Molecular Psychiatry (2020) 25:1323–1333
https://doi.org/10.1038/s41380-018-0283-2

ARTICLE

Effects of the KCNQ channel opener ezogabine on functional connectivity of the ventral striatum and clinical symptoms in patients with major depressive disorder

Aaron Tan[1,2] · Sara Costi[1] · Laurel S. Morris[1] · Nicholas T. Van Dam[1,3] · Marin Kautz[1] · Alexis E. Whitton[4] · Allyson K. Friedman[5] · Katherine A. Collins[1] · Gabriella Ahle[6] · Nisha Chadha[7] · Brian Do[8] · Diego A. Pizzagalli[4] · Dan V. Iosifescu[9,10] · Eric J. Nestler[2,11,12] · Ming-Hu Han[2,11,12] · James W. Murrough[1,2,12]

Received: 28 March 2018 / Revised: 14 August 2018 / Accepted: 3 October 2018 / Published online: 1 November 2018

Abstract

Major depressive disorder (MDD) is a leading cause of disability worldwide, yet current treatment strategies remain limited in their mechanistic diversity. Recent evidence has highlighted a promising novel pharmaceutical target—the KCNQ-type potassium channel—for the treatment of depressive disorders, which may exert a therapeutic effect via functional changes within the brain reward system, including the ventral striatum. The current study assessed the effects of the KCNQ channel opener ezogabine (also known as retigabine) on reward circuitry and clinical symptoms in patients with MDD. Eighteen medication-free individuals with MDD currently in a major depressive episode were enrolled in an open-label study and received ezogabine up to 900 mg/day orally over the course of 10 weeks. Resting-state functional magnetic resonance imaging data were collected at baseline and posttreatment to examine brain reward circuitry. Reward learning was measured using a computerized probabilistic reward task. After treatment with ezogabine, subjects exhibited a significant reduction of depressive symptoms (Montgomery–Asberg Depression Rating Scale score change: -13.7 ± 9.7, $p < 0.001$, $d = 2.08$) and anhedonic symptoms (Snaith–Hamilton Pleasure Scale score change: -6.1 ± 5.3, $p < 0.001$, $d = 1.00$), which remained significant even after controlling for overall depression severity. Improvement in depression was associated with decreased functional connectivity between the ventral caudate and clusters within the mid-cingulate cortex and posterior cingulate cortex ($n = 14$, voxel-wise $p < 0.005$). In addition, a subgroup of patients tested with a probabilistic reward task ($n = 9$) showed increased reward learning following treatment. These findings highlight the KCNQ-type potassium channel as a promising target for future drug discovery efforts in mood disorders.

These authors contributed equally: Aaron Tan, Sara Costi

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41380-018-0283-2) contains supplementary material, which is available to authorized users.

✉ James W. Murrough
james.murrough@mssm.edu

[1] Mood and Anxiety Disorders Program, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[2] Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[3] Melbourne School of Psychological Sciences, University of Melbourne, Melbourne, VIC, Australia

[4] Department of Psychiatry, Harvard Medical School, Belmont, MA, USA

[5] Department of Biological Sciences, Hunter College, The City University of New York, New York, NY, USA

[6] Department of Psychology, Thomas Jefferson University, Philadelphia, PA, USA

[7] Department of Ophthalmology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[8] Roski Eye Institute, Keck School of Medicine at the University of Southern California, Los Angeles, CA, USA

[9] Department of Psychiatry, New York University School of Medicine, New York, NY, USA

[10] Nathan Kline Institute for Psychiatric Research, Orangeburg, NY, USA

[11] Department of Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[12] Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Introduction

Depression is a leading cause of disability worldwide [1]. Available treatments, however, are only partially effective

for many patients [2] and are associated with additional limitations, including a slow onset of therapeutic action and undesirable side effects [3, 4]. Currently, the Food and Drug Administration (FDA)-approved treatments for depression, mostly consisting of serotonergic and noradrenergic agents, largely share the same basic pharmacology and mechanism of action based on decades-old discoveries [5]. This lack of mechanistic diversity leaves little opportunity for improved patient outcomes or personalized treatment approaches. In contrast, rational drug discovery based on a mechanistic understanding of disease pathology promises to deliver more effective, targeted therapies [6].

Recent preclinical evidence has highlighted the KCNQ-type voltage-gated potassium channel as a promising novel molecular target for the treatment of depression in a well-validated mouse model of depression—chronic social defeat stress (CSDS) [7, 8]. CSDS produces two distinct phenotypes—susceptible and resilient—determined by the defeated mouse's willingness to interact with a novel mouse. CSDS results in an increased firing rate of ventral tegmental area (VTA) dopamine neurons projecting to the nucleus accumbens (NAc) within the ventral striatum (VS) in rodents expressing a pro-depressive phenotype [7, 9]. Critically, resilient animals also exhibit dysregulation within this circuit; however, they are able to actively counteract VTA–NAc hyperactivity by upregulating the KCNQ3 potassium channel within the VTA [7], which serves to rebalance dopaminergic firing [10]. Replicating this active mechanism in susceptible mice via viral overexpression of KCNQ3 within the reward circuit reverses the depressive behaviors, leading to a more resilient phenotype in rodents. Of translational importance, systemic injection of ezogabine, a KCNQ-selective potassium channel opener, also led to the amelioration of depressive behaviors in susceptible mice [8]. Taken together, this work supports the hypothesis that enhancing activity at KCNQ channels within the reward circuit may represent a novel mechanistic approach to antidepressant treatment discovery.

Based on these findings, we conducted a 10-week open-label pilot of ezogabine with the aim of determining whether ezogabine significantly engages the reward system in human patients with MDD. Connectivity of the reward system was measured using resting-state functional magnetic resonance imaging (fMRI) with the VTA and VS as regions of interest for resting-state functional connectivity (RSFC) analysis. In addition, clinical anhedonia and reward learning were examined using the Snaith–Hamilton Pleasure Scale (SHAPS) and the probabilistic reward task (PRT) [11], respectively. Consistent with the hypothesis that ezogabine functions to strengthen resilience to stress [12], changes in resilience were measured using the Connor–Davidson Resilience Scale (CD-RISC) [13]. We hypothesized that modulation of the KCNQ potassium channel in humans would parallel the antidepressant effects found in animals, especially within the domain of reward system function, and would additionally normalize the connectivity of brain circuitry involved in depressive symptomatology. In order to assess the specificity of the effect of ezogabine on brain circuitry, we conducted a parallel analysis of changes in VTA and VS RSFC associated with antidepressant response to the glutamate *N*-methyl-D-aspartate receptor antagonist ketamine in a separate group of adult patients with MDD.

