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Effect of Reboxetine Pretreatment on the Forced Swimming Test-induced Gene Expression Profile in the Rat Lateral Septum

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Abstract

The forced swim test (FST) is the most widely used model for assessing potential antidepressant activity. Although it has been shown that lateral septum is involved with the FST-related behavior, it is not clear whether antidepressant treatments could alter the FST-induced gene expression profile in the lateral septum. In the present study, the gene expression profiles in response to FST and reboxetine pretreatment were observed in the lateral septum of rats. Reboxetine is known as a most selective serotonin norepinephrine reuptake inhibitor. In addition, we compared the changes in gene expression profile between reboxetine response and nonresponse groups, which were determined by counting FST-related behavior. After FST, lateral septum from controls and reboxetine pretreated group were dissected and gene expression profiles were assessed using an Affymetrix microarray system containing 15,923 genes. Various genes with different functions were changed in reboxetine response group compared with reboxetine nonresponse group, In particular, pleiotrophin, orexin receptor 2, serotonin 2A receptor, neuropeptide Y5 receptor and thyroid hormone receptor β were decreased in reboxetine response group, but Lim motif-containing protein kinase 1 (Limk1) and histone deacetylase 1 (HDAC1) were increased. Although further studies are required for direct roles of these genes in reboxetine response, the microarray may provide tools to find out potential target genes and signaling pathways in antidepressant response.

Keywords: Microarray, Forced swimming test, Reboxetine, G-protein coupled receptor, Norepinephrine

Depression is one of the most common psychiatric disorders and an estimated 10-15% of people may become depressed during their lives[1]. Antidepressants are used to treat depressed patient, but one third or more of patients do not respond to treatment[2]. As with other diseases, approximations of both the disorder and the actions of corrective medications in laboratory animals are essential for the development of new effective drugs. In the field of experimental depression research, the forced swimming test (FST) is a widely used behavioral paradigm, which predicts the efficacy of antidepressant treatments[3]. This is largely due to its ease of use, reliability across laboratories, ability to detect a broad spectrum of antidepressants, and its capacity to meet the high-throughput demands of the pharmaceutical industry[4]. However, the major drawback of the traditional FST is that it is unreliable in the detection of the effects of selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SSRIs)[5], which are the most widely prescribed antidepressant drugs today. In an effort to enhance the sensitivity of the traditional FST in the rat to detect the efficacy of SSRI, several simple procedural modifications have been made[5,6]. These developments include increasing the water depth to 30 cm from traditional depths of 15-18 cm, and using a time sampling technique to rate the predominant behavior over a 5-s interval. Moreover, it is possible to predict whether tested antidepressants act through

norepinephrine or serotonin, based on the FST-related specific behavior. In fact, antidepressants that primarily potentate 5-HT-mediated neurotransmission increases swimming behavior whereas those with primary actions through catecholamines (such as norepinephrine) increase climbing behavior.

Lateral septum plays an important role in regulating mood and motivation[7]. It has been shown that the lateral septum is activated by various types of stress as indicated by increased expression of c-Fos, activity-regulated cytoskeleton-associated protein (ARC) and other transgene[8-11]. Among brain regions that are related to FST, lateral septum is also one of the most prominent regions in which FST increases c-Fos and 2-deoxyglucose uptake (2-DG)[12]. Interestingly, FST-induced increase in 2-DG uptake in lateral septum was blocked by imipramine treatment[12]. In addition, repeated paroxetine treatment also inhibits the FST-induced increase in c-Fos in the lateral septum[13]. These results suggest that the lateral septum is one of relevant areas in the FST-related behavior and that antidepressant pretreatment prior to FST may exert antidepressant effect via blocking the changes of gene expression in the lateral septum. This possibility is further supported by the fact that vasopressin V1b receptor antagonist exert it antidepressant drug action through V1b receptors located in the lateral septum[14].

However, it is not clear at present whether antidepressant pretreatment prior to the FST changes the gene expression profile in the lateral septum. In particular, changes in gene expression profile of antidepressant response have not been studied. Reboxetine is antidepressant and is known as the most selective norepinephrine reuptake inhibitor[15]. In the present study, we treated animals with reboxetine prior to FST and then divided animals into the response and nonresponse groups based on behavioral response during FST. To understand the gene expression profile of the lateral septum in each group in detail, we performed cDNA microarray using Affymetrix oligonucleotide microarrays.

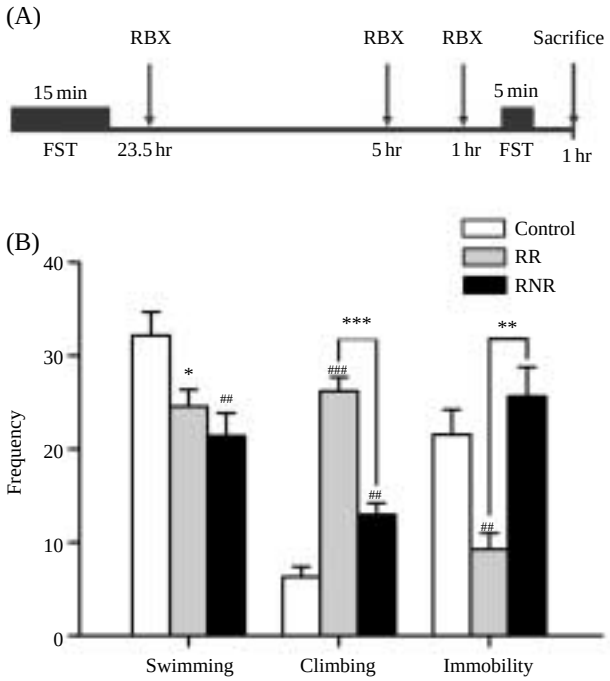

Figure 1. Effects of reboxetine pretreatment on forced swimming test (FST)-induced behavior. A: Schematic figure of reboxetine pretreatment and FST procedures. Pretest session of FST was applied for 15 min at the first day and test session of FST was tried 24 h later. Reboxetine (10 mg/kg, i.p.) was injected at 23.5, 5 and 1 h before test session of FST (5 min) and the predominant behavior at 5-s interval for 5 min was measured during the FST. Controls received saline instead of reboxetine prior to test session of FST. B: The data represent mean ± standard error of mean of swimming, climbing and immobility (n=6 per group). $^{\#}P<0.05$, $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ vs. controls, as assessed by one-way ANOVA followed by the *post hoc* Fisher's least significant difference (LSD) tests. $^{**}P<0.01$ and $^{***}P<0.001$ between RR and RNR groups. Abbreviations used: RR, reboxetine response group; RNR, reboxetine nonresponse group.

