Iowa Initiative for Artificial Intelligence

Final Report

Project title:	Molecular Antidepressant Signatures from Single Cell Transcriptomics in Human Induced Pluripotent Stem Cell-derived Human Cortical Spheroids
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Were specific aims fulfilled:	No
Readiness for extramural proposal?	No
If yes … Planned submission date	
Funding agency	
Grant mechanism	
If no … Why not? What went wrong?	We were not able to complete the single cell RNA-seq experimentation within the designated time frame due to the COVID-19 research ramp down and subsequent staffing limitations, i.e. a postdoctoral fellow tasked with working on this project left the laboratory in February 2021.

Brief summary of accomplished results:

The preponderance of data presented below is gene expression data for the NPC line, a fibroblast-derived human induced pluripotent stem cell (hiPSC) line induced along a neural lineage but not terminally differentiated. We culled and analyzed L1000 expression data for the following antidepressants (all 10 μM, 24 hour exposures) within the given classes: tricyclic antidepressants (TCAs) (9) – amitriptyline, amoxapine, clomipramine, desipramine, dibenzepin, doxepin (3 datasets), maprotiline, nortriptyline, and trimipramine; selective serotonin reuptake inhibitors (SSRIs) (5) – escitalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline; serotonin norepinephrine reuptake inhibitors (SNRIs) (2) – duloxetine and venlafaxine; and monoamine oxidase inhibitors (MAOIs) (2) – bifemelane and isocarboxazid. 3 genes were upregulated (*HLA-DMA*, *PAK6*, and *PIP42K*), and 3 genes were downregulated (*CRK*, *GJA1*, and *NUP86*) by ≥5/9 TCAs (including multiple representations in the 3 doxepin datasets). 10 genes were upregulated (including *HLA-DMA*), and 11 genes were down-regulated by ≥3/5 available SSRIs. For the NEU line, a terminally differentiated neuronal cell line, 2 genes were upregulated (*GRB10* and *TSPAN8*), and 5 genes were downregulated (*CA2*, *CD40*, *EPHA3*, *PEL1*, and *RFC5*) by both fluoxetine and fluvoxamine. Only one gene was upregulated (*TSTA3*) and downregulated (*SSBP2*) by the SNRIs duloxetine and venlafaxine. For the NPC line, only one gene was upregulated (*PRKX*) and downregulated (*IGFBP*) by the MAOIs bifemelane and isocarboxazid. On the other hand, in the NEU line, 9 and 8 genes were up- and downregulated, respectively, by both MAOIs.

Research report:

Aims (provided by PI):

Synaptic plasticity in key affective brain regions, *e.g.* the prefrontal cortex and hippocampus, is causally linked to the efficacy of antidepressant medications, neuromodulatory therapies and evidence-based psychotherapies in major depression. Changes in gene expression are necessary for synaptic plasticity, *e.g.* increased neocortical and hippopcampal expression of brain-derived neurotrophic factor (BDNF). Global gene expression changes in response to antidepressants have been studied in model organisms and peripheral human tissue but not in the human brain. Moreover, antidepressant-induced gene expression changes are likely cell-type specific with both spatial and temporal components. As a result, there is a critical need to study transcriptional effects in human brain tissue on the single cell-level in response to antidepressant medications, both classical monoaminergic and novel, rapid-acting agents.

Specific Aim 1#: The Development of Antidepressant Signature(s) ("Score") Based on Available Transcriptomic Data. In collaboration with the IAII, we will query existing databases, e.g. LINCS Data Portal (http://lincsportal.ccs.miami.edu/datasets/), for human transcriptomic data from known antidepressants at physiologically-relevant doses. In this framework, the input variables (X) are antidepressant medications ("perturbagens"), *e.g.* SSRIs, and the response or outcome variables (Y) are publicly-available gene expression data. In these training sets, we will create an antidepressant "score" based on transcriptomic profiles, *e.g.* if a gene is up- or down-regulated by numerous known antidepressant medications, this would increase the probability of mechanistic importance, and, conversely, if a gene is not differentially expressed or lacks consistent directionality, this would decrease its likelihood of playing a mechanistic role.

Specific Aim 2: Single-Cell Transcriptomics in hiPSC-Derived Cortical Spheroids (hCSs). We next aim to use single cell RNA expression data (scRNA-Seq) (X) to determine the antidepressant propensity of individual neurons (Y) from hiPSC-derived hCSs exposed to both traditional and rapid-acting antidepressants, *e.g.* racemic ketamine, bioactive ketamine metabolites [S-ketamine/esketamine, (2R,6R)-hydroxynorketamine (HNK), *etc*.] and scopolamine. Due to the large number of cells (10,000-100,000) and coverage (15,000-50,000 reads/cell) offered by droplet capture and single-cell chromium RNA library preparation (10X Genomics, Pleasanton, CA, U.S.A.), we anticipate obtaining ample transcriptional data for both validation and test sets.

