Hippocampal transcriptional and neurogenic changes evoked by combination yohimbine and imipramine treatment

Basma Fatima Anwar Husain, Ishira N. Nanavaty, Swananda V. Marathe, Rajeev Rajendran, Vidita A. Vaidya

Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, Maharashtra, India

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Adjunct α₂-adrenergceptor antagonism is a potential strategy to accelerate the behavioral effects of antidepressants. Co-administration of the α₂-adrenergceptor antagonist yohimbine hastens the behavioral and neurogenic effects of the antidepressant imipramine. We examined the transcriptional targets of short duration (7 days), combination treatment of yohimbine and imipramine (Y + I) within the adult rat hippocampus. Using microarray and qPCR analysis we observed functional enrichment of genes involved in intracellular signaling cascades, plasma membrane, cellular metal ion homeostasis, multicellular stress responses and neuropeptide signaling pathways in the Y + I transcriptome. We noted reduced expression of the α₂α₆-adrenergceptor (Adra2α), serotonin 5HT₂C Receptor (Htr2c) and the somatostatin receptor 1 (Sstr1), which modulate antidepressant action. Further, we noted a regulation of signaling pathway genes like inositol monophosphatase 2 (Impa2), iodothyronine deiodinase 3 (Dio3), regulator of G-protein signaling 4 (Rgs4), alkaline ceramidase 2 (Acer2), doublecortin-like kinase 2 (Dclk2), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (Nfκbα) and serum/glucocorticoid-regulated kinase 1 (Sgk1), several of which are implicated in the pathophysiology of mood disorders. Comparative analysis revealed an overlap in the hippocampal regulation of Acer2, Nfκbα, Sgk1 and Impa2 between Y + I treatment, the fast-acting electroconvulsive seizure (ECS) paradigm, and the slow-onset chronic (21 days) imipramine treatment. Further, Y + I treatment enhanced the quiescent neural progenitor pool in the hippocampal neurogenic niche similar to ECS, and distinct from chronic imipramine treatment. Taken together, our results provide insight into the molecular and cellular targets of short duration Y + I treatment, and identify potential leads for the development of rapid-action antidepressants.

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1. Introduction

α₂-adrenergceptors are considered putative targets for rapid-action antidepressants (Blier, 2003). Several studies have indicated enhanced α₂-adrenergceptor expression, binding and functional responses within limbic brain regions, including the hippocampus, in both patients with major depressive disorder and animal models of depression (Escribá et al., 2004; Flüge et al., 2003; Rivero et al., 2014; Valdizán et al., 2010). Chronic antidepressant treatments evoke a downregulation or desensitization of the α₂-adrenergceptor, a process suggested to influence the duration of therapeutic lag associated with pharmacological antidepressants (Invernizzi and Garattini, 2004; Subhash et al., 2003).

Clinical studies indicate that antidepressants such as mirtazapine, which exhibit α₂-adrenergceptor antagonism, exert faster behavioral effects when administered alone or in conjunction with a classical antidepressant (Carpenter et al., 2002; Quitkin et al., 2001). The α₂-adrenergceptor antagonist yohimbine when co-administered with the antidepressant fluoxetine hastens antidepressant responses in patients with major depressive disorder (Sanacora et al., 2004), as well as in animal models (Dhir and Kulkarni, 2007). Preclinical studies (Yanpallewar et al., 2010) also indicate that combination treatment with the α₂-adrenergceptor antagonist yohimbine and the tricyclic antidepressant, imipramine (Y + I) elicits neurogenic, neutrotrophic and behavioral changes within 7 days, as compared to imipramine treatment which requires 3 weeks of treatment to evoke similar responses. These studies suggest that α₂-adrenergceptor antagonism is a putative target for stand-alone or combination antidepressant therapy.

Currently, the molecular and cellular consequences of treatments such as the combination Y + I treatment, that bring together α₂-adrenergceptor antagonism with a classical pharmacological antidepressant treatment, remain poorly elucidated. To gain an understanding of the global gene expression changes that accompany the rapid
behavioral outcomes of the Y + I treatment paradigm, we carried out microarray studies to assess the transcriptional targets of Y + I treatment within the hippocampus, a brain region that is known to be a target of antidepressant therapy. Further, we also evaluated the specific progenitor stage within the hippocampal neurogenic niche that is targeted by Y + I treatment, as hippocampal neurogenesis has been strongly linked to the behavioral effects of antidepressants. Finally, we addressed whether the transcriptional and neurogenic targets of the short duration Y + I treatment are also influenced by distinct antidepressant treatments such as the fast-acting electroconvulsive seizure (ECS) paradigm and the long duration (21 days) treatment with the classical antidepressant imipramine (I) with a view to identifying common molecular signatures across diverse antidepressant therapeutic strategies.