Materials and methods

Ezogabine's pharmacology, brain exposure, safety, and tolerability

Ezogabine is a first-in-class KCNQ-selective potassium (K+) channel opener approved by the U.S. FDA for the adjunctive treatment of partial-onset seizures. Ezogabine selectively binds to and activates KCNQ transmembrane K+ ion channels, thereby enhancing transmembrane potassium currents mediated by the KCNQ (Kv7.2–7.5; e.g., KCNQ2/3) family of ion channels. By activating KCNQ channels, ezogabine is thought to stabilize the resting membrane potential and reduce brain excitability, likely leading to the observed anticonvulsant effect. Ezogabine is rapidly absorbed and readily crosses the blood–brain barrier at physiological concentrations and is metabolized via glucuronidation and acetylation with a half-life between 7 and 11 h. The efficacy of ezogabine as adjunctive therapy in partial-onset seizures was established in three multicenter, randomized, double-blind, placebo-controlled studies in 1239 adult patients. The most common adverse effects of ezogabine are dizziness (23%), drowsiness (22%), and fatigue (15%). Skin discoloration around the lips or nail bed, either blue or gray, has been reported and is estimated to affect up to 10% of patients treated for >2 years. Ezogabine carries a black box warning regarding the potential for retinal abnormalities following treatment with ezogabine. Other side effects include urinary retention, neuropsychiatric symptoms (confusional state, psychotic symptoms, and hallucinations), and withdrawal seizures [14–17].

Study participants and design

The current study recruited 18 subjects aged 18–65 years with a primary diagnosis of MDD as assessed by the Mini-International Neuropsychiatric Interview [18]. Additional inclusion criteria were a score of ≥21 on the Montgomery–Asberg Depression Rating Scale (MADRS) [19] and a score of at least 20 on the SHAPS [20], indicating

moderate depression and anhedonia severity, respectively. Individuals were excluded if they had a lifetime history of schizophrenia, bipolar or psychotic disorder, substance use disorder in the preceding 6 months, unstable medical condition, retinal abnormalities, active suicidal or homicidal ideation, or current use of any psychotropic medications, except as specified below. Every subject underwent physical examination, clinical hematological and biochemical screening, urine toxicology testing, and ECG. Study participants were free of concomitant psychotropic medications for at least 2 weeks (4 weeks for fluoxetine) prior to commencement and for the duration of the study, with exceptions being a stable dose of zolpidem 10 mg nightly for sleep or a benzodiazepine for sleep or anxiety (dosage equivalent to lorazepam ≤1 mg daily). As part of the screening procedures and during the course of the study (midway through the treatment with ezogabine and 2 weeks post-cessation of the drug), participants underwent an ophthalmological exam according to the FDA recommendation. In addition, an ECG was completed midway through and at the end of the study in order to monitor for QT interval prolongation, which is reported to occur with the study drug.

All study procedures were conducted at the Icahn School of Medicine at Mount Sinai in New York City. The institutional review board at Icahn School of Medicine at Mount Sinai approved the study, and written informed consent was obtained from all participants prior to any study procedure. Participants were compensated for their time and effort. The study is registered at clinicaltrials.gov (NCT02149836). Following screening, study subjects completed a pretreatment assessment that included the PRT [11] and fMRI scanning (details below). Participants who completed the pretreatment assessment and continued to meet all inclusion/exclusion criteria entered the treatment period. During this phase, ezogabine was titrated following the FDA guidelines until reaching the maximum study protocol-defined target dose of 300 mg three times daily (900 mg/day) at week 4. Subjects were required to tolerate a minimum dose of 600 mg/day in order to continue in the study. At each visit, participants completed self-report and clinician-administered rating scales performed by trained raters and met with a study psychiatrist. The treatment period consisted of eight study visits, which culminated in the primary posttreatment visit, where participants received a second and final PRT assessment and fMRI scan. Following this visit, participants were instructed to taper the study medication over the following 3 weeks based on FDA-recommended guidelines and returned to the clinic for a final study exit visit. The primary clinical outcome of the study was depression severity as measured by the MADRS . Depression was additionally measured using the Quick Inventory of Depressive Symptomatology–Self Report (QIDS-SR) [21] and global illness severity was measured using the Clinical Global Impression-Improvement/Severity (CGI-I/S) [22]. Anhedonia was measured using the SHAPS and the Temporal Experience of Pleasure Scale (TEPS) [23]. Resilience was measured by the CD-RISC [13]. Safety and tolerability were assessed at each study visit by frequency of adverse events (AEs) and suicidal ideation and behavior measured using the Columbia Suicide Severity Rating Scale (C-SSRS) [24]. Medication compliance was calculated via medication reconciliation forms and pill count. AEs were reported according to the Medical Dictionary for Regulatory Activities system [25].

Statistical analysis of clinical data

Demographic data and clinical characteristics were analyzed using summary statistics. Given the small sample size, last observation carried forward was used for intent-to-treat (ITT) analysis. Paired two-sided t tests between pretreatment (week 0) and posttreatment (week 10) were performed on self-report questionnaires and clinician-administrated scales. For instruments with more than two time points available for analysis, repeated-measures analysis of variance (RM-ANOVA) was performed. A $p < 0.05$ was considered statistically significant and Bonferroni correction was utilized for RM-ANOVA analyses. No adjustment was made for multiplicity across clinical measures. The proportion of patients who achieved response and remission criteria was also computed, defined as a 50% reduction in MADRS score at the end of the study compared to pretreatment or a MADRS score <10, respectively.

fMRI data acquisition, processing, and analysis

Imaging data were acquired using a Siemens 3 T Connectome Skyra scanner (Siemens, Erlangen, Germany) with a 32-channel headcoil. A T1-weighted anatomical image was acquired at 0.8 mm isotropic resolution (TR = 2400 ms, TE = 2.07 ms, Flip Angle = 8°). Resting-state fMRI data were acquired as a set of 600 gradient-echo echo-planar images with 70 axial slices (2.1 mm isotropic resolution, no gap, TR = 1000 ms, TE = 35 ms, flip angle = 60°, multiband factor = 7) for 10 min with eyes open. Resting-state fMRI data were processed using a combination of AFNI [26] and FSL [27] tools. Data were despiked, motion corrected, and co-registered to their respective anatomical image. Independent component analysis-based motion denoising was performed with ICA-AROMA [28]. Signal from white matter and cerebrospinal fluid were regressed out [29]. Functional data were normalized to Montreal Neurological Institute (MNI) space and spatially smoothed with a 6 mm full-width-at-half-maximum kernel. Finally,

volumes were band-pass filtered between 0.01 and 0.1 Hz and detrended.

Seed-to-whole-brain RSFC was computed at pretreatment and posttreatment for three regions of interest: VTA, defined functionally [30], and bilateral ventral caudate (vCa) and NAc, defined anatomically by the Harvard–Oxford Atlas distributed with FSL (see Fig. 2d for seeds). Within the reward circuit, we focused on the striatal seeds, in part due to limited signal availability at the level of the VTA [31]. For these analyses, we primarily examined RSFC changes that were associated with clinical improvement [(posttreatment − pretreatment RSFC) × (posttreatment − pretreatment MADRS/SHAPS)]. We secondarily examined (a) RSFC changes from pretreatment to posttreatment; (b) pretreatment RSFC correlations with pretreatment clinical measures; and (c) pretreatment RSFC correlations with changes in clinical measures. These analyses were performed as paired or one-sample two-sided *t* tests with clinical measures as covariates of interest and sex and age as nuisance covariates. For seed-to-whole-brain analyses, cluster-defining thresholds were computed at an alpha level of <0.05 using AFNI's method of permutation testing. For voxelwise $ps < 0.01$, this method adequately controls the false positive rate <5% and addresses the concerns that have been raised with significance testing of fMRI data [32, 33]. We reported findings as significant for a voxelwise $p < 0.005$, for which the minimum significant cluster size was determined to be 137 voxels. For the primary aim, Bonferroni correction was performed for a total of four comparisons (two seeds vs. change in MADRS/SHAPS). Pearson's correlations between the mean RSFC of significant clusters and clinical measures were computed and then converted to corrected *p* values.

