(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
02 August 2024 (02.08.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/158914 A1

(51) International Patent Classification:
G01N 33/50 (2006.01) *G01N 33/543* (2006.01)
G01N 33/74 (2006.01) *G01N 21/64* (2006.01)

(21) International Application Number:
PCT/US2024/012787

(22) International Filing Date:
24 January 2024 (24.01.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/481,367 24 January 2023 (24.01.2023) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:
— *with international search report (Art. 21(3))*

WO 2024/158914 A1

(54) Title: OPTICAL SENSOR PROBES

(57) Abstract: The present disclosure features a probe that includes an optical sensor. The optical sensor includes a plurality of particles (e.g., cells) that include a ligand receptor coupled to an optical label (e.g., a molecular sensor, e.g., a GPCR fused to a fluorescent protein). The plurality of particles is immobilized on the probe, e.g., on a lens or optical fiber attached to or incorporated within the probe, e.g., at or near a distal end of the probe.

OPTICAL SENSOR PROBES

STATEMENT AS TO FEDERALLY FUNDED RESEARCH

This invention was made with government support under Grant Nos. AT010971, DK131642, GM007753, GM144273, HL007901, and DA048790 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

Traditional methods of sampling and analyzing biological fluids, such as microdialysis, mass spectrometry, and voltammetry, suffer from limitations including low temporal resolution, excessive perturbation of the system being studied, and limited range of accessible analytes, respectively. New devices and methods for improved multiplexed molecular tracking to overcome these challenges are needed.

SUMMARY OF THE DISCLOSURE

In one aspect, featured is a probe that includes an optical sensor. The optical sensor includes a plurality of particles that include a ligand receptor coupled to an optical label. The plurality of particles is immobilized on the probe, e.g., on a lens or optical fiber attached to or incorporated within the probe, e.g., at or near a distal end of the probe.

In some embodiments, the particles are microbeads, liposomes, lipid nanoparticles, or micelles.

In some embodiments, the ligand receptor is encapsulated within the microbeads, liposomes, lipid nanoparticles, or micelles.

In some embodiments, the ligand receptor is coated on the microbeads, liposomes, lipid nanoparticles, or micelles.

In some embodiments, the particles are cells expressing the ligand receptor.

In some embodiments, the cells are human or non-human cells, such as, e.g., Chinese hamster ovary cells, BALB/c mouse myeloma cells, human retinoblasts, monkey kidney cells, human embryonic kidney cells, baby hamster kidney cells, mouse Sertoli cells, human cervical carcinoma cells, canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor cells, or human hepatoma cells. In some embodiments, the human embryonic kidney cells are HEK293T cells.

In some embodiments, the cells are treated with colchicine, e.g., to stabilize the microtubules and prevent degradation or destabilization of the cells.

In some embodiments, the lens is a gradient refractive index lens.

In some embodiments, the lens has a diameter of from 100 μm to 1 mm (e.g., 100 μm, 200 μm, 300 μm, 400 μm, 500 μm, 600 μm, 700 μm, 800 μm, 900 μm, or 1 mm).

In some embodiments, the plurality of particles is encapsulated matrix, such as a hydrogel matrix.

In some embodiments, the hydrogel matrix includes collagen.

In some embodiments, the ligand receptor is a G-protein coupled receptor (GPCR).

In some embodiments, the optical label is a fluorescent label.

In some embodiments, the fluorescent label is a fluorescent protein.

In some embodiments, the fluorescent protein is a green fluorescent protein (GFP), a red fluorescent protein (RFP), or a yellow fluorescent protein (YFP).

In some embodiments, the GPCR is fused to the fluorescent protein., e.g., a GFP or RFP. In some embodiments, the GFP is a circular permuted GFP.

In some embodiments, the probe includes a plastic tubing disposed around the distal end of the probe.

In some embodiments, the plastic tubing includes polyimide.

In some embodiments, the probe includes an array of optical sensors disposed on the lens. For example, the probe may include an array that includes a first optical sensor to detect a first ligand and a second optical sensor to detect a second ligand. The probe may include a plurality of different optical sensors (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more different optical sensors), where each optical sensor is configured to detect a distinct ligand.

In some embodiments, the probe further includes a sensor for tracking physiological indicia. For example, the probe may further include a blood flow sensor, a temperature sensor, a heart rate sensor, a blood pressure sensor, or an oxygen saturation sensor.

A second aspect features a method for detecting a ligand (or a plurality of ligands, such as, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more different ligands) in a biological sample. The method includes contacting the biological sample containing the ligand with the optical sensor of a probe as described herein, e.g., of any of the above embodiments. The method further includes allowing the ligand to bind to the ligand receptor of the optical sensor, in which the optical label emits an optical signal upon binding of the ligand to the ligand receptor. The method further includes detecting the optical signal.

In some embodiments, the detecting step includes imaging the plurality of cells, e.g., with two-photon imaging.

In some embodiments, a microscope is employed to image the cells. The microscope may include, for example, a tunable lens (e.g., an electrically tunable lens).

In some embodiments, the optical label includes a fluorescent protein that emits fluorescence.

In some embodiments, the biological sample includes a cerebrospinal fluid (CSF), blood, saliva, lymph, interstitial fluid, bone marrow, or urine.

In some embodiments, the ligand is a peptide (e.g., a hormone, a cytokine, a chemokine, a regulatory protein, or a neuropeptide). In some embodiments, the peptide is arginine vasopressin (AVP), oxytocin (OXT), somatostatin (SST), corticotropin-releasing factor (CRF), vasoactive intestinal peptide (VIP), cholecystokinin (CCK), corticotropin-releasing factor (CRF), neuropeptide Y (NPY), parathyroid hormone (PTH), urocortin (Uro), neurotensin (NTS), sphingosine-1 phosphate (S1P), or insulin.

In some embodiments, the ligand is a small molecule (e.g., a neurotransmitter or a metabolite). In some embodiments, the small molecule is serotonin (5-HT), norepinephrine (NE), dopamine (DA), acetylcholine (ACh), histamine (HA), melatonin, adenosine (Ado), adenosine triphosphate (ATP), melatonin, or glucose.

In some embodiments, the imaging is performed in vivo. In some embodiments, the optical sensor of the probe is disposed in vivo through a cannula (e.g., a stainless-steel cannula).

In some embodiments, the optical sensor is disposed into a lateral ventricle, blood vessel, kidney, intraperitoneal region, spine, bone marrow, or bladder of a subject.

In some embodiments, the method detects a concentration of the ligand(s) in the biological sample.

In some embodiments, the method is used to detect a disease or disorder or to monitor a treatment regimen in a subject having a disease or disorder.

In some embodiments, the disease or disorder is autism spectrum disorder, Prader-Willi syndrome, Schaaf-Yang Syndrome, Fragile X syndrome, diabetes, obesity, atherosclerosis, high blood pressure, or high cholesterol.

In some embodiments, the method of detection is used in subject that has previously received intranasal administration of AVP.

A third aspect features a method of detecting a concentration of a ligand in a biological sample. The method includes contacting the biological sample containing the ligand with the optical sensor of a probe as described herein, e.g., of any of the above embodiments, and allowing the ligand to bind to the ligand receptor. The optical label emits an optical signal upon binding of the ligand to the ligand receptor. The method further includes detecting the optical signal; and correlating the optical signal with a reference signal of a control biological sample to determine the concentration.

In some embodiments, the control sample corresponds to a biological sample from a healthy subject or a subject having a disease or disorder (e.g., autism spectrum disorder, Prader-Willi syndrome, Schaaf-Yang Syndrome, Fragile X syndrome, diabetes, obesity, atherosclerosis, high blood pressure, or high cholesterol). In some embodiments, the probe includes a plurality of different optical sensors (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more optical sensors).

A fourth aspect features a kit that includes a probe as described herein, e.g., a probe as defined in any of the above embodiments. The kit may further include a receptacle for a biological sample. The kit may further include instructions for use of the probe. In some embodiments, the kit further includes one or more of a lens, a plasmid, particles (e.g., liposomes, lipid nanoparticles, micelles, plastic (e.g., polystyrene) particles, or cells), a matrix material (e.g., a hydrogel matrix and/or components thereof), and a cannula.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

The accompanying drawings are included to illustrate embodiments of the disclosure and further an understanding of its implementations.

FIG. 1 is a schematic drawing showing an example molecular sensor (here, comprising a ligand receptor for dopamine, DA) composed of the D2 dopamine receptor (transmembrane protein)) fused to green fluorescent protein (shown as two discs). When the ligand binds its endogenous receptor, this causes a conformational change in green fluorescent protein (GFP)that increases its fluorescence. Fluorescence intensity scales with ligand concentration (hexagons) and can be calibrated against

standards to obtain a mapping of fluorescence to ligand concentration. Subsequently, dynamic measurements can be made many times each second, and read out in real time.

FIGS. 2A-2E show an outline of an experimental setup. F**IG. 2A** is a diagram outlining the generation of an optical sensor of a probe. HEK293T cells are transfected with a different fluorescent sensor in each well, then mixed together and fixed to the end of a gradient refractive index (GRIN) lens (500 µm diameter) with hydrogel. **FIG. 2B** is a diagram showing two-photon imaging of HEK cells in microplate wells with varying concentrations of each ligand. **FIG. 2C** is an exemplary field of view from a 5-plex sensor. **FIG. 2D** is a diagram showing fluorescence responses over time as the lens is moved across all wells. **FIG. 2E** is an image of the microscope objective that dips sequentially in each well for 3D, sub-micron imaging.

FIG. 3 is a set of images showing results of dipping the probe containing the optical sensor into different wells containing sub-micromolar to nanomolar concentrations of different signals. Center image shows baseline fluorescence of a mixed set of six different molecular cell sensors. Left and right panels show fractional change in fluorescence in response to dipping the optical sensor of the probe sequentially into wells each containing one ligand at saturating concentrations. Note the different patterns of molecular sensor cells responding to each ligand.

FIGS. 4A and **4B** are fluorescence images and graphs showing sensor expression and ligand sensing. **FIG. 4A** shows results from a serotonin sensor cell. Left: Molecular sensor cells (HEK-293T cells) (e.g., circled cell) expressing a fluorescent serotonin molecular sensor (e.g., comprising a ligand receptor that binds to serotonin). Right: After dipping the sensor array into various known concentrations of serotonin (5-HT), the sigmoid sensitivity curve was determined and an EC_{50} concentration of 35.7 nM was estimated – in the range of levels of serotonin in mouse CSF. **FIG. 4B** shows results from a vasopressin molecular sensor cell (e.g., red circled cell). The vasopressin sensor was sensitive, with many-fold change in fluorescence in arginine vasopressin (AVP) bound vs. unbound state, and high selectivity to AVP.

FIG. 5 is a schematic drawing showing a diagram of in vivo experimental design. Two stainless steel guide cannulas (ID 0.62 mm) are implanted in adult mice (P56-70; one vertical cannula in the anterior portion of left lateral ventricle, another in the right lateral ventricle, occipital angle). After two weeks of recovery, mice are head-fixed for two-photon imaging and a 0.5 mm diameter gradient refractive index (GRIN) lens decorated with fluorescent sensor expressing HEK293T cells is acutely introduced into the anterior guide cannula such that the optical sensor contacts the cerebrospinal fluid (CSF). Optionally, a catheter is introduced into the contralateral cannula for intracerebroventricularly (ICV) infusions. Two-photon imaging of the HEK293T cells is then performed through the GRIN lens while the mouse runs on a running wheel and is subjected to sensory or pharmacological stimuli.

FIG. 6A is a fluorescence image showing the time course (right panel) of fluorescence from a single serotonin molecular sensor cell (e.g., expressing a molecular sensor comprising a ligand receptor that binds serotonin) (red circle) expressing GRAB-5HT2h in an awake, head-fixed mouse receiving a tail pinch at minute 45 and contralateral intracerebroventricularly (ICV) infusion of a saturating dose of 5 mM serotonin in cerebrospinal fluid (CSF) (30 µL at 5 µL/min) at minute 95.

FIG. 6B is a set of images showing matching sensor identity across *in vivo* and *in vitro* characterization. Left: baseline two-photon image of mixture of molecular sensor cells *in vivo*. Middle,

right: Fractional change in fluorescence (dF/F) images of a molecular sensor array response to a saturating dose of norepinephrine (5 mM, 30 µL at 5 µL/min) administered ICV to a mouse in vivo (middle) and by dipping the same lens into 1 µM norepinephrine (NE) in PBS in vitro following removal from the mouse (right). Yellow arrows indicate NE-responsive cells.

FIG. 6C is a fluorescence image and graph showing a timecourse (right panel) of fluorescence of an arginine vasopressin (AVP) molecular sensor cell (circle) within a six-sensor array (left), in response to 1 mg/kg intraperitoneal (IP) delivery of AVP (right) in an awake mouse.

FIG. 7A is a fluorescent image showing identified serotonin (5HT) (red circle), arginine vasopressin (AVP) (green circle), and oxytocin (OXT) (blue circle) molecular sensor cells identified within a single field of view in an awake mouse.

FIG. 7B is a graph showing a timecourse of the fluorescence (ΔF (scaled)) of the 5HT (red lines), AVP (green lines), and OXT (blue lines) molecular sensor cells of **FIG. 7A**. A 15 second tail shock (0.4 mA, 5 ms pulse, 50 Hz) was administered to the awake mouse at 60 minutes; a 30 second tail shock was administered at 90 minutes; AVP was administered intraperitoneally at 120 minutes; OXT was administered intraperitoneally (IP, 200 µL/sec) at 165 minutes; and norepinephrine was administered intracerebroventricularly (ICV, 5 µL/min) at 210 minutes.

FIGS. 8A-8D are schematic drawings showing tracking of arginine vasopressin (AVP) levels in a lateral septum. AVP levels are tracked in lateral septum using fiber photometry recordings (**FIGS. 8B** and **8C**) following lateral septum viral expression of a novel AVP sensor. At the same time, increasing levels of AVP are delivered to the lateral ventricle (**FIGS. 8A** and **8C**), inversely proportional to the current AVP levels, which may be estimated using real-time feedback from the online photometry recordings (**FIGS. 8B** and **8C**).

FIG. 9A is a schematic drawing showing a hydrogel-based design using an optical fiber instead of a gradient refractive index (GRIN) lens for real-time tracking of a single cerebrospinal fluid (CSF) component in freely moving animals.

FIG. 9B is a graph showing cerebrospinal fluid (CSF) serotonin (5HT) exhibits rapid fluctuations on a timescale of tens of seconds, correlated with mouse running speed. Inset compares 5HT-specific signal in blue (465 nm excitation) to 5HT-nonspecific movement artifact (405 nm excitation, isosbestic point of GRAB-HT2h sensor), confirming that the signal is not due to movement artifact. (right) Serotonergic neurons in the mouse lateral ventricle wall, putative secretors of CSF 5HT.

DEFINITIONS

As used herein, the term “about” means +/- 10% of the recited value.

The term “biological fluid” is meant a liquid or solution containing analytes (e.g., proteins or small molecules) of a living organism (e.g., a human) into which a probe or optical sensor of the disclosure can be placed (e.g., either in vivo or in vitro). The biological fluid can be, or can contain analytes from, e.g., blood, serum, plasma, urine, saliva, wound fluid (e.g., drains), amniotic fluid, cerebrospinal fluid (CSF), lung fluid (e.g., lung wash fluid collected from a bronchoalveolar lavage), abdomen fluid (e.g., ascites or fluid collected from peritoneal lavage), tissue (e.g., placental or dermal), pancreatic fluid, chorionic villus sample, and cells). The biological fluid can be one that is taken from a subject (e.g., a human or non-human animal) or can be one that is present in a subject (e.g., a human or non-human animal). A

biological fluid may be further purified or separated into different components. In a particular embodiment, whole blood is separated into serum and plasma components.

As used herein, "GFP" refers to a protein that exhibits fluorescence (e.g., green fluorescence) when exposed to light of an appropriate wavelength (e.g., in the blue to ultraviolet range). In some embodiments, a GFP is a circular permuted GFP (cpGFP). In some embodiments, a cpGFP includes a changed order of amino acids in its peptide sequence, e.g., such that the resulting GFP includes a protein structure with different connectivity, but overall similar three-dimensional (3D) shape.

A "GPCR" or "G protein-coupled receptor" is also known as a seven-(pass)-transmembrane domain receptor, 7TM receptor, heptahelical receptor, serpentine receptor, and G protein-linked receptor (GPLR). Ligands can bind either to the extracellular N-terminus and loops (e.g., glutamate receptors) or to the binding site within transmembrane helices (rhodopsin-like family) of a GPCR. When a ligand binds to the GPCR it causes a conformational change in the GPCR. The biggest change is an outward movement of the cytoplasmic part of the 5th and 6th transmembrane helix (TM5 and TM6). In some embodiments, a GPCR is used as a ligand receptor, as described herein. In some embodiments, a GPCR forms part of a molecular sensor. In some embodiments, a GPCR is coupled to an optical label. In some embodiments, the conformational change in the GPCR upon binding of a ligand results in an optically detectable change in the optical label.

A "gradient refractive index lens," "gradient-index lens," or "GRIN lens," as used herein, refers to a lens including a gradient in refractive index of the material. In some embodiments, a gradient refractive index lens may have flat surfaces. In some embodiments, a gradient refractive index may be optically aligned to an optical fiber.

An "isolated" protein or peptide is one which has been separated from a component of its natural environment. In some embodiments, a protein or peptide is purified to greater than 95% or 99% purity as determined by, e.g., electrophoresis (e.g., SDS-PAGE, isoelectric focusing (IEF), or capillary electrophoresis) or chromatography (e.g., ion exchange or reverse phase high-performance liquid chromatography (HPLC)).