2. Methods

2.1. Animals

Adult male Wistar rats (250–400 g, 2–3 months of age) and adult male nestin-GFP transgenic mice (25–35 g, 2–3 months of age) generated as previously described (Yu et al., 2005) were group housed. Animals were maintained on a 12 h light/dark cycle with access to food and water ad libitum. Nestin-GFP reporter mice were a kind gift from Dr. Steven Kernie (Columbia University, New York, USA) (Yu et al., 2005). Nestin-GFP mice were maintained on a C57BL/6J background and express green fluorescent protein (GFP) under the control of the nestin promoter, thus facilitating the identification of distinct stages of neural precursor development. All animal procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the TIFR Institutional Animal Ethics committee.

2.2. Animal treatments

2.2.1. Drug treatments

To assess the global transcriptional changes evoked within the hippocampus by the fast-acting antidepressant combination of α2-adrenoceptor blockade with yohimbine, along with the classical antidepressant imipramine, animals received yohimbine (2 mg/kg; Sigma, St Louis, MO, USA) 30 min prior to administration of imipramine (20 mg/kg; Sigma) (Y + I group) or received vehicle treatment (Ctl group), once a day for seven consecutive days. Two independent Y + I treatment groups were processed for microarray (n = 3 per group) and qPCR analysis (n = 9–10 per group). To determine hippocampal gene expression changes elicited by chronic antidepressant treatment, rats received imipramine (10 mg/kg) or vehicle treatment (Ctl) once daily for 21 days, and were sacrificed 2 h following the final treatment (n = 8–10 per group). To assess the hippocampal transcriptional changes evoked by 7 days of imipramine treatment, rats received imipramine (20 mg/kg) or vehicle treatment (Ctl) once daily for 7 days, and were sacrificed 2 h following the final treatment (n = 10–11 per group). To examine the effects of Y + I treatment on quiescent neural precursor populations (QNPs) in the hippocampal neurogenic niche, nestin-GFP reporter mice received Y + I or vehicle treatment (n = 5–6 per group) as described above and were sacrificed 24 h after the last injection. To assess the effects of 21 day imipramine treatment on the QNP pool, nestin-GFP mice were treated with imipramine (20 mg/kg) or vehicle once daily for 21 days and sacrificed 24 h after the last injection (n = 5–6 per group). The vehicle for all drugs was 0.9% saline, except for yohimbine in which case the vehicle was 10% DMSO. The choice of drug doses was based on previous studies (Sairanen et al., 2005; Yanpalwe et al., 2010), and all drugs were administered by intraperitoneal injection. All animals for gene expression analysis were sacrificed 2 h after the final injection. Nestin-GFP mice used for analysis of quiescent neural precursor populations were sacrificed 24 h following the last treatment.

2.2.2. Electroconvulsive seizure

To address the effects of electroconvulsive seizure (ECS) therapy on hippocampal gene expression, rats received ECS once daily via bilateral spring loaded ear clip electrodes (ECT unit, UGO Basile, Comerio, Italy) (Frequency: 100 pulses/s; pulse width: 0.9 ms; pulse duration: 0.5 s; current: 70 mA) or sham treatment (application of ear clip electrodes without electrical stimulation) for 7 consecutive days (n = 8–10 per group). All animals showed tonic clonic seizures in response to ECS administration. For gene expression analysis, rats were sacrificed 2 h after the final seizure administration. To examine the effects of ECS treatment on quiescent neural precursors (QNP), nestin-GFP mice (n = 3–4 per group) received either a single ECS or sham treatment and were sacrificed 24 h later.