In order to assess whether the primary findings are specific to the pharmacology of ezogabine, parallel analyses were performed on a group of 15 adult individuals with MDD who received a single intravenous infusion of ketamine (0.5 mg/kg) in the context of a separate clinical trial (clinicaltrials.gov; NCT01880593). These subjects received pretreatment and posttreatment scans with identical acquisition parameters, preprocessing pipelines, and analytic strategy as the subjects in the ezogabine protocol. Likewise, subjects in the ketamine protocol were free of concomitant antidepressant treatment and in a current major depressive episode (MDE). See Supplemental Material for further detail.

Probabilistic reward task

The PRT is a signal detection test that provides an objective assessment of reward learning [11]. During this computer-based task, subjects are asked to discriminate between two ambiguous stimuli—a short (11.5 mm) vs. long (13 mm) mouth displayed rapidly (100 ms) in a schematic face—in order to receive a monetary reward of 20¢. Unbeknownst to the subjects, correct identification of one stimulus (the "rich stimulus") is reinforced three times more frequently than the other stimulus (the "lean stimulus"). Under these experimental circumstances, healthy subjects reliably develop a response bias for the rich stimulus, regardless of which stimulus was actually presented. Contrarily, subjects with MDD fail to develop this bias for the more frequently reinforced stimulus and tend to respond similarly to both stimuli, reflecting decreased responsiveness to rewards. Discriminability was also calculated as a measure of more general task performance. Response bias and discriminability were computed using the following formulae:

$$\text{Response bias} : \log b = \frac{1}{2}\log\left(\frac{\text{Rich}_{\text{correct}} * \text{Lean}_{\text{incorrect}}}{\text{Rich}_{\text{incorrect}} * \text{Lean}_{\text{correct}}}\right)$$

$$\text{Discriminability} : \log b = \frac{1}{2}\log\left(\frac{\text{Rich}_{\text{correct}} * \text{Lean}_{\text{incorrect}}}{\text{Rich}_{\text{incorrect}} * \text{Lean}_{\text{correct}}}\right)$$

The task consisted of three 100-trial blocks and was programmed in E-Prime (Version 1.1; Psychology Software Tools, Inc., Pittsburgh, PA). To avoid practice effects in repeated-measures designs, two separate versions of the PRT were used and randomly assigned. The parameters were identical except that the target stimuli were discriminated by mouth size or nose size [34].

Prior to data analysis, PRT data underwent a quality-control check wherein trials with below chance accuracy and/or >10% reaction time outliers were excluded from analysis. The measure of interest was change in response bias across the three Blocks of the task, as a function of Time (pretreatment to posttreatment), as analyzed by repeated-measures *Block*×*Time* ANOVA. Change in discriminability was also examined in order to ensure that increases in response bias were not associated with general improvements in task performance [11, 35]. Pearson's correlations between pretreatment response bias (averaged over the latter two blocks of the task, after learning has occurred) and changes in clinical measures were computed to test for associations with treatment response.

Results

Sample characteristics

Of the 26 subjects assessed for eligibility, 18 met all inclusion/exclusion criteria and were enrolled and constituted the ITT sample (age 51.1 ± 9.1 years, 13 males). Subjects were in their current MDE for an average of 5 years and had a median of three lifetime MDEs; 13 had

Table 1 Demographic and clinical characteristics of the sample ($n = 18$)

Demographic characteristics	
Age, mean (SD)	51.1 (9.1)
Male, *n* (%)	13 (72.2)
Race/ethnicity, *n* (%)	
White/Caucasian	6 (33.3)
Black/African American	10 (55.5)
Hispanic/Latino	2 (11.1)
Employment, *n* (%)	
Full-time	2 (11.1)
Part-time	5 (27.8)
Retired	1 (5.6)
Unemployed	10 (55.6)
Educational attainment, *n* (%)	
Not graduated from High School	1 (5.6)
High School	2 (11.1)
Some College (some college+2 year college)	7 (38.9)
College	2 (11.1)
Some Graduate/Professional	2 (11.1)
Graduate/Professional	3 (16.7)
Relationship status, *n* (%)	
Widowed	1 (5.6)
Divorced/separated	5 (27.8)
Single, never married	12 (66.7)
Depression characteristics	
Baseline QIDS-SR, mean (SD)	13.8 (3.2)
Baseline MADRS, mean (SD)	29.5 (4.9)
Number of depressive episodes, mean (SD)	3.1 (2.2)
Age at first depressive episode, mean (SD)	31.2 (16.5)
Current depressive episode	
Age at onset, mean (SD)	45.9 (10.1)
Duration in months, mean (SD)	61.4 (85.2)
Chronic depression, *n* (%)	10 (55.6)
Recurrent depression, *n* (%)	13 (72.2)
Psychiatric characteristics	
Anxiety disorder, *n* (%)	7 (38.9)
PTSD, *n* (%)	2 (11.1)
Past EtOH use disorder, *n* (%)	4 (22.2)

EtOH ethyl alcohol, *MADRS* Montgomery–Åsberg Depression Rating Scale, *PTSD* post-traumatic stress disorder, *QIDS-SR* Quick Inventory of Depressive Symptomatology-Self-Report

experienced recurrent MDD (Table 1). Pretreatment MADRS score ranged from 21 to 38, and pretreatment SHAPS score ranged from 27 to 51. Of the 18 individuals, 17 completed all study visits. One participant elected to discontinue the study after 6 weeks of treatment for unspecified reasons; all other participants completed the study. Across the whole sample, compliance with study medication as measured by pill count was 97%.

Symptom change and tolerability

Depressive symptoms decreased significantly from pretreatment (week 0) to posttreatment (week 10) (MADRS mean change: -13.7 ± 9.6, $t_{17} = -6.01$, $p < 0.001$, Cohen's $d = 2.08$) and throughout the study as a function of time (RM-ANOVA: $F_{3,52} = 21.96$, $p < 0.001$, partial-$\eta^2 = 0.56$; Fig. 1a). Pairwise comparisons showed a significant improvement from week 3 onwards (all ps < 0.001, Bonferroni adjusted), relative to pretreatment. Overall, after 10 weeks of treatment, patients showed a 45% (SD = 28.9%) reduction in MADRS score from pretreatment to posttreatment. Likewise, QIDS-SR score was significantly reduced at the end of the study compared to pretreatment (mean change: -5.72 ± 4.4, $t_{17} = -5.53$, $p < 0.001$, Cohen's $d = 1.64$). Eight out of the 18 (44%) and 5 out of the 18 (28%) patients met response and remission criteria, respectively.