As used herein, an "isolated" nucleic acid or plasmid refers to a nucleic acid molecule or plasmid that has been separated from a component of its natural environment. An isolated nucleic acid or plasmid includes a nucleic acid molecule or plasmid contained in cells that ordinarily contain the nucleic acid molecule or plasmid, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

A "ligand" is a molecule (e.g., a small molecule or biological molecule, e.g., a peptide) that forms a complex with a biomolecule, e.g., a receptor, e.g., a ligand receptor. In some embodiments, ligands include molecules in biological fluids. In some embodiments, a ligand may be a hormone, neurotransmitter, neuromodulator, or metabolite. In some embodiments, a ligand may be serotonin (5-HT), cholecystokinin (CCK), arginine vasopressin (AVP), oxytocin (OXT), corticotropin-releasing factor (CRF), vasoactive intestinal peptide (VIP), histamine (HA), neuropeptide Y (NPY), adenosine (Ado), norepinephrine (NE), dopamine (DA), acetylcholine (ACh), parathyroid hormone (PTH), urocortin (Uro), neurotensin (NTS), somatostatin (SST), sphingosine-1 phosphate (S1P), adenosine triphosphate (ATP), or melatonin.

A "ligand receptor" is a biological molecule, e.g., a protein, that receives and transduces signals that may be integrated into biological systems upon binding of a ligand. In some embodiments, binding of a ligand to the ligand receptor results in a conformational change in the ligand receptor. In some embodiments, a ligand receptor is coupled to an optical label (e.g., a fluorescent protein, such as GFP) to form a molecular sensor (e.g., binding of the ligand to the ligand receptor induces a conformational change in an associate fluorescent protein, such that the fluorescent protein produces a detectable signal). In some embodiments, a ligand receptor is a GPCR. In some embodiments, the ligand receptor is a modified GPCR. In some embodiments, the ligand receptor has been modified to bind to a ligand that is found in a biological fluid of interest. In some embodiments, the ligand receptor has been modified to contain a fluorescent protein.

As used herein, "mCherry" refers to a member of the mFruits family of monomeric red fluorescent proteins (mRFPs) derived from DsRed of *Discosoma* sea anemones. mCherry absorbs light between 540-590 nm and emits light in the range of 550-650 nm.

A "molecular sensor" refers to a sensing moiety that can be used to detect the presence, absence, or concentration of a ligand. In some embodiments, a molecular sensor described herein includes a ligand receptor and an optical label. In some embodiments, molecular sensors are intensiometric, such that the fluorescence output of the molecular sensor (e.g., of the optical label of the molecular sensor) is proportional to the concentration of ligand in the surrounding fluid or liquid. In some embodiments, molecular sensors exhibit ligand-dependent changes in fluorescence lifetime. In some embodiments, molecular sensors may have operable lifetimes of hours to days. In some embodiments, a molecular sensor is a G-protein coupled receptor-activation-based (GRAB) molecular sensor. In some embodiments, a GRAB sensor includes an endogenous, membrane-targeted G-protein coupled receptor (GPCR) fused to a circular-permuted GFP (cpGFP). In some embodiments, a molecular sensor is useful for the detection of a ligand that corresponds to the target of its ligand receptor. In some embodiments, a type of molecular sensor refers to one or more molecular sensors including the same ligand receptor. In some embodiments, a type of molecular sensor refers to one or more molecular sensors including the same ligand receptor (e.g., ligand receptors binding to the same ligand) and the same optical label.

A "molecular sensor cell" is a cell, e.g., a genetically modified cell, that expresses a molecular sensor. In some embodiments, a molecular sensor cell expresses only one type of molecular sensor. In some embodiments, a molecular sensor cell may be any genetically modified cell described herein. In some embodiments, a molecular sensor cell may be a HEK293T cell.

An "optical label" is a molecule that generates an optical signal. For example, the optical signal may be a fluorescence signal. In some embodiments, an optical label may generate a fluorescence signal. In some embodiments, an optical label is a dye (e.g., a fluorescent dye) or a fluorescent protein. In some embodiments, the fluorescent protein is a green fluorescent protein (GFP), a red fluorescent protein (RFP), a yellow fluorescent protein (YFP), or derivative thereof. In some embodiments, the protein is a circular-permutated GFP (cpGFP) or a split GFP. In some embodiments, the optical label is a cpGFP.

"Percent (%) sequence identity" with respect to a reference polynucleotide or polypeptide sequence is defined as the percentage of nucleic acids or amino acids in a candidate sequence that are identical to the nucleic acids or amino acids in the reference polynucleotide or polypeptide sequence,

after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid or amino acid sequence identity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, percent sequence identity values may be generated using the sequence comparison computer program BLAST. As an illustration, the percent sequence identity of a given nucleic acid or amino acid sequence, A, to, with, or against a given nucleic acid or amino acid sequence, B, (which can alternatively be phrased as a given nucleic acid or amino acid sequence, A that has a certain percent sequence identity to, with, or against a given nucleic acid or amino acid sequence, B) is calculated as follows:

100 multiplied by (the fraction X/Y)

where X is the number of nucleotides or amino acids scored as identical matches by a sequence alignment program (e.g., BLAST) in that program's alignment of A and B, and where Y is the total number of nucleic acids in B. It will be appreciated that where the length of nucleic acid or amino acid sequence A is not equal to the length of nucleic acid or amino acid sequence B, the percent sequence identity of A to B will not equal the percent sequence identity of B to A.

As used herein, the terms "subject" and "patient" are used interchangeably, and refer to an animal, such as a mammalian species (e.g., mouse, monkey, ape, or human) or an avian species (e.g., bird). Mammals include, but are not limited to, mice, simians, humans, farm animals, sport animals, and pets. The term avian as used herein includes poultry.

"Two-photon imaging" or "two-photon microscopy" refers to a non-linear fluorescence microscopy technique, in which simultaneous absorption of two photons is used to excite a fluorophore. In some embodiments, two-photon imaging may be used to visualize the optical labels of molecular sensors.

DETAILED DESCRIPTION

The present disclosure includes devices and methods of use for detecting a ligand in a biological fluid. The present disclosure features a probe with an optical sensor. The optical sensor includes a plurality of particles (e.g., cells) that include a ligand receptor coupled to an optical label (e.g., a GPCR fused to a fluorescent protein (e.g., a GRAB sensor)). The plurality of particles can be immobilized on the probe, e.g., on a lens or optical fiber attached to or incorporated within the probe, e.g., at or near a distal end of the probe. The optical sensor can be used to detect the ligand by employing a ligand receptor coupled to the optical label. When the ligand binds the ligand receptor, the receptor undergoes a conformational change that may be transduced to the optical label (e.g., a fluorescent protein). The optical label may emit a greater intensity of signal upon binding of the ligand to the ligand receptor, thus allowing the optical label to signal a presence or concentration of a ligand. Accordingly, the probes described herein provide extremely sensitive ligand detection capabilities with enhanced spatiotemporal tracking of a ligand in a biological fluid (e.g., in vivo or in vitro). Furthermore, the probes described herein can employ a plurality of distinct optical sensors such that multiple ligands can be detected in a single

fluid (e.g., simultaneously and/or in real time). Such detection capabilities allow the probes described herein to be used for the detection of a disease or disorder in a subject or to monitor a treatment regimen in a subject undergoing a treatment regimen for a disease or disorder, e.g., in which the concentration of a ligand is indicative of a disease state or responsiveness to a treatment thereof.

Probes

Featured is a probe that includes an optical sensor(s). The optical sensor includes a plurality of particles that include a ligand receptor coupled to an optical label. The plurality of particles may be immobilized on a lens or an optical fiber attached to or incorporated within the probe, e.g., at or near a distal end of the probe. The particles may be any suitable particles that bind or incorporate the ligand receptor coupled to the optical label. For example, the particles may include beads (e.g., microbeads), liposomes, lipid nanoparticles, micelles, or cells. The beads may be polymeric beads (e.g., plastic beads, e.g., polystyrene beads). The plurality of particles includes the ligand receptor encapsulated therein or coated thereon. The particles may be cells expressing the ligand receptor (e.g., molecular sensor cells). As ligand receptors are membrane bound proteins, the ligand receptor may be incorporated into a lipid bilayer, e.g., of a liposome or a cell. The probe can have any suitable size or shape, e.g., to be disposed in biological sample in vivo or in vitro. The probe may contain a cylindrical shape.

The probes described herein may include an optical fiber. In some embodiments, an optical fiber may be between 1-15 mm in length (e.g., between 5-10 mm; between 1-5 mm, between 10-15 mm, between 3-7 mm, between 8-11 mm, between 12-15 mm, or between 8-9 mm in length; e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 mm in length; e.g., about 8.85 mm in length). In some embodiments, an optical fiber may be between 50-2000 μm in diameter (e.g., between 50-500 μm, between 500-1000 μm, between 1000-1500 μm, between 1500-2000 μm, between 300-800 μm, between 800-1300 μm, between 1300-1800 μm, between 250-750 μm, or between 100-1000 μm in diameter; e.g., about 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 μm in diameter; e.g., about 500 μm in diameter). In some embodiments, the optical fiber includes polymer-based (e.g., PDMS, polycarbonate, etc.) and/or silica-based materials (e.g., glass, quartz, fused silica, borosilicate glass, etc.).

The probes described herein may include a lens. The lens may be a gradient refractive index (GRIN) lens. In some embodiments, a GRIN lens may be between 1-15 mm in length (e.g., between 5-10 mm; between 1-5 mm, between 10-15 mm, between 3-7 mm, between 8-11 mm, between 12-15 mm, or between 8-9 mm in length; e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 mm in length; e.g., about 8.85 mm in length). In some embodiments, a GRIN lens is about 5-10 mm in length. In some embodiments, a GRIN lens may be between 50-2000 μm in diameter (e.g., between 50-500 μm, between 500-1000 μm, between 1000-1500 μm, between 1500-2000 μm, between 300-800 μm, between 800-1300 μm, between 1300-1800 μm, between 250-750 μm, between 300-1000 μm, or between 100-1000 μm in diameter; e.g., about 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 μm in diameter; e.g., about 500 μm in diameter). In some embodiments, a GRIN lens is between 300-1000 μm in diameter. The lens may include polymer-based (e.g., PDMS, polycarbonate, etc.) and/or silica-based materials (e.g., glass, quartz, fused silica, borosilicate glass, etc.).

The probes described herein may be contacted with a plastic tubing, such that the distal end of the probe is in contact with the plastic tubing. The plastic tubing may include or be composed of a polymer (e.g., polyimide, polyethylene or polyethylene derivatives, such as cyclic olefin copolymers (COC), polymethylmethacrylate (PMMA), polydimethylsiloxane (PDMS), polycarbonate, polystyrene, polypropylene, polyvinyl chloride, polytetrafluoroethylene, polyoxymethylene, polyether ether ketone, polycarbonate, polystyrene, polyamide, etc.). The plastic tubing may include polyimide. The plastic tubing may be modified to allow attachment to a plurality of cells, e.g., encapsulated in a hydrogel. The plastic tubing may be modified by or coated with a thin layer (e.g., about 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 μm) of a silicone-based elastomer to generate an elastomeric surface. For example, the elastomer surface can be etched, e.g., with oxygen plasma treatment and/or a 2.5% potassium hydroxide solution. In some embodiments, the plastic tubing, e.g., the modified plastic tubing, e.g., the elastomeric surface, further includes an additional material to enhance adhesion (e.g., poly-L-lysine (Sigma), glutaraldehyde (Sigma), gelatin (Sigma), or chitosan (Heppe Medical Chitosan Gmbh)) of an optical sensor to the plurality of particles (e.g., cells) encapsulated in a hydrogel. A hydrogel may be polymerized on the plastic tubing, e.g., at or near the distal end of the plastic tubing.

In some embodiments, the probe includes an array of optical sensors, e.g., disposed on the lens. For example, the probe may include an array that includes a first optical sensor to detect a first ligand and a second optical sensor to detect a second ligand. The probe may include a plurality of optical sensors (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more), where each optical sensor is configured to detect a distinct ligand.

The probe may further include a sensor for tracking physiological indicia. For example, the probe may further include a blood flow sensor, a temperature sensor, a heart rate sensor, a blood pressure sensor, or an oxygen saturation sensor.

Particles

The particles incorporated into the optical sensor may be any suitable particles that can attach to or incorporate the ligand receptor coupled to the optical label. For example, the particles may include beads (e.g., microbeads), liposomes, lipid nanoparticles, micelles, or cells. The beads may be polymeric beads (e.g., plastic beads, e.g., polystyrene beads). The plurality of particles includes the ligand receptor encapsulated therein or coated thereon. In some embodiments, the particles are cells expressing the ligand receptor (e.g., molecular sensor cells).

Cells

The probe described herein may employ particles that are cells, e.g., genetically modified cells. Genetically modified cells useful in the methods and systems of the disclosure, e.g., in any of the probes disclosed herein, include a variety of cells. In some embodiments, the cell is an isolated cell. The cell may be in cell culture or a co-culture of two or more cell types. In some embodiments, the cell is ex vivo. In some embodiments, the cell is obtained from a living organism and maintained in a cell culture. In some embodiments, the cell is a single-cellular organism.

The cell may be a prokaryotic cell. In some embodiments, the cell is a bacterial cell or derived from a bacterial cell. In some embodiments, the cell is an archaeal cell or derived from an archaeal cell.

The cell may be a eukaryotic cell. In some embodiments, the cell is a plant cell or derived from a plant cell. In some embodiments, the cell is a fungal cell or derived from a fungal cell. In some embodiments, the cell is an animal cell or derived from an animal cell. In some embodiments, the cell is an invertebrate cell or derived from an invertebrate cell. In some embodiments, the cell is a vertebrate cell or derived from a vertebrate cell. In some embodiments, the cell is a mammalian cell or derived from a mammalian cell. In some embodiments, the cell is a human cell. In some embodiments, the cell is a rodent cell. In some embodiments, the cell is synthetically made, sometimes termed an artificial cell.

In some embodiments, the cells are Chinese hamster ovary cells, BALB/c mouse myeloma cells, human retinoblasts, monkey kidney cells, human embryonic kidney cells, baby hamster kidney cells, mouse Sertoli cells, human cervical carcinoma cells, canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor cells, or human hepatoma cells. The cell may be derived from a cell line. A wide variety of cell lines for suitable for genetic modification are known in the art. Examples of cell lines include, but are not limited to, HEK293T, MF7, K562, HeLa, Chinese hamster ovary (CHO), and transgenic varieties thereof. Cell lines are available from a variety of sources known to those with skill in the art (see, e.g., the American Type Culture Collection (ATCC) (Manassas, Va.)). The cell may be an immortal or immortalized cell.

The cells may be treated with colchicine or other microtubule stabilizing/depolymerizing agent to prevent cell movement and proliferation.

Synthetic particles

The probes described herein may employ particles that are synthetic particles (e.g., non-cellular particles), such as beads. The bead may be polymeric beads (e.g., plastic beads, e.g., polystyrene beads). A particle, e.g., a bead, may be porous, non-porous, hollow, solid, semi-solid, semi-fluidic, fluidic, and/or a combination thereof. In some instances, a particle, e.g., a bead, may be dissolvable or degradable. In some cases, a particle, e.g., a bead, may not be degradable.

A particle, e.g., a bead, may include natural and/or synthetic materials. For example, a particle, e.g., a bead, can include a natural polymer, a synthetic polymer or both natural and synthetic polymers. Examples of natural polymers include proteins and sugars such as deoxyribonucleic acid, rubber, cellulose, starch (e.g., amylose, amylopectin), proteins, enzymes, polysaccharides, silks, polyhydroxyalkanoates, chitosan, dextran, collagen, carrageenan, ispaghula, acacia, agar, gelatin, shellac, sterculia gum, xanthan gum, corn sugar gum, guar gum, gum karaya, agarose, alginic acid, alginate, or natural polymers thereof. Examples of synthetic polymers include acrylics, nylons, silicones, spandex, viscose rayon, polycarboxylic acids, polyvinyl acetate, polyacrylamide, polyacrylate, polyethylene glycol, polyurethanes, polylactic acid, silica, polystyrene, polyacrylonitrile, polybutadiene, polycarbonate, polyethylene, polyethylene terephthalate, poly(chlorotrifluoroethylene), poly(ethylene oxide), poly(ethylene terephthalate), polyethylene, polyisobutylene, poly(methyl methacrylate), poly(oxymethylene), polyformaldehyde, polypropylene, polystyrene, poly(tetrafluoroethylene), poly(vinyl acetate), poly(vinyl alcohol), poly(vinyl chloride), poly(vinylidene dichloride), poly(vinylidene difluoride), poly(vinyl fluoride) and/or combinations (e.g., co-polymers) thereof. Beads may also be formed from materials other than polymers, including lipids, micelles, ceramics, glass-ceramics, material composites, metals, other inorganic materials, and others.

Particles, e.g., beads, may be of uniform size or heterogeneous size. In some cases, the diameter of a particle, e.g., a bead, may be at least about 1 μm, 5 μm, 10 μm, 20 μm, 30 μm, 40 μm, 50 μm, 60 μm, 70 μm, 80 μm, 90 μm, 100 μm, 250 μm, 500 μm, 1 mm, 2 mm, 3 mm, 4 mm, 5 mm, 6 mm, 7 mm, 8 mm, 9 mm, 10 mm, or greater. In some cases, a particle, e.g., a bead, may have a diameter of less than about 1 μm, 5 μm, 10 μm, 20 μm, 30 μm, 40 μm, 50 μm, 60 μm, 70 μm, 80 μm, 90 μm, 100 μm, 250 μm, 500 μm, 1 mm, 2 mm, 3 mm, 4 mm, 5 mm, 6 mm, 7 mm, 8 mm, 9 mm, 10 mm or less. In some cases, a particle, e.g., a bead, may have a diameter in the range of about 40-75 μm, 30-75 μm, 20-75 μm, 40-85 μm, 40-95 μm, 20-100 μm, 10-100 μm, 1-100 μm, 20-250 μm, or 20-500 μm, 500 μm-1 mm, 1 mm-2 mm, 1-5 mm, or 1-10 mm.

Particles may be of any suitable shape. Examples of particles, e.g., beads, shapes include, but are not limited to, spherical, non-spherical, oval, oblong, amorphous, circular, cylindrical, and variations thereof.

