Supplemental Information

SEP-363856, a novel psychotropic agent with a unique, non-D_2 receptor mechanism of action

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Supplemental Material and Methods

SmartCube® System for Behavioral Phenotypic Screening

The SmartCube® system is a mouse-based behavioral screening platform that combines proprietary hardware, computer vision algorithms and machine learning based data mining tools (Roberds *et al.*, 2011; Alexandrov *et al.*, 2015; Shao *et al.*, 2016). In order to create a reference database of therapeutic class signatures, various doses of clinically approved CNS drugs used to treat schizophrenia, depression, anxiety and other psychiatric disorders were screened. During a 45-minute automated test session, where mice are presented with multiple challenges, the behavioral responses of adult male C57Bl/6 mice (Taconic, Germantown, NY) treated with vehicle or test compounds were captured and analyzed using computer vision software and proprietary algorithms. During each test session over 2000 features are captured and the behavioral responses following treatment were compared to the database of reference drugs. SEP-856 was dissolved in vehicle (5% Pharmasolve; 30% PEG 200/ PEG 400/ propylene glycol; 65% saline) and administered i.p. (10 ml/kg) at dose levels of 0.1, 0.3, 1 and 10 mg/kg. Testing was initiated 15 minutes post-dosing.

PCP-Induced Hyperactivity

PCP-induced hyperactivity was assessed in open field chambers (27.3 x 27.3 x 20.3 cm; Med Associates Inc., St Albans, VT) surrounded by infrared photo beams (16 x 16 x 16) to measure horizontal and vertical activity. Distance travelled was measured from horizontal beam breaks. Adult, male C57Bl/6J mice (Jackson Laboratories, Bar Harbor, ME) were acclimatized to the experimental testing room for at least 1 hour prior to

testing. Mice were treated with vehicle (p.o.), SEP-856 (0.3, 1 and 3 mg/kg, p.o.) or clozapine (positive control; 1 mg/kg, i.p.) and placed in the open field chambers for 30 min measurement of baseline activity. Mice were then injected with either water or PCP (5 mg/kg, i.p.) and placed back in the open field chambers for a 60 min session. SEP-856 was dissolved in 20% hydroxypropyl-beta-cyclodextrin and clozapine in 10% DMSO. PCP was dissolved in sterile injectable water. The dosing volume for all treatments was 10 ml/kg. The open field chambers were cleaned following each test. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

Prepulse Inhibition (PPI)

The acoustic startle measures an unconditioned reflex response to external auditory stimulation. PPI, consisting of an inhibited startle response (reduction in amplitude) to an auditory stimulation following the presentation of a weak auditory stimulus or prepulse, has been used to assess deficiencies in sensory-motor gating, such as those seen in schizophrenia. Mice were placed in the PPI chambers (Med Associates) for a 5-minute session of white noise (70 dB) habituation after which the test session was automatically started. The session started with a habituation block of 6 presentations of the startle stimulus alone, followed by 10 PPI blocks of 6 different types of trials. Trial types are: null (no stimuli), startle (120 dB), startle plus prepulse (4, 8 and 12 dB over background noise i.e. 74, 78 or 82 dB) and prepulse alone (82 dB). Trial types were presented at random within each block. Each trial started with a 50 ms null period during which baseline movements are recorded. There was a subsequent 20 ms period

during which prepulse stimuli were presented and responses to the prepulse measured. After further 100 milliseconds the startle stimuli were presented for 40 milliseconds and responses recorded for 100 milliseconds from startle onset. Responses were sampled every ms. The inter-trial interval was variable with an average of 15 seconds (range from 10 to 20 seconds). In startle alone trials the basic auditory startle was measured, and in prepulse plus startle trials the amount of inhibition of the normal startle was determined and expressed as a percentage of the basic startle response (from startle alone trials), excluding the startle response of the first habituation block.

Adult, male C57Bl/6J mice (Jackson Laboratories, Bar Harbor, ME) were acclimatized to the experimental room for at least 1 hour prior to testing. SEP-856 (0.3, 1, 3, 10 and 30 mg/kg, p.o.) was formulated in 20% hydroxypropyl-beta-cyclodextrin (vehicle) and haloperidol (1 mg/kg, i.p.) in 10% DMSO. The dosing volume for all treatments was 10 ml/kg and animals were dosed 30 min prior to testing. The PPI enclosures were cleaned following each test. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

<u>PCP-Induced Deficits in Social Interaction</u>

For five days prior to test, male Sprague-Dawley rats (~ 150 g on arrival from Harlan Laboratories, IN) were injected twice daily with either PCP (2 mg/kg; s.c.) or saline (s.c.). On day 6, after acute pretreatment (30 min) with either water (p.o.), clozapine (2.5 mg/kg, i.p.) or SEP-856 (1, 3 and 3 mg/kg, p.o.) a pair of unfamiliar rats, receiving the same treatment were placed in a white plexiglass open field arena (24" x 17" x 8") and allowed to interact with each other for 6 minutes. Social interactions ('SI') included:

sniffing the other rat; grooming the other rat; climbing over or under or around the other rat; following the other rat; or exploring the ano-genital area of the other rat. Passive contact and aggressive contact were not considered a measure of social interaction. The time the rats spent interacting with each other during the 6-minute test was recorded by a trained observer blinded to drug treatment and condition. The social interaction chambers were thoroughly cleaned after each test session. Twenty animals were tested in each group for a final number of ten interactions. PCP, clozapine and SEP-856 were dissolved in saline, 5%PEG/5%Tween80 in saline, and sterile injectable water respectively. All treatments were administered at a dosing volume of 1ml/kg. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

Forced Swim Test

The forced swim test consisted of one 6-minute session of forced swimming in individual opaque cylinders (15 cm tall x 10 cm wide, 1000 ml beakers) containing fresh tap water at a temperature of 23 ± 2 °C and a depth of 12 cm (approximately 800 ml) for each test animal. The time the animal spent immobile was recorded over the 6-minute trial. Every one minute, the cumulative immobility time was recorded from the start of the session and noted on the study data record sheet. Immobility was defined as the postural position of floating in the water. The animals are generally observed with the back slightly hunched and the head above water with no movements or with small stabilizing movements of the limbs. Sometimes the back is arched with the animal stretched across the sides of the beaker, and in this posture, immobility was recorded only if the animal was not struggling. Adult, male BalbC/J mice (Jackson Laboratories,

Bar Harbor, ME) were tested 60 minutes post administration of vehicle (sterile injectable water; p.o.), or SEP-856 (0.3, 1, 3 and 10 mg/kg, p.o.) and 30 minutes post sertraline (positive control; 20 mg/kg, i.p.) injection. All treatments were formulated in sterile injectable water and administered at a dosing volume of 10 ml/kg. Data are represented as the time the mice spent immobile during the 6-minute trial. Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

Catalepsy

Catalepsy was assessed using the bar test in adult, male C57Bl/6J mice (Jackson Laboratories, Bar Harbor, ME). The front paws of a mouse were placed on a horizontal metal bar raised 2" above a Plexiglas platform and time was recorded for up to 30 seconds per trial. The test ended when the animal's front paws returned to the platform or after 30 seconds. The test was repeated three times and the average of the trials is reported as the intensity index of catalepsy. Mice were acclimatized to the experimental room for at least one hour prior to testing. Catalepsy was assessed at 30 minutes and 90 minutes following vehicle (20% hydroxypropyl-beta-cyclodextrin, p.o.), haloperidol (1 mg/kg, i.p.) or SEP-856 (100 mg/kg, p.o.) administration. Haloperidol and SEP-856 were dissolved in 20% hydroxypropyl-beta-cyclodextrin and 10% DMSO respectively and administered at a volume of 10 ml/kg. At the end of each trial, the apparatus was thoroughly cleaned with 70% ethanol. Data were analyzed using two-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

Electroencephalograph (EEG) Recordings

Seven male Sprague-Dawley rats (300 ± 25 g; Charles River, Wilmington, MA) were implanted with chronic recording devices for continuous recordings of EEG, electromyograph (EMG), core body temperature (Tb), and locomotor activity (LMA) via telemetry. Under isoflurane anesthesia (1–4%), a dorsal midline incision on top of the head and a midventral incision along the linea alba through the peritoneum were made. Sterile miniature transmitters (F40-EET, Data Sciences Inc., St Paul, MN) were inserted through the peritoneal incision and sewn to the musculature with a single stitch of silk suture (4-0). Four biopotential leads from the transmitters were inserted subcutaneous into the neck and head region. Two holes were drilled through the skull, one at –5.0 mm AP and 4.0 mm ML and the other at 2.0 mm AP and 2.0 mm ML from bregma. The two biopotential leads used as EEG electrodes were inserted into the holes and affixed to the skull with dental acrylic. The two biopotential leads used as EMG electrodes were sutured into the neck musculature. The incision was closed with suture (silk 4-0) and antibiotics were administered topically. Pain was relieved by a long-lasting analgesic (buprenorphine) administered intramuscularly postoperatively. After surgery, animals were placed in a clean cage and observed until they recovered. EEG, EMG, Tb, and LMA were recorded via telemetry using DQ ART 4.1 software (Data Sciences Inc., St Paul, MN). Animals were acclimated to the handling procedures and were given two separate 1 ml administrations of vehicle by oral gavage, one 7 days and the other 3 days before the first experimental day. Following completion of the data collection, expert scorers determined states of sleep and wakefulness in 10 second (s) epochs by examining the recordings visually using NeuroScore software (Data Sciences Inc., St