The Effects of Reboxetine on the FST-related Behaviors

Reboxetine pretreatment significantly increased the climbing frequency in both reboxetine response (RR) and reboxetine nonresponse (RNR) groups ($F_{2,15}=61.724$, $P<0.001$). Climbing frequency in RR group was significantly higer than that in RNR group, with both being higher than control group (Figure 1B). Reboxetine pretreatment significantly decreased immobility frequency in RR group, whereas it did not decrease immobility frequency in RNR group. The decrease in immobility frequency in RR group was caused by increased climbing frequency, since swimming frequency in RR group was lower than that in control group (Figure 1B).

Generation of Microarray Gene Expression Data

Changes in rat lateral septum gene expression during FST test were broadly evaluated using oligonucleotide-based Affymetrix microarrays representing close to 15,923 named genes. These arrays were used to probe labeled cRNA derived from microdissected lateral septum samples from control, RR and RNR groups. We used Genespring array tools software to filter the 15,923 genes. After sample scanned with a laser scanner, primary image condensation was per-

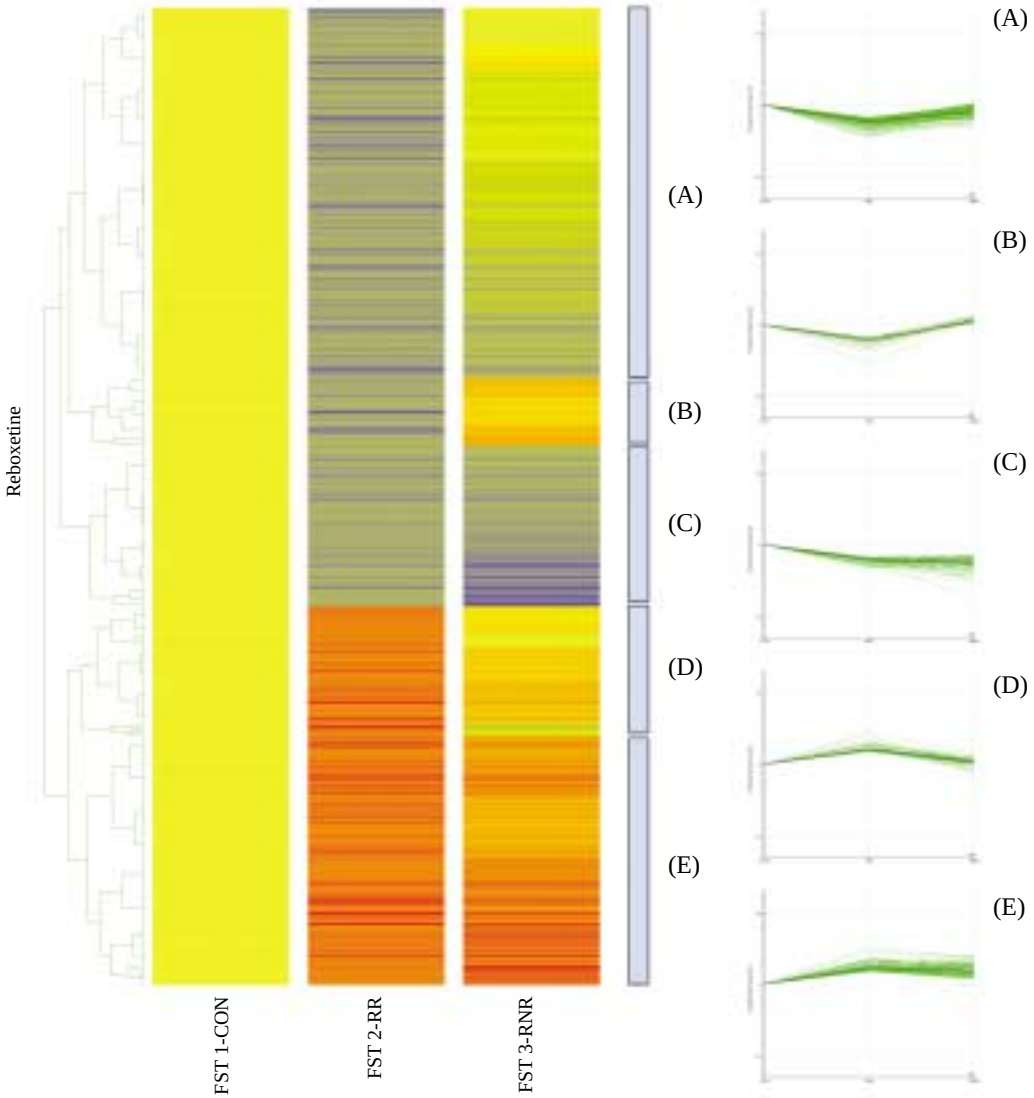

Figure 2. Cluster analysis of gene expression profiles in the lateral septum of saline and reboxetine treated rats. 485 genes were grouped into five clusters (A-E) according to their pattern of expression. For each gene, the log ratio of expression at the indicated RR and RNR after reboxetine pretreatment is represented by the pseudo-color scale at the bottom of the figure. The dendrogram on the left side of the cluster shows the statistical relatedness of the genes in the cluster, with shorter branches representing closer relationships between genes. The black line graphs on the right show the average ratio profiles for the genes and green line graphs on the right show the ratio profiles for the genes in the corresponding cluster (n=6 per group).

formed with the Genechip software version 4.0 (Affymetrix), and expression values for all chips were scaled to a target intensity of 200. Samples were evaluated for quality by comparison of percentage present values as well as 5′ to 3′ ratios of glyceraldehyde-3-phosphate dehydrogenase and actin. Gene probe sets were removed if they were called absent at all four separate experiments in FST. We found 9,507 genes that were hierarchical cluster algorithm.

