Changes in hippocampal gene expression by 7-nitroindazole in rats submitted to forced swimming stress

Effect of 7-NI on hippocampal gene expression

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Abstract and Keywords

Nitric oxide (NO) is an atypical neurotransmitter that has been related to the pathophysiology of major depression disorder (MDD). Increased plasma NO levels have been reported in depressed and suicidal patients. Inhibition of neuronial nitric oxide synthase (nNOS), on the other hand, induces antidepressant effects in clinical and pre-clinical trials. The mechanisms responsible for the antidepressant-like effects of nNOS inhibitors, however, are not completely understood. In the present study genomic and proteomic analysis were used to investigate the effects of the preferential nNOS inhibitor 7-nitroindazole (7-NI) on changes in global gene and protein expression in the hippocampus of rats submitted to forced swimming test (FST). Chronic treatment (14 days, i.p.) with imipramine (15 mg/Kg/daily) or 7-NI (60 mg/Kg/daily) significantly reduced immobility in the FST. Saturation curves for Serial analysis of gene expression (SAGE) libraries showed that the hippocampus of animals submitted to FST presented a lower number of expressed genes compared to non-FST stressed groups. Imipramine, but not 7-NI, reverted this effect. GeneGo analyses reveled that genes related to oxidative phosphorylation, apoptosis and survival controlled by HTR1A signaling and cytoskeleton remodeling controlled by Rho GTPases were significantly changed by FST. 7-NI prevented this effect. In addition, 7-NI treatment changed the expression of genes related to transcription in the CREB pathway. Therefore, the present study suggests that changes in oxidative stress and neuroplastic processes could be involved in the antidepressant-like effects induced by nNOS inhibition.

Keywords: Major depression; nitric oxide; oxidative stress; neurogenesis; neuroplasticity.

Introduction

Nitric oxide (NO) is an atypical neurotransmitter involved in pathological processes related to major depression disorder (MDD) (Baranano et al., 2001). NO is synthesized from L-arginine by three different isoforms of NO synthase: neuronal (nNOS), inducible and endothelial NOS (eNOS)(Magarinos & Mcewen, 1995). nNOS is widely but unevenly distributed in the mammalian brain, where it accounts for more than 90% of NO production (Abkevich *et al.*, 2003, Joca *et al.*, 2007).

The involvement of NO-mediated neurotransmission in depression is supported by several pieces of evidence. For example, a higher number of nNOS containing neurons in the hippocampus was described in post-mortem studies of depressive patients (Oliveira et al., 2008), and increased plasma NO levels have been reported in depressed patients with suicide attempts (Khovryakov *et al.*, 2010, Kim *et al.*, 2006). Moreover, systemic or intra-hippocampal treatment with 7-nitroindazole (7-NI), a preferential nNOS inhibitor, induces antidepressant-like effects in the forced swimming test (FST) (Joca & Guimaraes, 2006, Yildiz *et al.*, 2000). Furthermore, exposure of laboratory animals to uncontrollable or severe stressors, which can induce several physiological and behavioral changes that resemble human depression such as anedonia, memory impairments and excessive glucocorticoids (GC) secretion, induces nNOS expression in brain structures related to this disorders, including the hippocampus, amygdala and cortex (De Oliveira *et al.*, 2000, Oliveira *et al.*, 2008).

However, despite the evidence linking NO to biological processes associated with depression such as oxidative stress (Bergstrom *et al.*, 2007, Packer *et al.*, 2005), monoamine functions (Kiss & Vizi, 2001, Yamamoto *et al.*, 2001), S-nitrosilation signaling (Evans et al., 2002), and gene expression changes (Datson et al., 2001a), the molecular mechanisms responsible for the antidepressant-like effects of NOS inhibitors are still not completely understood. Therefore,

the present study was aimed at unveiling possible neuromolecular pathways associated with antidepressant-like effects of nNOS inhibition. To achieve this goal, we measured total protein and gene expression in the hippocampus of rats treated chronically with a preferential nNOS inhibitor 7-NI (Alderton et al., 2001) or the standard antidepressant imipramine and submitted to the forced swimming test (FST), an animal model that is widely used for detecting antidepressant-like effects (Nestler & Hyman, 2010).