Paul, MN). All doses of SEP-856 (1, 3 and 10 mg/kg), caffeine (10 mg/kg), and vehicle were administered by oral gavage with a minimum of 3 days between successive treatments. Doses were administered at the start of Zeitgeber hour 7 (ZT7; i.e. 6 hours after light on) and the following 6 hours of continuous EEG and EMG recordings were analyzed. EEG and EMG data were scored visually in 10 second epochs for wakes (W), REM, and non-REM (NREM). Scored data were analyzed and expressed as time spent in each state per hour. Latency to NREM onset for each rat was calculated from the time of drug administration to the first six continuous 10 second epochs scored as NREM. Latency to REM onset for each rat was calculated from the time of drug administration to the first three continuous 10 second epochs scored as REM. Cumulative time spent in W, NREM, and REM, as well as the REM:NREM ratios, were calculated for the 6-hour recording period. Tb and LMA (counts per minute) were analyzed as mean values per hour (hourly means).

In Vivo Microdialysis

Male Sprague-Dawley rats (240-325 g; Harlan, Frederick, MD) were allowed to acclimate to the facility for at least 5 days before surgery.

Microdialysis experiment: Two days prior to test article administration, rats were anesthetized with a mixture of ketamine/xylazine (70 mg/kg/6 mg/kg, i.m) and placed in a stereotaxic instrument. Microdialysis probes (CMA/12; 4 mm membranes) were then implanted in the striatum and/or prefrontal cortex. The coordinates from bregma for striatum were +0.9 mm anterior-posterior, +3 mm medio-lateral; -7.5 mm dorso-ventral and for prefrontal cortex, +3.4 mm anterior-posterior, -0.6 mm medio-lateral; -5.5 mm

dorso-ventral. Probes were secured to the skull with cranioplastic cement (Henry Schein) and three screws. Once conscious, rats received a single dose of ketoprofen (10 mg/kg, s.c) for analgesia. 24 to 28 hours after the surgery, rats were placed under light isoflurane anesthesia in order to connect the microdialysis probes to the perfusion pumps using sterile polyethylene tubing. Rats were then placed in microdialysis chambers (with free access to food and water) and artificial cerebrospinal fluid (aCSF; CMA) was perfused through the probes at a flow rate of 0.5 µL/min overnight. Approximately 14-16 hours later, on the day of test article administration, the probe perfusion flow rate was increased to 1.5 µL/min and a 2-hour equilibration period was allowed before sample collection began. Microdialysate samples were collected every 30 minutes using tubes containing 10 µL of formic acid (0.5M) in refrigerated fraction collectors, to prevent monoamine degradation. Four baseline samples were collected at 30-minute intervals over a 2-hour period before rats were dosed orally (2 mL/kg, via gavage) with SEP-856 or vehicle. SEP-856 was administered at 3, 10 and 30 mg/kg p.o. in saline. Eight samples were collected at 30-minute intervals over a 4-hour period after test article administration. Animals were awake and freely moving throughout the experiment. Rats were returned to their home cage at the end of the microdialysis experiment and sacrificed by decapitation within 72 hours. Brains were immediately removed and frozen using isopentane in dry ice and stored at -80ºC until histological verification of probe placement.

Sample analysis: Monoamine levels in perfusate samples from either brain area were analyzed by HPLC-EC detection within 24 hours of the microdialysis experiment. On

every experimental day, HPLC instruments were calibrated using freshly prepared standards containing known concentrations of dopamine (DA) and serotonin (5HT) (0; 0.05; 0.1; 1; 2 and 5 pg/μL of aCSF). For each concentration, 45 μL of standards in aCSF were added to 10 μL of formic acid (0.5M) in order to reproduce the dialysate sample conditions. DA and 5HT levels in the striatum were measured by transferring the dialysate samples to an autosampler (ESA, Inc. Model 540). 27 μL were injected onto a Capcell Pak column (250 × 1.5 mm, 3 μm particle size; ESA MD-160). DA and 5HT were eluted using a mobile phase consisting of 150 mM ammonium acetate and 140 μM EDTA in 15% methanol and 5% acetonitrile, pH 6.0. DA and 5HT were detected with a glassy carbon target analytical cell (ESA 5041) at a potential of 220mV using a Coulochem III detector (ESA). Chromatography data was acquired on a PC and analyzed using the EZ Chrome Elite software (Agilent technologies). DA and 5HT were measured in dialysate samples from the prefrontal cortex using the Alexys 100 system (Antec Leyden). Samples were transferred onto an autosampler (AS 100Antec Leyden) and a 30 μL injection was split equally between 2 columns. DA and 5HT were eluted with a C18 column (50 × 1 mm, 3 μm particle size; Antec Leyden ALB-105). The mobile phase for either column consisting in 50 mM phosphoric acid, 8 mM KCl, 0.1 mM EDTA and 500 mg/L OSA in 4 to 7% MeOH, pH 6.0. DA and 5HT were detected with a 0.7 mm glassy carbon electrode cell (VT-03, Antec Leyden) at a potential of 300 mV. Chromatography data were acquired on a PC and analyzed using the Alexys software (Antec Leyden).

In Vivo Pharmacokinetic Studies

Pharmacokinetic studies were conducted in male ICR mice (21.2 to 24.4 g; Shanghai Laboratory Animal Center), male Sprague-Dawley rats (212 to 235 g; Shanghai Laboratory Animal Center) and male rhesus monkeys (~ 3.75 years of age). In all studies SEP-856 was formulated in phosphate buffered saline (pH 7 – 7.4) for oral or i.v. administration to animals that were fasted overnight. To determine brain penetration, mice or rats were dosed orally with 10 mg/kg SEP-856 and blood and brain collected at various timepoints from 15 min to 24 hours (n = 3/timepoint) after drug administration. Blood samples were collected by cardiac puncture following euthanasia by CO_2 inhalation, placed into K_2EDTA tubes and centrifuged at 8,000 rpm for 6 minutes at 4^0C, and the plasma extracted and frozen at -80°C. Brains were removed, placed on ice and rinsed with saline prior to freezing at -80°C. The PK properties of SEP-856 were determined in rats dosed with 10 mg/kg (i.v.) or 50 mg/kg (p.o.) SEP-856 and in rhesus monkeys dosed with 5 mg/kg SEP-856 (i.v. or p.o.). Serial blood samples were collected at various timepoints ranging from 5 min to 24 hours post dose (n = 3 subjects). Concentrations of SEP-856 were determined by high performance liquid chromatography with mass spectrometric detection. The lower limits of quantification were 2.5 ng/ml for plasma and 5 ng/g for brain and pharmacokinetic parameters were calculated using WinNonlin Pro version 5.0 or 5.2 (Pharsight Corporation, USA). Any concentrations that were below the limit of quantification were omitted from the calculation of pharmacokinetic parameters in individual animals.

In Vitro Autoradiography

In vitro autoradiography was used to determine the effects of SEP-856 on [^{3}H]-8-OH-DPAT binding to 5-HT_{1A} receptors in brain sections of adult Sprague-Dawley rats (Harlan, Frederick, MD). Briefly, slide mounted rat brain sections (12 µm) consisting of prefrontal cortex (PFC), cortex (motor and somatosensory), septum, striatum, dorsal hippocampus and hypothalamus were preincubated for 30 minutes in 50 mM tris buffer (pH 7.5) at room temperature. Sections were then incubated in the same buffer containing 4 mM $CaCl_2$; 0.1% ascorbic acid pH 7.5 containing 2 nM [^{3}H]-8-OH-DPAT in the absence (total binding) and presence of SEP-856 (100 nM, 1 uM or 10 µM) for one hour at room temperature (22 -25 ˚C). Non-specific binding was defined by 10 µM 5-HT. Following incubation, slices were briefly rinsed, then washed in ice cold incubation buffer for 2 x 10 minutes. Brain sections were then rinsed in ice cold H_2O and dried under a stream of cool air. Rat brain sections were imaged by placing the tissue sections in a Biospace β-Imager for 6 hours. Afterwards, [^{3}H]-8-OH-DPAT binding in the absence and presence of SEP-856 was quantified using software provided by Biospace and the effect of SEP-856 on [^{3}H]-8-OH-DPAT binding to 5-HT_{1A} receptors was determined. In a follow up experiment, the above experimental design was repeated using rat brain sections containing cortex and septum. Following image quantification, Graphpad Prism software was used to determine the IC_{50} of SEP-856.