Gene Expression Differences in RR and RNR Rat

We divided animals into RR and RNR groups according to their responses to FST. We filtered out genes which did not vary at least 1.5-fold from the log of the mean of the first filter in at least 60% of the gene expressed. Initially, we found 485 genes that were significantly affected by the RR following the FST. These genes were clustered using Genespring 7.0 (Figure 2). We relied on the ontology database (Go

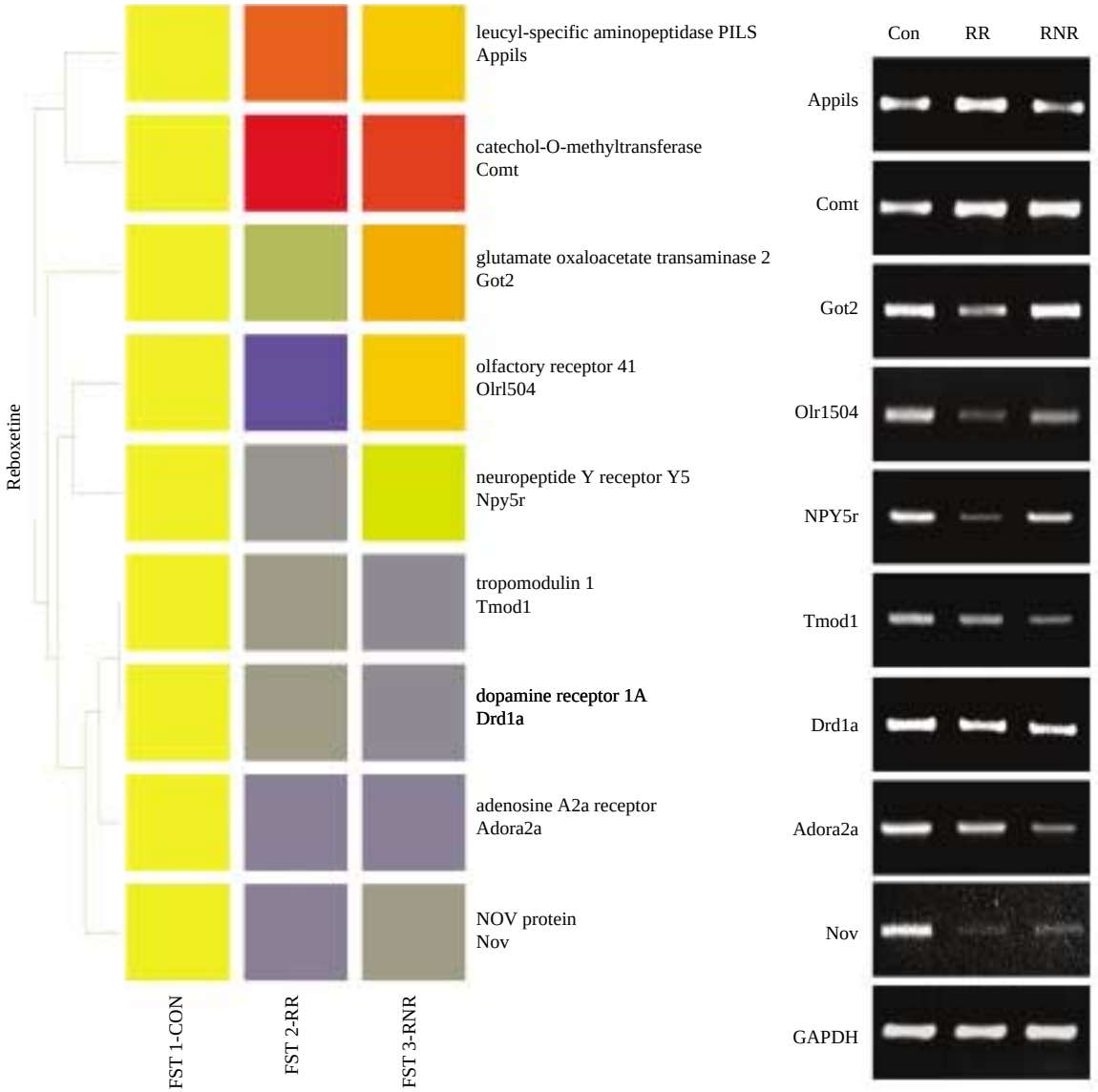

Figure 3. Gene tree containing the 9 genes that were differentially regulated after reboxetine pretreatment (left panel). Reverse transcriptase-polymerase chain reaction (RT-PCR) amplification of 9 genes from lateral septum RNA after reboxetine pretreatment (right panel). The PCR products obtained from control group (lane 1, Con), reboxetine response group (lane 2, RR), and reboxetine nonresponse group (lane 3, RNR) were separated on an agarose gel.

database). Although the ontology system provides information that can be used to quantify over- or under-representation of identified genes relative to total microarray genes within a functional category, it does not provide a functional designation for all genes. All genes differentially expressed were clustered based on biological relevance.

Custer A included 184 genes that were significantly down regulated in the RR group, but were not changed or decreased in the RNR group. Among genes changed, 45 genes based on functional relevance are described in Table 1. In brief, various functions were involved in cluster A such as apoptosis, metabolism, cell adhesion, cell communication, synaptic transmission, regulation of synaptic plasticity, proliferation, transport, development (including nervous system development), regulation of cell growth, neuron differentiation, response to stress, and signal transduction.

Cluster B included 33 genes that were significantly down regulated in the RR group, but not significantly regulated in the RNR group. Among genes changed, 15 genes based on functional relevance are described

Table 1. List of significantly down regulated genes in reboxetine response group (cluster A) after reboxetine treatment.

