Pattern Separation, BDNF, and the Mechanisms of Vortioxetine

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Background and rationale

The pathophysiology of Major depressive disorder (MDD) is likely to involve multiple brain regions including the hippocampus, nucleus accumbens, prefrontal cortex and hypothalamus (Krishnan and Nestler, 2010). A fundamental challenge in developing new treatments is identifying circuit-specific contributions to pathophysiology of MDD and elucidating the circuit-based mechanisms underlying actions of novel antidepressants. The identification of circuit-based endophenotypes that are objectively quantified and non-invasively imaged will permit stratification of risk for disease, prediction and monitoring of treatment responses using circuit-based readouts as biomarkers and promote innovation of new antidepressants (Kheirbek et al., 2012).
Antidepressants influence neural circuits in multiple ways including stimulation of adult neurogenesis in the dentate gyrus (DG)-CA3 circuit of the hippocampus in rodents and humans (Malberg et al., 2000; Boldrini et al., 2009; Boldrini et al., 2012; Boldrini et al., 2013). Blocking adult hippocampal neurogenesis in rodents abrogates some of the behavioral effects of antidepressants (Santarelli et al., 2003) (Li et al., 2008; Walker et al., 2014). Importantly, we have recently found that in a rodent model of depression, enhancing adult hippocampal neurogenesis is sufficient to confer resilience against chronic stress (Hill et al, submitted). Since adult hippocampal neurogenesis generates new neurons in the DG throughout life in humans (Spalding et al., 2013), it may represent a mechanism by which antidepressants exert their therapeutic effects. **Understanding how adult neurogenesis affects DG-CA3 structure and functions is critical to defining the actions of antidepressants.**

Work by us and others has demonstrated that adult hippocampal neurogenesis plays a critical role in fundamental encoding functions of the DG-CA3 circuit such as pattern separation, a mnemonic process by which similar inputs are made more distinct (Clelland et al., 2009; Sahay et al., 2011b; Sahay et al., 2011a; Nakashiba et al., 2012; Niibori et al., 2012). Blockade of adult hippocampal neurogenesis impairs pattern separation, whereas enhancement of adult hippocampal neurogenesis is sufficient to improve pattern separation. Pattern separation is essential for optimal encoding of contextual information so as to ensure that new experiences are stored without overlapping or interfering with those previously encoded. Pattern separation also serves to constrain pattern completion, a process by which full memories are retrieved based on partial cues. Although this central function of adult hippocampal neurogenesis in pattern separation (minimization of interference) is critical for formation of new episodic memories, it may also play a critical role in regulation of affect. This is possible in several different ways. First, optimal encoding of emotional cues gates adaptive activation of fear and stress circuits. Decreased levels of neurogenesis may result in inappropriate encoding of fearful stimuli and the excessive activation of hypothalamic circuits and production of stress hormones such as glucocorticoids.

Second, impaired pattern separation of cues in the environment may underlie the negative cognitive bias seen in MDD by increased pattern completion of strongly encoded aversive memories. Alternatively, negative response bias may arise from increased pattern separation of cues with a negative valence that can lead to excessive deliberation on aversive outcomes. Third, impaired pattern separation may affect processing of cue-contingency relationships and abnormal perception of salience and reward resulting in increased anxiety and anhedonia. Recent studies in rodents suggest that genetic blockade of adult hippocampal neurogenesis increases anhedonia (Snyder et al., 2011; Walker et al., 2014). **Thus, enhancing adult hippocampal neurogenesis through augmentation of pattern separation may reverse abnormal activation of emotional circuits in the amygdala, hypothalamus, prefrontal cortex and nucleus accumbens to modulate expression of fear and stress reactivity, executive functions and anhedonia** (**Figure 1**).

Human imaging studies have suggested that the DG-CA3 circuit's functions in pattern separation are conserved between mice and men (Yassa and Stark, 2011). Using fMRI in combination with an incidental encoding task in which subjects are presented a series of objects of varying similarity, several groups have found that the DG-CA3 circuit is most likely to be engaged when object similarity is high (Yassa and Stark, 2011). Thus, the DG may perform input-output transformations similar to that seen in rodents (Leutgeb et al., 2007). Interestingly, using a behavioral proxy of the pattern separation imaging based task, preliminary studies suggest that pattern separation of neutral, but not negative items, is impaired in individuals with depressive symptoms (Dery et al., 2013; Shelton and Kirwan, 2013). In contrast, discrimination of pattern separation-completion balance may represent a circuit-based endophenotype in MDD. However, despite a proposed role for pattern separation in antidepressant action (Sahay and Hen, 2007), *it is not known whether antidepressants enhance pattern separation in individuals with MDD*. Addressing this fundamental gap in our understanding of antidepressant action will enable stratification of risk for MDD, prediction and monitoring of treatment responses using pattern separation as a biomarker and promote development of new antidepressants.