D_2 Receptor Occupancy in Rats

The occupancy of D_2 receptors after i.p. administration of SEP-856 was evaluated in male Sprague-Dawley rats (216-232 g; Harlan, Frederick, MD). SEP-856 (10 mg/kg, 2

ml/kg) or vehicle (20% HPβCD) were administered i.p. at t = 0. [^{3}H]-raclopride (60 μCi/kg) was administered i.v. at t = 30 min and rats were killed by rapid decapitation at t = 60 min. Brains were frozen in isopentane (previously cooled on dry ice) and stored at -80ºC until required for cryosectioning. Blood samples were processed to obtain plasma samples which were then stored at -80ºC until required for exposure analysis. For cryosectioning, brains were removed from the -80ºC freezer and allowed to thaw to -20ºC. Coronal sections (20 μm) of the striatum (region of interest) and cerebellum (reference region) were cut and thaw mounted onto glass microscope slides. Slide mounted tissue sections (of striatum and cerebellum) were placed in the Biospace β-Imager and imaged for 6 hours. Images were quantitated using software (Betavision plus) provided by Biospace and a signal:noise (striatum:cerebellum) value was determined for each animal. The percent receptor occupancy was determined using the following equation:

$$\%RO = 100 \times [(\text{average } S{:}N_{Vehicle}) - S{:}N_{Subject}] / [(\text{average } S{:}N_{Vehicle}) - 1]$$

A satellite group of rats was also used for determination of SEP-856 exposures in the plasma and brain. Rats received SEP-856 (10 mg/kg, 2 ml/kg, i.p.). 60 minutes later, rats were euthanized by CO_2 inhalation. Rats were then decapitated, and brains and plasma samples collected. Brain and plasma samples were analyzed for drug concentration using LC/MS analysis.

D_2 Receptor Occupancy in Nonhuman Primates

Positron Emission Tomography (PET) imaging was conducted in two nonhuman primate female baboons (*Papio anubis*; ~18 kg) to study the effect of SEP-856 on D_2

receptors: one dose (~7.25 mg/kg) was tested in duplicate. Prior to injection, quality control of the radiopharmaceutical was performed to ensure purity, identity, strength and sterility. A blockade protocol design was used to estimate the receptor occupancy where SEP-856 was administered 30 minutes prior to [^{18}F]-fallypride injection. A baseline study (no blocking agent) was also conducted with [^{18}F]-fallypride for each animal. The animals were fasted for 18–24 hours before the study and were anesthetized with intramuscular ketamine at 10 mg/kg and glycopyrrolate at 0.01 mg/kg (at 2 hours prior to radiopharmaceutical injection for the imaging studies), transferred to the PET camera for the imaging studies, and intubated with an endotracheal tube for continued anesthesia with 2.5% isoflurane administered through a rebreathing circuit. Body temperature was kept at 37 °C using a heated water blanket. Vital signs, including heart rate, blood pressure, respiration rate, oxygen saturation and body temperature, were monitored every 10 to 20 min during the study. An intravenous line was placed and used for injection of the radiopharmaceutical [^{18}F]-fallypride and the test article SEP-856 for the blockade studies. For the latter, the animal was injected with SEP-856 as a bolus over 5 min at T = 0 min and with the radiotracer [^{18}F]-fallypride at T = 30 minutes. Following the intravenous injection of [^{18}F]-fallypride as a bolus over 3 min at T = 30 minutes, a series of 45 dynamic 3D PET scans were obtained continuously on a Biograph mCT camera over three hours (T = 30 – 210 minutes) as follows: 6 x 30 seconds, 3 x 1minutes, 2 x 2 minutes, 34 x 5 minutes. The dynamic series were subsequently reconstructed using iterative reconstruction with corrections for random, scatter, and attenuation provided by the camera manufacturer. Reconstructed PET image data volumes were transferred to the image processing PMOD software package

(PMOD Technologies, Zurich, Switzerland) where the images were realigned onto the monkey MR to apply a volume of interest (VOI) template comprising the following regions: caudate, putamen, globus pallidus and cerebellum. Average activity concentration (kBq/cc) within each VOI was determined and time activity curves (TAC) were generated for each study, depicting the regional brain activity concentration over time (total uptake = specific plus non-displaceable). Time activity curves were expressed in SUV units (g/mL) by normalizing by the animal weight and the injected dose. The non-invasive reference region models SRTM, SRTM2 and Logan noninvasive using the cerebellum as reference region were applied to the regional time activity curves with the PMOD software to determine the binding potential BPND for each region mentioned above. For SRTM2, k'2 was estimated by doing a coupled fit across the caudate, putamen and globus pallidus. For Logan, the k'2 obtained from the SRTM2 coupled fit was used. The occupancy was determined using the binding potential BPND as follows: Occ = ((BP_{ND}Baseline – BP_{ND}Drug)/BP_{ND}Baseline). Similar results were obtained with all three non-invasive reference region models (SRTM, SRTM2 and Logan noninvasive). Thus, only the values obtained with the SRTM model are shown in the results.

Whole-Cell Patch Clamp Recordings in the DRN and VTA

Male C57BL/6 mice (4-16 weeks,15-35 g) were humanely killed by terminal anesthesia with isoflurane, cervical dislocation, and decapitation. The brain was then removed and 300 μm thick sagittal slices containing the VTA and/or the DRN were sectioned using a Leica VT1000S. After brain removal and throughout slicing the tissue was submerged in

ice cold (< 4°C) 'high sucrose' artificial cerebrospinal fluid (aCSF) of the following composition (in mM): Sucrose, 154; KCl, 1.9; KH_2PO_4, 1.2; $CaCl_2$, 0.1; $MgCl_2$, 3.6; $NaHCO_3$, 26; D-glucose, 10; L-ascorbic acid, 0.3; equilibrated with 95% O_2-5% CO_2. Once slices were cut, they were transferred to a beaker containing 'standard' aCSF and left at room temperature for a minimum of one hour before commencing electrophysiological recordings. After this period, individual slices were transferred to a custom-built recording chamber continuously perfused with 'standard' aCSF at a rate of 4–10 ml/min. 'Standard' aCSF composition (in mM): NaCl, 127; KCl, 1.9; KH_2PO_4, 1.2; $CaCl_2$, 2.4; $MgCl_2$, 1.3; $NaHCO_3$, 26; D-glucose, 10; equilibrated with 95% O_2-5% CO_2. Whole-cell patch-clamp recordings were performed at room temperature using the 'blind' version of the patch-clamp technique with either Axopatch 1D or Multiclamp 700B amplifiers. Patch pipettes were pulled from thin-walled borosilicate glass with resistances of between 3 and 8 MΩ when filled with intracellular solution of the following composition (mM): potassium gluconate, 140; KCl, 10; EGTA-Na, 1; HEPES, 10; Na2-ATP, 2, 0.3 GTP with pH and osmolarity compensated with KOH and sucrose, respectively. Recordings were monitored on an oscilloscope and a PC running Axon pClamp software and digitized at 2-10 kHz. At the beginning of each whole-cell patch-clamp recording, a current/voltage (IV) relationship was performed to identify active conductances in the recorded neuron, before testing the effects of SEP-856 and TAAR1/5-HT_{1A} receptor ligands. Changes in electrophysiological parameters including membrane potential and neuronal firing rate were analyzed using Axon pClamp and Microsoft Excel software to measure the effects of test compounds. To attempt neuronal phenotype characterization, DRN neurons were classified as being either sensitive or

insensitive to administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT. In addition, after the determination of repeatable responses, administration of SEP-856 was repeated in the presence of the selective TAAR1 antagonist EPPTB and/or the selective 5-HT_{1A} receptor antagonist WAY-100635. Compound concentrations were chosen based on the results of pilot concentration-response whole-cell patch clamp recordings in the VTA and DRN. Changes in neuronal properties are presented as mean ± S.E.M and, in some cases, calculated as a percentage of control by calculating the mean of the normalized response. Where appropriate, statistical comparisons have been performed using the paired student's t-test, with $P < 0.05$ taken to indicate statistical significance.