Liposomes

The particles described herein may be liposomes, e.g., containing or coated with the ligand receptor coupled to the optical label. The composition of the liposome is usually a combination of phospholipids, usually in combination with, e.g., cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

A liposome described herein may include a phospholipid, such as a glycerophospholipid, e.g., a phosphatidylserine. A phosphatidylserine is a glycerol molecule having two hydroxyl groups substituted with fatty acid ester moieties and one hydroxyl group substituted with a phosphodiester moiety that is covalently bonded to serine side chain. A typical structure of a phosphatidylserine is RO-CH_2-CH(OR)-CH_2-OP(O)(OH)-$OCH_2CH(COOH)NH_2$, or a salt thereof, where each R is independently a fatty acid acyl. Additionally, or alternatively, a liposome described herein may include, e.g., a lysophospholipid, e.g., a lysophosphatidylserine. A lysophosphatidylserine is a phosphatidylserine missing one of its two fatty acid ester moieties. A typical structure of a lysophosphatidylserine is RO-CH_2-CH(OR)-CH_2-OP(O)(OH)-$OCH_2CH(COOH)NH_2$, or a salt thereof, where one R is a fatty acid acyl, and the other R is H. Thus, in certain embodiments, a liposome described herein includes RO-CH_2-CH(OR)-CH_2-OP(O)(OH)-$OCH_2CH(COOH)NH_2$, or a salt thereof, where each R is H or a fatty acid acyl, provided that at least one R is a fatty acid acyl.

One major type of liposomal composition includes phospholipids other than naturally derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Cationic liposomes possess the advantage of being able to fuse to the cell membrane. Non-limiting examples of cationic lipids include N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N--(I-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(I-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC),

1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyetetrahydro- 3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yeethylazanediyedidodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid can include, for example, from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). The ionizable/non-cationic lipid can be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), cholesterol, 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (sodium salt, DOPS), or a mixture thereof. The non-cationic lipid can be, for example, from about 5 mol % to about 90 mol %, about 10 mol %, or about 58 mol % if cholesterol is included, of the total lipid present in the particle. In some embodiments, an ionizable/non-cationic lipid can be a combination of lipids described above, e.g., a combination of lipids including DOPC, DOPS, Chol, and DOPE.

The conjugated lipid that inhibits aggregation of liposomal particles can be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate can be, for example, a PEG-dilauryloxypropyl (C_{12}), a PEG-dimyristyloxypropyl (C_{14}), a PEG-dipalmityloxypropyl (C_{16}), or a PEG-distearyloxypropyl (C_{18}). The conjugated lipid that prevents aggregation of particles can be, for example, from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle. In some embodiments, the liposome composition further includes cholesterol at, e.g., about 10 mol % to about 60 mol % or about 50 mol % of the total lipid present in the particle.

Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol. Examples of other methods to introduce liposomes into cells in

vitro and in vivo include U.S. Pat. No. 5,283,185; U.S. Pat. No. 5,171,678; WO 94/00569; WO 93/24640; WO 91/16024; Feigner, (1994) J. Biol. Chem. 269:2550; Nabel, (1993) Proc. Natl. Acad. Sci. 90:11307; Nabel, (1992) Human Gene Ther. 3:649; Gershon, (1993) Biochem. 32:7143; and Strauss, (1992) EMBO J. 11:417.

Lipid Nanoparticles

The particles described herein may be lipid nanoparticles, e.g., containing or coated with the ligand receptor coupled to the optical label. The ligand receptors may be fully encapsulated in a lipid formulation, e.g., a lipid nanoparticle (LNP). The LNPs may have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic.

Non-limiting examples of cationic lipids include DODAC, DDAB, DOTAP, DOTMA, DODMA, DLinDMA, DLenDMA, DLin-C-DAP, DLin-DAC, DLin-MA, DLinDAP, DLin-S-DMA, DLin-2-DMAP, DLin-TMA.Cl, DLin-TAP.Cl, 1DLin-MPZ, DLinAP, DOAP, DLin-EG-DMA, (DLin-K-DMA or analogs thereof, ALN100, MC3, Tech G1, or a mixture thereof. The cationic lipid can include, for example, from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

The ionizable/non-cationic lipid can be an anionic lipid or a neutral lipid including, but not limited to, DSPC, DOPC, DOPS, DPPC, DOPG, DPPG, DOPE, POPC, POPE, DOPE-mal, DPPE, DMPE, DSPE, 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, SOPE, cholesterol, or a mixture thereof. The non-cationic lipid can be, for example, from about 5 mol % to about 90 mol %, about 10 mol %, or about 60 mol % if cholesterol is included, of the total lipid present in the particle.

The conjugated lipid that inhibits aggregation of particles can be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate can be, for example, a PEG-dilauryloxypropyl (C_{12}), a PEG-dimyristyloxypropyl (C_{14}), a PEG-dipalmityloxypropyl (C_{16}), or a PEG-distearyloxypropyl (C_{18}). The conjugated lipid that prevents aggregation of particles can be, for example, from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle.

In some embodiments, the LNP further includes cholesterol at, e.g., about 10 mol % to about 60 mol % or about 50 mol % of the total lipid present in the particle.

Micelles

The particles described herein may be micelles, e.g., containing or coated with the ligand receptor coupled to the optical label. Micelles are a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. Micelles may be made of lipids. The micelle phase is caused by the packing behavior of single-tail lipids in a bilayer. The difficulty filling all the volume of the interior of a bilayer, while accommodating the area per head group forced on the molecule by the hydration of the lipid head group, leads to the formation of the micelle. This type of micelle is known as a normal-phase micelle (oil-in-water micelle). Inverse micelles have the head groups at the center with the tails extending out (water-in-oil micelle).

Micelles are approximately spherical in shape. Other phases, including shapes such as ellipsoids, cylinders, and bilayers, are also possible. The shape and size of a micelle are a function of the molecular geometry of its surfactant molecules and solution conditions such as surfactant concentration, temperature, pH, and ionic strength. The process of forming micelles is known as micellization and forms part of the phase behavior of many lipids according to their polymorphism.

Matrix

The particles (e.g., cells) of the optical sensor may be encapsulated within, coated on, or mixed with, a suitable matrix, such as a hydrogel matrix. The matrix containing the particles (e.g., cells) may be deposited on the lens of the probe. For example, the optical sensor may include a plurality of cells encapsulated in a hydrogel matrix. The hydrogel matrix may include one or more polymers, e.g., collagen, hyaluronic acid, MATRIGEL®, chitosan, dextran, agarose, gelatin, alginate, protein polymers, methylcellulose, acrylamide, bis-acrylamide, polyacrylamide and derivatives thereof, poly(ethylene glycol) and derivatives thereof (e.g. PEG-acrylate (PEG-DA), PEG-RGD), gelatin-methacryloyl (GelMA), methacrylated hyaluronic acid (MeHA), polyaliphatic polyurethanes, polyether polyurethanes, polyester polyurethanes, polyethylene copolymers, polyamides, polyvinyl alcohols, polypropylene glycol, polytetramethylene oxide, polyvinyl pyrrolidone, polyacrylamide, poly(hydroxyethyl acrylate), and poly(hydroxyethyl methacrylate), etc. The hydrogel matrix may be a mixture of two or more polymers described herein.

Ligands and Ligand Receptors

The probes described herein employ a ligand receptor coupled to an optical label (e.g., to form a molecular sensor) to detect a ligand in a sample (e.g., a biological fluid). Any suitable ligand or ligand receptor may be employed for detection in a sample. The ligand receptor may be a naturally occurring ligand receptor. The ligand receptor may be an engineered ligand receptor. In some embodiments, the ligand receptor is a G-protein coupled receptor (GPCR) (e.g., a GRAB sensor).

The ligand may be a peptide (e.g., a hormone, a cytokine, a chemokine, a regulatory protein, or a neuropeptide). The peptide may be arginine vasopressin (AVP), oxytocin (OXT), somatostatin (SST), corticotropin-releasing factor (CRF), vasoactive intestinal peptide (VIP), cholecystokinin (CCK), corticotropin-releasing factor (CRF), neuropeptide Y (NPY), parathyroid hormone (PTH), urocortin (Uro), neurotensin (NTS), sphingosine-1 phosphate (S1P), or insulin.

The ligand may be a small molecule (e.g., a neurotransmitter or a metabolite). In some embodiments, the small molecule is serotonin (5-HT), norepinephrine (NE), dopamine (DA), acetylcholine (ACh), histamine (HA), melatonin, adenosine (Ado), adenosine triphosphate (ATP), or glucose.

Ligand Receptors Coupled to Optical Labels

Suitable optical sensors described herein include a ligand receptor coupled to an optical label. For example, the ligand receptor may be covalently attached to an optical label, e.g., to form a molecular sensor. Molecular sensors that may be useful in the probes and methods described herein include those described in, e.g., Jing et al., *Nat Methods.* 17(11):1139-1146, 2020 (e.g., molecular sensor to acetylcholine, e.g., GPCR and cpGFP), Wang et al., *bioRxiv.* 2022.03.26.485911, 2022 (e.g., molecular

sensor to somatostatin, cholecystokinin, corticotropin-releasing factor, neuropeptide Y, neurotensin, and vasoactive intestinal peptide, e.g., GPCR and cpGFP), Qian et al., IBRO Reports. 6:S392, 2022 (e.g., a molecular sensor to somatostatin), Wu et al., *Neuron*. 110(5):770-782, 2022 (e.g., molecular sensor to ATP, e.g., GPCR and cpGFP), Peng et al., *Science*. 369(6508):eabb0556, 2020 (e.g., molecular sensor to adenosine, e.g., GPCR and cpGFP), Qian et al., *bioRxiv*. 2022.02.10.480016, 2022 (e.g., molecular sensor to oxytocin, e.g., GPCR and cpGFP), Sun et al., *Nat Methods*. 17(11):1156-1166, 2020 (e.g., molecular sensor to dopamine, e.g., GPCR and cpGFP), Dong et al., *bioRxiv*. 2022.08.19.504485, 2022 (e.g., molecular sensor to histamine, e.g., GPCR and cpGFP), Feng et al., *Neuron*. 102(4):745-761, 2019 (e.g., molecular sensor to norepinephrine, e.g., GPCR and cpGFP), Marvin et al., *Nat. Methods*. Nov;15(11):936-939, 2018 (e.g., molecular sensor to glutamate, e.g., glutamate/aspartate import solute-binding protein (GltI) and circularly permuted superfolder GFP), Marvin et al., Nat Methods. 16(8):763-770, 2019 (e.g., molecular sensor to γ-aminobutyric acid (GABA)), Unger et al., *Cell*. 183(7):1986-2002, 2020 (e.g., molecular sensor to serotonin), Patriarchi et al., *Science*. 360(6396):eaat4422, 2018 (e.g., molecular sensor to dopamine; e.g., GPCR and cpGFP), Duffet et al., *Nat. Methods*. 19(2):231-241, 2022 (e.g., molecular sensor to orexin; e.g., GPCR and cpGFP), Chen et al., *Nature*. 499(7458):295-300, 2013 (e.g., molecular sensor to calcium; e.g., calmodulin and GFP), each of which is incorporated herein by reference in its entirety. Molecular sensors that may be useful in the probes and methods described herein additionally include those described in, e.g., PCT Publication No. WO 2019/062744 and U.S. Patent Publication No. US20200400567A1 (e.g., molecular sensor to epinephrine, norepinephrine, acetylcholine, serotonin, dopamine, histamine, isoproterenol, oxytocin, ; e.g., GPCR and GFP, YFP, BFP, YFP, or RFP), each of which is herein incorporated by reference in its entirety.

Optical Labels

A ligand receptor as described herein may be covalently attached to an optical label (e.g., to form a molecular sensor). Theoptical label may be a fluorescent molecule. A fluorescent molecule may be used in the probes as described herein to detect the presence and/or concentration of a ligand by fluorimetry and/or by direct visualization using fluorescence microscopy. Exemplary fluorescent molecules that can be conjugated to a ligand receptor of the disclosure include green fluorescent protein (GFP), cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), red fluorescent protein, phycoerythrin, allophycocyanin, hoescht, 4',6-diamidino-2-phenylindole (DAPI), propidium iodide, fluorescein, coumarin, rhodamine, tetramethylrhoadmine, and cyanine. Additional examples of fluorescent molecules suitable for conjugation to the ligand receptors of the disclosure are well-known in the art and have been described in detail in, e.g., U.S. Patent Nos. 7,417,131 and 7,413,874, each of which is incorporated by reference herein. The optical label may be an engineered variant of GFP, CFP, YFP, RFP or another fluorescent protein. For example, in some embodiments, the green fluorescent protein is a circular permuted GFP or enhanced GFP (eGFP). In some embodiments, the RFP is DsRed, DsRed-Express, Ds-Red-Express2, or mCherry. Other fluorescent proteins are well known to the skilled artisan.

Linkers

The ligand receptors described herein may be conjugated to an optical label (e.g., a fluorescent protein) via a linker. The linker may be a peptide linker or a chemical linker.

A peptide linker may be, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, or more amino acids long. The linker may be, e.g., from about 1 to about 500 amino acids (e.g., about 1 to about 400, about 1 to about 300, about 1 to about 20, about 5 to about 300, about 5 to about 200, about 5 to about 100, about 5 to about 50, about 5 to about 30, about 10 to about 30, about 10 to about 20) long.

Suitable peptide linkers are known in the art, and include, for example, peptide linkers containing flexible amino acid residues such as glycine and serine.

The peptide linker may include the amino acid sequence of any one of (GS)x, (GGS)x, (GGGGS)x, (GGSG)x, (SGGG)x, wherein x is an integer from 1 to 50 (e.g., 1-40, 1-30, 1-20, 1-10, or 1-5). In some embodiments, the peptide linker has the amino acid sequence $(GGGGS)_x$, wherein x is an integer selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In some embodiments, a peptide linker contains only glycine residues, e.g., at least 4 glycine residues (e.g., 4-200, 4-180, 4-160, 4-140, 4-40, 4-100, 4-90, 4-80, 4-70, 4-60, 4-50, 4-40, 4-30, 4-20, 4-19, 4-18, 4-17, 4-16, 4-15, 4-14, 4-13, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7, 4-6 or 4-5 glycine residues) (e.g., 4-200, 6-200, 8-200, 10-200, 12-200, 14-200, 16-200, 18-200, 20-200, 30-200, 40-200, 50-200, 60-200, 70-200, 80-200, 90-200, 100-200, 120-200, 140-200, 160-200, 180-200, or 190-200 glycine residues). In certain embodiments, a linker has 4-30 glycine residues (e.g., 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 glycine residues).

Methods

The present disclosure includes methods for generating the molecular sensors and molecular sensor cells described herein and methods for in vitro and in vivo detecting of ligands in a biological sample, e.g., by using the probes described herein.

Methods for Generating Molecular Sensors

Molecular sensors of the disclosure (e.g., including a ligand receptor and an optical label) can be generated using techniques known in the art, e.g., by using recombinant methods and compositions. A molecular sensor may be generated by combining any one of the ligand receptors described herein with any optical label described herein. In some embodiments, a molecular sensor includes a ligand receptor coupled to an optical label.

A molecular sensor can include a ligand receptor that binds to a target of interest (e.g., a molecule that is a component of a biological fluid (e.g., cerebrospinal fluid (CSF), interstitial fluid, blood, lymph, saliva, bone marrow, or urine); e.g., corticotropin-releasing factor (CRF), vasoactive intestinal peptide (VIP), histamine (HA), neuropeptide Y (NPY), adenosine (Ado), norepinephrine (NE), dopamine (DA), acetylcholine (ACh), parathyroid hormone (PTH), urocortin (Uro), neurotensin (NTS), somatostatin (SST), sphingosine-1 phosphate (S1P), adenosine triphosphate (ATP), and melatonin) and that is coupled to an optical label, e.g., a green fluorescent protein (GFP), a red fluorescent protein (RFP), a yellow fluorescent protein (YFP), a circular-permuted fluorescent protein (e.g., a circular-permuted GFP (cpGFP)), a split fluorescent protein (e.g., a split GFP), or derivative thereof)

A ligand receptor can be coupled to an optical label by fusing the optical label at a suitable site on the ligand receptor. For example, the optical label can be fused at a suitable site on the ligand receptor,

such that a conformational change in the ligand receptor that results from the binding of the ligand receptor to a ligand causes a conformational change in the optical label, thereby generating an optical signal from the optical label.

Methods for Generating Molecular Sensor Cells

Methods for generating molecular sensor cells, e.g., cells that express molecular sensors (e.g., including a ligand receptor and an optical label) include, e.g., recombinant methods and compositions. For example, isolated nucleic acids encoding a molecular sensor described herein can be provided and used to transfect a cell of interest (e.g., HEK293T cells). The isolated nucleic acids may further encode mCherry. Such isolated nucleic acids may encode a ligand receptor described herein and/or an optical label described herein. One or more vectors (e.g., expression vectors) and/or one or more plasmids including the isolated nucleic acid(s) can be used to transfect the cell(s) to produce the sensor cells. An isolated nucleic acid and/or vector or plasmid may encode a molecular sensor (i.e., a ligand receptor coupled to an optical label) and an mCherry.

The molecular sensor can be expressed in a cell. In some embodiments, a cell expresses only one type of molecular sensor. In some embodiments, a cell expresses one or more copies of one type of molecular sensor. In some embodiments, a molecular sensor cell is a genetically modified cell. Any suitable cell known in the art and/or disclosed herein may be used to generate a molecular sensor cell. A cell used to generate a molecular sensor cell may be derived from a cell line. A wide variety of cell lines suitable for genetic modification are known in the art. Examples of cell lines include, but are not limited to, HEK293T, MF7, K562, HeLa, Chinese hamster ovary (CHO), and transgenic varieties thereof. Cell lines are available from a variety of sources known to those with skill in the art (see, e.g., the American Type Culture Collection (ATCC) (Manassas, Va.)). The molecular sensor cell may be generated using an immortal or immortalized cell. The molecular sensor cell may be a HEK293T cell.

Methods for In Vitro Detection of Ligands

The disclosure also includes methods for performing *in vitro* measurements for detecting a ligand (or multiple ligands) in a biological sample (e.g., a biological fluid) using, e.g., an optical sensor as described herein. The methods can include the detection of multiple ligands simultaneously.

