Identifying Potential Novel Pathways and Therapeutic Targets of Major Depressive Disorder with Functional Genomics

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Identifying Potential Novel Pathways and Therapeutic Targets of Major Depressive Disorder with Functional Genomics

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Abstract

Background: Major depressive disorder (MDD) is a debilitating illness and a leading cause of disability, but its pathophysiology remains to be completely elucidated. Resistance to traditional antidepressant treatment is highly prevalent within patient populations. Ketamine, a rapid-acting antidepressant that has shown success in treating resistant patients, is hypothesized to function by modulating excitatory and inhibitory neurotransmitters. This has led to a paradigm shift from the monoaminergic hypothesis, towards a glutamate and neuroplasticity hypothesis, though this picture may still be incomplete. The purpose of this study is to identify novel systems potentially implicated in MDD and suggest potential therapeutic mechanisms.

Methods: An integrative genomics and systems biology approach was used to identify genes and pathways that may be relevant to MDD. Starting with genes implicated in current, well-established paradigms, a correlation analysis was used to query publicly available microarray datasets to identify potential genes that may be relevant to MDD. Systems and pathways of interest were identified through functional enrichment. Through a manual review, genes of systems and pathways potentially relevant to MDD were used to generate a protein interaction network. Interesting genes identified from the network were functionally enriched and clustered to identify new high-level themes. Genes from the protein interaction network were then classified into sets based on different paradigms using functional annotations. Genes belonging to multiple sets – and thus being involved in multiple different paradigms relevant to MDD – were identified for future research.

Results: The highest-ranked enrichment cluster contained GABAergic signaling, retrograde endocannabinoid signaling, and morphine addiction. Other interesting pathways identified were T cell receptor signaling, cocaine addiction, and nicotine addiction. Other broad themes identified in other clusters were calcium, serotonin, the immune system, TGF beta, glutamate, telomeres, the guanine nucleotide exchange factor, the extracellular matrix, and DNA regulation. Potential genes of interest are listed in Table 3.

Conclusions: Throughout the study, interesting genes, groups of genes, and systems were identified manually and systematically, some of which validate existing literature and some give possible directions for future research. Further experiments are required to confirm causal links.

Introduction

Major depressive disorder (MDD) is a leading cause of disability worldwide, affecting over 264 million people [1] and 7.1% of adults in the United States [2]. Despite its prevalence, the pathophysiology of depression is still not well understood. The first major paradigm was the monoamine hypothesis, which proposed that lower levels of monoaminergic neurotransmitters – serotonin, dopamine, and norepinephrine – contribute to the pathogenesis of MDD. Traditional antidepressants, which include tricyclics, selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), and monoamine oxidase inhibitors (MAOIs), generally function to increase levels of these neurotransmitters.

Confirming the role of serotonin, the kynurenine pathway (KP) and its metabolites have also been a major interest of current research. The pathway metabolizes tryptophan, the precursor to serotonin, and produces quinolinic acid, an NMDA agonist that can induce excitotoxicity [3]. It is theorized that depressed patients have increased KP activity, resulting in less available tryptophan for serotonin synthesis, and consistent with this, a meta-analysis shows that depressed patients consistently have less tryptophan and kynurenic acid and higher levels of quinolinic acid than controls [4]. Naturally, this leads to questions about the upstream causes and downstream effects of KP dysregulation.

One potential upstream cause is the excess release of glucocorticoids. Tryptophan 2,3-dioxygenase controls the rate-limiting step of the KP and is induced by glucocorticoids [5]. Consistent with the psychopathogenesis of depression as a result of chronic stress, glucocorticoids are one of the major hormones released during the stress response. Another enzyme that controls the rate-limiting step of the KP is indoleamine-2,3-dioxygenase, which is induced by inflammatory factors [6,7].

The downstream effects of the KP point towards a role of the NMDA receptor in the biology of MDD, and more broadly neuroplasticity. One of the biggest shortcomings of the monoamine hypothesis is the long delay before traditional antidepressants produce therapeutic effects. Treatment-resistance to traditional antidepressants is also highly prevalent, suggesting that the monoaminergic hypothesis is incomplete. With the discovery of ketamine as an efficacious, rapid-acting antidepressant [8], the predominant paradigm in the current field of depression research has shifted towards a neuroplasticity hypothesis [9] and investigating the roles of glutamate and GABA. Ketamine's mechanism of action is an ongoing area of research, but broadly it increases neuroplasticity by acting as an NMDAR antagonist – protecting against neurotoxicity – and inducing local synaptic protein synthesis [10]. However, this picture may not be entirely complete. Other NMDA antagonists have failed to produce antidepressant effects to the same extent as ketamine [108], which suggests that there are mechanisms unique to ketamine beyond modulation of glutamatergic and GABAergic systems.

The purpose of this study is to identify novel systems potentially implicated in the pathophysiology of depression as areas of future research and to propose possible therapeutic mechanisms based on molecular function.

Methodology

The following genes were chosen as representatives from the major current paradigms as a starting point:

SLC6A4, serotonin hypothesis – The serotonin transporter that mediates reuptake of serotonin from the synapse back into the presynaptic neuron. SSRIs inhibit this transporter to increase available synaptic serotonin levels.

HTR1A, serotonin hypothesis – Serotonin autoreceptor that is a part of negative feedback regulation of serotonin, preventing the release of serotonin in the presence of excess synaptic serotonin. Decreased HTR1A activity, through downregulation or desensitization, is associated with antidepressant effects [11].

TDO2, serotonin hypothesis - Tryptophan 2,3-dioxygenase, one of the enzymes of the rate-limiting step of the kynurenine pathway.

BDNF, neuroplasticity hypothesis – Brain-derived neurotrophic factor, plays a major role in neuroplasticity and the formation of new synapses.

GRIN2B, glutamate hypothesis – Subunit for the NMDA receptor. This subunit in particular has been implicated in depression [12].

PTGS2, inflammation hypothesis – COX2, a major component of the inflammatory response and a target of NSAIDs. Celecoxib, a selective COX2 inhibitor and NSAID, may have antidepressant effects [13].

CRHR1 & CRHR2, HPA axis – CRH receptors that play an important role in mediating the stress response.

NR3C1, HPA axis – Glucocorticoid receptor that mediates transcription and downstream effects of glucocorticoids.

The Allen Human Brain Atlas [109] was used to find genesets correlating with the starting genes. The correlate and starting genes were enriched with DAVID 6.8 [110, 111] for KEGG pathways [112]. Manually, interesting pathways were identified from the literature, and the genes in these pathways along with the starting genes comprised the list of candidate genes for subsequent analysis. Candidate genes were visualized in networks with a variety of methods to determine connectivity and structure. STRING 11.0 [113] was used to create a protein-protein interaction (PPI) network that was further analyzed using Cytoscape 3.8.0 [114] and organized and filtered using MCODE 1.6.1 [115] and clusterMaker 1.3.1 [116]. EnrichmentMap 3.3.0 [117] was used

to construct a pathway interaction network with enriched pathways, and NetworkAnalyst 3.0 [118] was used to create a drug-protein interaction network with the candidate genes.

The candidate gene PPI networks were used to identify further interesting genes to add to the candidate gene list. This new candidate gene list was enriched again with DAVID to identify gene clusters.

Enrichment annotations were then used to group genes from a filtered PPI network into sets based on their involvement in different broad theories of depression: monoamine, neuroplasticity, immune system, neurotrophin, behavioral responses, glucocorticoid, DNA, cholinergic, and endocrine. UpSetR [119] was used to identify intersections between these gene sets.

