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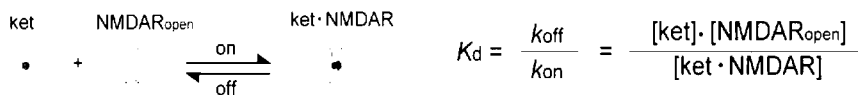


FIG. 13D

(57) Abstract: A method for improving the antidepressant effects of an NMDAR pore blocker is provided, comprising: (1) prior to, or within a plasma half-time after, administering the NMDAR pore blocker, carrying out behavioral, pharmaceutical or physical measures to allow ion channels of a greater proportion of the NMDAR molecules in the lateral habenula (LHb) to be opened; and optionally (2) after administering the NMDAR pore blocker, carrying out behavioral, pharmaceutical or physical measures to prevent or reduce the untrapping of the NMDAR pore blocker molecules from the NMDAR. Therapeutic regimens and pharmaceutical combinations for effectively treating depression are also provided. The disclosure further provides a drug delivery system including an implantable unit that is capable of realizing a controllable, repeated, precise and in vivo delivery of an NMDAR pore blocker and a priming and/or enhancing agents in the LHb region of the subject's brain to thereby achieve an improved therapeutic effects.

METHODS AND DEVICES FOR TREATING DEPRESSION

BACKGROUND

Depression is a mood disorder that causes a persistent feeling of sadness and loss of interest, and it is estimated that around 3.8% of the population, or 280 million people, experience depression worldwide. There have been a wide variety of medications developed for treating depression (e.g. selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, N-methyl-D-aspartate (NMDA) receptor antagonists, etc.), yet the less-ideal efficacy and serious side effects that are frequently found during the treatment process have seriously limited the clinical application of these antidepressants.

SUMMARY

In order to address the issues associated with existing antidepressant medications, the present disclosure provides methods for increasing the duration of the therapeutic effects (i.e. antidepressant effect) of an NMDAR pore blocker (e.g. ketamine) administered to a subject with depression, therapeutic regimens, pharmaceutical combinations, and implantable drug delivery systems that are capable of efficiently delivering such drugs with durable therapeutic effects.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1L show results that single injection of ketamine causes sustained antidepressant effects and prolonged suppression of LHb bursting activity. FIG. 1A, Brain concentration of ketamine after a single i.p. injection of ketamine in CRS mice, as measured by LC-MS/MS. Behavioral effects at different time points, as measured in (FIGS. 1B and 1C) and FIGS. 2A-2D, are marked. Dotted line indicates the half-life of ketamine, which is about 13 min. FIGS. 1B and 1C, Behavioral effects at 1 h and 24 h after a single i.p. injection of ketamine in CRS mice in the FST (FIG. 1B) and the SPT (FIG. 1C). Sal: saline. Ket: ketamine. FIG. 1D, Experimental paradigm for LHb slice recording after i.p. injection of ketamine (10 mg kg⁻¹) in CRS mice. FIG. 1E,

Representative traces showing spontaneous activity of three LHb neuron types, burst-firing, tonic-firing and silent. FIG. 1F, Pie charts illustrating the percent abundance of the three types of LHb neurons in CRS mice at different time points after saline or ketamine i.p. injection. FIG. 1G, Experimental paradigm for *in vivo* recording after a single injection of ketamine (i.p., 10 mg kg⁻¹) in CRS mice. FIG. 1H, Illustration of *in vivo* single-unit recording in the LHb. FIG. 1I, An example recording site stained with DAPI. White dotted lines demarcate MHb and LHb. White arrows indicate the electrode tracks. Scale bar, 100 μ m. LHb: lateral habenula. MHb: medial habenula. FIG. 1J, Example traces showing *in vivo* neuronal activity recorded in CRS mice before, 1 h and 24 h after saline (green, left) or ketamine (blue, right) administration. Bursts (pink shades) are identified by the ISI method (see Methods). FIGS. 1K and 1L, Bar graphs illustrating the bursting spike frequency (FIG. 1K) and bursts per minute (FIG. 1L) in CRS mice at different time points after saline or ketamine i.p. injection. Note that data is not normally distributed so nonparametric test is used for statistics. * $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$; NS, not significant. Error bars indicate SEMs.

FIGS. 2A-2D show results that single injection of ketamine no longer causes antidepressant effects on day 3 or day 7, related to FIGS. 1A-1L. FIG. 2A, Schematic of LC-MS/MS (left) and experimental paradigm for behavioral tests (right) after i.p. injection of 10 mg kg⁻¹ ketamine. FIG. 2B, Plasma concentration of ketamine after a single i.p injection of ketamine in CRS mice, as measured by LC-MS/MS. Dotted line indicates the half-life of ketamine. FIGS. 2C and 2D, Behavioral effects at 3 d and 7 d after a single i.p. injection of ketamine in CRS mice in the FST (FIG. 2C) and the SPT (FIG. 2D). Sal: saline. Ket: ketamine. NS, not significant.

FIGS. 3A-3K show results that single injection of ketamine causes prolonged suppression of LHb bursting activity, related to FIGS. 1A-1L. FIG. 3A, Pie charts illustrating the percent abundance of the three types of LHb neurons in naïve mice. FIG. 3B, Pie charts illustrating the percent abundance of the three types of LHb neurons in CRS mice 3 d after saline or ketamine i.p. injection. FIGS. 3C and 3D, Bar graphs illustrating the bursting spike frequency (number of bursting spikes per second, FIG. 3C) and bursts per min (number of bursts per minute, FIG. 3D) in CRS mice at different time points after saline or ketamine i.p. injection. FIG. 3E, Percentage of

blockade of bursting spike frequency or bursts per min at each time point calculated as (saline value - ketamine value)/saline value. FIG. 3F, Recording sites of electrodes in LHb. Black lines indicate location of habenula, green dots indicate recording sites of saline group, blue dots indicate recording sites of ketamine group. FIG. 3G, Example traces showing *in vivo* neuronal activity recorded in CRS mice 3 d after saline (green, top) or ketamine (blue, bottom) administration. Bursts (pink shades) are identified by the ISI method (see Methods). FIGS. 3H and 3I, Bar graphs illustrating the bursting spike frequency (FIG. 3H) and bursts per minute (FIG. 3I) in CRS mice 3 d after saline or ketamine i.p. injection. FIGS. 3J and 3K, Percentage of blockade of bursting spike frequency (FIG. 3J) and bursts per minute (FIG. 3K) at each time point calculated as (baseline value - value of each time point)/baseline value. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant.

FIGS. 4A-4D show results that ketamine preferentially inhibits LHb neurons with high bursting spike frequency, related to FIGS. 1A-1L. FIG. 4A, Histogram distribution of baseline bursting spike frequency of all recorded LHb units. Pie graph showing percentage of neurons with baseline bursting spike frequency larger than 2 Hz. FIGS. 4B and 4C, Top: Scatter plots of the bursting spike frequency of recorded LHb units at baseline state plotted against bursting spike frequency at 0-5 min, 5-10 min, 20-30 min and 1 h after i.p. injection of saline (FIG. 4B) or ketamine (FIG. 4C). Green/blue, grey and red dots indicate neurons showing significant inhibition, no change and significant increase in bursting spike frequency, respectively. Pie graphs show the percentage of inhibited (green in saline group, FIG. 4B; blue in ketamine group, FIG. 4C), excited (red) and unchanged (grey) LHb neurons. Bottom: histogram distribution of baseline bursting spike frequency of the three LHb neuron types. FIG. 4D, Percentage of LHb neurons inhibited by either saline (green) or ketamine (blue) in the “< 2 Hz” and “> 2Hz” (baseline bursting spike frequency) groups. Numbers in each box are percentages of inhibited neurons. Note that a significantly larger fraction of neurons is inhibited by ketamine in the “> 2 Hz group”. * $P < 0.05$; ** $P < 0.01$; **** $P < 0.0001$; NS, not significant.

FIGS. 5A-5R show results that single injection of ketamine in CRS mice causes prolonged inhibition of NMDAR currents in LHb. FIG. 5A, Experimental paradigm for slice recording after

i.p. injection of ketamine (10 mg kg^{-1}) in CRS mice. FIG. 5B, Schematic of the whole-cell recording of evoked synaptic responses in sagittal LHb slices. FIGS. 5C and 5I, Example traces of evoked AMPAR-eEPSCs (-70 mV , measured at the peak) and NMDAR-eEPSCs ($+40 \text{ mV}$, measured at 35 ms after stimulation, dotted line) in LHb neurons in presence of picrotoxin (PTX) at 1 h (FIG. 5C) and 24 h (FIG. 5I) after i.p. injection of saline or ketamine in CRS mice. FIGS. 5D and 5J, Ratios of NMDAR-eEPSCs and AMPAR-eEPSCs (recorded at 1.5 mA stimulation intensity) at 1 h (FIG. 5D) and 24 h (FIG. 5J) after i.p. injection of saline or ketamine in CRS mice. FIGS. 5E, 5G, 5K and 5M, Stimulus-response (input-output) curves of NMDAR-eEPSCs (FIGS. 5E and 5K) and AMPAR-eEPSCs (FIGS. 5G and 5M) of LHb neurons at 1 h (FIGS. 5E and 5G) and 24 h (FIGS. 5K and 5M) after i.p. injection of saline or ketamine in CRS mice. FIGS. 5F, 5H, 5L and 5N, Bar graphs of NMDAR-eEPSCs (FIGS. 5F and 5L) and AMPAR-eEPSCs (FIGS. 5H and 5N) recorded at 1.5 mA stimulation intensity at 1 h (FIGS. 5F and 5H) and 24 h (FIGS. 5L and 5N) after i.p. injection of saline or ketamine in CRS mice. FIGS. 5O and 5Q, Example traces of evoked NMDAR-eEPSCs ($+40 \text{ mV}$, measured at the peak) of LHb neurons in presence of PTX and NBQX at 1 h (FIG. 5O) and 24 h (FIG. 5Q) after i.p. injection of saline or ketamine in CRS mice. FIGS. 5P and 5R, Stimulus-response (input-output) curves of NMDAR-eEPSCs (isolated by application of PTX and NBQX under voltage clamp at $+40 \text{ mV}$) of LHb neurons at 1 h (FIG. 5P) and 24 h (FIG. 5R) after i.p. injection of saline or ketamine in CRS mice. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; NS, not significant.

FIGS. 6A-6G show results that single injection of ketamine in CRS mice causes prolonged inhibition of NMDAR currents in LHb, related to FIGS. 5A-5R. FIGS. 6A, Left: example traces of LHb eEPSCs evoked at $+40 \text{ mV}$ in presence of PTX (black), PTX + NBQX (blue) or PTX + NBQX + AP5 (red) in ACSF. Right: Amplitudes of eEPSCs measured at 35 ms after stimulation onset. Note that eEPSC amplitudes at 35 ms post stimulation are not significantly affected by NBQX blockaded of AMPARs, but are greatly reduced by AP5 blockaded of NMDARs, indicating that the major component at this time point is NMDAR-eEPSC. FIGS. 6B-6C, Bar graphs showing percentage of NMDAR-eEPSCs smaller or larger than 10 pA at 1 h (FIG. 6B) and 24 h (FIG. 6C) after treatment. FIGS. 6D-6F, Bar graphs of ratios of NMDAR-eEPSCs and AMPAR-eEPSCs (FIG.

6D), NMDAR-eEPSCs (FIG. 6E) and AMPAR-eEPSCs (FIG. 6F) of LHb neurons at 1.5 mA stimulation intensity from brain slices prepared at 3 d after i.p. injection of saline or ketamine in CRS mice. FIG. 6G, Percentage of blockade of NMDAR-eEPSCs at each time point calculated as (saline value - ketamine value)/saline value. * $P < 0.05$; **** $P < 0.0001$; NS, not significant.

FIGS. 7A-7M demonstrate the prolonged blockade of LHb NMDARs after ketamine wash off. FIG. 7A, NMDAR-eEPSCs (normalized by baseline) during incubation and wash-out of vehicle, memantine (100 μ M), or ketamine (100 μ M). NMDAR-eEPSCs are isolated by application of PTX and NBQX in Mg^{2+} free ACSF under voltage clamp at -70 mV. FIG. 7B, Bar graphs showing NMDAR-eEPSCs at the end of the 10 min perfusion period (left) and at 50-60 min (right). Mem: memantine. Ket: ketamine. FIG. 7C, Experimental paradigm for behavioral testing after local bilateral infusion of ketamine (100 mM, 0.1 μ l each side) or memantine (100 mM, 0.1 μ l each side) into the LHb in CRS mice. FIG. 7D, Illustration of bilateral implantation of cannulae in the LHb of CRS mice. Hippo, hippocampus. MHb: medial habenula. LHb: lateral habenula. White dashed lines demarcate MHb and LHb. Scale bar, 500 μ m. FIG. 7E, Infusion sites of drugs verified by cholera toxin subunit B (CTB). FIGS. 7F-7H, Behavioral effects at 1 h and 24 h after local bilateral infusion of memantine into the LHb in CRS mice in the FST (FIG. 7F, FIG. 7G) and SPT (FIG. 7H). FIGS. 7I-7L, Behavioral effects at 24 h (FIG. 7I, FIG. 7J) and 7 d (FIG. 7K, FIG. 7L) after local bilateral infusion of ketamine into the LHb in CRS mice in the FST (FIG. 7I, FIG. 7K) and SPT (FIG. 7J, FIG. 7L). FIG. 7M, Behavioral effects at 14 d after local bilateral infusion of ketamine into the LHb in CRS mice in the FST. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant.

FIGS. 8A-8C show the input resistance of recorded neurons, related to FIG. 7A. Input resistance of recorded neurons during baseline, wash-in (at 10 min) and wash-out (at 50 min) periods in ket- (FIG. 8A), mem- (FIG. 8B) and vehicle- (FIG. 8C) treated groups. NS, not significant.

FIGS. 9A-9E demonstrate the recovery of LHb NMDAR-eEPSCs after washout of ketamine in presence of endocytosis blocker Dyngo-4a, related to FIG. 7A and FIG. 10A. FIG. 9A, Dyngo-4a successfully blocks LFS (1Hz, 15min)-induced, endocytosis-based long-term depression of

NMDAR-eEPSCs in hippocampal CA1 neurons. NMDAR-eEPSCs are isolated by application of PTX and NBQX in Mg^{2+} free ACSF under voltage clamp at -70 mV. In the Dyngo-4a group, 30 μM Dyngo-4a is additionally added in ACSF throughout the recording. FIGS. 9B and 9D, In presence of Dyngo-4a, LHb NMDAR-eEPSCs (normalized by baseline) still do not show recovery after wash-out of 100 μM ketamine (FIG. 9B) or 10 μM ketamine (FIG. 9D) in LHb neurons. FIGS. 9C and 9E, Bar graphs showing NMDAR-eEPSCs at the end of the 10 min perfusion period (left) and at 50-60 min (right). Ket: ketamine. The data of vehicle group in (FIG. 9B) was from the data of ket- group in FIG. 7A and the data of vehicle group in (FIG. 9D) was from the data of “no stim” group in FIG. 10A. NS, not significant.

FIGS. 10A-10B show results that neural activity untraps ketamine from NMDARs. FIG. 10A, Top left: a “kick-off” protocol used to untrap ketamine, including five 3-s pairings of presynaptic stimulation (1 Hz) with postsynaptic depolarization (to +10 mV) delivered over a 5 min period. Bottom: NMDAR-eEPSCs (normalized by baseline) during incubation and wash-out of ketamine (10 μM) under three conditions. No stim: no extra stimulation. “Kick-off”: two blocks of “kick-off” activity given after ketamine wash-out. Pre alone: two blocks of presynaptic stimulation alone without postsynaptic depolarization given after ketamine wash-out. Top right: Input resistance of recorded neurons during baseline, wash-in (at 10 min) and wash-out (at 50 min) periods in three different conditions. FIG. 10B, Bar graphs showing NMDAR-eEPSCs at 50-60 min. ** $P < 0.01$; NS, not significant.

FIGS. 11A-11D show the potential reverse correlation between the level of initial NMDAR blockade and the level of recovery. FIG. 11A, NMDAR-eEPSCs (normalized by baseline) during incubation and wash-out of ketamine (10 μM or 100 μM). NMDAR-eEPSCs are isolated by application of PTX and NBQX in Mg^{2+} free ACSF under voltage clamp at -70 mV. Bin is 1 min. Each line represents one recorded cell. The red line shows an example cell with no recovery and the green line shows an example cell with 29 % recovery. The black line is the average of all recorded cells. FIG. 11B, Illustration of the calculation of maximal blockade, blockade at 50-60 min and recovery percentage. The maximal blockade is averaged by the 10-min values during maximal blockade period (there is some variation among individuals, but mostly between 0 min to 20 min).

Recovery percentage is calculated as: maximal blockade – blockade at 50-60 min. FIG. 11C, Bar graph showing the maximal blockade and blockade at 50-60 min of each cell after ketamine perfusion. Each color represents one recorded cell. FIG. 11D, Recovery percentage plots against maximal blockade of LHb-NMDAR-eEPSCs. Each color represents one recorded cell.

FIG. 12 shows that “Kick off” protocol does not induce NMDAR-LTP. NMDAR-eEPSCs (normalized by baseline) after kick off protocol. NMDAR-eEPSCs are isolated by application of PTX and NBQX in Mg^{2+} free ACSF under voltage clamp at -70 mV.

FIGS. 13A-13L show results that activation of LHb input pathway at different ambient ketamine levels regulates ketamine's sustained antidepressant effects bidirectionally. FIG. 13A, Schematic of viral construct, viral injection in the LH and optic fiber implantation in the LHb of CRS mice. FIG. 13B, An example showing bilateral viral injection sites in the LH (top) and viral expression in axon terminals in the LHb as well as canular sites for optic fiber implantation (bottom). LH: lateral hypothalamus. EP: entopeduncular nucleus. LHb: lateral habenula. Scale bar, 200 μm . FIG. 13C, RTPA induced by constant stimulation (635 nm, 40 Hz, 2 ms pulse) of the LH-LHb axon terminals. Left, representative heat maps; Right, percentage of time spent in light-stimulated chamber. FIG. 13D, Equation of dynamic equilibrium for the ketamine-NMDAR interaction. K_d : dissociation constant. FIG. 13E, When ambient ketamine level is lower than K_d , the interaction between ketamine and NMDAR favors more unbinding. FIG. 13F, Experimental paradigm for stimulating LH-LHb terminals at low ambient ketamine level to untrap ketamine from NMDARs. After CRS, RTPA is conducted to confirm the effectiveness of LH-LHb stimulation. 1 h after a single injection of ketamine (i.p., 10 mg kg^{-1}), optical stimulation of LH-LHb terminals (635 nm, 40 Hz, 2 ms pulse, 3 min) is delivered, when ketamine's plasma concentration has dropped to 0.23 μM . Electrophysiological recording or behavioral testing are conducted 23 h later. FIG. 13G, NMDAR-eEPSCs of LHb neurons recorded at 24 h after ketamine or saline injection from experiment in (FIG. 13F). FIG. 13H, Behavioral effects (FST) at 24 h after ketamine or saline injection from experiment in (FIG. 13F). FIG. 13I, When ambient ketamine level is higher than K_d , the interaction between ketamine and NMDAR favors more binding. FIG. 13J, Experimental paradigm for stimulating LH-LHb terminals at high ambient ketamine level to trap more ketamine

into NMDARs. Immediately after a single injection of ketamine (i.p., 5 mg kg⁻¹), optical stimulation of LH-LHb terminals (635 nm, 40 Hz, 2 ms pulse, 3 min) is delivered, when ketamine's plasma concentration is above 6 μM. Electrophysiological recording or behavioral testing are conducted 24 h later. FIG. 13K, NMDAR-eEPSCs of LHb neurons recorded at 24 h after ketamine or saline injection from experiment in (FIG. 13J). FIG. 13L, Behavioral effects (FST) at 24 h after ketamine or saline injection from experiment in (FIG. 13J). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; NS, not significant.

FIGS. 14A-14G show that 40 Hz LH-LHb stimulation induces LHb burst firing and kicks off trapped ketamine *in vivo*. FIG. 14A, Schematic of viral construct, viral injection in the LH of CRS mice. FIG. 14B, Experimental paradigm. FIG. 14C, Schematic of whole-cell recording of LHb neurons *in vitro* and representative trace showing burst firing elicited by pulsed light stimulation (635 nm, 40 Hz, 2 ms pulse, 0.5 mW) of LH terminals in LHb brain slices. FIG. 14D, Experimental paradigm for electrophysiological recording immediately after optical stimulation in CRS mice. Optical stimulation (635 nm, 40 Hz, 2 ms pulse, 3 min) of LH-LHb terminals or no stimulation is delivered at 1 h after ketamine injection. FIGS. 14E-14G, NMDAR-eEPSCs (FIG. 14E), AMPAR-eEPSCs (FIG. 14F) and NMDA/AMPA ratio (FIG. 14G) of LHb neurons recorded immediately after optical stimulation from experiment in (FIG. 14D). * $P < 0.05$, NS, not significant.

FIG. 15 show the brain concentration of ketamine after a single i.p injection of 5 mg kg⁻¹ ketamine in CRS mice, as measured by LC-MS/MS. As noted by the dotted line, the half-life of ketamine is 13 mins.

FIG. 16 illustrates a block diagram of a drug delivery system according to various embodiments. Blocks boxed with dotted lines represent optional features according to certain embodiments.

DETAILED DESCRIPTION

The present disclosure substantially provides methods for improving, or more specifically increasing the duration of, the therapeutic effects (i.e. antidepressant effect) of an NMDAR pore blocker that is administered to a subject in need thereof (e.g. patients with depression, etc.),

therapeutic regimens and pharmaceutical combinations, and implantable drug delivery systems that are capable of efficiently delivering such drugs with durable therapeutic effects.

Throughout the disclosure, the term "subject" refers to a mammal that is susceptible to depression, which may include a human subject, but may also include other primates (e.g. monkeys, apes, etc.) rodents (e.g. mouse, rat, etc.), or other species of mammals; the term "N-methyl-D-aspartate receptor (NMDAR)" refers to the ionotropic glutamate receptor present on the cell membranes of neurons in the brain of a subject, whose activation relies on the binding of agonistic ligands (e.g. glutamate, glycine and NMDA, etc.) and the depolarization of neurons and results in the opening of the ion channel that is nonselective to cations (Ca^{2+} , Na^{+} , K^{+} , etc.); the term "NMDAR pore blocker" or "NMDAR channel blocker", refers to a class of compounds that can block the ion channel (i.e. pore) of NMDAR, which optionally comprises one or more of ketamine, memantine, dextromethorphan (AXS-05), dizocilpine, amantadine, lanicemine, D-methadone, etc., or pharmaceutically acceptable derivatives thereof; the phrase "pharmaceutically acceptable" means that a molecular entity or a composition is generally believed to be physiologically tolerable and does not typically produce intolerable adverse effects (e.g. toxicity, etc.) when administered to a subject; the term "derivative" refers to any pharmaceutically acceptable salt, solvate, or prodrug of a compound which, upon administration, is capable of providing (directly or indirectly) the compound or an active metabolite or residue thereof. Such derivatives are recognizable to those skilled in the art, without undue experimentation. Derivatives are described, for example, in Burger's Medicinal Chemistry and Drug Discovery, 5th Edition, Vol 1: Principles and Practice, which is incorporated herein by reference to the extent of teaching such derivatives.

In a first aspect, a method for increasing the duration of the therapeutic effect of NMDAR pore blocker is provided, comprising at least one of: (1) prior to, or within a first time period after, administering the NMDAR pore blocker or a pharmaceutically acceptable salt thereof to the subject, carrying out a first procedure such that ion channels of a greater proportion of the NMDAR molecules in the lateral habenula (LHb) of the subject are opened to be thereby accessible to molecules of the NMDAR pore blocker compared to when the first procedure is absent; or (2) after administering the NMDAR pore blocker or a pharmaceutically acceptable salt thereof to the subject,

carrying out a second procedure such that untrapping of the molecules of the NMDAR pore blocker from the NMDAR is prevented or reduced.

The term "pharmaceutically acceptable salt" as used herein refers to a form of a therapeutically active agent consisting either of a cationic form of the agent in combination with a suitable anion, or of an anionic form of the agent in combination with a suitable cation. The pharmaceutically acceptable salts for each of the NMDAR pore blockers listed above are well-known to artisans working in the field. For example, the pharmaceutically acceptable salts of ketamine include: (R, S)-ketamine hydrochloride, (R, R)-ketamine hydrochloride, (S, S)-ketamine hydrochloride, ketamine derivatives hydrochloride, etc. Herein optionally, administration of the NMDAR pore blocker or a pharmaceutically acceptable salt thereof can be by means of systemic or local administration. The term "systemic administration" as used herein refers to a route of administration of a pharmaceutical agent into the circulatory system (e.g. vascular system) of a subject such that the entire body of the subject is affected. Examples of a systemic administration include: enteral (i.e. gastrointestinal route) or parenteral route (i.e. any route that is not enteral, including intravenous or intra-articular infusion, intramuscular injection, subcutaneous injection, etc.). The term "local administration" as used herein refers to a route of administration of a pharmaceutical agent directly to a region of interest (e.g. the LHb in the brain) in the body such that only the region of interest is affected while other part of the body is minimally affected. Examples of a local administration include implantation of an implantable drug delivery device or insertion of an indwelling catheter.

When systemic administration is applied, the first time period is the plasma elimination half-time of the NMDAR pore blocker in the recipient subject. Specifically for a human subject, when the NMDAR pore blocker is ketamine, memantine, dextromethorphan (AXS-05), dizocilpine, amantadine, lanicemine or D-methadone, the first time period is approximately 3 hours, 60 hours, 29.5 hours, 16 hours, 9 hours and 33 hours, respectively.

When local administration is applied, it can be by means of a catheter or a controllable implantable device. As used herein, the term "catheter" refers to a thin tube made of a biocompatible material that is inserted or embedded in the body and intended for local drug delivery at a region of interest; optionally the catheter may further comprise a sensing module that is capable of detecting

certain physiological or physicochemical parameters (e.g. temperature, pH, concentration of certain molecules, etc.). When a catheter is employed, it can be arranged such that its drug delivery outlet is disposed within, or in the proximity of, the LHb of the subject. The catheter may be directly utilized for drug delivery, but optionally may be further operably connected to a distally arranged reservoir, such as an Ommaya reservoir (Zubair A et al. 2023; Liu HG et al. 2021), which can be disposed beneath a scalp or elsewhere of the subject and allows for the repeated filling of the injectable contents (i.e. formulations of the NMDAR pore blocker). The term "controllable implantable device" refers to a drug delivery device implanted within the body and designed to realize a controllable drug delivery following a preset program or upon receiving an external instruction. When a controllable implantable device is applied, the device is configured to be capable of local delivery in a controllable manner. Optionally, the implantable device may be wholly embedded within, or in a proximity of, the LHb of the subject. Optionally the implantable device may have Ommaya reservoir-like configuration, comprising a catheter module and a reservoir module that are distally arranged in the body. The catheter module has a drug delivery outlet that is arranged within, or in the proximity of, the LHb of the subject, and is further operably connected the reservoir module accommodates the NMDAR pore blocker.

As used herein, the phrase "ion channels of a greater proportion of the NMDAR molecules in the lateral habenula (LHb) of the subject are opened to be thereby accessible to molecules of the NMDAR pore blocker compared to when the first procedure is absent" in (1) means that the proportion of the NMDAR molecules in the LHb that are opened in the presence of the first procedure is at least 20% (e.g. 20%, 40%, 60%, 80%, 100%, etc.) more than the proportion of the NMDAR molecules in the LHb that are opened in the absence of the first procedure; the phrase "untrapping of the molecules of the NMDAR pore blocker from the NMDAR is prevented or reduced" in (2) means that in the presence of the second procedure, the number of the NMDAR pore blocker molecules previously trapped in the pores of the NMDAR in the LHb that are untrapped or displaced thereoff is zero ("prevented") or smaller ("reduced") than compared to otherwise (i.e. in the absence of the second procedure).

In any of the above embodiments of the method, each of the first and second procedures may comprise behavioral (or psychological), pharmaceutical (or chemical), physical measures or any of their combinations.