The method includes contacting a probe, e.g., a probe containing one or more optical sensors, e.g., a plurality of optical sensors (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more optical sensors, such as optical sensors coated on or embedded in particles, such as by, e.g., molecular sensor cells), with one or more biological samples, e.g., a biological fluid. The biological sample (e.g., biological fluid) may include one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) ligands. In some embodiments, the one or more biological samples (e.g., biological fluids) may each contain only one ligand. In some embodiments, the one or more biological samples (e.g., biological fluids) may each include more than one ligand (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more ligands). The ligand may be a peptide or a small molecule. The peptide may be a neuropeptide. The small molecule may be a neurotransmitter. In some embodiments, the ligand is corticotropin-releasing factor (CRF), vasoactive intestinal peptide (VIP), histamine (HA), neuropeptide Y (NPY), adenosine (Ado), norepinephrine (NE), dopamine (DA),

acetylcholine (ACh), parathyroid hormone (PTH), urocortin (Uro), neurotensin (NTS), somatostatin (SST), sphingosine-1 phosphate (S1P), adenosine triphosphate (ATP), or melatonin.

The optical sensor may emit an optical signal upon contact of the optical sensor with a biological sample (e.g., biological fluid) containing a ligand that binds to the ligand receptor of the optical sensor. In some embodiments, an optical sensor with one or more molecular sensors emits an optical signal upon contacting the optical sensor with a biological sample (e.g., biological fluid) with the corresponding ligand. Detecting an optical signal from an optical sensor may indicate that the biological sample (e.g., biological fluid) includes a ligand corresponding to the ligand receptor contained in the molecular sensor of the molecular sensor cells of the optical sensor. In some instances, an optical sensor is contacted with one or more fluids, each containing a single ligand. In some instances, the optical sensor is contacted with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more fluids, each containing a single ligand. In some instances, each fluid contains a different ligand from the other fluids. In some instances, a molecular sensor cell emits an optical signal after contacting a fluid containing a ligand. In some instances, a molecular sensor cell in the optical sensor only emits an optical signal after contacting one fluid of a set of fluids containing one ligand of a set of ligands and does not emit an optical signal after contacting any of the other fluids of the set of fluids, e.g., containing a different ligand. In some instances, a molecular sensor cell is configured to allow detection of one ligand of a set of ligands by emitting an optical signal after contacting the fluid containing the ligand. In some embodiments, one or more molecular sensing cells are each configured to allow detection of a distinct ligand in a set of ligands by sequentially contacting the sensor with one fluid in a set of fluids. A probe with multiple optical sensors can be used to simultaneously detect each of multiple different ligands (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more ligands) to which the multiple optical sensors are directed.

The strength of the detected optical signal may correlate with the concentration of the detected ligand in the biological sample (e.g., biological fluid). In some embodiments, the optical signal is a fluorescence signal. In some embodiments, contacting a molecular sensor containing a ligand receptor and an optical label causes a conformational change in the ligand receptor, which causes the emission of a fluorescence signal by the optical label. In some embodiments, the optical signal is detected using microscopy. In some embodiments, the optical signal is detected using fluorescence microscopy. In some embodiments, the optical signal is detected using two-photon (e.g., two-photon fluorescence) microscopy. In some embodiments, fluorescence intensity scales with ligand concentration can be calibrated against standards to obtain a mapping of fluorescence to ligand concentration. Subsequently, dynamic measurements can be made many times each second, and read out in real time.

One or more biological samples (e.g., biological fluids) can be added to one or more corresponding receptacles. The molecular sensor may be inserted into the one or more receptacles so that the molecular sensor contacts the one or more biological samples. The molecular sensor may be inserted into a receptacle for a duration of between 1-120 seconds (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, or 120 seconds). In some embodiments, the molecular sensor is inserted into a receptacle for a duration of about 60 seconds.

A receptacle may contain about 0.1, 0.5, 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, 1000, 2000, 3000, 4000, 5000, 10000 μL, or more of a biological fluid. In some embodiments, a receptacle contains about 20 μL of a biological fluid.

Methods for In Vivo Detection of Ligands

The disclosure provides methods for performing *in vivo* measurements for detecting a ligand in a biological sample, e.g., a biological fluid, e.g. using an optical sensor described herein.

The method may include contacting a probe, e.g., an optical sensor, e.g., a plurality of molecular sensor cells with a biological sample, e.g., a biological fluid, e.g., in a subject. The subject may be a mouse, a primate, or a human. In some instances, the biological sample (e.g., biological fluid) includes one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) ligands. In some embodiments, the biological sample, e.g., biological fluid may be or include cerebrospinal fluid (CSF), interstitial fluid, blood, lymph, saliva, bone marrow, or urine. In some embodiments, the ligand is a peptide or a small molecule. In some embodiments, the peptide is a neuropeptide. The small molecule may be a neurotransmitter. In some embodiments, the ligand is corticotropin-releasing factor (CRF), vasoactive intestinal peptide (VIP), histamine (HA), neuropeptide Y (NPY), adenosine (Ado), norepinephrine (NE), dopamine (DA), acetylcholine (ACh), parathyroid hormone (PTH), urocortin (Uro), neurotensin (NTS), somatostatin (SST), sphingosine-1 phosphate (S1P), adenosine triphosphate (ATP), or melatonin.

The probe containing the optical sensor(s) can be inserted in vivo directly into the biological fluid and used to detect the presence of, or changes in, one or more target ligands over time (e.g., in real time). The sensing of multiple ligands can be performed simultaneously.

If desired, the optical sensor can be contacted with the biological sample (e.g., biological fluid) for between 0-72 hours, or more (e.g., about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, 21, 24, 36, 48, 60, or 72 hours, or more). The optical sensor can be contacted with the biological sample within a subject (e.g., a mouse, a primate, or a human). In some embodiments, a tube (e.g., a cannula) is inserted into the subject, and a probe (e.g., including the optical sensor) is inserted into the tube (e.g., the cannula) such that the probe (e.g., the optical sensor) is contacted with the biological sample, e.g., the biological fluid. The tube (e.g., the cannula) may be inserted into a subject (e.g., a mouse, a primate, or a human) at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or more days prior to the insertion of the probe into the tube. The cannula may be made with any suitable material known in the art, e.g., a bioplastic or stainless-steel. The cannula and/or probe (e.g., optical sensor) may be inserted into an appropriate region of the subject to be placed into contact with the biological sample (e.g., into a blood vessel, intraperitoneal region, spine, bone marrow, bodily orifice, or organ). The cannula and/or probe (e.g., optical sensor) is inserted into a brain, e.g., a region of the brain. The cannula and/or probe (e.g., optical sensor) may be inserted into a lateral ventricle. Alternatively, the probe containing the optical sensor(s) may be inserted into another organ or tissue (e.g., the lungs, the heart, the liver, a kidney, the bladder, the uterus, the vagina, a blood vessel, the stomach, the gallbladder, the spleen, or the intestines) and used to monitor the presence of, or changes in, one or more ligands in the organ or tissue.

An optical sensor emits an optical signal upon contacting the optical sensor with a biological sample (e.g., biological fluid). In some embodiments, an optical sensor including one or more molecular sensor cells including a molecular sensor including a ligand receptor emits an optical signal upon contacting the optical sensor with a biological sample (e.g., biological fluid) including the corresponding ligand. Detection of an optical signal from an optical sensor after contacting the optical sensor with the biological sample (e.g., biological fluid) indicates that the biological sample (e.g., biological fluid) includes a ligand corresponding to the ligand receptor contained in the molecular sensor. In some embodiments, the strength of the detected optical signal correlates with the concentration of the detected ligand in the biological sample (e.g., biological fluid). The optical signal may be, for example, a fluorescence signal. In some embodiments, contacting a molecular sensor containing a ligand receptor and an optical label causes a conformational change in the ligand receptor, which causes the emission of a fluorescence signal by the optical label. In some embodiments, the optical signal is detected using microscopy. In some embodiments, the optical signal is detected using fluorescence microscopy. In some embodiments, the optical signal is detected using two-photon (e.g., two-photon fluorescence) microscopy.

Prior to the use of a probe or optical sensor to perform an in vivo measurement, the probe or optical sensor may be calibrated according to the following method. In some embodiments, an optical sensor (or probe containing the optical sensor) can be contacted with one or more fluids, each containing a single ligand. The optical sensor may be contacted with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more fluids, each containing a single ligand. Each fluid may contain a different ligand from all of the other fluids. A molecular sensor cell in the optical sensor may emit an optical signal after contacting a fluid containing a ligand. A molecular sensor cell in the optical sensor may only emit an optical signal after contacting one fluid of a set of fluids containing one ligand of a set of ligands and does not emit an optical signal after contacting any of the other fluids of the set of fluids containing any of the other ligands of the set of ligands. A molecular sensor cell in the optical sensor is determined to detect one ligand of a set of ligands after emitting an optical signal after contacting the fluid containing the ligand. In some embodiments, a calibration is performed in which one or more molecular cells are each configured to allow detection of a ligand among a set of ligands after sequentially contacting the optical sensor with a fluid in a set of fluids. The optical sensor may be contacted with one or more fluids (e.g., 1, 2, 3, 4, 5, 6, or more fluids), each containing the same ligand, but with different concentrations. Contacting the optical sensor with the one or more fluids containing the same ligand at different concentrations may cause the optical label of the molecular sensors of the molecular sensor cells to emit an optical signal at different intensities. The optical signal intensity may correlate to the ligand concentration of the fluid. In some embodiments, a concentration standard curve may be calculated by contacting the optical sensor with one or more fluids (e.g., 1, 2, 3, 4, 5, 6, or more fluids), each containing the same ligand, at a different known concentration. The one or more fluids may include a dilution series of a ligand. The one or more fluids may include one or more dilution series (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 dilution series) corresponding to one or more ligands (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more ligands).

Methods for Detecting and Treating a Disease or Disorder

The probes described herein can be used to detect a disease or disorder or to monitor a treatment regimen of a disease or disorder. For example, a probe containing an optical sensor with a ligand receptor coupled to an optical label can be used to detect the concentration of a ligand in a biological sample.

In one example, arginine vasopressin (AVP), the level of which has been linked to prosocial behaviors, can be monitored. People with lower CSF levels of AVP may show pronounced reduction in social function, and impairments in social behavior in autism spectrum disorder (ASD) may be associated with low AVP levels. For example, the likelihood of ASD may increase over 1,000-fold with decreasing CSF AVP levels, and low neonatal CSF AVP levels can be used to predict a future ASD diagnosis. AVP signaling may also be associated with other diseases, such as Prader-Willi and Fragile X syndrome. Accordingly, a probe as described herein equipped with an optical sensor containing a ligand receptor (e.g., GPCR) specific for AVP and coupled to a fluorescent label (e.g., a fluorescent protein, such as GFP or RFP) may be used to detect a concentration of AVP in CSF levels of a subject (e.g., in real-time) to determine whether the subject has or is at risk of developing a disease or disorder, such as ASD, Schaaf-Yang Syndrome, Prader-Willi syndrome, or Fragile X syndrome.

Concentrations of a ligand, such as AVP, may be compared to the concentration of the ligand in a normal subject and/or in other subjects suffering from the disease or disorder (e.g., a control sample) in order to establish a threshold concentration(s) or ranges of concentrations associated with healthy and/or diseased states. If a subject is undergoing treatment, the concentration of the ligand can be monitored over time to determine whether the subject's disease state is improving (e.g., approaching or reaching normal ligand concentrations in a fluid sample) or requires a treatment (e.g., the ligand concentration is not within a normal ligand concentration of a healthy subject).

A treatment regimen (e.g., of intranasal AVP) may be adjusted based on ligand detection. For example, if a subject is nonresponsive to a treatment, e.g., as determined based on the concentration of ligand in a fluid sample, the dosage of a treatment regimen may be increased in amount and/or frequency, and the corresponding change in the ligand concentration in the fluid may be ascertained. If the subject is responsive to a treatment regimen, e.g., as determined based on the concentration of ligand in a fluid sample, the treatment regimen may be discontinued, or the dose may be decreased in amount and/or frequency.

The probes and methods described herein may be used to detect or treat any disease or disorder in which a concentration of a ligand (e.g., a biomarker) may be used to track the presence of a disease state. The disease or disorder may be, for example, autism spectrum disorder, Prader-Willi syndrome, Schaaf-Yang Syndrome, Fragile X syndrome, diabetes, obesity, atherosclerosis, high blood pressure, high cholesterol, stroke, seizure, and trauma (e.g., traumatic brain injury).

Exemplary diseases or disorders, ligands, animal models, and sensors are shown in **Table 1**.

Table 1. Exemplary Diseases, Ligands, and Optical Sensors

Disease or Disorder	Ligand(s) to be detected	Animal/human model references	Ligand Sensor
Autism Spectrum Disorder	Vasopressin, oxytocin	1–4	27
Metabolic disease	Insulin, glucagon, ghrelin, cholecystokinin, leptin, gastrin, therapeutics (Exendin-4, PrRP31, others)	5–9	10, 36
Epilepsy/seizure	Lactate, glutamate (CSF)	11	12, 34
Alzheimer's disease	Amyloid beta, Tau, insulin	13,14	10,15–17
Myocardial infarction	Troponin	18	19
Stroke	Neurofilament, tau, myelin basic protein, GFAP (CSF)	20	15
Traumatic brain injury	Neurofilament, tau (CSF)	21	15
Neurocysticercosis	Aspartate, glutamate, GABA, glutamine	22	34, 35
Cancers	Chemotherapeutics, e.g., platinum complexes		23
Chronic pain, anesthesia	Opioids, non-opioid pain medications, e.g., intrathecal delivery	24	25,26
Atherosclerosis	C-reactive protein (CRP)	28	29
High cholesterol	LDL, HDL, cholesterol	30	31, 32
Hypertension	CRP	33	29

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Systems and Kits

The disclosure also features systems and kits for use with any of the probes disclosed herein. The system or kit may further include a receptacle for a biological sample. The kit may further include instructions for use. The kit or system may further include one or more of a lens, a plasmid, particles (e.g., liposomes, lipid nanoparticles, micelles, plastic (e.g., polystyrene) particles, magnetic particles, or cells), cells, hydrogel matrix components, a cannula, a microscope, a computational system, an image acquisition system, a closed-loop system, and other suitable components.

Image Acquisition Systems

The disclosure provides an image acquisition system suitable for use with an in vivo or in vitro detection method disclosed herein. The image acquisition system may include a microscope. The image acquisition system may include one or more light sources (e.g., lasers). The image acquisition system may be a two-photon microscope system. The microscope may include a lens, e.g., a tunable lens, e.g., an electrically tunable lens. The image acquisition system may include a controller (e.g., a computer) capable of controlling the image acquisition system to perform any of the detection methods disclosed herein.

The image acquisition system may be a microendoscope of a fiber two-photon microscope.

Computational Systems

The disclosure also provides a computational system capable of calculating the kinetics of ligands detected in a biological sample, e.g., a biological liquid. In some embodiments, the computational system includes a computing device (e.g., a computer). In some embodiments, the computing device implements an algorithm for identifying the ligands corresponding to each molecular sensor cell detected using a method disclosed herein. In some embodiments, the computing device implements an algorithm for converting the optical system obtained from an image acquisition system into a concentration of a detected ligand.

Closed-Loop Systems

The disclosure provides a closed-loop system, e.g., for coupling any of the methods of ligand detection disclosed herein with a pharmacological delivery system and/or a sensory stimulation system. The closed-loop system may be a positive- or negative-feedback system. The closed-loop system may include pumps (e.g., syringe, peristaltic, diaphragm pumps, or vacuum), heat pads, audio devices (e.g., speakers, bells, etc.), display devices (e.g., screens or monitors), shock pads, or other devices suitable for delivering a pharmacological substance and/or a sensory stimulus. The closed loop system provides internal feedback to maintain regular delivery of a pharmacological agent in order to maintain a desired dosage level of the pharmacological agent or to maintain a desired level of a particular ligand in a fluid by regulating administration of the pharmacological agent (see, e.g., **FIGS. 8A-8D**).

Standards for In Vitro *Quantification*

The disclosure provides kits for creating standards for in vitro quantification of ligands using any of the probes and methods described herein and/or for using any of the devices described herein. The kit may include one or more ligands that may be bound by a ligand receptor described herein. the kit may include salts, reagents, buffers, liquid handlers, multiwell plates.

Kits for Generating Molecular Sensor Cells

The disclosure provides kits for generating any of the molecular sensor cells described herein. The kit may include nucleic acids, vectors, and/or plasmids useful for expressing in a cell any of the molecular sensors described herein. The kit may provide reagents, e.g., transfection reagents. In some embodiments, the kit includes cells, e.g., HEK293T cells.

Kits for Construction of Probe

The disclosure provides kits for constructing a probe. The kit may include polymers (e.g., for creating a plastic tube (e.g., polyimide, polyethylene or polyethylene derivatives, such as cyclic olefin copolymers (COC), polymethylmethacrylate (PMMA), polydimethylsiloxane (PDMS), polycarbonate, polystyrene, polypropylene, polyvinyl chloride, polytetrafluoroethylene, polyoxymethylene, polyether ether ketone, polycarbonate, polystyrene, polyamide, etc.) or for creating hydrogels (e.g., collagen, hyaluronic acid, MATRIGEL®, chitosan, dextran, agarose, gelatin, alginate, protein polymers, methylcellulose, acrylamide, bis-acrylamide, polyacrylamide and derivatives thereof, poly(ethylene glycol) and derivatives thereof (e.g. PEG-acrylate (PEG-DA), PEG-RGD), gelatin-methacryloyl (GelMA), methacrylated hyaluronic acid (MeHA), polyaliphatic polyurethanes, polyether polyurethanes, polyester polyurethanes, polyethylene copolymers, polyamides, polyvinyl alcohols, polypropylene glycol, polytetramethylene oxide, polyvinyl pyrrolidone, polyacrylamide, poly(hydroxyethyl acrylate), and poly(hydroxyethyl methacrylate), etc.).

The kit may include a fiber optic cable or a lens. The fiber optic cable and/or lens may be created from any suitable material, including polymer-based (e.g., PDMS, polycarbonate, etc.) and/or silica-based materials (e.g., glass, quartz, fused silica, borosilicate glass, etc.). The lens may be a gradient refractive index (GRIN) lens described herein.

EXAMPLES

The following examples are to illustrate the disclosure. They are not meant to limit the disclosure in any way.

Example 1. Generation of Molecular Sensors and Molecular Sensor Cells

Molecular sensors including ligand receptors (e.g., GPCRs) and optical labels (e.g., circular-permuted GFPs (cpGFP), as well as an mCherry reporter were generated.