Results

Identifying Potential Candidate Genes and Pathways

The Allen Human Brain Atlas was used to find genes that are expressed in correlation to the starting genes. Correlate genes with Pearson correlation coefficient $\geq .65$ from all 9 genes were compiled into a gene list. Running the gene set through DAVID, the genes in the GAD disease class for depression from the set were identified and listed in Table 1. DAVID was also used to identify KEGG pathways enriched in the gene list, which are listed in Table 2.

disrupted in schizophrenia 1(DISC1).
prostaglandin-endoperoxide synthase 2(PTGS2).
glutamate metabotropic receptor 7(GRM7).
neurotrophic receptor tyrosine kinase 2(NTRK2).
cholinergic receptor muscarinic 2(CHRM2).
CD3e molecule(CD3E).
cadherin 13(CDH13).
phospholipase A2_group IVA(PLA2G4A).
glutamate ionotropic receptor AMPA type subunit 3(GRIA3).
corticotropin releasing hormone receptor 1(CRHR1).
aralkylamine N-acetyltransferase(AANAT).
brain derived neurotrophic factor(BDNF).
potassium two pore domain channel subfamily K member 2(KCNK2).
myelin transcription factor 1 like(MYT1L).
lymphotoxin alpha(LTA).
empty spiracles homeobox 2(EMX2).
neuronal PAS domain protein 2(NPAS2).
CREB binding protein(CREBBP).
5-hydroxytryptamine receptor 2A(HTR2A).
Wnt family member 7A(WNT7A).
aryl hydrocarbon receptor nuclear translocator like 2(ARNTL2).
neuropeptide Y(NPY).
nuclear receptor subfamily 3 group C member 1(NR3C1).

Table 1

Term	Count	PValue	Genes	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
hsa04080:Neuroactive ligand-receptor interaction	48	8.16E-12	C5H1, MCHR1, AVPR2, MCHR2, GABRB3, THRB, GABRB1, TSHB, NR3C1, GABBR2, GHRHR, S1PR2, HRH1, PTGIR, GRIN2B, HRH2, GALR3, TAAR5, HTR1F, GPR156, GABRA2, PTGER2, PTH2R, GABRA4, RXFP1, GABRA3, GRIN1, GABRA5, GRIN2A, HTR4, GRIA3, NPY1R, NPY5R, P2RX5, CRHR1, GRM5, SSTR3, ADRB1, CHRM3, GRIA2, CHRM2, GRM7, CHRM1, TBXA2R, GHSR, NMBR, LHB, HTR2A	277	6879	3.010	2.007E-09	2.007E-09	1.057E-08
hsa04921:Oxytocin signaling pathway	28	8.07E-08	MEF2C, ADCY2, PTGS2, PPP3R1, CACNB1, KCNJ12, KCNJ3, PPP3CB, PLCB1, CAMK2A, PRKCA, CACNA2D1, CAMK1G, GNAO1, ROCK1, CACNG8, PRKAB2, PRKCG, CACNG3, CACNA2D3, PRKCB, KCNJ4, MAPK1, PLA2G4A, KCNJ6, CAMK1, CALM3, GUCY1B3	150	6879	3.243	1.985E-05	9.923E-06	1.045E-04
hsa04724:Glutamatergic synapse	24	8.70E-08	PRKCA, DLGAP1, GNAO1, ADCY2, GRIN1, GRIN2A, PPP3R1, PRKCG, GRIA3, HOMER1, SHANK2, KCNJ3, PRKCB, SLC17A7, GRM5, MAPK1, PLA2G4A, SLC1A2, GRIN2B, GRIA2, GRM7, PPP3CB, PLCB1, SLC1A1	114	6879	3.657	2.141E-05	7.138E-06	1.128E-04
hsa04723:Retrograde endocannabinoid signaling	22	1.87E-07	PRKCA, GABRA2, ADCY2, GNAO1, GABRB3, PTGS2, GABRA4, GABRA3, GABRB1, GABRA5, PRKCG, GRIA3, KCNJ3, PRKCB, SLC17A7, GRM5, MAPK1, NAPEPLD, KCNJ6, GRIA2, MAPK13, PLCB1	101	6879	3.784	4.602E-05	1.150E-05	2.424E-04
hsa05031:Amphetamine addiction	16	3.37E-06	PRKCA, STX1A, ARC, GRIN1, PPP3R1, GRIN2A, GRIA3, PRKCG, PRKCB, GRIN2B, GRIA2, PPP3CB, CALM3, CREB3L1, SLC18A1, CAMK2A	66	6879	4.211	8.278E-04	1.656E-04	4.362E-03
hsa04020:Calcium signaling pathway	27	9.55E-06	ADCY2, PPP3R1, ITPKA, ATP2B1, HRH1, HRH2, PPP3CB, NOS2, PLCB1, CAMK2A, PRKCA, SLC8A1, GRIN1, HTR4, GRIN2A, PRKCG, PRKCB, P2RX5, GRM5, ADRB1, CHRM3, CHRM2, CHRM1, TBXA2R, CALM3, CACNA1E, HTR2A	179	6879	2.620	2.348E-03	3.917E-04	1.238E-02
hsa05033:Nicotine addiction	12	9.91E-06	SLC17A7, GABRA2, GRIN2B, GRIA2, GABRB3, GABRA4, GABRA3, GABRB1, GRIN1, GABRA5, GRIN2A, GRIA3	40	6879	5.211	2.435E-03	3.483E-04	1.284E-02
hsa04720:Long-term potentiation	15	1.67E-05	PRKCA, GRIN1, CREBBP, PPP3R1, GRIN2A, PRKCG, PRKCB, GRM5, MAPK1, GRIA2, GRIN2B, PPP3CB, CALM3, PLCB1, CAMK2A	66	6879	3.948	4.107E-03	5.143E-04	2.168E-02
hsa04024:cAMP signaling pathway	28	2.10E-05	ADCY2, GABBR2, CNGB1, ATP2B1, BDNF, GRIN2B, CREB3L1, RAPGEF4, CAMK2A, HTR1F, PIK3R1, PTGER2, ROCK1, GRIN1, CREBBP, HTR4, GRIN2A, GRIA3, PDE4C, NPY1R, MAPK1, ADRB1, NPY, GRIA2, CHRM2, CHRM1, CALM3, GHSR	198	6879	2.457	5.146E-03	5.731E-04	2.717E-02
hsa04728:Dopaminergic synapse	21	3.57E-05	PRKCA, GNAO1, KIF5A, PPP2R5C, GRIN2A, PRKCG, GRIA3, KCNJ3, PRKCB, KCNJ6, GRIN2B, GRIA2, MAPK13, PPP3CB, CALM3, CREB3L1, SLC18A1, PLCB1, PPP2R2B, CAMK2A, PPP2R2C	128	6879	2.850	8.743E-03	8.778E-04	4.625E-02
hsa05032:Morphine addiction	17	5.03E-05	PRKCA, GABRA2, ADCY2, GNAO1, GABRA4, GABRB3, GABRA3, GABRB1, GABRA5, PRKCG, PDE4C, GABBR2, KCNJ3, PRKCB, KCNJ6, PDE2A, PDE5B	91	6879	3.245	1.231E-02	1.125E-03	6.521E-02
hsa04725:Cholinergic synapse	19	5.30E-05	PRKCA, ADCY2, GNAO1, PRKCG, KCNJ12, KCNJ3, PRKCB, KCNQ5, KCNJ4, MAPK1, KCNQ3, KCNJ6, CHRM3, CHRM2, CHRM1, CREB3L1, PLCB1, CAMK2A, PIK3R1	111	6879	2.973	1.295E-02	1.086E-03	6.864E-02
hsa04713:Circadian entrainment	17	8.63E-05	PRKCA, ADCY2, GNAO1, GRIN1, GRIN2A, GRIA3, PRKCG, KCNJ3, PRKCB, MAPK1, KCNJ6, GRIN2B, GRIA2, CALM3, GUCY1B3, PLCB1, CAMK2A	95	6879	3.109	2.101E-02	1.632E-03	1.118E-01
hsa04010:MAPK signaling pathway	30	2.57E-04	MEF2C, FGF8, PPP3R1, CACNB1, FGF13, BDNF, PAK2, SOS2, PPP3CB, FGF3, PRKCA, CACNA2D1, CACNG8, NLK, PRKCG, CACNG3, CACNA2D3, MECOM, PRKCB, MAPK1, PLA2G4A, DUSP2, RASGEF2, MAPK13, IKBKG, NTRK2, CACNA1E, DUSP8, DUSP7, DUSP6	253	6879	2.060	6.120E-02	4.500E-03	3.321E-01
hsa04261:Adrenergic signaling in cardiomyocytes	20	3.11E-04	PRKCA, CACNA2D1, ADCY2, CACNG8, PPP2R5C, CACNB1, CACNG3, CACNA2D3, TNNT2, ATP2B1, MAPK1, ADRB1, MAPK13, CALM3, CREB3L1, RAPGEF4, PLCB1, PPP2R2B, CAMK2A, PPP2R2C	138	6879	2.518	7.370E-02	5.091E-03	4.025E-01
hsa04726:Serotonergic synapse	17	5.48E-04	PRKCA, GNAO1, PTGS2, GABRB3, GABRB1, HTR4, PRKCG, KCNJ3, PRKCB, MAPK1, PLA2G4A, KCNJ6, SLC18A1, PLCB1, HTR3B, HTR1F, HTR2A	111	6879	2.660	1.261E-01	8.390E-03	7.076E-01
hsa04360:Axon guidance	18	8.84E-04	NGEF, ROCK1, EFNB3, PPP3R1, NTN4, NTN2G, LRRC4C, SLIT1, NCK2, SEMA5B, EPHA4, MAPK1, EPHB6, RND1, PAK2, SEMA4G, SEMA4C, PPP3CB	127	6879	2.462	1.956E-01	1.272E-02	1.140E+00
hsa04911:Insulin secretion	14	1.02E-03	PRKCA, KCNMA1, STX1A, ADCY2, FFAR1, PRKCG, KCNMB1, PRKCB, CHRM3, KCNN1, CREB3L1, RAPGEF4, PLCB1, CAMK2A	85	6879	2.861	2.217E-01	1.383E-02	1.312E+00
hsa04750:Inflammatory mediator regulation of TRP channels	15	1.31E-03	PRKCA, PTGER2, ADCY2, PRKCG, PRKCB, PLA2G4A, HRH1, MAPK13, IL1RAP, CALM3, PLA2G6, PLCB1, CAMK2A, PIK3R1, HTR2A	98	6879	2.659	2.760E-01	1.686E-02	1.687E+00
hsa04730:Long-term depression	11	1.98E-03	CRHR1, PRKCA, MAPK1, PLA2G4A, GNAO1, GRIA2, GRIA3, GUCY1B3, PRKCG, PLCB1, PRKCB	60	6879	3.185	3.856E-01	2.406E-02	2.533E+00

