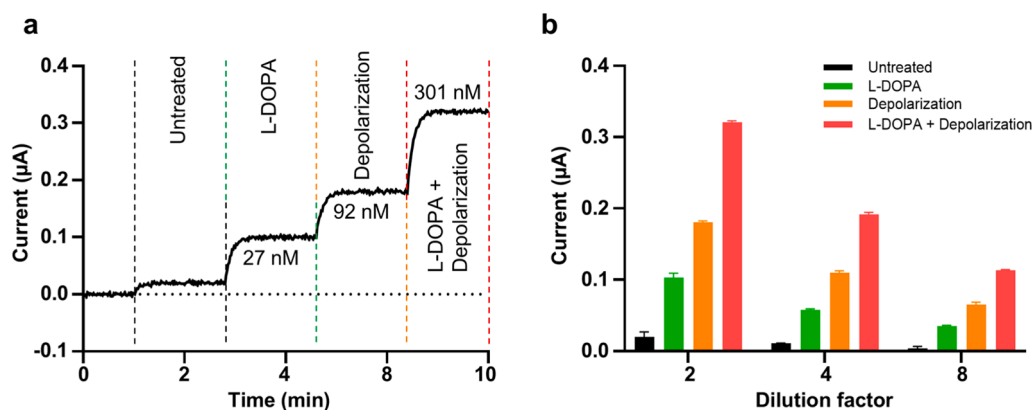


Fig. 4. Electrochemical detection of stimulus-dependent dopamine release from SH-SY5Y cells. (a) Representative amperometric responses of the dopamine-sensing electrode to conditioned media collected from SH-SY5Y cells under different stimulation conditions, including untreated, L-DOPA-treated, depolarized, and combined L-DOPA plus depolarization. (b) Quantitative comparison of current responses at different dilution factors (1/2, 1/4, and 1/8) for each condition. Data presented as the mean $\pm$ SD ($n = 3$ electrodes). The concentration values indicated in the trace were estimated from the corresponding in vitro calibration equations.

distinguishing graded, stimulus-dependent dopamine release from neuronal cells.

3.4. In vivo individual chemical sensing using the multichannel LIG probe

The in vivo sensing capability of the implanted multichannel LIG probe was evaluated in mice by monitoring electrochemical signals from individual sensing electrodes following systemic administration of target analytes. As illustrated in Fig. 5a, each neurochemical (glucose, lactate, and cocaine for dopamine induction) was independently administered, and the corresponding electrochemical response was

recorded from the designated sensing electrode.

Following probe implantation and baseline stabilization, current changes were continuously recorded under physiological conditions. For glucose sensing, a glucose solution was intravenously administered at a dose of 3 g/kg body weight. Immediately after injection, a transient decrease in current was observed, followed by gradual recovery and a sustained increase over time. The current reached approximately 2 μ A above baseline by the end of the recording period, indicating successful in vivo detection of glucose-associated changes (Fig. 5b). Based on the in vitro calibration curve, the corresponding glucose concentration at the representative measurement point was estimated to be approximately