Plasmids, each including one molecular sensor and mCherry, were expressed in HEK293T cells. Molecular sensor cells were either (1) HEK293T cells (ATCC) acutely transfected with the plasmid encoding a GRAB sensor or (2) HEK293 cells containing 1+ stably-integrated copies of a GRAB sensor-encoding plasmid. All cells were cultured in Denville 35 mm cell culture dishes (Thomas Scientific) with

DMEM (high glucose + GLUTAMAX® supplement; ThermoFisher Scientific) medium containing 10% fetal bovine serum (FBS; R&D Systems) and 1% penicillin/streptomycin (P/S) solution (Sigma) and stored in a standard tissue culture incubator set to 37 °C, 95% humidity and 5% CO^2. After being thawed, cells used for experiments were maintained in culture for up to 8 passages before being discarded. HEK293T cells being acutely transfected were detached from the culture dish using mechanical force, resuspended at a density of 300,000-500,000 cells/mL, and seeded in each well of a poly-D-lysine-treated (Sigma) 24-well plate (Corning) at 150,000-170,000 cells/well. Wells were then treated with a standard EFFECTENE® transfection mixture (Qiagen) optimized separately for each GRAB sensor plasmid according to the instructions provided by the supplier. Each well was transfected with a encoding a different GRAB sensor as well as mCherry (for ratiometric normalization). After 24 hours, the expression level of the sensor cells were screened and the cells were treated with colchicine (1 μM) or another microtubule stabilizing/depolymerizing agent for one hour to prevent cell movement and proliferation. HEK293 cells stably-expressing GRAB sensors were generated using the same acute transfection procedure discussed above followed by a week-long G418 Geneticin (Thermo Fisher) treatment. Stably-expressing sensor cells to be used in experiments were seeded in a 24-well plate (Corning) at 150,000-170,000 cells/well for 24 hours and treated with colchicine for 1 hour.

Example 2. Development of Devices and Methods for Multiplexed Molecular Tracking

Generation of Molecular Sensors

G-protein coupled receptor-activation-based ("GRAB") optical molecular sensors were generated by fusing an endogenous, membrane-targeted G-protein coupled receptor (GPCR) to a circular-permuted GFP (cpGFP). These sensors are intensiometric such that the fluorescence output of the cpGFP is proportional to the concentration of ligand in the surrounding fluid, and some also exhibit ligand-dependent changes in fluorescence lifetime, allowing for stable long-term recordings over hours to days (Sun et al. *Cell.* 174(2):481-496; 2018; Wan et al. *Nat Neurosci.* 24(5):746-752, 2021; Ablikim, *Phys. Rev. Lett.* 123(12):122003, 2019) (**FIG. 1**). A large panel of such sensors, each of which is specific to a different hormone, neurotransmitter, neuromodulator or metabolite, has been developed (Sun et al. *Cell.* 174(2):481-496; 2018; Wan et al. *Nat Neurosci.* 24(5):746-752, 2021; Ablikim, *Phys. Rev. Lett.* 123(12):122003, 2019). The presently used GRAB sensors are specific for: serotonin (5-HT), cholecystokinin (CCK), arginine vasopressin (AVP), oxytocin (OXT), corticotropin-releasing factor (CRF), vasoactive intestinal peptide (VIP), histamine (HA), neuropeptide Y (NPY), adenosine (Ado), norepinephrine (NE), dopamine (DA), acetylcholine (ACh), parathyroid hormone (PTH), urocortin (Uro), neurotensin (NTS), somatostatin (SST), sphingosine-1 phosphate (S1P), adenosine triphosphate (ATP), and melatonin (Sigma, Bachem). These probes can track real-time changes in the concentration of all of these ligands simultaneously.

Construction of the Probe

Molecular sensor HEK293T cells were separately transfected with different molecular sensors (e.g., coupled ligand receptors and optical labels; **FIG. 1**) with plasmids, each encoding a single GRAB sensor and mCherry, using standard EFFECTENE® (Qiagen) transfection reagent. After 24 hours, the cells were treated with colchicine or another microtubule stabilizing/depolymerizing agent to prevent cell

movement and proliferation. The day of an experiment, colchicine-treated cells were detached, washed with PBS and centrifuged twice for 10 minutes at 500xg to remove any colchicine, debris, or media. After the second spindown, the supernatant was aspirated, and the cells were encapsulated in a hydrogel containing variable concentrations of MATRIGEL® (Corning), collagen I (Corning), hyaluronic acid (Advanced BioMatrix) and/or alginate (Sigma) at a concentration of 250,000-1,000,000 cells/uL. In one example, the hydrogel included 25-50% MATRIGEL® (stock concentration ~20 mg/mL) and 50-75% collagen I (stock concentration ~10 mg/mL). To prepare this hydrogel, the cell pellet was suspended in 5-10 uL of MATRIGEL® (thawed from -20 ºC on ice for 25-30 minutes) and 10-15 µL of collagen I (kept on ice) and the solution was thoroughly homogenized with a pipette. Because the polymerization of MATRIGEL® and collagen was temperature-sensitive, the cell/hydrogel solution was immediately placed on ice.

In the cold room, the hydrogel/MATRIGEL® mixture, encapsulating the molecular sensor cells transfected with different molecular sensors, was drawn into a pulled capillary tube and 100-200 nL of the mixture was applied to the end of a probe including a 500 µm diameter gradient refractive index (GRIN) lens, which can be implanted into biological tissue and/or used for deep-tissue two-photon imaging (**FIG. 2A**). The lens was then transferred to a humidifying chamber kept at 37 °C in a tissue culture incubator for gel polymerization. Gel polymerization took about 10 minutes.

In particular, a GRIN lens (singlet, 8.85 mm length, 500 µm diameter, GRINTECH) was inserted in polyimide tubing extending 0.3 mm beyond the tip to form a receptacle that was then coated with a thin layer (~50 µm) of Kwik-Sil (World Precision Instruments), a silicone-based elastomer. The elastomer surface was then etched with oxygen plasma treatment and/or a 2.5% potassium hydroxide solution (Sigma), washed in phosphate buffered saline (PBS, ThermoFisher) and (optionally) coated with an additional layer to enhance adhesion (such as poly-L-lysine (Sigma), glutaraldehyde (Sigma), gelatin (Sigma) or chitosan (Heppe Medical Chitosan Gmbh)). The hydrogel mixture was then applied to the receptacle (~200 µm thick) and allowed to form a gel that stably adheres to the receptacle. The different molecular sensors all included optical labels including green fluorescence protein (GFP).

Immediately after polymerization of the hydrogel and adherence to the distal end of the probe, the probe was immersed in the same media used to culture cells (DMEM + 10% FBS + 10% P/S for 1-2 hours. Finally, the probes were briefly washed in PBS before being inserted into an animal. Liposomal particles prepared with sensor proteins embedded in their membranes as well as microbeads covalently or non-covalently coated with purified soluble sensor proteins can be similarly encapsulated in such a hydrogel for probe development.

The probe was sequentially dipped into different wells of a multiwell plate including dilution series of different ligands and imaged via two-photon imaging (**FIG. 2B**) using a two-photon microscope (Neurolabware, Spectra-Physics) in order to (1) spatially demultiplex sensor signals and (2) establish quantitative dose-response curves for each ligand. Molecular sensor cells within the hydrogel/ MATRIGEL® mixture exhibited fluorescence when dipped into wells corresponding to the ligand receptor of the corresponding molecular sensor cell (**FIG. 2C**). Fluorescence signal intensity depended on the concentration of the ligand within the well (**FIG. 2D**). Each well, e.g., including a particular ligand at a particular concentration, increased the fluorescence of only one type of molecular sensor cells (e.g., including a molecular sensor including the corresponding ligand receptor) in a manner proportional to

ligand concentration (in a sigmoidal manner consistent with the use of endogenous receptors for that signal), allowing for calibration of quantitative dose-response curves for each ligand and spatial demultiplexing of the detected sensor signals (e.g., based on different ligand-molecular sensor cell pairs).

During the dipping, volumetric imaging of these gel-embedded sensor arrays (2 vol/s) was performed using a two-photon microscope with an electrically tunable lens (imaging green and red fluorescence to allow ratiometric normalization to a control mCherry tag). The resulting fluorescence patterns provided a fingerprint for the identity and level of each signal, corresponding to each sensor cell within the imaging area of the probe. To perform this probe calibration, the GRIN lens was placed in a custom holder secured in focus below the microscope objective (Thorlabs), and motors were programmed to move the hydrogel at the front of the lens through sequential dips into the wells of a 384-well plate (20 µL of fluid per well, 1 minute/well and 15 sec/transfer). Calibration wells contain buffered saline or 1 of 6 logarithmically spaced concentrations of each signal (12 sensors x 12 signals = 144 wells). Additional wells containing samples of biological fluids (e.g., blood, plasma, cerebrospinal fluid (CSF)) can be added and analyzed in parallel at high throughput. An average of 10 fluorescence volumes in each well (30 z-planes, 5 µm/plane, 0.5 vol/s, ~500x500 µm2 FOV; electrically tunable lens, 980 nm excitation <30 mW, red and green emission collected for ratiometric analyses of GRAB:mCherry) was acquired. Concurrently, 2-photon fluorescence lifetime estimates were obtained for each plane via the GRIN lens (Lutas et al. *Cell Rep.* 38(4):110297, 2022; Zhang et al. Nature. 597(7875):245-249, 2021), which were sensitive to GRAB ligand binding (Ma et al. bioRxiv. 2022.09.28.510014, 2022) but insensitive to photobleaching. Volumes were non-rigidly aligned using custom software we developed previously (MATLAB) (Shipley et al., *Neuron.* 108(4)623-639, 2020) and fluorescence time courses were extracted and corrected for out-of-focus fluorescence. The distinct spatial patterns are illustrated in **FIG. 3** for response patterns of an array of sensors during sequential delivery of saturating levels of dopamine, adenosine, norepinephrine, histamine, acetylcholine, and melatonin.

The ability to estimate the concentration of ligands was demonstrated in **FIG. 4A** for serotonin and **FIG. 4B** for arginine vasopressin (AVP) sensors upon dipping into various concentrations of ligand. The EC50 concentrations of the device (e.g., of the molecular sensors) were found to be in the 10s to 100s of nM range and were similar to the sensitivities of the endogenous GPCRs upon which each molecular sensor was built, ensuring sensing of a biologically relevant range of signal concentrations.

Example 3. Real Time, Multiplexed Tracking of Cerebrospinal Fluid Components in Awake, Behaving Mice

The method and device described in Example 2 was modified and tested *in vivo* in awake mice implanted with a chronic cannula in the lateral ventricle. A gradient refractive index (GRIN) lens and sensor array was acutely inserted each day, together with contralateral intracerebroventricular injection (ICV) delivery of arginine vasopressin (AVP) or other signals (**FIG. 5**). Two stainless steel guide cannulae (inner diameter (ID) 0.62 mm) were implanted in adult mice (P56-70; one vertical cannula in the anterior portion of left lateral ventricle, another in the right lateral ventricle, occipital angle). After two weeks of recovery, mice were head-fixed for two-photon imaging and a 0.5 mm diameter GRIN lens decorated with fluorescent sensor expressing HEK293T cells was acutely introduced into the anterior guide cannula such that the cells contact the cerebrospinal fluid (CSF). Optionally, a catheter was introduced into the

contralateral cannula for ICV infusions. Two-photon imaging of the HEK293T was then performed through the GRIN lens while the mouse ran on a running wheel and was subjected to sensory or pharmacological stimuli. Each session involved a 30-minute acclimation period, followed by 2-3 hours of two-photon imaging (same parameters as in probe calibration step). In each session, changes in signal levels during voluntary locomotion, changes in arousal indexed by pupil area, and changes in facial expressions were tracked.

FIG. 6A shows data from an example session in which CSF levels of serotonin was optically measured in a head-fixed mouse running on a treadmill (following multiple habituation sessions). Stable recordings were observed across two hours. Fast changes in CSF serotonin in response to a tail pinch (e.g., at 45 minutes), as well as to a saturating dose of serotonin (5 mM serotonin in artificial CSF (aCSF) (30 µL at 5 µL/min)) delivered ICV (e.g., at 95 minutes) were detected using the probe. Multiple molecular sensors could be tracked and discriminated between *in vivo*. **FIG. 6B**, left, shows an array of sensors imaged in vivo. **FIG. 6B**, middle, shows responses to ICV delivery of norepinephrine (NE; 5 mM, 30 µL at 5 µL/min) in vivo. The molecular sensor array (e.g., probe) was then removed from the mouse and imaged the same cells *in vitro* during dipping in various standards. The same pattern of cells sensitive to NE *in vivo* were again selectively activated by NE delivered *in vitro* (1 µM NE in PBS). A similar multisensory experiment showed a clear time course of elevated CSF AVP *in vivo* following intraperitoneal (IP) AVP delivery (**FIG. 6C**).

Multiplexed molecular sensor cell tracking was also demonstrated in a head-fixed awake mouse through parallel tracking of fluorescence change in serotonin (5HT), arginine vasopressin (AVP), and oxytocin (OXT) molecular sensor cells (**FIG. 7A)**. A 15 second tail shock (0.4 mA, 5 ms pulse, 50 Hz) was administered to the awake mouse at 60 minutes; a 30 second tail shock was administered at 90 minutes; AVP was administered intraperitoneally at 120 minutes; OXT was administered intraperitoneally (IP, 200 µL/sec) at 165 minutes; and norepinephrine was administered intracerebroventricularly (ICV, 5 µL/min) at 210 minutes. The change in fluorescence in the 5HT, AVP, and OXT molecular sensor cells following these stimuli was plotted as a timecourse for each of the molecular sensor cells (**FIG. 7B**). The timecourses for each type of molecular sensor cells were consistent, further demonstrating the robustness of this technique to multiplexing.

These data demonstrate that peripherally-delivered AVP and OXT traffick into and accumulate in CSF within 20 minutes of administration. This contrasts with common assumptions that peripheral signaling molecules are generally restricted from the brain. Instead, these results show that acute rises and drops in blood components can be rapidly reflected in the CSF. It was also observed that 5HT levels in CSF spontaneously rose during the first 30 minutes of the recording (before any tail shocks or injections) and precipitously dropped over the following two hours. These dynamics could reflect the yet-unexplored possibility that CSF-borne 5HT serves a signaling role in the brain to modulate neural activity and/or regulate CSF volume/composition. In sum, these results demonstrate the measurement of real-time fluctuations in the absolute and relative concentrations of a large and ever-expanding panel of neuromodulators, peptides, and metabolites in biological fluids, non-destructively and directly inside the animal. The molecular sensor cells' unique combination of rapid temporal resolution with scalable molecular resolution allowed for the study of previously unobservable dynamics in the composition of

minute fluid volumes, such as live mouse CSF, and has already augmented and revised longstanding assumptions in the field.

Example 4. Real-Time Tracking of Neuromodulator Dynamics During Intranasal AVP Therapy and Social Stimulation in Autism Spectrum Disorder Mouse Model

Mouse model and rigor: Male Magel2$^{+/-p}$ mice (C57BL/6J background, Jax #009062) are bred to female Magel2$^{+/+}$ mice to produce phenotypically mutant Magel2$^{+/-p}$ and control Magel2$^{+/+}$ offspring. 8M/8F KO and control mice/condition (Postnatal (P)45-P90) will be used. Sex is considered as a biological variable. Power analyses is used to estimate optimal group sizes once initial data are collected. Analyses do not assume equal variance and are corrected for multiple comparisons. To reduce bias, experimenters are blind to experimental conditions, and analyses are automated using MATLAB when possible.

The sensor array (i.e., probe) described herein is to quantitatively and simultaneously track time-varying concentrations of CSF levels of 12 signals, each of which have been recently validated, and each implicated in autism spectrum disorder (ASD): arginine vasopressin (AVP), oxytocin (OXT), somatostatin (SST), dopamine (DA), norepinephrine (NE), serotonin (5-HT), acetylcholine, histamine, melatonin, corticotropin-releasing factor, vasoactive intestinal peptide, and adenosine. In particular, in Magel2$^{+/-p}$ mice, AVP, OXT, and SST levels are reduced, and DA and 5-HT signaling is impaired. CSF signals are tracked in head-fixed mice (after extended habituation to restraint) across 2-hr daily sessions, at baseline (early light or dark cycle), during presentation of novel social/non-social odors (volatile fraction of urine or peanut butter), and during intranasal or intracerebroventricular AVP delivery. Facial videography is used to relate sensor signals with facial expressions [36], changes in arousal (pupil diameter), and locomotion across manipulations, using machine learning and generalized linear models. Novel or salient social odors in head-fixed mice should drive diverse changes in AVP and other neuromodulators in healthy mice but not in Magel2$^{+/-p}$ mice. Titrated intranasal delivery of AVP (at 0.67, 1.33, or 2.66 µg/mouse across sessions) to Magel2$^{+/-p}$ mice prior to social stimuli should normalize levels of both AVP and other signals to those observed in control mice.

Example 5. Closed-Loop, High-Dimensional Control of Neuropeptidergic Tone on a Mouse Model of Autism Spectrum Disorder

Closed-loop CSF delivery methods are developed in freely moving mice, to match the dynamics of AVP released into the lateral septum of Magel2$^{+/-p}$ mice to those in control mice, both at baseline and during social and non-social interactions. A paradigm involving novel and non-novel social interactions in Magel2$^{+/-p}$ and control mice identical to that used in Borie et al. (Borie et al. *J. Clin. Invest.* 131(2):e144450, 2021) is used. However, instead of rescuing behavior and EEG arousal patterns as in Borie et al., using delivery of nonphysiological levels of AVP or optogenetic stimulation of AVP axons in lateral septum, AVP is delivered ICV in lateral ventricle adjacent to lateral septum in closed loop, to attain a desired level of AVP (e.g., at a level of 40% above baseline) (see **FIGS. 8A-8D**). The goal is to determine the minimal dosing and optimal dynamics of ICV delivery to maintain consistent rescue of social interactions without side effects or adaptation due to high dose, bolus administration, in order to inform future intranasal and ICV delivery approaches.