Data:

We downloaded the L1000 Connectivity Map perturbational profiles data for GSE92742 from the Gene Expression Omnibus (GEO) web site (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE92742) based on the Python tutorial provided on the cmapPy GitHub repository (https://github.com/cmap/cmapPy/blob/master/tutorials/cmapPy_pandasGEXpress_tutorial.ipynb). The data includes the following data files: GSE92742_Broad_LINCS_gene_info.txt, GSE92742_Broad_LINCS_sig_info.txt, and GSE92742_Broad_LINCS_Level5_COMPZ.MODZ_n473647x12328.gctx. From those data files, we were able to export CSV files that allowed us to compare different signatures for perturbations and genes.

AI/ML Approach:

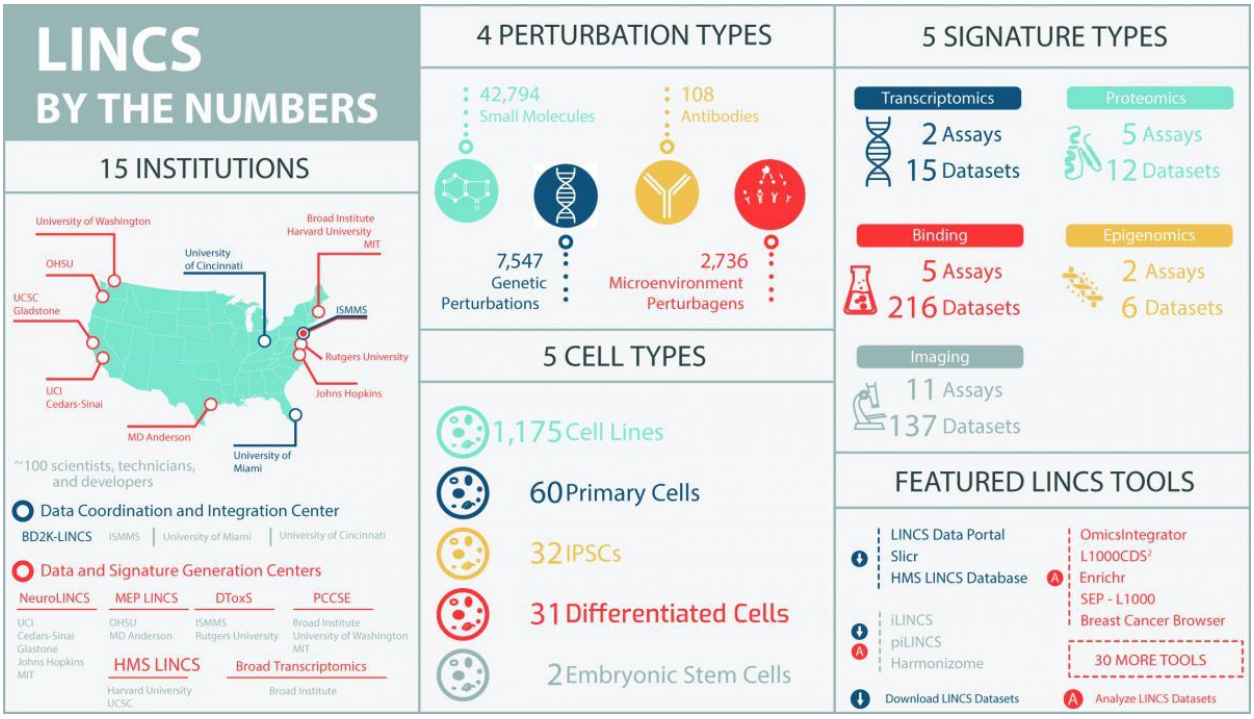
Not applicable (NA)

Experimental methods, validation approach:

