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Wireless, battery-free push-pull microsystem for membrane-free neurochemical sampling in freely moving animals

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Extensive studies in both animals and humans have demonstrated that high molecular weight neurochemicals, such as neuropeptides and other polypeptide neurochemicals, play critical roles in various neurological disorders. Despite many attempts, existing methods are constrained by detecting neuropeptide release in small animal models during behavior tasks, which leaves the molecular mechanisms underlying many neurological and psychological disorders unresolved. Here, we report a wireless, programmable push-pull microsystem for membrane-free neurochemical sampling using cellular spatial resolution in freely moving animals. In vitro studies demonstrate the sampling of various neurochemicals with high recovery (>80%). Open-field tests reveal that the device implantation does not affect the natural behavior of mice. The probe successfully captures the pharmacologically evoked release of neuropeptide Y in freely moving mice. This wireless push-pull microsystem creates opportunities for neuroscientists to understand where, when, and how the release of neuropeptides modulates diverse behavioral outputs of the brain.

INTRODUCTION

Neurological disorders, such as depression, anxiety, and many others, affect the quality of life of more than 100 million people worldwide (1). High molecular weight neurochemicals, such as neuropeptides and other polypeptide neurochemicals, play critical roles in various aspects of these neurological disorders (2–5). However, in many neural circuits in the brain, we have a limited understanding of how, where, and when the release of neuropeptides/other proteins modulate diverse behavioral outputs of the brain, at the cellular or circuit level. This is, in part, because current measurement technologies, such as microdialysis or fast-scan cyclic voltammetry (FSCV), have limited spatial and temporal precision or molecular specificity to detect these larger molecules (6–8). FSCV, a widely used electrochemical method, can measure redox-active neurochemicals with high spatiotemporal precision (9, 10). Neuropeptides, however, are often chemically inert (nonoxidizable). The in vivo neurochemical sampling and offline multiplex analysis using immunoassay, liquid chromatography, or mass spectrometry are of broad interest for neuroscience studies (11). The current in vivo sampling technique, microdialysis, relies on tubing connected to syringe pumps and is widely used for sampling small molecules through a semipermeable membrane (11–13). This method enables the detection of as many as 70 analytes from a single sample (14). This system, however, has limited spatial resolution due to the large probe size (150 to 440 μm in diameter) (15). Furthermore, current microdialysis probes suffer from the limited capability to sample neuropeptides and proteins due to their ultralow concentration (1 to 100 pM), the presence of a diffusion barrier membrane, and the nonspecific adsorption to the membrane.

Recent studies offer microdialysis probes with a high molecular weight cutoff membrane (16), modified membrane (17), or push-pull perfusion systems (18–20) that bypass certain drawbacks associated with the traditional microdialysis probe, but most microdialysis probes are still constrained by the following: (i) low recovery, (ii) sample loss due to the adsorption to dialysis membrane (16), (iii) poor temporal resolution (>1 min, typically ~10 min) (17), (iv) limited spatial resolution due to its large size (150 to 440 μm in diameter) (16, 17), (v) prolonged brain tissue damage and inflammation due to the mechanical mismatch (21, 22), (vi) physical constraints on the natural movements of the animals (16, 19, 20), or (vii) large device size (>75 mm by 70 mm in size) and bulky construction (>255 g) (23). These limitations challenge the rapid interrogations of the molecular mechanisms underlying many neurological diseases in small animal models, such as mice.

Here, we report a wireless, programmable push-pull microsystem for membrane-free sampling of neurochemicals, including potassium ions, small molecules such as dopamine (DA), and large molecules such as neuropeptide Y (NPY) with high recovery (>80%) and cellular-scale spatial resolution. The miniaturized device design, lightweight construction (~0.2 g), wireless, and battery-free operation of the push-pull microsystem enable in vivo neuropeptide sampling with pharmacological stimulation in awake, freely moving mice. The technology has the potential to allow investigations of the spatiotemporal dynamics of neurochemicals, especially for large molecule neuropeptides in freely moving mice.

RESULTS

Design and working principle of the wireless, programmable push-pull microsystem for membrane-free neurochemical sampling

The wireless, programmable push-pull microsystem includes four interconnected subsystems (Fig. 1A): (i) Four miniaturized push-pull
micropumps consist of platinum (Pt)–coated gold interdigitated electrodes (fig. S1, A and B), micropump chambers to house the electrolyte solution (Pt nanoparticles, PtNPs, dispersed in 50 mM NaOH) (fig. S1C), and a Pt (~5 nm thick)–coated polystyrene-block-polybutadiene-block-polystyrene (SBS) flexible membrane (120 μm thick) (fig. S1D); (ii) a refillable fluid reservoir to house the perfusion solution (volume, ~0.5 to 1.5 μl); (iii) a soft microfluidic probe (four channels, each with cross-sectional area of 10 μm by 10 μm; total thickness, ~150 μm; width, 350 μm) for the delivery of perfusion solution into the targeted brain regions and sampling of perfused fluid; and (iv) a collection of electronics and hardware for wireless power harvesting and independent activation over four micropumps. Figure 1A shows a schematic diagram and scanning electron microscope image of the tip end of a device that includes four microchannels for membrane-free fluid perfusion and collection. While the overall dimension of the microfluidic probe (four channels; total thickness, ~150 μm; width, 350 μm) is in the same dimensional range as that of conventional microdialysis probes (150 to 440 μm in diameter), the small size (cross-section: 10 μm by 10 μm) of each microfluidic channel determines the spatial resolution for fluid sampling (Fig. 1A, right), thereby making it possible to sample large-molecule neurochemicals with a cellular-scale spatial resolution. Figure 1 (B and C) shows the optical images of the frontside view of the wireless push-pull microsystem, which includes four miniaturized push-pull micropumps and a soft microfluidic probe, as well as the images of the backside view, which includes the radio frequency (RF) coil antenna and electronics for wireless power harvesting and control of the device. Similar to other thin-film neural probes (24–28), the soft mechanics (modulus, ~3 MPa) and thin geometry (~150 μm) of the microfluidic probe enable adaptation to brain micromotion associated with movement, respiration, and blood flow (29, 30). These properties can minimize mechanical irritation or tissue damage during in vivo chronic study (28, 31).