Antidepressants may influence pattern separation in DG-CA3 through direct stimulation of adult hippocampal neurogenesis and indirectly via regulation of growth factors such as brain derived neurotrophic factor or BDNF (Waterhouse et al., 2012).

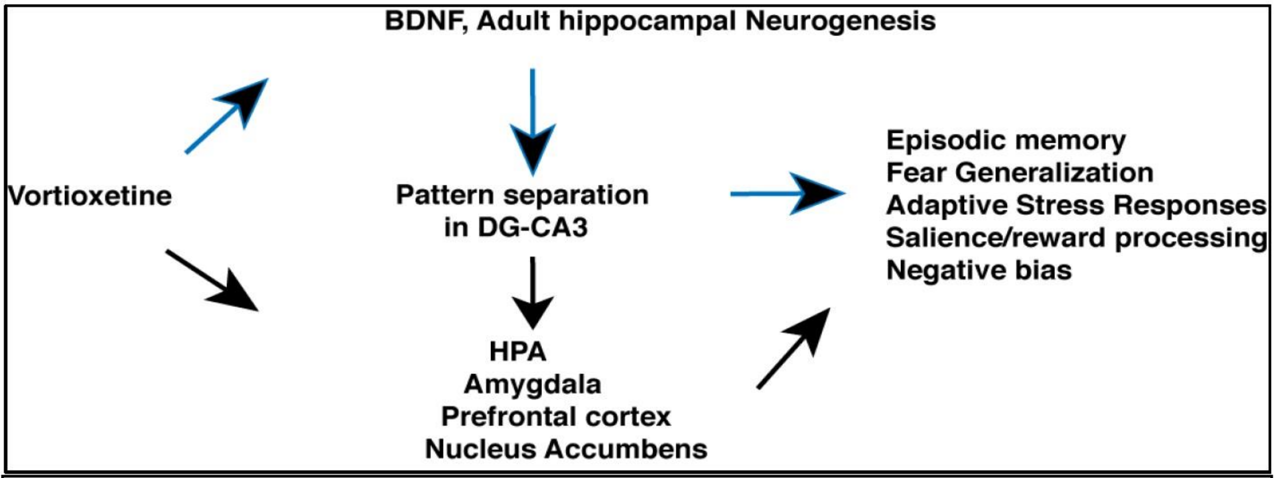

Figure 1. Schema outlining potential mechanisms underlying vortioxetine's effects on cognition and mood. We hypothesize that vortioxetine enhances pattern separation in DG-CA3 through increasing BDNF and adult hippocampal neurogenesis to produce cognitive benefit and modulation of mood (Blue arrows). Alternatively, vortioxetine may produce its effects on mood independently of pattern separation in DG-CA3 circuit (Black arrows).

Interestingly, BDNF levels are upregulated in the DG during encoding and contribute to pattern separation (Bekinschtein et al., 2013). In addition to enhancing the survival of adult-born neurons, BDNF stimulates synaptogenesis and dendritic growth (Duman and Monteggia, 2006; Sen et al., 2008; Waterhouse et al., 2012). Thus, at a structural level the increased integration of new neurons into the DG-CA3 circuit is accompanied changes in grey matter. How changes in DG-CA3 structural properties link relate to change in pattern separation is poorly understood. Furthermore, the impact of antidepressants on grey matter in DG-CA3 has been difficult to assess owing to a lack of studies employing high resolution structural imaging.

Here, we propose to bridge these important gaps in our understanding of vortioxetine's effects on cognition and mood by interrogating the impact of vortioxetine on pattern separation. In addition, we will examine changes in peripheral BDNF levels. **Together, these studies should generate unprecedented insights into mechanisms of action of vortioxetine and establish pattern separation and peripheral BDNF levels as biomarkers for antidepressant response.**

The central hypotheses are:

1. MDD participants at baseline will show an imbalance in pattern separation-completion that is reflected in behavioral tasks. Subjects' pattern separation performance will statistically significantly improve with vortioxetine.
2. MDD participants will have a statistically significant improvement in cognitive measures that will be mediated by changes in blood BDNF levels, and pattern

separation-completion tasks.

Study Objectives

We will implement a high throughout behavioral pattern separation task to probe the effects of vortioxetine on pattern separation in individuals with MDD. This study will measure pattern separation in patients with MDD pre and post treatment.