2.3. Microarray

Microarray analysis was performed to examine global transcriptional changes in the hippocampi of Y + I treated animals as compared to vehicle-treated controls (n = 3 per group). Hippocampi were dissected, frozen in liquid nitrogen, and stored at −80 °C prior to use. Hippocampal RNA was extracted using an RNeasy Minikit (Qiagen, The Netherlands) with RNA quality control assessment performed using the optical density ratio of 260/280 nm (NanoDrop spectrophotometer, NanoDrop Technologies, GE Healthcare, UK) and RNA integrity analysis performed using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). RNA was labeled using the Agilent Quick Amp labeling kit (Agilent Technologies). The labeled RNA sample (800 ng) was fragmented and hybridized to a custom Rattus norvegicus array 8 × 60k (Agilent microarray design identifier: 028279). The hybridized slides were washed as per manufacturer’s instructions and scanned using the Agilent microarray scanner G, model G2505C, at 3 μm resolution. Data extraction was performed using the Agilent Technologies Feature Extraction software (version 10.5.1.1.). Data were then analyzed using GeneSpring GX, version 7.3.1, software from Agilent Technologies. Normalization of the data was done in GeneSpring GX using the one color per chip and per gene, data were as follows: (1) transformation: set measurements <0.01 to 0.01; (2) per chip: normalize to 50th percentile; (3) per gene: normalize to specific samples (control). Differentially regulated genes were filtered with a cutoff of >1.5 for upregulation and <0.66 for downregulation. Statistical analysis was done using a t test with a significance level of 0.05 and corrected for multiple comparisons using the Benjamini and Hochberg method (Benjamini and Hochberg, 1995). Hierarchical clustering was done based on fold change values for each gene using the GeneSpring software. Array data from Y + I treated animals as compared to vehicle treated controls have been deposited in the NCBI's Gene Expression Omnibus under the GEO series accession number GSE61301. Functional analysis of genes regulated by the Y + I treatment versus control was done using DAVID (Database for Annotation, Visualization, and Integrated Discovery; http://david.abcc.ncifcrf.gov/) functional annotation tool (Dennis et al., 2003; Huang et al., 2009).

2.4. Quantitative PCR

qPCR was performed to (1) validate candidate genes from our microarray analysis in an independent cohort of Y + I treated animals, and (2) to compare gene expression profiles following Y + I treatment with those evoked by the fast acting antidepressant, ECS and the slow-onset pharmacological antidepressant, imipramine (21 days I). Hippocampal tissue was dissected and RNA extraction was performed followed by quality control analysis as described in Section 2.3. RNA was then subjected to reverse transcription and subsequently qPCR analysis (Applied Biosystems, Thermo Fisher Scientific Corporation, USA). Quantification was performed using the ddCT method as described previously.
(Bookout and Mangelsdorf, 2003). For analysis of gene expression, data from all groups was normalized to the average of two endogenous housekeeping genes, namely Gapdh and Actb (β-actin), which were unaffected by all treatments used in the study. All primer sequences used in this study are listed in Supplementary Table 1. Results were compared to the respective control group (vehicle or sham) and reported as fold change ± S.E.M.

2.5. Immunofluorescence

Nestin-GFP reporter mice were sacrificed by transcardial perfusion with 4% paraformaldehyde (PFA) and their brains were removed and post fixed in PFA. Serial coronal sections (50 μm) across the rostro-caudal extent of the hippocampus (bregma: −1.58 to −2.80; Paxinos and Watson, 1998) were generated using a vibratome (Leica Microsystems, Wetzlar, Germany). For GFP/GFAP double immunofluorescence, four sections per animal from within similar bregma regions were taken. The sections were incubated in blocking solution containing 10% horse serum in 0.3% Triton X-100 in 0.1 M phosphate buffer (PB) for 2 h. The sections were then incubated in a primary antibody cocktail composed of rabbit anti-GFP (1:500; Invitrogen, Carlsbad, CA, USA) and mouse anti-GFAP (1:1000; Sigma) in 0.1 M PB containing 0.3% Triton X-100, overnight at room temperature. The secondary antibodies used were donkey anti-rabbit Alexa-488 (1:250; Molecular Probes, Invitrogen, Carlsbad, CA, USA) and donkey anti-mouse Alexa-547 (1:250; Molecular Probes). Sections were mounted in Vectashield (Vector Laboratories, Inc., Burlingame, CA, USA) and imaged on a confocal microscope (Zeiss LSM 5 exciter, Carl Zeiss, Oberkochen, Germany).