According to some embodiments, the first procedure may comprise a first behavioral measure, which may include applying an aversive stimulus to the subject. The term "aversive stimulus" as used herein refers to a noxious or unpleasant stimulus or occurrence intended to elicit aversion, avoidance, and/or withdrawal responses from a subject, which may be realized by negative reinforcement or positive punishment. An aversive stimulus may comprise a stressor, which can be a chemical or biological agent, an environmental condition, an external stimulus or an event that cause stress to an individual or that individual might consider demanding, challenging and/or threatening. Herein, applying an aversive stimulus may include providing one or more unpleasant cues (e.g. visual, auditory, haptic, olfactory, gustatory cues or any combination thereof) to the subject. For example, a human subject may be provided with a virtual reality (VR) device, TV, a computer monitor or a projector, that displays images, sounds or videos, or may be forced in a specially designed environment (e.g. a room with horror or disturbing scenes or on a special device such as a maze, etc.), that can arouse unpleasant, horrified, painful, and/or aversive feelings, and/or recall bad memories/experiences to the subject. A rodent subject may be treated for restrained stress, forced swimming, tail suspension, air puff, social attack, bitter taste, olfactory cue (fox urine, 2,5-Dihydro-2,4,5-trimethylthiazoline (TMT), *ect.*), sleep deprivation, water deprivation, footshock, noise, or looming visual stimulus.

According to some embodiments, the first procedure may comprise a first chemical measure, comprising providing at least one priming agent to the subject. The term "priming agent" as used herein refers to a chemical/biological agent that is capable of facilitating pores/channels of the NMDAR molecules in the LHb to open to thereby become more accessible to the NMDAR pore blocker(s) compared to when the priming agent is absent. Herein, the at least one priming agent may comprise an NMDAR agonist, which may be of any of the following classes: (1) aspartate or derivatives (e.g. D-aspartate, NMDA, L-aspartate, etc.); (2) glutamate or derivatives (e.g. tetrazolylglycine, homocysteic acid, ibotenic acid, quinolinic acid, etc.); (3) glycine or derivatives

(e.g. D-alanine, L-alanine, milacemide, sarcosine, D-serine, L-serine, etc.); (4) polyamine (e.g. spermindine, spermine, etc.); or (5) positive allosteric modulators (e.g. nebotinell, etc.). The at least one priming agent may be systemically or locally delivered.

According to some embodiments, the first procedure may comprise a first physical measure capable of at least one of: (a) increasing the permeability of brain-blood barrier in a brain of the subject so as to increase a local concentration of the molecules of the NMDAR pore blocker in the LHb; or (b) stimulating the activity of the NMDAR in the LHb. For example, focused ultrasound can be utilized to thereby provide an acoustic measure to increase the permeability of brain-blood barrier in the LHb of the subject. Focused ultrasound-mediated blood brain barrier has been applied for localized delivery of drugs, genes or other therapeutical agents in the treatment of other central nervous system diseases (Gorick CM et al. 2022 and Wang J et al. 2022). In addition, a surgical measure can also be applied to realize (a) as described above, whereas a first electrophysical measure, by means of an electrode or an implantable device capable of electrically stimulating the activity of the NMDAR in the LHb can be applied to realize (b) as described above.

According to some embodiments, the second procedure comprises a second behavioral measure, which includes providing an environment to the subject such that the subject is capable of obtaining pleasant feelings (e.g. by providing one or more visual, auditory, haptic, olfactory, and/or gustatory cues that can arouse pleasant feelings) and/or avoiding negative emotional episodes. Herein, the providing one or more such pleasant cues can be by any means, such as by means of a VR device.

According to some embodiments, the second procedure comprises a second chemical measure, comprising providing at least one enhancing agent to the subject. The term "enhancing agent" as used herein refers to chemical/biological agent that is capable of enhancing the therapeutic effects of NMDAR pore blocker(s) as described above compared to when the agent is absent, and as such the enhancing agent may work by antagonizing the NMDAR activity and/or by suppressing the neuronal activity in the LHb of the subject. Optionally, the at least one enhancing agent comprises at least one first reagent, each capable of antagonizing the NMDAR activity, and selected from one of the following classes: (1) NMDAR open channel blocker (e.g. memantine, or dextromethorphan (AXS-05), dizocilpine, amantadine, lanicemine, or D-methadone, etc.); or (2) NMDAR competitive

antagonist (e.g. antagonist-2-amino-7-phosphonoheptanoic acid (AP7), antagonist 2-amino-5-phosphonopentanoic acid (AP5), 4-[(2E)-3-Phosphono-2-propenyl]-2-piperazinecarboxylic acid (CPPene), agonist-1-aminocyclopropanecarboxylic acid (ACPC), rapastinel (GLYX-13), L-4-chlorokyurenine or 4-CI-KYN (AV-101), D-cycloserine, Ro 25-6981, (1S,2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol (CP-101606), ifenprodil, rislenemdaz hydrochloride (MK-0657), etc.). Further optionally, the at least one enhancing agent comprises at least one second reagent, each capable of suppressing the neuronal activity in the LHb of the subject, selected from one of the following classes: (1) Gamma-aminobutyric acid type A receptor (GABA_AR) agonists (e.g. Muscimol, *etc.*); (2) α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor (AMPA) antagonist (e.g. antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)-quinoxaline (NBQX), 6-cyano-7-Nitroquinoxaline-2,3-dione (CNQX), *etc.*); or (3) sodium channel blockers (e.g. tetrodotoxin (TTX), *etc.*).

According to some embodiments, the second procedure comprises a second physical measure capable of suppressing the activity of the NMDAR in the LHb, which can comprise a second electrophysical measure by means of an electrode or an implantable device capable of electrically suppressing the activity of the NMDAR in the LHb.

In a second aspect, a therapeutic regimen for treating a subject with depression is further provided, the therapeutic regimen comprising: (1) administering an NMDAR pore blocker or a pharmaceutically acceptable salt thereof to the subject; and (2) prior to, or within a first time period after, administering an NMDAR pore blocker or a pharmaceutically acceptable salt thereof to the subject, carrying out a first procedure such that ion channels of a greater proportion of the NMDAR molecules in a lateral habenula (LHb) of the subject are opened to be thereby accessible to molecules of the NMDAR pore blocker compared to when the first procedure is absent.

Optionally, the administering of the NMDAR pore blocker or a pharmaceutically acceptable salt thereof comprises systemic administration, and the first time period is the plasma elimination half-time of the NMDAR pore blocker. Optionally, the administering of the NMDAR pore blocker or

a pharmaceutically acceptable salt thereof comprises local administration, which can be by means of a controllable implantable device or by means of a catheter. The different NMDAR pore blockers and their respective plasma elimination half-times, as well as the different embodiments for the local administration means, have been detailed above in the first aspect of the disclosure.

Optionally, the first procedure comprises a first behavioral measure, which comprises applying an aversive stimulus, e.g. providing one or more unpleasant cues (including visual, auditory, haptic, olfactory, gustatory cues or any combination thereof), to the subject. Optionally, the first procedure comprises a first chemical measure, comprising providing at least one priming agent to the subject, wherein each of the at least one priming agent is capable of opening pores of the NMDAR molecules in the LHb. Optionally, the first procedure comprises a first physical measure capable of at least one of: (a) increasing the permeability of brain-blood barrier in a brain of the subject so as to increase a local concentration of the molecules of the NMDAR pore blocker in the LHb; or (b) stimulating the activity of the NMDAR in the LHb. The different embodiments for the first behavioral, chemical and physical measures can reference the first aspect of the disclosure as described above.

According to some embodiments, the therapeutic regimen as described above further comprises: (3) after administering the NMDAR pore blocker or a pharmaceutically acceptable salt thereof to the subject, carrying out a second procedure such that untrapping of the NMDAR pore blocker molecules from the NMDAR is prevented or reduced.

Optionally, the second procedure comprises a second behavioral measure, comprising providing an environment to the subject such that the subject is capable of obtaining pleasant feelings and/or avoiding negative emotional episodes. Optionally, the second procedure comprises a second chemical measure, comprising providing at least one enhancing agent to the subject. Optionally, the second procedure comprises a second physical measure capable of suppressing the activity of the NMDAR in the LHb. The different embodiments for the second behavioral, chemical and physical measures can reference the first aspect of the disclosure as described above.

In a third aspect, a pharmaceutical combination for treating a subject with depression is further provided, comprising: (1) at least one NMDAR pore blocker or a pharmaceutically acceptable salt thereof; and one or both of: (2) at least one priming agent, each capable of opening pores of the

NMDAR molecules in the LHb; or (3) at least one enhancing agent, each capable of suppressing neuronal activity in the LHb. The different embodiments for the NMDAR pore blocker, the priming agent, and the enhancing agent can reference the first aspect of the disclosure as described above.

In a fourth aspect, the present disclosure further provides a drug delivery system, which is capable of realizing a controlled local release of an NMDAR pore blocker within a lateral habenula (LHb) of a subject.

FIG. 16 illustrates a block diagram of a drug delivery system according to some embodiments of the disclosure. As shown, the drug delivery system 001 comprises an implantable unit 1000, and optionally further comprises an external control unit 2000, and/or an external power unit 3000. The implantable unit 1000 is wholly or partially embedded within, or in the proximity of the LHb region in the brain of the subject, which can be implanted via surgery, and is configured to realize a controllable or programmable *in vivo* local drug delivery. The implantable unit 1000 can be designed to substantially comprise a miniaturized multi-module microdevice actuating the passive or on-demand *in vivo* drug release, which can be realized by means of a microchip and/or a microelectromechanical systems (MEMS). The term "module" as used herein refers to an assembly of functionally inter-connected hardware components (i.e. structural, electronic, mechanical, and/or MEMS components) that is designed to carry out a specific functionality, and may optionally also comprise software programs that control these hardware components to operate.

Specifically, the implantable unit 1000 comprises a first reservoir module 400, a dispensing module 300, a control module 200 and a power source module 100. The first reservoir module 400 accommodates a pharmaceutically acceptable formulation of the NMDAR pore blocker. The dispensing module 300 is operably coupled to the first reservoir module 400, and is configured, upon receiving a first activation command from the control module 200, to dispense the NMDAR pore blocker out of the first reservoir module 400. The control module 200 is communicatively coupled to the dispensing module 300 and is configured to control the dispensing module 300 for activation. The power source module 100 is electrically connected, and configured to provide power to the dispensing module 300 and the control module 200 respectively. Herein, the dispensing mechanism by which the dispensing module dispenses the NMDAR pore blocker out of the first reservoir module

can be by means of a microchip (Hilt JZ et al. 2005; Stevenson CL et al. 2012), a micropump (Lo R et al. 2009; Joshitha C et al. 2017), a microvalve (Nafea M et al. 2018), a microprobe (Spieth S et al. 2014), a microneedle (Kim YC et al. 2012), a cantilever (Cheong HR et al. 2018), or a shape memory alloy (Reynaerts D et al. 1997; Ali MSM et al. 2011). The disclosures of these above cited references are incorporated herein by reference in their entireties.

As used herein, the term "pharmaceutically acceptable formulation" refers to a formulation of a therapeutically active agent that is suitable for storage in a chosen medical device, delivery by a chosen route of administration, and is generally tolerated by the subject and exhibits an acceptable toxicity profile when administered at an appropriate dose. As such, a pharmaceutically acceptable formulation may comprise a therapeutically active agent and one or more pharmaceutically acceptable excipients (i.e. substances formulated alongside with the therapeutically active agent for storage, pharmacokinetic, pharmacodynamic, or other considerations), and/or one or more pharmaceutically acceptable carriers (i.e. substances formulated alongside with the therapeutically active agent for efficient drug delivery consideration; e.g. liposome). In one example, the pharmaceutical acceptable formulation of ketamine contained in the first reservoir module of the implantable unit of the drug delivery system disclosed herein may be a liquid injectable solution comprising ketamine hydrochloride diluted in sterile water, 0.9% sodium chloride, or 5% dextrose. Herein, the pharmaceutical acceptable formulation of the therapeutically active agent may be physically (i.e. via different packages) or mechanistically (i.e. dispensed from one single package) divided into a plurality of dosage forms, each containing one single dose.

According to some embodiments of the drug delivery system 001, the first reservoir module 400 in the implantable unit 1000 comprises a plurality of reservoirs, each accommodating one therapeutically effective dose of the NMDAR pore blocker, and the plurality of reservoirs can be arranged in an array or in a matrix. The dispensing module 300 is configured, upon receiving the first activation command from the control module 200, to dispense the NMDAR pore blocker out of the plurality of reservoirs in the first reservoir module 400, one per time. Each reservoir is provided with a capping membrane made of a biocompatible composition, and the dispensing module 300 comprises a dispensing mechanism configured to break the capping membrane so as to allow the one

therapeutically effective dose of the NMDAR pore blocker to be released out of the each of the plurality of reservoirs. As such the drug delivery system 001 can realize a precise local release of the NMDAR pore blocker (i.e. therapeutically active agent) to the LHb region of the subject in a controllable and repeated manner. There can be different dispensing mechanisms. Optionally, the dispensing mechanism comprises an electrothermal activation mechanism, and the capping membrane has a biocompatible and heat-responsive composition such as titanium (Ti), platinum (Pt) or gold (Au), etc. Optionally, the dispensing mechanism comprises an electrochemical activation mechanism, and the capping membrane can have a composition of gold (Au). These above two dispensing mechanisms have been reportedly utilized in the implantable drug delivery device to electrothermally (Maloney JM et al. 2005; Yang YJ et al. 2009; Prescott JH et al. 2006) or to electrochemically (Li Y et al. 2004; Santini JT et al. 1999) dissolve these metallic membranes capping the drug-accommodating reservoirs so as to realize an on-demand local drug delivery. The disclosures of these above cited references are incorporated herein by reference in their entireties.

According to some other embodiments of the drug delivery system 001, the first reservoir module 400 in the implantable unit 1000 comprises one single reservoir, and the dispensing module 300 is configured, upon receiving the first activation command from the control module 200, to operate on the first reservoir module 400 such that the NMDAR pore blocker is released out of the one single reservoir, one therapeutically effective dose per time. There can be different dispensing mechanisms for the dispensing module 300, including the use of a micropump (e.g. infusion type, peristaltic type, thermopneumatic type, osmotic type, or positive displacement type; disclosed in Lo R et al. 2009; Sheybani R et al. 2015; Meng E et al. 2012; Au AK et al. 2011; and Fong J et al. 2015) a microvalve (piezoelectric type or thermoresponsive type; disclosed in Rahimi R et al. 2011; Li PY et al. 2010; Samad MF et al. 2014; Nafea M et al. 2018; and Au AK et al. 2011). The disclosures of these above cited references are incorporated herein by reference in their entireties. By means of the micropumps or microvalves, the amount of each dose of the NMDAR pore blocker formulation can be precisely controlled, and optionally can also be flexibly adjusted.

Optionally, the implantable unit 1000 may further comprise a second reservoir module 420 accommodating a pharmaceutically acceptable formulation of a priming agent. The dispensing

module 300 is further operably coupled to the second reservoir module 420, and is further configured, upon receiving a second activation command from the control module 200, to dispense the priming agent out of the second reservoir module 420. According to some embodiment, the control module 200 is configured to control the dispensing module 300 to sequentially dispense the priming agent out of the second reservoir module 420 (so as to maximally open the pores of the NMDAR protein complexes in the LHb region for better access by the NMDAR pore blocker molecules) and dispense the NMDAR pore blocker out of the first reservoir module 400 (so as to locally provide the NMDAR pore blocker molecules to achieve the anti-depression effects on the recipient subject).

Optionally, the implantable unit 1000 may further comprise a third reservoir module 440 accommodating a pharmaceutically acceptable formulation of an enhancing agent. The dispensing module 300 is further operably coupled to the third reservoir module 440, and is further configured, upon receiving a third activation command from the control module 200, to dispense the enhancing agent out of the third reservoir module 440. According to some embodiment, the control module 200 is configured to control the dispensing module 300 to sequentially dispense the NMDAR pore blocker out of the first reservoir module 400 (so as to locally provide the NMDAR pore blocker molecules to achieve the anti-depression effects) and dispense the enhancing agent out of the third reservoir module 440 (so as to increase the duration of the therapeutic effects of the NMDAR pore blocker molecules).

According to some embodiments of the drug delivery system 001, the implantable unit 1000 comprise all of the first, second and third reservoir modules 400, 420 and 440 as described above, and the control module 200 is configured to control the dispensing module 300 to sequentially dispense the priming agent out of the second reservoir module 420 (so as to maximally open the pores of the NMDAR protein complexes in the LHb region), dispense the NMDAR pore blocker molecules out of the first reservoir module 400 (so as to locally provide the NMDAR pore blocker molecules), and dispense the enhancing agent out of the third reservoir module 440 (so as to increase the duration of the therapeutic effects of the NMDAR pore blocker molecules).

It is noted that in any of the embodiments of the drug delivery system 001 as described above, the detailed description and the various embodiments of the NMDAR pore blocker, the priming agent,

and the enhancing agent can reference to the first aspect of the disclosure provided above. In addition, each of the second or third reservoir module 420 and 440 may exist as one single reservoir or may be divided into a plurality of reservoirs, each containing one therapeutically effective dose of the priming agent or enhancing agent, and the dispensing module 300 is further provided with a corresponding dispensing mechanism to thereby realize a controllable delivery of the priming agent and/or the enhancing agent. Relevant description can reference to the description of the first reservoir module 400 provided above.

According to some embodiment as diagrammed in FIG. 16, the implantable unit 1000 may further comprise a catheter module 500 with a drug delivery outlet arranged within, or in the proximity of, the LHb of the subject, and the catheter module 500 is distally and operably connected to the first reservoir module 400 (optionally also to the second reservoir module 420, and/or to the third reservoir module 440). Herein optionally, the first, second and/or reservoir module 400, 420 and 440, along with other modules of the implantable unit 1000 (i.e. the power source module 100, the control module 200, and the dispensing module 300), may be arranged in a distal position relative to the drug delivery outlet of the catheter module 500, such as beneath a scalp of the subject. As such, the configuration of this embodiment of the implantable unit 1000 is similar to the Ommaya reservoir (Zubair A et al. 2023; Liu HG et al. 2021). This distributed arrangement has multiple advantages. First, because the drug reservoir module(s), e.g. first, second, and/or third reservoir modules 400/420/440 are close to the body surface, it brings about the convenience for refilling the drug reservoirs in a minimally invasive manner. Second, because the power source module 100 is close to the body surface, it allows for convenient battery replacement if a non-rechargeable battery is employed, or allows for optimized wireless energy transmission if a power receiver for wireless energy transmission is employed. Third, because the control module 200 is close to the body surface, the external control of, i.e. the transmission of external control instructions to, the control module is more convenient.

There could be different embodiments for the power source module 100 of the implantable unit 1000 in the drug delivery system 001 provided herein.

According to some embodiments, the power source module 100 comprises a first energy sub-module capable of storing energy, which can be a supercapacitor (Sheng H et al. 2021; Rita AA et al. 2021; US20150287544A1) or a battery (Bock DC et al. 2012; Yang SY et al. 2021). Herein, the battery can preferably include high-capacity, high-energy-density and long-lasting battery. Examples can include lithium batteries (e.g. lithium-iodine (Li-I₂) battery, lithium-thionyl-chloride (Li/SOCl₂) battery, lithium-silver vanadium oxide (Li-SVO) battery, lithium-carbon monofluoride (Li-Cfx) battery, or lithium-manganese oxide (Li-MnO₂), etc.), silver oxide batteries, or lithium ion batteries. Optionally the battery is rechargeable (e.g. lithium ion batteries). In other words, the drug delivery system 001 provided herein comprises an energy-storage device that can directly provide power source to other functional modules (i.e. the control module 200 and the dispensing module 300).

According to some other embodiments, the power source module 100 comprises a second energy sub-module capable of receiving power wirelessly from an external source, e.g. via electromagnetic waves or ultrasonic waves. In other words, by means of the second energy sub-module, the drug delivery system 001 disclosed herein can be configured to realize a wireless power transfer/transmission (WPT; disclosed in Khan SR et al. 2020), by which the implantable unit 1000 embedded in the recipient subject's brain can wireless receive power from outside, such as from a corresponding external power unit 3000 (see below for more details).

According to yet some other embodiments, the power source module 100 comprises a third energy sub-module capable of harvesting energy from body of the subject, e.g. electrochemically (e.g. biofuel cells that can self-generate energy by glucose oxidation, disclosed in Zebda A et al. 2013; and Maity D et al. 2023), thermoelectrically (e.g. thermoelectric energy generators providing solid-state energy by converting temperature differences into usable electricity; Janes T et al. 2021), or piezoelectrically (e.g. energy generators providing energy harvested from heartbeats, blood flow or breathing; Jiang D et al. 2020).

According to some embodiments, the drug delivery system 001 further comprises an external power unit 3000 configured to wirelessly transfer energy to the power source module 100 in the implantable unit 1000. Herein optionally, the wireless energy transfer is by means of electromagnetic

energy transfer (i.e. via the electromagnetic waves), and as such, the power source module 100 in the implantable unit 1000 may comprise a power receiver, the external power unit 3000 may correspondingly comprise a power transmitter, and the power receiver and the transmitter substantially form a pair of coupling antennas allowing the energy to be wirelessly transmitted from the external power unit 3000 to the implantable unit 1000, i.e. realizing the wireless power transmission (WPT). Herein optionally, the WPT comprises electromagnetic power transfer, which can further comprise one or more of a near-field (<100 mm), a mid-field (100-500 mm; disclosed in Poon A et al. 2007), or a far-field (>500 mm; disclosed in Huang FJ et al. 2011) WPT, each realized by a corresponding power transmitter-power receiver pair. Preferably, the WPT comprises a near-field WPT, which further optionally comprise at least one of an inductive coupling WPT (disclosed in Manoufali M et al. 2018; Feng P et al. 2018), a capacitive coupling WPT (disclosed in Takhti M et al. 2011; Hassan A et al. 2015), or a magnetic resonance coupling WPT (disclosed in Kurs A et al. 2007; Ramrakhyani AK et al. 2010). Alternatively, the wireless energy transfer is by means of ultrasonic energy transfer (i.e. via the ultrasonic waves), and as such, the power receiver in the power source module 100 in the implantable unit 1000 and the power transmitter of the external power unit 3000 may both comprise a piezoelectric material, with the power transmitter electrically excited to generate the ultrasound, and the power receiver converting the ultrasound into electrical energy. Such ultrasonic energy transfer can transmit energy in a range of a few mm up to about 100 mm (Agarwal K et al. 2017; Amar CH et al. 2015), and has been applied in the implantable medical devices (Johnson BC et al. 2018; Taalla RV et al. 2018; and Rosa BMG et al. 2016; Maleki T et al. 2011).

In any of the embodiments of the drug delivery system 001 described above, there can be different control mechanisms for the control module 200. According to some embodiments, the control module 200 is programmed to periodically send activation commands to the dispensing module 300 to thereby activate the dispensing module 300 for the delivery of the NMDAR pore blocker from the first reservoir module 400, and optionally of the priming agent from the second reservoir module 420, and/or of the enhancing agent from the third reservoir module 440 according to a program stored in the control module 200. Optionally the program can be modified. According to

some embodiments, the drug delivery system further comprises an external control unit 2000, which is communicatively coupled, and configured to send control instructions, to the control module 200 of the implantable unit 1000, and the control module 200 is further configured, upon receiving one control instruction from the external control unit 2000, to send a corresponding activation command to the dispensing module 3000. Herein optionally, the external control unit 2000 is communicatively coupled to the implantable unit 1000 by means of a corresponding pair of antennas, one arranged on the external control unit 2000 and the other one on the implantable unit 1000.

According to some embodiments, there is only one-way communication between the implantable unit 1000 and the external control unit 2000, i.e., the control module 200 of the implantable unit 1000 is configured to only receive control instructions from the external control unit 2000, and as such, the antenna in the control module 200 can be configured only as a signal receiver, whereas the antenna in the external control unit 2000 is configured as a signal transmitter. Herein, the external control module 2000 may further comprise a user interface allowing the subject or a doctor to manually prescribe the control instructions (e.g. instructions to deliver the NMDAR pore blocker). Optionally, the external control module 2000 may be a mobile phone or an exclusive device.

According to some other embodiments, there can be two-way communications between the implantable unit 1000 and the external control unit 2000 (as illustrated by the double-headed arrow), i.e. in addition to the control instructions transmitted from the external control unit 2000 to the control module 200 of the implantable unit 1000, the control module 200 may be configured to optionally transmit the state information that is collected from a sensing module 600 to the external control unit 2000 to thereby realize a data telemetry. As such, the antenna in each of the control module 200 and the external control unit 2000 is configured both as a signal receiver and a signal transmitter. Herein, the sensing module 600 may optionally comprise an electrode for detecting the electrophysiological state of the LHb neurons, and/or may optionally comprise a sensor (e.g. fiber optical sensor) capable of qualitative and/or quantitative detecting certain molecules in the LHb region. Herein, the external control module 2000 may similarly comprise a user interface allowing the subject or a doctor to manually prescribe the control instructions and allowing the state information collected by the sensing module 600 to be displayed to the subject or the doctor.

Optionally, the external control module 2000 may be further communicatively connected to a cloud so as to upload the state information of the subject to the cloud for centralized processing. Optionally, there is no manual prescription, and the external control module 2000 or the cloud server that communicatively connected to the external control module 2000 may, based on the state information collected from the sensing module 600, automatically make the prescription. For example, when the sensing module 600 detects that the LHb region of the subject undergoes an increase of burst firing that indicates an episode of depression attack, the external control module 2000 may send the control instruction to the control module 200 of the implantable unit 1000 to activate the sequential local drug delivery of the priming agent, the NMDAR pore blocker and the enhancing agent, so as to effectively control depression.

In any embodiments of the drug delivery system as described above, the implantable unit 1000 can be manufactured by fabricating the various components on a common platform (e.g. a printed circuit board (PCB) substrate or a silicon substrate) using sophisticated microelectronic integrated circuits technology, such as photolithography, etching, and bonding.

In the following, examples are provided with ketamine as one NMDAR pore blocker. Two remarkable characteristics of ketamine that make it attractive as a new antidepressant treatment are its rapid onset, and sustained activity. A single intravenous infusion of a sub-anesthetic dose of ketamine in depressed patients produces antidepressant and anti-suicidal responses as quickly as an hour, and the effects can last for days (Berman RM et al. 2000). Regarding the rapid onset of ketamine's actions, much progress has been made in understanding its mechanism. One recent study found that ketamine instantly blocks NMDAR-dependent bursting activity in the lateral habenula (LHb) (Yang Y et al. 2018), a signature neural activity in multiple animal models of depression (Hu H et al., 2020). As the brain's anti-reward center, LHb inhibits the downstream aminergic reward center (Matsumoto M et al. 2007) and is hyperactive in the depressive state (Cerniauskas I et al. 2019). Consequently, ketamine's rapid blockade of LHb bursting can potentially disinhibit the downstream dopaminergic and serotonergic neurons to quickly improve mood (Yang Y et al. 2018).

In comparison to the rapid effects, mechanisms underlying ketamine's sustained antidepressant effects have not been understood. Ketamine's elimination half-life is only approximately 3 h in

humans (Clements JA et al. 1982) and 13 min in mice (Maxwell CR et al. 2006), yet, its antidepressant activities can last for 3~14 d in humans (Berman RM et al. 2000; Newport DJ et al. 2015) and for at least 24 h in mouse models of depression (Maeng S et al. 2008; Autry AE et al. 2011). This is in dramatic contrast to ketamine's anesthetic effects, which quickly wear off in a few hours (White PF et al. 1982), and to other classical antidepressants, which require daily intake to maintain an effective blood concentration (Hiemke C et al. 2000). As sustained efficacy will reduce repeated drug administrations and unwanted side effects, how ketamine's antidepressant effects can long outlast its plasma elimination is thus not only an interesting basic biological question, but also has important clinical implications.

Previously, ketamine's sustained effects were attributed to long-term plastic mechanisms (Maeng S et al. 2008; Autry AE et al. 2011), especially new spine formation (Li N et al. 2010) or the ketamine metabolite (2R, 6R)-hydroxynorketamine (HNK) (Zanos P et al. 2016). However, ketamine-induced spine growth in mice cannot be detected until 12 hours post treatment (Moda-Sava RN et al. 2019), and the half-life of (2R, 6R)-HNK is still less than 30 minutes (Zanos P et al. 2019), not much longer than that of ketamine. On the other hand, a much simpler mechanism involving direct blockade of NMDARs has not yet been explored. For this direct-blockade mechanism, the key questions are: does blockade of NMDARs continue to exist long after ketamine elimination in the brain? And if yes, how is this prolonged blockade achieved, and does it contribute to ketamine's sustained antidepressant effects? In the current study, building on knowledge of ketamine's rapid effects in the LHb, we set out with both *in vitro* and *in vivo* electrophysiological recordings to investigate whether persistent blockade of NMDAR and burst firing in the LHb may provide a foundation for ketamine's sustained antidepressant actions.