Specific Aims.

Aim 1. To assess if vortioxetine improves pattern separation in patients with MDD.

Hypothesis: MDD participants at baseline will show an imbalance in pattern separation- completion that is reflected in behavioral tasks. Subjects' pattern separation performance will statistically significantly improve with vortioxetine

Aim 2. To determine how vortioxetine increases cognitive functioning.

Hypothesis: MDD participants will have a statistically significant improvement in cognitive measures that will be mediated by changes in blood BDNF levels, and behavioral pattern separation-completion

Inclusion and Exclusion Criteria

***Inclusion criteria*.**

1. Meets criteria for DSM-IV and V Major Depressive Disorder.
2. QIDS-C ≥ 16
3. Men and women Age > 18 and < 65.

Exclusion criteria.

1. The following DSM-IV diagnoses (any current or past history, except substance abuse disorders):
    a. bipolar disorder
    b. schizophrenia
    c. schizoaffective disorder
    d. obsessive compulsive disorder
    e. organic mental disorder
    f. substance use disorders, including alcohol, active within the last 6 months

g. delusional disorder
h. psychotic disorder not elsewhere classified
i. patients with mood congruent or mood incongruent psychotic features
2. Unable to follow instructions or otherwise unable to participate in the trial.
3. Pregnant women or women of child bearing potential who are not using a medically accepted means of contraception (defined as oral contraceptive pill or implant, condom, diaphragm, spermicide, IUD, s/p tubal ligation, partner with vasectomy)
4. Patients who, in the investigator's judgment, pose a current, serious suicidal or homicidal risk. These individuals will be referred immediately to appropriate clinical treatment
5. Serious or unstable medical illness including cardiovascular, hepatic, renal, respiratory, endocrine, neurologic, any history of seizure disorder or hematologic disease.
6. History of multiple adverse drug reactions or allergy to the study drugs.
7. Current use of other psychotropic drugs except benzodiazepines.
8. Clinical or laboratory evidence of hypothyroidism.
9. Patients who have had at least two failed trials of an antidepressant during their current major depressive episode.
10. Patients who have ever had electroconvulsive therapy (ECT)
11. Concomitant use of serotonergic agents
12. Chronic MDD – current depressive episode lasting longer than 2 years

Study Design and Schedule of Procedures

Behavioral Pattern Separation Task.

We will implement a rapid, high throughput behavioral task that captures the input-output transformation function characteristic of pattern separation processes (Stark et al., 2013). In this task, participants are shown a series of every-day objects as described above and are asked to identify the objects as indoors or outdoors. Immediately following encoding of this task, the subjects are asked to call the objects as "old" if they have seen the objects before in the task, "new" or "similar". As previously done by Stark and colleagues (Stark et al., 2013), a third of the objects in the testing phase are "old", "similar" and "new". Identifying a "similar' object correctly conveys pattern separation, whereas, incorrectly identifying it as "old" conveys pattern completion. By plotting responses as a function of object similarity, we can generate an input-output transfer curve. This task will serve the purpose of rapid assessment of putative changes in pattern separation-like mechanisms.

Study Design.

We propose a proof-of-concept 6-week open trial of vortioxetine for 20 patients with MDD. Each participant will use 10-20mg of vortioxetine daily. Because Abrupt discontinuation of this drug can lead to adverse side effects, the subjects will be encouraged to consult a primary care physician, or a study clinician in follow-up care to discontinue the vortioxetine safely; if the subject chooses to discontinue the vortioxetine after the study drug phase, the study clinicians will follow the vortioxetine's suggested titration guidelines.

The rationale for an open trial is that we are particularly interested in the heterogeneity of effect of vortioxetine on the biological and behavioral parameters, especially comparing responders to nonresponders. The open trial will also facilitate recruitment and completion.

Screening measures will include a diagnostic assessment and psychiatric history (MINI), depressive symptoms (QIDS), physical exam, vital signs, height, weight, urine toxicology, urine pregnancy (for females), and screening labs (11.5mL of blood will be drawn). Baseline assessment measures will include an assessment of adverse events, the QIDS, DSST, RAVLT learning and memory, pattern separation tasks and assessment of blood levels of BDNF (8mL of blood will be drawn). Patients will be assessed at weeks 2, 4, and 6 with the QIDS and will be asked if they have experienced any adverse events. Cognitive testing (DSST and RAVLT), and pattern separation tasks and assessment of blood levels of BDNF (8mL of blood will be drawn) will be done again at week 6 (see Table 1: Study Schedule of Assessments).