Active, independent fluid perfusion and collection through four microfluidic channels are controlled through the elastic expansion and recovery of the SBS membrane, resulting in push-pull operation. More specifically, following a wirelessly triggered operation modality, a low-power microcontroller (μC) in the electronics module activates a selected push-pull micropump to initiate a water electrolysis reaction (\(2\text{H}_2\text{O} \rightarrow \text{H}_2 + \text{O}_2\)) (32). The volume expansion associated with the generation of gas bubbles deforms the flexible SBS membrane and drives the flow of perfusion solution into targeted brain regions through the microfluidic channels. The perfused fluid is then retracted back to the reservoir upon the recovery of deformed flexible SBS membrane, which is induced by the recombination of hydrogen and oxygen bubbles in the presence of PtNPs (2H₂ + O₂ → Pt + 2H₂O) (33). This membrane-free, push-pull operation places the perfusion solution in direct contact with the extracellular fluid, thereby allowing for fast diffusion of targeted neurochemicals into the perfusion solution. Figure S2 shows the deformation of a flexible membrane, caused by the operation of micropumps, before and after the membrane was stretched (15.5% strain) 1000 times within 500 min, suggesting the longevity of the flexible SBS membrane.

To demonstrate the push-pull operation, we first delivered a red color perfusion solution. The perfused solution was then collected back upon the recovery of the flexible SBS membrane (Fig. 1D and movie S1). The coil antenna on the device allows for wireless power harvesting from a transmission antenna using magnetic inductive resonant coupling at the frequency of 13.56 MHz (Fig. 1, C and E) (28). This wireless power supply is compatible with many environments such as laboratory-made cages, water pools, or mazes (27). Figure 1F shows a piece of chicken breast inside a wireless power-harvesting enclosure. The infrared (IR) thermal characterization suggests that magnetic coupling has no heating effect on the chicken breast after 10 min of RF operation with 6 W power applied to the transmission antenna, and hence, no thermal damage is expected in similar biological tissues during in vivo studies. Programmable operation of multiple push-pull micropumps occurred through the μC (34). The complete system includes a laptop with a customized control software interface, a multicomponent power distribution control box, an auto antenna tuner, and a customizable transmission antenna (NeuroLux Inc.; fig. S3). The lightweight construction (~0.2 g; fig. S4) and battery-free operation enable the applications in awake and freely moving animals for programmable neurochemical sampling. Depending on the volume of loaded perfusion solution, the maximum device weight is around 0.3 g, which is still much lighter than that of wireless optofluidic probes (~1.8 g) (24), chronic neurophenotyping and photostimulation probe (2 g) (26), and conventional neural probes (e.g., fiber photometry system: 9.88 g) and comparable to the state-of-the-art neural probes, such as wireless photometry system (0.5 g) (35).

**Electrochemical characteristics of push-pull micropump**

The push-pull micropump is based on the water electrolysis and recombination reactions (Fig. 2A). The water electrolysis reaction is initiated when a driving voltage is applied to a pair of interdigitated electrodes in contact with an electrolyte solution (NaOH, 50 mM), resulting in the generation of oxygen and hydrogen gases (32). The standard potential for water electrolysis is 1.23 V (25°C) (36), and a higher potential is preferable for gas bubble formation and detachment from electrode surface (32). Here, a driving voltage of 3.0 V was used to actuate the electrolysis reaction. Upon removal of the driving voltage, the generated gases recombined into liquid water in the presence of Pt catalysts (33). These electrolysis and recombination reactions caused an elastic expansion and recovery of the flexible SBS membrane, thereby enabling the push-pull operation of fluid housed in the perfusion solution reservoir (Fig. 2A). Here, we investigated several approaches to improve the recovery efficiency of the deformed flexible membrane; this efficiency is defined as \((1 - h_2/h_1) \times 100\%\), in which \(h_1\) is the maximum height of the deformed membrane in the electrolysis reaction, and \(h_2\) is the height of the recovered membrane during the recombination reaction (fig. S5). First, the surface of interdigitated Au electrodes was coated by a Pt layer via the electrochemical plating method. The coated Pt layer increased the recovery efficiency from 8.97 ± 1.35% to 17.24 ± 0.93% within 3 min after removing the driving voltage (Fig. 2B). To further improve the recovery efficiency, Nafion-coated PtNPs were dispersed in the electrolyte solution. The micropumps, which used Nafion-coated PtNPs (2 mg/ml), showed a much higher membrane recovery efficiency within 3 min (62.30 ± 2.71%) (Fig. 2C, orange line) compared with Pt-coated Au electrodes (Fig. 2C, black line). Because of poor water solubility, the generated gases tended to escape from the surface of the interdigitated electrodes and accumulated on the surface of the flexible SBS membrane. These accumulated gas bubbles could further recombine into liquid water if a Pt layer is coated on the SBS membrane (Fig. 2C, blue line). A micropump with the Pt-coated Au electrodes, PtNPs, and Pt-coated...
Fig. 1. Design and operation of the wireless, programmable push-pull microsystem for membrane-free neurochemical sampling. (A) Schematic diagram of wireless push-pull microsystem and SEM image of the injectable tip end of the microsystem that includes four microchannels for membrane-free fluid perfusion and sampling. The wireless push-pull microsystem includes four push-pull micropumps, four perfusion solution reservoirs, a soft microfluidic probe (four channels), and a collection of electronics and hardware. The push-pull operation is based on water electrolysis and gas recombination reactions. (B) Frontside view and (C) backside view of the wireless push-pull microsystem. The small size (1 cm by 0.5 cm) and lightweight construction (~0.2 g) enable the applications in small animal models, such as mice. (D) Demonstration of fluid perfusion and membrane-free sampling via a soft microfluidic probe at different time points (t = 0 s, t = 5 s, t = 15 s, t = 35 s, and t = 50 s). Scale bar, 300 µm. Arrow indicates the fluid flow direction. (E) Electrical schematic diagram of the neurochemical sampling, control, and wireless power harvesting. (F) The images of a piece of chicken breast inside a cage with 6 W RF power applied to the transmission antenna. The thermal images show heatmaps before and after 10 min operation. Photo credit: Guangfu Wu, University of Connecticut.
SBS membrane shows a further enhanced membrane recovery efficiency of 97.59 ± 0.85% within 3 min (Fig. 2C, green line). The small variation in the recovery efficiency between the first and second uses demonstrates the reusability and repeatability of the push-pull microsystem (Fig. 2D). The orientation of the device may vary in in vivo studies due to the normal locomotor (e.g., rearing, walking, and exploring) and social (e.g., sniffing and climbing) behaviors of animals. Therefore, we investigated the effect of device orientation on recovery efficiency. The micropumps facing upward on the stage exhibited a slightly higher recovery efficiency than the micropumps facing downward, probably due to a higher recombination efficiency when gas molecules contacted with Pt-coated SBS membrane (upward) than that of Pt-coated Au electrodes (downward) (Figs. 2, E and F).