Methods

Animals

Male Wistar rats (200–220 g) were housed in pairs in a temperature-controlled room (24±11^{o}C) under standard laboratory conditions with free access to food and water (12 h light/12 h dark cycle). Procedures were conducted under approval by the Ethical Committee of FMRP-USP, in compliance with International laws and policies.

Drugs

Imipramine hydrochloride (15 mg/Kg; Sigma Chemical, Saint Louis, Missouri, USA), 7-nitroindazole (60 mg/Kg; Sigma Chemical, Saint Louis, Missouri, USA) or vehicle (DMSO:saline, 1:1; 2 mL/Kg) was daily-injected i.p. for 14 days (10:00 - 14:00 h). The doses and treatment schedule were based on previous studies describing antidepressant-like effects of 7-NI(Yildiz et al., 2000). The five experimental groups (n= 5-8 animals/group) were: naïve (NAI) animals not-submitted to FST stress, and treated with vehicle, (VEH + NAI); naïve animals treated with 7-NI (7-NI + NAI); animals treated with vehicle and submitted to FST (VEH + FST); animals treated with 7-NI and submitted to FST (7-NI + FST); animals treated with imipramine and submitted to FST (IMI + FST).

On the day before the last injection animals from the FST groups were submitted to the 15 min forced swimming pretest section (Porsolt et al., 1977). Twenty-three h later they received the last injection and 1 h after were submitted to the forced swimming session where immobility time, meaning the period in which the animal remained immobile or with minimal movements necessary for floating, together with the latency for the first immobility episode, were recorded for 5 min. Two hours later the animals were sacrificed under deep anesthesia (Urethane 25% 1mg/kg) and hippocampus were dissected and stored at -80^{o}C for later analyses.

Total RNA samples and Serial analysis of gene expression (SAGE) analysis

Total RNA was prepared from the hippocampus using a polytron homogenizer and TRIzol LS Reagent (Invitrogen Corporation; Carlsbad, CA) according to manufacturer's instructions. A pool of 3-4 animal samples, resulting in 30 μg of total RNA, was used for the SAGE procedure.

SAGE was carried out using the I-SAGE^{TM} Kit (Invitrogen Corporation; Carlsbad, CA) following the manufacturer's protocol. The tag frequency counting was obtained by SAGE^{TM} analysis software, and genes identification was extracted from CGAP SAGE Genie (http://cgap.nci.nih.gov/SAGE). The SAGE methodology was chosen in the present study due to its similar sensitivity compared to microarrays, with the advantage of being potentially able to detect changes in genes not present in the array (Feldker *et al.*, 2003, Martins-De-Souza *et al.*, 2010, Weinreb *et al.*, 2007). Moreover, since this method is based on relative frequency, SAGE libraries from independent studies could be more easily compared (Czibere *et al.* 2011). Also, new genes can be identified by re-analyses of unpredicted tags using Gene Banks.

In order to validate SAGE libraries, five genes of interest were chosen for quantitative real time (qRT) PCR using cyber green of PCR master mix (Applied Biosystem, California, CA) on the basis of previous reports showing expression changes after stress exposure(Hill & Gorzalka, 2005, Holmes *et al.*, 1995, Mclaughlin *et al.*, 2007, Mongeau *et al.,* Pandey *et al.,* 2010, Thome *et al.*, 2001). They included the cannabinoid receptor 1 (CNR1), synaptophysin (SYP), glutathione S-transferase (GST), neurotrophic tyrosine kinase receptor type 2 (NTRK2), and serotonin receptor type 2C (5-HT_{2c}) genes.

Protein sample and MS/MS mass spectrometry analysis

Total protein was isolated from the organic phase of RNA TRIzol of the same samples used for SAGE analysis, following manufacturer's instructions with modifications. The protein content was determined by Bradford (Life Science, CA) reagent method, and stored at -80^{o}C for later use.

In each experimental group three bi-dimensional gels were prepared using 1mg of total protein per gel, from a 3-4 pooled animal sample. The isoeletric focalization was performed using 18cm 3-10 pH gradient (Amersham, SE) in the Ettan IPGPhor 3 (GE Healthcare, CA), and the second dimension was run at SDS Ettan DALT six (GE Healthcare, CA).