In Vivo Extracellular Single-Unit Recordings in the DRN

Male Sprague-Dawley rats (275-400 g; Harlan, IN) were anesthetized with Urethane (initial dose at 1.2 to 1.6 g/kg, i.p.) and were surgically implanted with two catheters, one for femoral vein (drug administration) and one for femoral artery (blood sampling). The animals were then mounted on a stereotaxic apparatus (David Kopf instrument) in a flat skull position. Proper surgical anesthesia was maintained throughout the experiment by administration of supplemental doses of the anesthetic. Core temperature was maintained at 37ºC by a heating pad. Borosilicate glass micropipette electrodes (3 mm OD, 2 mm ID, Sutter Instrument) were pulled by PE-22 micro-electrode puller (Narishige Group) and then filled with 0.5% sodium acetate in 2% Pontamine Skyblue (Sigma). The electrodes had in vitro impedances of 1-3 MΩ. To gain access to dorsal raphe nucleus recording site the micro-electrode was advanced by a single axis in vivo

micromanipulator (Scientifica, United Kingdom) mounted on Kopf stereotaxic holders. One burr hole was drilled on the skull with stereotaxic coordinate of AP-7.8 mm, lateral 0.8 mm. The dura was carefully removed to expose the cortical surface. The recording electrode was inserted into brain through the hole at a 10-degree angle towards midline and advanced to reach the target coordinate of raphe nucleus (5.1-6.1 mm below the brain surface).

Extracellular single-unit activities were amplified (x1000), filtered (low pass 3 KHz and high pass at 300 Hz), displayed on the oscilloscope and stored in a computer equipped with the Spike 2 analysis system (Cambridge Electronic Design, UK) for off-line analysis. The recorded neurons location was histologically confirmed. Based on previous reports, the neurons which met the following criteria were included for the study: Slow firing rate (0.1 to 5 Hz), exhibiting a long duration (2-4 ms), single or bursting patterned action potentials with biphasic or triphasic extracellular waveforms (Aghajanian *et al.*, 1978; Clifford *et al.*, 1998; Hajós *et al.*, 2007). The baseline firing activity of the neuron was recorded for at least 10 min prior to the compound administration. SEP-856 was tested at 1, 2, and 5 mg/kg by i.v. injection. After clear inhibitory effects were observed (3-5 minutes after compound administration), WAY-100635 (0.08 mg/kg, i.v.) was given to determine whether it could antagonize the inhibitory effect of SEP-856. This dose range was previously reported to reverse the inhibitory effects of 8-OH-DPAT (Martin *et al.*, 1999). All test substances were dissolved in sterile saline and administered i.v. at a dose volume of 1 ml/kg. Blood samples were taken 30 min following compound administration. At the end of each experiment, the recording site was marked by the microiontophoresis of pontamine skyblue (-20 μA, 15

min). Each rat was then given an overdose of urethane. The brains were immediately removed, were frozen on dry ice, and were cut into 40 μM thick coronal sections using a cryostat. The sections were mounted on gelatin-coated slides and stained with cresyl violet in order to determine the location of the recording sites.

<u>In Vitro Binding Studies</u>

Equilibrium radioligand binding was performed using the following incubation conditions:

$5\text{-}HT_{1A}$: Membranes from HEK-293 cells expressing the human recombinant $5\text{-}HT_{1A}$ receptor were incubated in the presence of 0.3 nM [^{3}H]-8-OH-DPAT for 60 minutes at 22°C.

$5\text{-}HT_{1B}$: Rat cerebral cortex membranes were incubated with 0.1 nM [^{125}I]-CYP for 120 minutes at 37°C.

$5\text{-}HT_{1D}$: Membranes from CHO cells expressing the rat recombinant $5\text{-}HT_{1D}$ receptor were incubated in the presence of 1 nM [^{3}H]-5-HT for 60 minutes at 22°C.

$5\text{-}HT_{2A}$: Membranes from HEK-293 cells expressing the human recombinant $5\text{-}HT_{2A}$ receptor were incubated in the presence of 0.1 nM [^{125}I]-(±)DOI for 60 minutes at 22°C.

$5\text{-}HT_{2B}$: Membranes from HEK-293 cells expressing the human recombinant $5\text{-}HT_{2B}$ receptor were incubated in the presence of 0.2 nM [^{125}I]-(±)DOI for 60 minutes at 22°C.

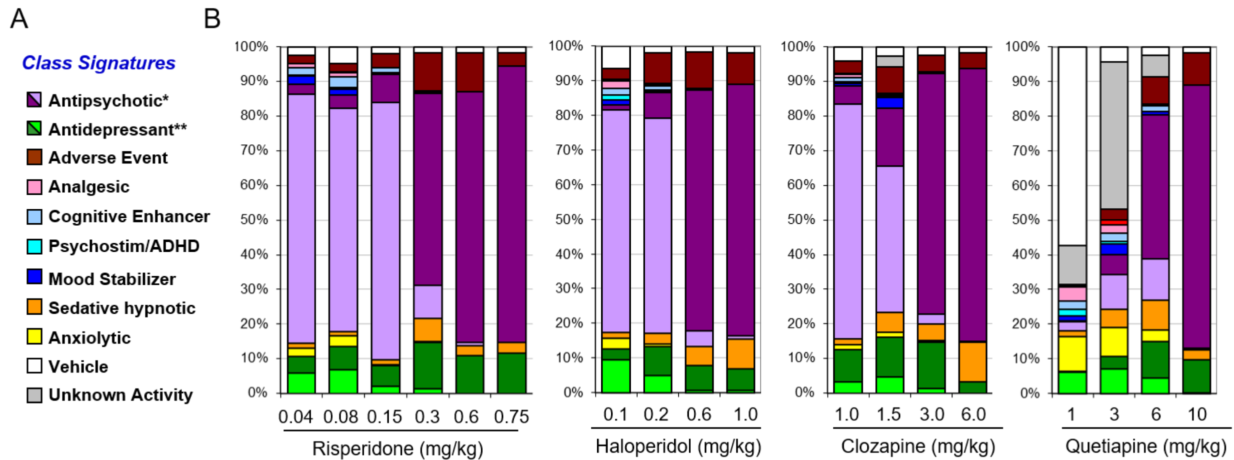
$5\text{-}HT_{2C}$: Membranes from HEK-293 cells expressing the human recombinant $5\text{-}HT_{2C}$ receptor were incubated in the presence of 0.1 nM [^{125}I]-(±)DOI for 60 minutes at 37°C.

$5\text{-}HT_{7}$: Membranes from HEK-293 cells expressing the human recombinant $5\text{-}HT_{7}$ receptor were incubated in the presence of 4 nM [^{3}H] LSD for 120 minutes at 22°C.

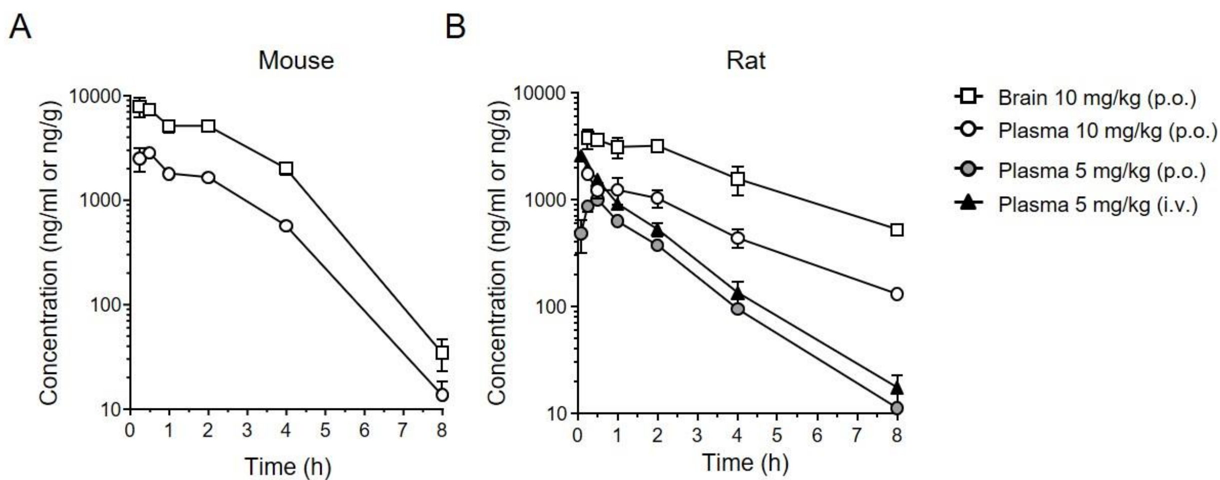
D_2: Membranes from HEK-293 cells expressing the human recombinant D_2 receptor were incubated in the presence of 0.3 nM [^{3}H]-methylspiperone for 60 minutes at room temperature.

α_{2A}: Membranes from HEK-293 cells expressing the human recombinant α_{2A} receptor were incubated in the presence of 0.3 nM [^{3}H]-methylspiperone for 60 minutes at room temperature.