Programmable fluid perfusion and collection of the push-pull microsystem

We investigated the dynamics of fluid perfusion and sampling under different operating conditions (Fig. 3, A to D). A driving voltage of 3.0 V was applied to the micropumps for 10 s; the droplet size was then recorded for 300 s. For the micropumps with the Au electrodes only, the generated droplet through a microfluidic probe reached a maximum size within ~120 s and maintained its size after another 180 s (Fig. 3A, top), suggesting that negligible fluid was
retracted back by using Au electrodes within 3 min (movie S2). For the micropumps with Pt-coated Au electrodes, Pt-coated flexible SBS membrane, and PtNPs (40 mg/ml) in the electrolyte solution, the generated droplet reached its maximum size and achieved ~100% recovery after another ~35 s (Fig. 3A, bottom). Figure 3B shows the changes of normalized droplet size as a function of time for the push-pull microsystem by using Au electrodes (gold), only Pt-coated Au electrodes (Pt@Au, black), only Pt-coated SBS membrane (Pt@SBS, blue), Pt-coated Au electrodes, PtNPs (40 mg/ml), and Pt-coated SBS membrane (green; PtNPs, Pt@SBS, and Pt@Au). n = 4. The time point when the droplet reached the maximum size was set as 0 s. (C) Normalized droplet size as a function of time under various PtNP concentrations (40, 8, 6, 4, and 2 mg/ml). (D) The dynamics of fluid sampling as a function of PtNP concentrations. (E) Time-dependent normalized droplet size change induced by gas recombination for the first, second, and third uses. (F) Variation of droplet size compared with the first use when using PtNPs (40 mg/ml). Variation (%) here is defined as \((D_n - D_1)/D_1 \times 100\) (n = 2 for the second use; n = 3 for the third use) at each time; \(D_n\) represents the diameter of the droplet. The calculation is based on the data in (E).

Fig. 3. Programmable fluid perfusion and sampling of the push-pull microsystem. (A) Top: The changes of droplet size at \(t = 0, 120,\) and 300 s with Au electrodes only. Bottom: The changes of droplet size at \(t = 0, 15,\) and 50 s with Pt@Au, Pt@SBS, and PtNPs (C = 40 mg/ml). (B) The normalized droplet size as a function of time for the push-pull microsystem by using only Au electrodes (gold), only Pt-coated Au electrodes (Pt@Au, black), only Pt-coated SBS membrane (Pt@SBS, blue), Pt-coated Au electrodes, PtNPs (40 mg/ml), and Pt-coated SBS membrane (green; PtNPs, Pt@SBS, and Pt@Au). n = 4. The time point when the droplet reached the maximum size was set as 0 s. (C) Normalized droplet size as a function of time under various PtNP concentrations (40, 8, 6, 4, and 2 mg/ml). (D) The dynamics of fluid sampling as a function of PtNP concentrations. (E) Time-dependent normalized droplet size change induced by gas recombination for the first, second, and third uses. (F) Variation of droplet size compared with the first use when using PtNPs (40 mg/ml). Variation (%) here is defined as \((D_n - D_1)/D_1 \times 100\) (n = 2 for the second use; n = 3 for the third use) at each time; \(D_n\) represents the diameter of the droplet. The calculation is based on the data in (E).
Electrical and thermal characterization of the wireless push-pull microsystem

We first measured the power consumptions for the operation of red indicator light-emitting diode (LED), µC, and push-pull micropumps, respectively. On the basis of our measurements, the µC consumed a power of ~2.1 mW during the operation (Fig. 4A). The peak power of simultaneously operating red indicator LED (20 Hz frequency and 10% duty cycle) and µC was 2.7 mW as shown in Fig. 4A and fig. S13 (5 Hz and 10% duty cycle). The voltage-power characteristics for push-pull micropumps are summarized in Fig. 4B. A power of 0.6 mW was needed to initiate the operation of the push-pull micropump. Therefore, a total power of 3.3 mW was needed for the robust operation of wireless push-pull microsystems. We then systematically measured the harvested power from the receiving antenna of the push-pull microsystem at nine different locations and three different heights (1, 3, and 5 cm) in a mouse home cage (18 cm by 25 cm) and a behavior box (19.2 cm by 42.1 cm), which were surrounded by an optimized double-loop transmission antenna at heights of 4 and 11 cm (Fig. 4, C to F, and fig. S14). With 10 W RF power applied to the optimized double-loop transmission antenna, the receiving antenna of the push-pull microsystem harvested at least 14.3 mW of power when placed at the center of a behavior box (Fig. 4E), the location with the lowest magnetic field intensity. Harvested power from the receiving antenna of the push-pull device changes with the angle between the transmission loop antenna and the receiving antenna in the device, thereby causing a decrease of harvested power with an increasing angle in some in vivo studies. For example, the normal locomotor (e.g., rearing) and social (e.g., climbing) behaviors may lead to a short period (~1 to 5 s) of angular mismatch. To study whether the receiving antenna can provide sufficient power for the wireless operation of push-pull micropumps, we operated the devices and measured the received power at different angular mismatches (0°, 15°, 30°, 45°, 60°, and 75°) in the mouse home cage (18 cm by 25 cm) and the behavior box (19.2 cm by 42.1 cm).