hsa04727:GABAergic synapse	13	3.15E-03	PRKCA, GABRA2, GNAO1, ADCY2, GABRA4, GABRB3, GABRA3, GABRB1, GABRA5, PRKCG, GABBR2, PRKCB, KCNU6	85	6879	2.657	5.403E-01	3.633E-02	4.011E+00
hsa04970:Salivary secretion	13	3.48E-03	PRKCA, ATP2B1, KCNMA1, ADCY2, ADRB1, CHRM3, AQP5, CALM3, GUCY1B3, PRKCG, TRPV6, PLCB1, PRKCB	86	6879	2.626	5.759E-01	3.824E-02	4.418E+00
hsa04022:cGMP-PKG signaling pathway	19	3.98E-03	KCNMA1, MEZC, SLC8A1, MEF2A, ADCY2, ROCK1, PPP3R1, CNGB1, IRS1, KCNMB1, ATP2B1, MAPK1, ADRB1, PDE2A, PPP3CB, CALM3, CREB3L1, GUCY1B3, PLCB1	158	6879	2.089	6.247E-01	4.171E-02	5.031E+00
hsa04014:Ras signaling pathway	24	5.14E-03	PRKCA, FGF8, GRIN1, GRIN2A, PRKCG, FGF13, HGF, PRKCB, RASAL2, RASAL1, MAPK1, PLA2G4A, PAK2, GRIN2B, RASGRF2, ETS2, IKBKKG, SOS2, CALM3, PLA2G6, SYNGAP1, SHC3, FGF3, PIK3R1	226	6879	1.845	7.183E-01	5.142E-02	6.450E+00
hsa05030:Cocaine addiction	9	6.23E-03	CDK5R1, BDNF, GRIN2B, GRIA2, GRIN1, GPSM1, GRIN2A, CREB3L1, SLC18A1	49	6879	3.191	7.850E-01	5.964E-02	7.778E+00
hsa04925:Aldosterone synthesis and secretion	12	6.23E-03	PRKCA, ADCY2, CAMK1G, PDE2A, STAR, CAMK1, CALM3, CREB3L1, PRKCG, PLCB1, CAMK2A, PRKCB	81	6879	2.574	7.852E-01	5.743E-02	7.781E+00
hsa04270:Vascular smooth muscle contraction	15	6.87E-03	PRKCA, KCNMA1, RAMP2, ADCY2, ROCK1, PRKCG, KCNMB1, PRKCB, MAPK1, PTGIR, PLA2G4A, PLA2G6, CALM3, GUCY1B3, PLCB1	117	6879	2.227	8.164E-01	6.085E-02	8.542E+00
hsa05014:Amotrophic lateral sclerosis (ALS)	9	7.06E-03	SLC1A2, GRIN2B, GRIA2, MAPK13, GRIN1, PPP3CB, PPP3R1, GRIN2A, NEFM	50	6879	3.127	8.248E-01	6.031E-02	8.766E+00
hsa04370:VEGF signaling pathway	10	7.52E-03	PRKCA, MAPK1, PLA2G4A, PTGS2, MAPK13, PPP3CB, PPP3R1, PRKCG, PIK3R1, PRKCB	61	6879	2.848	8.440E-01	6.205E-02	9.321E+00
hsa04971:Gastric acid secretion	11	8.40E-03	PRKCA, ADCY2, CHRM3, HRH2, CALM3, PRKCG, GAST, PLCB1, CAMK2A, CNK2C, PRKCB	73	6879	2.618	8.745E-01	6.684E-02	1.035E+01
hsa04015:Rap1 signaling pathway	22	8.91E-03	PRKCA, FGF8, ADCY2, GNAO1, MAGI1, TLN2, GRIN1, GRIN2A, PRKCG, FGF13, HGF, RGS14, PRKCB, MAPK1, GRIN2B, LPAR5, MAPK13, CALM3, RAPGEF4, PLCB1, PIK3R1, FGF3	210	6879	1.820	8.894E-01	6.856E-02	1.095E+01
hsa04068:FoxO signaling pathway	16	9.61E-03	NLK, CREBBP, PRKAB2, SMAD3, HOMER1, IRS1, MAPK1, G6PC, CDKN1B, PLK2, CCND2, MAPK13, FOXG1, SOS2, AGAP2, PIK3R1	134	6879	2.074	9.069E-01	7.151E-02	1.176E+01
hsa04012:ErkB signaling pathway	12	1.06E-02	PRKCA, MAPK1, NCK2, CDKN1B, NRG3, PAK2, SOS2, PRKCG, SHC3, CAMK2A, PIK3R1, PRKCB	87	6879	2.396	9.271E-01	7.630E-02	1.289E+01
hsa05214:Glioma	10	1.13E-02	PRKCA, MAPK1, E2F3, SOS2, CALM3, PRKCG, SHC3, CAMK2A, PIK3R1, PRKCB	65	6879	2.672	9.395E-01	7.919E-02	1.374E+01
hsa04916:Melanogenesis	13	1.16E-02	PRKCA, MAPK1, TYRP1, GNAO1, ADCY2, CREBBP, CALM3, CREB3L1, PRKCG, PLCB1, WNT7A, CAMK2A, PRKCB	100	6879	2.258	9.437E-01	7.891E-02	1.406E+01
hsa04310:Wnt signaling pathway	16	1.24E-02	PRKCA, NLK, CREBBP, PPP3R1, PRKCG, PRKCB, DKK4, SOST, PRICKLE1, CCND2, PRICKLE2, PPP3CB, WIF1, PLCB1, WNT7A, CAMK2A	138	6879	2.014	9.539E-01	8.190E-02	1.496E+01
hsa04012:GnRH signaling pathway	12	1.46E-02	PRKCA, MAPK1, PLA2G4A, ADCY2, MAPK13, GNRH2, SOS2, CALM3, PLCB1, CAMK2A, LHB, PRKCB	91	6879	2.291	9.731E-01	9.312E-02	1.735E+01
hsa05142:Chagas disease (American trypanosomiasis)	13	1.56E-02	MAPK1, GNAO1, CD3E, MAPK13, IKBKKG, SMAD3, NOS2, PLCB1, PPP2R2B, IFNGR2, PPP2R2C, PIK3R1, TLR9	104	6879	2.171	9.790E-01	9.669E-02	1.842E+01
hsa04611:Platelet activation	15	1.66E-02	ADCY2, ROCK1, TLN2, GP9, MAPK1, PTGIR, PLA2G4A, MAPK13, COL27A1, TBXA2R, FCER1G, GUCY1B3, PLCB1, COL24A1, PIK3R1	130	6879	2.004	9.839E-01	1.004E-01	1.954E+01
hsa04520:Adherens junction	10	1.96E-02	PTPRJ, MAPK1, NLK, WASF1, CREBBP, LMO7, SMAD3, ACTN1, WASL, CTNNA2	71	6879	2.447	9.922E-01	1.144E-01	2.258E+01
hsa04960:Aldosterone-regulated sodium reabsorption	7	2.25E-02	PRKCA, MAPK1, PRKCG, IRS1, SLC9A3R2, PIK3R1, PRKCB	39	6879	3.118	9.963E-01	1.276E-01	2.553E+01
hsa04915:Estrogen signaling pathway	12	2.59E-02	MAPK1, GNAO1, ADCY2, KCNJ6, SOS2, CALM3, CREB3L1, GABBR2, PLCB1, SHC3, KCNJ3, PIK3R1	99	6879	2.106	9.984E-01	1.424E-01	2.881E+01