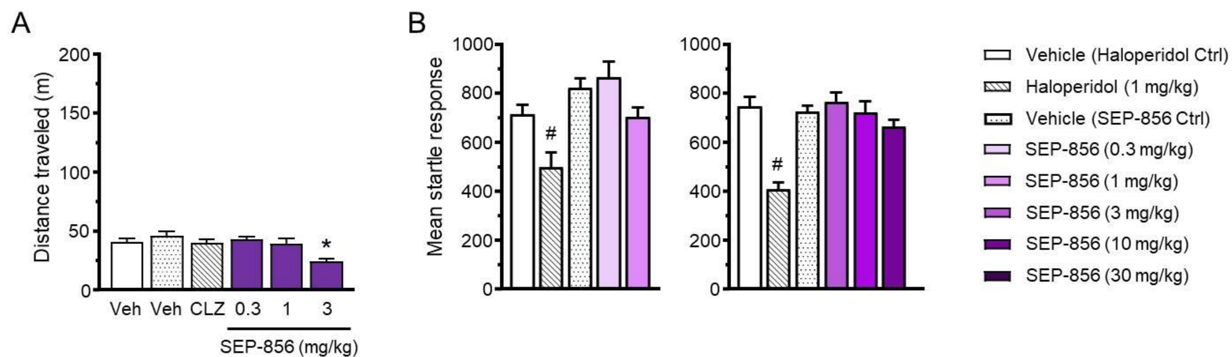
Supplemental Figures



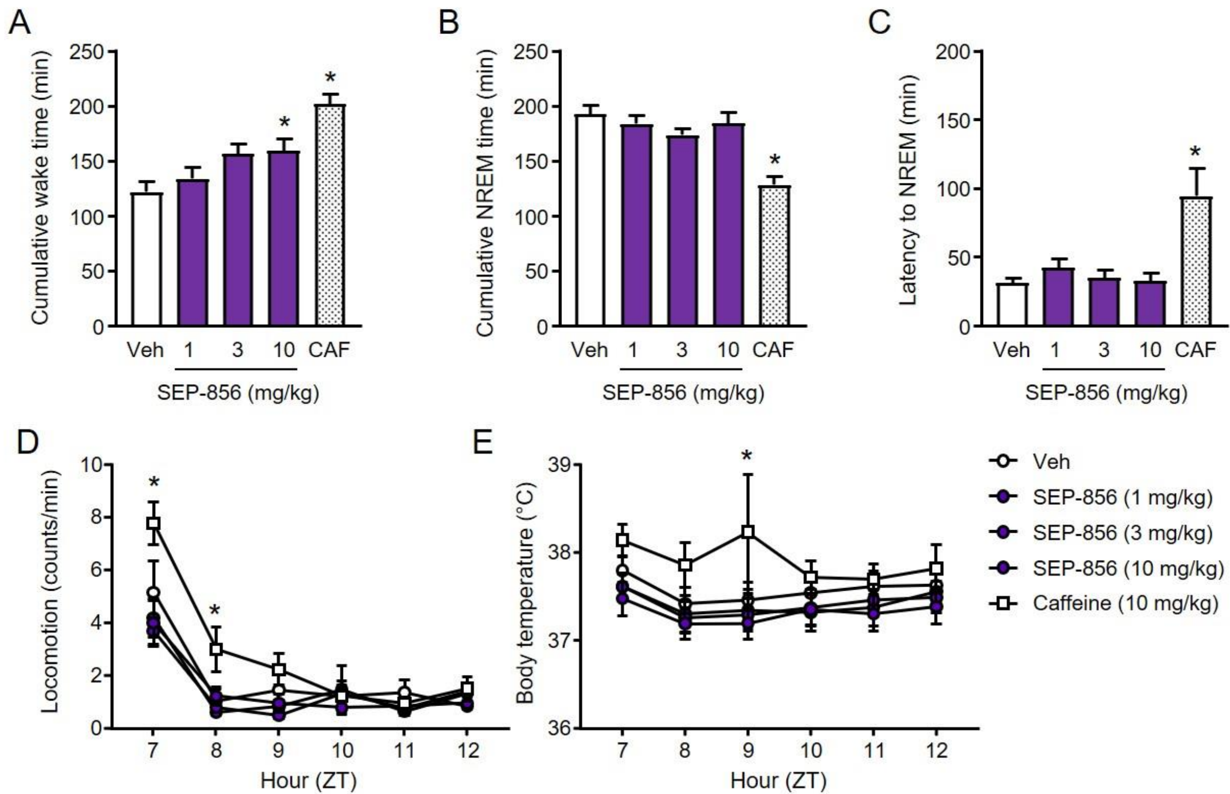
Supplemental Figure 1. SmartCube® behavioral signatures of typical and atypical antipsychotic drugs in mice. (A) Behavioral class signature color key representing 15 classes (*antipsychotic (purple) and high-dose antipsychotic (dark purple); **antidepressant (green) and *high-dose antidepressant (dark green)). (B) The predominant purple color is characteristic of an antipsychotic signature as shown for different doses of risperidone, haloperidol, clozapine and quetiapine (administered i.p. 15 min before testing, n = 6-8/dose).



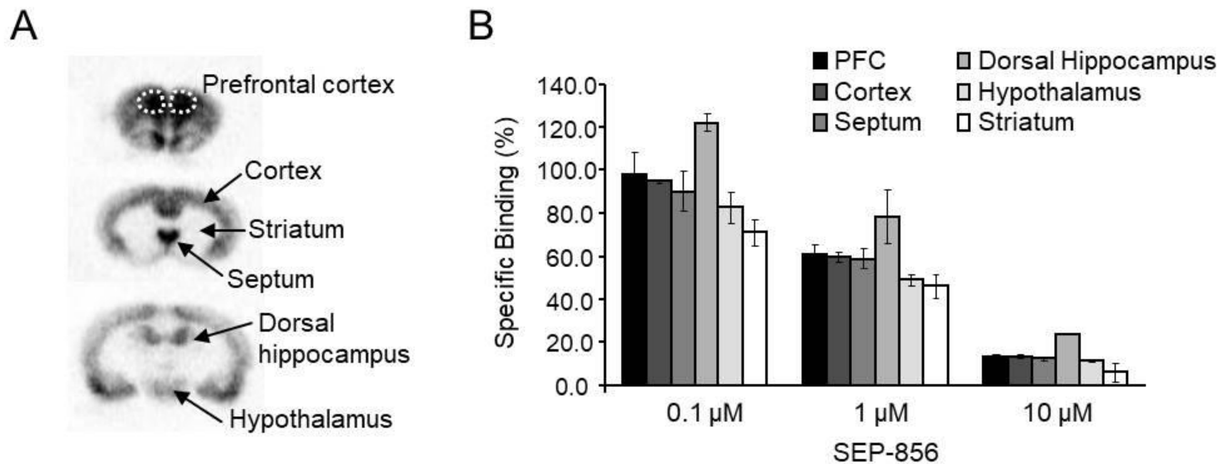
Supplemental Figure 2**.** SEP-856 exhibits good brain penetrance and rapid absorption in rodents**.** SEP-856 was administered orally and/or intravenously to male (A) ICR mice and (B) Sprague-Dawley rats. Plasma and brain samples were taken from n = 3 animals at each of the time points indicated. The brain-to-plasma AUC ratios for the 10 mg/kg dose ranged from 2.58 to 3.54 and 2.1 to 3.9 in mice and rats, respectively. Data are shown as mean ± s.e.m.