Chip No.	Description	Common name	Function class	Fold change[a]	
				RR	RNR
1369084_a_at	Bcl-2-related ovarian killer protein	Bok	apoptosis	0.604	0.766
1387166_at	aryl hydrocarbon receptor-interacting protein-like 1	Aipl1	apoptosis	0.498	0.651
1373062_at	Sulfatase 1	Sulf1	apoptosis, metabolism	0.637	0.901
1369407_at	tumor necrosis factor receptor, superfamily member 11b (osteoprotegerin)	Tnfrsf11b	apoptosis, signal transduction	0.652	1.012
1371588_at	Parvin, alpha	Parva	cell adhesion	0.665	0.732
1374529_at	Thrombospondin 1	Thbs1	cell adhesion	0.536	0.893
1373717_at	Opioid-binding protein/cell adhesion molecule-like	Opcml	cell adhesion	0.579	0.869
1369609_at	claudin 11	Cldn11	cell adhesion, transport	0.66	0.921
1368926_at	semaphorin 4f	Sema4f	cell communication, nervous system development	0.64	0.881
1368912_at	thyrotropin releasing hormone	Trh	cell communication, signal transduction	0.665	0.91
1388057_a_at	discs, large (Drosophila) homolog-associated protein 1	Dlgap1	cell communication, synaptic transmission	0.613	1.001
1368924_at	growth hormone receptor	Ghr	cell differentiation	0.517	0.814
1388999_at	Transcription factor 12	Tcf12	immune response, development	0.546	0.869
1387538_at	acetyl-coenzyme A carboxylase alpha	Acaca	metabolism	0.656	0.821
1370370_at	hyaluronidase 2	Hyal2	metabolism	0.64	0.947
1368095_at	adenylate kinase 3	Ak3	metabolism	0.604	0.823
1367829_at	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	Echs1	metabolism	0.577	0.739
1367806_at	glutaminase	Gls	metabolism	0.587	0.679
1369968_at	pleiotrophin	Ptn	nervous system development	0.609	0.957
1369351_at	contactin 3	Cntn3	nervous system development	0.593	0.825
1387036_at	hairy and enhancer of split 1 (Drosophila)	Hes1	nervous system development	0.497	0.722
1376734_at	Nephroblastoma overexpressed gene	Nov	regulation of cell growth	0.445	0.555
1369886_a_at	calcium binding protein 1	Cabp1	regulation of synaptic plasticity	0.581	0.906
1369271_at	protein kinase, AMP-activated, beta 2 non-catalytic subunit	Prkab2	response to stress, signal transduction	0.587	0.813
1373443_a_at	tyrosine hydroxylase	Th	response to stress, synaptic transmission	0.614	1.014
1369377_at	hypocretin (orexin) receptor 2	Hcrtr2	signal transduction	0.665	0.908
1369124_at	5-hydroxytryptamine (serotonin) receptor 2A	Htr2a	signal transduction	0.665	0.883
1368506_at	regulator of G-protein signaling 4	Rgs4	signal transduction	0.654	0.716
1368849_at	casein kinase 1, gamma 3	Csnk1g3	signal transduction	0.642	0.765
1387484_at	transforming growth factor, beta receptor III	Tgfbr3	signal transduction	0.638	0.882
1369917_at	neurotrophin receptor associated death domain	Nradd	signal transduction	0.603	0.995
1386963_at	thyroid hormone receptor interactor 10	Trip10	signal transduction	0.594	0.815
1388080_a_at	histamine receptor H3	Hrh3	signal transduction	0.498	0.581
1369860_a_at	5-hydroxytryptamine (serotonin) receptor 2C	Htr2c	signal transduction	0.469	0.558
1369102_at	mitogen activated protein kinase 10	Mapk10	signal transduction	0.421	0.562
1387497_at	neuropeptide Y receptor Y5	Npy5r	synaptic transmission, signal transduction	0.5	0.849
1387569_at	synaptic vesicle glycoprotein 2c	Sv2c	transport	0.647	0.741
1369500_at	potassium channel, subfamily K, member 1	Kcnk1	transport	0.624	0.767
1370464_at	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	Abcb1a	transport	0.58	0.789

Table 1. Continued.

Chip No.	Description	Common name	Function class	Fold change[a]	
				RR	RNR
1398855_at	ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit b, isoform 1	Atp5f1	transport	0.557	0.698
1387941_s_at	phospholipase A2, group VI	Pla2g6	transport	0.498	0.69
1369700_at	chloride channel 7	Clcn7	transport	0.39	0.517
1369798_at	ATPase, Na^+/K^+ transporting, beta 2 polypeptide	Atp1b2	transport	0.619	0.841
1387441_at	potassium channel, subfamily K, member 3	Kcnk3	transport	0.527	0.712
1370602_at	ATPase, Ca^{++} transporting, plasma membrane 4	Atp2b4	transport, metabolism	0.558	0.796

[a]The fold change was calculated as 1.5^{SLR}, with SLR being the signal log ration

Table 2. List of significantly down regulated genes in reboxetine response group (cluster B) after reboxetine treatment.

Chip No.	Description	Common name	Function class	Fold change[a]	
				RR	RNR
1370770_s_at	kit ligand	Kitl	cell proliferation	0.613	1.212
1387644_at	betacellulin	Btc	cell proliferation	0.651	1.276
1368167_at	cathepsin E	Ctse	immune response	0.661	1.056
1387983_at	thyroid hormone receptor beta	Thrb	metabolism	0.63	1.179
1387491_at	glycerol kinase	Gyk	metabolism	0.549	1.074
1389871_at	glutamate oxaloacetate transaminase 2	Got2	metabolism	0.66	1.34
1371184_x_at	Tropomyosin 3, gamma	Tpm3	regulation of muscle contraction	0.627	1.17
1370412_at	troponin T1, skeletal, slow	Tnnt1	regulation of muscle contraction	0.629	1.174
1373112_at	Muscle, intestine and stomach expression 1	Mist1	signal transduction	0.528	1.091
1375789_at	Parathyroid hormone receptor 1	Pthr1	signal transduction	0.523	1.224
1387488_a_at	calcitonin receptor	Calcr	signal transduction	0.661	1.229
1388091_at	olfactory receptor 1500	Olr1500	signal transduction	0.289	1.14
1370079_at	Rhesus blood group CE and D	Rhced	transport	0.649	1.049
1370076_at	potassium inwardly-rectifying channel, subfamily J, member 16	Kcnj16	transport	0.41	1.26
1368636_at	cytochrome P450, family 27, subfamily b, polypeptide 1	Cyp27b1	transport	0.492	1.336

[a]The fold change was calculated as 1.5^{SLR}, with SLR being the signal log ration

in Table 2. These genes are involved with diverse aspects of biological functions such as cell proliferation, immune response, transport and signal transduction.