Protein expression levels were estimated by average of relative spot volumes obtained from two gels that presented spot location reproducibility higher than 95% by Image Master Software (Amersham, SE, version 5.0). Protein spots differently expressed were excised from the gel for MS/MS spectrometry in MALDI-TOF. To identify the proteins, the tryptic hydroxyoate peptide fingerprint was analyzed using Mascot method and identification by National Center for Biotechnology Information (NCBI) protein database (http://prospector.ucsf.edu/csfhtml4.0/msfit.htm). Proteins with Mascot score (MS) identity levels ≥ 36 were taken to be reliable identified.

Biological network analysis using MetaCore™

Results from SAGE and 2-DE-peptide mass fingerprint were analyzed by MetaCore software (v.5.1 build 16271, GeneGo, St. Joseph, MI, USA). This gene enrichment analysis approach is one of the methods that can be used to extract meaningful biological information from large genomic data set (Subramanian *et al.*, 2005), grouping genes that share common biological function (Nikolsky *et al.*, 2005).

Statistical analysis

The forced swimming test results were compared by one-way ANOVA followed by Duncan *post hoc* test. The sensitivity of the SAGE genome expression, reflecting the extent of the detected genes, was performed by calculating the ratio between new tags and total sequenced tags identified in each sequencing round. The saturation curves were compared by ANOVA. Hierarchical clustering, using GeneCluster 2.0 (Cambridge, MA) was performed in order to evaluate the level of similarity-divergence among the SAGE libraries. Genes differentially expressed in the SAGE libraries were identified by confidence interval test (SAGEic) as previously described(Abkevich et al., 2003). The pairs of SAGE libraries and 2D protein spot volumes were compared as follow: VEH + NAI group against VEH + FST; VEH + FST group against 7NI + FST, and VEH + FST group against IMI + FST. The libraries VEH + NAI and VEH + 7NI were not compared by this method because the hierarchical analyze showed high similarity between these groups. The general level of gene activity was estimated by Kruskal-Wallis test for averages of fold change between the groups mentioned above. The qRT results were compared by one-way ANOVA, followed by Duncan post hoc test. Spearman correlation test was also used to evaluate the relationship between mean values of gene expression levels obtained by qRT and SAGE. Proteins spots were considered to be

differently expressed when the relative volume between one spot and its homologue in another treated group was higher than two times and the *t* test resulted in a value of $P < 0.05$. The statistical method used by MetaCore software is based on gene ontological categorization to identify processes regulated by genes or proteins differentially expressed. The *P* values represent the statistical relevance of the ontological matches calculated as the probability of a match occurring by chance, given the size of the database. Lower *P* values indicated that more genes belong to a same pathway. The *P* value was calculated as previously described (Pan *et al.*). Only ontological pathways with *P* value < 0.05 were considered on present study.

Results

Behavioral effects of imipramine and 7-NI

Imipramine and 7-NI induced an antidepressant-like effect in the FST (Yildiz *et al.*, 2000), significantly decreasing immobility time ($F_{2,18}$=15.83, *P*=0.0001) and increasing the latency for the first immobility episode ($F_{2,18}$=9,66 *P*=0.0014, Fig 1*a*).

SAGE analyses

A total of 61,272; 62,031; 60,471; 61,390 and 60,754 gene tags were sequenced, with 26,406; 26,256; 19,270; 19,072 and 24,936 genes mapped and quantified in the hippocampus of VEH + NAI, VEH + 7-NI, VEH + FST, VEH + 7-NI and VEH + IMI groups, respectively. Analysis of the saturation curves showed that all SAGE libraries fitted into a hyperbolic curve with saturation on the abscissa (R2 > 0.99, Fig 1*b*), suggesting a high covering of hippocampal transcriptome using the SAGE method, as previously reported (Anisimov, 2008). ANOVAs of SAGE libraries curves revealed statistical differences between the saturations curves ($F_{(4, 34)}$ = 23.01; $P < 0.0001$). These analysis suggest that SAGE libraries of

VEH + NAI, 7-NI + NAI and IMI + FST presented a higher variety of gene expressed, compared to libraries VEH + FST and 7-NI + FST.