Eleven out of the 18 (61%) patients were classified as "much improved" or "very much improved" according to the CGI-I (Fig. 1b). There was a significant improvement in anhedonia from pretreatment to posttreatment (SHAPS mean change: -6.1 ± 5.3, $t_{17} = 4.8$, $p < 0.01$, Cohen's $d = 1.00$) and throughout the study as a function of time ($F_{5,85} = 11.84$, $p < 0.001$, partial-$\eta^2 = 0.41$), which remained after controlling for depression severity measured by the change in MADRS score calculated without the item related to anhedonia (Item 8: Inability to feel) ($F_{5,80} = 3.03$, $p = 0.015$, partial-$\eta^2 = 0.16$). Finally, study participants showed an improvement in resilience (CD-RISC mean change: 8.2 ± 12.2, $t_{17} = 2.9$, $p < 0.01$; pairwise comparison $F_{3,52} = 3.61$, $p = 0.02$, partial-$\eta^2 = 0.17$, Bonferroni adjusted) (Table 2).

The most common AE was dizziness, which occurred in eight subjects. Less frequent AEs were confusion and headache that were reported in three and two participants, respectively. Owing to the occurrence of AEs, three study participants failed to achieve the highest dose (900 mg/day) and remained at 750 mg/day ($n = 1$) and 600 mg/day ($n = 2$). No subjects discontinued the treatment protocol because of AEs, and no serious AEs (SAEs) occurred during the course of the study. No incidents of retinal abnormalities were observed during the study. A summary of AEs is reported in Table S1. No increase in suicidal ideation as measured by the C-SSRS was reported and no participants experienced emergence of suicidal behavior during the study trial.

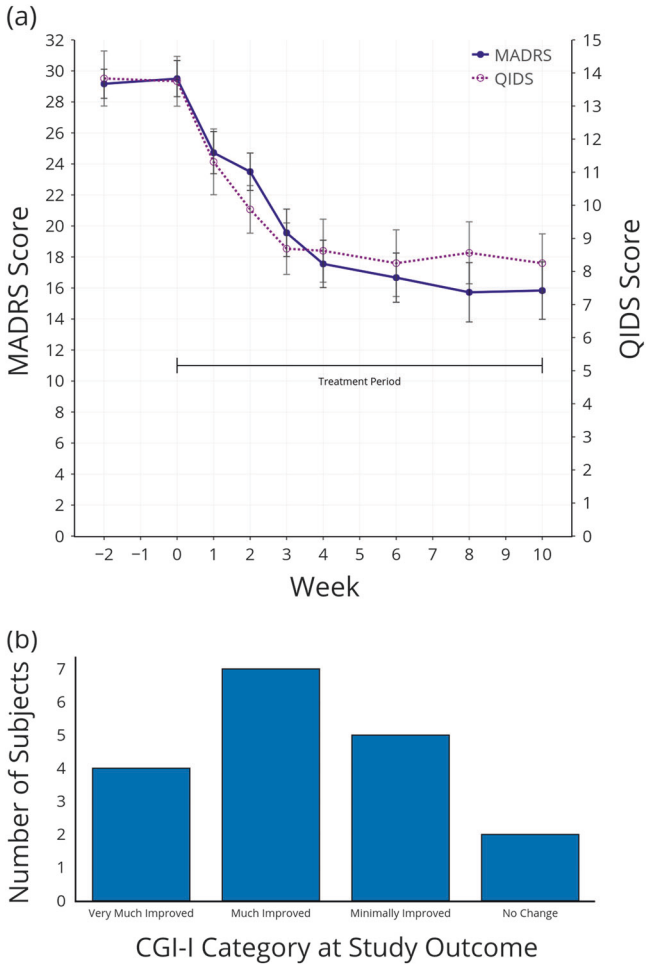

Fig. 1 Change in clinical outcomes in patients with major depressive disorder treated with the KCNQ channel opener ezogabine. **a** Mean MADRS and QIDS-SR score (±SEM) over time during the course of ezogabine treatment. MADRS score decreased significantly from pretreatment (week 0) to posttreatment (week 10) (mean change: -13.7 ± 9.6, $t_{17} = -6.01$, $p < 0.001$, Cohen's $d = 2.08$) and throughout the study as a function of time (RM-ANOVA: $F_{3,52} = 21.96$, $p < 0.001$, partial-$\eta^2 = 0.56$). Likewise, QIDS-SR score was significantly reduced at the end of the study compared to pretreatment (mean change: -5.72 ± 4.4, $t_{17} = -5.53$, $p < 0.001$, Cohen's $d = 1.64$). **b** CGI-I category at study posttreatment. Eleven out of the 18 (61%) patients were classified as "much improved" or "very much improved" according to the CGI-I. CGI-I Clinical Global Impression—Improvement, MADRS Montgomery-Asberg Depression Rating Scale, QIDS-SR Quick Inventory of Depression-Self Report

Table 2 Additional outcome measures ($n = 18$)

Measure	Mean change[a]	Standard deviation	Statistic	p Value	Cohen's d
SHAPS	−6.06	5.34	$t_{17} = -4.81$	<0.001	1.00
TEPS-ANT	5.06	6.49	$t_{17} = 3.03$	0.004	0.50
TEPS-CON	5.28	7.58	$t_{17} = 2.96$	0.009	0.62
CGI-S	−1.56	1.25	$t_{17} = -5.29$	<0.001	1.80
CGI-I	−1.72	0.96	$t_{17} = -7.62$	<0.001	2.54
CD-RISC	8.22	12.16	$t_{17} = 2.87$	0.011	0.49

CD-RISC Connor-Davidson Resilience Scale, *CGI-I/S* Clinical Global Impression—Improvement/Severity, *MADRS* Montgomery–Åsberg Depression Rating Scale, *QIDS* Quick Inventory of Depressive Symptomatology, *SHAPS* Snaith–Hamilton Pleasure Scale, *TEPS-ANT* Temporal Experience of Pleasure Scale—Anticipatory, *TEPS-CON* Temporal Experience of Pleasure Scale—Consummatory

[a]Mean change = end of the study − baseline

Resting-state functional connectivity

Of the 18 subjects enrolled, 16 had a pretreatment fMRI scan, and 14 had both a pretreatment and posttreatment fMRI scan. In our primary analysis, we found that improvement in depressive symptoms (MADRS) from pretreatment to posttreatment was significantly associated with a reduction in connectivity between vCa and clusters within the mid-cingulate cortex (MCC) (peak $z = -4.29$, $k = 189$, corrected $p = 0.008$) and the posterior cingulate cortex (PCC)/precuneus (peak $z = -3.82$, $k = 170$, corrected $p = 0.008$; Fig. 2a). Improved anhedonia (SHAPS) was similarly associated with reduced connectivity between vCa and both MCC (peak $z = -4.87$, $k = 411$, corrected $p = 0.004$) and PCC (peak $z = -3.78$, $k = 182$, corrected $p = 0.13$; Fig. 2b). All results except the association between change in vCa–PCC RSFC and change in SHAPS survive Bonferroni correction. In the ketamine group, there were no significant associations between changes in vCa RSFC and changes in symptoms at the whole-brain level. Furthermore, there were no significant associations between changes in vCa RSFC with the clusters reported above and respective changes in clinical measures (all $ps > 0.4$) (Supplementary Material Figure S2).