**Fig. 4.** Electrical characteristics of the wireless push-pull microsystem. (A) Power consumption of a red indicator LED (20 Hz frequency and 10% duty cycle) and a microcontroller in a behavior box with 10 W applied to the transmission antenna. The peak value of power consumption for the simultaneous operations of the red indicator LED and microcontroller was 2.7 mW. The measurement was performed when a 5.6 kilohm resistor was connected in series with the red indicator LED. (B) Voltage-power characteristics of the push-pull micropump. A voltage of 1.95 V (power consumption of 0.6 mW) resulted in the generation of gas bubbles to push the perfusion solution. (C) Photographs of the wireless push-pull microsystems with the simultaneous operation of an indicator LED and a push-pull micropump at different locations with a height of 3 cm and a 75° angular misalignment in a mouse behavior box (19.2 cm by 42.1 cm). The input power of the transmission antenna was 10 W. For the push-pull operation, a fluid labeled with red dye was delivered from the perfusion solution reservoir to the tip end of the microfluidic probe and then retracted back to the perfusion reservoir. (D) Schematic illustration of a mouse behavior box (19.2 cm by 42.1 cm) mounted with a double-loop antenna (4 cm and 11 cm). (E and F) Harvested power of receiving antenna at nine different locations of the behavior box with different angular misalignments (0°, 15°, 30°, 45°, 60°, and 75°) as a function of the height (1, 3, 5 cm). The input power of the transmission antenna was 10 W. The load resistance was 3.2 kilohm. Dotted lines indicate the minimum required power (3.3 mW) to operate a wireless push-pull microsystem. Photo credit: Guangfu Wu, University of Connecticut.
Both the push-pull micropumps and the CMA 7 probe exhibited the capability of sampling \( K^+ \) and DA, while the overall recovery was difficult to be sampled by using the CMA 7 probe (Fig. 5C). The pull microsystem showed significantly improved performance (>80% recovery) for sampling the large molecule, NPY, which is commonly found neurochemicals in the brain, including norepinephrine, serotonin, \( \gamma \)-aminobutyric acid, and uric acid, demonstrating the high selectivity of measuring DA (fig. S25). The wireless push-pull microsystem showed significantly improved performance (>80% recovery) for sampling the large molecule, NPY, which is difficult to be sampled by using the CMA 7 probe (Fig. 5C). The high recovery for the push-pull microsystem is attributed to the high selectivity of measuring DA (fig. S25). The wireless push-pull microsystem was further highlighted by the red indicator LED when a freely moving mouse implanted with the push-pull device was located near the center of a behavior box (19.2 cm by 42.1 cm) with an angular misalignment (fig. S21). For applications in which increasing number of multiple operation channels are needed (e.g., for multisite sampling), and hence, more power is required. One of the possible solutions is programming the microcontroller with certain duty cycles and frequencies so that these channels are operated on different time domain. In that case, at a specific time, only one channel is operating, which could meet this power limitation. In addition, a larger antenna could be designed to accommodate this increasing power requirement. We also studied the thermal characteristics for the operation of the wireless push-pull microsystem. Figure S22 shows minor temperature changes for the operation of push-pull micropumps. The study of thermal imaging suggests no heating effect of the wireless microsystem on the brain tissue for the in vivo study.

**Wireless push-pull operation for in vitro sampling \( K^+ \), DA, and NPY**

To benchmark and evaluate the performance of the wireless push-pull microsystem, we used it to sample three representative neurochemicals (ion: \( K^+ \); small molecule: DA; and large molecule: NPY) in phantom brain tissues (0.6% agarose gel), which were soaked in \( K^+ \), DA, or NPY solution with different concentrations for 24 hours (fig. S23). Potassium is an important electrolyte in the brain and plays a critical role in regulating neural activities such as the maintenance of transmembrane potential and the excitability/spiking of neurons (37). DA is a major monoamine and plays an important role in rewarding signaling, decision-making, motor control, sleeping, and food intake (38–39). Figure 5 demonstrates the capability of the wireless push-pull microsystem to sample neurochemicals, including \( K^+ \), DA, and NPY. Both the push-pull micropumps and the CMA 7 probe exhibited the capability of sampling \( K^+ \) and DA, while the overall recovery achieved by using the wireless push-pull micropump was higher than that by using the CMA 7 probe. Here, the recovery is defined as the concentration of \( K^+ \), DA, or NPY in collected samples, measured by using a commercial potassium meter, a laboratory-customized DA sensor [aptamer-enhanced graphene field-effect transistor (AeG-FET)], and a commercial enzyme-linked immunosorbent assay (ELISA) NPY test kit, over its known concentration in the phantom brain model. This customized DA sensor showed a significantly higher sensor response when the sensor was exposed to DA (20 nM) compared to exposing to three orders of magnitude higher concentration (100 \( \mu \)M) of commonly found neurochemicals in the brain, including norepinephrine, serotonin, \( \gamma \)-aminobutyric acid, and uric acid, demonstrating the high selectivity of measuring DA (fig. S25). The wireless push-pull microsystem showed significantly improved performance (>80% recovery) for sampling the large molecule, NPY, which is difficult to be sampled by using the CMA 7 probe (Fig. 5C). The high recovery for the push-pull microsystem is attributed to the...
membrane-free operation and the direct contact of the perfusion solution with NPY molecules in phantom brain gel, thereby enabling fast diffusion and collection. In contrast, the slow diffusion through the semipermeable membrane of the CMA 7 probe showed a lower recovery. Until now, there has not been a way to sample NPY, an important representative neuropeptide with a large molecular weight, in freely moving animals with a high spatial resolution (cellular sampling area). For this reason, the wireless push-pull microsystem developed here opens new avenues for sampling neurochemicals with larger molecular weights while maintaining high spatial resolution and fast dynamics.

**Device implantation impacts on animal behaviors**

To study the impact of device implantation on the animal behaviors (movement and spatial preferences), we implanted the device into the lateral hypothalamus (LHA; M/L: medial/lateral, 1.00 mm; A/P: anterior/posterior, −1.32 mm; D/V: dorsal/ventral, −4.70 mm). Figure S26 shows the procedures of the device implantation. Note that the soft microfluidic probe was attached onto a needle-shaped polyimide substrate (width, ~350 μm; thickness, 75 μm); such a thickness offers sufficient mechanical stiffness for the device implantation while providing adequate mechanical flexibility to minimize the irritation at the device-tissue interfaces (27). Movie S3 shows a freely moving mouse implanted with our wireless push-pull microsystem.