2.6. Cell counting analysis

To study the influence of Y + I, ECS or 21 day imipramine treatment was compared to their respective controls on the population of quiescent neural precursors (QNPs), the percent colocalization of nestin-GFP positive progenitors with the radial glial marker, glial fibrillary acidic protein (GFAP) was assessed using confocal analysis with z-plane sectioning at 1 μm steps on a Zeiss LSM 5 exctic confocal microscope. The percent colocalization of nestin-GFP and GFAP within the subgranular zone (SGZ) and granule cell layer (GCL) of the dentate gyrus (DG) hippocampal subfield was determined within the population of 35–40 GFP-immunopositive precursors selected at random per animal (4 sections/animal). All cell counting analysis was performed on coded sections by an experimenter blind to the treatment conditions.

2.7. Statistical analysis

Statistical analysis was performed using the software Instat (Graphpad Software Inc., USA). Experiments were subjected to the unpaired Student’s t test, with parametric distribution determined using the Kolmogorov–Smirnov test. Statistical significance was determined at p < 0.05. Welch corrections were applied to the t test for specific cases where the standard deviation was significantly different between groups.

3. Results

3.1. Short duration, combination yohimbine and imipramine treatment alters the hippocampal transcriptome

Short duration (7 days) combination treatment with the α2-adrenoceptor antagonist, yohimbine (Y) and the antidepressant, imipramine (I) has been previously reported to accelerate the neurogenic, neurotrophic and behavioral effects of imipramine (Yanpallewar et al., 2010). To examine the molecular targets that might underlie the rapid antidepressant-like cellular and behavioral effects of Y + I treatment, we performed a microarray analysis on hippocampi derived from Y + I treated animals (Fig. 1A). Y + I treatment resulted in the downregulation of 441 genes and upregulation of 155 genes in the hippocampus (Fig. 1B), with a preponderance of downregulated genes noted in the array. To identify the functional categories of genes enriched in the Y + I regulated hippocampal transcriptome, we subjected the microarray results to DAVID analysis (Dennis et al., 2003; Huang et al., 2009). DAVID analysis identified enriched gene sets under the functional categories of plasma membrane, intracellular signaling cascade, cellular metal ion homeostasis, neuropeptide signaling pathway and multicellular response to stress.

3.2. qPCR validation of hippocampal transcriptional changes evoked by Y + I treatment

The Y + I mediated regulation of several genes was validated using qPCR analysis in an independent cohort of Y + I treated animals.
Notably, we observed the downregulation of the mRNA expression of the α2A-adrenoceptor (Adra2a) and serotonin 5-HT2C receptor (Htr2c), which have both been linked to the speed of antidepressant response (Blier, 2003; Opal et al., 2013; Sanacora et al., 2004; Yanpallewar et al., 2010). Further, the inhibitory autoreceptor for somatostatin (Sstr1) which has been linked to antidepressant responses and mood-related behavior (Faron-Górecka et al., 2013; Nilsson et al., 2012) was downregulated in the hippocampi of Y + I treated animals. We also noted the downregulation of genes such as the regulator of G protein signaling 4 (Rgs4), which is implicated in determining the speed of desensitization of Go- and Gi-mediated signaling pathways (Diversé-Pierluissi et al., 1999). Other intracellular signaling pathway genes linked through clinical and preclinical studies to the pathogenesis of depression or suggested as targets for antidepressants and mood stabilizers such as inositol (myo)-1(or 4)-monophosphatase 2 (Impa2) (Jiménez et al., 2013); serum/glucocorticoid regulated kinase 1 (Sgk1) (Anacker et al., 2013; Conti et al., 2007; Frodl et al., 2012) and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (Nfkbia) (Gray et al., 2013) were found to be significantly changed in our qPCR studies. We noted a downregulation of iodothyronine deiodinase 3 (Dio3), which catalyzes the inactivation of thyroid hormone, a neurohormone that has been used in combination with antidepressants to accelerate and augment their therapeutic effects (Lifschytz et al., 2006). We observed the regulation of genes involved in cell proliferation, dendritic remodeling and synaptic reorganization such as alkaline ceramidase 2 (Acer2) (Mao and Obeid, 2008) and doublecortin-like kinase 2 (Dclk2) (Shin et al., 2013). The fold changes observed in the qPCR and microarray analysis and their significance levels are shown in Fig. 2. We noted a subset of genes, such as interleukin 1 beta (Il1b), phospholipase C epsilon (Plce1), RCAN3 family member 3 (Rcan3), and sema domain, immunoglobulin domain (Ig), short basic domain, secreted (Semadc),