In our secondary analyses, we found that greater pretreatment connectivity between vCa and MCC was

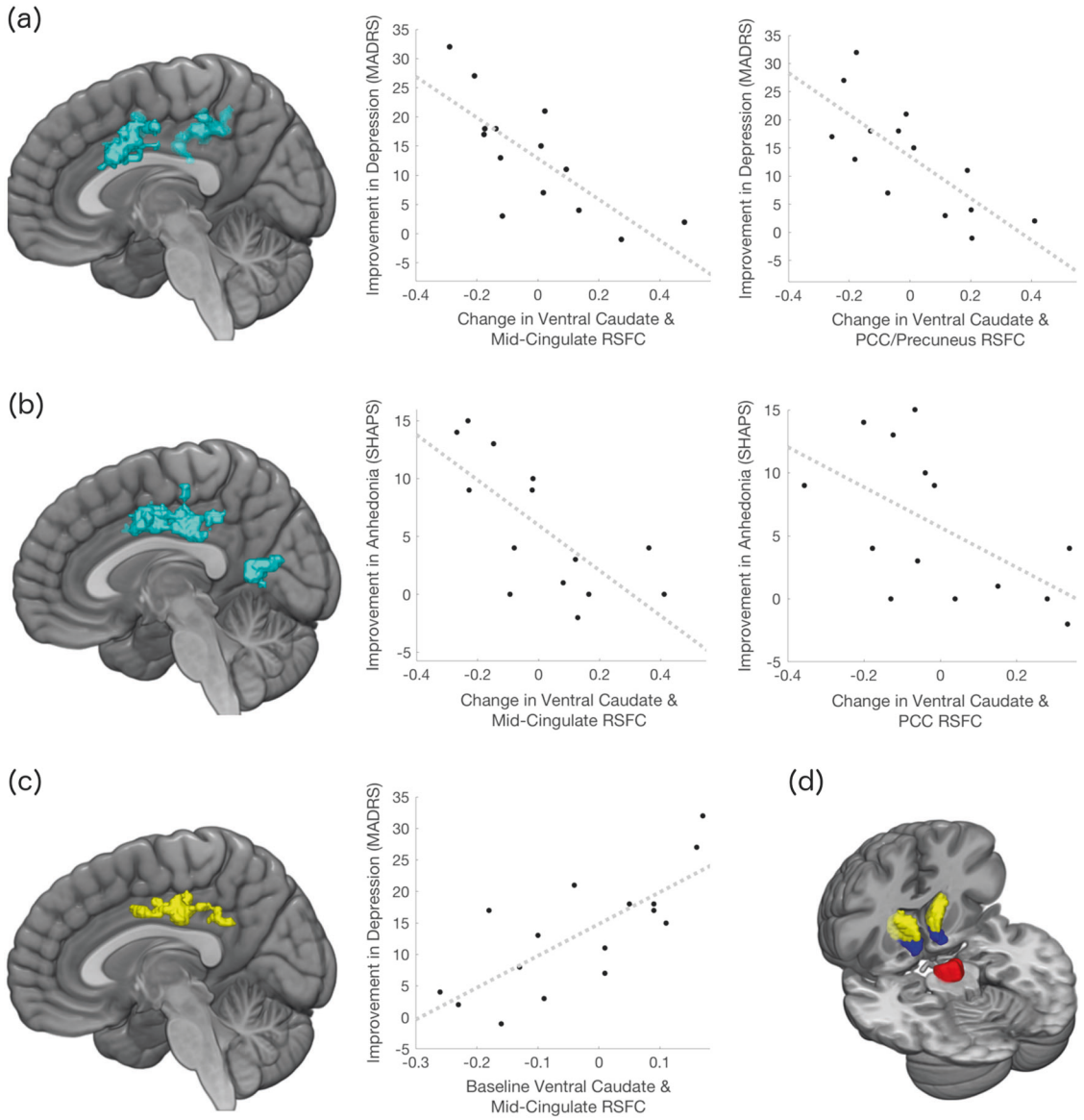

Fig. 2 Functional connectivity of the ventral caudate in patients with major depressive disorder treated with the KCNQ channel opener ezogabine. **a** Clusters where reduction of RSFC with the vCa significantly correlated with reduction in MADRS score. **b** Clusters where reduction in RSFC with the vCa significantly correlated with reduction in SHAPS score. **c** Cluster where increased pretreatment RSFC with the vCa significantly correlated with reduction in MADRS scores. **d** vCa, NAc, and VTA seeds, 3D view. Yellow: vCa, blue: NAc, red: VTA. MADRS Montgomery–Asberg Depression Rating Scale, NAc nucleus accumbens, PCC posterior cingulate cortex, RSFC resting-state functional connectivity, SHAPS Snaith–Hamilton Pleasure Scale, vCa ventral caudate, VTA ventral tegmental area

significantly associated with greater improvement in depressive symptoms from pretreatment to posttreatment (peak $z = 3.79$, $k = 164$; Fig. 2c) (all voxel-wise $p < 0.005$, cluster-wise $\alpha < 0.05$; Fig. 2; MNI coordinates of cluster peaks are reported in Supplementary Material Table S2).

There were no significant associations between NAc or VTA connectivity changes and symptom changes, and there were no significant connectivity changes from pretreatment to posttreatment or associations between pretreatment connectivity and pretreatment clinical measures.

Reward learning

Sixteen subjects completed the PRT at both pretreatment and posttreatment. Of these, 12 had valid pretreatment data and 9 had valid pretreatment and posttreatment data. The *Block*×*Time* ANOVA revealed a main effect of *Block* ($F_{2,16} = 5.38$, $p = 0.02$, $\eta^2 = 0.40$), where across both time points, response bias increased from blocks one to three of the task. A main effect of *Time* also emerged ($F_{1,8} = 6.34$, $p = 0.04$, $\eta^2 = 0.44$), where across blocks, response bias was found to be significantly higher at posttreatment compared to