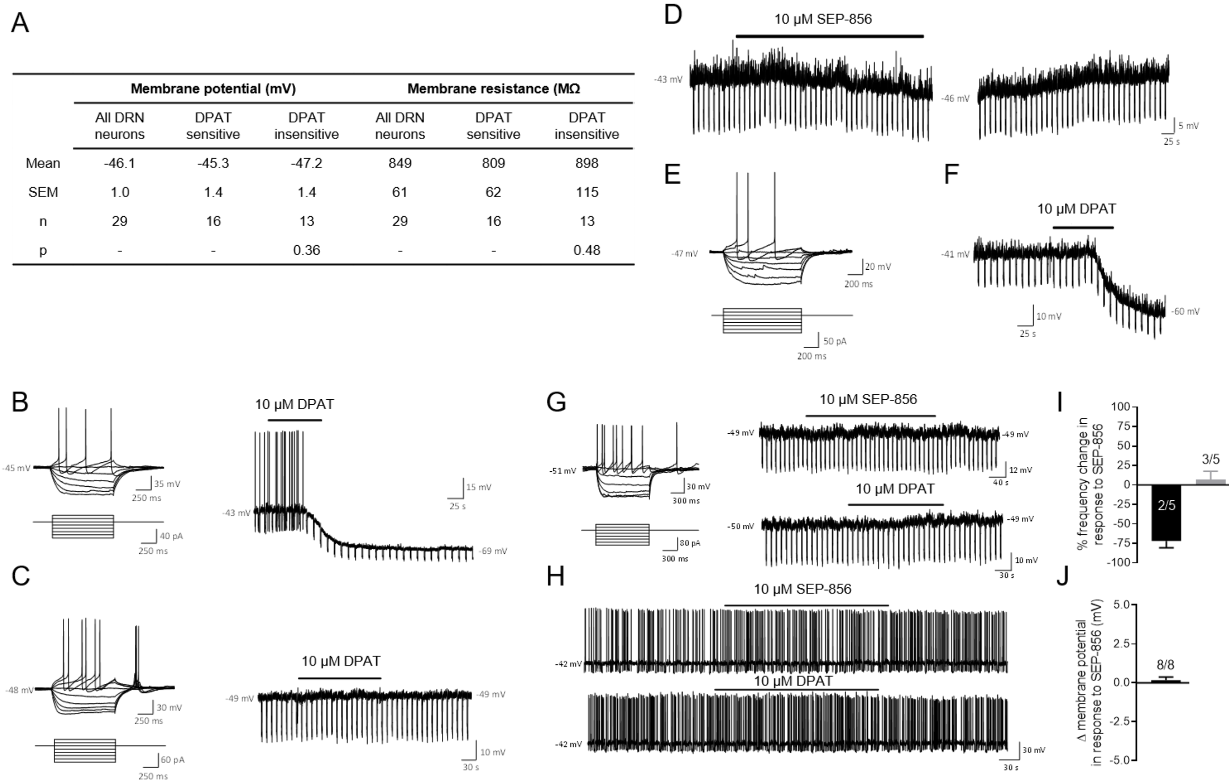
Supplemental Figure 3. Effect of SEP-856 on baseline locomotion and the acoustic startle response in C57Bl/6J mice. (A) At the highest tested dose (3 mg/kg), oral SEP-856 administration led to a slight reduction in overall activity during the open field test (one-way ANOVA $F_{(5, 59)} = 5.5$, $p = 0.0003$; Tukey's post-hoc test, * $p < 0.05$ vs. Veh (second bar from the left)). Clozapine (CLZ) tested at 1 mg/kg i.p. had no significant effect on locomotion. First vehicle (20% cyclodextrin, i.p.); second vehicle (20% cyclodextrin, p.o.). (B) The acoustic startle response, measured during the PPI test, was not significantly affected by SEP-856 (p.o.) compared to the respective vehicle control. In contrast, Haloperidol (i.p.) produced a significant reduction in the mean startle response (one-way ANOVA for the first experiment: $F_{(4, 42)} = 8.5$, $p < 0.0001$; second experiment: $F_{(5, 49)} = 11.5$, $p < 0.0001$; Tukey's post-hoc test, * $p < 0.05$ vs. Veh; n = 8-10/group). Vehicle for haloperidol (10% DMSO, i.p.), vehicle for SEP-856 (20% cyclodextrin, p.o.). Data are shown as mean ± s.e.m.



Supplemental Figure 4. Effects of SEP-856 on sleep/wake architecture in Sprague-Dawley rats. (A) Acute, oral administration of SEP-856 led to a significant increase in cumulative wake time at 10 mg/kg over the 6-hour recording period (one-way repeated-measures ANOVA + two-tailed t-test, *p < 0.05 vs. Veh). Cumulative NREM (B), latency to NREM (C), locomotor activity (D) and body temperature (E) were not significantly affected by SEP-856 during the 6-hour recording period (two-way repeated-measures ANOVA + two-tailed t-test, *p < 0.05 vs. Veh). The positive control caffeine (CAF, 10 mg/kg, p.o.) produced an expected increase in cumulative wake time, decrease in cumulative NREM time, increase in the latency to NREM as well as an increase in locomotion and body temperature. Dosing occurred in the middle of the resting phase, at the beginning of Zeitgeber time 7. N = 7. Data are shown as mean ± s.e.m.



Supplemental Figure 5. SEP-856 binds to 5-HT_{1A} receptors in rat brain slices. (A) Autoradiographs of coronal brain sections depicting binding of the 5-HT_{1A} agonist [^{3}H]-8-OH-DPAT. SEP-856 displaced [^{3}H]-8-OH-DPAT in a concentration-dependent manner, in all brain regions tested. N = 4 brains, 2 sections/brain. Data are shown as mean ± s.e.m.



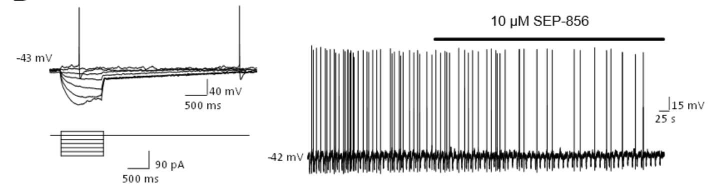
	Membrane potential (mV)			Membrane resistance (MΩ		
	All DRN neurons	DPAT sensitive	DPAT insensitive	All DRN neurons	DPAT sensitive	DPAT insensitive
Mean	-46.1	-45.3	-47.2	849	809	898
SEM	1.0	1.4	1.4	61	62	115
n	29	16	13	29	16	13
p	-	-	0.36	-	-	0.48

Supplemental Figure 6. Effects of SEP-856 on DRN firing. (A) Summary of the general properties of neurons recorded from the DRN. DRN neurons were classified based on their electrophysiological properties and response to administration of the 5-HT_{1A} receptor agonist [^{3}H]-8-OH-DPAT (DPAT). (B) Representative current/voltage relationship (left) of a DRN neuron in which DPAT induced a marked membrane hyperpolarization (right). (C) Representative current/voltage relationship (left) of a DRN neuron in which DPAT was without effect on membrane potential (right). Example of a current-clamp recording (D) and current/voltage relationship (E) of a quiescent, DPAT-sensitive DRN neuron in which SEP-856-induced membrane hyperpolarization. (F) Current-clamp recording of the DRN neuron shown in D and E during DPAT administration. SEP-856 had little effect in most DPAT-insensitive, quiescent (G) and spontaneously active (H) DRN neurons. (I, J) Quantifications of SEP-856 response (based on changes in membrane potential (mV) and/or firing rate (Hz) and expressed relative to baseline) in DPAT-insensitive DRN neurons. Solid bars indicate the timeframe of compound administration to the slice. Two-tailed t-test, * $p < 0.05$. Data are shown as mean ± s.e.m.

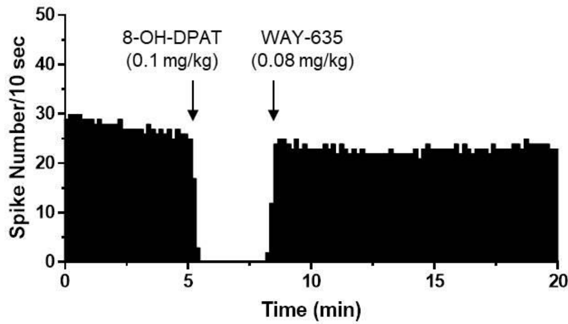
A

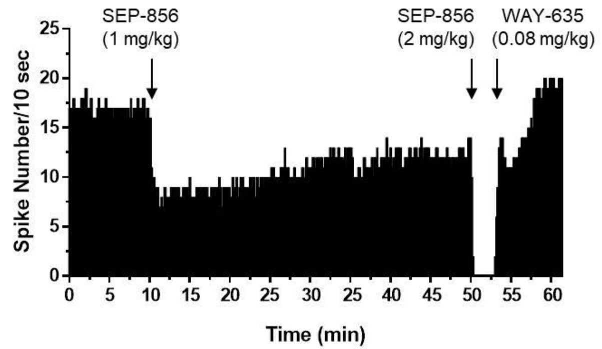
	Membrane potential (mV)	Membrane resistance (MΩ)	Firing rate (Hz)
Mean	-40.7	935	1.97
SEM	0.6	104	0.6
n	23	23	