Hierarchical clustering identified at least three distinct groups. The first one corresponded to animals submitted to stress by FST that received vehicle or 7-NI. The second one included non-FST stressed animals, treated or not with 7-NI, and the last one consisted of animals treated with imipramine (Fig 1*c*).

To evaluate the pattern of hippocampal global gene expression induced by FST and drug treatment we compared the SAGE libraries of VEH + FST group against VEH + NAI, 7-NI + FST and IMI + FST groups. Comparison between VEH + NAI and VEH + FST groups showed changes in the expression of 1469 genes. Among them, 1312 genes (89.3%) showed increased expression in the VEH + FST group against only 157 gens (10.7%) in the VEH + NAI. 7-NI and IMI, on the other hand, induced changes in the expression of 1201 and 1687 genes, respectively. 7-NI and IMI decreased the expression of 1078 (89.8%) and 1514 (89.8%) genes, respectively, compared to the VEH + FST group, whereas only 123 (10.2%) and 173 (10.3%) genes had their expression increased by these drugs (Table 1). Finally, stressed animals treated with vehicle (VEH + FST) presented a higher global gene expression compared to the other groups (Fig 1*d*; Kruskal-Wallis test $P<0.0001$, followed by *post hoc* Dunn's test, $P<0.05$).

To identify the biological processes preferentially modified by FST stress and detected by SAGE, the genes differently expressed between the VEH + NAI and VEH + FST groups were submitted to enrichment MetaCore analysis. The analysis indicated that a significant number of genes involved in oxidative phosphorylation, apoptosis signaled by 5-HT_{1A} receptor and cytoskeleton remodeling processes were statistically changed (Table 2).

Among the genes involved in oxidative phosphorylation, 25 were up-regulated while 4 were down-regulated by FST. The former included cytochrome-c oxidase polypeptide 6A1

mitochondrial, NADH dehydrogenase and ATP synthesis mitochondrial, which showed more than nine fold increase after FST compared to the control group (Supplement S1).

Genes of at least three pathways involving 5-HT_{1A} signaling were modified according to the GeneGO analyze. The first one is associated with activation of an alpha inhibitory G- protein (Gi), adenylate cyclase and regulatory protein PP2A, which modulates the BAX protein (Gi-ADC-PP2A-BAX). The second one involves AKT and ERK2-MAPK1. The last one is also related to AKT and NF-κB (Supplement S2, S3). FST also modified the expression of several genes involved in cytoskeletal and neurophilament remodeling. FST (VEH + FST) stress induced more them 14 fold [0; 1.7]ci increase in the expression of glutamate receptor subunit GluR2 (Rn.91361) compared to VEH + NAI group (Supplement S4).

Comparison between vehicle and 7-NI treated groups submitted to FST stress performed by enrichment analysis showed that genes related to physiological processes associated to oxidative phosphorylation, cytoskeleton remodeling, cell adhesion and genes related with transcriptional factors responding to beta-adrenergic receptors and cAMP response element-binding (CREB) were significantly modified by 7-NI treatment (Table 2).

7-NI prevented the increase in the expression of several genes related to respiratory complex I, succinil dehydrogenase, and ATPases. Moreover, 7-NI prevented the induction of subunits 6 and 8 of the cytochrome complex and increased the expression of eighth Cox subunit (Supplement S5). Similar effects were observed for cytoskeleton remodeling signaled by FKA and cell adhesion genes, with 7-NI preventing the up-regulation of genes such as $G_{(0)}$-protein, CDC42 and LIMK. This drug also increased the expression of the cytoskeleton genes beta-actin and actin (Supplement S6).

Regarding the physiological process related to CREB, 7-NI treatment prevented the increase in gene expression of calcium channel, Erk (MAPK1/3), c-Jun, c-Fos and AP1, and increased the expression of calmodulin 1 (Calm1) (Supplement S7, S8).