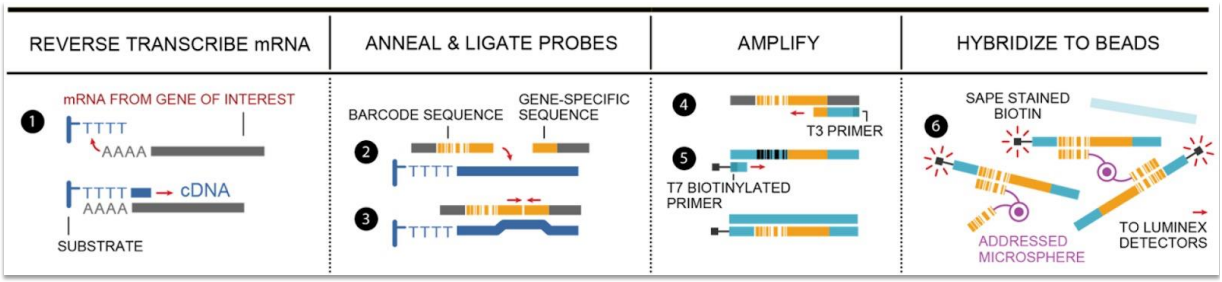
The L1000 database is a publicly-accessible repository of high-throughput expression data released by the Library of Integrated Network-based Cellular Signatures (LINCS) consortium and downloadable from the Gene Expression Omnibus (GEO) (Figure 1A). The L1000 gene expression pipeline directly measures the expression of 978 genes ("landmark genes") in each experiment and then estimates the remainder (>22,000 genes) with ~82% accuracy using National Center for Biotechnology Information (NCBI) modeling (Figure 1B).

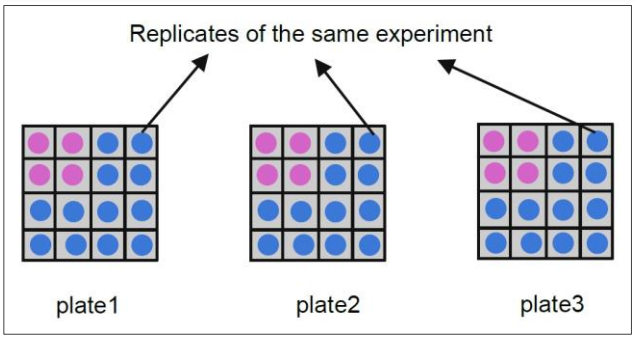
Figure 1. A. "LINCS by the Numbers" schematic (adapted from https://commonfund.nih.gov/lincs) and B. LINCS Experimental Pipeline

A.



B.





Control replicate Experiment replicate

This database was initially filtered by "Antidepressant(s)" medications (peturbagens) and further filtered by antidepressant class, *i.e.* TCAs, SSRIs, *etc*.; dose (10 μM); length of exposure (24 hours); and cell line (the neuronal-like lines described above). Five separate datasets were concatenated into a single milieu with the open-source Juptyer Notebook, a web-based environment for cloud computing. In the L1000 database, gene expression data are reported as Z-scores, and, for stratification, we used a Z-score cut-off of ≥2 and ≤2 as significant up- and downregulation, respectively. Majority up- and down-regulated genes were complied with subsequent functional correlation.

Results:

Table 1. A. Up- and B. Downregulated Genes by SSRIs (NPC)

A. Upregulated*

	# genes	*INP 4B*	*RAC 2*	*TM4 SF1*	*AKR 1C3*	*HLA-DMA*	*MAP 7*	*PECR*	*SATB 1*	*STEAP 1*	*TBC1 D31*
Escitalopram	85	X	X	X				X		X	
Fluoxetine	44	X	X				X			X	
Fluvoxamine	208	X	X	X	X	X		X	X		X
Paroxetine	987				X	X	X	X	X	X	X
Sertraline	31			X	X	X	X		X		X

*Z-score ≥2 with at least 3/5 SSRIs

B. Downregulated**

	# genes	*CSRP1*	*PCNA*	*PUF60*	*IGFBP2*	*ILK*	*ITGAE*	*KIF20A*	*MYL9*	*NT5DC2*	*PFN1*	*TUBB6*
Escitalopram	58		X	X	X			X				
Fluoxetine	32	X	X		X		X		X	X		
Fluvoxamine	147	X	X	X		X		X			X	X
Paroxetine	626	X	X		X	X	X	X	X	X	X	X
Sertraline	17	X		X		X	X		X	X	X	X

**Z-score ≤2 with at least 3/5 SSRIs

Table 2. A. Up- and B. Downregulated Genes by SSRIs (NEU)

A. Upregulated*

	# genes	*GRB10*	*TSPAN8*
Fluoxetine	52	X	X
Fluvoxamine	18	X	X

*Z-score ≥2 with both SSRIs

B. Downregulated**

	# genes	*CA2*	*CD40*	*EPHA3*	*PEL1*	*RFC5*
Fluoxetine	71	X	X	X	X	X
Fluvoxamine	40	X	X	X	X	X

**Z-score ≤2 with both SSRIs

Table 3. A. Up- and B. Downregulated Genes by SNRIs (NPC)