AVP levels are tracked in lateral septum using fiber photometry recordings (**FIG. 8A** and **8B**) following lateral septum viral expression of a novel AVP sensor. At the same time, increasing levels of AVP are delivered to the lateral ventricle (**FIG. 8C**), inversely proportional to the current AVP levels estimated using real-time feedback from the online photometry recordings (**FIG. 8D**). Increases in AVP levels are titrated proportional to the degree of inappropriate behavioral and cortical EEG responses during encounters with novel or familiar mice or objects (behavior measured using online analyses of high-speed videography). Briefly, the number of social investigations per minute is measured, and the delivery rates of AVP are updated each minute using a staircase procedure scaled to the degree of social impairment. To achieve these goals, a closed-loop system (based on Bonsai software; open-ephys.org) has been developed for ICV delivery of signals including AVP. A real-time place preference assay has also been developed in which ICV delivery is coupled to the animal's location (easily extendable to more complex online analyses of social behavior using real-time, open-source behavioral analysis tools). These closed loop approaches are expected to restore social behaviors and EEG arousal in social settings with greater consistency across the day and requiring lower overall doses of AVP as compared to the acute, bolus dosing used in prior studies, establishing a blueprint for more sophisticated delivery methods in humans with ASD. Closed-loop systems may be similarly constructed by combining quantification of biomolecules in other biological fluids and by delivering of suitable pharmacological and/or sensory (e.g., visual, olfactory, or sensory) stimuli.

Example 6. Single Ligand Monitoring Using Fiber Optic Cable

Multiplexed monitoring is not always required. For applications to monitor a single fluid component, an analogous hydrogel approach may be used using a 400 μm diameter optical fiber (length 6-7 mm, Doric) instead of a GRIN lens, achieving real-time tracking of a single GRAB sensor by fiber photometry in freely moving mice (**FIG. 9A**). Using this approach, natural fluctuations in CSF serotonin was measured on a timescale of tens of seconds, correlated with running speed, which was elicited by neural activity of the dense plexus of serotonergic axons lining the walls of the brain ventricular system (**FIG. 9B**). These results further demonstrated the potential discoveries possible with our probe and sets the stage for an in-depth study of neuromodulation at unprecedented temporal and molecular resolution.

Example 7. Ligand Sensing in Humans

A wide range of potential translational applications may be feasible for this technology. For example, in some applications a drug should be delivered at an optimal dosing regimen to achieve its intended effects. Real-time drug and signaling molecule tracking in blood, CSF, interstitial fluid, and other locations in the body dramatically improves time resolution of bioavailability studies, allow for drug effects on endogenous signaling processes to be tracked in parallel, and allow for drug delivery to be dynamically tuned to maintain any number of parameters within desirable ranges. For example, a blood insulin sensor combined with a standard glucometer could be used for quantitative regulation of insulin doses in closed loop delivery systems. Additionally, parallel tracking of other metabolic hormones such as CCK, ghrelin, and others, improves insulin dosing and effect tracking. A number of ligands for which the molecular sensors have generated (e.g., AVP, CCK, melatonin, and others) could benefit from such refined dosing.

Another outstanding clinical problem related to pharmacodynamics is optimal dosing of sedatives and hypnotics during anesthesia. Inflexible dosing regimens may lead to suboptimal recovery timelines due to longer drug aftereffects and side effects as well as cognitive effects. Real-time anesthetic dose and effect tracking in blood and other bodily fluids complements all other real-time physiology recorded in the OR (pulse, blood pressure, etc.) to inform personalized dosing for each patient. Besides drugs and hormones, other solutes in bodily fluids that are highly informative at real-time resolution include damage signals, such as troponin related to a heart attack, lactate released into CSF during a seizure, and other damage-associated signals characteristic of stroke, trauma, and other acute conditions. Real-time monitoring of such signals in bodily fluids of individuals prone to recurrent damage events allow for substantially earlier detection and treatment of such acute insults when every second counts.

Shunts, catheters, and other long-term implantable devices are commonly applied in both inpatients and outpatients. For instance, many hospitalized patients have a Foley catheter placed for urine collection, but there are no real-time readouts of urine composition, and every analysis requires a new sample. Monitoring the composition of urine dynamically would allow for acute changes in kidney function and other readouts to be detected and acted upon immediately, minimizing lasting damage. CSF shunts are often placed in neurosurgical procedures and left to drain passively without any assessment of the fluid, which almost certainly reflects important real-time information like drug bioavailability in the CSF, acute damage signals reflecting complications such as hemorrhage, infection, and others.

These and other applications to real-time monitoring of bodily fluids in humans could radically improve the timelines of disease detection and treatment as well as refine existing treatments to maximize benefits and minimize side effects.

OTHER EMBODIMENTS

All publications, patents, and patent applications mentioned in this specification are incorporated herein by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the disclosure has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations following, in general, the principles and including such departures from the disclosure that come within known or customary practice within the art to which the disclosure pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Other embodiments are within the claims.

CLAIMS

1. A probe comprising an optical sensor, the optical sensor comprising a plurality of particles comprising a ligand receptor coupled to an optical label, wherein the plurality of particles is immobilized on a lens or optical fiber attached at or near a distal end of the probe.

2. The probe of claim 1, wherein the particles are microbeads, liposomes, lipid nanoparticles, or micelles.

3. The probe of claim 2, wherein the ligand receptor is encapsulated within the microbeads, liposomes, lipid nanoparticles, or micelles.

4. The probe of claim 2 or 3, wherein the ligand receptor is coated on the microbeads, liposomes, lipid nanoparticles, or micelles.

5. The probe of claim 1, wherein the particles are cells expressing the ligand receptor.

6. The probe of claim 5, wherein the cells are Chinese hamster ovary cells, BALB/c mouse myeloma cells, human retinoblasts, monkey kidney cells, human embryonic kidney cells, baby hamster kidney cells, mouse Sertoli cells, human cervical carcinoma cells, canine kidney cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor cells, or human hepatoma cells.

7. The probe of claim 6, wherein the human embryonic kidney cells are HEK293T cells.

8. The probe of any one of claims 5-7, wherein the cells are treated with colchicine.

9. The probe of any one of claims 1-8, wherein the lens is a gradient refractive index lens.

10. The probe of any one of claims 1-9, wherein the lens has a diameter of from 100 μm to 1 mm.

11. The probe of any one of claims 1-10, wherein the plurality of particles is encapsulated in a matrix, such as a hydrogel matrix.

12. The probe of claim 11, wherein the hydrogel matrix comprises collagen.

13. The probe of any one of claims 1-12, wherein the ligand receptor is a G-protein coupled receptor (GPCR).

14. The probe of any one of claims 1-13, wherein the optical label is a fluorescent label.

15. The probe of claim 14, wherein the fluorescent label is a fluorescent protein.

16. The probe of claim 15, wherein the fluorescent protein is a green fluorescent protein (GFP), a red fluorescent protein (RFP), or a yellow fluorescent protein (YFP).

17. The probe of claim 15 or 16, wherein the GPCR is fused to the fluorescent protein.

18. The probe of claim 17, wherein the fluorescent protein is GFP or RFP.

19. The probe of claim 18, wherein the GFP is a circular permuted GFP.

20. The probe of any one of claims 1-19, wherein the probe comprises a plastic tubing disposed around the distal end of the probe.

21. The probe of claim 20, wherein the plastic tubing comprises polyimide.

22. The probe of any one of claims 1-21, comprising an array of optical sensors disposed on the lens.

23. The probe of claim 22, wherein the array comprises a first optical sensor to detect a first ligand and a second optical sensor to detect a second ligand.

24. The probe of claim 22 or 23, comprising a plurality of optical sensors, wherein each optical sensor is configured to detect a different ligand.

25. The probe of any one of claims 1-24, further comprising a sensor for tracking physiological indicia.

26. The probe of claim 25, wherein the sensor is a blood flow sensor, a temperature sensor, a heart rate sensor, a blood pressure sensor, or an oxygen saturation sensor.

27. A method of detecting a ligand in a biological sample, the method comprising:

(a) contacting the biological sample comprising the ligand with the optical sensor of the probe of any one of claims 1-26;

(b) allowing the ligand to bind to the ligand receptor, wherein the optical label emits an optical signal upon binding of the ligand to the ligand receptor; and

(c) detecting the optical signal.

28. The method of claim 27, wherein step (c) comprises imaging the plurality of cells.

29. The method of claim 28, wherein the imaging comprises two-photon imaging.

30. The method of claim 28 or 29, wherein a microscope is employed to image the cells.

31. The method of claim 30, wherein the microscope comprises a tunable lens.

32. The method of claim 31, wherein the tunable lens is an electrically tunable lens.

33. The method of any one of claims 27-32, wherein the optical label comprises a fluorescent protein that emits fluorescence.

34. The method of any one of claims 27-33, wherein the biological sample comprises a cerebrospinal fluid (CSF), blood, saliva, lymph, interstitial fluid, bone marrow, or urine.

35. The method of claim 34, wherein the biological sample comprises CSF.

36. The method of any one of claims 27-35, wherein the ligand is a peptide.

37. The method of claim 36, wherein the peptide is a hormone, a cytokine, a chemokine, a regulatory protein, or a neuropeptide.

38. The method of claim 36 or 37, wherein the peptide is arginine vasopressin (AVP), oxytocin (OXT), somatostatin (SST), corticotropin-releasing factor (CRF), vasoactive intestinal peptide (VIP), cholecystokinin (CCK), corticotropin-releasing factor (CRF), neuropeptide Y (NPY), parathyroid hormone (PTH), urocortin (Uro), neurotensin (NTS), sphingosine-1 phosphate (S1P), or insulin.

39. The method of any one of claims 27-35, wherein the ligand is a small molecule.

40. The method of claim 39, wherein the small molecule is a neurotransmitter or a metabolite.

41. The method of claim 39 or 40, wherein the small molecule is serotonin (5-HT), norepinephrine (NE), dopamine (DA), acetylcholine (ACh), histamine (HA), melatonin, adenosine (Ado), adenosine triphosphate (ATP), melatonin, or glucose.

42. The method of any one of claims 27-41, wherein the imaging is performed in vivo.

43. The method of claim 42, wherein the optical sensor of the probe is disposed in vivo through a cannula.

44. The method of claim 43, wherein the cannula is a stainless-steel cannula.

45. The method of any one of claims 42-44, wherein the optical sensor is disposed into a lateral ventricle, blood vessel, kidney, intraperitoneal region, spine, bone marrow, or bladder of a subject.

46. The method of any one of claims 27-45, wherein the method detects a concentration of the ligand in the biological sample.

47. The method of claim 46, wherein the method is used to detect a disease or disorder or to monitor a treatment regimen in a subject having a disease or disorder.

48. The method of claim 47, wherein the disease or disorder is autism spectrum disorder, Prader-Willi syndrome, Schaaf-Yang Syndrome, Fragile X syndrome, diabetes, obesity, atherosclerosis, high blood pressure, or high cholesterol.

49. A method of detecting a concentration of a ligand in a biological sample, the method comprising:

(a) contacting the biological sample comprising the ligand with the optical sensor of the probe of any one of claims 1-26;

(b) allowing the ligand to bind to the ligand receptor, wherein the optical label emits an optical signal upon binding of the ligand to the ligand receptor;

(c) detecting the optical signal; and

(d) correlating the optical signal with a reference signal of a control biological sample to determine the concentration.

50. The method of claim 49, wherein the control sample corresponds to a biological sample from a healthy subject or a subject having a disease or disorder.

51. The method of claim 50, wherein the disease or disorder is autism spectrum disorder, Prader-Willi syndrome, Schaaf-Yang Syndrome, Fragile X syndrome, diabetes, obesity, atherosclerosis, high blood pressure, or high cholesterol.

52. A kit comprising the probe of any one of claims 1-26 and a receptacle for a biological sample.

53. The kit of claim 52, further comprising one or more of a lens, a plasmid, particles, cells, hydrogel matrix components, and a cannula.