(Fig. 2), indicating a strong reproducibility of the microarray results. Notably, we observed the downregulation of the mRNA expression of the α2A-adrenoceptor antagonist, yohimbine (Y) and the antidepressant, imipramine (I) (S, time-point for sacrifice). (B) Quantitative PCR (qPCR) analysis was performed on hippocampi derived from an independent cohort of Y + I and vehicle treated control animals (n = 9–10 per group) to confirm a subset of the hippocampal transcriptional changes observed with microarray analysis. Shown are the fold changes and p values for each gene based on the qPCR analysis and on the microarray data. Data are represented as mean ± S.E.M. of the fold change. Significance for qPCR results was determined at p < 0.05, unpaired Student’s t test, Welch corrections were applied for those genes where the standard deviations varied significantly between the two groups. Significance for microarray analysis was determined at p < 0.05, t test corrected for multiple comparisons.
that were regulated in the microarray but were not validated using qPCR analysis in an independent cohort.

3.3. Chronic ECS and chronic imipramine treatment also regulate a subset of Y + I transcriptional targets in the hippocampus

Given that the short duration, combination Y + I treatment has been previously reported to evoke antidepressant-like behavioral effects within 7 days of treatment, we next addressed whether the transcriptional targets of Y + I treatment in the hippocampus were also regulated by another fast-acting antidepressant treatment, chronic electroconvulsive seizure (ECS, 7 days). We performed qPCR on hippocampi derived from ECS and sham treated animals, and addressed the regulation of specific Y + I target genes validated by qPCR analysis in the ECS treated cohort as compared to their sham treated controls (Fig. 3).

Similar to Y + I treatment, chronic ECS also led to the downregulation of 5HT2c and an increased expression of Acer2 and Nfkbia. While the regulation of Impa2 and Sgk1 did not reach significance, we noted a strong trend towards downregulation for Impa2 (p = 0.055) and upregulation for Sgk1 (p = 0.064). Notably, the genes we observed transcriptionally repressed (5HT2c, Impa2) or enhanced (Acer2, Nfkbia, Sgk1) by Y + I treatment also showed a similar pattern of regulation following chronic ECS, with the exception of Rgs4 which was reduced following Y + I treatment and was enhanced by chronic ECS. We did not observe any change in the regulation of Adra2a, Dclk2, Dio3 or Sstr1 following chronic ECS. Taken together, these results indicate that specific genes which are regulated by short duration Y + I treatment are also transcriptionally regulated by the fast-action antidepressant paradigm of ECS.

We next examined whether the validated gene targets of short duration Y + I treatment are also responsive to regulation following chronic (21 days) or short duration (7 days) administration of imipramine. We performed qPCR analysis on hippocampi derived from chronic imipramine (21 days or 7 days) and their respective vehicle treated controls. For the 21 day chronic imipramine treatment we noted a significant decline in hippocampal Impa2 expression, with a trend towards a decline observed in Adra2a (p = 0.061) (Fig. 4A, B). We also observed a significant upregulation in Acer2, Nfkbia and Sgk1 expression (Fig. 4A, B) following both 21 day imipramine treatment. However, other validated Y + I target genes (Dclk2, Dio3, Htr2c, Rgs4, Sstr1) were not observed to be altered following chronic imipramine treatment (Fig. 4A, B). qPCR analysis revealed that 7 day imipramine treatment similar to Y + I treatment showed an upregulation of Acer2, Dclk2, Nfkbia and Sgk1, and in contrast to Y + I treatment evoked an opposing effect of an upregulation in Sstr1 gene expression (Fig. 4C, D). While Y + I treatment resulted in the robust downregulation of the transcripts of several genes (Adra2a, Dio3, Htr2c, Impa2, Rgs4) (Fig. 2) these were found to be unaffected by 7 day imipramine treatment (Fig. 4C, D). Interestingly, the pattern of regulation of the genes observed following 7 or 21 day imipramine treatment showed a degree of overlap with that observed following Y + I treatment. These results indicate that a subset of the Y + I target genes in the hippocampus are modulated in the same fashion following short duration (7 days) or chronic imipramine (21 days) treatment.