Materials and Methods

Animals. Male adult (8–16 weeks of age) C57BL/6 mice (SLAC or Shanghai Jihui) were used. Mice were group-housed four per cage under a 12-h light-dark cycle (light on from 7 a.m. to 7 p.m.) with free access to food and water *ad libitum*. All animal studies and experimental procedures were approved by the Animal Care and Use Committee of the animal facility at Zhejiang University.

Chronic restraint stress (CRS). Mice were subjected to chronic-restraint stress by placement in 50-ml conical tubes with holes for air flow for 2–4 h per day for 14 consecutive days (Yang Y et al. 2018).

Systemic drug delivery for antidepressant. All drugs were dissolved in 0.9% saline, and injected intraperitoneally (i.p.). The dosage of ketamine (Gutian Pharma Co. or Beikang Pharma Co.) was 10 mg kg⁻¹ or 5 mg kg⁻¹. The ketamine used in this study was a mixture of R-ketamine and S-ketamine. 1 h, 24 h, 3 d and 7 d after drug delivery, animals were subjected to behavioral tests and *in vivo* recording or sacrificed for *in vitro* electrophysiology studies.

Viral vectors. AAV2/9-hSyn-ChrimsonR-tdTomato-WPRE-SV40-pA (titre: 2.27×10^{13} v.g. ml⁻¹, 1:10 dilution, 0.1–0.2 µl bilateral into LH, Taitool), AAV2/9-hSyn-mCherry-WPRE-pA (titre: 1.53×10^{13} v.g. ml⁻¹, 1:10 dilution, 0.1–0.2 µl bilateral into LH, Taitool) were aliquoted and stored at -80 °C until use.

Surgery. Mice were deeply anaesthetized by 1% sodium pentobarbital (100 mg kg⁻¹ body weight, Sigma) and placed in a stereotactic frame (RWD Instruments). The virus was bilaterally injected into the LH (0.1–0.2 µl) (AP, - 0.82 mm from bregma; ML, ± 1.08 mm; DV, - 4.90 mm from the dura) using a pulled glass capillary with a pressure microinjector (Picospritzer III, Parker) at a rate of 0.1 µl min⁻¹. The injection needle was withdrawn 10 min after the end of the injection. Optical fibers (200 µm width) were implanted above LHb (AP, - 1.72 mm from bregma; ML, ± 1.14 mm; DV, - 2.40 mm from the dura) at a 15° angle in the ML direction. After surgery, mice recovered from anaesthesia on a heat pad. Mice were euthanized after all experiments to verify the sites of viral injection and optical fiber implantation. Brain sections were cut at 60 µm thickness (Leica CM1950) and counterstained with DAPI or Hoechst. Fluorescent image acquisition was performed with the Olympus VS120 virtual microscopy slide scanning system. Only data from mice with correct injection sites were used.

Forced swim test (FST). Forced swim test were used to model behavioral despair, as previous study (Yang Y et al. 2018). Mice were individually placed in a cylinder (12 cm diameter, 25 cm height) of water (23–25 °C) and swam for 6 min. The test was performed in normal light conditions (30–35 lux). Water depth was set to prevent animals from touching the bottom with their tails or hind limbs. Animal behaviors were videotaped from the side. The immobile duration during the 2–6 min

test was counted offline by an observer blinded to animal treatment. The immobile duration was defined as the time when animals remained floating or motionless with only movements necessary for keeping balance in the water.

Sucrose preference test (SPT). Sucrose preference test was used to model anhedonia or inability to feel pleasure, as previous study (Yang Y et al. 2018). Mice were single housed and habituated with two bottles of water for 2 days, followed by two bottles of 2% sucrose for another 2 days. Then after a 24 h water deprivation, animals were exposed to one bottle of 2% sucrose and one bottle of water for 2 h in the dark phase. Bottle positions were switched 1 h after test started. Total consumption of each fluid was measured and sucrose preference was defined as the average of sucrose consumption ratios during the first and second hours. The sucrose consumption ratio was calculated by dividing the total consumption of sucrose by the total consumption of both water and sucrose.

Real-time place aversion (RTPA). Mice were placed in a white open chamber (52 cm × 26 cm × 23 cm) consisting of two chambers, and allowed to freely move between chambers. During the following 20-min test, a stimulated side was assigned. Laser stimulation (635 nm, 40 Hz, 2 ms pulse, 250 μW) was delivered as soon as mice entered the stimulated side and terminated once mice crossed to the non-stimulated side. A video camera positioned above the chamber recorded each trial. Mouse locations and velocity were tracked and analyzed using Any-maze software (Stoelting). The time in stimulated (light-on) and non-stimulated (light-off) chambers were then calculated.

Cannula infusion experiment. A double guide cannulae (centre-to-centre distance 1 mm, RWD) was placed, and inserted bilaterally into the LHb (AP, - 1.80 mm from bregma; ML, ± 0.50 mm; DV, - 2.65 mm from the dura) of mice. A double dummy cannulae (RWD), secured with a dust cap, was inserted into the guide cannula to prevent clogging during the recovery period. After mice had recovered for at least 7 days, drugs were microinjected with a double injector cannula, while mice were anaesthetized with isoflurane (RWD) on an anaesthetic machine. The extensions were manually sharpened before insertion. Ketamine and memantine (Sigma) were dissolved in 0.9% saline. Before the local drug infusion, tip-sharpened double injector cannulae were inserted into the guide cannulae to ensure clear passage and then pulled out. One microlitre of the drug was infused (0.1 μl per 2.5 min) into each side through another set of tip-sharpened double injector cannulae, which were connected

to the microsyringe (baige). The injector cannulae were left in place for an additional 5 min to minimize the spread of the drug along the injection track. Behavioral tests were performed 1 h or 24 h after memantine infusion and 24 h, 7 d or 14 d after ketamine infusion. To verify the drug infusion sites, 0.2 μ l CTB-488 (Invitrogen) was injected to each side of the LHb after all behavioral tests. Mice were then euthanized 30 min after CTB injection for immunostaining. Brain slices were counterstained with Hoechst before mounting on the slides. Fluorescent image acquisition was performed with the Olympus VS120 virtual microscopy slide scanning system. Only data from mice with correctly sited injections were used.

LC–MS/MS measurement of drug concentration. Male mice were anaesthetized by isoflurane and subsequently decapitated 2 min, 5 min, 10 min, 30 min, 1 h, 4 h and 24 h after ketamine administration (i.p.). Blood was collected in EDTA-containing (30 mg ml⁻¹) tubes and centrifuged at 2000rpm for 10 min (4 °C). Plasma was collected and stored at -80 °C until analysis. Whole brain tissues (about 0.4-0.5 g) were immediately collected in the EP tubes. The tissues were immediately frozen in liquid nitrogen and stored at -80 °C until analysis. After samples were thawed, 100 mg brain tissues or 100ul plasma were measured, 1 ml solution (ddH₂O: acetonitrile = 9:1, v/v) and steel balls were added to homogenize samples for 3 min at 60 Hz with a tissue grinder. 2.5 ul of ketamine of different concentrations (0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 50 ng ml⁻¹) was mixed with 47.5 μ l blank brain tissue to establish the standard calibration curve. The quantification of ketamine was accomplished by calculating area ratios using verapamil solution (2 ng ml⁻¹ solution) as the internal standard. 50 ul of the tested brain tissue was mixed with 200 μ l acetonitrile with verapamil solution (2 ng ml⁻¹) for vortex mixing. After centrifugation for 15 min at 20,000 r.p.m. at 4 °C, the upper layer was injected into the chromatographic system. The concentrations of ketamine were determined by achiral LC–MS/MS following a previously described method(Zanos P et al. 2016; Yang Y et al. 2018), with slight modifications. The analysis was accomplished using a Waters ACQUITY UPLC BEH C18 column (2.1 mm $\times$ 50 mm ID, 1.7 μ m; Waters). The mobile phase consisted of 0.1% formic acid buffer as component A and acetonitrile as component B at a flow rate of 0.35 ml/min, temporized at 10 °C (injection volume: ketamine 10 μ l). A linear gradient was run as follows: 0-0.5 min, 10% B; 0.5-1.5 min, from 10% B increased to 90% B; 1.5-2.5 min, 90% B; 2.5-2.51 min, from

90% B decreased to 10% B; 2.51-3.5 min, 10% B. The MS/MS analysis was performed using waters TQ-S micro. Positive electrospray ionization data were acquired using multiple reaction monitoring (MRM) using the following transitions for (R, S)-ketamine studies: 238.096 → 124.987.

Optogenetic light delivery and protocols. For *in vitro* experiments, LHb brain slices were made from mice expressing ChrimsonR (Chuong et al. 2014) in the LH, a 635 nm, 40 Hz, 0.5 mW, 2 ms duration pulsed (4 pulses) red light was delivered to activate LH-LHb axon terminals. For RTPA test, in mice expressing ChrimsonR/mCherry in the LH, a 635 nm red light was bilaterally delivered into the LHb through optical fibers at 40 Hz, 2 ms pulse, 250 μ W by laser (Inper) when mice entered the stimulated-side chamber. For *in vivo* ketamine binding and unbinding experiments, same optogenetic protocol (635 nm, 40 Hz, 2 ms pulse, 250 μ W) was delivered for 3 min either immediately after ketamine (5 mg kg⁻¹)/saline injection or 1 h after ketamine (10 mg kg⁻¹)/saline injection.

LHb brain slice preparation. Mice were anaesthetized by 1% sodium pentobarbital (100 mg kg⁻¹ body weight, Sigma), and then perfused with 20 ml ice-cold ACSF (oxygenated with 95% O₂ + 5% CO₂) containing (mM): 125 NaCl, 2.5 KCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂ and 25 glucose, with 1 mM pyruvate added. The brain was removed as quickly as possible after decapitation and put into chilled and oxygenated ACSF. Coronal (for spontaneous neuronal activity recording) or sagittal (for eEPSCs recording) slices containing the LHb or hippocampal CA1 were sliced into 300 μ m sections in cold ACSF using the Leica VT1200S vibratome and then transferred to ACSF at 32 °C for incubation and recovery. ACSF was continuously gassed with 95% O₂ and 5% CO₂. Slices were allowed to recover for at least 1 h before recording. CRS mice and naïve mice both went through an FST at least 1 day before brain slice recording and we only used the CRS animals that showed high immobile duration (>120 s) and the naïve animals that showed low immobile duration (<120 s) in the FST for slice recording.

In vitro electrophysiological recordings. For LHb neuron recordings, currents were measured under whole-cell patch-clamp using pipettes with a typical resistance of 4-8 M Ω filled with internal solution containing (mM): 105 K-gluconate, 30 KCl, 4 Mg-ATP, 0.3 Na-GTP, 0.3 EGTA, 10 HEPES and 10 Na-phosphocreatine, with pH set to 7.25-7.30 (for spontaneous neuronal activity recording) or with internal solution containing (mM): 115 CsMeSO₃, 20 CsCl, 10 HEPES, 2.5 MgCl₂, 4

Na-ATP, 0.4 Na-GTP, 10 Na-phosphocreatine, 0.6 EGTA and 5 QX-314, with pH set to 7.25-7.30 (for eEPSCs recording). The external ACSF solution containing (mM): 125 NaCl, 2.5 KCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂ and 25 glucose. Cells were visualized with infrared optics on an upright microscope (BX51WI, Olympus). A MultiClamp 700B amplifier controlled by DigiData 1550 digitizer and pCLAMP10 software were used for electrophysiology (Axon Instruments). The series resistance and capacitance were compensated automatically after a stable Gigaseal was formed. Recordings were typically performed between 3 and 10 min after the break-in. The spontaneous neuronal activity was recorded under current clamp ($I = 0$ pA). LHb neurons show three modes of spontaneous activity at resting conditions. Silent cells showed no spike activity during recording. Tonic cells spontaneously generated tonic trains of action potentials. Burst cells spontaneously generated clusters of spikes with an initially high but progressively declining intra-burst firing frequency in each burst. After membrane potential stabilized, 3 minutes of data were collected to calculate bursting spike frequency and bursts per min. Bursting spike frequency was calculated as the spike number of bursting spikes per second. Bursts per min was calculated as the number of bursts per minute. The percentage of blockade of bursting spike frequency or bursts per min at each time point were calculated as below:

$$\text{Percentage of blockade} = (\text{average value of saline group} - \text{value of ketamine group}) / \text{average value of saline group} * 100\%$$

Evoked EPSCs were recorded under voltage clamp at -70 mV or +40 mV in sagittal LHb slices by stimulating the input stria medullaris fiber in a modified extracellular ACSF solution with GABA_AR blocker picrotoxin (PTX, 100 μ M, TOCRIS). Stimulation pulse (0.25-1.50 mA; 0.2 ms; step by 0.25 mA) were delivered every 6-10 s. Cells were first held at -70 mV to record electrically evoked fast AMPAR-mediated currents (AMPA-eEPSCs). Subsequently, cells were held at +40 mV to record a combination of AMPAR- and slower NMDAR-mediated currents (NMDAR-eEPSCs) (Mameli M et al. 2011; Maroteaux M et al. 2012). More than three traces were averaged at each stimulation intensity and holding potential. AMPAR-eEPSCs were determined by the peak current amplitude at -70 mV. NMDAR-eEPSCs were determined by the current amplitude 35 ms after stimulation onset at +40 mV. NMDA/AMPA ratios were determined by dividing the

NMDAR-eEPSCs by the AMPAR-eEPSCs at 1.5 mA stimulation intensity. The percentages of neurons with > 10 pA NMDAR-eEPSCs were calculated at 1.5 mA stimulation intensity. The percentage of blockade of NMDAR-eEPSCs at each time point was calculated as below:

$$\text{Percentage of blockade} = (\text{average value of saline group} - \text{value of ketamine group}) / \text{average value of saline group} * 100\%$$

AMPA receptors in the LHB mostly lack the GluR2 subunit, and consequently AMPAR-eEPSCs show strong inward rectification and fast decay (Cerniauskas I et al. 2017), much faster than NMDAR-eEPSCs. To confirm the NMDAR component of the recorded current at 35 ms after stimulation onset, mixed NMDAR- and AMPAR-eEPSCs were recorded at +40 mV with ACSF containing PTX (100 μ M, TOCRIS). 10 μ M NBQX (AMPA blocker, Sigma) was then perfused into the recording solution to block AMPAR currents. Afterwards 50 μ M AP5 (NMDAR blocker, Sigma) was further added and perfused into the recording solution to block NMDAR currents. The current amplitude detected at 35 ms at +40 mV was significantly blocked by AP5, but showed no difference in the presence or absence of NBQX, suggesting that AMPAR-eEPSCs have mostly decayed by 35 ms (FIG. 6A). The pure NMDAR-eEPSCs in LHB neurons 1 h or 24 h after ketamine i.p. injection were recorded at +40 mV by pharmacological isolation method (with NBQX and PTX in recording ACSF to block AMPARs and GABA_ARs respectively). NMDAR-eEPSCs were calculated by the peak amplitude at this recording condition.

For ketamine washout experiments, evoked NMDAR-eEPSCs were recorded under voltage clamp at -70 mV in a modified extracellular ACSF solution with NBQX (10 μ M, Sigma) to block AMPARs and PTX (100 μ M, TOCRIS) to block GABA_ARs. Recordings were made in ACSF containing no added Mg²⁺ to reduce the Mg²⁺ blockade of NMDARs. Stimulation intensity (0.1 ~ 0.3 ms, 0.1 ~ 5 mA) was adjusted for each cell to produce adequate responses. LHB neurons with an NMDAR-eEPSCs less than 10 pA were not used in the wash-out experiments. Stimulation pulses were delivered every 10 s. After 5 min stable baseline recording, 100 μ M ketamine or 10 μ M ketamine or 100 μ M memantine was washed into the recording ACSF and then washed out 10 min later to watch for the recovery of NMDAR-eEPSCs in the next 50 min. The vehicle group was carried out as the control. NMDAR-eEPSCs were normalized by the baseline before drugs application. The

normalized NMDAR-eEPSCs at the end of drug perfusion (at 10 min) were calculated to show the degrees of drug blockade (FIG. 7B) and the averaged normalized NMDAR-eEPSCs at 50-60 min to show the degrees of responses recovery (FIGS. 7B and 10B). Amount of blockade was calculated in a 10-min bin and the maximal one was taken as the maximal blockade. Note that due to trapping effect, the maximal blockade could occur after the end of the 10 min wash-in period (FIGS. 11A-11D). The percentage of recovery was calculated as: maximal blockade - blockade at 50-60 min (FIGS. 11A-11D). The input resistance during recording was monitored by 20 mV potential injection.

To prove the efficacy of Dyngo-4a in blocking endocytosis, we tested it on an LFS-induced LTD protocol in hippocampal CA1 neurons (Montgomery, J. M., 2005). Sagittal slices containing hippocampal CA1 were prepared and NMDAR-eEPSCs were recorded under voltage clamp at -70 mV as described above by stimulating the schaffer collaterals. After 5 min stable baseline recording, LFS (1 Hz, 15 min, 900 pulses) was performed, and then returned to the recording frequency of 0.1 Hz for 35 min. For blockade of the NMDAR endocytosis, 30 μ M Dyngo-4a (ApexBio) was additionally added to the recording ACSF in LFS-induced LTD of NMDAR experiments and washout experiments (Dankovich TM et al. 2021). For *in vitro* “kick off” experiments, 10 min after ketamine washout, two “kick off” sessions (pairing presynaptic 1 Hz electrically stimulation with 3 s postsynaptic depolarization to +10 mV, 3 s pairing per min, 5 min per session, 30 spikes total) with 4 min interval were performed to cells in the “kick off” group. For cells in the pre-alone group, during the same time window (10 min after ketamine washout), two sessions of presynaptic 1 Hz electrically stimulation alone were performed. The average normalized NMDAR-eEPSCs after 50 min were calculated to show the degrees of responses recovery. As control, two “kick off” sessions were applied to cells without ketamine-treatment.

In vivo electrophysiology. For *in vivo* single-unit recording, a custom-made screw-driven microdrive consisting of sixteen nickel chromium alloy wire (California Fine Wire Company, CFW, .0014", Stablohm 650) single electrode was implanted into the LHb (AP, -1.72 mm; ML, 0.47 mm; DV, -2.50 mm from the dura) of CRS mice. Silver wires were attached to two screws on the skull as ground. The microdrive was secured to the skull with dental cement. After recovery for more

than one week, mice were allowed to adapt to the recording headstage for 30 min before recording. Spontaneous spiking activity and wideband electronic signals (0.1-7500 Hz) were recorded by a neural recording system (Plexon Inc.) and digitized at 40 kHz with a gain of 1000 $\times$. Spontaneous spiking signals were band-pass filtered between 300 and 6000 Hz. Common median reference (CMR) was assigned as a digital reference. The amplitude threshold for the spike capture was adjusted for each unit according to the signal-to-noise ratio. Spontaneous spiking signals of the mice were recorded for 10 min after habituation in their home cages as the baseline. Spiking signals were continuously recorded for 1 h after ketamine treatment (10 mg kg⁻¹, i.p.) with headstage un-removed. Paired statistical method was used for the 0-1 h data. For data collected at 24 h or 3 d after ketamine treatment, since animals need to return to homecage for rest, headstage was removed and remounted. Once the headstage is removed and remounted, there is some probability of slight shift of the recording electrode, and the number of units may also change. Therefore, we defined the units before and after the headstage reset as different units and used an unpaired statistical method for the 24 h and 3 d data. The electrodes were lowered in steps of 62.5 μ m after each recording session, followed by at least a 3-day recovery. If mice received a second ketamine injection, at least a one-week interval was introduced before the next recording session. The CRS animals showing high immobile duration (>120 s) in the FST were used in *in vivo* recording. The positions of the electrodes were verified at the end of all experiments and only data from mice with correct electrode positions were used.

Spike sorting. All waveforms recorded from each electrode were imported in Offline Sorter V3/4 (Plexon Inc.). Single units were manually identified by threshold crossing and principal component analysis (PCA). Spikes with inter-spike interval (ISI) less than the refractory period (1.4 ms) were excluded. Cross correlograms were plotted to ensure that no cell was discriminated more than once on the overlapping electrode.

Data analysis. Data was analyzed using Neuroexplorer4/5 (Plexon Inc.) and MATLAB. We defined *in vivo* bursting as clusters of spikes beginning with a maximal inter-spike interval of 20 ms and ending with a minimal inter-spike interval of 50 ms. The minimal intra-burst interval was set at 50 ms and the minimal number of spikes in a burst was set at 2. Bursts per minute and bursting spike frequency were analysed by Neuroexplorer4/5 (Plexon Inc.) and Excel 2013. For the data of 1 h

continuous recording after ketamine treatment, the inhibited or excited units (colored dots in FIGS. 4A-4D) were statistically analysed by z-score transformation (bursting spike frequency). Post-injection z-score of each unit was calculated as:

$$Z = (\bar{x} - \mu) / \sigma$$

where $\bar{x}$ was the mean of all the 100 s-bin values during the post-injection period (i.e., 20-30 min after drug injection), μ and σ were the mean and the standard deviation (SD) of all the 100 s-bin values during the baseline period (i.e., 10 min before drug injection).

The inhibition or excitation of a unit was identified when its post-injection z-score (bursting spike frequency) was ≤ -1.67 or ≥ 1.67 ($p < 0.05$) (Courtin J et al. 2014), respectively. And we divided the units into two groups: basal bursting spike frequency > 2 Hz group and basal bursting spike frequency < 2 Hz group. The percentages of inhibited units in each group were calculated. The percentage of blockade of bursting spike frequency or bursts per min at each time point was calculated as below:

$$\text{Percentage of blockade} = (\text{baseline value} - \text{value of each time point}) / \text{baseline value} * 100\%.$$

Statistical analysis. Required sample sizes were estimated on the basis of our previous experience performing similar experiments. Mice were randomly assigned to treatment groups. Analyses were performed in a manner blinded to treatment assignments in all behavioral experiments. Statistical analyses were performed using GraphPad Prism software v7. By pre-established criteria, values were excluded from analysis if the drug delivery sites or virus injection sites or electrodes implant sites were out of the LHb. All statistical tests were two-tailed, and significance was assigned at $p < 0.05$. Normality and equal variances between group samples were assessed using the D'Agostino & Pearson omnibus normality test and Brown-Forsythe tests, respectively. When normality and equal variance between sample groups were achieved, paired or unpaired t-test was used. When normality or equal variance of samples failed, the Mann-Whitney test or Wilcoxon matched pairs test was performed. Linear regression test, Chi-square test, RM one-way ANOVA or two-way ANOVA Multiple comparisons were used in appropriate situations.

Results

Example 1: Sustained behavioral effects of ketamine

We first mapped the antidepressant time course of a single systemic injection of ketamine in the chronic restraint stress (CRS) mouse model of depression. After exposure to 14 d of restraint stress, mice were injected with ketamine (intraperitoneal (i.p.), 10 mg kg⁻¹). At different time points (1 h, 24 h, 3 d and 7 d) after ketamine injection, we then measured both plasma and brain concentrations of ketamine using liquid chromatography-tandem mass spectrometry (LC-MS/MS), and tested mice for depressive-like behaviors (FIGS. 1A, 2A and 2B). Depressive-like behaviors were measured by both the forced swim test (FST), which models behavioral despair, and the sucrose preference test (SPT), which models anhedonia or the inability to feel pleasure. Compared to saline group, after ketamine injection the immobility time in the FST was significantly decreased and the preference for sucrose water in the SPT significantly increased at 1 h ($P = 0.0005$ for FST, and $P = 0.011$ for SPT, Mann-Whitney test), as well as 24 h ($P = 0.033$ for FST, and $P = 0.02$ for SPT, unpaired t test) (FIGS. 1B and 1C). At 3 d post ketamine injection, there was an antidepressant trend in the FST ($P = 0.081$, unpaired t test) but not SPT (FIG. 2C and 2D). At 7 d post injection, the antidepressant effects became insignificant (FIG. 2C and 2D). Notably, LC-MS/MS showed that both the plasma and brain concentrations of ketamine dropped quickly after injection (FIGS. 1A and 2B). By 1 h, it had dropped to a level (0.23 μ M in the brain) too low to inhibit NMDARs ($IC_{50} = \sim 5 \mu$ M in presence of magnesium (Kotermanski SE et al. 2009)); yet the antidepressant effects remained significant until 24 h (FIGS. 1B and 1C). These paradoxical results confirmed previous reports on ketamine's short half-life and sustained behavioral effects, calling for a mechanistic explanation.

Example 2: Sustained LHb suppression by ketamine

Since blockade of LHb bursting mediates the rapid antidepressant effects of ketamine (Yang Y et al. 2018), we wished to examine whether it may also underlie the sustained effects of ketamine. We first examined how long a single i.p. injection of ketamine can suppress the bursting activity of LHb neurons (FIG. 1D). We made coronal LHb brain slices of CRS mice at 1 h, 24 h or 3 d after mice were i.p. injected with ketamine, and recorded spontaneous neuronal activity at resting conditions (FIG. 1D). As previously shown (Yang Y et al. 2018; Hu H et al. 2020; Cui Y et al. 2019), LHb neurons are intrinsically active and can be categorized into silent, tonic firing and burst firing types (FIG. 1E). The proportion of bursting neurons was significantly higher in CRS mice

compared with naïve mice (Yang Y et al. 2018) (FIG. 1F, FIG. 3A). In slices prepared 1 h after ketamine injection, the percentage of bursting neurons dropped from 40% in saline group to 13% in ketamine group ($P < 0.0001$, Chi-square test; FIG. 1F). Notably, in slices prepared 24 h after ketamine injection, the percentage of bursting neurons was still significantly reduced (44% in saline group vs 24% in ketamine group, $P = 0.012$, Chi-square test; FIG. 1F). By 3 d after ketamine injection, the difference was no longer significant (40% in saline group vs 33% in ketamine group, $P = 0.48$, FIG. 3B). The bursting spike frequency (defined as number of bursting spikes per second) and bursts per minute (defined as number of bursts per minute) of recorded LHb neurons showed a similar trend of change: significantly suppressed at 1 h and 24 h but not at 3 d after ketamine injection (FIGS. 3C-3E). We next tested whether single-ketamine-injection-induced sustained suppression of LHb bursting also occurs *in vivo*. For that, we performed single-unit recording in the LHb of freely moving CRS mice before and at different time points after i.p. injection of ketamine (FIGS. 1G-1L, FIGS. 3F-3K). Suppression of LHb bursting activity, as measured by bursting spike frequency and bursts per minute, was significant as early as the first 5 min, maximized at 5-10 min, and persisted to 24 h after ketamine injection (FIGS. 1K and 1L). By 3 d after ketamine injection, the suppression of LHb bursting activity was not significant (FIGS. 3H and 3I). In comparison, saline treatment produced no alteration of LHb bursting activity during long-term *in vivo* recording in CRS mice (FIGS. 1K, 1L, and 3H-3K). Further analysis revealed that the bursting spike frequency of LHb neurons had a bimodal distribution and ketamine preferentially affected the population with a higher basal bursting spike frequency (FIGS. 4A-4D). In particular, although only 27% (26/97) LHb neurons had bursting spike frequency larger than 2 Hz (> 2 Hz group) (FIG. 4A), however, a majority (e.g. 76.9% at 1 h) of ketamine-inhibited neurons was in this “> 2 Hz” group (FIGS. 4B-4D). Such activity-dependent inhibition is consistent with ketamine’s use-dependent nature of blockade--blocking the NMDAR channel only when it is in the open state (MacDonald JF et al. 1987). Both *in vitro* and *in vivo* recording data demonstrated that a single systemic injection of ketamine in depressive-like mice elicited sustained inhibition of LHb bursting activity, in a time course paralleling the behavioral effects.