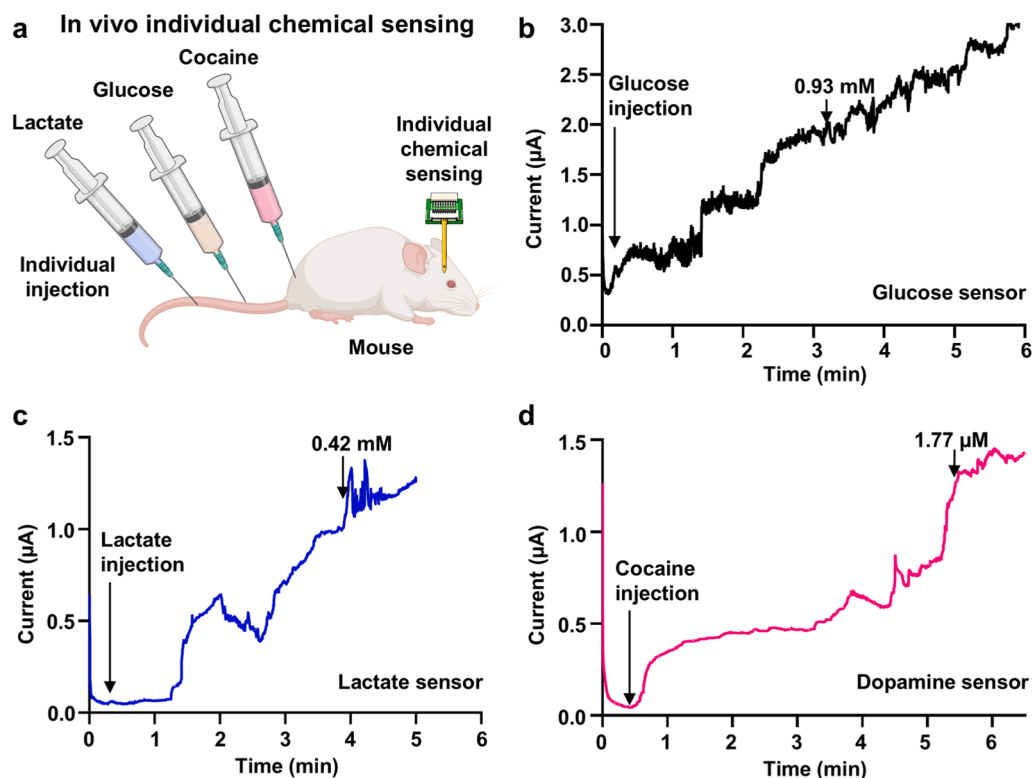


Fig. 5. In vivo individual neurochemical sensing using the multichannel LIG probe. (a) Schematic illustration of the in vivo experimental setup, in which glucose, lactate, and cocaine (for dopamine induction) are independently administered and electrochemical responses are recorded from the implanted LIG probe. (b) Amperometric response of the glucose-sensing electrode following intravenous glucose injection. (c) Amperometric response of the lactate-sensing electrode following lactate administration. (d) Amperometric response of the dopamine-sensing electrode following cocaine administration. The concentration values indicated in the traces were estimated from the corresponding in vitro calibration equations.

0.93 mM, which is within the range reported for extracellular brain glucose in previous implantable biosensor studies [35,37].

For lactate sensing, systemic lactate administration induced a gradual increase in current after the initial stabilization phase. Compared with the glucose response, the signal exhibited a slower rise and showed several transient fluctuations during the recording period. The overall current change reached approximately 1.2 μ A, demonstrating that the lactate-responsive electrode remained electrochemically active in the brain environment (Fig. 5c). The corresponding lactate concentration was estimated to be approximately 0.42 mM, which is consistent with previously reported extracellular brain lactate concentrations [35,37].

Dopamine sensing was evaluated via intraperitoneal administration of cocaine (15 mg/kg), which is known to elevate extracellular dopamine levels. To independently verify dopamine dynamics under the same experimental conditions, fiber photometry was first performed using a virally expressed dopamine sensor in the mPFC. Cocaine administration induced a clear increase in fluorescence intensity, confirming enhanced dopamine release in vivo (Fig. S7 and Fig. S8). Consistent with these optical measurements, the dopamine-sensing electrode of the implanted LIG probe exhibited a progressive increase in current following cocaine injection, with distinct signal transitions observed during the later phase of recording. The response reached approximately 1.3 μ A by the end of the measurement period, while the baseline prior to stimulation remained stable. This pronounced increase in current is consistent with pharmacologically induced dopamine release (Fig. 5d). Based on the in vitro calibration, the corresponding dopamine concentration was estimated to be approximately 1.77 μ M. This value is comparable to previously reported real-time electrochemical measurements showing dopamine elevations ranging from hundreds of nanomolar to micromolar levels following cocaine administration or electrical stimulation [44,45].

Taken together, the distinct current responses observed from the glucose-, lactate-, and dopamine-responsive electrodes demonstrate that the multichannel LIG probe retains its electrochemical functionality after implantation and enables real-time in vivo monitoring of

individual neurochemical signals. The estimated neurochemical concentrations further support that the measured current responses correspond to physiologically relevant in vivo neurochemical dynamics. These results highlight the capability of a single probe to simultaneously detect multiple neurochemical species in vivo.