Cluster C included 80 genes that were down-regulated by reboxetine, suggesting that these genes are regulated by reboxetine irrespective of antidepressant response during FST. Among genes changed, 31 genes are described in Table 3. The down-regulated genes in cluster C are involved in different functions such as nervous system development, metabolism, signal transduction, cell proliferation, apoptosis, transport, immune response and response to stress.

Cluster D included 64 genes that were significantly up-regulated in the RR group, but were not significantly changed in the RNR group. Among genes changed, 13 genes based on functional relevance are described in Table 4. The up-regulated genes in the RR group are involved in different functions such as apoptosis, cell adhesion, development (including nervous system development), cell proliferation, immune response, metabolism, signal transduction and transport.

Cluster E included 124 genes that were up-regulated by reboxetine irrespective of antidepressant response. This result suggests that these genes are regulated by reboxetine pretreatment, but did not represent the genes which are related to antidepressant

Table 3. List of significantly down regulated genes in reboxetine response and reboxetine nonresponse groups (cluster C) after reboxetine treatment.

Chip No.	Description	Common name	Function class	Fold change[a]	
				RR	RNR
1368771_at	sulfatase 1	Sulf1	apoptosis, metabolism	0.528	0.549
1369309_a_at	tachykinin 1	Tac1	cell communication, synaptic transmission	0.582	0.422
1368301_at	adenosine A2a receptor	Adora2a	cell communication, transport	0.431	0.441
1387270_at	hematopoietically expressed homeobox	Hhex	cell differentiation	0.63	0.631
1369025_at	CD5 antigen	Cd5	cell proliferation, signal transduction	0.611	0.573
1371017_at	T-cell receptor gamma chain	Tcrg	immune response	0.602	0.526
1387707_at	solute carrier family 2 (facilitated glucose transporter), member 3	Slc2a3	metabolism, transport	0.506	0.565
1371108_a_at	ATPase, Na^+/K^+ transporting, alpha 1 polypeptide	Atp1a1	metabolism, transport	0.602	0.582
1393480_at	protein phosphatase 1, regulatory (inhibitor) subunit 2	Ppp1r2	nervous system development	0.498	0.354
1374235_at	Down syndrome critical region gene 1-like 1	Dscr1l1	nervous system development	0.649	0.646
1369544_a_at	homeo box A1	Hoxa1	nervous system development	0.655	0.654
1368479_at	dopamine receptor 1A	Drd1a	nervous system development, synaptic transmission	0.539	0.477
1368982_at	protein kinase inhibitor, alpha	Pkia	regulation of protein kinase activity	0.655	0.541
1369078_at	mitogen activated protein kinase 1	Mapk1	response to stress, signal transduction	0.539	0.515
1387241_at	G-protein coupled receptor 88	Gpr88	signal transduction	0.643	0.528
1369129_at	RAS guanyl releasing protein 1	Rasgrp1	signal transduction	0.559	0.591
1368319_a_at	homer homolog 1 (Drosophila)	Homer1	signal transduction	0.518	0.464
1369614_at	RAP2B, member of RAS oncogene family	Rap2b	signal transduction	0.66	0.431
1369674_at	purinergic receptor P2X, ligand-gated ion channel, 5	P2rx5	signal transduction	0.615	0.639
1369882_at	prodynorphin	Pdyn	synaptic transmission	0.42	0.302
1369541_at	tropomodulin 2	Tmod2	synaptic transmission	0.596	0.644
1387720_at	calsyntenin 2	Clstn2	synaptic transmission	0.526	0.542
1387054_at	ATP-binding cassette, sub-family G (WHITE), member 1	Abcg1	transpor	0.665	0.68
1369099_at	solute carrier family 30 (zinc transporter), member 1	Slc30a1	transport	0.63	0.503
1368400_at	translocase of inner mitochondrial membrane 8 homolog a (yeast)	Timm8a	transport	0.479	0.487
1388059_a_at	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	Slc11a2	transport	0.608	0.532
1368864_at	synaptoporin	Synpr	transport	0.652	0.572
1388172_at	integral membrane transport UST1r	Ust1r	transport	0.61	0.626
1371103_at	RAB6A, member RAS oncogene family	Rab6a	transport	0.643	0.623
1370121_at	adducin 1 (alpha)	Add1	transport	0.659	0.615
1370662_a_at	adaptor-related protein complex 2, beta 1 subunit	Ap2b1	transport	0.585	0.573

[a]The fold change was calculated as 1.5^{SLR}, with SLR being the signal log ration

response. Among genes changed, 23 genes based on functional relevance are described in Table 5. These genes in cluster E are involved in different functions such as apoptosis, signal transduction, cell adhesion, cell differentiation, immune response, development (including nervous system development), metabolism, signal transduction and transport.

Taken together, these results suggest that genes in

Table 4. List of significantly upregulated genes in reboxetine response group (cluster D) after reboxetine treatment.

Chip No.	Description	Common name	Function class	Fold change[a]	
				RR	RNR
1388193_at	huntingtin interacting protein 1	Hip1	apoptosis	1.62	1.043
1388761_at	histone deacetylase 1 (predicted)	Hdac1_predicted	apoptosis, development	1.51	1.079
1388140_at	RAB13, member RAS oncogene family	Rab13	cell adhesion, signal transduction	1.574	1.091
1376425_at	Transforming growth factor, beta 2	Tgfb2	cell proliferation, immune response	1.58	1.122
1367786_at	proteosome (prosome, macropain) subunit, beta type 8	Psmb8	immune response	1.827	1.18
1399161_a_at	type 1 tumor necrosis factor receptor shedding aminopeptidase regulator	Arts1	immune response, cell differentiation	1.968	1.161
1387566_at	phospholipase A2, group IVA (cytosolic, calcium-dependent)	Pla2g4a	metabolism	1.511	1.11
1386985_at	glutathione S-transferase, mu 1	Gstm1	metabolism	1.634	1.021
1370385_at	phospholipase A2, group VI	Pla2g6	metabolism, transport	1.841	0.985
1369149_at	LIM motif-containing protein kinase 1	Limk1	nervous system development, signal transduction	1.878	1.176
1374324_at	Prostaglandin E receptor 1	Ptger1	signal transduction	1.812	0.78
1388078_a_at	amiloride-sensitive cation channel 2, neuronal	Accn2	transport	1.655	1.166
1367688_at	secretory carrier membrane protein 4	Scamp4	transport	1.569	1.022

[a]The fold change was calculated as 1.5^{SLR}, with SLR being the signal log ration

cluster B and cluster D are specifically changed in reboxetine response group, whereas genes in other clusters represent genes regulated by reboxetine pretreatment irrespective of antidepressant response during the FST.