Taken together, these results indicate that amongst the Y + I validated gene targets are a subset of genes that also serve as targets for other behaviorally relevant antidepressant treatment regimes, suggesting certain common molecular pathways in the hippocampus that are influenced similarly by all three antidepressant treatments used in our study namely Y + I, ECS and 21 day I treatment (Fig. 5).

3.4. Y + I treatment, similar to ECS, enhances the quiescent neural precursor pool in the hippocampal neurogenic niche

Given that we observed an overlap between Y + I, ECS and 21 day I treatment in the pattern of transcriptional regulation evoked in the hippocampus, we next sought to address whether these treatments influence similar neural precursor pools within the hippocampal neurogenic niche. Previous studies indicate that while ECS enhances the Type 1/quiescent neural precursor (QNP) pool in the hippocampal neurogenic niche, pharmacological antidepressants do not appear to influence the QNPs and rather exert their effects on the Type 2/transit amplifying neural precursors (ANP) (Encinas et al., 2006; Segi-Nishida et al., 2008). We compared the influence of short duration Y + I treatment, acute ECS and 21 day I treatment on the QNP pool using nestin–GFP transgenic reporter mice. Nestin marks the quiescent neural precursors (QNPs) as well as transient amplifying neural precursors (ANPs), while GFAP (Glial Fibrillary Acidic Protein) is a radial glial marker co-expressed with nestin in the QNP pool (Kempermann et al., 2004). We assessed the percentage of GFP/GFAP double-positive precursors to estimate the QNP population (Fig. 6A).
We noted a significant increase in the percentage of nestin-GFP/GFAP double positive cells following 7 day Y + I treatment as compared to controls (Fig. 6B). Y + I treatment has been reported to enhance the proliferation of hippocampal progenitors (Yanpallewar et al., 2010), and our results extend these findings to indicate that Y + I treatment enhances the QNP pool. Further, acute ECS also enhances the QNP population as shown previously (Segi-Nishida et al., 2008), whereas a 21 day I treatment does not appear to influence this particular precursor population (Fig. 6C, D). Taken together, these findings indicate that Y + I treatment and ECS overlap in their effects on the QNP pool, distinct from chronic imipramine treatment.

4. Discussion

In the present study, we performed microarray analysis to determine the hippocampal transcriptional targets of the fast-acting combination treatment with the \( \alpha_2 \)-adrenoceptor antagonist yohimbine and the tricyclic antidepressant imipramine (Y + I). The Y + I hippocampal transcriptome revealed functional enrichment of genes associated with plasma membrane and intracellular signaling cascades, neuropeptide signaling pathways, cellular metal ion homeostasis, and multicellular responses to stress. A comparative analysis with fast-acting antidepressants like ECS and both a short duration (7 days) and chronic (21 days) imipramine treatment revealed a certain degree of overlap in gene regulation. Furthermore, Y + I treatment enhanced the number of QNPs within the hippocampal neurogenic niche, an effect similar to that evoked by ECS (Segi-Nishida et al., 2008). Together, our results provide novel evidence of global gene expression changes induced within the hippocampus following Y + I treatment, and highlight the existence of common transcriptional targets with both the fast-acting ECS treatment, as well as the slow-onset, chronic imipramine treatment.