The enrichment analysis of the genes differentially expressed in animals treated with vehicle against those treated with imipramine and submitted to FST showed that drug-induced gene expression modifications also occurred in biological pathways associated with oxidative phosphorylation, apoptosis/prolipheration signaled by 5-HT_{1A} receptor, cytoskeleton remodeling, and transcriptional control (Table 2).

Imipramine prevented the increased expression of most genes related to oxidative phosphorylation (Supplement S9). It also prevented the induction of genes related to 5-HT_{1A} signaling promoted by FST stress. Within this pathway, imipramine inhibited the increased expression of NF-κB, STAT3 and caspase regulator factor PARP-1. The expression of PPA2 protein was also inhibited. In addition, imipramine treatment decreased the expression of the apoptosis-related genes c-Raf-1 and Map2K2. Moreover, it reduced the expression of G-protein potassium channel (Kcnj5), and induced the expression of ionotropic glutamate receptor alpha 3 (Gria3) when compared to VEH + FST group (Supplement S10, S11).

Similarly to 7-NI, cytoskeleton-related genes were also significantly changed in animals treated with imipramine. This drug inhibited their expression although, for at last three of them, the precursor of activator of tissue plasminogen (PLAT), cytoskeleton actin (Actg1), and myosin heavy head type II (MyHC), the expression was enhanced. Different from 7-NI, imipramine changed the expression of genes related to DNA methylation such as a family of protein 14-3-3 (over 60 fold increase) and class II histone deacetilases and histone H3 (14 and 19 fold decrease, respectively). Chronic imipramine treatment also decreased the expression of G-beta/gamma protein compared to animals treated with vehicle (Supplement S12).

The enrichment analyze for gene function was also performed for the non-stressed animals comparing vehicle- versus 7-NI treated animals. The MetaCore database indicated only major effects in cytoskeleton remodeling and cell adhesion gene. There were no significant changes in oxidative phosphorylation, cell signaling mediated by CREB, pathways signaled by 5-HT_{1A}

or beta adrenergic receptor related genes (Table 2).

Quantitative PCR analysis (qRT)

The SAGE libraries were validated using an independent method. qRT was performed for genes that showed changes in the SAGE analysis. They included genes related to oxidative stress (GST), neuronal plasticity (CNR1, SYN), and serotonin-mediated neurotransmission ($5\text{-}HT_{2C}$) (Supplement S13). Spearman test showed positive correlation ($r=0.609$, $P<0.001$) between gene expression analyses by SAGE and qRT, suggesting a reproducibility of SAGE method (Fig 2).

Proteomic analysis

The analysis of gels by Image Master software allowed us to identify a different number of protein spots in each five experimental groups (VEH + NAI: 340; 7-NI + NAI: 296; VEH + FST: 616; 7-NI + FST: 446; IMP + FST: 496, Fig 3). The increase in protein spots in the stressed group and their decrease by drug treatment is similar to the global gene expression changes found by SAGE analysis. To identify the proteins that had their expression modified by FST stress, the gels of animals treated with VEH + NAI were compared to those of VEH + FST animals. From a total of 65 protein spots, 32 showed increased expression, while 33 were down-regulated in VEH + FST group when compared to VEH + NAI. Among them 28 proteins were sequenced by MaldTofe method (Supplement 14).

When the proteins of VEH + FST rats were compared to 7-NI + FST animals it was found that 10 protein spots had their expression enhanced and 97 decreased at least two fold by the drug (Table 1). From this total 28 proteins were sequenced and characterized by MS/MS spectrometry (Supplement S15). A similar comparison with imipramine-treated animals showed enhanced expression of 43 proteins spots and reduction of 48 (Table 1). From this

total, 54 protein proteins were sequenced and characterized by MS/MS spectrometry (Supplement S16).

Finally, the comparison of 7-NI + NAI and VEH + NAI groups showed that drug treatment alone was able to increase or decrease the expression of only 12 or 15 proteins, respectively. Thus, due to the low number of proteins differently expressed between these groups (7-NI + NAI and VEH + NAI), a trend also observed in hierarchical clustering performed in SAGE libraries, they were not sequenced.

The enrichment analysis by MetaCore method for screening proteins differently expressed between the groups VEH + NAI and VEH + FST indicated that, among the functional pathways significantly represented by these genes, there were the oxidative phosphorylation ($P = 4.59^{-3}$), oxidative stress ($P = 3.72^{-2}$), and prevention of apoptosis induced by ROS ($P = 3.97^{-2}$).