3.5. In vivo multimodal recording of neurochemical and electrophysiological signals

We next evaluated the capability of the multichannel LIG probe for simultaneous multimodal recording, integrating multiplexed neurochemical sensing with electrophysiological measurements. To enable concurrent neural signal acquisition, additional electrode sites were opened and modified with black Pt to reduce impedance and enhance signal quality. SEM confirmed the successful deposition of nanostructured, protrusive black Pt on the porous LIG surface (Fig. S9). The electroplated electrodes exhibited an impedance of 0.91 ± 0.05 M Ω at 1 kHz (Fig. S10), which is suitable for neural recording [46]. The multichannel LIG probe was implanted into the mPFC, allowing simultaneous monitoring of neurochemical and electrical activity in vivo under identical experimental conditions (Fig. 6a).

The in vivo electrochemical responses of the implanted probe were evaluated following cocaine administration, which is known to elevate extracellular dopamine levels. Current signals were continuously recorded from the glucose-, lactate-, and dopamine-responsive channels under physiological conditions (Fig. 6b).

The glucose-responsive channel exhibited a gradual increase in current following cocaine injection, reaching approximately 0.25 μ A above baseline by the end of the recording period. This glucose-associated response is consistent with previous studies reporting cocaine-induced extracellular glucose increases in the brain [47,48]. In contrast, the lactate-responsive channel remained near baseline throughout the measurement, with total fluctuations limited to approximately 0.05 μ A or less. No obvious lactate response was detected following cocaine administration. This may reflect the dynamic balance between lactate production, transport, and neuronal consumption,

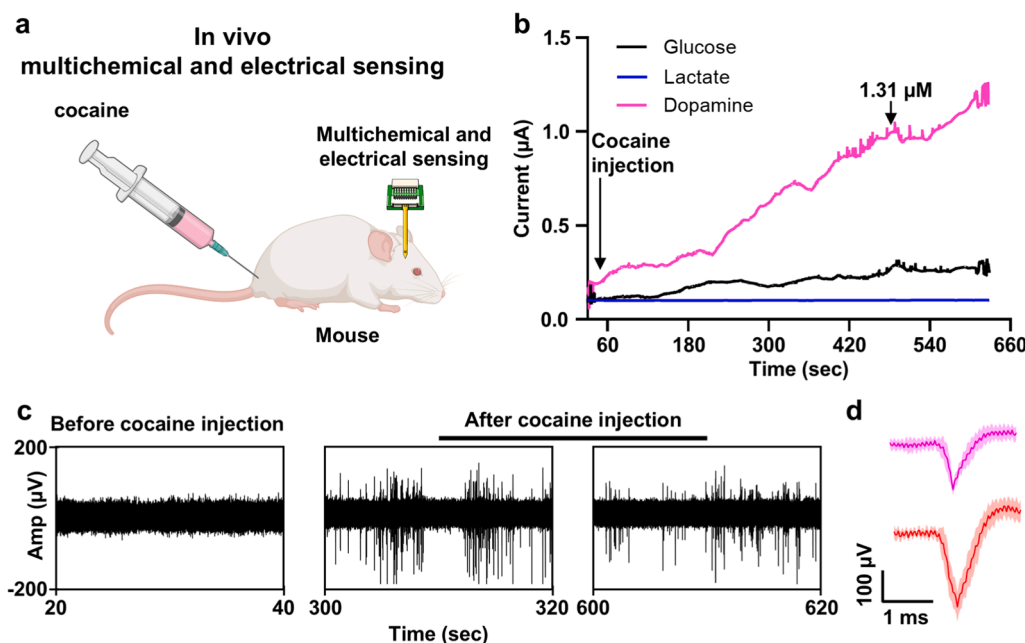


Fig. 6. In vivo multimodal recording of neurochemical and electrophysiological signals using the LIG probe. (a) Schematic illustration of the in vivo multimodal sensing setup, in which cocaine is administered and simultaneous neurochemical and electrophysiological signals are recorded from the implanted probe in the mouse brain. (b) Simultaneous amperometric recordings from glucose-, lactate-, and dopamine-responsive channels following cocaine injection. (c) Representative neural recording traces from a single electrode before and after cocaine administration. (d) Representative spike waveforms recorded after cocaine administration. The concentration value indicated in the trace was estimated from the corresponding in vitro calibration equations.

rather than the absence of lactate-related metabolic activity [3]. Together, these results suggest distinct metabolic responses to cocaine under the present conditions, with a modest glucose-associated increase and negligible lactate variation.