A. Upregulated*

	# genes	*TSTA3*
Duloxetine	9	X
Venlafaxine	26	X

*Z-score ≥2 with both SNRIs

B. Downregulated**

	# genes	*SSBP2*
Duloxetine	7	X
Venlafaxine	20	X

**Z-score ≤2 with both SNRIs

Table 4. A. Up- and B. Downregulated Genes by TCAs (NPC)

A. Upregulated*

	# genes	*HLA-DMA*	*PAK6*	*PIP42K*	*EGFR*	*MAN2B1*	*PDIA5*	*CD58*	*CEBPA*	*CPNE3*	*DNM1*	*HES1*	*HIST1H2BK*	*LPL*	*STEAP1*
Amitriptyline	25	X		X		X	X		X			X	X		X
Amoxapine	22	X	X			X					X				
Clomipramine	48	X	X	X			X			X			X		
Desipramine	59	X		X	X			X			X			X	X
Dibenzepin	28		X	X	X				X	X				X	
Doxepin	32/30/34	XXX	XX			X	X								
Maprotiline	15	X	X							X					
Nortriptyline	25		X		X				X			X			
Trimipramine	102			X	X	X	X	X	X		X	X	X	X	X

*Z-score ≥2 in at least 3/9 TCAs (excluding cases of 3 in which one is partial with doxepin)
Highlighted = majority

B. Downregulated**

	# genes	*CRK*	*GJA1*	*NUP85*	*OSBP10*	*HACD3*	*VIM*	*GTPBP8*	*LAP3*
Amitriptyline	20	X				X		X	
Amoxapine	25	X	X	X		X	X		X
Clomipramine	57	X	X	X					
Desipramine	46	X	X		X			X	X
Dibenzepin	31		X	X	X				
Doxepin	21/21/30	XXXX		XX		XX	XX		
Maprotiline	20	X	X	X	X				
Nortriptyline	21			X			X		
Trimipramine	85		X		X	X	X	X	X

**Z-score ≤2 in at least 3/9 TCAs (excluding cases of 3 in which one is partial with doxepin)
Highlighted = majority

Table 5. A. Up- and B. Downregulated Genes by MAOIs (NPC, NEU)

A. Upregulated*

NPC		
	# genes	*PRKX*
Bifemelane	17	X
Isocarboxazid	17	X

NEU										
	# genes	*ACKR1*	*GALE*	*GPX3*	*GSTM3*	*HAL*	*IKZF1*	*LEPR*	*RBP3*	*STAT3*
Bifemelane	130	X	X	X	X	X	X	X	X	X
Isocarboxazid	57	X	X	X	X	X	X	X	X	X

*Z-score ≥2 with both MAOIs

B. Downregulated**

NPC		
	# genes	*IGFBP2*
Bifemelane	11	X
Isocarboxazid	16	X

NEU									
	# genes	*BEX1*	*KHL9*	*MLLT11*	*PLXNA1*	*PNMA2*	*RNH1*	*TRIP6*	*UBE2A*
Bifemelane	93	X	X	X	X	X	X	X	X
Isocarboxazid	41	X	X	X	X	X	X	X	X

*Z-score ≤2 with both MAOIs

Ideas/aims for future extramural project:

Potential next steps include the identification of gene expression signatures of other antidepressant "perturbagens" in the L1000 database, including those with alternative mechanisms to those reported here, *e.g.* bupropion (norepinephrine-dopamine reuptake inhibitor and nicotine receptor antagonist), mirtazapine (serotonin, histamine, and alpha-2 adrenergic receptor antagonist), and trazodone/nefazodone (serotonin 2A receptor antagonists).

Consistent with Specific Aim #2, we also seek to validate our gene expression findings for with the above antidepressants, as well as and extend into rapid-acting antidepressants with alternative mechanisms of action, *e.g.* the N-methyl-D-aspartate receptor and glutamate modulator (+/-)-ketamine and its bioactive metabolites and the muscarinic receptor antagonist scopolamine, in hiPSC-derived hCSs [Sloan *et al.* (2018) *Nat Protoc* **13**(9):2062-2085, PMID: 30202107], via scRNA-Seq.

Publications resulting from project:

To date, there have been no publications resulting from this project. However, the results from this project will be presented at the 2021 American College of Neuropsychopharmacology (ACNP) annual meeting (hybrid meeting with in-person option currently scheduled from December 5-8, 2021 in San Juan, Puerto Rico):

Niciu, M.J., Kinnaird, B., Lee, K.-P. (2021) Neuronal monoaminergic antidepressant response in the L1000 database.