Example 3: Prolonged LHb-NMDAR block by ketamine

In order to understand why ketamine can cause sustained suppression of LHb bursting, and because LHb bursting activity depends on the NMDARs (Yang Y et al. 2018), we next measured in LHb brain slices how long ketamine can continue to block the NMDAR currents after a single i.p. injection (FIGS. 5A-5R, FIGS. 6A-6G). We made sagittal LHb brain slices of CRS mice at 1 h, 24 h or 3 d after mice were injected with ketamine (i.p., 10 mg kg⁻¹), placed a stimulating electrode close to the input stria medullaris (SM) fiber, and performed whole-cell patch-clamp to record evoked excitatory post-synaptic currents (eEPSCs) (FIGS. 5A and 5B). In LHb slices from both saline- and ketamine-treated mice, we isolated AMPAR- and NMDAR- mediated excitatory postsynaptic currents (AMPA-eEPSCs and NMDAR-eEPSCs respectively) based on their temporal characteristics (see Methods, FIGS. 5C and 5I, FIG. 6A). In LHb slices prepared 1 h after ketamine injection, there was a significant decrease in the ratio of NMDAR-eEPSCs and AMPAR-eEPSCs (N/A ratio, 0.30 ± 0.06 , saline; 0.03 ± 0.01 , ketamine; $P < 0.0001$, Mann-Whitney test; FIG. 5D). To further distinguish whether this reduction was due to a change of NMDAR-eEPSCs or AMPAR-eEPSCs, we analyzed the input-output curves of NMDAR- and AMPAR-eEPSCs respectively. NMDAR-eEPSCs of the ketamine group showed strongly reduced amplitudes across a range of stimulation intensities compared to the saline group (FIG. 5E). For example, the amplitude of NMDAR-eEPSCs recorded at 1.5 mA stimulation intensity was 41.3 ± 8.6 pA from the saline group ($n = 29$ in 3 mice) and 7.6 ± 1.8 pA from the ketamine group ($n = 24$ in 3 mice, $P < 0.0001$, Mann-Whitney test; FIG. 5F). The prevalence of neurons with large-amplitude NMDAR-eEPSCs (> 10 pA) was notably lower in ketamine-treated group (69%, saline; 29%, ketamine, $P < 0.0001$, Chi-square test; FIG. 6B). In contrast, the input-output curves and the amplitude of AMPAR-eEPSCs were not significantly different between saline and ketamine groups ($P = 0.11$, Mann-Whitney test; FIGS. 5G and 5H). Strikingly, in LHb slices prepared 24 h after ketamine injection, strong inhibition of NMDARs (under 1.5 mA stimulation, 39.09 ± 5.77 pA, saline; 17.58 ± 4.41 pA, ketamine; $P = 0.001$, Mann-Whitney test; FIGS. 5K-5N) and decrease of NMDA/AMPA ratios (0.35 ± 0.08 , saline; 0.12 ± 0.04 , ketamine; $P = 0.004$, Mann-Whitney test; FIG. 5J) persisted. By 3 d after ketamine injection, the blockade was no longer significant (FIGS. 6D-6F). The levels of NMDAR-eEPSC inhibition (see Methods for calculation)

in the LHb at 1 h, 24 h and 3 d after ketamine injection were $81.5 \pm 4.4 \%$, $55.0 \pm 11.3 \%$, and $-1.2 \pm 25.8\%$, respectively (FIG. 6G), again, paralleling the time course of ketamine's behavioral effects. To further confirm the persistent blockade of NMDAR by ketamine, we pharmacologically isolated the pure NMDAR-eEPSCs in the LHb of CRS mice in the presence of both GABA_AR blocker picrotoxin (PTX) and AMPAR blocker NBQX. The pharmacologically isolated NMDAR-eEPSCs also showed a significant blockade at 24 h after ketamine i.p. injection (FIGS. 5O-5R).

Example 4: NMDAR blockade persists after ketamine wash-out

We next investigated the potential mechanism by which ketamine may continue to block NMDARs long after its elimination in the brain. It is of interest to note that, in the slice recording paradigm in FIGS. 5A-5R, even for the 1 h time point, LHb slices had been incubated and perfused in ketamine-free ACSF solutions for hours, and the residual ketamine in the tissue from i.p. injection should have been washed off by the time of recording. The fact that NMDARs still showed strong inhibition suggests that ketamine may be trapped in the NMDAR channel, which prevents it from clearance. Indeed, as an open channel blocker, after binding to the channel, ketamine can be trapped in the channel pore, getting released only when the channel is open again (MacDonald JF et al. 1991; Mealing GA et al. 1999). To test the trapping hypothesis more directly, we continuously monitored NMDAR-eEPSC responses in LHb brain slices while washing in and out ketamine or memantine, another pore-blocking-type NMDAR inhibitor with similar affinity but faster off-rate compared to ketamine (Parsons CG et al. 1995) (FIG. 7A). NMDAR-eEPSCs were pharmacologically isolated at -70 mV (in the absence of magnesium to remove magnesium blockade of NMDARs, see Methods, FIG. 7A). After a 5 min recording of stable baseline, ketamine (100 μ M) or memantine (100 μ M) was perfused into the recording ACSF and then washed out 10 min later to watch for the recovery of NMDAR-eEPSCs in the next 50 min (FIG. 7A). As control, NMDAR-eEPSCs did not change in vehicle-treated group during this whole length of recording (FIG. 7A) and the input resistance of recorded neurons remained constant throughout the recording period (FIGS. 8A-8C), indicating a stable recording. At the end of the 10 min drug wash-in period, NMDAR-eEPSCs showed an equal level of strong reduction by the two drugs (ketamine: $85.1 \pm 3.7\%$ reduction, $n = 6$ cells; memantine: $84.3 \pm 3.5\%$ reduction, $n = 6$ cells; FIG.

7B). After the drug wash-out, the memantine-blocked NMDAR-eEPSCs quickly recovered, and by 50 min, only $19.9 \pm 18.2\%$ reduction remained (FIG. 7B). In contrast, the ketamine-blocked NMDAR-eEPSCs continued to be blocked after ketamine washout, and by 50 min, the reduction of NMDAR-eEPSCs currents was still as large as $82.7 \pm 4.7\%$ (FIG. 7B). Since the treatment-relevant peak concentration of ketamine in the brain is around $16 \mu\text{M}$ (FIG. 1A), we then repeated the above washout experiment using the treatment-relevant concentration, $10 \mu\text{M}$ ketamine (FIGS. 10A-10B). While the NMDAR-eEPSC responses decayed slower during the 10 min perfusion period in $10 \mu\text{M}$ ketamine, compared with $100 \mu\text{M}$ ketamine condition, significant blockade of NMDAR response ($73.8 \pm 7.1\%$ reduction, $n = 6$ cells) still lasted at least for 50 min (black, FIGS. 10A-10B). To eliminate the possibility of NMDARs endocytosis, we introduced Dyngo-4a, an NMDAR endocytosis inhibitor, which could effectively suppress low-frequency stimulation (LFS)-induced endocytosis of hippocampal NMDAR-eEPSCs (FIG. 9A) (Montgomery, J. M., 2005). In the presence of Dyngo-4a, LHb NMDAR-eEPSCs still did not recover after either $100 \mu\text{M}$ or $10 \mu\text{M}$ ketamine washout (FIGS. 9B-9E), suggesting that the persistent suppression of NMDAR-eEPSCs in the LHb after ketamine washout is not due to endocytosis. Together, these results suggest that LHb NMDARs can be continuously blocked long after ketamine's clearance in plasma. Interestingly, when we analyzed individual neurons recorded in FIG. 7A and FIG. 10A, a reverse correlation was observed between the level of initial NMDAR blockade by ketamine and the level of recovery: neurons with a higher percentage of blockade after ketamine wash-in tend to have a lower level of recovery at the end of 50-min wash-out ($R^2 = 0.50$, $P = 0.01$, linear regression, FIGS. 11A-11D), suggesting that the more complete the blockade, the slower recovery of the NMDAR currents. This reverse correlation has also been reported in hippocampal neurons, where it reflects the contribution of a reservoir pool of extrasynaptic NMDARs in recovery (McQuate A et al. 2020).

Example 5: Local ketamine produces extended antidepressant effects

If prolonged blockade of NMDARs in the LHb contributes causally to ketamine's sustained antidepressant effects, we expect that local infusion of ketamine into the LHb should mimic the sustained effects of systemic ketamine injection. To test that, we performed bilateral infusion of either ketamine (100 mM , $0.1 \mu\text{l}$ each side) or memantine (100 mM , $0.1 \mu\text{l}$ each side) into the LHb

of CRS mice through dual-guide cannulae, and measured depressive-like behaviors days later (FIGS. 7C-7M). Consistent with its rapid wash-out in LHb brain slice recordings (FIGS. 7A-7B), infusion of memantine did not produce antidepressant effects in CRS mice at 24 h (FIGS. 7F-7H). In contrast, lasting antidepressant effects were present in both the FST and SPT at 24 h, and even 7 d, after ketamine infusion into the LHb (FIGS. 7I-7M). We previously found that local infusion of ketamine into the LHb causes antidepressant effects as early as 1 h (Yang et al. 2018). Therefore, local infusion of ketamine in the LHb is sufficient to recapitulate both the rapid (1 h) and sustained (24 h) antidepressant effects of systematic ketamine injection.

Example 6: Neural activity untraps ketamine

We next reasoned that, if trapping of ketamine in the channel pore indeed accounts for the prolonged blockade of NMDARs, untrapping it should quickly bring back NMDAR responses in the brain slice wash-out experiments (FIG. 10A). One way to rapidly release a voltage-dependent trapping blocker is to open the NMDAR channel again by agonist binding and accelerate its release by voltage depolarization (Huettner JE et al. 1988; Tovar KR et al. 2002). Therefore we designed a “kick-off” protocol, by pairing presynaptic 1 Hz electrical stimulation (to release glutamate) with postsynaptic current injection to depolarize neurons (3 s at +10 mV per min for 5 min per session, 30 spikes total) (Tovar KR et al. 2002). 10 min after ketamine washout, we introduced two such “kick-off” sessions of neuronal activity, and found that as predicted, NMDAR-eEPSC recovered to $80.0 \pm 11.3\%$ of baseline level by 50 min (FIG. 10A). In contrast, a protocol with 1 Hz presynaptic stimulation alone did not result in such recovery (FIGS. 10A-10B). The “kick-off” protocol itself did not cause any sustained change in the NMDAR-eEPSCs of LHb neurons without ketamine treatment (FIG. 12), suggesting that the recovered NMDAR-eEPSC was not due to long-term potentiation.

Example 7: Activation of LHb at low [Ket] shortens antidepressant

The above *in vitro* experiment indicated the potential to “kicking off” ketamine from the NMDARs in LHb neurons through neural activity. Subsequently, we explored whether this understanding combined with ketamine’s pharmacokinetics (PK) could be harnessed to modulate the duration of ketamine’s behavioral effects *in vivo* (FIGS. 13A-13L). Unlike the *in vitro*

recording condition, there is magnesium and it is not possible to voltage-clamp neurons in the *in vivo* environment. Instead, in order to induce LHb neural activity and open NMDARs *in vivo*, we stimulated a major LHb input pathway, the lateral hypothalamus (LH), to induce LHb burst firing, through which short-interval spikes can produce concomitant glutamate release and postsynaptic depolarization required for NMDAR opening (FIGS. 14A-14C). To stimulate the LH input to LHb, we expressed the AAV-ChrimsonR-tdTomato in the LH of CRS mice, and through bilateral optic fibers, stimulated the LH terminals in the LHb (635 nm, 40 Hz, 2 ms pulse, 250 μ W, see Methods, FIGS. 13A-13B). As the LH-LHb stimulation is known to be aversive (Lecca S et al. 2017; Stamatakis AM et al. 2016; Zheng Z et al. 2022), we tested the 40 Hz protocol on inducing real-time place aversion (RTPA, FIG. 13C) in every animal going through the behavioral protocol (FIGS. 13F and 13J), to confirm that the stimulation was effective. As a use-dependent open channel blocker, ketamine's interaction with the NMDARs depends on the channels' open state, which we can manipulate using the above optogenetic stimulation protocol. When NMDARs are opened and accessible to ketamine entry, according to the dynamic equilibrium, the binding and unbinding of ketamine will be strongly regulated by its ambient level: when ambient ketamine level is higher than the dissociation constant (K_d), the interaction favors more binding; when ambient ketamine level is lower than K_d , it favors more unbinding (FIGS. 13D, 13E and 13I). Therefore, for the *in vivo* "kick off" or unbinding experiment, we tried to induce NMDAR opening at a low ambient ketamine level, by stimulating the LH-LHb terminals (40 Hz, 3 min) at 1 h after ketamine injection, when its ambient level had dropped to 0.23 μ M (FIG. 1A), several fold below the K_d ($\sim$ 0.8 μ M) (Parsons CG et al. 1995) (FIGS. 13E-13F, FIG. 14D). We first examined the "kick off" effect by recording NMDAR-eEPSCs of LHb brain slices prepared right after the optical stimulation (FIGS. 14D-14G). Compared with the non-stimulated group, there was a significant increase in NMDAR-eEPSCs, suggesting that ketamine had been released from the NMDARs by the *in vivo* LH-LHb optical stimulation (FIGS. 14D-14G). To test the effects of such "kick off" protocol on sustained antidepressant effects, we then measured both the LHb-NMDAR responses and behavioral effects of LH stimulation at 24 h after ketamine administration. In control mCherry-expressing mice, which do not have light-induced activity in the LHb, ketamine still

suppressed the NMDAR-eEPSCs and reduced immobility in the FST at 24 h after ketamine injection (FIGS. 13G-13H). In ChrimsonR-expressing mice, which have light-induced activity in the LHb, however, ketamine no longer had such effects at the 24 h time point (FIGS. 13G-13H). These results suggest that *in vivo* induced activity in the LHb at a time point with low ambient ketamine level eliminates ketamine's sustained effects on both LHb NMDARs and antidepressant behaviors.

Example 8: Activation of LHb at high [Ket] extends antidepression

For clinical relevance, it would be desirable to achieve the opposite from the above -- to extend the duration of ketamine's antidepressant effects, or, achieve the same duration with a lower dosage. When the dosage of ketamine i.p. injection was lowered from 10 to 5 mg kg⁻¹, the antidepressant effects were still observed at 1 h (Kim JW et al. 2021) but no longer at 24 h (Franceschelli A et al 2015) (FIG. 13L). One explanation for this shortened effect is that at this lower dose, more of the blocked LHb-NMDARs may have recovered by 24 h (FIG. 13K). Given the reverse correlation between the level of initial ketamine blockade and the level of recovery (FIGS. 11A-11D), we reasoned that if we could block more LHb-NMDARs upon ketamine treatment, we may slow down its recovery and extend the therapeutic effects of ketamine at 5 mg kg⁻¹. For more ketamine blockade, we chose to stimulate the LH-LHb pathway to open more NMDARs at a time when ambient ketamine level is high (FIGS. 13I-13J). We injected CRS mice with ketamine at 5 mg kg⁻¹, and then stimulated the LH-LHb pathway (635 nm, 3 min, 40 Hz) immediately after the ketamine injection, when ambient ketamine level was above 6 μ M (FIG. 15), several fold above the K_d (FIG. 13J). Compared with the mCherry-expressing group, ChrimsonR-expressing group was expected to favor more ketamine-NMDAR binding after light stimulation and a slower recovery of NMDAR responses (FIG. 13J). Indeed, 24 h after ketamine injection, in the control mCherry-expressing mice, 5 mg kg⁻¹-ketamine-treated group did not show difference with saline-treated mice; in the ChrimsonR-expressing mice, however, ketamine-treated mice showed more suppressed NMDAR responses and reduced immobility in the FST (FIGS. 13K-13L). There were also significant differences between ketamine-treated ChrimsonR- and the mCherry-expressing group (FIGS. 13K-13L). These results suggest that *in vivo* induced activity in the LH-LHb pathway at a time

point with high ambient ketamine extended ketamine's effects on both inhibiting LHb NMDARs and reducing depression-like behaviors.

Discussion

In the present work, we examine whether the sustained anti-depressant efficacy of ketamine is a direct consequence of its unique pharmacodynamics feature. We demonstrate that, even though ketamine concentrations drastically decay in the plasma and the brain, and fall below the IC₅₀ within 30 minutes, ketamine continues to block NMDARs and suppress burst firing in the LHb, for up to 24 h in mice (FIGS. 1A-1L and 5A-5R). The sustained inhibition of NMDARs by ketamine is not due to endocytosis, and can be displaced by neural activity (FIGS. 7A-7M, 9A-9E and 10A-10B). By activating the LH-LHb circuit at different time points after ketamine treatment, we were able to either shorten or extend ketamine's sustained antidepressant effects (FIGS. 13A-13L). These findings support a hypothesis that long-term blockade of NMDARs within the LHb region mediates ketamine's sustained antidepressant effects, at least within 24 h. Beyond the 24 h time point, it is possible that neural plasticity and other secondary mechanisms may kick in to account for the sustained effects at an even longer time scale (Maeng S et al. 2008; Li N et al. 2010; Autry AE et al. 2011; Zanos P et al. 2016; Kim JW et al. 2021; Moda-Sava RN et al. 2019). Nevertheless, our study illustrates a case where a biophysical channel block mechanism extended to extraordinarily long times by the *in vivo* physiological parameters may explain an important therapeutic function.

At the molecular level, it is a surprising discovery that a single dose of ketamine administration blocks the NMDAR channel for as long as 24 hours. This duration is orders-of-magnitude longer than its reported dissociation time (5 ~ 13 s) (Mealing GA et al. 1999), which were measured *in vitro* in steady-state NMDARs in dissociated cells in the constant presence of saturating amount of agonists (10 μ M glycine, 10 μ M NMDA) (Clements JD et al. 1992). In the physiological synaptic environment, however, upon a single stimulation, the post-synaptic NMDARs are shown to be transiently exposed to glutamate for only 1 ~ 2 ms (Clements JD et al. 1992), and the duration of channel open time is less than 10 ms (Buck DP et al. 2000). In addition, without stimulation, the intrinsic open probability of NMDARs in synapses is negligible ($P_o = 0.04$) (Rosenmund C et al. 1995). Therefore it is highly plausible that under low-level neural activity *in vivo*, the trapped

ketamine can not be efficiently released from the NMDARs. Another factor that could possibly contribute to ketamine's extended action time *in vivo* is delayed diffusion (Katz B et al. 1973). Unlike transmitters which have transporter-based clearance mechanism, ketamine could likely rebind to unbound NMDARs receptors multiple times as it is released and diffuses out of the synaptic clefts or extracellular spaces where astrocytic endfeet tightly wrap around neurons (Cui Y et al. 2018). Given the difference in wash off experiments between memantine and ketamine (FIGS. 7A-7M), such lateral diffusion seems unlikely to be the major explanation for the sustained effects, but may nevertheless contribute to the extended action time. As a consequence of the above factors, the apparent k_{off} of ketamine *in vivo* can be much longer than the previously measured in-solution k_{off} . Therefore once bound, a significant amount of ketamine is trapped, isolating it from metabolic degradation and sustaining its antidepressant effects.

Currently, there is an intense debate about whether the antidepressant effects of ketamine are mediated by the NMDARs at all. One major argument against an NMDAR-based mechanism is that, unlike ketamine, many other NMDAR inhibitors failed to show comparable clinical efficacy (Newport DJ et al. 2015). However, this notion was directly challenged by a recent phase III clinical trial demonstrating that, in conjunction with a compound to slow down its metabolism, AXS-05, another pore-trapping-type NMDAR inhibitor effectively improved patients' depression condition (Iosifescu DV et al. 2022). The success of AXS-05 strongly indicated that the root cause of failure for other NMDAR antagonists is not the target, but rather the properties of compounds themselves. Different NMDAR inhibitors have distinct inhibition mechanisms (e.g. competitive binding, allosteric binding vs. pore trapping) (Lodge D et al. 1990; Traynelis SF et al. 2010), pharmacokinetic and pharmacodynamic features. As illustrated in this study, even an NMDAR inhibitor that shares similar trapping mechanism and similar binding affinity as ketamine but a faster off rate – memantine (Parsons CG et al. 1995) – can have less optimal and less lasting antidepressant effects (Gideons ES et al. 2014; Zarate CA et al. 2006) (FIGS. 7A-7M). These results demonstrate that the unique pharmaceutical features of ketamine are critical for its antidepressant effects, and optimization of these properties is a promising new direction for developing new antidepressant treatments.

Our discoveries have several direct clinical implications. Firstly, we found that local application of ketamine in the LHb produced even more long-lasting antidepressant effects than systemic application, which were 7 days in mice (FIGS. 7K and 7L) and 14 days in rats (data not shown). This may be explained by the fact that, by locally targeting the LHb region, we were able to administer ketamine at a much higher concentration (e.g. mM range), which ensured a larger fraction of NMDARs being blocked and, therefore, a longer duration of NMDAR inhibition (FIGS. 11A-11D). This high local concentration can not be achieved by systemic administration because of the well-known side effects on peripheral organs (Chu PS et al. 2008). Clinically, local administration at the LHb could be achieved, possibly through liposome delivery combined with focused ultrasound (Elias WJ et al. 2016), or before deep brain stimulation (DBS) protocol in the region (Sartorius A et al. 2010). Secondly, our data suggest that the effective duration of ketamine treatment is subjected to regulation and interference by local neural activity: activating LHb at low ambient ketamine level shortens, whereas activating LHb at high ambient ketamine level extends, ketamine's antidepressant effects (FIGS. 13A-13L). Since aversive stimuli can effectively activate the LHb under physiological conditions (Hu H et al. 2020), these data suggest two appealing, clinically testable strategies to extend ketamine's efficacy: one is to apply ketamine during a depressive episode, or, to activate the LHb during ketamine administration by moderate aversive stimuli such as noise or other acute stressor, to open more LHb-NMDARs for more ketamine trapping; the other is to minimize negative emotional episodes after ketamine treatment to slow down the ketamine untrapping.

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CLAIMS

1. A method for increasing duration of a therapeutic effect of an N-methyl-D-aspartate receptor (NMDAR) pore blocker administered to a subject in need thereof, comprising at least one of:

prior to, or within a first time period after, administering the NMDAR pore blocker or a pharmaceutically acceptable salt thereof to the subject, carrying out a first procedure such that ion channels of a greater proportion of the NMDAR molecules in a lateral habenula (LHb) of the subject are opened to be thereby accessible to molecules of the NMDAR pore blocker compared to when the first procedure is absent; or

after administering the NMDAR pore blocker or a pharmaceutically acceptable salt thereof to the subject, carrying out a second procedure such that untrapping of the NMDAR pore blocker molecules from the NMDAR is prevented or reduced.

2. The method of claim 1, wherein the NMDAR pore blocker comprises at least one of ketamine, memantine, or dextromethorphan (AXS-05), dizocilpine, amantadine, lanicemine, or D-methadone.

3. The method of claim 1 or claim 2, wherein the administering the NMDAR pore blocker or a pharmaceutically acceptable salt thereof comprises systemic administration.

4. The method of claim 3, wherein the first time period is the plasma elimination half-time of the NMDAR pore blocker.

5. The method of claim 3 or 4, wherein the subject is a human subject, wherein one of the following is met:

(1) the NMDAR pore blocker comprises ketamine, and the first time period is approximately 3 hours;

(2) the NMDAR pore blocker comprises memantine, and the first time period is approximately 60 hours;

(3) the NMDAR pore blocker comprises dextromethorphan (i.e. AXS-05), and the first time period is approximately 29.5 hours; or

(4) the NMDAR pore blocker comprises amantadine, and the first time period is approximately 16 hours;

(5) the NMDAR pore blocker comprises lanicemine, and the first time period is approximately 9 hours; or

(6) the NMDAR pore blocker comprises D-methadone, and the first time period is approximately 33 hours.

6. The method of claim 1 or claim 2, wherein the administering the NMDAR pore blocker or a pharmaceutically acceptable salt thereof comprises local administration.

7. The method of claim 6, wherein the local administration is by means of a controllable implantable device capable of local delivery to the LHb of the subject.

8. The method of claim 7, wherein the controllable implantable device is wholly embedded within, or in a proximity of, the LHb of the subject.

9. The method of claim 7, wherein the controllable implantable device comprises a catheter module with a drug delivery outlet arranged within, or in the proximity of, the LHb of the subject, and the implantable device further comprises a distally arranged reservoir module operably connecting the catheter module, wherein the reservoir module accommodates the NMDAR pore blocker or a pharmaceutically acceptable salt thereof.

10. The method of claim 6, wherein the local administration is by means of a catheter with a drug delivery outlet arranged within, or in the proximity of, the LHb of the subject.

11. The method of claim 10, wherein the catheter is operably connected to a distal reservoir.

12. The method of claim 11, wherein the distal reservoir is Ommaya reservoir arranged beneath a scalp of the subject.

13. The method of any of claims 1-12, wherein the first procedure comprises a first behavioral measure, comprising applying an aversive stimulus to the subject.

14. The method of claim 13, wherein the subject is a human subject, wherein the applying an aversive stimulus to the subject comprises providing one or more unpleasant cues to the human subject, wherein the one or more unpleasant cues comprises a visual cue, an auditory cue, a haptic cue, an olfactory cue, a gustatory cue, or any combination thereof.

15. The method of claim 14, wherein the providing one or more unpleasant cues to the human subject is by means of a virtual reality (VR) device.

16. The method of claim 13, wherein the subject is a rodent subject, wherein the applying an aversive stimulus or a stressor to the subject comprises performing one of the following tasks to the rodent subject: restrained stress, forced swimming, tail suspension, air puff, social attack, bitter taste, olfactory cue (fox urine, 2,5-Dihydro-2,4,5-trimethylthiazoline (TMT), *ect.*), sleep deprivation, water deprivation, footshock, noise, or looming visual stimulus.

17. The method of any of claims 1-16, wherein the first procedure comprises a first chemical measure, comprising providing at least one priming agent to the subject, wherein each of the at least one priming agent is capable of opening pores of the NMDAR molecules in the LHb.

18. The method of claim 17, wherein the at least one priming agent comprises an NMDAR agonist, wherein the NMDAR agonist is of one of the following classes:

- (1) aspartate or derivatives thereof (e.g. D-aspartate, NMDA, L-aspartate, etc.);
- (2) glutamate or or derivatives thereof (e.g. tetrazolylglycine, homocysteic acid, ibotenic acid, quinolinic acid, etc.);
- (3) glycine or or derivatives thereof (e.g. D-alanine, L-alanine, milacemide, sarcosine, D-serine, L-serine, etc.);
- (4) polyamine (e.g. spermindine, spermine, etc.); or
- (5) positive allosteric modulator (e.g. nebotinel, etc.).

19. The method of any of claims 1-18, wherein the first procedure comprises a first physical measure capable of at least one of:

- increasing the permeability of brain-blood barrier in a brain of the subject so as to increase a local concentration of the molecules of the NMDAR pore blocker in the LHb; or
- stimulating the activity of the NMDAR in the LHb.

20. The method of claim 19, wherein the first physical measure comprises an acoustic measure capable of increasing the permeability of brain-blood barrier in the LHb of the subject, wherein the acoustic measure is by means of focused ultrasound.

21. The method of claim 19, wherein the first physical measure comprises a surgical measure.

22. The method of claim 19, wherein the first physical measure comprises a first electrophysical measure by means of an electrode or an implantable device capable of electrically stimulating the activity of the NMDAR in the LHB.
23. The method of any of claims 1-22, wherein the second procedure comprises a second behavioral measure, comprising providing an environment to the subject such that the subject is capable of obtaining pleasant feelings and/or avoiding negative emotional episodes.
24. The method of claim 23, wherein the providing an environment to the subject comprises providing one or more pleasant cues to the human subject, wherein the one or more pleasant cues comprises a visual cue, an auditory cue, a haptic cue, an olfactory cue, a gustatory cue, or any combination thereof.
25. The method of claim 24, wherein the providing one or more pleasant cues to the human subject is by means of a virtual reality (VR) device.
26. The method of any of claims 1-25, wherein the second procedure comprises a second chemical measure, comprising providing at least one enhancing agent to the subject, each capable of enhancing the therapeutic effect of the NMDAR pore blocker.
27. The method of claim 26, wherein the at least one enhancing agent comprises at least one first reagent, each capable of antagonizing the NMDAR activity, and selected from one of the following classes:
- (1) NMDAR open channel blocker (e.g. memantine, or dextromethorphan (AXS-05), dizocilpine, amantadine, lanicemine, or D-methadone, etc.); or
 - (2) NMDAR competitive antagonist (e.g. antagonist-2-amino-7-phosphonoheptanoic acid (AP7), antagonist 2-amino-5-phosphonopentanoic acid (AP5), 4-[(2E)-3-Phosphono-2-propenyl]-2-piperazinecarboxylic acid (CPPene), agonist-1-aminocyclopropanecarboxylic acid (ACPC), rapastinel (GLYX-13), L-4-chlorokynurenine or 4-Cl-KYN (AV-101), D-cycloserine, Ro 25-6981, (1S,2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol (CP-101606), ifenprodil, rislenemdaz hydrochloride (MK-0657), etc.).