Strikingly, our array and qPCR results revealed a significant decline in the hippocampal expression of the \( \alpha_2A \)-adrenoceptor (\( \text{Adra2a} \)) and the serotonin 5-HT2C receptor (\( \text{Htr2c} \)) within 7 days of Y + I treatment. Both these monoaminergic receptors exhibit enhanced receptor expression and/or binding within limbic brain regions in patients with depression and animal models of depression (Pandey et al., 2006; Rivero et al., 2014; Valdizán et al., 2010), and are downregulated and/or desensitized following chronic antidepressant treatment (Barbon et al., 2011; Invernizzi and Garattini, 2004; Subhash et al., 2003). This reduction in \( \alpha_2A \)-adrenoceptor expression and function has been hypothesized to regulate the therapeutic lag associated with the behavioral effects of antidepressants (Blier, 2003). Antagonists of the \( \alpha_2 \)-adrenoceptor and 5-HT2C receptors exhibit antidepressant and anxiolytic effects, and are reported to evoke faster behavioral effects (Dwyer et al., 2010; Muguruza et al., 2013; Opal et al., 2013; Quitkin et al., 2001). Given the importance of these monoaminergic receptors in regulating depression-like behavior, it is tempting to speculate that a rapid reduction in hippocampal \( \text{Adra2a} \) and \( \text{Htr2c} \) expression may contribute to the faster antidepressant-like effects noted with short duration Y + I treatment (Yanpallewar et al., 2010). In addition to the decline in specific monoaminergic receptors, we also observed a downregulation of the somatostatin receptor 1 (\( \text{Sstr1} \)) following short duration Y + I treatment.
This observation is intriguing, since somatostatin autoreceptor (Sstr1) modulation could regulate the release of somatostatin, a neuropeptide that exerts powerful anxiolytic and antidepressant-like effects (Engin et al., 2008). Antidepressants enhance hippocampal somatostatin levels (Pallis et al., 2009), and somatostatin autoreceptor antagonists evoke synergistic antidepressant-like behavioral effects when co-administered with imipramine (Nilsson et al., 2012). It is noteworthy that a short duration 7 day imipramine treatment, that does not exert antidepressant like behavioral effects (Yanpallewar et al., 2010), is not sufficient to result in a downregulation of Adra2a and Htr2c, and with regard to Sstr1 evokes a significant upregulation in contrast to 7 day Y + I treatment.

Besides altered regulation of plasma membrane-associated receptor expression, we observed significant enrichment of intracellular signaling pathway genes, with a decline in Rgs4 and an upregulation of Sgk1, Nfkbia and Dclk2 expression. Rgs4 modulates antidepressant-like behaviors with a positive regulation of the behavioral effects of diverse classes of antidepressant drugs observed in Rgs4 deficient mice (Stratinaki et al., 2013). Further, we noted increases in the expression of stress-associated signaling pathway genes such as Sgk1 and Nfkbia; perturbations in serum and glucocorticoid regulated kinase-1 (Sgk1) and NFkB signaling have been reported in patients and models of depression (Frodl et al., 2012; Pace et al., 2006). A transcriptional target of short duration Y + I treatment that may mechanistically contribute to rapid antidepressant effects is the type 3 iodothyronine deiodinase (Dio3), which catalyzes the inactivation of thyroid hormone. A decline in hippocampal expression of Dio3, could translate to increased local availability of thyroid hormone, which has been suggested as a mode to accelerate and augment antidepressant effects (Abraham et al., 2006; Lifschytz et al., 2006). Further, thyroid hormone is known to exert robust positive effects on adult hippocampal neurogenesis (Desouza et al., 2005; Kapoor et al., 2012), a process that is also targeted by short duration Y + I treatment, which increases progenitor proliferation and maturation (Yanpallewar et al., 2010).

Our results highlight a degree of overlap in gene regulation across the diverse treatment paradigms of Y + I, ECS and 21 day I, with a similar pattern of change noted in Sgk1, Nfkbia, Impa2 and Acer2. A short duration 7 day imipramine treatment overlapped with 7 day Y + I treatment in the pattern of upregulation of Acer2, Dclk2, Nfkbia and Sgk1, but did not overlap with regard to the downregulation of gene expression following Y + I treatment. A limited degree of overlap was noted in the regulation of monoaminergic receptor expression, wherein Y + I and ECS exhibited a decline in Htr2c expression, and Y + I and 21, but not 7 day, I treatments, induced a downregulation of Adra2a mRNA levels. It is noteworthy that the directionality of change in gene expression for overlapping targets remained the same across all treatments, with the exception of Rgs4 and Sstr1 which showed an opposing pattern following Y + I and ECS treatment and Y + I and 7 day I treatment respectively. Amongst the genes robustly upregulated by all treatment paradigms is alkaline ceramidase Acer2, involved in the enzymatic breakdown of ceramides. Studies indicate that depressed individuals, as well as animal models of stress, exhibit abnormally high ceramide levels (Gracia-Garcia et al., 2011; Gubins et al., 2013). Ceramide formation pathways are downregulated by antidepressants, and ceramidase-deficient mice with elevated hippocampal ceramide levels display depression-like behaviors and decreased hippocampal neurogenesis (Gubins et al., 2013). Lowering ceramide production may be a putative target for antidepressant drug development (Kornhuber et al., 2014). It is noteworthy that we observe a robust upregulation of Acer2, in the diverse antidepressant treatment paradigms used in our study. Although alkaline ceramidase 2 per se has thus far not been reported to be targeted by antidepressant treatments, our results highlight that this enzyme is a major transcriptional target of diverse antidepressant treatments, suggesting the possibility that increased ceramide catabolism may contribute to antidepressant-like effects.