Primary endpoints.

1. Pattern separation tasks
2. Blood levels of BDNF
3. QIDS
4. DSST, RAVLT learning and memory

Statistical Analysis Plan

Target sample size is 20 on the basis of detecting a moderate effect size in all measures. Univariate comparisons for primary and secondary endpoints will be done with a paired T-test. Pearson correlation coefficients will be used to assess dyadic relationships. Logistic regression will be used to understand the relationship between differences in cognitive tests as the dependent variable with changes in BDNF, pattern separation tasks, QIDS, and cognitive tests.

Data Management and Analysis

Data Confidentiality and Security. Subjects will be identified by alphanumeric codes only; consistent with HIPPA procedures, names, initials and other possible identifiers will not be included in the electronic database. Hard copies of data that could identify subjects will be stored in locked file cabinets with restricted access; all subject-level data will be password protected.

Back-up and Storage. An archival record of all data that has passed through the above- noted quality checks will be maintained on a data entry hard disk and on a network drive and, for archival data for the study as a whole, by the database manager. Redundant copies of the CD-ROM archive disks will be stored in a separate offsite cabinet to ensure the survival of the data in case of fire or other disaster. Full network and CD ROM backups will be made weekly and monthly, respectively.

Data Access for Investigators. The database manager and, when necessary, Dr. Nierenberg will participate in all conference calls in which data management and analysis are discussed. Masking treatment assignment, the data base manager will generate biannual reports for the investigative team covering recruitment, subject progress, data flow, treatment adherence and retention, acute and follow-up outcomes and adverse events.

General Considerations for Data Analysis. We will perform descriptive statistics prior to testing our hypotheses. These statistics will include means (or frequencies and proportions for categorical variables), standard deviations, skewness and kurtosis, and odds-ratios. Variables with distributions violating assumptions of normality will be transformed. If this fails to improve the normality of the distribution, distribution-free statistics will be employed.

The study has baseline and post-treatment creating clustered data due to repeated measures. Thus, we will use established methods for such study designs to examine treatment differences. The two most popular approaches for longitudinal data modeling are the generalized estimating equations (GEE) and linear mixed-effects model approach (LMM). Both approaches accommodate missing data. While LMM explicitly models between- and within-subject variation using random effects, GEE ignores between-subject variability by treating subjects as independent units and basing model estimation and inference on the marginal distribution of the response of such units. Although LMM has the advantage of being able to separate between-subject variability from the total variability of responses, it requires parametric assumptions on observed and latent variables, rendering inference vulnerable to departures from normal distribution. The GEE, although incapable of modeling between-subject variation, can provide robust estimates. Although LMM will be the main analytic tool, particularly for estimating between-subject variability, we will also use GEE to assess the robustness of the population parameters such as treatment effects. All analyses will be conducted using the latest version of SAS, which includes procedures for LMM and GEE. We will apply these models for both the intent-to-treat and completer analyses. Inference based on GEE or LMM is valid provided that missing data are non-informative or “missingness” does not depend on the value of the unobserved outcome. Although missing data in many studies in mental health research are non-informative, we will perform sensitivity analyses to ensure that this is the case. In particular, we will entertain both parametric and semi-parametric models that assume some missingness mechanism and compare estimates obtained from such models with those from GEE and LMM. Such analyses will inform us whether the non-informative missingness assumption is violated and if so, to what extent. If the non-informative missingness assumption is deemed to be severely violated, we will report treatment effects using the methods that account for informative missingness.

Power Analysis and Sample Size Determination. The following sample size calculations pertain to the expected changes related the psychophysiological indices to be obtained. The sample size selected for this proposal is based on detection of between-group significant differences at an alpha of .05 and on published analysis of pattern separation-completion balance (Bakker et al., 2012).

Table 1: Schedule of Assessments

Week **Visit**	**Screening** V1	**Baseline** V2	**Week 2** V3	**Week 4** V4	**Week 6** V5
Procedure					
Consent	X				
Inclusion/Exclusion	X				
Adverse events		X	X	X	X
MINI	X				
QIDS-C	X	X	X	X	X
Physical Exam	X				
Concomitant Medications	X	X	X	X	X
Progress Note	X	X	X	X	X
Vitals	X				
Height & weight	X				
Urine tests: toxicology	X				
Urine tests: pregnancy	X				
Blood tests: Screening Labs	X				
Blood tests: BDNF		X			X
Cognitive tests: DSST		X			X
Cognitive tests: RAVLT		X			X
Pattern separation tests		X			X

* Baseline visits will occur within 4 days to 10 days of the screening visits.

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