28. The method of claim 26 or claim 27, wherein the at least one enhancing agent comprises at least one second reagent, each capable of suppressing the neuronal activity in the LHb of the subject, selected from one of the following classes:

- (1) Gamma-aminobutyric acid type A receptor (GABA_AR) agonists (e.g. Muscimol, *etc.*);
- (2) α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor (AMPA) antagonists (e.g. antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)-quinoxaline (NBQX), 6-cyano-7-Nitroquinoxaline-2,3-dione (CNQX), *etc.*); or
- (3) sodium channel blockers (e.g. tetrodotoxin (TTX), *etc.*).

29. The method of any of claims 1-28, wherein the second procedure comprises a second physical measure capable of suppressing the activity of the NMDAR in the LHb.

30. The method of claim 29, wherein the second physical measure comprises a second electrophysical measure by means of an electrode or an implantable device capable of electrically suppressing the activity of the NMDAR in the LHb.

31. A therapeutic regimen for treating a subject with depression, comprising:

administering an NMDAR pore blocker or a pharmaceutically acceptable salt thereof to the subject; and

prior to, or within a first time period after, administering an NMDAR pore blocker or a pharmaceutically acceptable salt thereof to the subject, carrying out a first procedure such that ion channels of a greater proportion of the NMDAR molecules in a lateral habenula (LHb) of the subject are opened to be thereby accessible to molecules of the NMDAR pore blocker compared to when the first procedure is absent.

32. The therapeutic regimen of claim 31, wherein the NMDAR pore blocker comprises at least one of ketamine, memantine, or dextromethorphan (AXS-05), dizocilpine, amantadine, lanicemine, or D-methadone.

33. The therapeutic regimen of claim 31 or claim 32, wherein the administering an NMDAR pore blocker or a pharmaceutically acceptable salt thereof comprises systemic administration, and the first time period is the plasma elimination half-time of the NMDAR pore blocker.

34. The therapeutic regimen of any one of claims 31-33, wherein the administering the NMDAR pore blocker or a pharmaceutically acceptable salt thereof comprises local administration, by means of a controllable implantable device capable of local delivery to the LHb of the subject or by means of a catheter with a drug delivery outlet arranged within, or in the proximity of, the LHb of the subject.

35. The therapeutic regimen of any of claims 31-34, wherein the first procedure comprises a first behavioral measure, comprising applying an aversive stimulus or a stressor to the subject.

36. The therapeutic regimen of claim 35, wherein the applying an aversive stimulus or a stressor to the subject comprises providing one or more unpleasant cues to the human subject, wherein the one or more unpleasant cues comprises a visual cue, an auditory cue, a haptic cue, an olfactory cue, a gustatory cue, or any combination thereof.

37. The therapeutic regimen of any of claims 31-36, wherein the first procedure comprises a first chemical measure, comprising providing at least one priming agent to the subject, wherein each of the at least one priming agent is capable of opening pores of the NMDAR molecules in the LHb.

38. The therapeutic regimen of claim 37, wherein the at least one priming agent comprises an NMDAR agonist, wherein the NMDAR agonist is of one of the following classes:

- (1) aspartate or derivative thereof (e.g. D-aspartate, NMDA, L-aspartate, etc.);
- (2) glutamate or derivatives thereof (e.g. tetrazolylglycine, homocysteic acid, ibotenic acid, quinolinic acid, etc.);
- (3) glycine or derivatives thereof (e.g. D-alanine, L-alanine, milacemide, sarcosine, D-serine, L-serine, etc.);
- (4) polyamine (e.g. spermindine, spermine, etc.); or
- (5) positive allosteric modulator (e.g. neostinel, etc.).

39. The therapeutic regimen of any of claims 31-38, wherein the first procedure comprises a first physical measure capable of at least one of:

- increasing the permeability of brain-blood barrier in a brain of the subject so as to increase a local concentration of the molecules of the NMDAR pore blocker in the LHb; or
- stimulating the activity of the NMDAR in the LHb.

40. The therapeutic regimen of claim 38, wherein the first physical measure comprises an acoustic measure, a surgical measure, or a first electrophysical measure.

41. The therapeutic regimen of any one of claims 31-40, further comprising: after administering the NMDAR pore blocker or a pharmaceutically acceptable salt thereof to the subject, carrying out a second procedure to keep activity of the LHb low such that untrapping of the NMDAR pore blocker molecules from the NMDAR is prevented or reduced.

42. The therapeutic regimen of claim 41, wherein the second procedure comprises a second behavioral measure, comprising providing an environment to the subject such that the subject is capable of obtaining pleasant feelings and/or avoiding negative emotional episodes.

43. The therapeutic regimen of claim 42, wherein the providing an environment to the subject comprises providing one or more pleasant cues to the human subject, wherein the one or more pleasant cues comprises a visual cue, an auditory cue, a haptic cue, an olfactory cue, a gustatory cue, or any combination thereof.

44. The therapeutic regimen of any one of claims 41-43, wherein the second procedure comprises a second chemical measure, comprising providing at least one enhancing agent to the subject, each capable of suppressing the neuronal activity, or of antagonizing the NMDAR activity, in the LHb of the subject.

45. The therapeutic regimen of claim 44, wherein the at least one enhancing agent comprises at least one first reagent, each capable of antagonizing the NMDAR activity, and selected from one of the following classes:

(1) NMDAR open channel blocker (e.g. memantine, or dextromethorphan (AXS-05), dizocilpine, amantadine, lanicemine, or D-methadone, etc.); or

(2) NMDAR competitive antagonist (e.g. antagonist-2-amino-7-phosphonoheptanoic acid (AP7), antagonist 2-amino-5-phosphonopentanoic acid (AP5), 4-[(2E)-3-Phosphono-2-propenyl]-2-piperazinecarboxylic acid (CPPene), agonist-1-aminocyclopropanecarboxylic acid (ACPC), rapastinel (GLYX-13), L-4-chlorokynurenine or 4-CI-KYN (AV-101), D-cycloserine, Ro 25-6981,

(1S,2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol (CP-101606), ifenprodil, rislenemdaz hydrochloride (MK-0657), etc.).

46. The therapeutic regimen of claim 44 or claim 45, wherein the at least one enhancing agent comprises at least one second reagent, each capable of suppressing the neuronal activity in the LHb of the subject, selected from one of the following classes:

- (1) Gamma-aminobutyric acid type A receptor (GABA_AR) agonists (e.g. Muscimol, *etc.*);
- (2) α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor (AMPA) antagonists (e.g. antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)-quinoxaline (NBQX), 6-cyano-7-Nitroquinoxaline-2,3-dione (CNQX), *etc.*); or
- (3) sodium channel blockers (e.g. tetrodotoxin (TTX), *etc.*).

47. The therapeutic regimen of any of claims 41-46, wherein the second procedure comprises a second physical measure capable of suppressing the activity of the NMDAR in the LHb.

48. The therapeutic regimen of claim 47, wherein the second physical measure comprises a second electrophysical measure by means of an electrode or an implantable device capable of electrically suppressing the activity of the NMDAR in the LHb.

49. A pharmaceutical combination for treating a subject with depression, comprising:

at least one NMDAR pore blocker or a pharmaceutically acceptable salt thereof; and
one or both of:

at least one priming agent, each capable of opening pores of the NMDAR molecules
in the LHb; or

at least one enhancing agent, each capable of suppressing neuronal activity in the
LHb.

50. The pharmaceutical combination of claim 49, wherein each of the at least one NMDAR pore blocker is selected from a group consisting of ketamine, memantine, or dextromethorphan (AXS-05), dizocilpine, amantadine, lanicemine, and D-methadone.

51. The pharmaceutical combination of claim 49 or claim 50, wherein each priming agent is an NMDAR agonist selected from one the following types: (1) aspartate or derivatives thereof (e.g. D-aspartate, NMDA, L-aspartate, etc.); (2) glutamate or derivatives thereof (e.g. tetrazolyglycine,

homocysteic acid, ibotenic acid, quinolinic acid, etc.); (3) glycine or derivatives thereof (e.g. D-alanine, L-alanine, milacemide, sarcosine, D-serine, L-serine, etc.); (4) polyamine (e.g. spermidine, spermine, etc.); (5) positive allosteric modulator (e.g. neostinel, etc.).

52. The pharmaceutical combination of any one of claims 49-51, wherein the at least one enhancing agent comprises at least one first reagent, each capable of antagonizing the NMDAR activity and selected from one of the following classes:

- (1) NMDAR open channel blocker (e.g. memantine, or dextromethorphan (AXS-05), dizocilpine, amantadine, lanicemine, or D-methadone, etc.); or
- (2) NMDAR competitive antagonist (e.g. antagonist-2-amino-7-phosphonoheptanoic acid (AP7), antagonist 2-amino-5-phosphonopentanoic acid (AP5), 4-[(2E)-3-Phosphono-2-propenyl]-2-piperazinecarboxylic acid (CPPene), agonist-1-aminocyclopropanecarboxylic acid (ACPC), rapastinel (GLYX-13), L-4-chlorokynurenine or 4-Cl-KYN (AV-101), D-cycloserine, Ro 25-6981, (1S,2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol (CP-101606), ifenprodil, rislenemdaz hydrochloride (MK-0657), etc.).

53. The pharmaceutical combination of any one of claims 49-51, wherein the at least one enhancing agent comprises at least one second reagent, each capable of suppressing the neuronal activity in the LHb of the subject, selected from one of the following classes:

- (1) Gamma-aminobutyric acid type A receptor (GABA_AR) agonists (e.g. Muscimol, *etc.*);
- (2) α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor (AMPA) antagonists (e.g. antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)-quinoxaline (NBQX), 6-cyano-7-Nitroquinoxaline-2,3-dione (CNQX), *etc.*); or
- (3) sodium channel blockers (e.g. tetrodotoxin (TTX), *etc.*).

54. A drug delivery system capable of realizing a controlled local release of an NMDAR pore blocker within a lateral habenula (LHb) of a subject, comprising an implantable unit, wherein the implantable unit comprises a first reservoir module, a dispensing module, a control module and a power source module, wherein:

the first reservoir module accommodates a pharmaceutically acceptable formulation of the NMDAR pore blocker;

the dispensing module is operably coupled to the first reservoir module, and is configured, upon receiving a first activation command from the control module, to dispense the NMDAR pore blocker out of the first reservoir module;

the control module is communicatively coupled to the dispensing module and is configured to control the dispensing module to be activated; and

the power source module is electrically connected, and configured to provide power, to the dispensing module and the control module respectively.

55. The drug delivery system of claim 54, wherein the first reservoir module comprises a plurality of reservoirs, each accommodating one therapeutically effective dose of the NMDAR pore blocker, wherein the dispensing module is configured, upon receiving the first activation command from the control module, to dispense the NMDAR pore blocker out of the plurality of reservoirs in the first reservoir module, one per time.

56. The drug delivery system of claim 55, wherein each of the plurality of reservoirs is provided with a capping membrane having a biocompatible composition, and the dispensing module comprises a dispensing mechanism that is configured to break the capping membrane so as to allow the one therapeutically effective dose of the NMDAR pore blocker to be released out of the each of the plurality of reservoirs.

57. The drug delivery system of claim 56, wherein the dispensing mechanism is an electrothermal activation mechanism, and the capping membrane has a heat-responsive composition selected from titanium (Ti), platinum (Pt) or gold (Au).

58. The drug delivery system of claim 56, wherein the dispensing mechanism is an electrochemical activation mechanism, and the capping membrane has a composition of gold (Au).

59. The drug delivery system of claim 54, wherein the first reservoir module comprises one single reservoir, wherein the dispensing module is configured, upon receiving the first activation command from the control module, to operate on the first reservoir module such that the NMDAR pore blocker is released out of the one single reservoir in the first reservoir module, one therapeutically effective dose per time.
60. The drug delivery system of claim 59, wherein the dispensing module comprises a micropump, having a type selected from an infusion type, a peristaltic type, a thermopneumatic type, an osmotic type, or a positive displacement type.
61. The drug delivery system of claim 59, wherein the dispensing module comprises a microvalve, having a piezoelectric type or a thermoresponsive type.
62. The drug delivery system of any one of claims 54-61, wherein the NMDAR pore blocker is selected from a group consisting of ketamine, memantine, or dextromethorphan (AXS-05), dizocilpine, amantadine, lanicemine, and D-methadone.
63. The drug delivery system of any one of claims 54-62, wherein the implantable unit further comprises a second reservoir module accommodating a pharmaceutically acceptable formulation of a priming agent, wherein the priming agent is capable of open pores of the NMDAR molecules to thereby allow molecules of the NMDAR pore blocker to get access thereto.
64. The drug delivery system of claim 63, wherein the priming agent comprises an NMDAR agonist, wherein the NMDAR agonist is of one of the following types:
- (1) aspartate or derivatives thereof (e.g. D-aspartate, NMDA, L-aspartate, etc.);
 - (2) glutamate or derivatives thereof (e.g. tetrazolylglycine, homocysteic acid, ibotenic acid, quinolinic acid, etc.);
 - (3) glycine or derivatives thereof (e.g. D-alanine, L-alanine, milacemide, sarcosine, D-serine, L-serine, etc.);
 - (4) polyamine (e.g. spermindine, spermine, etc.); or
 - (5) positive allosteric modulator (e.g. neostinel, etc.)
65. The drug delivery system of claim 63 or claim 64, wherein the dispensing module is further operably coupled to the second reservoir module, and is further configured, upon receiving a second

activation command from the control module, to dispense the priming agent out of the second reservoir module.

66. The drug delivery system of claim 65, wherein the control module is configured to control the dispensing module to sequentially:

dispense the priming agent out of the second reservoir module; and

dispense the NMDAR pore blocker out of the first reservoir module.

67. The drug delivery system of any one of claims 54-66, wherein the implantable unit further comprises a third reservoir module accommodating a pharmaceutically acceptable formulation of an enhancing agent, wherein the enhancing agent is capable of capable of antagonizing the NMDAR activity or suppressing the neuronal activity in the LHb of the subject.

68. The drug delivery system of claim 67, wherein the enhancing agent comprises a reagent selected from one of the following classes:

(1) NMDAR open channel blocker (e.g. memantine, or dextromethorphan (AXS-05), dizocilpine, amantadine, lanicemine, or D-methadone, etc.);

(2) NMDAR competitive antagonist (e.g. antagonist-2-amino-7-phosphonoheptanoic acid (AP7), antagonist 2-amino-5-phosphonopentanoic acid (AP5), 4-[(2E)-3-Phosphono-2-propenyl]-2-piperazinecarboxylic acid (CPPene), agonist-1-aminocyclopropanecarboxylic acid (ACPC), rapastinel (GLYX-13), L-4-chlorokynurenine or 4-CI-KYN (AV-101), D-cycloserine, Ro 25-6981, (1S,2S)-1-(4-hydroxyphenyl)-2-(4-hydroxy-4-phenylpiperidino)-1-propanol (CP-101606), ifenprodil, rislenemdaz hydrochloride (MK-0657), etc.);

(3) Gamma-aminobutyric acid type A receptor (GABA_AR) agonists (e.g. Muscimol, *etc.*);

(4) α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor (AMPA) antagonists (e.g. antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)-quinoxaline (NBQX), 6-cyano-7-Nitroquinoxaline-2,3-dione (CNQX), *etc.*); or

(5) sodium channel blockers (e.g. tetrodotoxin (TTX), *etc.*).

69. The drug delivery system of claim 67 or claim 68, wherein the dispensing module is further operably coupled to the third reservoir module, and is further configured, upon receiving a third

activation command from the control module, to dispense the enhancing agent out of the third reservoir module.

70. The drug delivery system of claim 69, wherein the control module is configured to control the dispensing module to sequentially:

dispense the NMDAR pore blocker out of the first reservoir module; and

dispense the enhancing agent out of the third reservoir module.

71. The drug delivery system of any one of claims 54-62, wherein the implantable unit further comprises a second reservoir module accommodating a pharmaceutically acceptable formulation of a priming agent and a third reservoir module accommodating a pharmaceutically acceptable formulation of an enhancing agent, wherein:

the priming agent capable of open pores of the NMDAR molecules to thereby allow molecules of the NMDAR pore blocker to get access thereto;

the enhancing agent is capable of capable of antagonizing the NMDAR activity or suppressing the neuronal activity in the LHb of the subject;

the dispensing module is further operably coupled to the second reservoir module, and is further configured, upon receiving a second activation command from the control module, to dispense the priming agent out of the second reservoir module; and

the dispensing module is further operably coupled to the third reservoir module, and is further configured, upon receiving a third activation command from the control module, to dispense the enhancing agent out of the third reservoir module.

72. The drug delivery system of claim 71, wherein the control module is configured to control the dispensing module to sequentially:

dispense the priming agent out of the second reservoir module;

dispense the NMDAR pore blocker out of the first reservoir module; and

dispense the enhancing agent out of the third reservoir module.

73. The drug delivery system of any of claims 54-72, wherein the power source module comprises a first energy sub-module capable of storing energy.

74. The drug delivery system of claim 73, wherein the first energy sub-module comprises a battery.

75. The drug delivery system of claim 74, wherein the battery is a high-capacity battery selected from a lithium-iodine (Li-I₂) battery, a lithium-thionyl-chloride (Li/SOCl₂) battery, a lithium-silver vanadium oxide (Li-SVO) battery, a lithium-carbon monofluoride (Li-Cfx) battery, or a lithium-manganese oxide (Li-MnO₂).
76. The drug delivery system of claim 74, wherein the battery comprises a rechargeable battery.
77. The drug delivery system of claim 73, wherein the first energy sub-module comprises a supercapacitor.
78. The drug delivery system of any of claims 54-77, wherein the power source module comprises a second energy sub-module capable of receiving power wirelessly from an external source.
79. The drug delivery system of claim 78, wherein the second energy sub-module receives energy from the external source by means of electromagnetic energy transfer.
80. The drug delivery system of claim 78, wherein the second energy sub-module receives energy from the external source by means of ultrasound energy transfer.
81. The drug delivery system of any of claims 54-77, wherein the power source module comprises a third energy sub-module capable of harvesting energy from body of the subject.
82. The drug delivery system of claim 81, wherein the third energy sub-module is capable of harvesting energy electrochemically.
83. The drug delivery system of claim 82, wherein the third energy sub-module comprises a fuel cell capable of harvesting energy through glucose oxidation.
84. The drug delivery system of claim 81, wherein the third energy sub-module is capable of harvesting energy thermoelectrically.
85. The drug delivery system of claim 81, wherein the third energy sub-module is capable of harvesting energy piezoelectrically.
86. The drug delivery system of any of claims 54-85, wherein the implantable unit further comprises a catheter module with a drug delivery outlet arranged within, or in the proximity of, the LHB of the subject, wherein the catheter module is distally and operably connected to the first reservoir module.
87. The drug delivery system of claim 86, wherein the first reservoir module is arranged beneath a scalp of the subject.

88. The drug delivery system of any of claims 54-87, wherein the control module is programmed to periodically send activation commands to the dispensing module to thereby activate the dispensing module.
89. The drug delivery system of any of claims 54-88, further comprising an external control unit, wherein the external control module is communicatively coupled, and configured to send control instructions, to the control module of the implantable unit, wherein the control module is further configured, upon receiving one control instruction from the external control unit, to send a corresponding activation command to the dispensing module.
90. The drug delivery system of claim 89, wherein the external control unit is communicatively coupled to the implantable unit by means of a corresponding pair of a transmitter and an antenna, wherein the antenna is arranged in the implantable unit and is communicatively connected to the control module.
91. The drug delivery system of any of claims 54-90, further comprising an external power unit, wherein the external power unit is configured to wirelessly transfer energy to the power source module in the implantable unit.
92. The drug delivery system of claim 91, wherein the external control unit comprises a power transmitter, and the implantable unit comprises a corresponding power receiver.

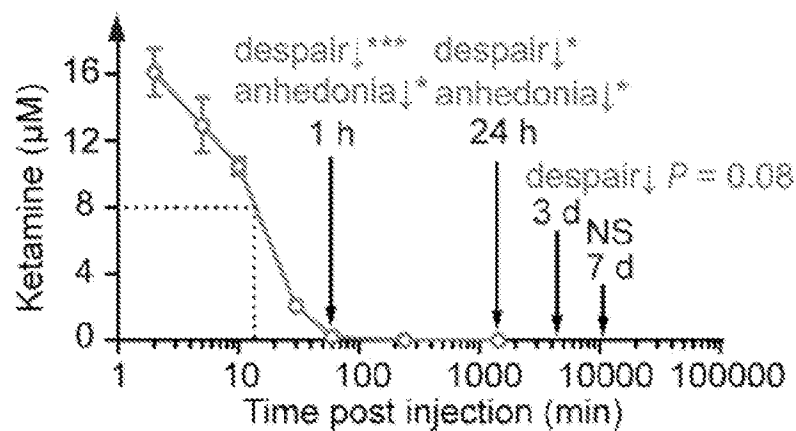


FIG. 1A

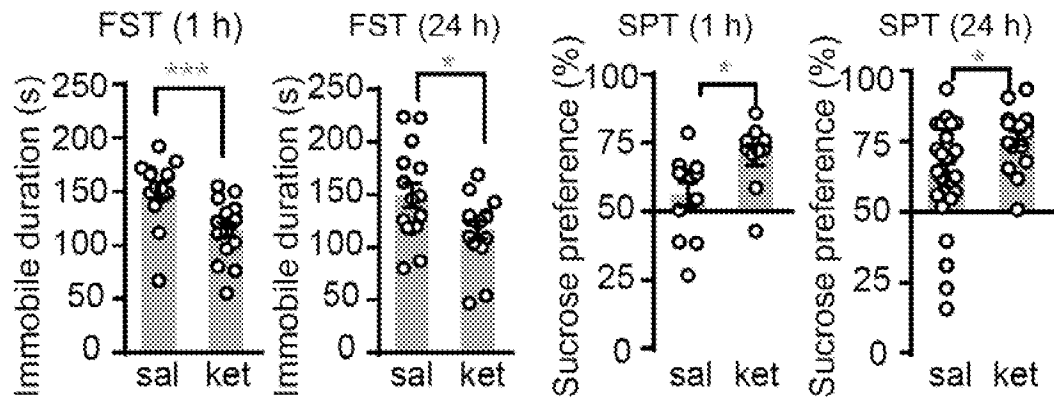


FIG. 1B

FIG. 1C

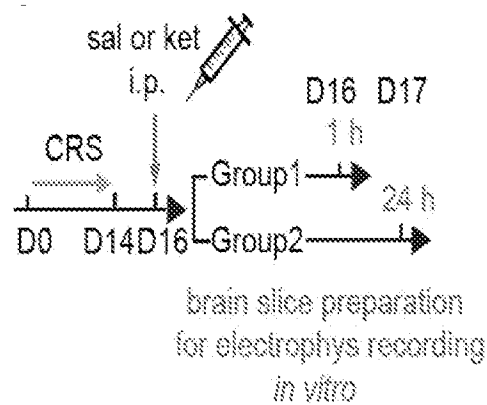


FIG. 1D

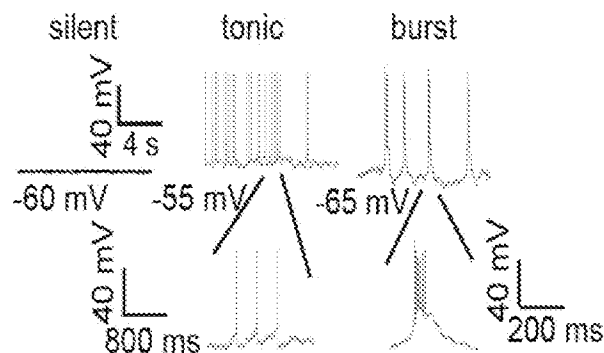


FIG. 1E

FIG. 1F

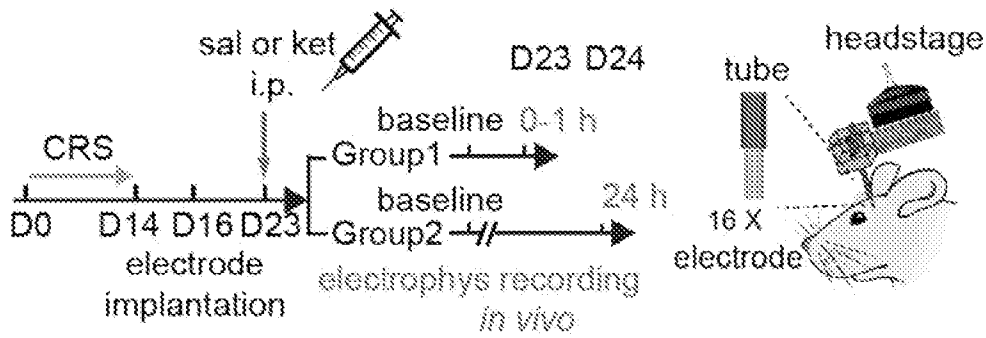
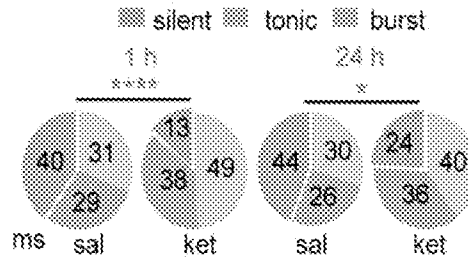
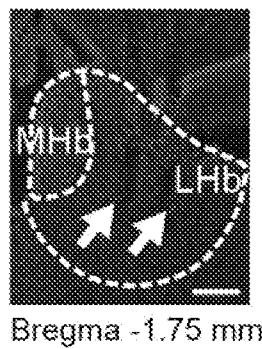