In stressed rats 7-NI significantly changed proteins related to apoptosis and/or proliferation associated to BAD ($P = 2.79^{-3}$), pathways driven by CREB ($P = 2.93^{-3}$) and cytoskeleton regulation and rearrangement ($P=9.19^{-4}$). Imipramine, on the other hand, modified proteins associated to GABA-A signaling ($P = 2.75^{-5}$), mitochondrial tricarboxil acid chain (TCA) ($P = 5.78^{-3}$), responses to oxidative stress and hypoxia ($P = 3.28^{-4}$), process linked to actin cytoskeleton ($P =2.19^{-2}$), and catalytic process of hydrogen peroxide ($P = 2.87^{-7}$).

Discussion

The preferential nNOS inhibitor 7-NI decreased immobility time in the FST, thus confirming its reported antidepressant-like effect (Joca & Guimaraes, 2006, Yildiz *et al.*, 2000). The primary mechanisms of this effect are not fully understood, although 7-NI shares some properties with imipramine such as the facilitation of neurogenesis and hipocampal neuroplasticity (Brown, 2010, Lu *et al.*, 1999). A genomic and proteomic analysis was

employed in the present study to further investigate these mechanisms. Although the SAGE strategy has a sensitivity similar to other genomic strategies such as microarray (Weinreb et al., 2007), it is considered to be an open approach, capable of detecting and quantifying unpredictable genes expressed at different biological samples(Martins-De-Souza *et al.* 2010). In addition to SAGE, proteomic analysis was also employed as a source of additional information to detect changes in protein expression levels.

Molecular analyses showed that forced swimming stress changed the pattern of gene and protein expression in about 4% to 5%. Hierarchical clustering analyses dividing the animals into two main groups, stressed and non-stressed, agreed with this finding, indicating a main effect of tress on gene expression pattern.

Moreover, in the present study, forced swimming stress modified, for the most part, the expression of genes related to cellular respiratory chain, oxidative stress responses, apoptosis control and neuroplasticity. Also, imipramine and 7-NI were able to attenuate FST-induced changes in the expression of most of these genes. For example, 7-NI prevented the stress-induced increase in the expression of a number of genes related to respiratory complex I-III, succinate dehydrogenase and ATP synthesis.

Functional analyses of both SAGE and proteomic libraries indicated that the expression of genes associated with oxidative phosphorylation, oxidative stress and prevention of apoptosis induced by ROS are consistently modified by the FST. These findings corroborate other reports from the literature showing changes in the expression of genes enrolled in mitochondrial oxidative chain and metabolism control in suicidal patients with history of depression or rodents after treatment with high doses of corticoids or exposure to the learned helplessness model of depression (Datson *et al.*, 2001b, Knapp & Klann, 2002, Rivas-Arancibia *et al.*, 2009). Depressive states have also been associated with an increase in oxidative damage in different brain sites, an effect that can be partially prevented by

antidepressant drugs (Bilici *et al.*, 2001, Eren *et al.*, 2007). In addition, a recent study using microarray has also found significant effects on metabolic related genes induced by different classes of antidepressant agents (Lee *et al.*, 2010). Finally, anti-oxidant drugs induce antidepressant-like effects in the FST (Berk *et al.*, 2008a, Ferreira *et al.*, 2008).

Together, these results corroborate the metabolic/oxidative stress hypotheses of mood disorders (Andreazza *et al.*, 2008, Berk *et al.*, 2008b, Hess *et al.*, 2005, Hroudova & Fisar, 2010); Moreover, several pieces of evidence indicate that NO could be an important mediator linking stress exposure, metabolic/oxidative changes and behavioral consequences, including 1) increases of NO production by nNOS following stress-related NMDA activation by glutamate (Rivas-Arancibia et al., 2009), although other NOS isoforms could also be involved (Fu *et al.,* 2010, Khovryakov *et al.* 2010); 2) increases in GC levels and neuronal energy demands (Lizasoain et al., 1996); 3) impairments of NADH formation due to inhibition of the respiratory chain by NO and ROS (Kamsler & Segal, 2003, Wang *et al.*, 2006); 4) joint effects of increased energy demands and inhibition of the respiratory chain, causing an imbalance between pro- and anti-oxidant mechanisms and increasing oxidative stress (Brown, Eren *et al.*, 2007, Herken *et al.*, 2007). As a final consequence, these mechanisms could lead to neuronal functional impairments (Meffert & Baltimore, 2005), dendritic remodeling, apoptosis and impairment of hipocampal neurogenesis (Kaltschmidt et al., 2005).