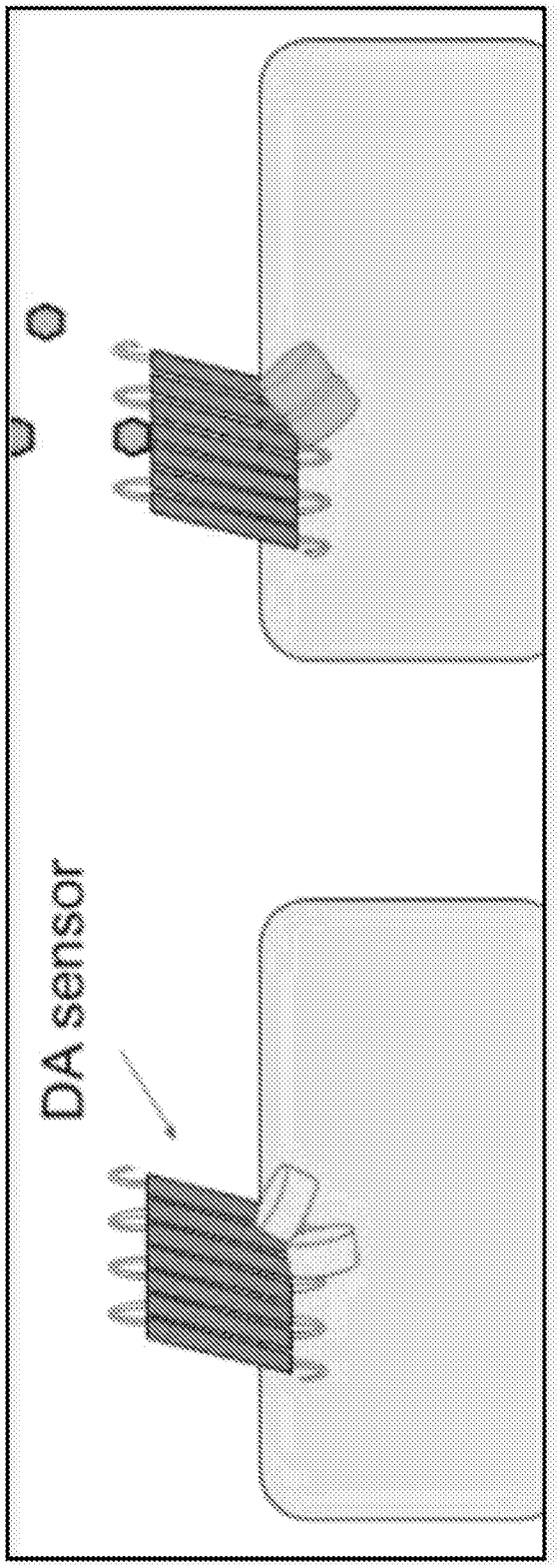


FIG. 1

FIG. 2A

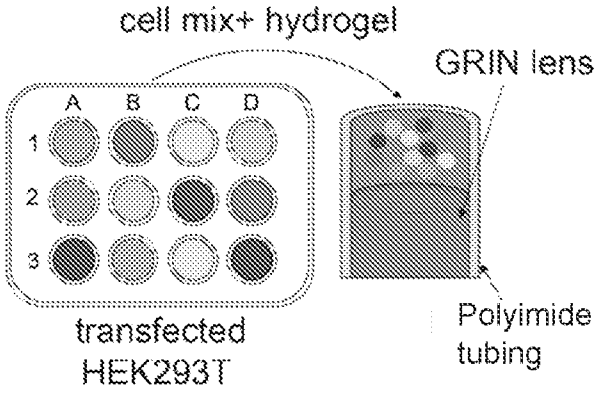


FIG. 2B

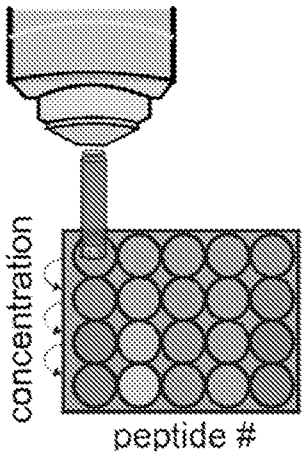


FIG. 2C

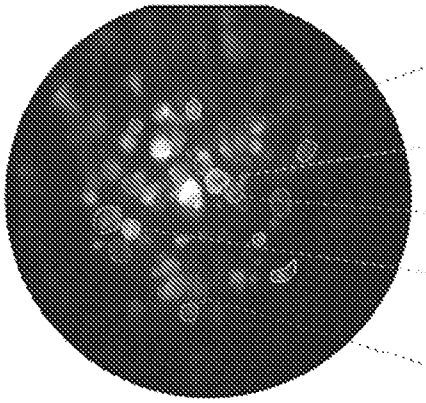


FIG. 2D

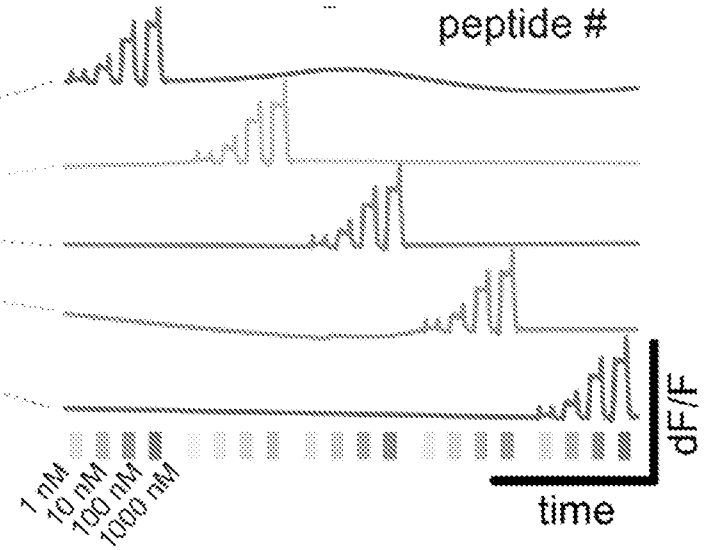


FIG. 2E

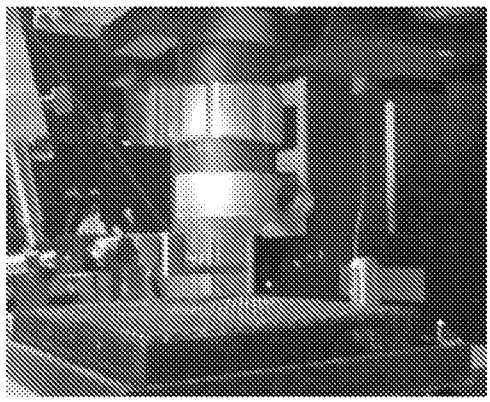


FIG. 3

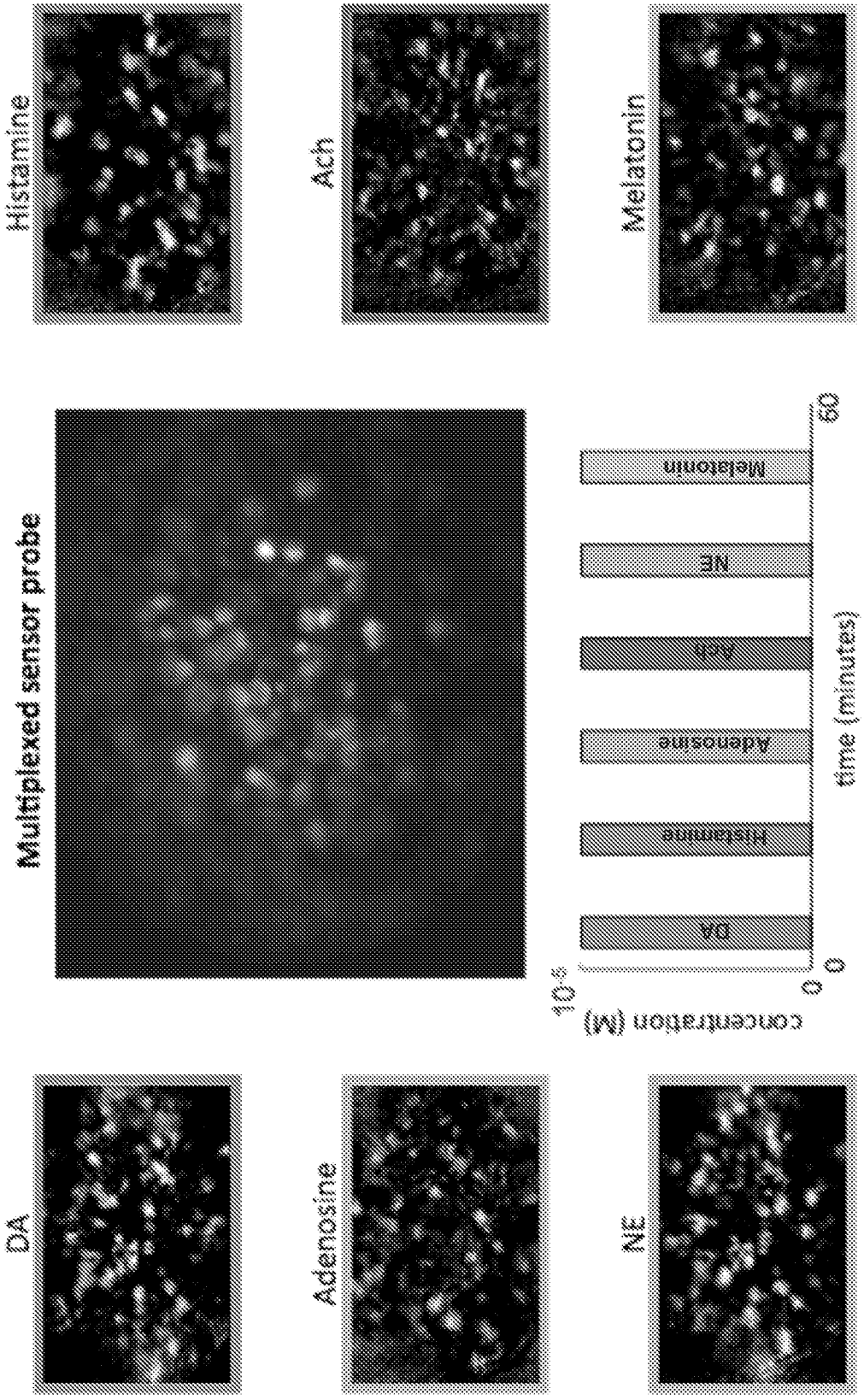

DA
Adenosine
NE
Multiplexed sensor probe
Histamine
Ach
Melatonin
concentration (M)
10-6
0
0
60
time (minutes)
DA
Histamine
Adenosine
Ach
NE
Melatonin

FIG. 4A

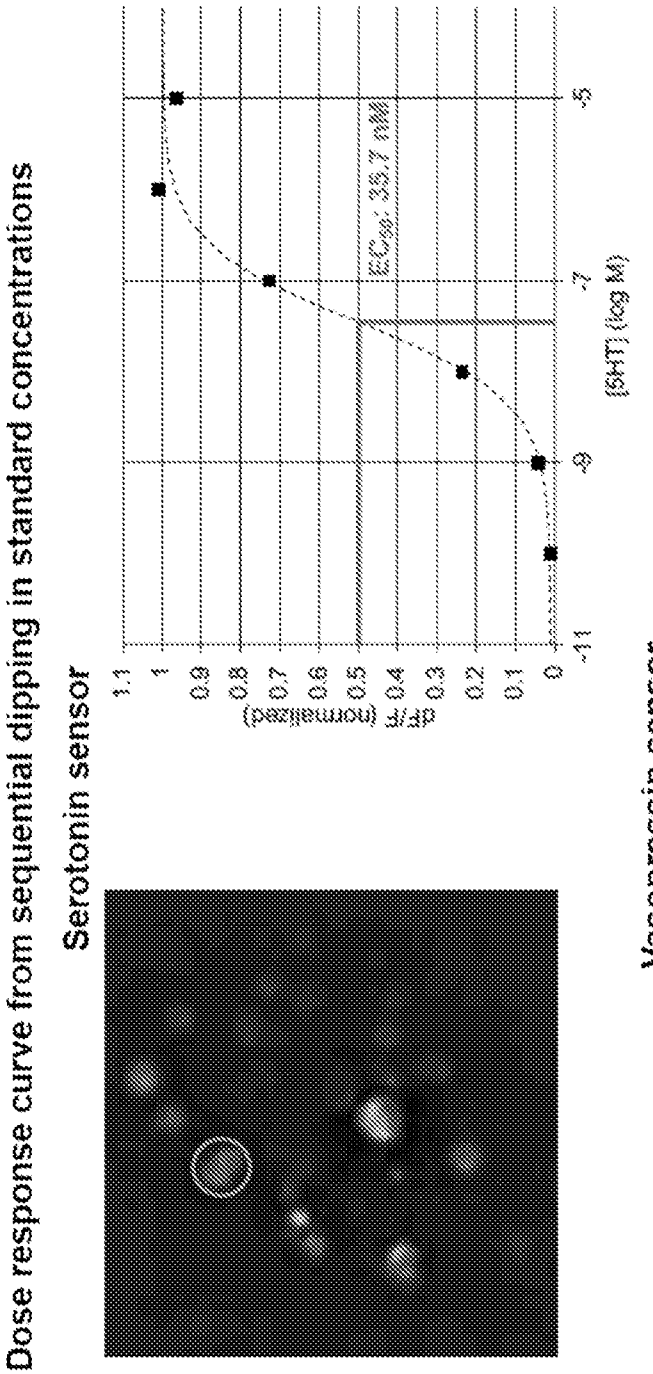

Dose response curve from sequential dipping in standard concentrations
Serotonin sensor
dF/F (normalized)
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-11
-9
-7
-5
[5HT] (log M)
EC50: 39.7 nM

FIG. 4B

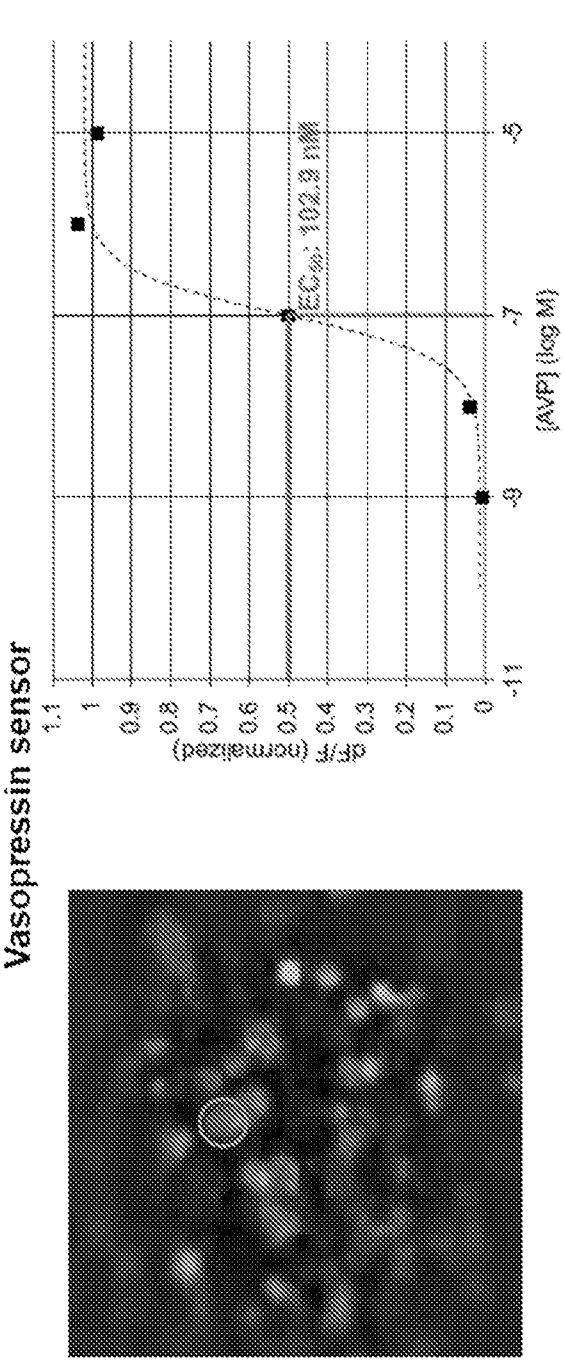

Vasopressin sensor
dF/F (normalized)
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-11
-9
-7
-5
[AVP] (log M)
EC50: 102.9 nM

FIG. 5

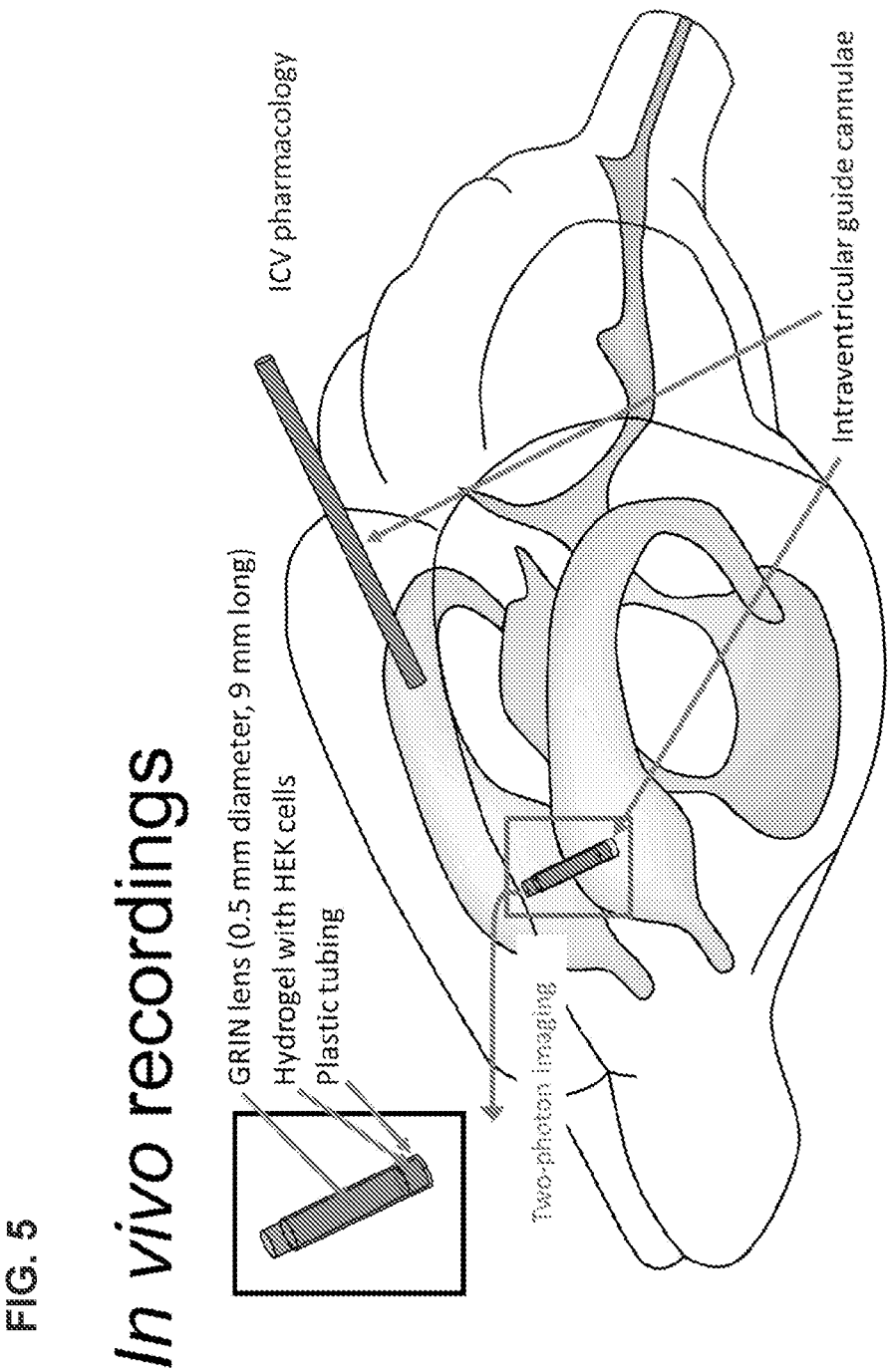

In vivo recordings
GRIN lens (0.5 mm diameter, 9 mm long)
Hydrogel with HEK cells
Plastic tubing
ICV pharmacology
Two-photon imaging
Intraventricular guide cannulae