Further, we also noted in common across all the treatment paradigms an upregulation of Sgk1 and Nfkbia. Sgk1, a serine/threonine protein kinase implicated in cellular stress responses, has previously been reported to show lowered expression in the hippocampi of patients with major depressive disorder, with a correlation between the degree of decline in expression and reduced hippocampal volume noted in these patients (Frodl et al., 2012). Our results corroborate a previous report that Sgk1 transcripts are enhanced following diverse antidepressant treatment modalities such as sleep deprivation, ECS and chronic fluoxetine administration (Conti et al., 2007). Nfkbia has a neuroprotective and anti-inflammatory role (Sharman et al., 2007) likely through the inhibition of NF-kappa-B, a transcription factor involved in cellular inflammatory stress responses. The regulation of this gene is interesting in light of evidence that NFkB-mediated inflammatory signaling is exacerbated in mood disorders (Bierhaus et al., 2003; Pace et al., 2006) and specific antidepressant treatments inhibit NFkB signaling in limbic brain regions (O'Sullivan and Ryan, 2009).

Short duration Y + I treatment has been reported to accelerate the neurogenic effects of the antidepressant imipramine (Yanpallewar et al., 2010). While distinct classes of antidepressants such as ECS and imipramine share in common a stimulatory effect on adult hippocampal

![Fig. 5. Comparison of transcriptional changes evoked by the Y + I, ECS and chronic imipramine treatments. Shown is a heat map to illustrate the directionality and significance of gene regulation evoked by Y + I, ECS and 21 day imipramine treatment. The data are expressed as fold change over respective control/sham treated groups. Genes that showed either a significant regulation ($p < 0.05$) or a strong trend towards significance ($p < 0.07$) are shown color coded to indicate nature of change in gene expression. Genes that did not show a significant change in mRNA expression have been represented as not regulated (white). Y + I, ECS and I treatment showed a strong overlap in the hippocampal regulation of Acer2, Impa2, Nfkbia, and Sgk1.](image-url)
neurogenesis (Malberg et al., 2000), a process implicated in their behavioral effects (Santarelli et al., 2003), the progenitor subtype influenced by these treatments differs. While acute and chronic ECS enhance the turnover of quiescent neural progenitors (QNPs), the putative stem cell of the hippocampal neurogenic niche (Segi-Nishida et al., 2008), pharmacological antidepressants target the transit amplifying progenitor pool (ANPs), which are lineage-determined progenitor cells with a limited proliferative capacity (Encinas et al., 2006). Strikingly, our results indicate that short duration Y + I treatment enhances the number of QNPs, similar to ECS but distinct from the slow-onset 21 day I treatment. We have previously shown that α2-adrenoceptor activation reduces the turnover of QNPs (Jhaeri et al., 2014), and our present findings indicate that combined α2-adrenoceptor blockade along with a classical antidepressants can result in enhanced turnover of QNPs in the adult hippocampal neurogenic niche. This is particularly intriguing since it suggests that such a combination treatment with adjunct α2-adrenoceptor antagonism shifts classical antidepressants towards targeting putative stem cells within the hippocampal neurogenic niche in common with the fast-acting ECS paradigm.

5. Conclusion

Taken together, our results provide novel evidence of the transcriptional targets of treatments that combine α2-adrenoceptor antagonism with a classical antidepressant drug. A comparative analysis to assess whether gene targets regulated by Y + I treatment are also regulated by the fast-acting ECS and the slow-onset chronic imipramine treatments highlighted specific targets (Sgk1, Nfkbia, Impa2 and Acer2) that were commonly regulated across all paradigms. Furthermore, Y + I treatment enhanced the numbers of QNPs within the hippocampal neurogenic niche, an effect in common with ECS. Our findings identify putative leads for the development of rapid action antidepressants, and raise the intriguing possibility that adjunct α2-adrenoceptor antagonism may shift classical antidepressant drugs towards exhibiting molecular and cellular actions in common with the fast-acting ECS paradigm.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.pnpbp.2015.03.004.

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