In addition to oxidative stress, changes in genes and proteins related to apoptosis/survival processes mediated by NF-κB/BAX-cytochrome C, cytoskeletal remodeling and neurophilament formation were also observed. The nuclear κB factor is a member of a transcriptional factor family composed by 5 elements that has been related to apoptotic mechanisms (Wang et al., 1998). Moreover, the expression of genes enrolled in apoptotic processes such as STAT3, cytochrome C, genes related to mitosis activated protein (MAPK) pathway, and several isoforms of Poly (ADP-ribose) polymerase and Ppp2r were also

changed by FST stress. This latter gene controls the expression of IκB (IKK), a inhibitor of NF-κB activation (Chang *et al.*, 2003, Shioda *et al.*, 2009). 7-NI and imipramine revert the changes in these apoptosis-related genes, suggesting that the common proliferative and neuroprotective effects previously described to 7-NI and imipramine (Castren *et al.*, 2007, Dranovsky & Hen, 2006) could be associated to inhibition of NF-κB/Ppar activity (Sarnico *et al.*, 2009, Veuger *et al.*, 2009).

The MAPK pathway, composed by the protein kinase regulated by extracellular signaling (ERK1/2), the Jun-N-terminal kinase (JNK), and MAPK p38, is also closely involved in processes such as cell proliferation, differentiation and stress response (Sousa et al., 2000). The MAPK p38 and ERK pathways are usually associated with cell death and citoprotection, respectively (Liu et al., 2010). The increased expression of MAPK1-3 found in stressed animals would favor the balance for apoptotic signaling (Harvey, 1964). In the present study only imipramine was able to prevent the changes in the MAPK pathway, indicating a distinct profile of gene expression changes compared to 7-NI.

The alteration of several isoforms of genes related to cytochrome C in animals submitted to FST that were, also for the most part, prevented by 7-NI and imipramine, suggests the involvement of apoptotic-related genes associated to the balance of anti-apoptotic Bcl and pro-apoptotic BAX proteins (Chung *et al.*, 2008, Yung *et al.*, 2004). Corroborating this proposal, 7-NI has been shown to prevent nitric oxide-induced cell death mediated by p53- and Bax-dependent pathway (Martin et al., 2011).Therefore, it is possible that exposure to the forced swimming test results in an oxidative stress insult, producing the engagement of pro- and anti-apoptotic factors, with antidepressive drugs changing the balance in favor of the latter.

Exposure to repeated restraint stress induces plastic changes in the hippocampus such as dendritic remodeling (O'kane *et al.*, 2004). Although the stress procedure (forced swimming)

used in the present work is much shorter than those reported to cause these changes, it did modify the expression of genes related to neurophilaments and cytoskeleton remodeling controlled by Rho GTPase. Rho belongs to a super protein family linked to GTPase named as Ras family (Rex et al., 2009), which also includes Ras and Ran proteins. They can be turned on-off by fast conformational shift when bound to GTP or GDP, being characterize as fast shift proteins (Sarandol et al., 2007). Through extracellular signaling, RhoA-GTPase regulates the function of LIM1 and 2 (LIMK1/2) proteins which, together Confilin, down-regulate the dynamic control of polymerization/depolarization of actin filaments (Chen *et al.*, 2006, Kreis & Barnier, 2009). The increased expression of several genes related to the RhoA/Rac/Cdc42 pathway in stressed animals (Cdc42, Cfl1, Pfn1, Cdc42), and its inhibition by 7-NI (Cdc42) and imipramine (Cdc42, Rho GDP) observed in the present study, corroborates the proposal that neurophilament remodeling induced by stress stimuli could be related to the behavioral changes observed in depressive patients (Abkevich et al., 2003).