FIG. 1G

FIG. 1H



Bregma -1.75 mm

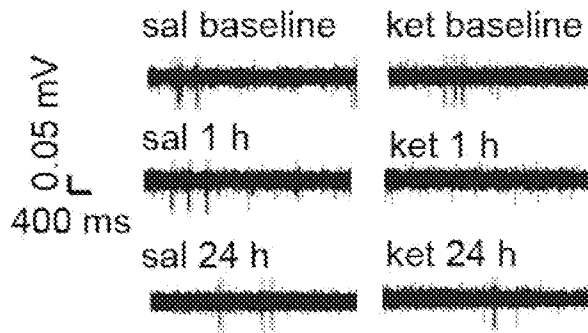


FIG. 1I

FIG. 1J

FIG. 1K

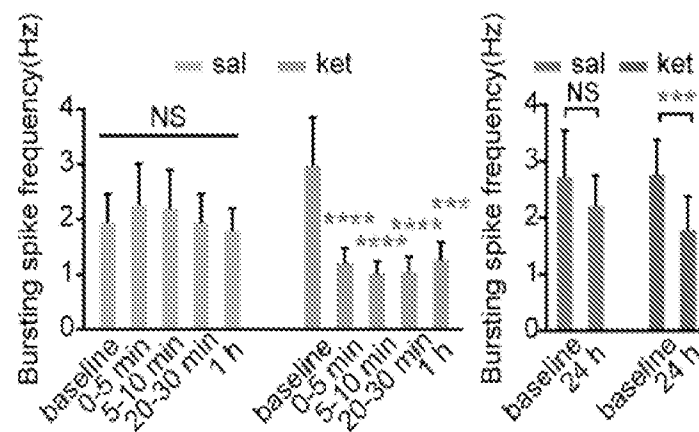


FIG. 1L

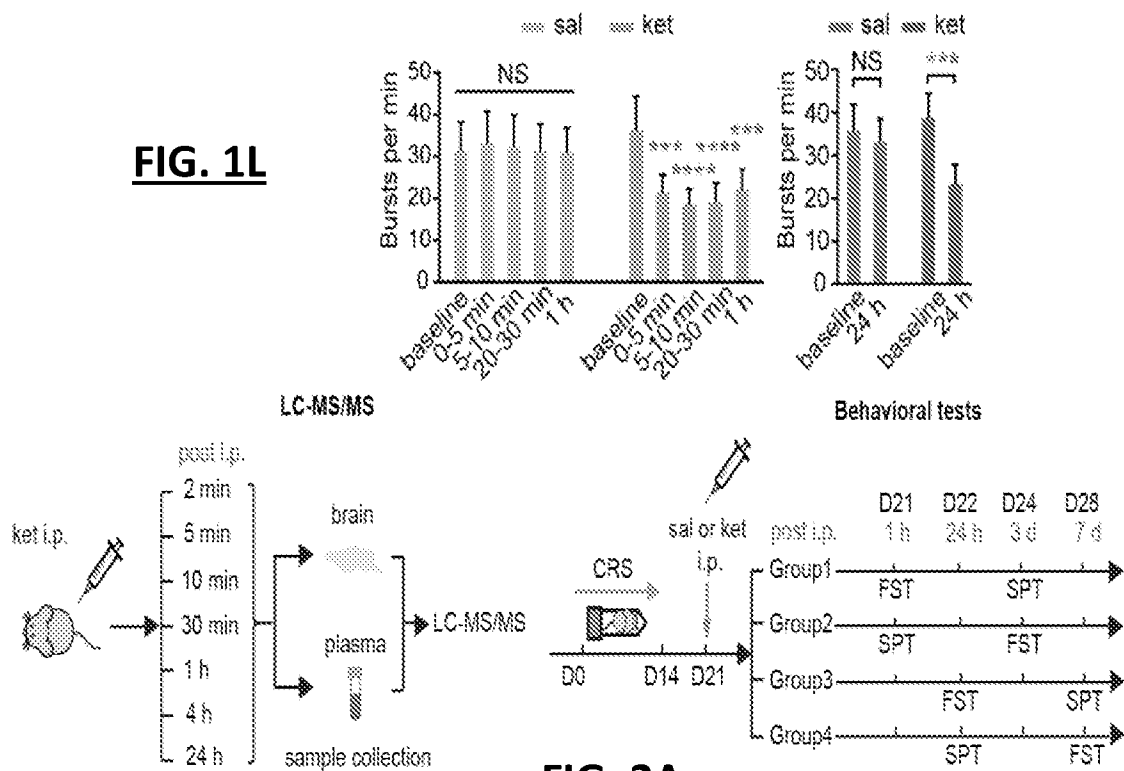


FIG. 2A

FIG. 2B

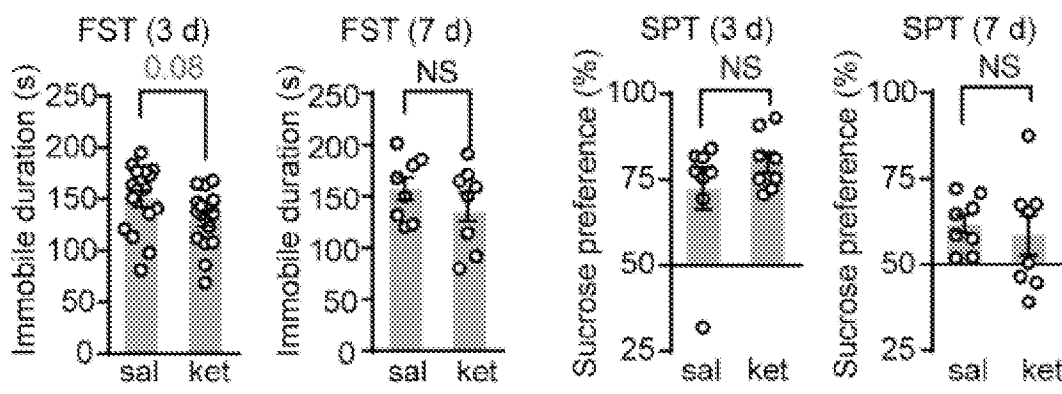
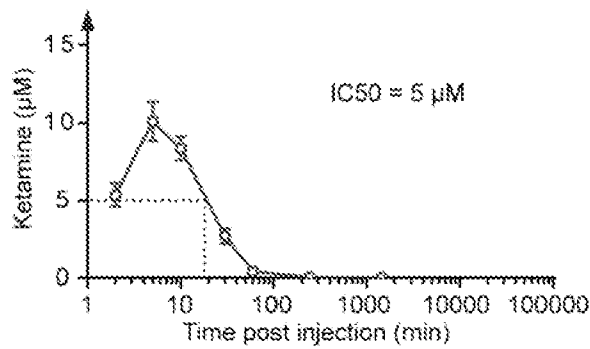
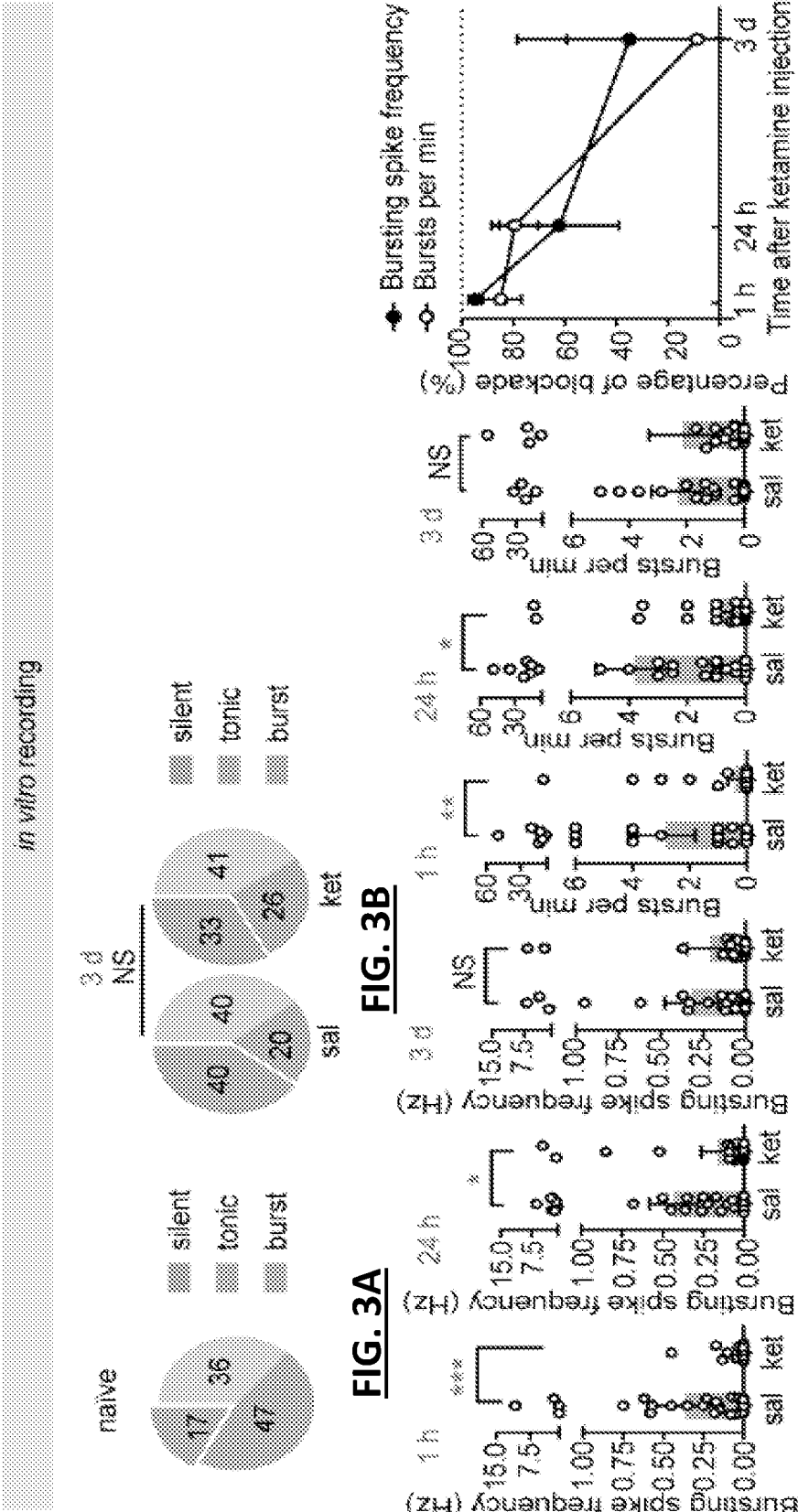


FIG. 2C

FIG. 2D



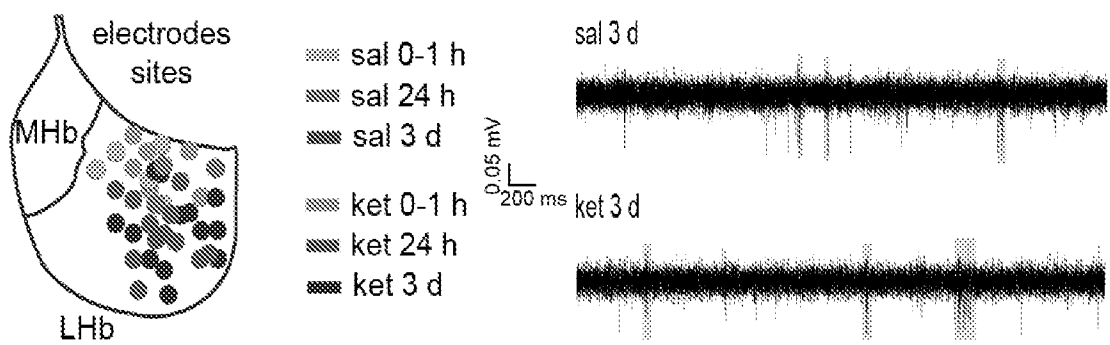


FIG. 3F

FIG. 3G

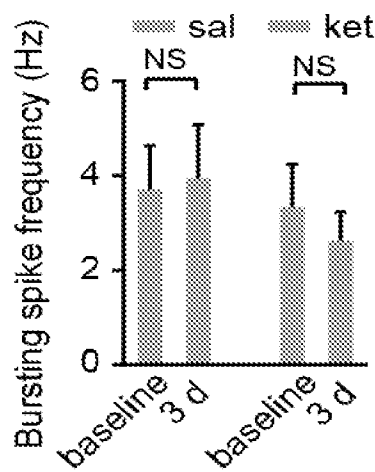


FIG. 3H

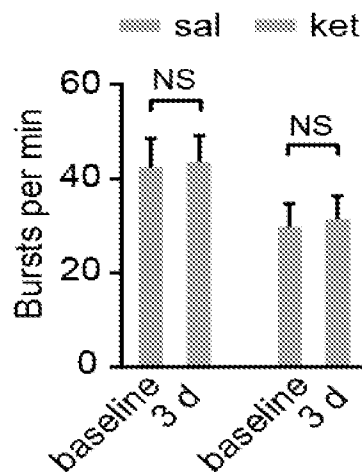


FIG. 3I

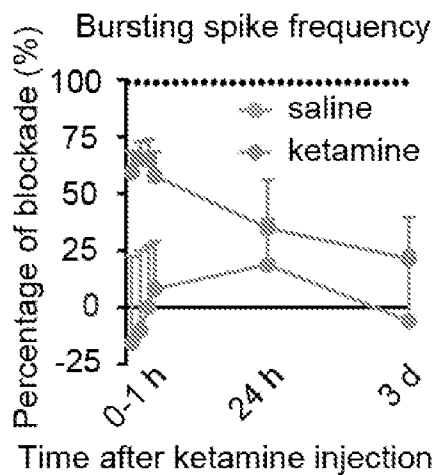


FIG. 3J

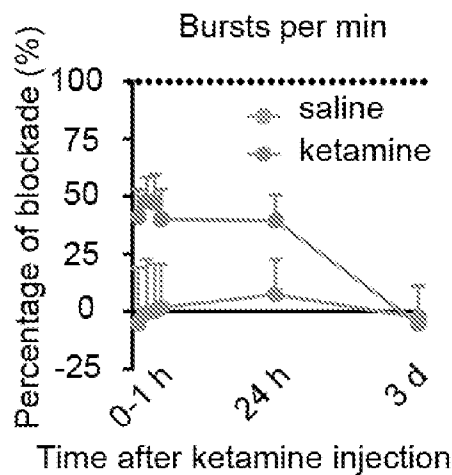
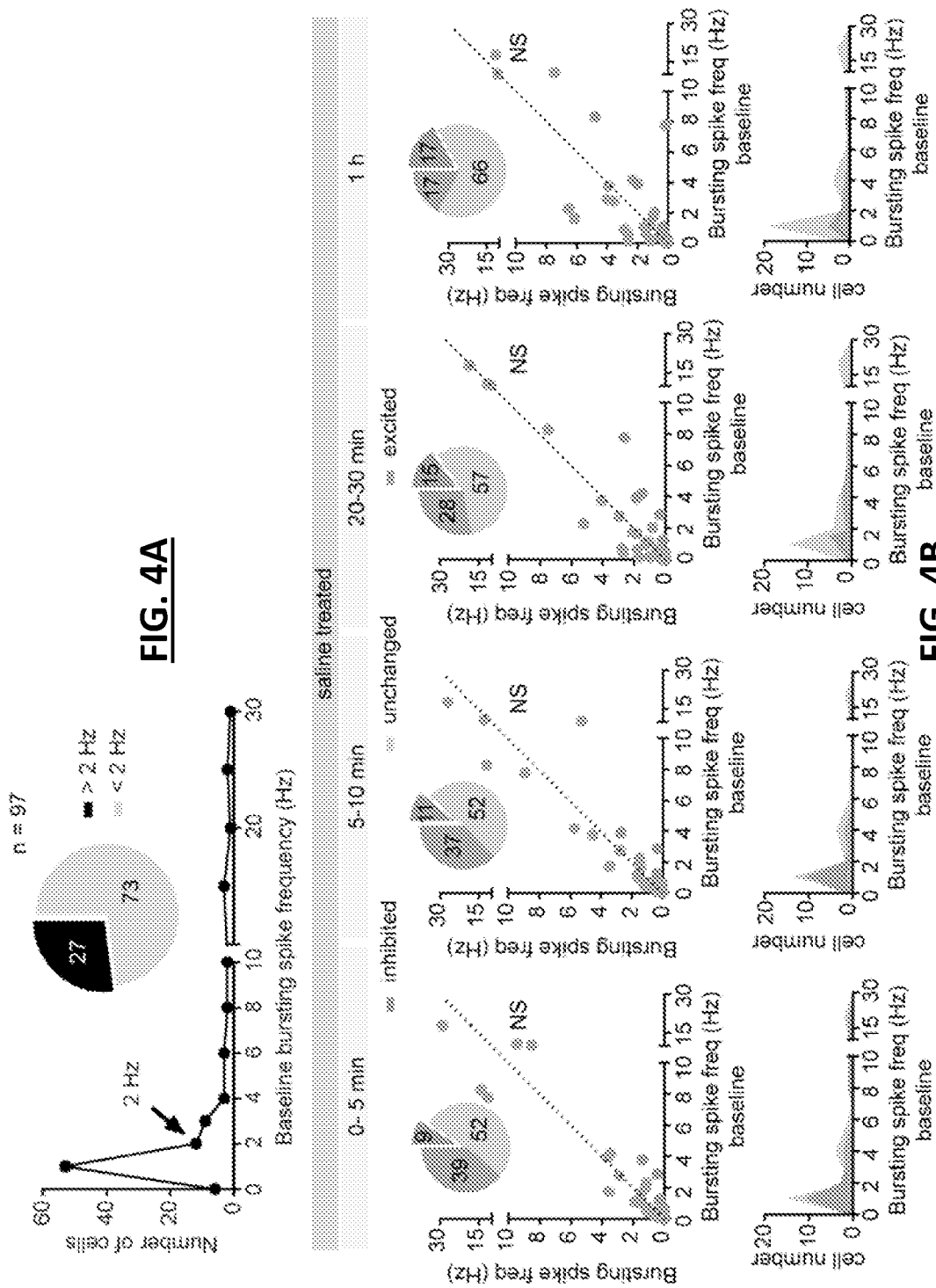
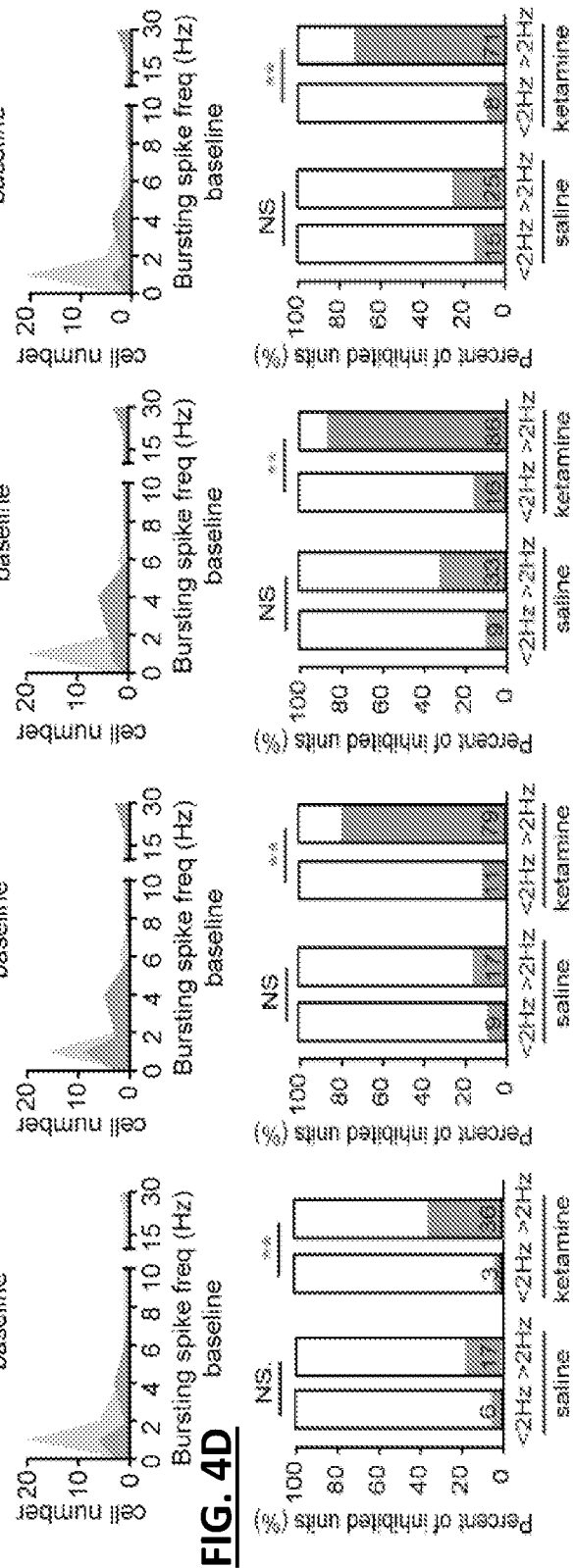
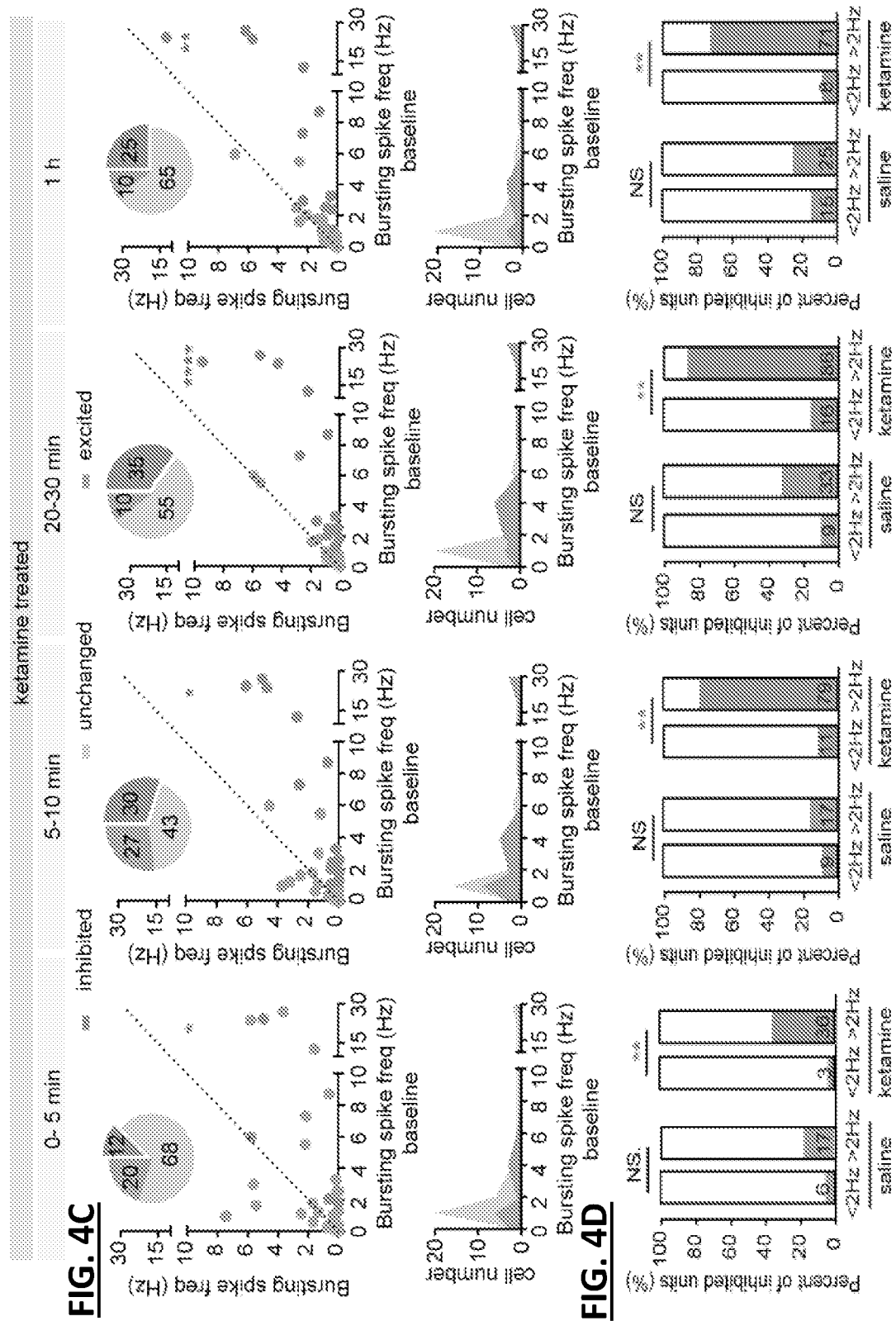
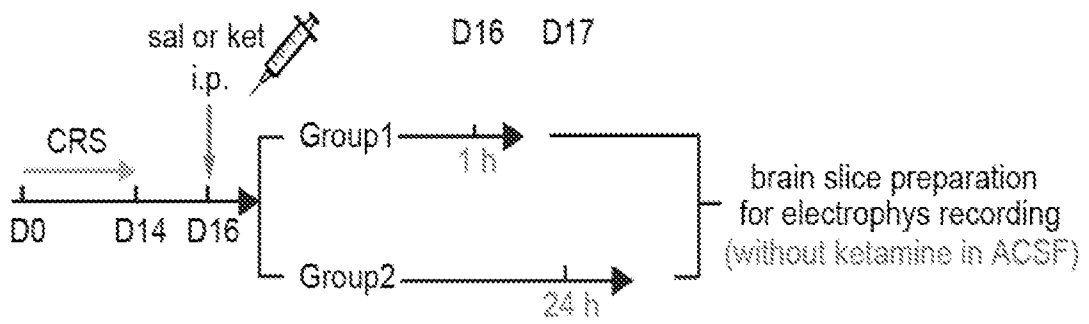
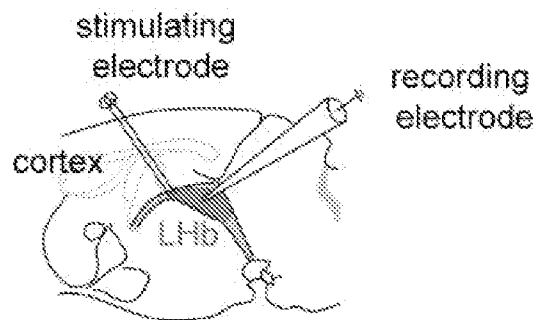


FIG. 3K





**FIG. 5A****FIG. 5B**

Temporally isolated AMPAR-eEPSCs and NMDAR-eEPSCs

1 h post ketamine

FIG. 5C

+PTX

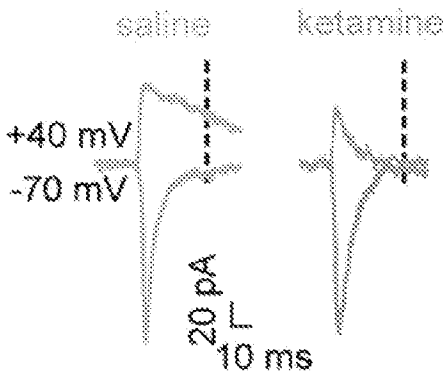


FIG. 5D

N/A ratio

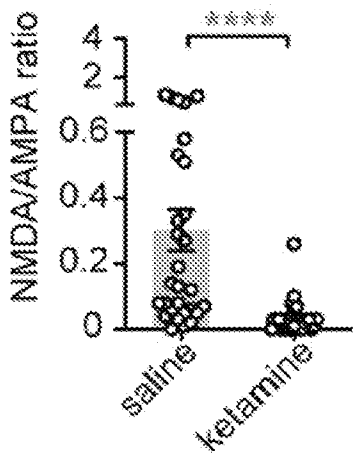


FIG. 5E

NMDA +40 mV

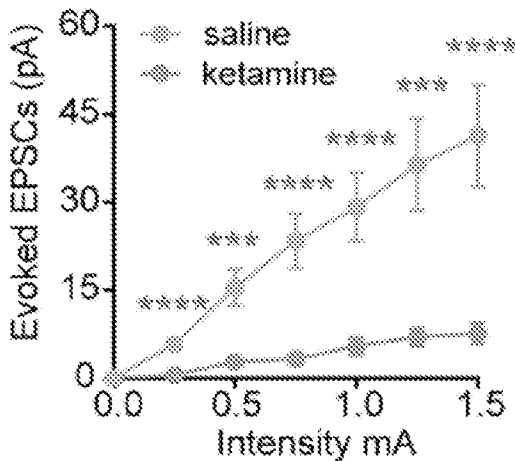


FIG. 5F

NMDA +40 mV

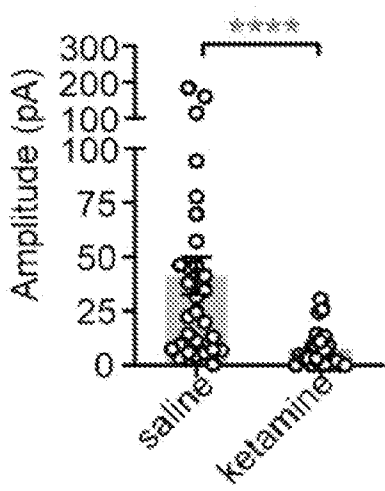


FIG. 5G

AMPA -70 mV

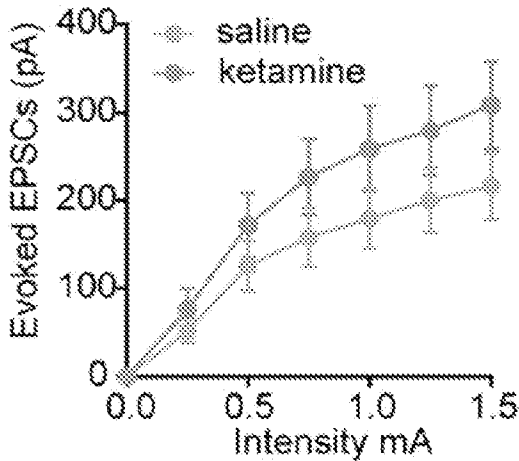
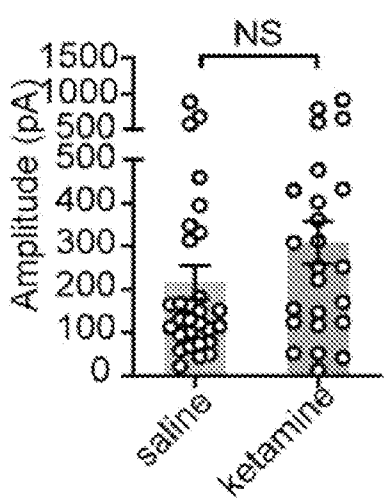