The open-field experimental paradigm for mice implanted with the device was recorded across multiple time points during pre- and post-implantation (1 day before device implantation; implantation day; 3 and 7 days after device implantation). Representative traces of 1 day before implantation and 3 days after device implantation reveal comparable behavioral patterns (Fig. 6A), suggesting no increased levels of anxiety or impairment in locomotion due to the device implantation. Further analysis shows no significant differences between 1 day before implantation and the implantation day for mice in total traveling distance, velocity, center distance, and center time (Fig. 6, B to F). Successful surgery recovery and unaffected mouse welfare are supported by the steady body weight and body temperature progression of the mice with surgery and the presence of the device (Fig. 6, G and H).

**In vivo NPY sampling in freely moving mice**

One of the most compelling uses of our device is its capability to sample large molecule neuropeptides. As a demonstration example, we choose NPY because it plays an important role in many physiological processes, including food intake, circadian rhythm, anxiety, and stress response (40, 41). However, the conventional microdialysis method is limited in its ability to collect this neuropeptide due to its large molecular weight (36 amino acids, 4 kDa) and ultralow concentration (16, 42), and thus, NPY will serve as a useful test case to represent the possibility of collecting additional similar-length peptides.

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**Fig. 6. Implantation impact on animal behaviors.**

(A) Open-field experiment with overlay traces of motion for preimplanted (1 day before implantation) and postimplanted (after 3 days) mice. (B) Total traveling distance. (C) Velocity. (D) Immobile time. (E) Center distance. (F) Time spent in the center of the arena for mice in an open-field assay at four different stages: 1 day before implantation, implantation day, postimplantation (after 3 days), and postimplantation (after 7 days). n = 5; *P < 0.05, one-way ANOVA with Tukey’s multiple comparisons test. ns indicates that the difference of the means is not significant at the 0.05 level. (G) Body weight of the mice as a function of time before and after implantation. (H) Variation of body temperature of the mice before and after implantation.
neuropeptides across the brain. NPY is distributed in different brain regions, including the LHA and the nucleus accumbens (NAc) (43). NPY receptors have been identified in perifornical hypocretin cells in the LHA, and direct administration of NPY agonists into the LHA increases Fos-like immunoreactivity in these hypothalamic neurons (44). NPY receptors are also highly concentrated in the NAc, which is an important region for regulating feeding behaviors (45). Therefore, in vivo NPY sampling upon pharmacological stimulation was performed in the LHA and the NAc of freely moving mice (Fig. 7). In this study, to facilitate the pharmacological stimulation and membrane-free neurochemical sampling in a single device platform in in vivo studies, the developed wireless, push-pull microsystem was integrated with miniaturized electrochemical micropumps (for neuropharmacology) reported in our previous study (28). More specifically, two miniaturized electrochemical micropumps were used to deliver drugs to the specific brain region of interest through a soft microfluidic probe (two channels) for neuropharmacology, while two push-pull micropumps were used to perfuse and sample the brain’s extracellular fluid of interest upon pharmacological stimulation through the same soft microfluidic probe (the other two channels) (fig. S27A). To sample neurochemicals, the interdigitated gold electrodes of two micropumps were coated with Pt layers via electrochemical plating (fig. S27B). The corresponding micropump chambers were used to house PtNP-dispersed electrolytes; the top of the micropump’s chamber was sealed with a Pt-coated flexible SBS membrane (fig. S27C).

Bicuculline, a competitive γ-aminobutyric acid type A (GABA_A) receptor antagonist, was infused into the LHA and the NAc to stimulate neurons in these regions through the removal of GABA_A receptor-mediated inhibition (46, 47) in awake and free-moving mice (wild-type C57BL/6J mice). The first push-pull micropump was used to push artificial cerebrospinal fluid (aCSF) into the LHA or the NAc for 1 min, and then the perfused fluid was gradually retracted back to the reservoir through the integrated microfluidic probe for 1 min (movie S4). Next, an electrochemical micropump was activated for 1 min to deliver bicuculline (15 mM, 0.5 μl) through the integrated microfluidic probe into the LHA or the NAc (movie S5). Three minutes later, another push-pull micropump was activated to deliver the perfusion solution (aCSF) into the LHA or the NAc for 1 min. The perfused fluid was then gradually retracted back to the reservoir through the microfluidic probe for 1 min (movie S6). In this study, we used a red LED, controlled by the μC, to indicate the operation status of our wireless device. More specifically, in our in vivo studies, blinking once per second of the red LED suggests the operation of push-pull micropump 1 for baseline NPY sampling (movie S4); blinking twice per second means the operation of an electrochemical micropump for bicuculline delivery (movie S5); blinking three times per second indicates the operation of push-pull micropump 2 for NPY sampling evoked by bicuculline delivery (movie S6). These sampled fluids were temporarily stored in the collection reservoirs of the wireless push-pull microsystem until a study session was finished. Note that the collection reservoirs do not need to be detached during the whole animal experiment as the device reported here offers multiple push-pull micropumps and the corresponding collection reservoirs. After finishing the animal experiment, the sampled fluids were quickly transferred from the collection reservoirs to polymerase

![Fig. 7. In vivo NPY sampling with pharmacological stimulation in freely moving mice.](image-url)
change area of 100 to 4 mm), while our wireless push-pull microsystem has an exchange membrane area of 0.44 mm outer diameter) for sampling large molecules, such as neuropeptides with high recovery and cellular-scale spatial resolution. This study reports a wireless, battery-free microsystem that enables push-pull sampling as currently available tools for direct in vivo sampling of neuropeptides with cellular spatial resolution are quite limited. Genetically encoded fluorescent sensors are receiving much attention, but at this point, most applications are for small molecule detection (48). Therefore, there are no reliable tools for validating the in vivo sampling of NPY. Overall, these in vivo data demonstrated that the wireless operation of our device can sample and detect a large molecule, specifically NPY, upon pharmacological stimulation in freely moving mice.