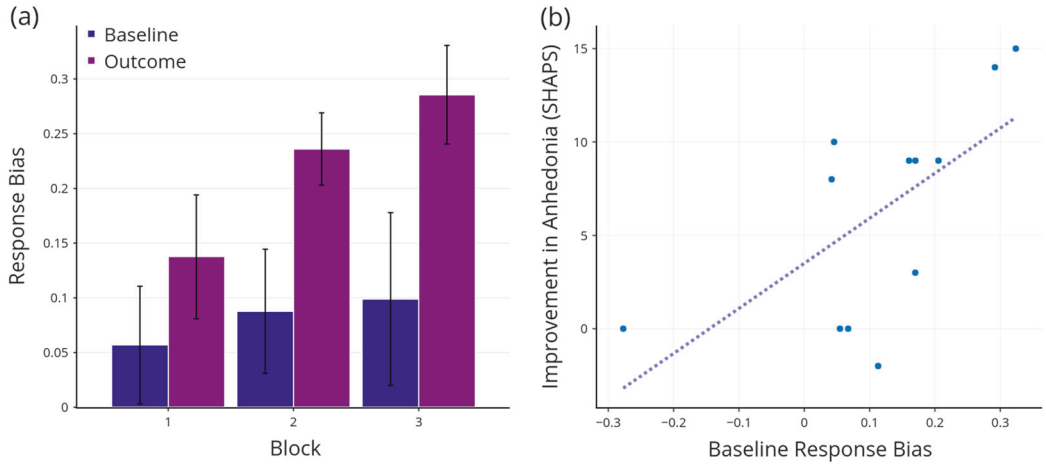

Fig. 3 Change in reward learning and association with anhedonia in patients with major depressive disorder treated with the KCNQ channel opener ezogabine. **a** Mean (±SEM) response bias across the three blocks of the PRT at pretreatment and posttreatment. The main effect of block was significant due to a significant increase in response bias across blocks, indicating that the asymmetrical reinforcement ratio was successful at inducing a behavioral response bias ($F_{2,16} = 5.38$, $p = 0.02$, $\eta^2 = 0.40$). Furthermore, the main effect of time was significant due to a significant increase in overall response bias from pretreatment to posttreatment, indicating that treatment improved the ability to modulate behavior based on prior reinforcement ($F_{1,8} = 6.34$, $p = 0.04$, $\eta^2 = 0.44$). **b** Higher pretreatment response bias averaged across blocks 2 and 3 was associated with greater improvements in anhedonic symptom severity on the SHAPS following treatment ($r = 0.64$, $p = 0.04$) SHAPS, Snaith–Hamilton Pleasure Scale

pretreatment (Fig. 3a), indicating improved reward learning. No significant interaction or main effects emerged from the *Time*×*Block* ANOVA on discriminability (a measure of subject's ability to discriminate between the stimuli that is unrelated to the asymmetrical reinforcement ratio), indicating that increases in response bias from pretreatment to posttreatment were not simply due to general improvements in task performance (all $ps > 0.05$). Finally, higher pretreatment response bias averaged across the latter two blocks of the task (after learning has taken place) was associated with a greater reduction in SHAPS score from pretreatment to posttreatment ($r = 0.64$, $p = 0.02$) (Fig. 3b). This association was not observed with changes in overall depression severity on the MADRS ($r = 0.46$, $p = 0.13$), suggesting that pretreatment response bias was specifically associated with treatment-related improvements in anhedonia but not in depressive symptoms more generally.

Discussion

In the current study, we report that ezogabine, a first-in-class KCNQ channel opener, is associated with improvement in symptoms of depression and anhedonia in patients with MDD in the context of an open-label design. The improvement in anhedonia remained significant after controlling for change in non-anhedonia depressive symptoms, indicating that ezogabine may specifically target the symptom of anhedonia. A significant increase in resilience was observed, consistent with the hypothesis that ezogabine functions to strengthen resilience to stress [12]. Ezogabine was well tolerated in this sample; no SAEs occurred and no subjects discontinued the treatment due to AEs. Improvements in depression and anhedonia were associated with a reduction in functional connectivity between the vCa and both the MCC and the PCC at the whole-brain level, corrected. Finally, subjects showed evidence of increased reward learning following treatment, which may indicate a potential reversal of a reward learning deficit known to be associated with depression [35, 36]. It is important to note that, due to the open-label nature of this study, we are unable to conclude whether these changes are due to the pharmacological effects of ezogabine or to non-specific factors, such as the placebo effect or natural variation in the underlying depression. In a separate group of patients with MDD who were treated with a single infusion of ketamine, we did not observe any changes in vCa connectivity associated with clinical improvement. These findings provide a preliminary indication that ezogabine may indeed be specifically targeting the reward system and subsequent randomized controlled trials examining the effects of ezogabine or other KCNQ channel potentiators on neurobehavioral measures linked to depression are thereby warranted.

The current findings are consistent with the recent preclinical work that provided the theoretical grounds for this study. The KCNQ potassium channel was selected as a pharmacological target for the treatment of depression because it was upregulated in VTA dopamine neurons exclusively in mice resilient to CSDS. Both susceptible and

resilient animals exhibit elevated VTA dopamine neuron excitability after CSDS, but only resilient animals actively engaged gene regulation mechanisms in order to reverse the phenotype [7–10]. This belies the common characterization of resilience as the lack of pathological alteration. Rather, one major feature of resilience may be to actively reverse the pathological effects of stress. Induction of this active resilience mechanism in previously susceptible mice—either via upregulation of the KCNQ channel expression with viral vectors or enhancing existing channels with a KCNQ potentiator such as ezogabine—was capable of reversing depressive symptoms. Our findings are also potentially consistent with preclinical studies that have demonstrated an association between increased brain-derived neurotrophic factor release and synaptogenesis within the VS/NAc and depressive behaviors [37, 38].

Effects of ezogabine appear to relate to modulation of a striatal–mid-cingulate network. Although it has been argued that affective functions are localized to the rostral cingulate, while cognitive and motor functions are localized to the MCC [39, 40], recent observations have implicated the MCC in a diverse array of affective functions including pain, negative affect, and social processing [41, 42]. The MCC is heavily connected with the caudate and the mid-brain dopaminergic system, which are implicated not only in the incentive salience of appetitive but also aversive stimuli [43–45]. Indeed, depressed patients show greater connectivity between the VS and MCC during loss vs. win and disappointment vs. win processing in a reward task [46]. Overall, the MCC seems to act as a hub that integrates affective information with cognitive control and motor centers for the expression of goal-directed behavior [41]. Taken together, our data suggest that ezogabine may function by decoupling between the ventral striatal reinforcement and MCC pain and negative affect systems.

The current study also implicated the PCC and precuneus in the neurobiological effects of ezogabine. These regions are the posterior elements of the default mode network and are thought to support episodic recollection while subjects engage in spontaneous cognition [47]. In a meta-analysis of resting-state fMRI studies in MDD, the anterior precuneus was found to be hyperactive [48]. Additionally, default mode dominance over task positive networks has been associated with a tendency toward maladaptive rumination as opposed to adaptive reflection [49]. Compared to healthy controls, depressed subjects exhibit elevated PCC response to emotional stimuli [50], and similar to the MCC, depressed subjects exhibit increased connectivity between the VS and precuneus during disappointment vs. win processing in a reward task [46]. As with the MCC, we propose that ezogabine may function by decoupling the ventral striatal reinforcement system from the PCC and precuneus, regions associated with maladaptive rumination and dysfunctional reward and emotional processing.