hsa05200:Pathways in cancer	33	2.71E-02	E2F3, FGF8, ADCY2, PTGS2, ARNT2, FGF13, LAMB3, SOS2, CSF3R, RARA, NOS2, PLCB1, TRAF5, FGF3, PIK3R1, PRKCA, PTGER2, ROCK1, KLK3, CREBBP, SMAD3, PRKCG, HGF, BIRC3, MECOM, PRKCB, CTNNA2, DAPK1, MAPK1, CDKN1B, LPAR5, IKBKKG, WNT7A	393	6879	1.459	9.989E-01	1.457E-01	3.000E+01
hsa04540:Gap junction	11	2.86E-02	PRKCA, GRM5, MAPK1, ADCY2, ADRB1, SOS2, GUCY1B3, PRKCG, PLCB1, PRKCB, HTR2A	88	6879	2.171	9.992E-01	1.497E-01	3.134E+01
hsa05231:Choline metabolism in cancer	12	2.95E-02	PRKCA, MAPK1, PLA2G4A, DGKB, SLC44A5, WASF1, SOS2, PRKCG, WASL, DGKI, PIK3R1, PRKCB	101	6879	2.064	9.994E-01	1.510E-01	3.216E+01
hsa04810:Regulation of actin cytoskeleton	20	3.22E-02	ARHGEF4, FGD1, FGF8, ROCK1, WASF1, DIAPH2, IQGAP3, AB12, ACTN1, FGF13, MAPK1, PAK2, CHRM3, CHRM2, CHRM1, SOS2, MOS, WASL, FGF3, PIK3R1	210	6879	1.654	9.997E-01	1.605E-01	3.456E+01
hsa04062:Chemokine signaling pathway	18	3.80E-02	ADCY2, ROCK1, NFKB1B, CX3CL1, CCL24, CCR9, MAPK1, CCL22, PPPB, CXCL14, CCL21, IKBKKG, SOS2, WASL, PLCB1, SHC3, XCR1, PIK3R1	186	6879	1.681	9.999E-01	1.837E-01	3.949E+01
hsa05143:African trypanosomiasis	6	3.83E-02	PRKCA, APOA1, PRKCG, PLCB1, PRKCB, TLR9	33	6879	3.158	9.999E-01	1.813E-01	3.970E+01
hsa04923:Regulation of lipolysis in adipocytes	8	3.99E-02	ADCY2, ADRB1, NPY, PTGS2, TSHB, NPY1R, IRS1, PIK3R1	56	6879	2.482	1.000E+00	1.848E-01	4.099E+01
hsa05223:Non-small cell lung cancer	8	3.99E-02	PRKCA, FHIT, MAPK1, E2F3, SOS2, PRKCG, PIK3R1, PRKCB	56	6879	2.482	1.000E+00	1.848E-01	4.099E+01
hsa04071:Sphingolipid signaling pathway	13	4.20E-02	PRKCA, S1PR2, MAPK1, ROCK1, MAPK13, PPP2R5C, FCER1G, PRKCG, PLCB1, PPP2R2B, PPP2R2C, PIK3R1, PRKCB	120	6879	1.882	1.000E+00	1.905E-01	4.268E+01
hsa00534:Glycosaminoglycan biosynthesis - heparan sulfate / heparin	5	4.57E-02	HS3ST3A1, HS3ST2, HS6ST3, EXT1L1, HS2ST1	24	6879	3.619	1.000E+00	2.019E-01	4.545E+01
hsa04650:Natural killer cell mediated cytotoxicity	13	4.68E-02	PRKCA, PPP3R1, PRKCG, PRKCB, MAPK1, ULBP1, SOS2, PPP3CB, FCER1G, SHC3, SH2D1B, IFNGR2, PIK3R1	122	6879	1.851	1.000E+00	2.028E-01	4.624E+01
hsa04066:HIF-1 signaling pathway	11	4.79E-02	PRKCA, MAPK1, CDKN1B, CREBBP, PRKCG, NOS2, CAMK2A, IFNGR2, PIK3R1, NPPA, PRKCB	96	6879	1.990	1.000E+00	2.036E-01	4.704E+01
hsa05222:Small cell lung cancer	10	5.39E-02	FHIT, E2F3, LAMB3, CDKN1B, PTGS2, IKBKKG, NOS2, BIRC3, TRAF5, PIK3R1	85	6879	2.044	1.000E+00	2.231E-01	5.123E+01
hsa04922:Glucagon signaling pathway	11	5.69E-02	CPTIC, G6PC, ADCY2, PRKAB2, CREBBP, PPP3CB, PPP3R1, CALM3, CREB3L1, PLCB1, CAMK2A	99	6879	1.930	1.000E+00	2.306E-01	5.321E+01
hsa04660:T cell receptor signaling pathway	11	6.02E-02	MAPK1, NCK2, PAK2, CD3E, MAPK13, NFKB1B, SOS2, IKBKKG, PPP3CB, PPP3R1, PIK3R1	100	6879	1.911	1.000E+00	2.386E-01	5.526E+01
hsa05161:Hepatitis B	14	7.26E-02	PRKCA, EGR3, E2F3, EGR2, CREBBP, YWHAB, PRKCG, PRKCB, STAT6, MAPK1, CDKN1B, IKBKKG, CREB3L1, PIK3R1	145	6879	1.677	1.000E+00	2.775E-01	6.232E+01
hsa04924:Renin secretion	8	7.27E-02	KCNMA1, PTGER2, ADRB1, PPP3CB, PPP3R1, CALM3, GUCY1B3, PLCB1	64	6879	2.171	1.000E+00	2.740E-01	6.240E+01
hsa05146:Amoebiasis	11	8.22E-02	PRKCA, ARG1, LAMB3, COL27A1, ACTN1, PRKCG, NOS2, PLCB1, COL24A1, PIK3R1, PRKCB	106	6879	1.803	1.000E+00	3.007E-01	6.710E+01
hsa04510:Focal adhesion	8	8.28E-02	PRKCA, ROCK1, TLN2, ACTN1, PRKCG, HGF, BIRC3, PRKCB, MAPK1, LAMB3, PAK2, CCND2, COL27A1, SOS2, COL6A3, SHC3, COL24A1, PIK3R1	206	6879	1.518	1.000E+00	2.983E-01	6.735E+01
hsa04722:Neurotrophin signaling pathway	12	8.29E-02	MAPK1, BDNF, MAPK13, NFKB1B, NTRK2, SOS2, CALM3, SHC3, CAMK2A, IRS1, PIK3R1, MATK	120	6879	1.737	1.000E+00	2.945E-01	6.741E+01
hsa04972:Pancreatic secretion	10	8.50E-02	PRKCA, ATP2B1, KCNMA1, ADCY2, CHRM3, PRKCG, PLCB1, RAB27B, PRKCB, CTRL	93	6879	1.868	1.000E+00	2.969E-01	6.835E+01
hsa04151:PI3K-Akt signaling pathway	27	9.10E-02	CSH1, FGF8, PPP2R5C, FGF13, LAMB3, COL27A1, SOS2, COL6A3, CSF3R, CREB3L1, PPP2R2B, PPP2R2C, FGF3, PIK3R1, PRKCA, YWHAB, HGF, IRS1, MAPK1, G6PC, CDKN1B, LPAR5, CCND2, CHRM2, CHRM1, IKBKKG, COL24A1	345	6879	1.359	1.000E+00	3.109E-01	7.094E+01
hsa04664:Fc epsilon R1 signaling pathway	8	9.37E-02	PRKCA, MAPK1, PLA2G4A, MAPK13, SOS2, FCER1G, PIK3R1, PRKCB	68	6879	2.044	1.000E+00	3.150E-01	7.207E+01
hsa04662:B cell receptor signaling pathway	8	9.95E-02	MAPK1, CR2, NFKB1B, SOS2, IKBKKG, PPP3CB, PPP3R1, PIK3R1	69	6879	2.014	1.000E+00	3.274E-01	7.428E+01