**DISCUSSION**

This study reports a wireless, battery-free microsystem that enables the push-pull operation for membrane-free sampling of neurochemicals with high recovery and cellular-scale spatial resolution (10 μm × 10 μm). One of the main advantages of this wireless microsystem is its ability to sample large molecules, such as neuropeptides, in freely moving animals. When compared with the commercial microdialysis probe (AtmosLM microdialysis probe; 1000 kDa cutoff, 0.44 mm outer diameter) for sampling large molecules, it offers much better spatial resolution. More specifically, the cylindrical AtmosLM microdialysis probe has an exchange membrane area of 1,381,600 to 5,526,400 μm² (outer diameter of 440 μm, length of 1 to 4 mm), while our wireless push-pull microsystem has an exchange area of 100 μm², which is at least 13,816-fold smaller than the AtmosLM probe.

Another key feature of our developed neural probe is its wireless operation, which allows for neurochemical sampling while offering complete freedom of physical or inertial motion constraints for mice. The wireless operation bypasses the limitations that conventional wires and tubing connected to external hardware have on the natural behavior of animals, which offers tremendous opportunities to link neurochemical release with animal behaviors. Furthermore, the thin geometry (~150 μm) and soft mechanics of the microfluidic probe make the push-pull microsystem adapt to brain micromotions resulting from movement, respiration, and blood flow. It can also minimize brain tissue damage and irritation at the biotic/abiotic interface due to mechanical interaction between the hardware and the surrounding soft moving tissues (28). Last, unlike the existing push-pull perfusion probes that use two capillaries (one push capillary is used to deliver the perfusion solution to the targeted brain tissue, and the perfused solution is then pulled back through pull capillary) (19, 20, 49, 50), in our wireless push-pull microsystems, a small fluid droplet is pushed to the external milieu to directly contact with the brain tissue and then recovered by reversing the flow direction in the same channel. Reducing the number of capillaries/channels used in the neural probe not only reduces its overall size and the resulting area of the lesioned brain tissue during the device implantation but also offers better control of fluid in the external milieu.

While a recent study developed a platform for microinvasive membrane-free biochemical sampling of brain interstitial fluid (23), our push-pull microsystem is distinct from the microinvasive platform in several ways: (i) The microinvasive platform is based on heating-induced contraction of shape memory alloy nitinol to drive the flow of perfusion solution, which requires a high temperature (70°C) and associated bulky battery to operate the device. The high temperature could cause the degradation of neuropeptides. In contrast, our membrane-free push-pull microsystem is based on the water electrolysis reaction and platinum-assisted gas recombination reaction to drive the operation of the push-pull micropumps (power consumption, ~0.6 mW; negligible temperature change). (ii) The large device size (>75 mm by 70 mm in size) and bulky construction (>255 g) of the microinvasive platform limit its application in small animals such as mice. In contrast, our membrane-free push-pull microsystem has a size of 1 cm by 0.5 cm and a weight of ~0.2 g, which are much smaller and lighter than those of the microinvasive platform. (iii) The rigid materials of microinvasive platform (borosilicate glass, with a modulus of several GPa) may induce prolonged brain tissue damage and inflammation due to mechanical mismatch between the implant and brain tissue. In addition, its large probe size (80 μm outer diameter, 50 μm inner diameter) limits the high spatial mapping of neurochemicals. In contrast, the soft mechanics (~3 MPa) of our push-pull microsystem could adapt to brain micromotion and minimize brain tissue damage and mechanical irritation at the biotic/abiotic interface. The small size (cross-section: 10 μm by 10 μm) of microfluidic channels also makes it possible to sample large molecule neurochemicals with cellular-scale spatial resolution. The membrane-free push-pull neurochemical sampling system is also distinct from our recent work in wireless optofluidic device (28), in which we focused on pharmacological and optogenetic neuromodulation.

The developed wireless push-pull microsystem opens the door for neuroscientists to understand where, when, and how the release of large-molecular weight neurochemical (neuropeptide/protein) modulates diverse outputs of the brain. For example, previous studies have demonstrated that the activation of LHA GABAergic neurons generates reinforcement and place preference behavior, likely through neuropeptide-mediated modulation of ventral tegmental area (VTA) DA neurons (51–54). However, LHA GABAergic neurons are highly heterogeneous (55), and little is known about the exact role of neuropeptide released by these neurons and their role in determining DA neuron activity during reinforcement and place preference behavior. The implementation of this wireless push-pull microsystem in mice to sample neuropeptides in vivo could further our understanding of the biology of LHA-VTA projections in this diverse population of neurons.

Compared with the continuous sampling of current microdialysis system, one limitation of this wireless push-pull microsystem is the lack of capability for time-sequential fluid sampling; it only allows capturing the neurochemical release before and after a behavior session and/or neuromodulation. For current microdialysis and push-pull sampling probes, the temporal resolution of continuous
sampling is limited by the Taylor dispersion in which the collected neurochemicals diffuse/spread in a liquid flowing tube. Recent studies couple microdialysis or push-pull sampling probes with droplet microfluidics to solve this sample dispersion issue (19, 20, 56). Time-subsequentially collecting samples into microfluidic reservoirs, where each fraction is harvested and stored into an independent reservoir immediately after sampling, can prevent the Taylor dispersion, making it possible to study time-dependent variation in neurochemical release. Therefore, a natural extension of this technology is the integration of one such time-sequential fluid sampling microfluidic device to enable continuous neurochemical sampling with subminute temporal resolution. Other areas for future development includes (i) optimizing the electronics design to involve additional supercapacitors (57) or multiple transmission antenna designs (34, 58) into the device platform and (ii) investigating various surface coatings and microfluidic designs to prevent the microfluidic channel clogging and scar encapsulation, thereby enabling long-term operations during animal’s lifetime (>6 months).

Overall, our work demonstrates a wireless, battery-free microsystem that includes four push-pull micropumps coupled to a soft microfluidic probe for membrane-free neurochemical sampling. This new device enables in vivo sampling of neuropeptides and other polypeptide neurochemicals in awake, freely moving animals. The developed wireless push-pull microsystem will facilitate the studies of the molecular mechanisms underlying many neurological and psychological disorders.