B

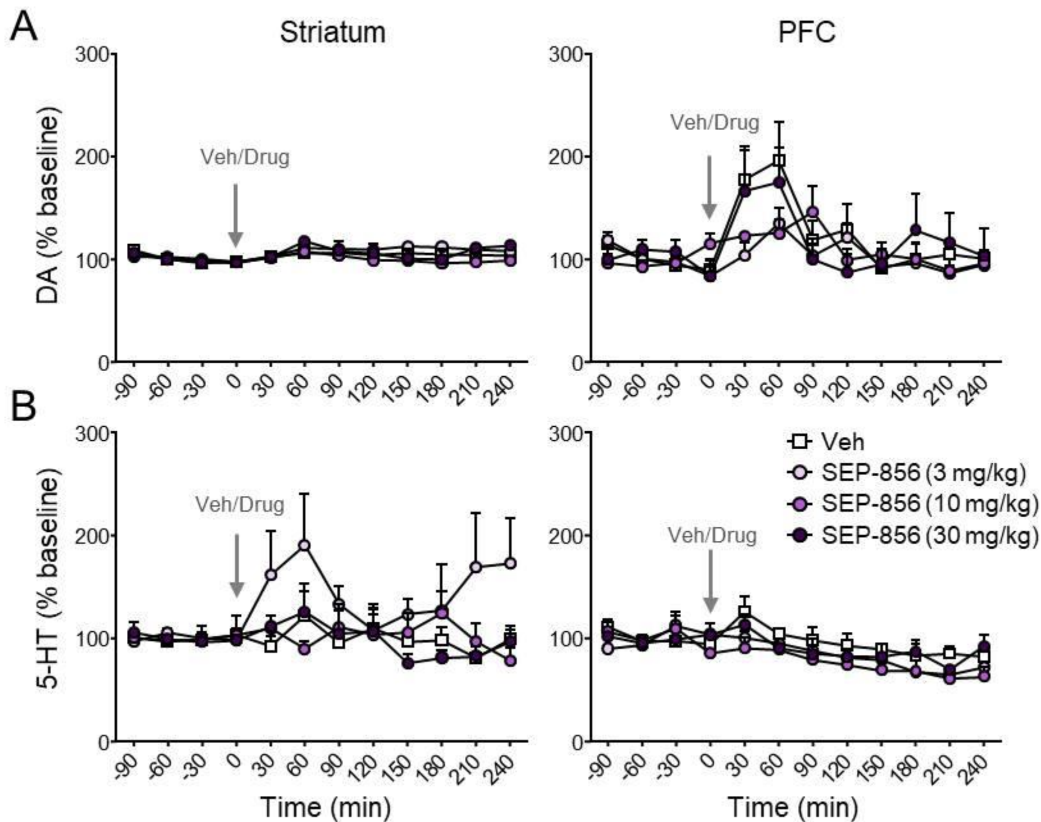


Supplemental Figure 7. Effects of SEP-856 on VTA firing. (A) Summary of the general properties of neurons recorded from the VTA. (B) Representative current/voltage relationship and current-clamp recording of a VTA neuron in which SEP-856 was without an effect. Solid bar indicates the timeframe of SEP-856 administration to the slice.





Supplemental Figure 8. SEP-856 inhibits firing of DRN in vivo through 5-HT_{1A}. (A) Single-unit discharges in DRN neurons were abolished following administration of the 5-HT_{1A} agonist 8-OH-DPAT (i.v.). The inhibition of firing was reversed by subsequent administration of the selective 5-HT_{1A} antagonist WAY-100635 (i.v., n = 1). (B) Rate histogram showing partial inhibitory effects of SEP-856 (1 mg/kg, i.v.) on single unit discharge of dorsal raphe nucleus. The inhibitory effect was time dependently reversed. Subsequent administration of SEP-856 at 2 mg/kg, i.v. resulted in a complete inhibition in discharge which was fully reversed by WAY-100635 (i.v., n = 1). Abbreviations: WAY-100635 (WAY-635).



Supplemental Figure 9. Oral SEP-856 administration does not alter extracellular serotonin (5-HT) and dopamine (DA) levels in the striatum and prefrontal cortex (PFC) of male Sprague-Dawley rats. All treatments were administered at time = 0, following 90 minutes of baseline-sample collection. Monoamine levels are expressed as percent change from baseline. (A) SEP-856 (p.o.) at all doses tested did not affect extracellular DA levels in the striatum (two-way repeated measures ANOVA: treatment effect $F_{3,\ 509} = 1.3$, $p = 0.1$; time x treatment interaction $F_{(33,509)} = 1.34$, $p = 0.1$; n = 11-13/group) and PFC (two-way repeated measures ANOVA: treatment effect $F_{(3,\ 343)} = 0.85$, $p = 0.5$; time x treatment interaction $F_{(33,343)} = 1.4$, $p = 0.3$; n = 8-10/group). (B) Similarly, 5-HT release in the striatum and PFC was not affected by oral SEP-856 treatment compared to vehicle control (two-way repeated measures ANOVA: striatum - treatment effect $F_{(3,\ 240)} = 3.0$, $p = 0.05$; time x treatment interaction $F_{(33,240)} = 1.1$, $p = 0.35$; n = 5-9/group / PFC - treatment effect $F_{(3,\ 91)} = 3.5$, $p = 0.06$; time x treatment interaction $F_{(33,91)} = 0.9$, $p = 0.6$; 3-4/group). Data are shown as mean ± s.e.m.

Supplemental Tables

Supplemental Table 1. Cerep Bioprint Targets

A1	ETB	M2	5-HT6
A2A	GABAA	M3	5-HT7
A2B	GABAB(1b)	M4	σ
A3	glucagon	NK1	sst1
α1A	AMPA	NK2	sst4
α 1B	kainate	Y1	GR
α 2A	NMDA	N neuronal α-BGTX-insensitive (α4β2)	ERα
α 2B	glycine (strychnine-insensitive)	N muscle-type	AR
α 2C	CXCR4	δ2 (DOP)	TR (TH)
1	TNF–α	κ(KOP)	UT
β 2	CCR2	μ (MOP)	VPAC1 (VIP1)
β 3	H1	PPARγ	V1a
AT1	H2	PAF	V2
AT2	H3	PCP	Ca2+ channel (L, dihydropyridine site)
APJ (apelin)	H4	EP2	Ca2+ channel (L, diltiazem site) (benzothiazepines)
BZD	I1	FP	Ca2+ channel (L, verapamil site) (phenylalkylamine)
BB3	BLT1 (LTB4)	IP (PGI2)	Ca2+ channel (N)
B2	CysLT1 (LTD4)	LXRβ	SKCa channel
CB1	MCH1	PCP	Na+ channel (site 2)
CB2	MC1	5-HT1A	Cl- channel (GABA-gated)
CCK1 (CCKA)	MC3	5-HT1B	norepinephrine transporter
CCK2 (CCKB)	MC4	5-HT1D	dopamine transporter
CRF1	MT1 (ML1A)	5-HT2A	GABA transporter
D1	MT3 (ML2)	5-HT2B	5-HT transporter
D2S	MAO-A	5-HT2C	
D3	motilin	5-HT3	
ETA	M1	5-HT4e	

Supplemental Table 2. Cerep Enzyme Screen Targets

COX1	HIV-1 protease	p38α kinase
COX2	neutral endopeptidase	acetylcholinesterase
PDE2A	MMP-1	COMT
PDE3A	MMP-2	xanthine oxidase/ superoxide O2-scavenging
PDE4D	MMP-9	ATPase (Na+/K+)
PDE5 (non-selective)	Abl kinase	5-HT transporter
PDE6 (non-selective)	CDK2 (cycE)	p38α kinase
ACE	ERK2) (P42mapk)	acetylcholinesterase
ACE-2	FLT-1 kinase (VEGFR1)	COMT
BACE-1 (β-secretase)	Fyn kinase	xanthine oxidase/ superoxide O2-scavenging
caspase-3	IRK (InsR)	ATPase (Na+/K+)
caspase-9	Lyn A kinase (h)	5-HT transporter