Table 2

Key:

Yellow – Pathways that are involved in neuroplasticity

Blue – Pathways involved in other systems hypothesized to contribute to the pathophysiology of depression

Red – Pathways that are potentially involved in depression but have limited and/or dated literature backing them

Purple— Gene sets containing genes associated with depression from Table 1

Several notable pathways appear. Of course, the glutamatergic synapse is one of them.

The mechanism of NMDAR-mediated excitotoxicity is excessive calcium intake into the postsynaptic neuron, and this is done by increased mGluR1/5 activity. mGluR1/5 has also been shown to upregulate NMDARs [14]. A possible therapeutic mechanism would be inhibition of mGluR1/5.

The role of AMPA receptors complicates the role of glutamate in neuroplasticity. Activation of AMPA receptors with glutamate actually increases BDNF release and neuroplasticity [15], and ketamine appears to increase prefrontal glutamate levels [16]. The disinhibition hypothesis states that ketamine's mechanism of action is actually the result of NMDAR inhibition on GABAergic neurons, which results in less inhibition and indirectly results in the release of more glutamate that activates AMPA receptors. Both mechanisms are possibly at play, with ketamine increasing activation of "good" glutamate receptors while inhibiting "bad" ones. Following this idea, several mechanisms increase synaptic glutamate: inhibition of mGluR2/3 or mGluR4/8, inhibition of KCNJ3 channels (labeled GIRK in the picture), or inhibition of SLC1A7/2 channels (labeled EAAT in the picture).

Several downstream protein cascades of BDNF receptor binding, including Ras, MAPK, and PI3K-Akt pathways, were also enriched.

Akt signaling activates mTOR, which then induces synaptic protein synthesis and neuroplasticity. This pathway is hypothesized to be a part of ketamine's mechanism of action [17,18]. Consistent with this idea and the neuroplasticity hypothesis, inhibiting mTOR should lessen ketamine's efficacy, but results have been contradictory. Inhibiting mTOR with rapamycin completely blocked ketamine-induced synaptogenesis in rats [17], yet in depressed patients rapamycin actually prolonged ketamine's antidepressant effects [19].

PI3K-Akt signaling is also activated by IL-2, IL-3, IL-4, IL-6, and IL-7, which also may be worth investigating as a connection to the immune system.

From Table 2, genes associated with depression that are in pathways of interest – gene sets highlighted in purple that also correspond to a highlighted pathway– along with the initial 9 genes make the list for candidate genes.

Interaction Networks

Pathway Interaction Network

All pathways in Table 2 were put into Cytoscape, and a network was constructed using EnrichmentMap. Connections are created between pathways if the similarity coefficient $\geq .63$. Lighter-colored nodes represent a higher corresponding q-value with a cutoff value of .05.

Protein Interaction Network

The initial 9 genes were used to build a protein-protein interaction network in STRING. The network includes first and second shell interactions and is based on experimental evidence using a combined score ≥ 0.40 . The networks were further evaluated in Cytoscape. Subnetworks that connect the products of the initial 9 genes were identified using Cytohubba.

Expanding the subnetwork to also include proteins that directly interact with the products of the initial 9 genes yields the network below.

Note how genes part of the serotonergic system (SLC6A4, HTR1A), glutamatergic system & neuroplasticity (BDNF, GRIN2B), neuroinflammation (PTGS2), and the stress response (NR3C1) are in the same subnetwork. This is not surprising because these are all systems in the

brain directly involved with the pathophysiology of depression. The HPA axis (CRH1, CRH2) – except for NR3C1, the glucocorticoid receptor – and the kynurenine pathway (TDO2) are not connected to the larger subnetwork. Again, this is not unexpected because the depressive effects of CRH and the metabolism of tryptophan are indirect.

The COP9 signalosome complex is still not well understood and an area of current research. The tricyclic antidepressant imipramine upregulates its expression in rats [20].

The mediator complex is a downstream target of the estrogen receptor, which may be involved in postpartum depression.

The 6th top MCODE subnetwork is composed of ribosomal genes. Dysfunction of ribosomal gene expression, possibly contributed to by dysfunctional glucocorticoid signaling, has shown to be associated with chronic social defeat [24].

STRING was also used to create a PPI network containing the list of candidate genes and first & second shell interactions. The network displayed below is a subnetwork that shows connections between the products of candidate genes as well as gene products that directly interact with them. The layout is arranged based on MCODE clusters using clusterMaker. Green colored nodes are candidate genes, and red colored nodes are the initial 9 genes. Red edges are connected to at least 1 initial gene. Darker edges are connections within MCODE clusters.

Network 1

Interesting genes from the MCODE subclusters were identified.

SHANK2 is a scaffolding protein in the postsynaptic density and may play a role in regulating mGluR activity. One proposed role of Shank proteins is to couple postsynaptic density to the endocytic zone, which recycles mGluR5 [25]. Triple knockdown of SHANK1/2/3 with concurrent mGluR5 agonism led to decreased downstream responses and lower spine mGluR5 levels, which as discussed earlier would decrease NMDAR levels. Confirming this, SHANK3 knockdown reduced NMDAR activity [26]. However, SHANK2 dysfunction has primarily been implicated in autism spectrum disorder and autistic-like social behavior – which may connect to the role of poor social behavior in the psychopathogenesis of depression – which is improved in mice by an NMDAR agonist that normalizes the hippocampal NMDA/AMPA ratio from a reduced value caused by SHANK2 knockout [27]. SHANK2 knockout mice also had lower hippocampal plasticity. Further complicating the role of SHANK2, a more recent study shows consistent increased hippocampal plasticity in SHANK2 knockout mice, though the knockout was achieved with exon 7 deletion (as opposed to exon 6 and 7 deletion in Won et al.) and NMDA/AMPA ratios were inconsistent [28]. SHANK2 knockout localized to Purkinje cells in mice increased repetitive and anxious behavior and impaired social behavior, probably due to decreased excitatory synapse activity brought about by the knockout [29, 30]. Deletion of exon 24 induced anhedonia and manic and reward-seeking behavior in mice that were treated with mood stabilizers in addition to social impairment [31]. Consistent with Won et al., a decreased NMDA/AMPA ratio was also observed, possibly due to an increase in NMDAR NR2A and NR2B (which is coded from GRIN2B) subunit levels. Thus, different SHANK2 mutations may have varying roles in multiple neuropsychiatric disorders, which may be an area of future research.