MATERIALS AND METHODS

Fabrication of power harvesting and control electronics

The fabrication of wireless power harvesting and control electronics is based on our previously reported method (28). Briefly, a flexible sheet of copper clad polyimide film (Cu/polyimide/Cu, 18/75/18 μm) was used as the substrate. An ultraviolet laser cutting machine (ProtoLaser U4, LPKF, Germany) was used to fabricate the RF coil, the leads and contact pads for electronic components, holes (diameter, 50 μm), and interdigitated electrodes for push-pull microfluidic probe. The interdigitated electrodes were then coated with gold via electroplating (thickness, 200 nm), and the holes were electroplated with copper to connect the top and bottom layers. Subsequently, electronic components, including capacitors (GRM0335C1H750GA01D, Murata Electronics; 02016D225MAT2A, AVX Corporation), rectifiers (SMS7621-040LF, Skyworks Solutions Inc.), red indicator LED (APG0603SURC-7T, Kingbright Company LLC), resistors, and a microcontroller (ATTINY84A-MUR, Microchip Technology), were attached to the device using a low-temperature solder (Indium Corporation). The possible delamination of the electronic components during device implantation and in vivo studies was prevented by applying an epoxy layer (thickness, ~500 μm). The electronics including the coil antenna and electronic components were encapsulated with a conformal bilayer of parylene C (Specialty Coating Systems) and polydimethylsiloxane (PDMS; dip coating) to prevent the biofluid penetration in vivo studies.

Fabrication of Pt-coated gold interdigitated electrodes

A Pt layer was electrochemically plated on the top of the gold (Au)–interdigitated electrodes to facilitate the recombination of the generated hydrogen and oxygen gases. The Pt-electroplating plating solution was obtained by dissolving the precursor- H₂PtCl₆ (0.1 g) (Sigma-Aldrich, St. Louis, MO, USA) in deionized (DI) water (37.8 ml) and magnetically stirred the solution for 24 hours at room temperature. Following this step, 2.2 ml of H₂SO₄ (Sigma-Aldrich, St. Louis, MO, USA) was added to the above solution to readjust the pH value (pH ~1.5). Before electrochemical plating, the gold interdigitated electrodes were washed with isopropanol and DI water to remove possible contaminants. A two-electrode setup was used to carry out the electrochemical plating under ambient conditions in which the gold interdigitated electrodes were used as the cathode and a Pt coil as the anode. The distance between the electrodes was 1 cm, and the electrical current for the electrochemical plating was fixed at 0.005 A for 1 min by using a DC power supply (2231A-30-3 Triple Channel DC Power Supply, Keithley Instruments, OH, USA). Last, the devices were washed with DI water three times and blow-dried with N₂ gas.

Preparation of Pt-coated flexible membrane

SBS (Sigma-Aldrich, MO, USA) was used to prepare the flexible membrane. SBS powder was first dissolved in toluene, yielding an SBS solution (4 g/40 ml). A silicon wafer was pretreated with mold release spray (Ease Release 200, Mann Release Technologies Inc., PA, USA) for 5 min, and then it was drop-cast with the SBS solution followed by annealing at 60°C for ~3 hours and then at 95°C overnight. Last, a thin layer of Pt (5 nm) was deposited onto the surface of SBS film via magnetic sputtering (Quorum Q150T Thin-Film Coater, Quorum Technologies Ltd., East Sussex, UK).

Synthesis of Nafion-modified Pt nanoparticles

One milliliter of Nafion dispersion (D2020 alcohol–based 1000 EW at 20% weight, Ion Power Inc., New Castle, DE, USA) was added to 39 ml of DI water. The solution was then stirred for 30 min and mixed with 1.5 ml of H₂PtCl₆ solution (20 mM), followed by another 10 min of stirring. Last, 50 ml of ethanol was added and the pH value of the solution was adjusted to 8.5 with NaOH. The mixture was refluxed at 84°C for 30 min, resulting in Nafion-modified Pt nanoparticles. After cooling down to room temperature, the resulting mixture was centrifuged (Jouan BR4i Centrifuge, Thermo Scientific, MA, USA) at a rate of 8000 rpm for 30 min. The resulted sediment was collected and washed with ethanol, annealed in an oven at 50°C overnight, and redispersed in 50 mM NaOH solution for use. The concentration of PtNPs in the NaOH solution was 2, 4, 6, 8, or 40 mg/ml.

Fabrication of soft microfluidic probe

The fabrication of soft microfluidic channels was based on our previously reported method (28). Briefly, the soft microfluidic probe consists of a defined microchannel layer and a capping layer, which are bonded by corona treatment. The microchannel layer was formed by casting and curing a PDMS layer (elastomer/curing agent ratio, 10:1; Sylgard 184, Dow Corning) (~80 μm) between a patterned silicon wafer and a supporting glass slide. Microfluidic channel patterns on the silicon wafer were created by a standard photolithography approach, followed by deep reactive ion etching (DRIE) (STS Pegasus ICP-DRIE; SPTS Technologies, Newport, UK). Poly(methyl methacrylate) (PMMA) A2 solution (2 g PMMA dissolved in 98 g anisole) was spin-coated at a rate of 3000 rpm for 1 min onto the patterned silicon wafer surface and cured at 185°C for 5 min resulting in an antiadhesion layer. The capping layer (thickness, ~70 μm) was prepared by spin-casting PDMS (elastomer/curing...
agent ratio, 10:1; Sylgard 184, Dow Corning) on a PMMA-coated glass slide followed by baking at 70°C for 45 to 60 min. The total thickness of the channel layer and the capping layer is ~150 μm.

Assembly of the device
The micropump chamber and perfusion solution reservoir were fabricated by milling a block of cyclic olefin polymer (thickness, 1 mm). The device assembly started with the bonding of micropump chamber with the interdigitated electrodes using a commercially available sealant (3M marine adhesive sealant fast cure 5200). The flexible SBS membrane was covered on the top of the micropump chamber with a pressure-sensitive adhesive (Adhesives Research Inc., EL-8932EE). The perfusion solution reservoir was then bonded on the top of the flexible membrane with the same sealant. The inlets of the soft microchannel layer were aligned and attached on the top surface of the perfusion solution reservoir using the same pressure-sensitive adhesive. Last, a pressure-sensitive adhesive was applied on the top of microchannel layer to finish the device assembly.