FIG. 5H

AMPA -70 mV



Temporally isolated AMPAR-eEPSCs and NMDAR-eEPSCs

24 h post ketamine

FIG. 5I

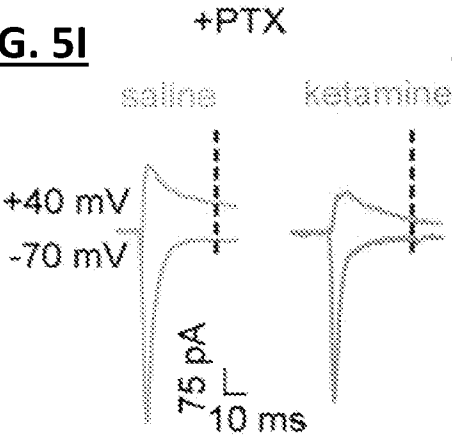


FIG. 5J

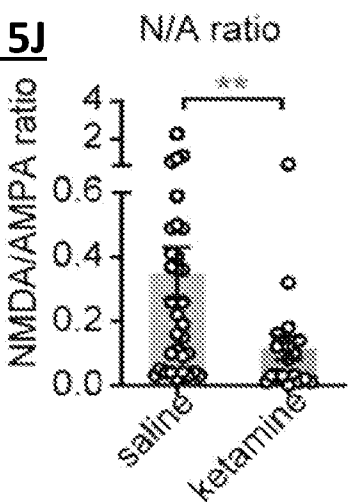


FIG. 5K

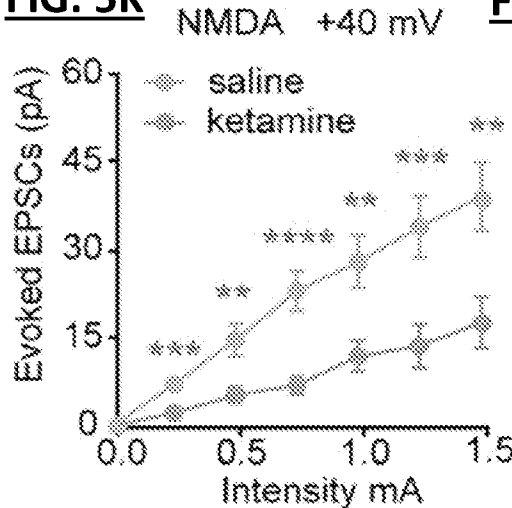


FIG. 5L

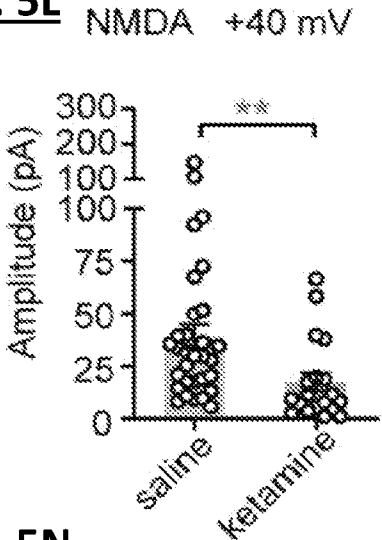


FIG. 5M

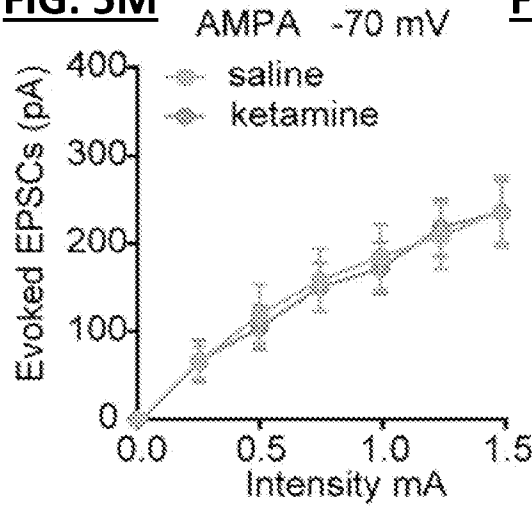
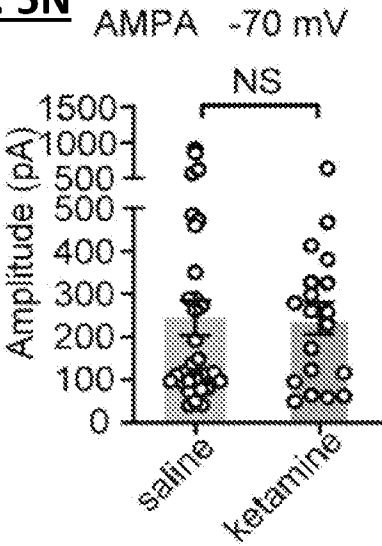


FIG. 5N



Pharmaceutically isolated pure NMDAR-eEPSCs

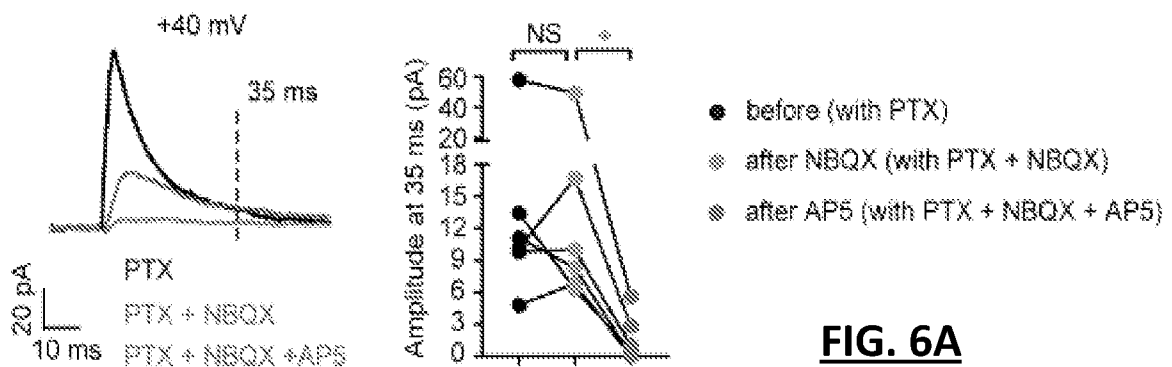
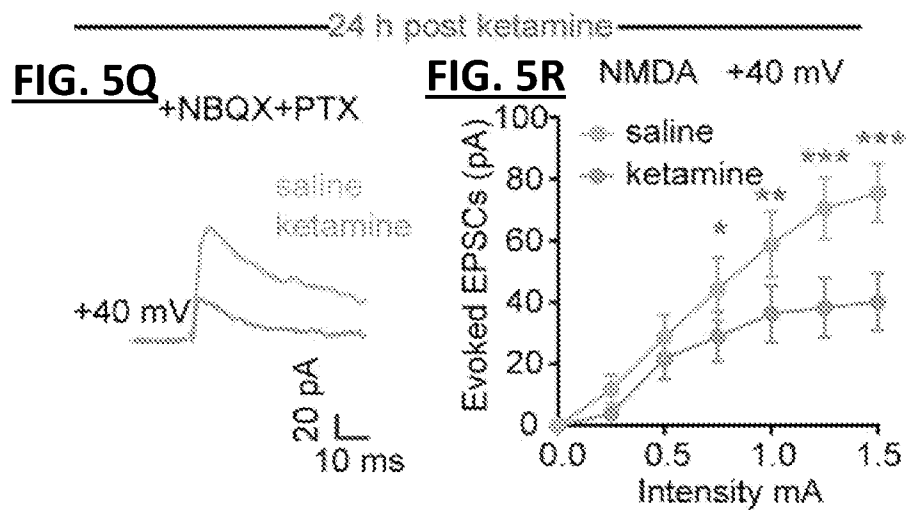
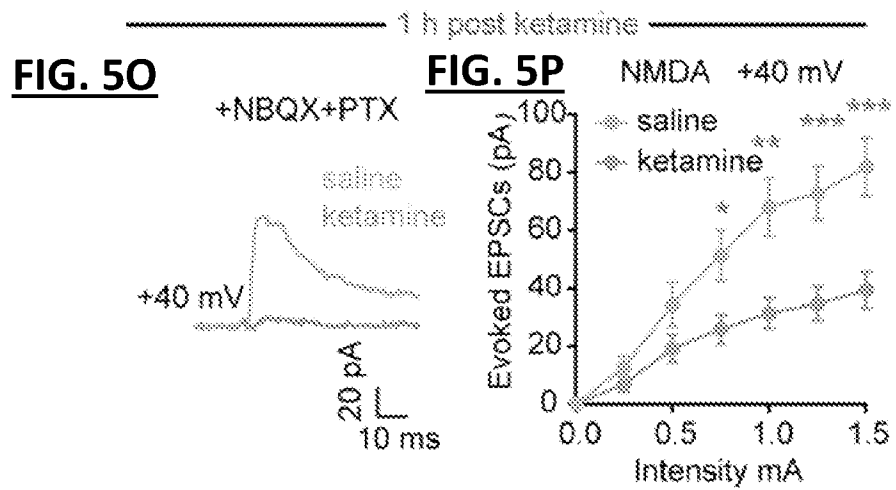


FIG. 6A

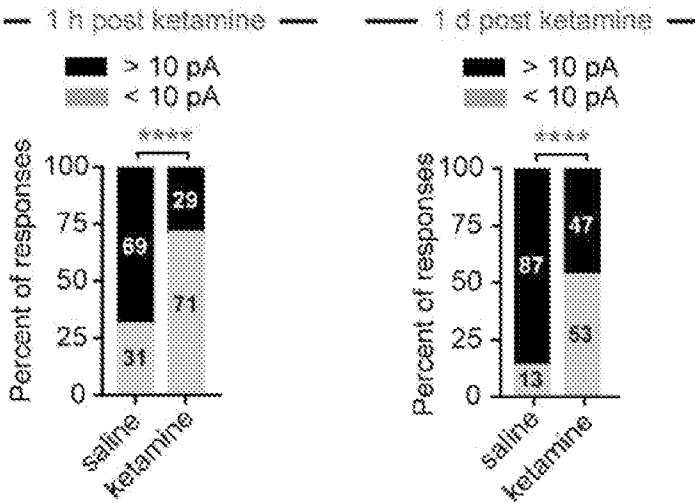


FIG. 6B

FIG. 6C

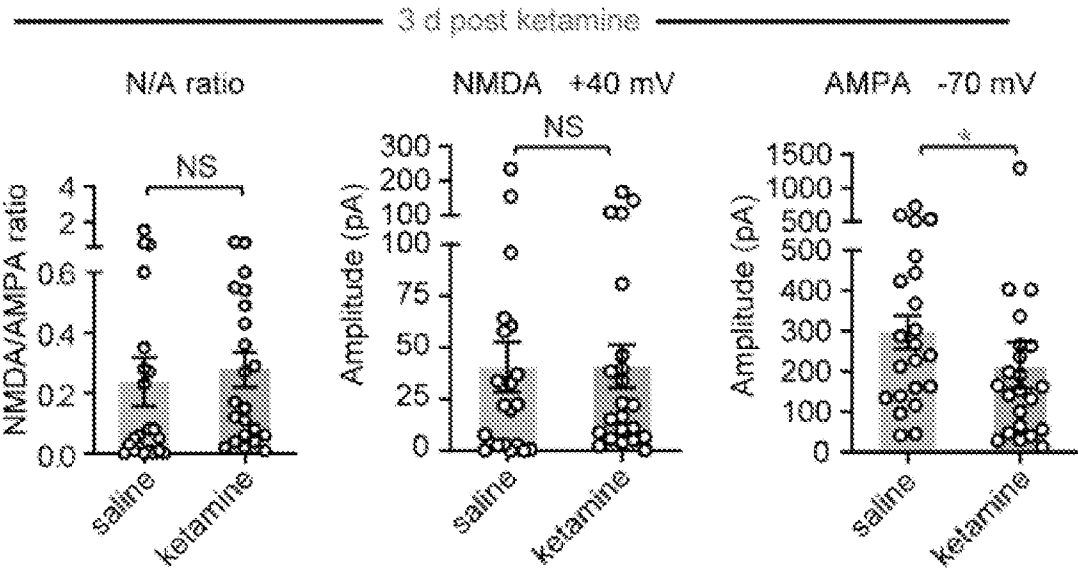
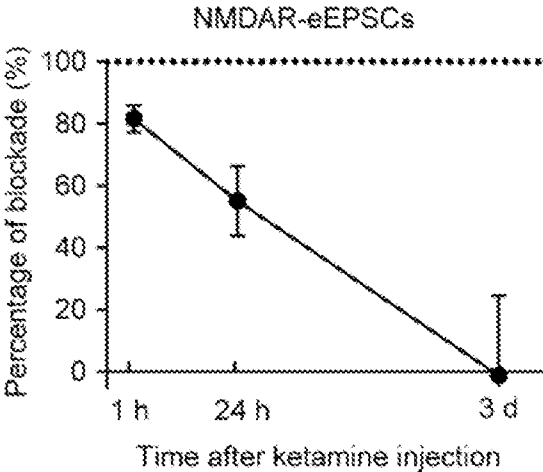


FIG. 6D

FIG. 6E

FIG. 6F

FIG. 6G



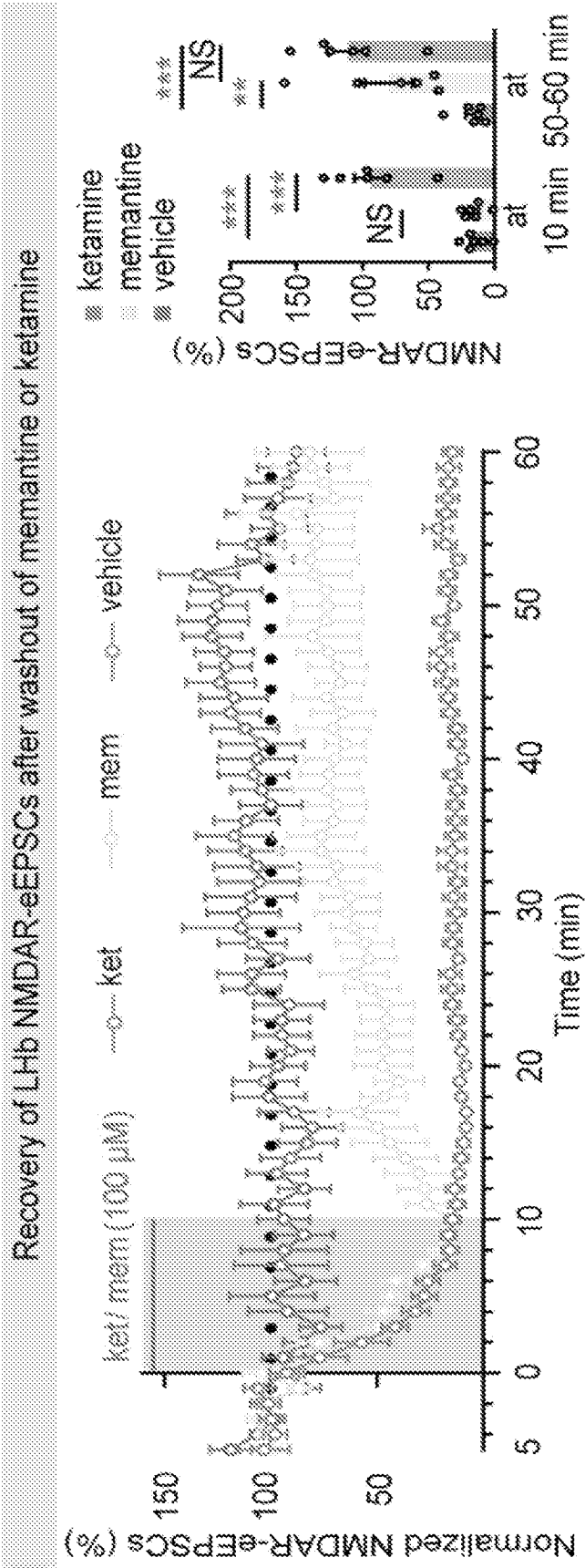


FIG. 7A

FIG. 7B

Behavioral effects by local LHb infusion of memantine or ketamine

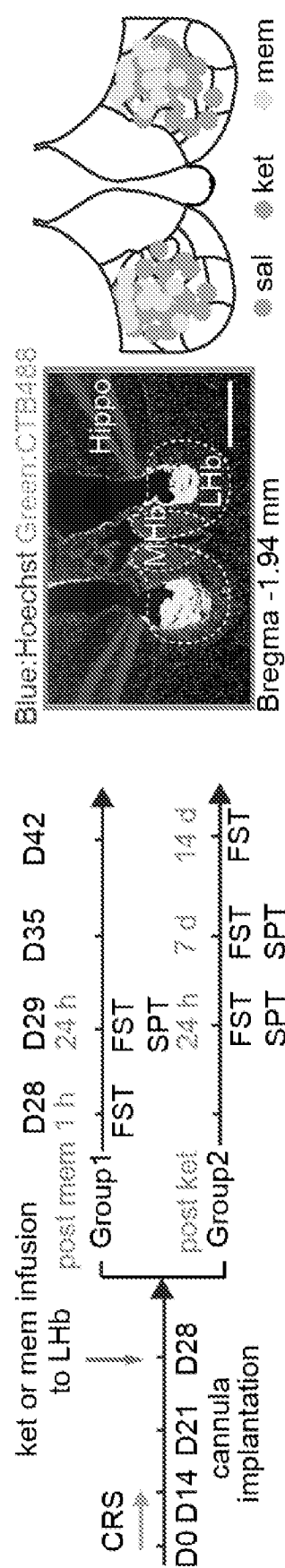


FIG. 7C

FIG. 7D

FIG. 7E

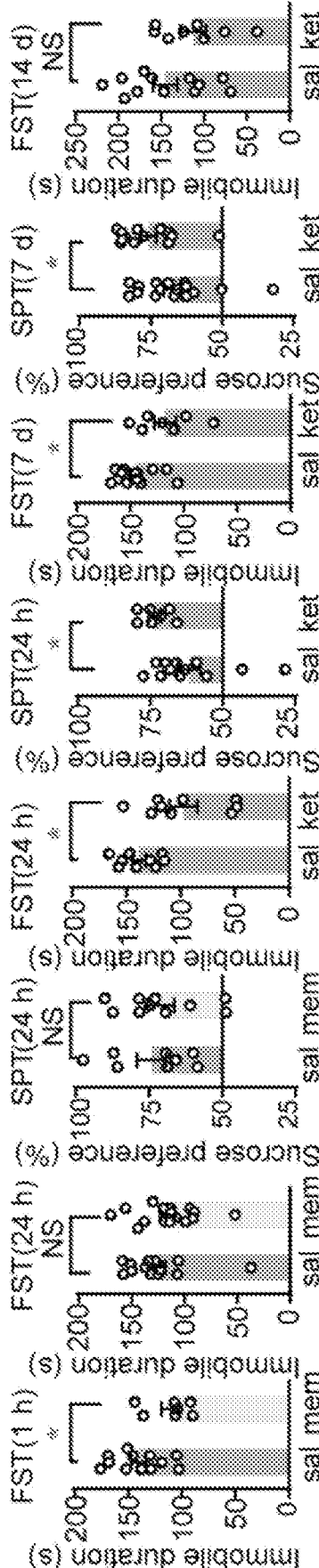


FIG. 7F

FIG. 7G

FIG. 7H

FIG. 7I

FIG. 7J

FIG. 7K

FIG. 7L

FIG. 7M

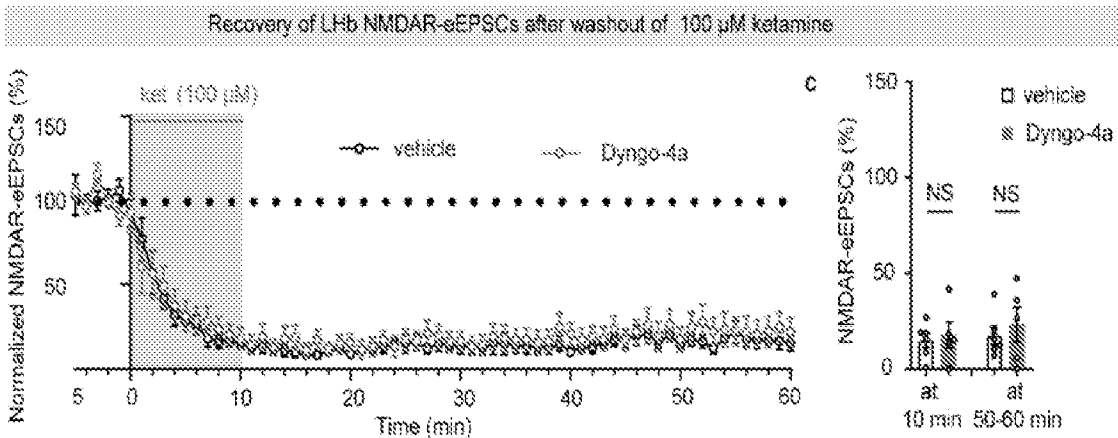
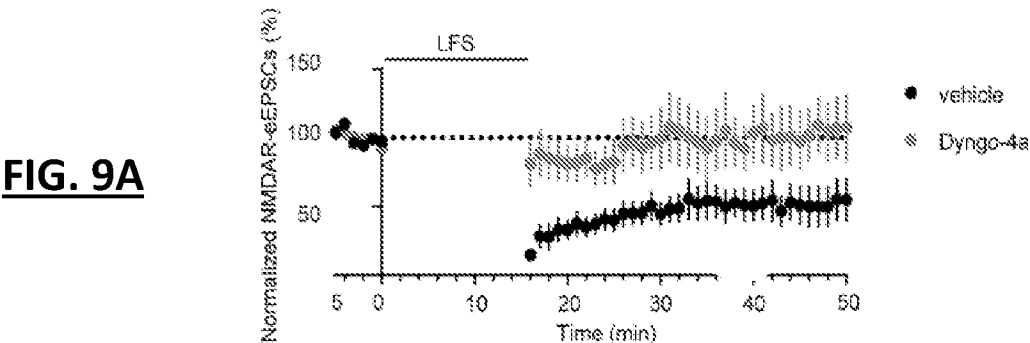
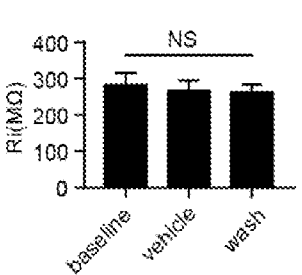
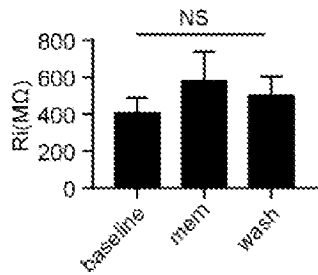
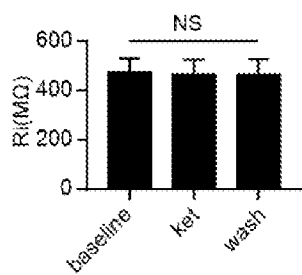


FIG. 9C

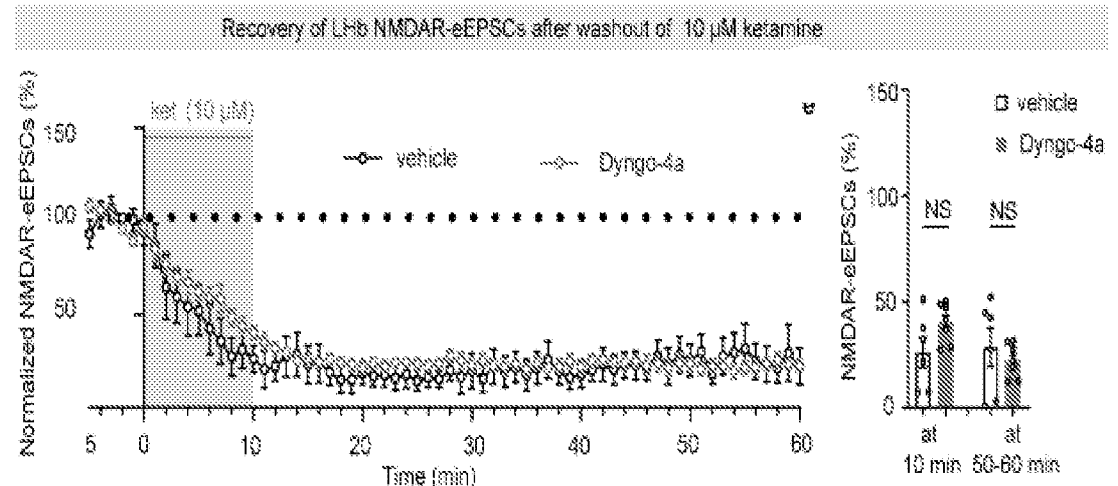
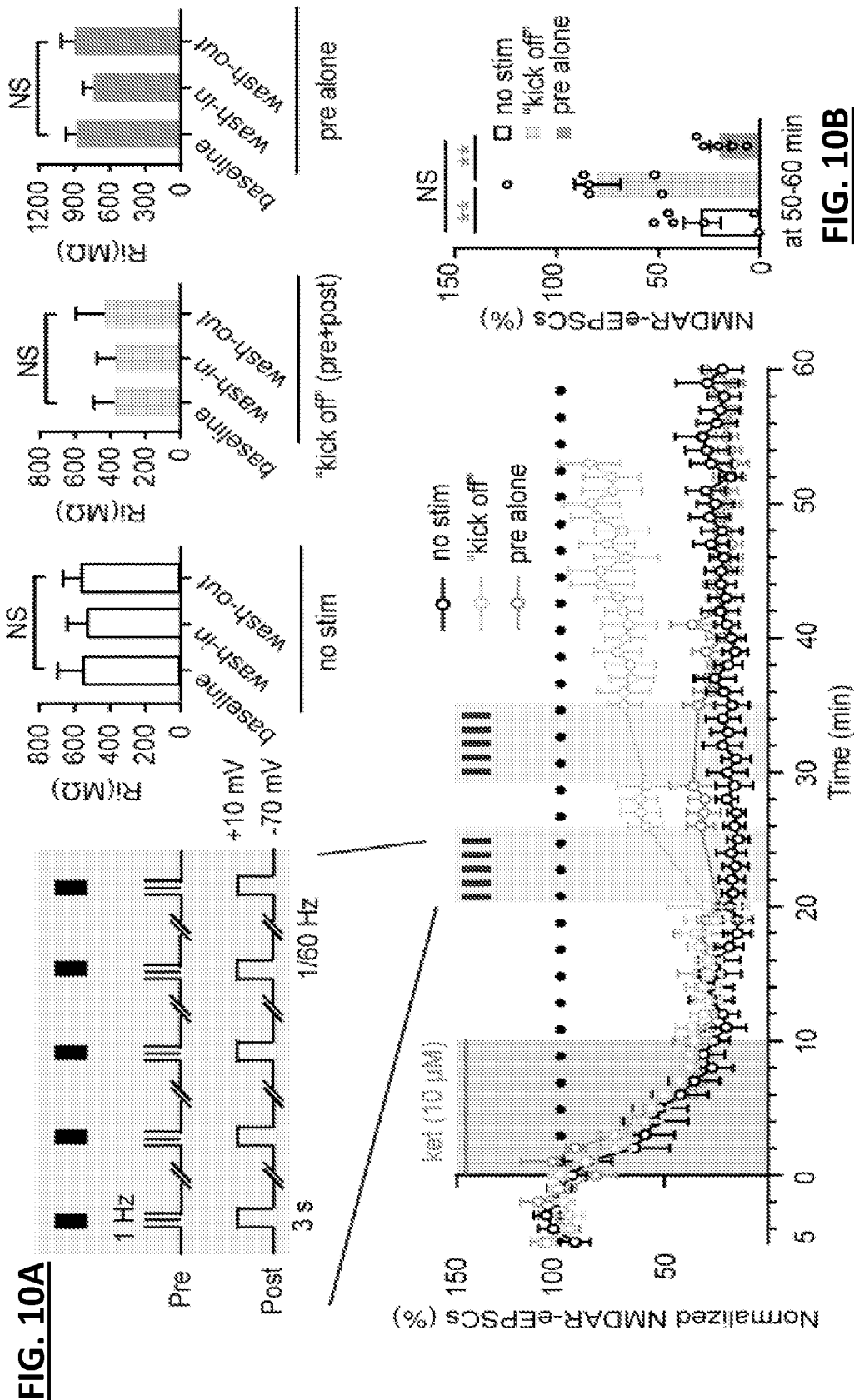