FIG. 6A

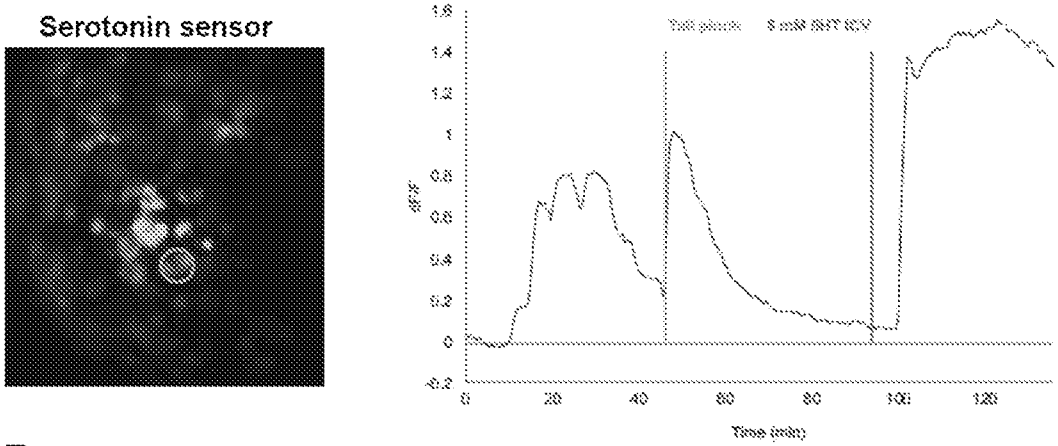


FIG. 6B

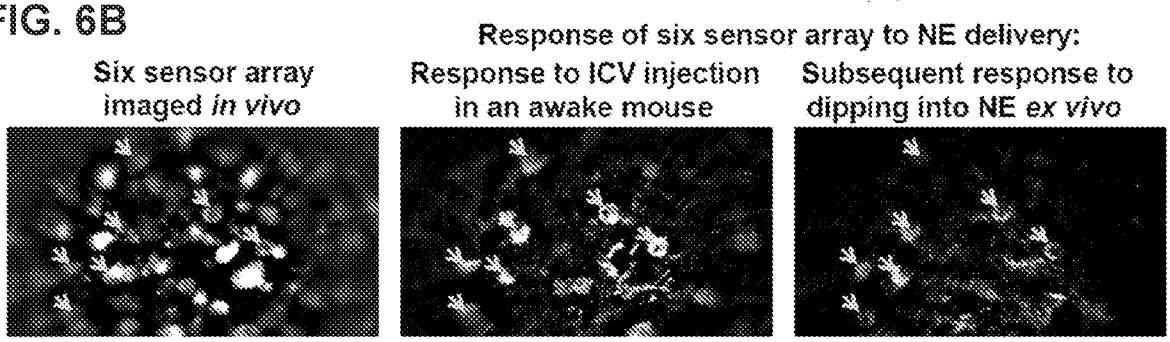


FIG. 6C

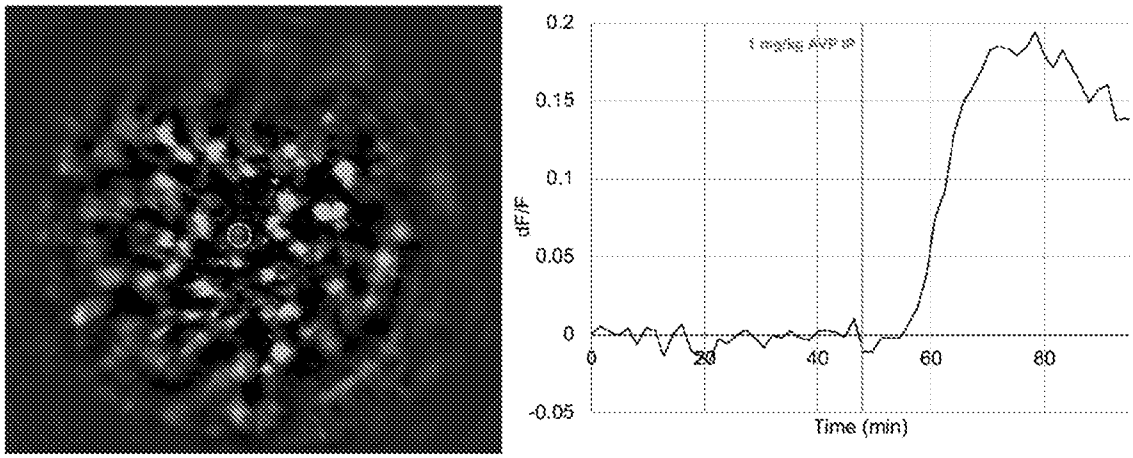


FIG. 7A

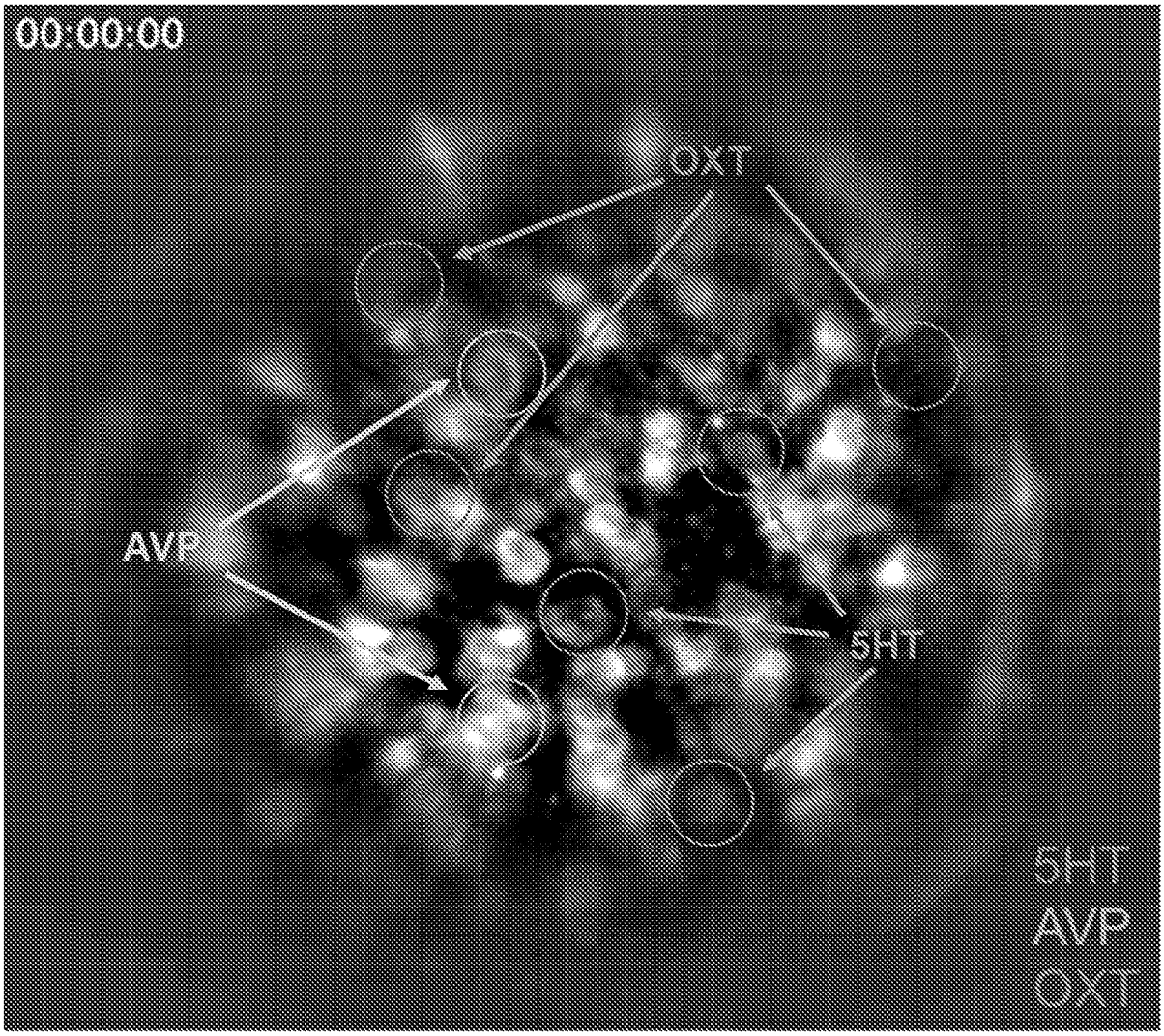
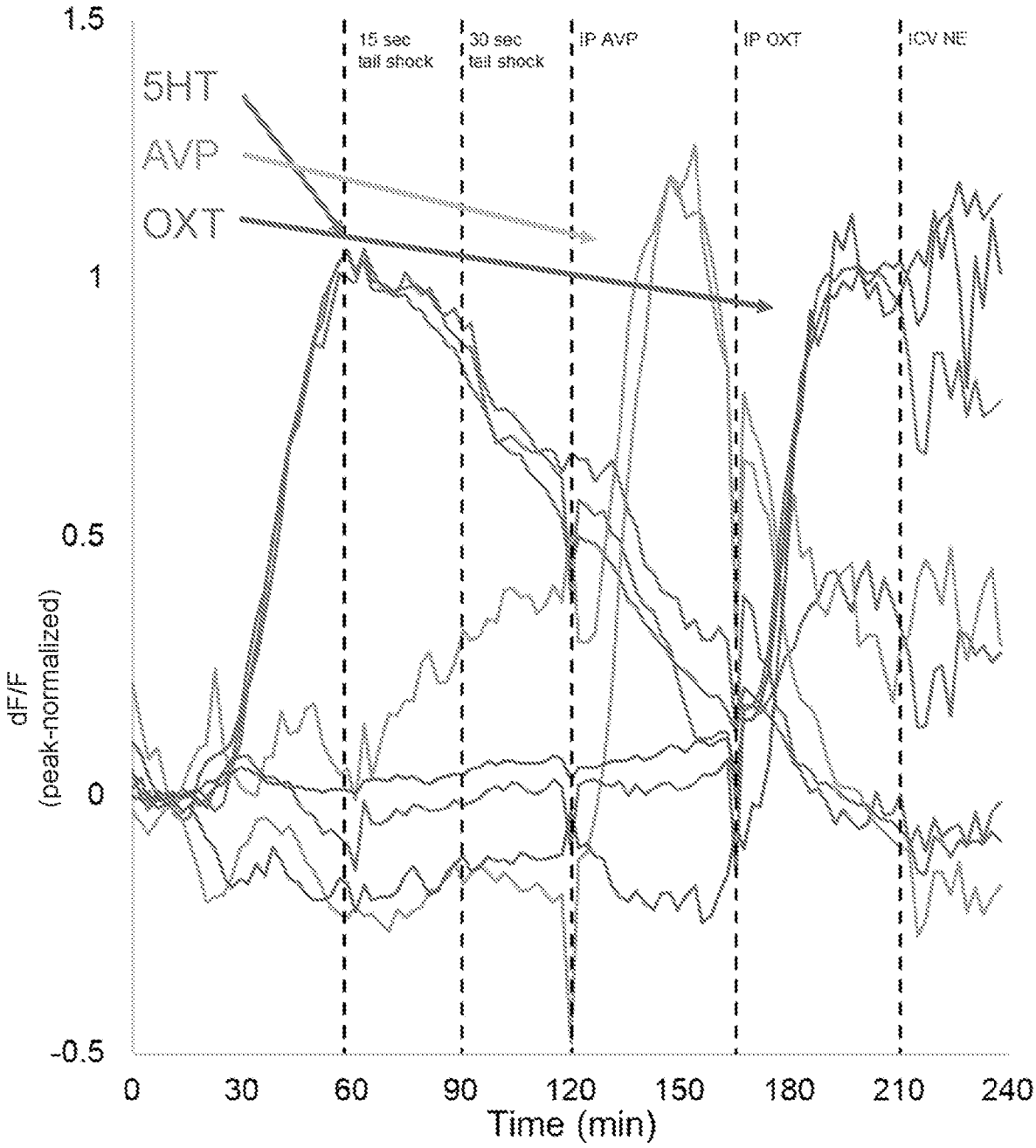


FIG. 7B


1.5
1
0.5
0
-0.5
dF/F
(peak-normalized)
15 sec
tail shock
30 sec
tail shock
IP AVP
IP OXT
ICV NE
5HT
AVP
OXT
0
30
60
90
120
150
180
210
240
Time (min)

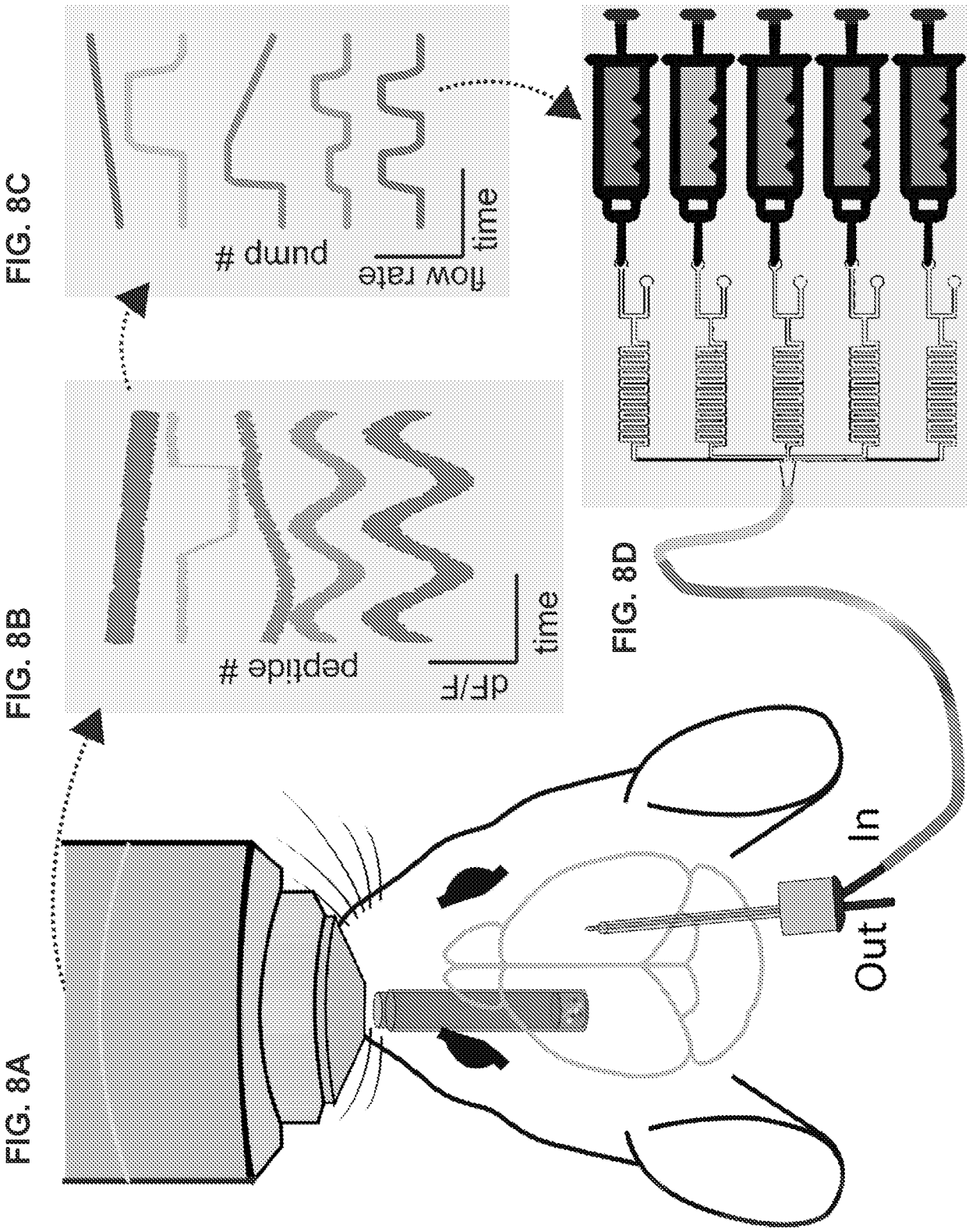


FIG. 8A
FIG. 8B
dF/F
time
peptide #
FIG. 8C
flow rate
time
pump #
FIG. 8D
In
Out

FIG. 9A

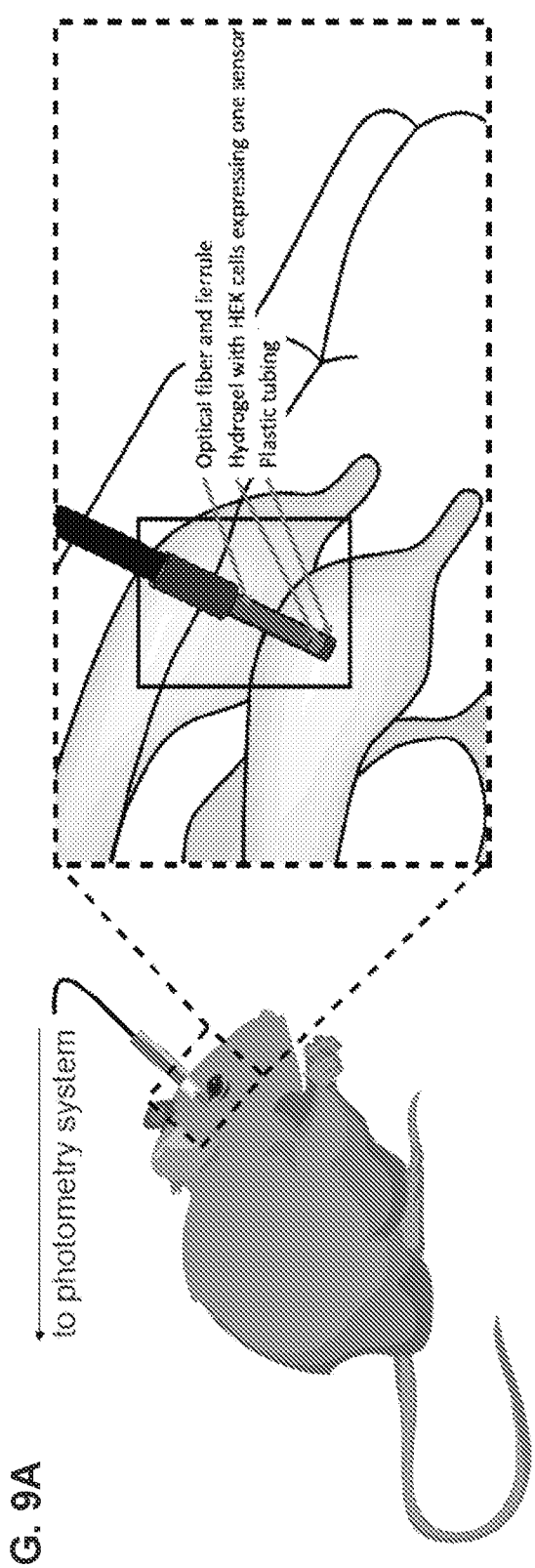
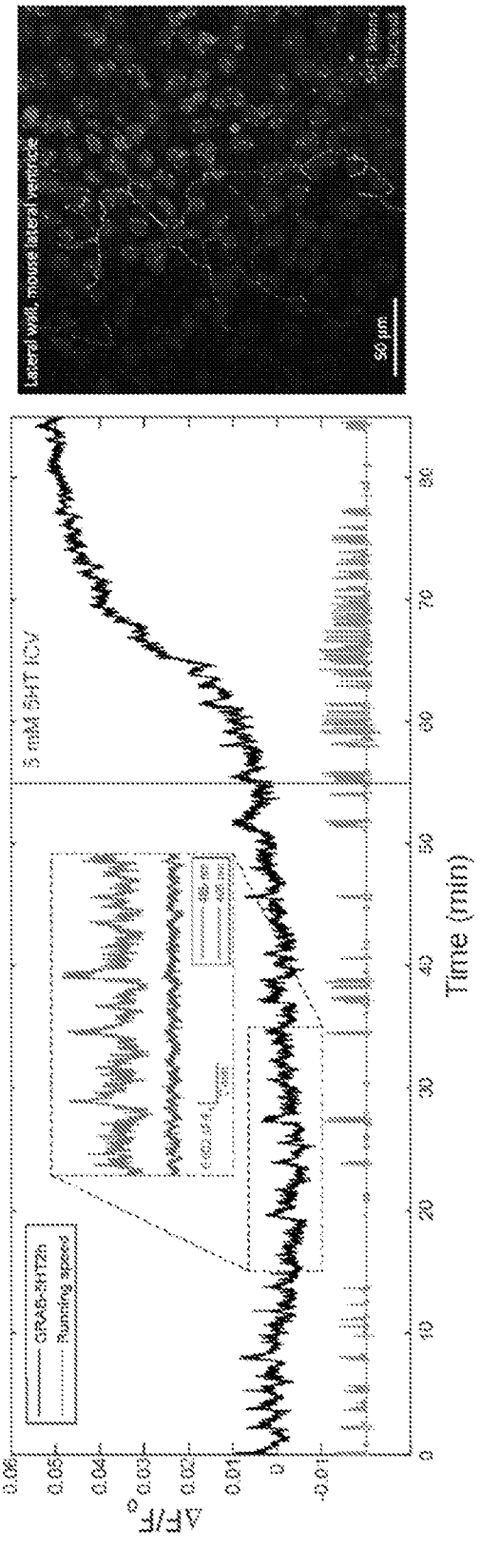

to photometry system
Optical fiber and ferrule
Hydrogel with HEK cells expressing ane sensor
Plastic tubing

FIG. 9B


Lateral ventricle
50 μm
3 mM 5HT ICV
GRAB-5HT2h
Running speed
ΔF/F
Time (min)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 24/12787

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. G01N 33/50 (2024.01)
ADD. G01N 33/74, G01N 33/543, G01N 21/64 (2024.01)

CPC - INV. G01N 33/5044
ADD. G01N 33/54366, G01N 2333/726, G01N 2333/43552

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2005/0158702 A1 (STUELPNAGEL et al.) 21 July 2005 (21.07.2005) para [0039]; [0054]; [0058]; [0062]; [0078]	1-2, 4/2, 5-7 --- 3, 4/3, 8
Y	WO 2000/064492 A1 (SCHULTZ et al.) 02 November 2000 (02.11.2000) abstract	3, 4/3
Y	US 2009/005328 A1 (VERDIN et al.) 01 January 2009 (01.01.2009) para [0005]; [0130]	8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 April 2024 (02.04.2024)	APR 24 2024
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 24/12787

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 9-53
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.