Ezogabine significantly improved reward learning as measured by the PRT. Subjects with MDD and healthy subjects with depressive symptoms show evidence of a more blunted response bias on this paradigm, reflecting a decreased responsiveness to reward. Thus evidence of a significant increase in the strength of this bias from pre-treatment to posttreatment is suggestive of an improvement in reward sensitivity and a greater ability to modulate behavior as a function of reinforcement. Likewise, we observed that a greater response bias at pretreatment was associated with greater improvement in anhedonic symptoms. This finding mirrors those from prior studies showing that greater response bias at pretreatment predicts response to 8 weeks of pharmacotherapy [36].

The current study had several limitations. First, the small sample size, broad age range, and lack of control or placebo group limit conclusions regarding efficacy and generalizability. Although we did find that changes in neurocircuitry were associated with symptom change, there was not a main effect of time. Without a placebo group, we are unable to distinguish between the specific pharmacological effects of ezogabine from non-specific factors. These non-specific factors may include patient expectation or natural variation in the depressive symptoms over time. Thus we are unable to conclude whether similar circuit changes would have been observed in the absence of drug treatment. To partially address the issue of the specificity of the observed effect of ezogabine, we conducted an analysis of changes in vCa circuitry associated with changes in depressive symptoms in a separate study of subjects who received a single infusion of ketamine as a treatment for MDD. This group demonstrated no association between changes in vCa connectivity and improvement in depression. While the inclusion of these results allows for a qualitative comparison to changes observed with ezogabine, caution is warranted in interpreting these results since these analyses do not represent a head-to-head comparison between the two treatments. In particular, subjects in the ketamine group were enrolled on the basis of having failed to respond to ≥2 adequate trials of an antidepressant medication and their course of treatment occurred on a different timescale. Additionally, we did not find any significant associations between VTA connectivity and clinical measures, which may have been due to a combination of our small sample size and the relatively poorer fMRI signal of midbrain structures. As a separate potential limitation, it was unexpected that the vCa rather than the NAc was the mediator of the effects of ezogabine. Reduced signal strength in the region of the NAc may have prevented us from detecting a true effect. However, given that the vCa also receives dopaminergic

innervation from the midbrain, it is possible that the vCa is an important mediator of ezogabine's antidepressant action. The striatum is organized as a series of spirals that proceed from a medial to lateral gradient, in which medial areas subserve limbic functions, central areas subserve cognitive functions, and lateral areas subserve motor functions [51]. The location of the vCa between medial and central areas implicates it in mediating between the limbic and cognitive systems [52].

In conclusion, this is the first study investigating the antidepressant effect of the KCNQ-selective channel potentiator ezogabine in subjects with MDD. Ezogabine was associated with an improvement in depressive and anhedonic symptoms and exhibited good tolerability. Based on the findings of this preliminary study, future randomized controlled trials of ezogabine in depressive disorders are warranted. Understanding ezogabine's mechanism of action in the human brain could lead to additional novel treatments of depression focused on promoting active biological mechanisms of resilience, rather than reversing the pathological changes associated with the syndrome, which has dominated antidepressant drug discovery efforts to date. These resilience-enhancing or "active antidepressant" strategies may open up new avenues of drug discovery for mood disorders.

Acknowledgements We would like to thank the Icahn School of Medicine at Mount Sinai research pharmacists, including Ivy Cohen, Alla Khodzhayeva, and Giuseppe Difiore, for their extensive work on this project.

Funding Funding for this study was provided by the Friedman Brain Institute and by the Ehrenkranz Laboratory for Human Resilience, both components of the Icahn School of Medicine at Mount Sinai. Additional research support was provided by Doris Duke Charitable Foundation (to JWM) and the National Institute of Mental Health (MH112081, to M-HH; K23MH094707, to JWM).

Compliance with ethical standards

Conflict of interest In the past 5 years, JWM has provided consultation services to Sage Therapeutics, Boehreinger Ingelheim, Novartis, Allergan, Fortress Biotech, Janssen Research and Development, Genentech, MedAvante-ProPhase, and Global Medical Education (GME) and has received research support from Avanir Pharmaceuticals, Inc. JWM is named on a patent pending for neuropeptide Y as a treatment for mood and anxiety disorders. The Icahn School of Medicine (employer of JWM) is named on a patent and has entered into a licensing agreement and will receive payments related to the use of ketamine if it is approved for the treatment of depression. JWM is not named on this patent and will not receive any payments. KCA has received consulting fees from MedAvante-ProPhase for services unrelated to this study. In the past 3 years, DAP has received consulting fees from Akili Interactive Labs, BlackThorn Therapeutics, Boehreinger Ingelheim, Pfizer, and Posit Science for activities unrelated to the present study. In the past 3 years, DVI has provided consultations to Alkermes, Axsome, MyndAnalytics (CNS Response), Jazz, Lundbeck, Otsuka, and Sunovion and has received research support (through his academic institutions) from Alkermes, Astra Zeneca, Brainsway, LiteCure, Neosync, Roche, and Shire. The other authors declare that they have no conflict of interest.

References

1. Ferrari AJ, Charlson FJ, Norman RE, Patten SB, Freedman G, Murray CJL, et al. Burden of depressive disorders by country, sex, age, and year: findings from the Global Burden of Disease Study 2010. PLoS Med. 2013;10:e1001547.
2. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, et al. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. Am J Psychiatry. 2006;163:1905–17.
3. Tollefson GD, Holman S. How long to onset of antidepressant action: a meta-analysis of patients treated with fluoxetine or placebo. Int Clin Psychopharmacol. 1994;9:245–50.
4. Ferguson JM. SSRI antidepressant medications: adverse effects and tolerability. Prim Care Companion J Clin Psychiatry. 2001;3:22–7.
5. Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci. 2006;7:137–51.
6. Millan MJ, Goodwin GM, Meyer-Lindenberg A, Ove Ogren S. Learning from the past and looking to the future: emerging perspectives for improving the treatment of psychiatric disorders. Eur Neuropsychopharmacol. 2015;25:599–656.
7. Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell. 2007;131:391–404.
8. Friedman AK, Juarez B, Ku SM, Zhang H, Calizo RC, Walsh JJ. et al. KCNQ channel openers reverse depressive symptoms via an active resilience mechanism. Nat Commun. 2016;7:11671
9. Chaudhury D, Walsh JJ, Friedman AK, Juarez B, Ku SM, Koo JW, et al. Rapid regulation of depression-related behaviours by control of midbrain dopamine neurons. Nature. 2013;493:532–6.
10. Friedman AK, Walsh JJ, Juarez B, Ku SM, Chaudhury D, Wang J, et al. Enhancing depression mechanisms in midbrain dopamine neurons achieves homeostatic resilience. Science. 2014;344:313–9.
11. Pizzagalli DA, Jahn AL, O'Shea JP. Toward an objective characterization of an anhedonic phenotype: a signal-detection approach. Biol Psychiatry. 2005;57:319–27.
12. Han MH, Nestler EJ. Neural substrates of depression and resilience. Neurotherapeutics. 2017;14:677–86.
13. Connor KM, Davidson JRT. Development of a new resilience scale: the Connor-Davidson Resilience scale (CD-RISC). Depress Anxiety. 2003;18:76–82.
14. Gunthorpe MJ, Large CH, Sankar R. The mechanism of action of retigabine (ezogabine), a first-in-class K+ channel opener for the treatment of epilepsy. Epilepsia. 2012;53:412–24.
15. Brodie MJ, Lerche H, Gil-Nagel A, Elger C, Hall S, Shin P, et al. Efficacy and safety of adjunctive ezogabine (retigabine) in refractory partial epilepsy. Neurology. 2010;75:1817–24.
16. French JA, Abou-Khalil BW, Leroy RF, Yacubian EM, Shin P, Hall S. et al. Randomized, double-blind, placebo-controlled trial of ezogabine (retigabine) in partial epilepsy. Neurology. 2011;76:1555–63.
17. Stafstrom CE, Grippon S, Kirkpatrick P. Ezogabine (retigabine). Nat Rev Drug Discov. 2011;10:729–30.
18. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured

diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 1998;59(SUPPL. 20):22–33.
19. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. Br J Psychiatry. 1979;134:382–9.
20. Snaith RP, Hamilton M, Morley S, Humayan A, Hargreaves D, Trigwell P. A scale for the assessment of hedonic tone. The Snaith-Hamilton Pleasure Scale. Br J Psychiatry. 1995;167:99–103.
21. Rush AJ, Trivedi MH, Ibrahim HM, Carmody TJ, Arnow B, Klein DN, et al. The 16-item Quick Inventory of Depressive Symptomatology (QIDS), clinician rating (QIDS-C), and self-report (QIDS-SR): a psychometric evaluation in patients with chronic major depression. Biol Psychiatry. 2003;54:573–83.
22. Busner J, Targum SD. The clinical global impressions scale: applying a research tool in clinical practice. Psychiatry. 2007;4:28–37.
23. Gard DE, Gard MG, Kring AM, John OP. Anticipatory and consummatory components of the experience of pleasure: a scale development study. J Res Pers. 2006;40:1086–102.
24. Posner K, Brown GK, Stanley B, Brent DA, Yershova KV, Oquendo MA, et al. The Columbia-suicide severity rating scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. Am J Psychiatry. 2011;168:1266–77.
25. Brown E, Wood L, Wood S. MedDRA - the medical dictionary for regulatory activities. Drug Saf. 2008;20:109–17.
26. Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res. 1996;29:162–73.
27. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TEJ, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage. 2004;23(SUPPL. 1):208–19.
28. Pruim RHR, Mennes M, van Rooij D, Llera A, Buitelaar JK, Beckmann CF. ICA-AROMA: a robust ICA-based strategy for removing motion artifacts from fMRI data. Neuroimage. 2015;112:267–77.
29. Jo HJ, Saad ZS, Simmons WK, Milbury LA, Cox RW. Mapping sources of correlation in resting state FMRI, with artifact detection and removal. Neuroimage. 2010;52:571–82.
30. Murty VP, Shermohammed M, Smith DV, Carter RMK, Huettel SA, Adcock RA. Resting state networks distinguish human ventral tegmental area from substantia nigra. Neuroimage. 2014;100:580–9.
31. Barry RL, Coaster M, Rogers BP, Newton AT, Moore J, Anderson AW, et al. On the origins of signal variance in FMRI of the human midbrain at high field. PLoS ONE. 2013;8:1–14.
32. Eklund A, Nichols TE, Knutsson H. Cluster failure: why fMRI inferences for spatial extent have inflated false-positive rates. Proc Natl Acad Sci. 2016;113:7900–5.
33. Cox RW, Chen G, Glen DR, Reynolds RC, Taylor PA. FMRI clustering and false positive rates. Proc Natl Acad Sci USA. 2017;114:E3370–1.
34. Whitton AE, Kakani P, Foti D, Van'T Veer A, Haile A, Crowley DJ, et al. Blunted neural responses to reward in remitted major depression: a high-density event-related potential study. Biol Psychiatry Cogn Neurosci Neuroimaging. 2016;1:87–95.
35. Pizzagalli DA, Iosifescu D, Hallett LA, Ratner KG, Fava M. Reduced hedonic capacity in major depressive disorder: evidence from a probabilistic reward task. J Psychiatr Res. 2008;43:76–87.
36. Vrieze E, Pizzagalli DA, Demyttenaere K, Hompes T, Sienaert P, De Boer P, et al. Reduced reward learning predicts outcome in major depressive disorder. Biol Psychiatry. 2013;73:639–45.
37. Berton O, McClung CA, DiLeone RJ, Krishnan V, Renthal W, Russo SJ, et al. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science. 2006;311:864–8.
38. Berton O, Mcclung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, et al. Essential role of BDNF in the social defeat. Stress. 2008;864:864–9.
39. Devinsky O, Morrell MJ, Vogt BA. Review article: Contributions of anterior cingulate cortex to behaviour. Brain. 1995;118:279–306.
40. Bush G, Luu P, Posner MI. Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn Sci. 2000;4:215–22.
41. Shackman AJ, Salomons TV, Slagter HA, Fox AS, Winter JJ, Davidson RJ. The integration of negative affect, pain and cognitive control in the cingulate cortex. Nat Rev Neurosci. 2011;12:154–67.
42. Apps MAJ, Rushworth MFS, Chang SWC. The anterior cingulate gyrus and social cognition: tracking the motivation of others. Neuron. 2016;90:692–707.
43. Jensen J, McIntosh AR, Crawley AP, Mikulis DJ, Remington G, Kapur S. Direct activation of the ventral striatum in anticipation of aversive stimuli. Neuron. 2003;40:1251–7.
44. Delgado MR, Li J, Schiller D, Phelps EA. The role of the striatum in aversive learning and aversive prediction errors. Philos Trans R Soc B Biol Sci. 2008;363:3787–800.
45. Brooks AM, Berns GS. Aversive stimuli and loss in the mesocorticolimbic dopamine system. Trends Cogn Sci. 2013;17:281–6.
46. Quevedo K, Ng R, Scott H, Kodavaganti S, Smyda G, Diwadkar V, et al. Ventral striatum functional connectivity during rewards and losses and symptomatology in depressed patients. Biol Psychol. 2017;123:62–73.
47. Raichle ME. The brain's default mode network. Annu Rev Neurosci. 2015;38:433–47.
48. Zhong X, Pu W, Yao S. Functional alterations of fronto-limbic circuit and default mode network systems in first-episode, drug-naïve patients with major depressive disorder: a meta-analysis of resting-state fMRI data. J Affect Disord. 2016;206:280–6.
49. Hamilton JP, Furman DJ, Chang C, Thomason ME, Dennis E, Gotlib IH. Default-mode and task-positive network activity in major depressive disorder: implications for adaptive and maladaptive rumination. Biol Psychiatry. 2011;70:327–33.
50. Ho TC, Connolly CG, Henje Blom E, LeWinn KZ, Strigo IA, Paulus MP, et al. Emotion-dependent functional connectivity of the default mode network in adolescent depression. Biol Psychiatry. 2015;78:635–46.
51. Haber SN, Fudge JL, McFarland NR. Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J Neurosci. 2000;20:2369–82.
52. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. Neuropsychopharmacology. 2010;35:4–26.