FIG. 9E



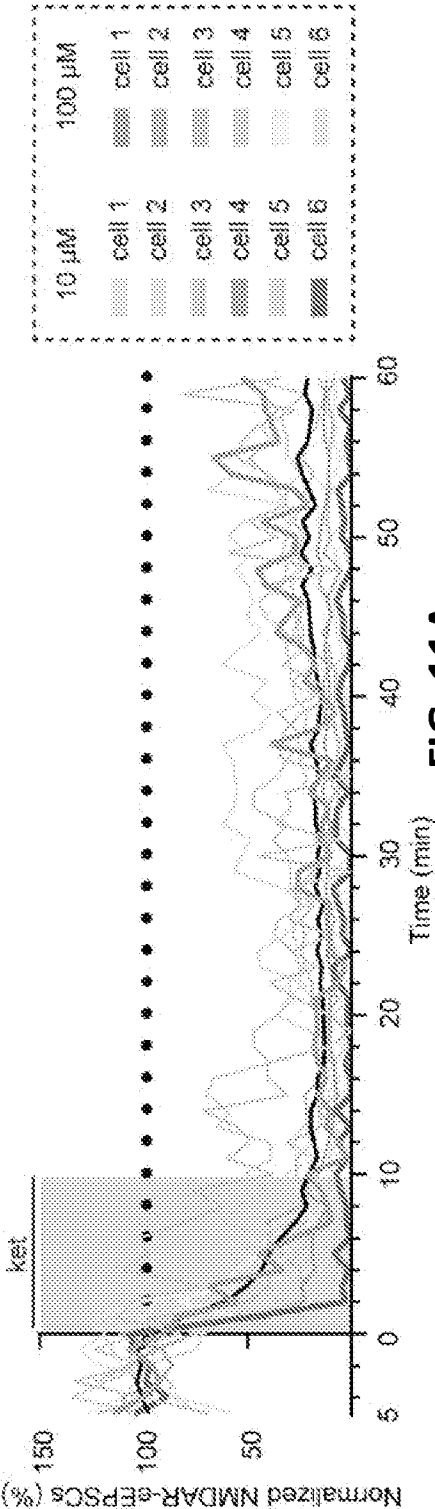


FIG. 11A

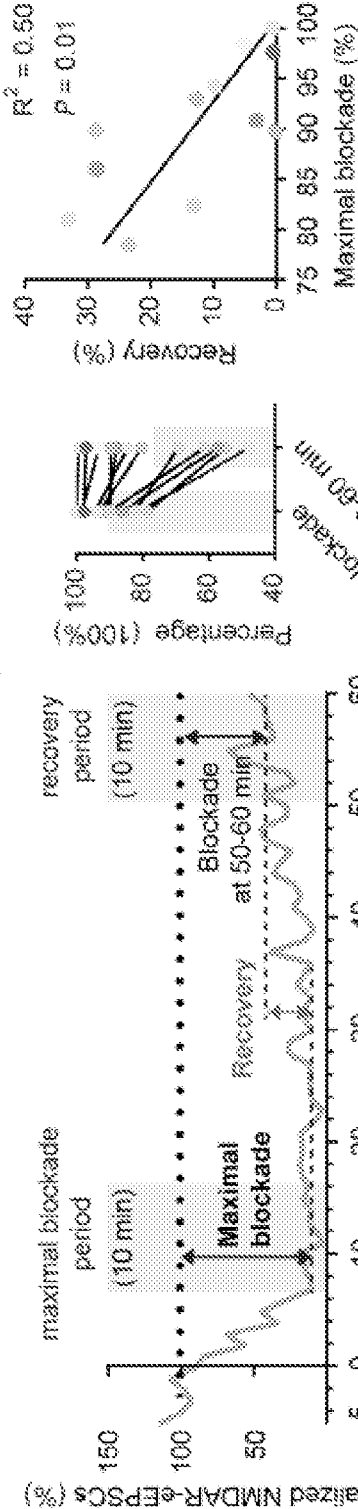


FIG. 11B

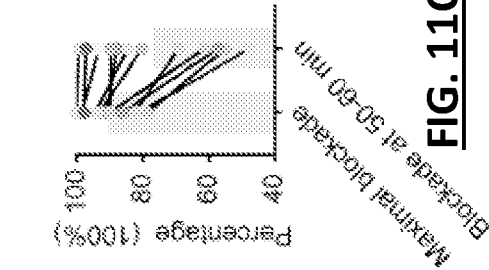


FIG. 11C

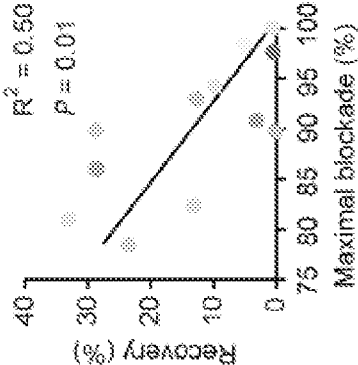


FIG. 11D

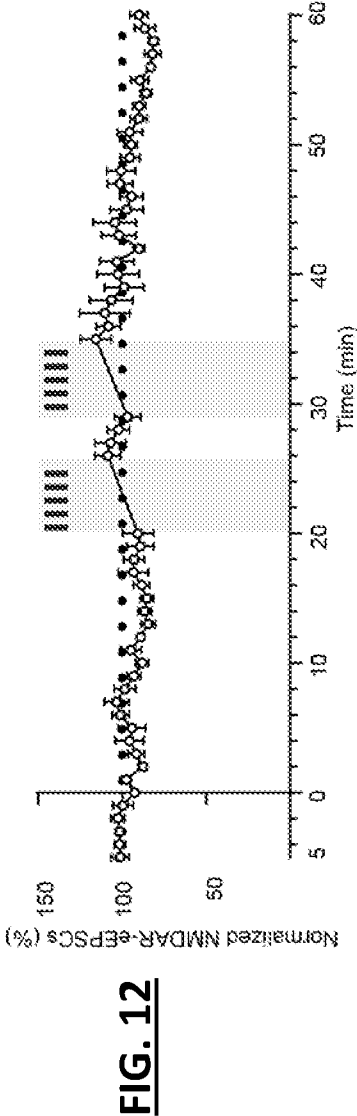


FIG. 13A

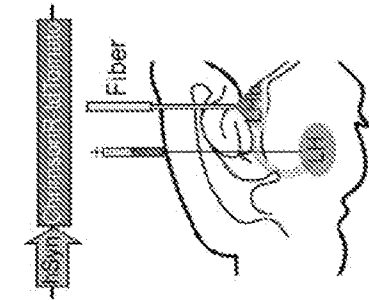


FIG. 13B

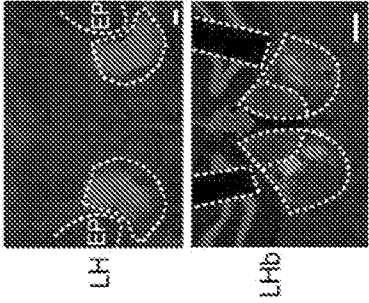


FIG. 13C

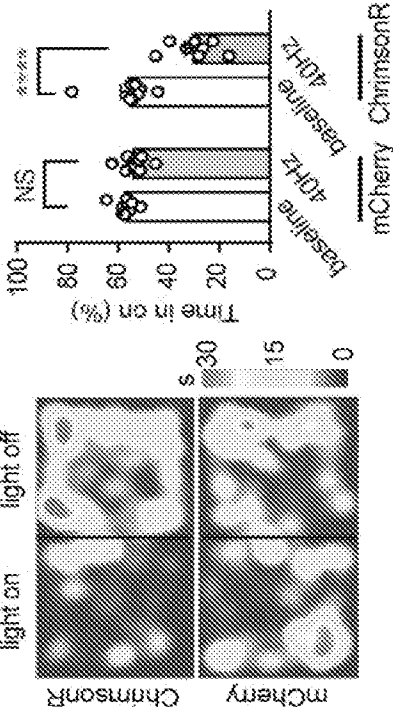
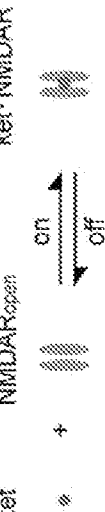


FIG. 13D



$$K_d = \frac{k_{\text{off}}}{k_{\text{on}}} = \frac{[\text{ket}] \cdot [\text{NMDAR}_{\text{open}}]}{[\text{ket} \cdot \text{NMDAR}]}$$

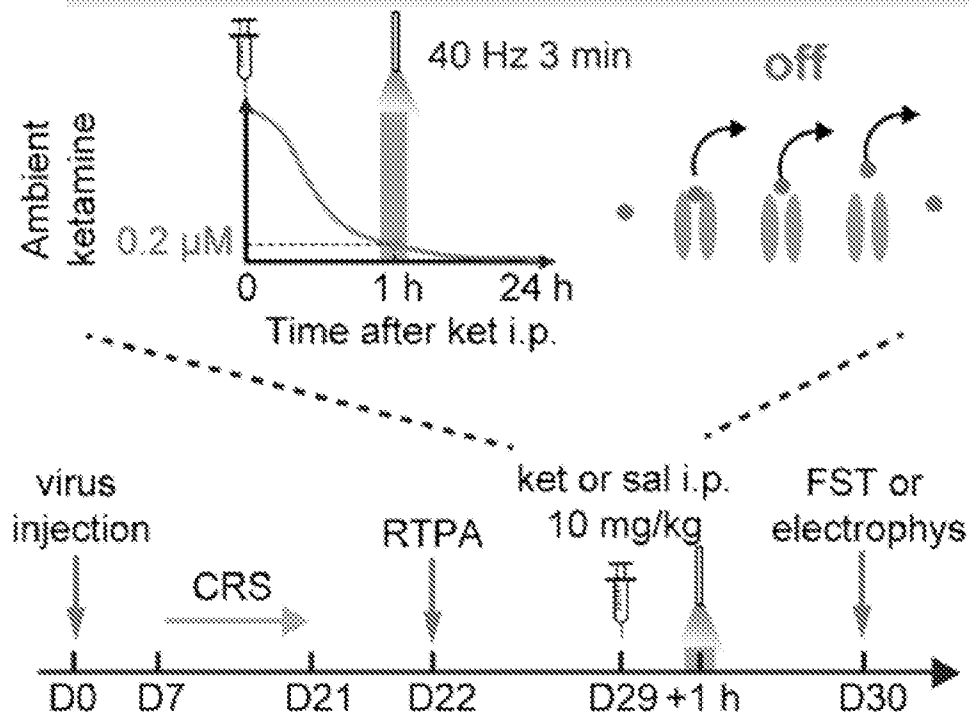
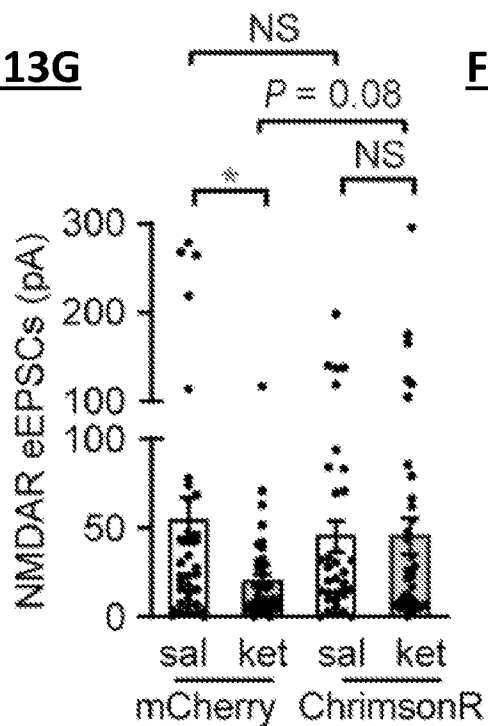
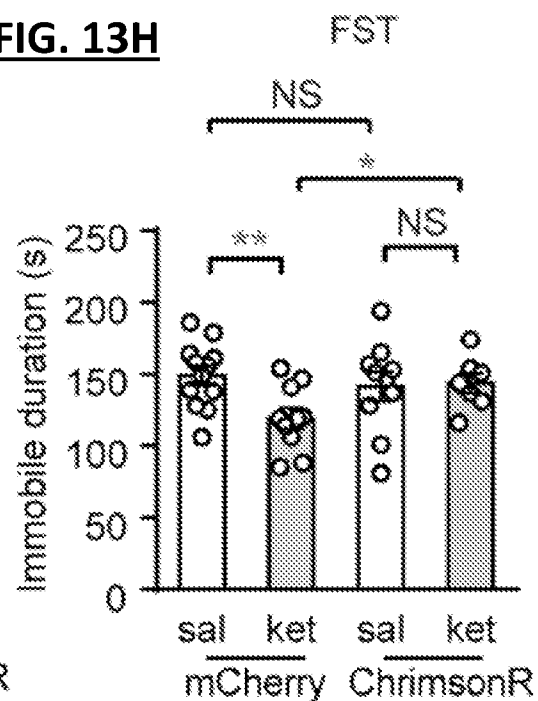
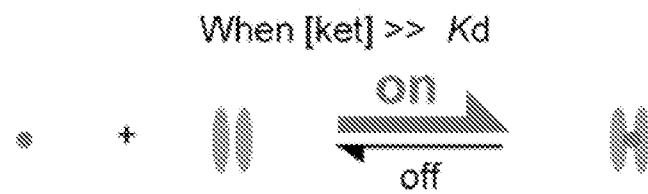
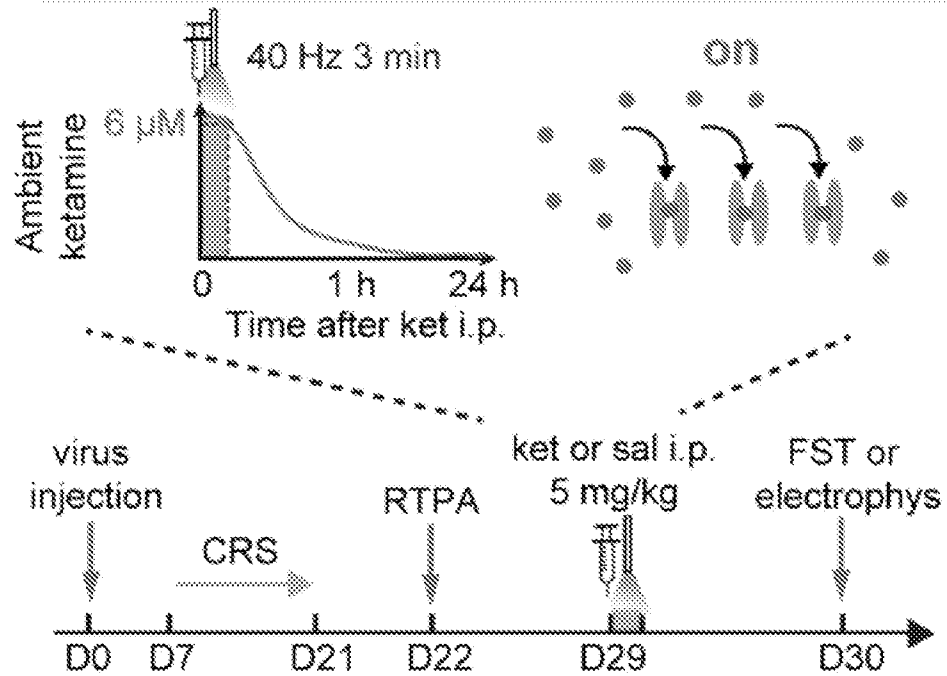
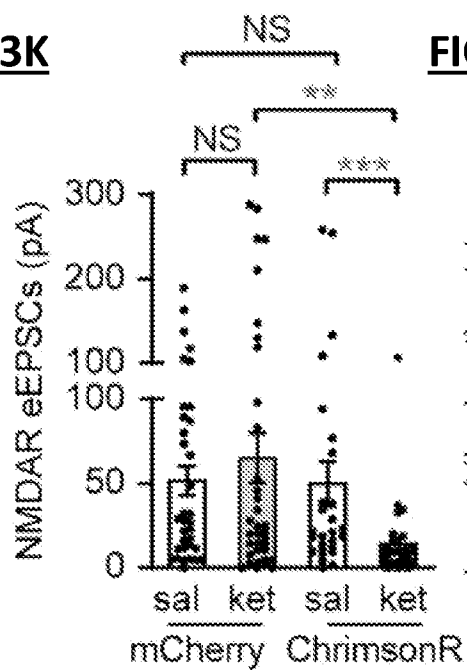
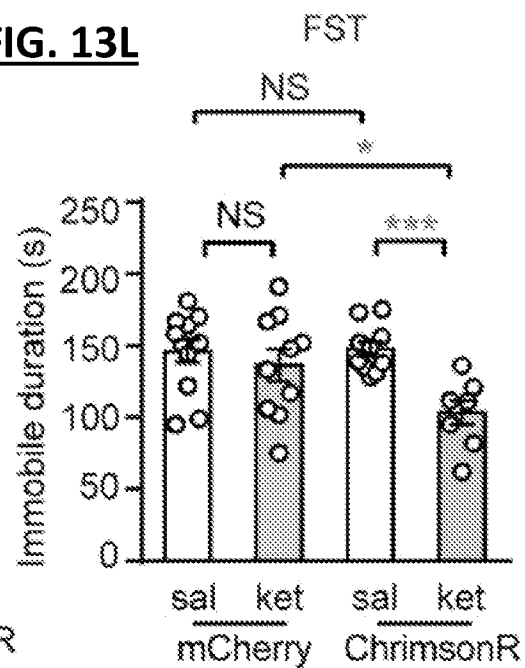
FIG. 13EWhen $[\text{ket}] \ll K_d$ **FIG. 13F**Stimulate LH-LHb at low $[\text{ket}]$ to untrap ketamine**FIG. 13G****FIG. 13H**

FIG. 13I**FIG. 13J** Stimulate LH-LHb at high [ket] to block more NMDARs**FIG. 13K****FIG. 13L**

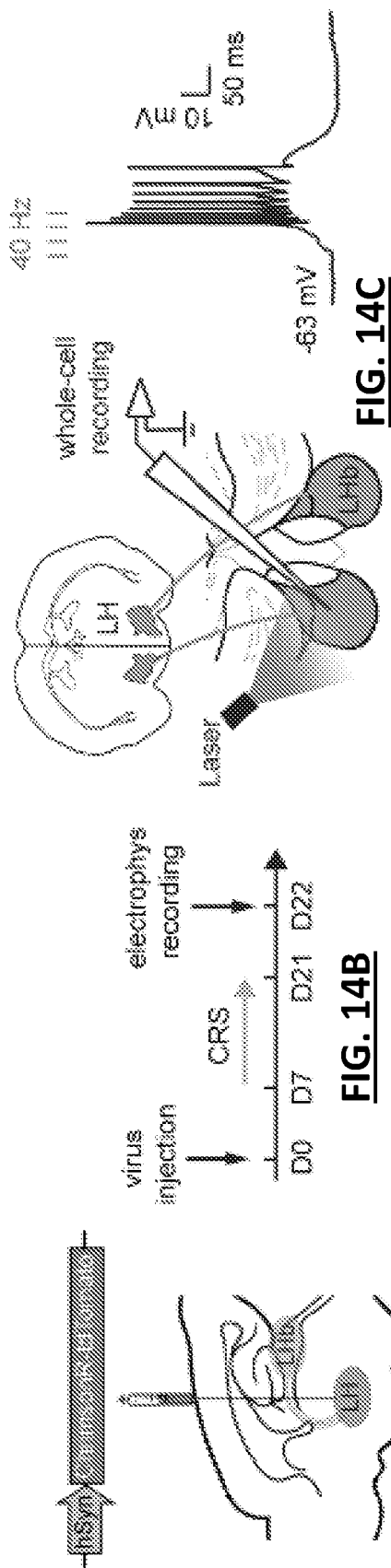


FIG. 14C

FIG. 14A

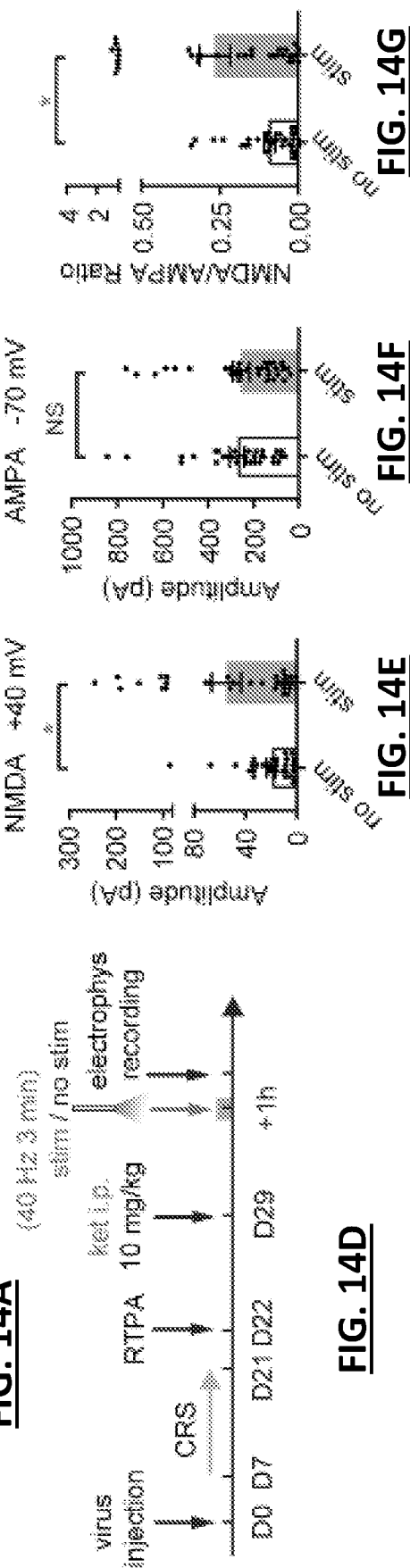


FIG. 14E

FIG. 14F

FIG. 14D

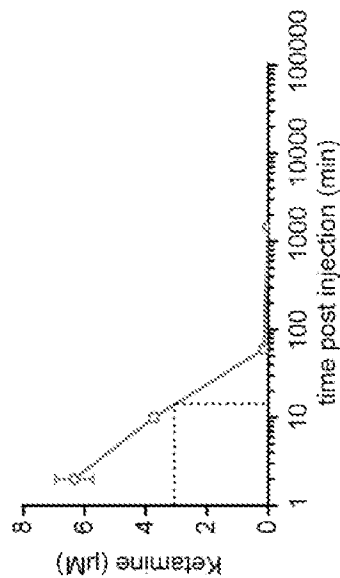


FIG. 15

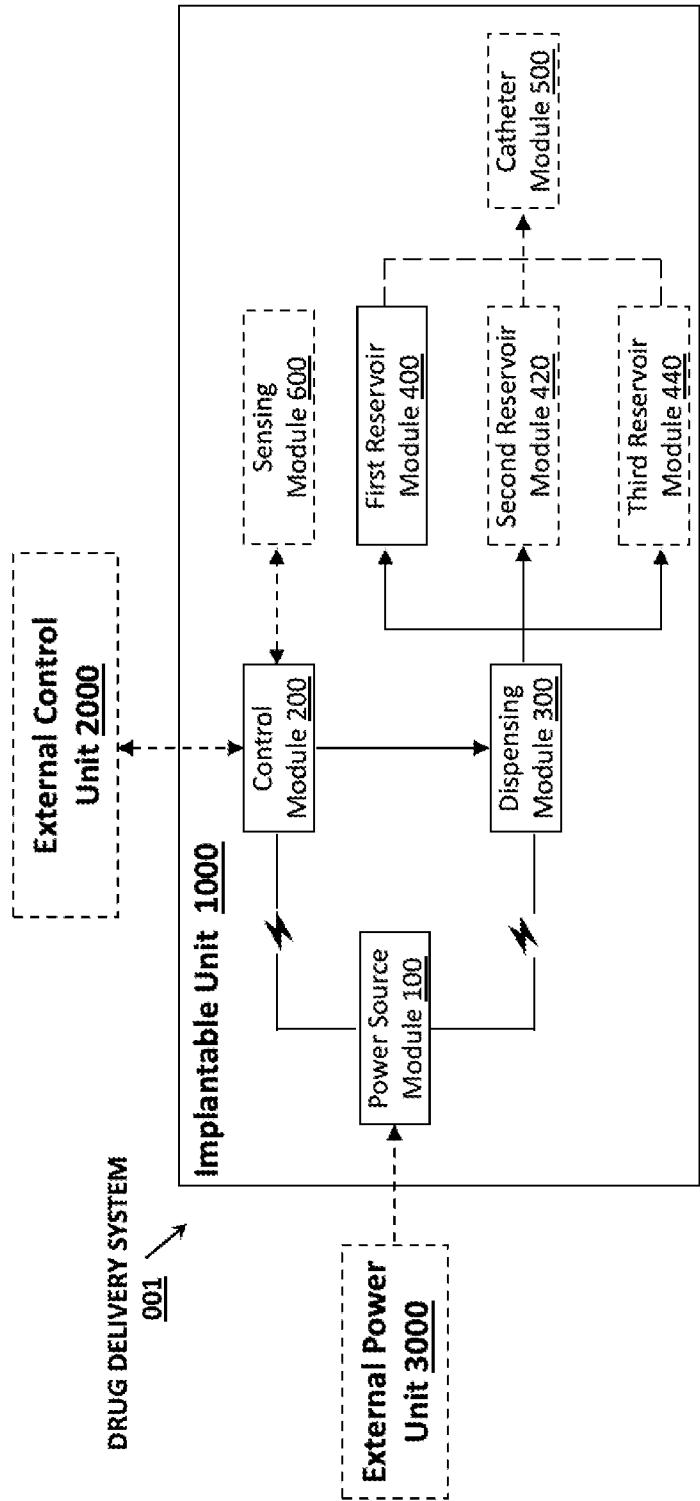


FIG. 16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2023/121712

A. CLASSIFICATION OF SUBJECT MATTER

A61K45/08(2006.01)i; A61K9/22(2006.01)i; A61P25/24(2006.01)i

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)

A61K, A61P

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

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

CNABS, CNTXT, DWPI, VEN, ENTXTC, ENTXT, CNKI, PUBMED, ISI Web of Science, Science Direct, STNext: LIANGZHU LABORATORY, ZHEJIANG UNIVERSITY, N-methyl-D-aspartate receptor, NMDAR, blocker, agonist, lateral habenula, ketamine, memantine, or dextromethorphan, AXS-05, dizocilpine, amantadine, lanicemine, methadone, GABA, AMPAR, sodium channel blocker

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015342947 A1 (UNIV. WEST VIRGINIA) 03 December 2015 (2015-12-03) the abstract	49,50,52
X	US 2017258779 A1 (LU R.B.) 14 September 2017 (2017-09-14) the abstract, claims 1-2	49,50,52
X	WO 2015171770 A1 (UNIV. NORTHWESTERN) 12 November 2015 (2015-11-12) the abstract, claims 1-47	49,50
A	WO 2018205935 A1 (UNIV. ZHEJIANG) 15 November 2018 (2018-11-15) the abstract, claims 1-20	1-92
A	WO 2012070034 A1 (UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG et al.) 31 May 2012 (2012-05-31) the abstract, claims 1-30	1-92
A	WO 2006121560 A2 (NEUROMOLECULAR PHARMACEUTICALS INC. et al.) 16 November 2006 (2006-11-16) the abstract, claims 1-49	1-92



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

19 December 2023

Date of mailing of the international search report

22 December 2023

Name and mailing address of the ISA/CN

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100088, China

Authorized officer

CUI,ChuanMing

Telephone No. (+86) 010-53961869

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2023/121712

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 108853510 A (UNIV. ZHEJIANG) 23 November 2018 (2018-11-23) the abstract, claims 1-10	1-92

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2023/121712

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.: **1-48**
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 1-48 relate to a method for increasing duration of a therapeutic effect of an N-methyl-D-aspartate receptor (NMDAR) pore blocker administered to a subject in need thereof or a therapeutic regimen for treating a subject with depression, and thus do not warrant an international search according to the criteria set out in PCT Rule 39.1(iv). The search has been carried out and based on the subject-matter of the manufacture of a medicament correspondingly.
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.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2023/121712

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