This does raise a question about the effects of ketamine on social behavior, and indeed ketamine does induce social-withdrawal in mice [32], which is reversed by a 5-HT7 agonist and memantine [33], which is also an NMDAR antagonist and further complicates the role of NMDARs in social behavior. No firm conclusions can be made on the effect of ketamine on social behavior on humans from animal model studies, but this does open an area for future research.

GRIA genes code for AMPAR subunits.

MAPK and MAPKK genes code for components of the ERK signaling pathway, a downstream target of BDNF that leads to protein synthesis and induces inflammation as part of the stress response [34]. This pathway seems to be essential for R-ketamine's antidepressant effects, as opposed to S-ketamine which requires mTORC1 signaling [35, 36], as well as other traditional antidepressants [37]. Not only does inhibiting MAPK prevent antidepressant effects, but it also induces depressive symptoms, suggesting it has a role in the pathogenesis of depression. MAPKs are also involved in synaptic plasticity and neuroinflammation [38, 39]. The p38 MAPK-MK2 complex induces neuroinflammation and the release of proinflammatory cytokines. Activation of

NMDARs causes p38 MAPK-mediated internalization of certain AMPARs, which underlies long-term depression [40], and AMPAR downregulation caused by NMDAR stimulation may be a mechanism for the pathogenesis of depression consistent with the disinhibition hypothesis. Consistent with this, ketamine reduced hippocampal p38 MAPK levels [36]. ERK1/p44 MAPK levels were also increased with ketamine treatment, which upregulates certain AMPARs [40], however ERK2/p42 MAPK also upregulates AMPARs but was decreased after ketamine treatment.

CALM1 is differentially expressed in depressed patients and is involved in dopamine signaling [41]. CaMK2 is activated by NMDAR-mediated calcium intake, which then activates Ras complexes which then activates MAPK signaling that upregulates surface AMPAR levels [40].

KCJN6 may play a role in rumination [42], which is a classic psychopathogenetic mechanism of depression.

NFkB is a transcription factor and a downstream target of PI3K-Akt signaling, and genes coding for NFkB subunits have been implicated in hippocampal synaptic plasticity. Knockout of NFkB1 (the p50 subunit) impaired hippocampal neurogenesis and cognition in mice [43]. On the other hand, chronic IKK-mediated activation of NFkB resulted in inflammation, neurodegeneration, and reduced BDNF levels in the hippocampus which was treated with deactivation of NFkB, which may suggest a therapeutic effect from the activation of NFkB1, including NFkBIB, and other agents inhibiting NFkB activity. This is consistent with the psychopathogenesis of depression as a result of chronic stress. Modulators of NFkB signaling have promise as novel antidepressants. The gabapentinoids pregabalin and gabapentin promote hippocampal neurogenesis and prevent depressive symptoms through an NFkB-dependent mechanism [44]. L-acetylcarnitine (LAC) is also a therapeutic of interest that also interacts with NFkB. In rats, LAC had antidepressant effects within days and lasted for 2 weeks after withdrawal [45]. Depressed rats had lower prefrontal and hippocampal BDNF levels and lower depolarization-evoked glutamate released, which were both treated with LAC. LAC levels are also lower in depressed patients [46]. A meta-analysis in humans found LAC to be a significantly effective monotherapy for depression treatment and even had fewer adverse effects than traditional antidepressants [47]. The mechanism underlying LAC is complex. LAC acetylates NFkB p65, which then promotes transcription of mGluR2 receptors [46, 48]. mGluR2 receptors have been observed to be upregulated, and this is prevented with NFkB inhibition [46]. Although LAC does appear to be well-tolerated, LAC's therapeutic effects are probably due to acute NFkB positive modulation, since chronic NFkB activation promoted depressive symptoms as discussed before; the effects of LAC treatment over a longer period should be further studied. In addition, upregulation of mGluR2 would cause depressive symptoms according to the disinhibition hypothesis. Previous studies have shown that elevated prefrontal mGluR2 levels are a characteristic of depressed patients [49] – which contrasts with Nasca et al., which observed downregulated prefrontal mGluR2 in depressed rats – and mGluR2 antagonism has

antidepressant effects [50, 51]. It is possible that LAC's antidepressant effects occur through another mechanism, and its basic mechanism and its clinical effects are areas of further research.

SLC1A2 is the glutamate transporter responsible for the majority of glutamate reuptake [52].

CACNA1C is associated with several psychiatric disorders, including bipolar disorder, depression, and schizophrenia. Complete CACNA1C knockout impaired neurogenesis, but knockdown and a heterozygous genotype had antidepressant effects [53]. A CACNA1C SNP predicts the development of depression after threatening life events [54]. Voltage-gated calcium channels in general may be associated with psychiatric disorders [55,56].

Protein-Drug Interaction Network

The list of candidate genes was put into NetworkAnalyst to create a network of protein-drug interactions using the DrugBank database. The largest subnetwork was visualized in Cytoscape.

Key:

Red nodes – Candidate genes

Light blue nodes – Drugs

Purple node & edges– Ketamine

Green nodes & edges– SSRIs

Orange nodes & edges – SNRIs

Dark blue node & edges – Tricyclics

No MAOIs were found.

Note how traditional antidepressants act on proteins of multiple pathways, not just monoaminergic systems. These interactions show other potential mechanisms that may contribute to these drugs’ antidepressant effects. They also provide evidence that the products of the candidate genes are possibly implicated in the pathophysiology of depression.

Quite nicely and without intention, ketamine is at the center of the network. Though ketamine is hypothesized to act primarily on the glutamatergic system to produce antidepressant effects, its mechanism of action may involve other systems as well, including the serotonergic system [57].

Other smaller subnetworks are shown below.

Enrichment Analysis

Functional Annotation Clustering

The genes identified from Network 1 and their isoforms are

SHANK1	SHANK2	GRIA2	GRIA3	GRIA4	MAPKAPK2	MAPKAPK3	MAP2K2	MAP3K14	MAPK3	MAP3K1	MAPK8	MAPK9	MAPK13	MAPK10	MAPK3K4
MAPKAPK5	MAP3K5	MAP3K7	MAP2K1	MAP2K6	MAPK1	MAPK14	CALM1	CALM3	CALM2	CAMK2G	CAMKK2	CAMK1	CAMKK1	CAMK1D	CAMK4
CAMK1G	CAMK2B	CAMK2D	CAMK2A	KCNJ5	KCNJ3	KCNJ9	KCNJ6	KCNJ12	KCNJ4	CACNA1C	CACNB1	CACNB2	CACNG2	CACNB3	CACNA1A
CACNA1S	CACNA2D1	CACNG1	CACNA1E	CACNA2D2	CACNA1B	CACNA1F	CACNA1D	CACNA2D3	NFKB2	NFKBIB	NFKB1	NFKBIA	SLC1A2		

The gene list above was combined with the candidate gene list. This combined gene list was run through DAVID for functional annotation clustering, and the resulting clusters are shown in the table below.

[illegible]

Several of these clusters have clear themes and highlight interesting molecular interactions and properties. The highest ranked cluster contains the following systems.

[illegible]

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Figure 1: Molecular mechanisms of morphine dependence.

The figure illustrates the molecular mechanisms of morphine dependence across four panels: **Control**, **Acute morphine**, **Chronic morphine**, and **Withdrawal**.

Control: A GABAergic neuron (Presynaptic terminal) releases GABA, which binds to GABA receptors on a Dopamine (DA) neuron (Postsynaptic cell). This results in a decrease in dopamine release. The diagram also shows the Prefrontal cortex (PFC), Polysubstance use nucleus (PTG), and Ventral tegmental area (VTA) in the brain.

Acute morphine: Morphine binds to MOR on the GABAergic neuron, leading to hyperpolarization and inhibition of GABA release. This results in disinhibition of the DA neuron and increased dopamine release. The diagram also shows the Polysubstance use nucleus (PTG) and Ventral tegmental area (VTA) in the brain.