RT-PCR and Pathway

Using reverse transcription-PCR (RT-PCR), we verified the significant changes of the expression of a subset of highly changed genes (Appls, Comt, Got2, Olr1500, Npy5r, Tmod1, Drd1a, Adora2a and Nov) in lateral septum. Similar changes to those observed in microarray system were observed. Primers used in this study are described in Table 6.

Discussion

FST has a great utility in dectecting known and novel antidepressant drugs[16,17]. However, few studies have examined neurochemical correlates of behavioral responses in the FST model. In the present study, we showed that reboxetine elicited an antidepressant activity in the FST as previously reported[18-21]. However, marked inter-individual differences were observed in the behavioral responses in the FST. RNR rats showed a significant increase in passive behavior (immobility) and a decrease in active behaviors (swimming and climbing), which was exactly opposite to RR rats. Similarly, during cDNA microarray procedures, a number of different genes were found to be responsive to reboxetine. The functional implication of regulation of several genes is described in more detail below.

First of all, several downregulated genes in cluster A and cluster B of RR group are interesting. Pleiotrophin is a member of neurite growth-promoting factor (NEGF) family that is highly expressed during embryonic and perinatal neural development[22]. Pleiotrophin is involved with neurite outgrowth promoting factor in rat brain and promotes the survival of dopaminergic neurons in embryonic mesencephalic cultures[23]. Moreover, pleiotropin promotes the production of dopaminergic neurons and increases tyrosine hydroxylase-positive neurons from embryonic stem cells[24]. On the contrary, remarkable upregulation of the enzymes of catecholamine biosynthetic pathway, including tyrosine hydoxylase, DOPA decarboxylase, and dopamine-β-hydroxylase but not phenylethanolamine-N-methyltransferase (PNMT) was observed in aorta of pleiotrophin knockout mice[25]. However, this upregulation of the enzymes of catecholamine biosynthetic pathway in pleiotrophin knockout mice may be the result of a compensatory response to the absence of norepinephrine[25]. Taken together, pleiotropin may be related to upregulate tyrosine hydroxylase in brain[24]. Thus, decreased tyrosine hydroxylase expression in RR group, in the present study, may reflect the decreased pleiotropin expression. In addition, as tyrosine hydroxylase is a rate-limiting enzyme in bio-

Table 5. List of significantly upregulated genes in reboxetine response and reboxetine nonresponse groups (cluster E) after citalopram treatment.

Chip No.	Description	Common name	Function class	Fold change[a]	
				RR	RNR
1369943_at	transglutaminase 2, C polypeptide	Tgm2	apoptosis	1.688	1.425
1390426_at	Notch gene homolog 1 (Drosophila)	Notch1	apoptosis, immune response	1.563	1.313
1387168_at	lymphocyte antigen 68	C1qr1	cell adhesion	1.567	1.405
1383075_at	cyclin D1	Ccnd1	cell differentiation	1.61	1.805
1370957_at	interleukin 6 signal transducer	Il6st	cell growth, immune response	1.563	1.857
1370105_at	lunatic fringe gene homolog (Drosophila)	Lfng	development	1.55	1.276
1368332_at	guanylate nucleotide binding protein 2	Gbp2	immune response	1.754	1.478
1371152_a_at	2′,5′-oligoadenylate synthetase 1, 40/46 kDa	Oas1	immune response	1.522	1.349
1387969_at	chemokine (C-X-C motif) ligand 10	Cxcl10	immune response, cell proliferation	1.846	2.148
1368826_at	catechol-O-methyltransferase	Comt	metabolism	2.722	2.34
1369663_at	epoxide hydrolase 2, cytoplasmic	Ephx2	metabolism	1.734	1.855
1387376_at	aldehyde oxidase 1	Aox1	metabolism, transport	1.753	1.693
1390682_at	Rnd2	rapostlin	nervous system development	1.662	1.623
1368065_at	regulator of G-protein signaling 19 interacting protein 1	Rgs19ip1	Signal transduction	1.509	1.227
1398778_at	proteasome (prosome, macropain) subunit, alpha type 1	Psma1	transport	1.875	1.35
1370031_at	golgi SNAP receptor complex member 2	Gosr2	transport	1.557	1.786
1369144_a_at	potassium voltage gated channel, Shal-related family, member 3	Kcnd3	transport	1.533	1.579
1369679_a_at	nuclear factor I/A	Nfia	transport	1.889	1.511
1392903_at	Synaptobrevin-like 1	Sybl1	transport	1.772	2.228
1371029_at	polycystic kidney disease 1 homolog	Pkd1	transport	2.127	1.794
1367636_at	insulin-like growth factor 2 receptor	Igf2r	transport, signal transduction	1.836	2.116

[a]The fold change was calculated as 1.5^{SLR}, with SLR being the signal log ration

synthesis of catecholamine such as dopamine and norepinephrine, decreased tyrosine hydroxylase expression in RR group may represent a compensatory mechanism in response to increased norepinephrine availability by reboxetine pretreatment before the FST, thus limiting norepinephrine turnover around the lateral septum. This result raise the possibility that increased norepinephrine levels around the lateral septum may be positively correlated the reboxetine response in the FST.