Supplemental Table 3. Ricerca Receptor Screen Targets

Adenosine A1	Estrogen ERα	N-Formyl Peptide Receptor FPR1	Tachykinin NK3
Adenosine A2A	Estrogen ERβ	N-Formyl Peptide Receptor-Like FPRL1	Thyroid Hormone
Adenosine A3	GPR103	Neuromedin U NMU1	TRH
Adrenergic α1A	GABAA, Chloride Channel, TBOB	Neuromedin U NMU2	TGF-β
Adrenergic α1B	GABAA, Flunitrazepam, Central	Neuropeptide Y Y1	Transporter, Adenosine
Adrenergic α1D	GABAA, Muscimol,	Neuropeptide Y Y2	Transporter, Choline
Adrenergic α2A	GABAB1A	Neurotensin NT1	DAT
Adrenergic α2C	Gabapentin	Nicotinic Acetylcholine	Transporter, GABA
Adrenergic β1	Galanin GAL1	Nicotinic Acetylcholine α1, Bungarotoxin	Transporter, Monoamine
Adrenergic β2	Galanin GAL2	Nicotinic Acetylcholine α7, Bungarotoxin	NET
Adrenergic β3	Glucocorticoid 314541	NPBW2/GPR8	SERT
Adrenomedullin AM	Glutamate, AMPA	Opiate δ1 (OP1, DOP)	TNF, Non-Selective
Adrenomedullin AM2	Glutamate, Kainate	Opiate κ(OP2, KOP)	Urotensin II
Androgen	Glutamate, NMDA, Agonism	Opiate μ(OP3, MOP)	Vanilloid
Angiotensin AT2	Glutamate, NMDA, Glycine	Orphanin ORL1	VIP1
APJ	Glutamate, NMDA, Phencyclidine	Phorbol Ester	Vasopressin V1A
Atrial Natriuretic Factor (ANF)	Glutamate, NMDA, Polyamine	PDGF	Vasopressin V1B
Bombesin BB1	Glycine, Strychnine-Sensitive	Potassium Channel [KATP]	Vasopressin V2
Bombesin BB2	Growth Hormone Secretagogue (GHS)	Potassium Channel [SKCA]	Vitamin D3
Bombesin BB3	Growth Hormone Secretagogue (GHS)	Potassium Channel hERG	
Bradykinin B1	Histamine H1	Progesterone PR-B	
Bradykinin B2	Histamine H2	Prostanoid CRTH2	
Calcitonin	Histamine H3	Prostanoid DP	
Calcium Channel L-Type, Benzothiazepine	Histamine H4	Prostanoid EP2	
Calcium Channel L-Type, Dihydropyridine	Imidazoline I2	Prostanoid EP4	
Calcium Channel L-Type, Phenylalkylamine	Inositol Trisphosphate IP3	Purinergic P2X	
Calcium Channel N-Type	Interleukin IL-1	Purinergic P2Y 314632	
Cannabinoid CB1	Interleukin IL-6	Retinoid X RXRα	
Chemokine CCR1	Leukotriene, BLT (LTB4)	Ryanodine RyR3	
Chemokine CCR2B	Leukotriene, Cysteinyl CysLT1	5-HT1A	
Chemokine CCR4	Leukotriene, Cysteinyl CysLT2	5-HT2B	
Chemokine CCR5	Melanocortin MC1	5-HT2C	
Chemokine CX3CR1	Melanocortin MC3	5-HT3	
Chemokine CXCR2 (IL-8RB)	Melanocortin MC4	5-HT4	
Colchicine	Melanocortin MC5	5-HT5A	
Corticotropin Releasing Factor CRF1	Melatonin MT1	5-HT6	
Dopamine D1	Melatonin MT2	Sigma σ1	
Dopamine D2S	10 μM -1	Sigma σ2	
Dopamine D3	252200 Motilin	Somatostatin sst1	
Dopamine D4	Muscarinic M1	Somatostatin sst2	
Dopamine D5	Muscarinic M2	Somatostatin sst3	
Endothelin ETA	Muscarinic M3	Somatostatin	
Endothelin ETB	Muscarinic M4	Somatostatin sst5	
Epidermal Growth Factor (EGF)	Muscarinic M5	Tachykinin NK1	

Supplemental Table 4. Ricerca Enzyme Screen Targets

Catechol-O-Methyltransferase (COMT)	PDE2
Cholinesterase, Acetyl, ACES	PDE2A
Monoamine Oxidase MAO-A	PDE3
Monoamine Oxidase MAO-B	PDE3A
Nitric Oxide Synthase, Endothelial (eNOS)	PDE4
Nitric Oxide Synthase, Inducible (iNOS)	PDE4A1A
Nitric Oxide Synthase, Neuronal (nNOS)	PDE5
PDE1	PDE5A
PDE10A2	PDE6
PDE1A	

Supplemental Table 5. Pharmacokinetic properties of SEP-856.

Male ICR mice, Sprague-Dawley rats and rhesus macaques were dosed with SEP-856 by p.o. and/or i.v. administration. Parameters were derived from mean plasma or brain concentrations for n = 3 animals per dose route. Data are shown as mean or mean ± s.d.

Parameters	Mouse		Rat				Monkey	
	p.o.		i.v.	p.o.			i.v.	p.o.
	Plasma	Brain	Plasma	Plasma	Plasma	Brain	Plasma	
Dose (mg/kg)	10	10	5	5	10	10	5	5
AUC_{0-t} (ng*h/ml or ng*h/g)	7256	22442	3275 ± 567	1910 ± 144	4955	15102	6563 ± 2202	4708 ± 1694
$AUC_{0-\infty}$ (ng*h/ml or ng*h/g)	7273	22483	3306 ± 588	1930 ± 144	5352	16854	6677 ± 2173	3754
CL (L/h/kg)	-	-	1.54 ± 0.26	-	-	-	0.797 ± 0.223	-
V_{ss} (L/kg)	-	-	2.57 ± 0.1	-	-	-	3.59 ± 1.96	-
$MRT_{(0-\infty}$ (h)	1.95	2.06	1.41 ± 0.22	1.68 ± 0.03	3.01	3.54	3.33 ± 1.15	5.90
$t_{1/2}$ (h)	0.847	0.808	1.17 ± 0.16	1.24 ± 0.1	2.10	2.33	3.14 ± 1.26	3.03
t_{max} (h)	0.50	0.25	0.083	0.42 ± 0.14	0.25	0.25	0.083	6.00 ± 2.83
C_{max} (ng/ml or ng/g)	2854 ± 298	7972 ± 2908	2578 ± 110	1056 ± 173	1750 ± 369	3762 ± 1324	2191 ± 194	431 ± 104
Bioavailability (%)	-	-	58 – 120				71.4 ± 1.59	

Supplemental Table 6. Plasma and brain exposure to SEP-856 in mice and rats following single oral administration in different behavioral tests. Data are shown as mean ± s.d.

	SEP-856 (mg/kg)	Plasma (ng/ml)	Brain (ng/g)
Mouse PCP hyperactivity test[1]	0.3	4.8 (2.5)	42.6 (0.9)
	1	13.1 (7.3)	90.1 (42.6)
	3	85.6 (50.2)	367.3 (177.0)
Mouse PPI/startle[2]	0.3	5.6 ± 1.8	79.1 ± 22.1
	1	20.3 ± 3.7	255.8 ± 50.2
	3	181.3 ± 73.8	711.9 ± 89.7
	10	781.0 ± 169.4	2962.5 ± 373.3
	30	1965.0 ± 308.6	6368.8 ± 1560.9
Rat PCP social interaction test[3]	1	56.6 ± 10.5	380 ± 20.1
	3	112.4 ± 58.9	868.8 ± 458.4
	10	280.5 ± 173.2	1939.5 ± 1152.8

[1]Samples were collected from n = 4 mice per group at the end of behavioral testing, approximately 90 minutes after SEP-856 administration

[2] Samples were collected from n = 4 mice per treatment group at the end of behavioral testing, approximately 55 minutes after SEP-856 administration

[3] Samples were collected from n = 4 rats per treatment group at the end of behavioral testing, approximately 40 minutes after administration of SEP-856

Supplemental Table 7. SEP-856 occupancy of D_2 receptors measured with $[^3H]$-raclopride in rats.

In vivo occupancy of i.p. SEP-856 (10 mg/kg) at D_2 receptors was assessed in male Sprague-Dawley rats. $[^3H]$-raclopride was administered i.v. 30 minutes post SEP-856/vehicle dosing. Striatal and cerebellar (reference region) brain sections were assessed 30 min later using autoradiography. Data are shown as mean ± s.e.m; n = 6/group.

Treatment (i.p.)	Signal:Noise	% RO	Plasma (ng/ml)	Brain (ng/g)	B:P
Vehicle	5.7 ± 0.1	0 ± 8.5	NA	NA	NA
10 mg/kg	5.7 ± 0.3	12.6 ± 6.4	1321.7 ± 55.8	7858.3 ± 284.7	6.0 ± 0.2

Supplemental Table 8. In vivo occupancy of SEP-856 at D_2 receptors measured with $[^{18}F]$-fallypride-PET in nonhuman primates (*Papio anubis*).

$[^{18}F]$-fallypride binding potentials (BPND) were determined at baseline and following SEP-856 treatment using the non-invasive reference region model SRTM. BPND were subsequently used to estimate the receptor occupancy in several brain regions. SEP-856 was administered i.v. 30 minutes prior to $[^{18}F]$-fallypride injection. 3D PET scans were obtained continuously over 3 hours (T = 30-210 minutes). Data are shown as mean ± s.e.m.

Brain region	$[^{18}F]$-fallypride BP_{ND}		Receptor Occupancy (%)	Plasma (ng/ml)	
	Baseline	~ 7.25 mg/kg		60 min	180 min
Caudate	16.6 ± 5.7	15.2 ± 5.8	9.1 ± 3.3		
Putamen	20.3 ± 4.8	19.4 ± 5.9	6.2 ± 7.0	2850 ± 250	1765 ± 125
Globus Pallidus	8.3 ± 2.2	7.7 ± 2.7	9.6 ± 8.8		

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