A distinct response was observed in the dopamine-responsive channel. Following cocaine injection, the current increased progressively over time, reaching approximately 1.0 μA above baseline. This response was substantially larger than that observed in the glucose channel and clearly distinguishable from the near-flat lactate trace, indicating selective detection of cocaine-induced dopamine elevation.

In parallel with the neurochemical measurements, electrophysiological recordings revealed modulation of neuronal activity following cocaine administration. Electrodes that showed little to no detectable signals prior to injection exhibited more pronounced irregular neural signal fluctuations after injection (Fig. 6c). The temporal evolution of the neural signals was consistent with the observed increase in dopamine levels, suggesting that dopaminergic modulation induced by cocaine is accompanied by enhanced neuronal excitability (Fig. 6d) [49, 50].

Taken together, these results demonstrate that cocaine administration induces a pronounced increase in dopamine, accompanied by modest glucose changes, negligible lactate variation, and increased neuronal firing activity. The multichannel LIG probe enables simultaneous, real-time multimodal monitoring of neurochemical and electrical signals in the living brain, providing a powerful platform for investigating neurochemical–electrophysiological coupling in vivo.

4. Conclusions

In this study, we developed a flexible LIG-based neural probe capable of multiplexed neurochemical sensing and electrophysiological recording within a single miniaturized platform. By employing a site-selective, sequential enzyme functionalization strategy, independent sensing interfaces for glucose, lactate, and dopamine were successfully integrated onto closely spaced electrodes. This approach enables reliable multi-analyte detection while minimizing cross-contamination between adjacent sensing sites, while preserving the activity of previously immobilized enzymes throughout the sequential functionalization process.

The fabricated probe exhibited selective, concentration-dependent electrochemical responses for each target analyte in vitro and in cellular environments. Electrochemical characterization confirmed successful enzyme immobilization on each sensing site, and negligible responses in adjacent non-target channels demonstrated effective suppression of crosstalk between neighboring electrodes. Furthermore, in vivo experiments demonstrated that the device maintained stable sensing performance after implantation and enabled real-time monitoring of individual neurochemical signals in the mouse brain. Simultaneous measurements of glucose, lactate, dopamine, and neural activity further highlight the probe's capability for multimodal recording, revealing distinct neurochemical responses and associated changes in neuronal firing following pharmacological stimulation.

Despite these advances, the current platform is limited by electrode density, which constrains spatial resolution for large-scale neural circuit analysis. Future work will focus on increasing electrode density through advanced fabrication approaches, such as fine patterning using ultraviolet (UV) laser processing, to achieve smaller feature sizes and enable high-density integration. This improvement is expected to facilitate simultaneous neurochemical and electrophysiological measurements across multiple brain regions and support large-scale data acquisition.

In addition, wireless systems will be integrated to enable multimodal signal acquisition in freely behaving animals. Such developments will provide a powerful platform for investigating the relationships among neurochemical dynamics, neural activity, and behavior, and for advancing our understanding of brain circuit function under both physiological and pathological conditions.

Overall, this work establishes a practical and scalable strategy for multimodal neural interfacing. By enabling simultaneous and integrative analysis of multiple neurochemical and electrophysiological signals within a compact platform, it provides a valuable tool for studying complex brain function and dynamics in vivo.

CRediT authorship contribution statement

Seungjun Lee: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Giheon Kim:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A-Hyeon Lee:** Writing – original draft, Investigation, Data curation. **Dae-Si Kang:** Writing – original draft, Investigation, Data curation. **Jeong Hun Kim:** Writing – original draft, Investigation, Data curation. **Ja Wook Koo:** Resources, Methodology. **Suk Won Lee:** Resources, Methodology. **Namsun Chou:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Hyogeun Shin:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2026.140518.

Data availability

Data will be made available on request.

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