Expression of orexin receptor 2 (Hctr2), 5-hydroxytryptamine (serotonin) receptor 2 (Htr2a), neuropeptide Y5 receptor (Npy5r) and olfactory receptor 1500 (Olr1500), known as G-protein coupled receptor (GPCR), was decreased in RR group compared with RNR group. Expression of other GPCRs such as histamine receptor H3 (Hrh3) and 5-hydroxytryptamine (serotonin) receptor 2C (Htr2c) was also decreased in RR group relative to RNR group. Downregulation of GPCRs is generally induced by repeated or prolonged activation of receptors[26]. This phenomenon is characterized by a reduction in the total number of specific receptor binding sites (B_{max}) without a change in apparent affinity (K_D). Among the neurotransmitters that is related to changes in receptor expression, orexin is involved with regulation of arousal and energy metabolism. Indeed, canine narcolepsy (daytime sleepiness) is caused by disruption of the orexin receptor 2 gene[27]. In this manner, age-related reduction in orexin receptor 2 gene may be involved with sleep disorder observed with aging[28]. Orexin shows dense immunoreactivity in noradrenergic locus coeruleus[29] which is the primary source of forebrain norepinephrine. Similarly, orexin receptor 2 is also located in norepinephrine and epinephrine cells in adrenal medulla[30]. Interestingly, orexin infusion into the locus coeruleus significantly increased norepinephrine release in the hippocampus[31]. As it is possible that downregulation of orexin receptor 2 gene may reflect increased orexin neurotransmission, this increased orexin neurotransmission would lead to increased norepinephrine neurotransmission around the

Table 6. PCR primer sequences for validation of microarray results.

Chip No.	Common	Primer	Sequence	PCR product bp
1399161_at	Appils	Sense Antisense	GCCTGAAGAACCACTGAAGC TGTCTGGCACAGCATACACA	479
1368826_at	Comt	Sense Antisense	TCCTGCTCTTGCGACACCTG CGTTGTCAGCTAGGAGCACT	584
1389871_at	Got2	Sense Antisense	ACTTCGTCGGCTCTAAACCA ACTTCGTCGGCTCTAAACCA	585
1388091_at	Olr1504	Sense Antisense	GCACCAAGTTCTGTGCTTCA TAGCCATGGCAATCTCCTTC	417
1387497_at	Npy5r	Sense Antisense	CGCCATCCAGTAAGGTCATT ACGAACTGGCATTCAGATCC	457
1387370_at	Tmod1	Sense Antisense	AGTACAAGCCTGTGCCTGAT TCTTCCTCACAAGGTCGTTG	518
1368479_at	Drd1a	Sense Antisense	GGACACCGAGGATGACAACT CCACACAAACACATCGAAGG	417
1368301_at	Adora2a	Sense Antisense	GAGAGGATGATGGCCAGGTA CCTCTTCTTCGCCTGTTTTG	593
1376734_at	Nov	Sense Antisense	ACCTGTGGCTCAGAGGAGAA CGTCTTCAGCTCCAGCTCTT	543

lateral septum. In addition, the expression of serotonin 2A and 2C receptor was decreased in RR group. In line with this result, desipramine, a similar selective norepinephrine reuptake inhibitor, decreased serotonin 2A receptor binding[32]. Decreased expression of 5-HT2A and 5-HT2C receptor in RR goup would reflect the increased availability of serotonin in the lateral septum[33]. In fact, selective norepinephrine reuptake inhibitor desipramine did not increase serotonin release, but significantly slowed serotonin reuptake, thus resulting in increased serotonin availability[34]. Growing evidence indicates that decreased 5-HT2A receptor function, either by selective 5-HT2A antagonist or antisense inhibition of 5-HT2A receptors decreases immobility in the FST[35,36]. Thus, decreased expression of 5-HT2A receptor may have positive effect to reboxetine response in RR group.

NPY coexists with neurotransmitters, especially with norepinephrine. As an inverse relationship between neuropeptide Y and NPY1 and NPY5 receptors was observed[37] similarly to other GPCRs, it is possible that reduced expression of NPY5 receptor may reflect increased NPY availability in the lateral septum. Moreover, chronic desipramine treatment reduced NPY2 receptor binding, confirming similar changes would occur at other subtypes of NPY receptor[38]. In this regard, it would be emphasized that intracerebroventricular injection of NPY induces antidepressant effect in the FST, which is thought to be mediated through NPY1 and NPY5 receptors[39,40]. Thus, this result suggests that the possible increase of NPY in the lateral septum, as expected from reduced NPY5 receptor gene expression, may enhance antidepressant effects of reboxetine. Further works are required to elucidate mechanisms underlying decreased GPCR expression in RR group. Another interesting aspect of gene regulation in RR group may be the changes in gene expression related to the thyroid function. As with other receptors, reduced expression of thyroid hormone receptor β (Thrb) and thyrotropin releasing hormone (TRH) may be related to increased thyroid hormones in rat brain. Previous studies also showed that TRH in cerebrospinal fluid was increased in depressed patients[41,42]. Moreover, depressed patients with low triiodothyronine (T3) levels predicted relapse[43]. It has been shown also that thyroid hormone is effective for rearactory depression[44,45]. Likewise, triiodothyronine (T3) exerts antidepressant in the FST in female rats[46]. Thus it would appear that increased thyroid hormone availability in the lateral septum may further contribute to reboxetine response in the FST.

Among genes upregulated in RR group, Lim motif-containing protein kinase 1 (Limk1) and histone deacetylase 1 (HDAC1) are interesting. Limk1, actin-based cytoskeleton, plays a key role in regulating spine structure, and actin reorganization in spines is critical for the maintenance of long term potentiation[47]. Limk1 belongs to serine/threonine kinases family, which is thought as potent regulators of the actin cytoskeleton through phosphorylation of ADF/cofilin[48,49]. Recent study shows that there was a downregulation of Limk1

in frontal cortex of learned helplessness model, one of the animal models of depression[50]. Thus, it would be interesting to note that Limk[1] expression may be decreased in depression, but may be increased by reboxetine pretreatment in the FST, suggesting that reboxetine response may be accompanied by increased synaptic plasticity in the lateral septum. In addition, evidence now indicates that long term modifications to histone proteins may contribute to neural plasticity[51]. In fact, DNA is associated with histone protein, which can be modified by acetylation or deacetylation. The acetylation and deacetylation of histone protein are carried by histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzymes, respectively. Through modifications of histone, DNA could be unwind and bind transcription factor, leading to gene activation. Recent studies have shown that repeated antidepressants treatment changes the expression of HDAC[52,53]. For example, repeated fluoxetine treatment for 10 days significantly increase histone deactylase 2 (HDAC2) in the striatum[52]. Moreover, repeated imipramine treatment selectively downregulates histone deacetylase 5 (HDAC 5) in hippocampus[53]. Although there is no evidence that HDAC1 is involved directly or indirectly with antidepressant actions at present, these results raise the possibility that regulation of different subtypes of histone deacetylase may be related to reboxetine response in the FST.