Chronic morphine: Repeated exposure to morphine leads to receptor internalization and desensitization, resulting in a decrease in dopamine release. The diagram also shows the Polysubstance use nucleus (PTG) and Ventral tegmental area (VTA) in the brain.

Withdrawal: After chronic morphine treatment, withdrawal leads to a decrease in dopamine release, which is associated with negative motivational effects. The diagram also shows the Polysubstance use nucleus (PTG) and Ventral tegmental area (VTA) in the brain.

Withdrawal: increased adrenergic tone: Increased adrenergic tone during withdrawal leads to a decrease in dopamine release, which is associated with persistent behavioral changes. The diagram also shows the Polysubstance use nucleus (PTG) and Ventral tegmental area (VTA) in the brain.

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As previously discussed, GABAergic neurons are implicated through the disinhibition hypothesis. Some other methods of reducing GABA release are inhibiting CACNA1A/B (labeled VGCC in the picture) or activating GABBR1/2 autoreceptors (labeled GABAB in the picture).

Endocannabinoids released by a local increase in calcium in postsynaptic dendritic spines inhibit further release of GABA and glutamate from the presynaptic neuron by binding to CB1 receptors. The endocannabinoid system is highly expressed in the hippocampus, a brain region implicated in depression, and is involved in the stress response [58]. CB1 receptors are present on both GABAergic and glutamatergic neurons, and CB1 receptor activation can lead to less release of both types of neurotransmitters downstream through depolarization-induced inhibition of excitation (DSE) and inhibition (DSI). According to the disinhibition hypothesis, this may produce conflicting effects with regards to depression treatment. High-affinity CB1R antagonists and agonists – since DSE and DSI are transient events – are both worth investigating for therapeutic effects, especially if they can selectively target different neuron types. Endocannabinoids may also play various roles in the synaptic plasticity of various brain regions [59].

Although morphine addiction may not appear directly relevant to depression, this pathway provides an interesting insight into the regulation of dopaminergic neurons with GABA. Additionally, acute morphine produces a similar effect as ketamine by inhibiting GABA receptors, which increases dopamine signaling for morphine – which may also occur with ketamine. This may also partially explain the potential for ketamine to become addictive, albeit to a lesser extent than morphine. Tianeptine, an atypical antidepressant, is a mu-opioid receptor (labeled MOR on the picture) agonist, similar to morphine. Interestingly, the mu-opioid receptor antagonist naltrexone has been shown to lessen the antidepressant effects of ketamine [60]. All of this suggests that the opioid system is involved in the pathophysiology of depression and the mechanism of action of ketamine. Buprenorphine, a partial mu-receptor agonist, has been shown to rapidly treat suicidal ideation in a randomized control trial [61]. Less potent opioids like buprenorphine in a controlled setting may have use as a last-line option for severe treatment-resistant depression. Regardless, the opioid system has potential as a novel target for depression treatment.

Clusters 8 and 11 have a theme of the immune response, and cluster 11 also includes neurotransmitter systems. T-cell signaling is also a reoccurring theme.

Several IL2 related functional annotations are in cluster 11, although they do not meet the threshold for statistical significance. IL2 is an anti-inflammatory cytokine, and it induces ITK which dephosphorylates the transcription factor that leads to the immune response, including the release of proinflammatory cytokines and TNF. IL2 may be a novel therapeutic that reduces the release of proinflammatory cytokines, particularly IL17 from Th17 cells, and by stimulating T regulator cells that control inflammation [62].

Cocaine addiction is in cluster 12. While cocaine is obviously not a practical therapeutic agent, the involved pathways reveal some interesting molecular interactions.

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In contrast to the hippocampus, the NAc of depressed patients is hypertrophic and has increased plasticity, which seems to be reversed by ketamine [63]. Stress increases VTA mesolimbic signaling which was treated with fluoxetine [64], thus BDNF release in the NAc is also increased and explains the increased plasticity. Ketamine reduces proBDNF – the precursor to BDNF – levels in the NAc (in contrast to the hippocampus, where it is increased) [36], which may explain ketamine’s therapeutic mechanism in the NAc. IKK levels were increased in the NAc, which mediated increased plasticity by increasing spine formation [66]. Depressive symptoms seem to be a result of increased plasticity due to BDNF-TrkB signaling rather than mesolimbic dopamine signaling in and of itself [65].

From the diagram, activation of mGlu2/3 results in less production of BDNF in the NAc. However with regards to reducing depressive symptoms, activating mGlu2/3 would produce theoretically conflicting effects in glutamatergic synapses. DRD1 inhibition and DRD2 activation would also decrease BDNF production in this context.

NICOTINE ADDICTION

A cholinergic hypothesis of depression is not new and supported by many lines of evidence [67]. It was developed when acetylcholinesterase inhibitors were observed to induce depressive symptoms [67, 68].

Consistent with the psychopathogenesis of depression, acute changes in acetylcholine levels due to acute stress can produce adaptive behaviors, but chronic changes can be responsible for maladaptive behaviors [70]. Interestingly, depressive symptoms in mice induced by an acetylcholinesterase inhibitor could even be reversed by nAChR antagonists and fluoxetine, which also increased acetylcholinesterase activity [71]. Also, increased acetylcholine signaling in the hippocampus particularly seems to contribute to depressive symptoms. Alpha-7 receptor agonists resulted in increased glutamate release and induced long-term potentiation in the hippocampus [72]. Clinical studies have shown that transdermal nicotine has antidepressant effects, notably also in older patients with late-life depression which is characterized by poor antidepressant response [73, 74], which is possibly due to the chronic desensitization of alpha-4 beta-2 nAChRs. Bupropion, a drug that supports smoking cessation, also functions as an antidepressant. It is an antagonist of alpha-4 beta-2 nAChRs [75], which possibly contributes to its antidepressant effects by reducing GABA inhibition of dopaminergic neurons. In postmortem subjects, beta-2 subunits were significantly less available in depressed subjects – possibly due to higher levels of acetylcholine – with the availability of the receptors significantly correlating with severity of depression [69].

However, a model of alpha-4 beta-2 nAChR stimulation inducing depression completely contradicts the previous NAc plasticity model. Decreasing alpha-4 beta-2 activity, whether through antagonism or desensitization, reduces inhibitory neuron activity and increases dopamine release, which increases NAc plasticity downstream. However, there is evidence that alpha-7 nAChR stimulation induces depressive symptoms [76] and cytosine, a selective alpha-4 beta-2 partial agonist, which is consistent with the NAc plasticity model [77]. However, there is contradictory evidence that alpha-7 nAChR knockout induced depressive symptoms and increased NAc synaptogenesis and BDNF levels [78]. It is clear that this system is complex and has some involvement in the pathophysiology of depression, but more research is needed before a consistent model can be created. Alpha-7 receptors are also present on macrophages, and when triggered inhibit the release of proinflammatory cytokines [79].

Addictive drugs, including morphine, nicotine, and cocaine decrease GABA inhibition of dopamine [80]. Acute stress also appears to decrease GABA inhibition through the glucocorticoid system, which is consistent with the NAc plasticity model. Annotations involving GABA are also clustered with nicotine addiction.

Consistent with the NAc plasticity model, chronic stress elevates the VTA AMPAR/NMDAR ratio and alpha-7 activation increases susceptibility to social stress [81]. In addition, chronic nicotine decreased subsequent VTA dopamine signaling in response to acute IV nicotine. This supports chronic nicotine patches as a treatment, if this result is due to globally decreased VTA dopamine signaling – which would lead to decreased NAc plasticity downstream. However, chronic nicotine also led to increased social avoidance.

Benzodiazepine receptor activity is also in the same cluster as nicotine addiction, which might also suggest another connection [82].

There are other clusters with consistent themes, which may or may not be involved in the pathophysiology of depression, and they were identified manually.

Clusters 4, 5, 20, and 30 involve calcium signaling.