In vitro neurochemical sampling
The devices deploying PtNPs dispersed NaOH solution (40 mg/ml), Pt-coated Au electrodes, and Pt-coated flexible SBS membrane were used to investigate in vitro fluid collection capabilities. The microreservoirs were filled with DI water as perfusion solution. The probe of wireless push-pull microsystem was implanted into the phantom brain gels containing KCl, DA, or NPY solution. The phantom brain gels were soaked in KCl, DA, or NPY solution for 24 hours. The power was supplied via micropumps, the indicator LED illuminated a red light, and a microdroplet was generated at the end of the probe. The time for push and pull operation of the push-pull microsystem in all experiments is 10 and 35 s, respectively.

The liquid in the micoreservoir was collected with a microsyringe for further analysis by using commercial potassium meter (Horiba Potassium LAQUAtwin K-11 Pocket Tester), laboratory-customized DA sensor (AeG-FET), and a commercial ELISA NPY test kit (catalog no. EZRMNPy-27K, MilliporeSigma).

DA concentration measurement with AeG-FET
The fabrication of G-FET was based on standard photolithography protocol. Briefly, source and drain electrodes were formed by magnetic sputtering of chromium and gold on a polyimide substrate (~76 μm thick). Chemical vapor deposition–grown graphene on Cu foil was transferred, patterned, and assembled with the fabricated source and drain electrodes. The G-FET device was encapsulated with a thin layer of SU-8 (~0.8 μm thick). The surface of graphene transistor was functionalized with a DA aptamer (5′/pyrene/CGA CGC CAG TTT GAA GGT TCG TTC GCA GGT GTG GAG TGA CGT CG-3′). The AeG-FET was exposed to DA concentration at the physiologically relevant range of 1 nM to 10 μM to establish the relationship between electrical current and DA concentration. The concentration of sampled DA solution with push-pull microsystem or CMA 7 dialysis probe was calculated by using the above calibration curve.

Surgery
Wild-type C57BL/6J mice at an age of 4 to 6 or 8 to 10 weeks old were used in this study. These mice accessed food pellets and water ad libitum. The procedure for device implantation is similar to that for implanting the conventional cannula or the optical fiber. For surgery, mice were anesthetized by isoflurane (1.6 to 2%, with 100% O2 at 500 cc/min) using vaporizer. The mice were then shaved on top of the head, followed by mounting the head of the mouse onto the stereotaxic apparatus, and the head was adjusted to horizontal level. Mouse body temperature was supplemented with a Thermostar heating pad. To implant device into the mouse brain, holes (2.0 mm in diameter) were drilled on the skull following the coordinates: M/L, 1.00 mm; A/P: −1.32 mm for the LHA; M/L, 1.00 mm; A/P, 1.04 mm for the NAc using a high-speed drill. The dura was then carefully removed with fine forceps. The push-pull microsystem was mounted on a stereotaxic arm and positioned. The device probe was inserted into coordinates for LHA (M/L, 1.00 mm; A/P, −1.32 mm; D/V, −4.70 mm) and NAc (M/L, 1.00 mm; A/P, 1.04 mm; D/V, −3.81 mm). The main body of the device adhered to the skull with tissue glue.

Behavior study
Open field was used to test the motor function and normal exploratory locomotion as previously described (60). Briefly, mice were given at least 30 min to habituate the open-field room. The mice were then put in the center of the open-field apparatus consisting of a clear, open Plexiglas box (46.6 cm by 38.5 cm by 25.6 cm). The movement of mouse over a 10 min period was monitored by an overhead camera controlled by ANYmaze software (Stoelting, IL). Total distance, velocity, total immobile time, and center time were quantified by ANYmaze software. The sensitivity for the detection of immobile time was set as 65% of the body area. The animal needed to maintain immobile for at least 5 s before it was considered as immobile by the software. To measure body temperature, the mice were restrained by hand and placed on a cage lid. Then, mouse tail was lifted, and a rectal temperature probe covered with Vaseline was gently inserted into the mouse to measure the body temperature.

In vivo NPY sampling in awake and free-moving mice
Wireless push-pull microsystems were implanted into the LHA or NAc of wild-type (C57BL/6J) mice. A few hours after the implantation, we loaded two perfusion fluid reservoirs with aCSF (0.5 μl in each reservoir) and one drug reservoir with bicuculline (0.5 μl; 15 mM), respectively. Upon the activation of first push-pull microsystem wirelessly through the graphical user interface, the perfusion solution of aCSF was delivered into the LHA or NAc, and the basal interstitial fluid was sampled (0.5 μl). Next, we delivered the bicuculline (15 mM) through the drug reservoir and associated microfluidic channel to evoke the release of NPY in LHA or NAc. Three minutes later, another push-pull microsystem was activated to sample the release of NPY (0.5 μl), evoked by bicuculline stimulation, into the corresponding reservoir. After the completion of in vivo experiments, the sampled fluids were collected in PCR tubes, while the mice were anesthetized. DPP-IV inhibitor was immediately added to the tubes and quickly stored in 4°C refrigerators for external analysis immediately. The NPY concentration in the sampled solution was measured by using an ELISA kit. All procedures were approved by the Animal Care and Use Committee of the University of Missouri-Columbia and conformed to U.S. National Institutes of Health guidelines.

NPY concentration determination with ELISA
A rat/mouse NPY ELISA kit (catalog no. EZRMNPy-27K, MilliporeSigma) including a 96-well plate assay was used for nonradioactive quantification
of NPY, following the standard 17-step NPY ELISA procedure (fig. S31). In general, the well plate was divided into multiple sections, including blank wells, standard sample wells, reconstituted standard wells, quality control 1 and 2 wells, and collected sample wells, in which standard sample wells, reconstituted standard wells, and quality control wells were used to validate the ELISA kit and calibrate the relationship between absorbance and concentration of standard samples. The 96-well plate was read with a plate reader at 450 nm.

**Statistics**

The software used for statistical analysis is OriginLab Pro 2019b and GraphPad Prism 8.4.1. For n sizes, the number of animals is provided. Data from failed devices were excluded from the analysis. All experimental data are expressed as the means ± SEM. For two-group comparisons, statistical significance was determined by paired t tests. For multiple-group comparisons, one-way ANOVA with Tukey’s multiple comparisons test was used. P < 0.05 was considered statistically significant.

**SUPPLEMENTARY MATERIALS**

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