In conclusion, the molecular mechanisms underlying antidepressant action are poorly understood, but expression profiling may offer a potential insight into the antidepressant mechanisms. In the present study, we demonstrated that microarrays provide an efficient means to monitor and to identify gene expression profile that arises from the exposure of FST to antidepressants. Although further studies are required for direct roles of these genes in reboxetine response, the microarray may provide tools to find out potential target genes and signalling pathways in antidepressant response.

Methods

Animals

Adult male Sprague-Dawley rats (280-300 g, Orient, Seoul, Korea) were allowed to acclimate to the housing conditions and were handled daily for a week before the experiment. The animals were kept in polypropylene cages at 21-22˚C, with a 12-h light/dark cycle (lights on at 6:00 AM). Food and water were provided to the rats *ad libitum*. Rats were randomly divided into a vehicle treatment control group (n=6) and reboxetine pretreatment group (n=17) with similar mean group body weight. All the procedures used in this study followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).

Forced-Swimming Test (FST)

On the 1st day of the FST, rats were placed in clear, 65 cm-tall by 25 cm-diameter cylinders filled to 30 cm with 25˚C water. After 15 min of forced swimming (pretest session), the rats were removed from the water, dried with towels, and placed in a warmed enclosure for 30 min. The cylinders were emptied and cleaned between rats. At 24 h after the pretest session of FST, rats were retested for 5 min (test session) under identical swim conditions. The FST data presented in the present report were collected during test sessions of FST, which were videotaped from the side of the cylinders. Reboxetine or saline was given three times at 1, 5 and 23.5 h prior to the test session. Videotapes were scored by raters unaware of the treatment condition. Rats were rated at 5-s intervals throughout the duration of the test session; at each 5-s interval, the predominant behavior was assigned to one of three categories: immobility, swimming or climbing. A rat was judged to be immobile if it was making only movements necessary to keep its head above water; climbing if it was making forceful thrashing movements with its forelimbs directed against the walls of the cylinder; swimming if it was actively making swimming movements that caused it to move within the center of the cylinder. Depending on behaviors during FST, we divided animals into reboxetine response (RR, n=6) and reboxetine nonresponse group (RNR, n=6) from 17 rats pretreated with reboxetine.

Isolation of Total RNA

Total RNA was isolated from snap frozen cells and tissue using Trizol. Each sample was dissolved in 1 mL Trizol reagent per 50-100 mg of tissue using a homogenizer (Tissue tearor, Model 985-370, Biospec products, Inc.) according to the manufacturer's instructions. Trizol was removed by addition of chloroform followed by isopropanol precipitation. The precipitates were washed using 75% ethanol. The amount and purity of RNA was quantified UV spectrometer by measuring the optical density at 260 and 280 nm and the integrity was checked by agarose gel electrophoresis.

Microarrays

Purified RNA (5 μg) derived from each lateral septum was reverse-transcribed using Life Technologies Superscript Choice System (Grand Island, NY). Dou-

ble-stranded cDNA (0.5-1.0 μg) was used as a template for synthesis of biotin-labeled cRNA using a BIOarray RNA Transcript labeling kit (Enzo Diagnostics, NY). Labeled cRNA was purified on RNeasy affinity resin (Qiagen, CA) and fragmented randomly (an average size; 50-100 bases) by incubation at 94˚C for 35 min in 100 mM potassium acetate and 300 mM magnesium acetate solution. A common reference pool was prepared by pooling equal amounts of c-RNA from all samples investigated. We analyzed 3 samples and common reference cRNA on GeneChips RAT Genome 230 Genechip (Affymetrix, CA). Labeled cRNA samples were hybridized to RAT Genome 230 Genechip at 45˚C for 16 h in a hybridization oven under constant rotation (60 rpm). After hybridization, arrays were washed, stained with streptavidin-phycoerythrin using an Affymetrix Fluidics station. The chips were scanned using a GeneArray scanner (Agilent, CA) and the readings from the quantitative scanning were analyzed by the Affymetrix Gene Expression Analysis microarray Suite Software (MAS) 5.0 (Affymetrix). All hybridization intensities were corrected by a set value for mean total intensity (set value=500). To qualitatively assess differences between control-FST and reboxetine-FST samples, the scattergram was generated and fold changes were calculated. Only the transcripts called present (P) in the experimental array were subjected to the comparison analysis to get the up and down regulated genes by reboxetine-FST. Logarithm base 2 (Log2) was used for data normalization, so data within each chip are in agreement with a normal distribution. The functional classification was performed based on revised annotation information in the Affymetrx NetaffixTM (http://www.affymetrix.com; for RAE230A index date for annotations 06-15-2006).

RT-PCR

To screen for expression of selected genes and to validate each pair of primers (Table 6), then we performed RT-PCR on pooling equal amounts of cRNA from all samples investigated. Total RNA (2 μg) was reverse-transcribed, and an equal aliquot of resulting RT product was amplified by PCR with specific primer set under the following conditions: an initial denaturation at 94˚C for 1 min, followed by 30 cycles of 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 1 min, and final extension at 72˚C for 5 min. PCR products were runned on 1% agarose gels and visualized by staining with ethidium bromide.

Statistics

With regard to FST-related behaviors, statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by *post hoc* Fisher's least significant difference (LSD) test. Significance was accepted for P-values < 0.05.

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