Cluster 23 involves serotonin.

Cluster 25 involves the immune system.

Cluster 26 involves TGF beta.

Cluster 36 involves telomere regulation. Depressed individuals appear to have shorter telomere length [83], and telomere length predicts SSRI response [84, 85].

Cluster 37 involves guanine nucleotide exchange factor.

Cluster 41 involves the extracellular matrix.

Cluster 45 involves DNA regulation.

Set Intersection

Every gene from the unfiltered PPI network from the end of part 2.II was put into DAVID and annotated with Gene Ontology terms. A python script was used to find certain keywords and genes associated with those keywords. In the table below, genes are grouped into sets based on which theory of depression they fall under, with the keyword used in the second row.

Genes belonging to 6 groups																			
UCN	KRAS	CRH																	
Genes belonging to 5 groups																			
PPARGC1A	NTRK1	MAPK1	MEF2C	BAD	BCL2														
Genes belonging to 4 groups																			
PTGS2	RAC1	ABL1	CTNNB1	GSK3B	NOS1	HDAC2	PTK2B	PPP3CB	PPP3CA	EGFR	MTOR	HRAS	NEDD4	HMGB1	PPARA	PLCB1	CREB1	AKT1	TNF
Genes belonging to 3 groups																			
HTR2A	CDK5	SNCA	PARK2	ADRB1	WNT1	DNM2	HIF1A	SIN3A	RARA	UBB	TRAF6	NFKB2	ARHGEF2	CAMK4	CAMK1D	NR1H4	TRAF2	TLR4	ILK
REL	GRIP1	IKBK	STAT3	CDC42	NCK2	IL1RAP	BCL6	TLR3	BRAF	GRIN1	RGS14	YWHAH	GATA1	PTPN11	SRC	NGFR	PDPK1	MAPKAPK2	MAPK13
RELA	NFKB1	SMAD3	MED1	MAPK3															

Table 3

For brevity, an overview will only be done on the genes with evidence supporting a connection to depression from the 6, 5, and 4-degree intersections.

Urocortin (UCN) is a high-level regulator of the stress response and within the same family as CRH. It promotes anxious behavior and decreases appetite [86], which is a symptom of depression, though its wide-reaching regulatory roles makes it an unlikely practical therapeutic target.

PGC-1 alpha (PPARGC1A) regulates mitochondrial biogenesis and gene expression. Activation of PGC-1 alpha through exercise redirects the kynurenine pathway towards the production of kynurenic acid, which is an NMDA antagonist [87]. Downregulated PGC-1 alpha results in oxidative stress, inflammation, and NFkB activation, and TNF decreases PGC-1 alpha expression [88].

TrkA (NTRK1) is the receptor for nerve growth factor and related to TrkB, the receptor for BDNF. The tricyclic antidepressant amitriptyline is a TrkA/B agonist [89].

MEF2C is a transcription factor. In mice, increased activity results in increased dendritic spine density and complexity in several brain regions, including the hippocampus [90].

In mice, decreasing NAc RAC1 expression induces social defeat behavior and anhedonia, which is reversed by upregulation [91].

ABL1 is a proto-oncogene for gastric cancer. Depression-induced oxidative stress exacerbates the prognosis of cancer patients through ABL1-involved pathways [92].

Beta-catenin (CTNNB1) is a key player in the canonical Wnt pathway, and WNT1 is a ligand initiating the pathway. Beta-catenin promotes transcription of certain genes, and direct nucleic beta-catenin insertion into the NAc promotes stress-resilience and produces antidepressant effects [93]. D2 dopamine receptor activation promotes beta-catenin mediated transcription [94]. In the hippocampus, canonical Wnt signaling has a positive role in neurogenesis [95]. Glutamate activation of NMDARs results in calpain-dependent cleavage of beta-catenin in the hippocampus [96], and cleaved beta-catenin resulted in greater transcription activity, which is paradoxical considering ketamine's antidepressant effects and mechanism as an NMDAR antagonist. However, the study was conducted with a focus on cancer rather than on neuronal cells, and

extranuclear interactions of beta-catenin may also be present [97]. Overall, it appears that increasing beta-catenin signaling produces antidepressant effects, and Wnt ligands may produce therapeutic effects.

GSK-3 β is also involved in canonical Wnt signaling. GSK-3 β ubiquitinates beta-catenin for proteolysis, and cleaved beta-catenin is resistant to this degradation [96]. Consistent with the hypothesis of beta-catenin having therapeutic effects, overactive GSK-3 β is associated with depressive symptoms [98]. Also, GSK-3 β mediates maladaptive NAc plasticity [99], and knockdown prevents depressive symptoms. GSK-3 β is also inhibited by lithium, and ketamine, which is necessary for its antidepressant effects [18]. Akt signaling leads to mTOR-mediated inhibition of GSK-3 β , which may explain the role of mTORC1 in the pathophysiology of depression. GSK-3 β inhibitors may be a novel antidepressant.

NOS1 codes for nitric oxide synthase 1. NO modulates norepinephrine, serotonin, dopamine, and glutamate, and inhibition of NOS prevented neurotoxicity resulting from NMDA agonism [100]. Many conventional and atypical antidepressants interact with NO pathways.

HDAC2 codes for histone deacetylase 2. Depression is associated with increased acetylation of histone H3 and decreased HDAC2 expression in the NAc, and HDAC2 also mediates hippocampal plasticity [101]. Direct inhibition of HDAC has antidepressant effects and reverses the effects of chronic defeat stress. Interestingly, the study also implicates genes involved in the effects of cocaine, as previously enriched.

Patients with lung cancer with EGFR mutations had higher TNF- α levels, which is normally associated with depression, but paradoxically tended to have lower depression severity [102].

Maternal immune activation during pregnancy leads to inflammation, which results in decreased NEDD4 activity that leads to decreased neurogenesis and synapse formation and is a risk factor for depression [103].

HMGB1 is a chromatin protein that aids in transcription. Upregulated HMGB1 is associated with increased presence and activity of kynurenine pathway enzymes [104].

PPAR- α (PPARA) is a transcription factor. PEA, an endogenous PPAR- α agonist, has antidepressant effects [105, 106]. PPARs may also be involved in the endocannabinoid system [107].

Conclusion

The purpose of this was to identify novel systems underlying the pathophysiology of depression as research directions and potential therapeutic targets. First, starting representative genes were selected from the major current paradigms of depression: the serotonin hypothesis (and kynurenine pathway), glutamate hypothesis, neuroplasticity, inflammation, the HPA axis, and the stress response. Using publicly available microarray datasets on Allen Brain Atlas, gene

correlates were identified and filtered for genes related to depression using DAVID to make a gene candidate list. Creating a protein interaction network from the starting genes, subnetworks originating from the glucocorticoid receptor coded by NR3C1 were identified based on high interconnectivity. These subnetworks had themes of histones, the COP9 signalosome, SWI/SNF chromatin remodeling complexes, the estrogen receptor, and ribosomes. A drug-protein interaction network was created to identify potential therapeutic agents for further investigation and to find interactions between currently established antidepressants with candidate genes. Ketamine was at the center of the network and connected to many of the candidate genes, thereby validating this methodology. Creating a protein interaction network with the candidate genes and organizing for highly interconnected subclusters, SHANK2, GRIA2/3/4, MAPK & MAPKK genes, CALM & CAMK genes, KCJN6, CACN genes, NFKB genes, and SLC1A2 were identified as genes of interest. The candidate genes and the genes in interesting clusters identified from the network were enriched with DAVID. The most notable enrichment clusters had themes of GABAergic regulation, endocannabinoid signaling, opioid signaling, cholinergic signaling, and T-cell and antibody signaling. Potential pathogenesis and therapeutic mechanisms involving these systems were proposed based on KEGG pathway illustrations and molecular interactions. Using Gene Ontology annotations, every gene from the candidate protein interaction network was classified into groups based on current theories of depression. Genes belonging to multiple groups are highly interesting and were identified. These genes may be the subject of future research and novel paradigms.

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Conflict of Interest

The author declares no conflict of interest.