The LIM-Confilin system is also controlled by Cdc42, acting synergically in the dynamic control of actin filaments. The Rho-GTPase-LIM-Confilin complex seems to be involved in dendritic retraction, with arrest of actin filaments to lamelipody and filipody formation, while Cdc42 would be linked to prolongation of growing cones and dendritic ramification (Chen et al., 2006). Inhibition of GTPase-Rho-kinase increases LTP amplitude and interfere with synaptic plasticity (Park *et al.*, 2007, Seo *et al.*, 2006). Recent studies have shown that cytoskeletal components associated to a sub-family of Rho protein are important for LTP formation. The RhoA-ROCL-LIM2 signaling is proposed to promote the initial stages of LTP formation while the Rac1-LIMK1 pathway acts on its consolidation and maintenance (Rex et al., 2009).

The changes in several genes related to cAMP response element-binding (CREB) induced by 7-NI and detected by both SAGE and proteomic analysis suggest that NO-mediated changes

in the CREB pathway could be related to the antidepressant-like proprieties of NOS inhibitors. CREB is a nuclear transcriptional factor crucial for the expression of several genes that affect the function of entire neural circuits (Carlezon et al., 2005). Stress exposure reduces CREB activation and the expression of brain derived neurotrophic factor (BDNF) and its receptor tyrosine kinase B (TrkB), affecting the neurotrophic signaling pathway and reducing hipocampal cell proliferation. On the other hand, antidepressant treatment increases CREB phosphorylation and CREB, BDNF and trkB expression (Gass & Riva, 2007), preventing plastic consequences of stress. It has been proposed that activation of BDNF-trkB-CREB pathway, with consequent alteration in hipocampal plasticity and neurogenesis, is a common key point to antidepressant effects (Castren *et al.*, 2007, Shirayama *et al.*, 2002). The increase in expression of CREB-related genes such as Calm1 and TrkB suggest that the antidepressant-like effects of imipramine could also be related to this pathway (Nibuya *et al.*, 1996, Tardito *et al.*, 2009).

Although sharing some common effects on gene expression, the present data suggest that imipramine and 7-NI effects also involve different mechanisms. For example, chronic treatment with 7-NI increased the expression of genes related to cell adhesion and cytoskeletal remodeling by FAK, while imipramine increased mainly genes linked to cytoskeletal remodeling and neurophysiological process signaled by the 5-HT1A receptor. Moreover, only imipramine changed the expression of genes related to DNA methylation, a mechanism that has been associated with epigenetic mechanisms related to stress consequences (Carlezon *et al.*, 2005, Tsankova *et al.*, 2006).

There are some limitations in the present study: 1. individual analysis of gene expression could have allowed for correlations between specific gene expression and behavior changes. However, most similar studies employing SAGE or microarray approaches have used pooled samples (Czibere *et al.*, 2011, George *et al.*, 2011, Guipponi *et al.* 2011**)**. Moreover, the

reliability of our SAGE approach was tested in individual samples by an independent method (qRT) (Supplement 13), which found a positive correlation ($r=0.609$, $P<0.001$) between means of individual results obtained by qRT and SAGE data (Fig 2); 2. even if all groups were submitted to the same administration procedure, the stress of the i.p. injections could have also affected gene expression. It is doubtful, however, that the present approach (SAGE) would have been sensitive enough to detect such changes. Corroborating this idea, a recent study using microarray that compared several antidepressant agents administrated orally or by i.p. injection (1, 3 or 7 days) failed to detect any difference in gene expression due to the drug administration route (Lee *et al.* 2010); 3. although no single drug administration group was included, the main objective of the study was to investigate gene expression changes induced by chronic treatment with the preferential inhibitor of neuronial oxide nitric synthase, 7-NI, in animals submitted to forced swimming test (FST). Rats (different from mice) are usually not sensitive to single antidepressant injections in this test, but only to subchronic (three injections in 24 h) treatment. Although this treatment regime could have been used in the present study, the technique emplyed might not be sensitive enough to detect small effects on gene expression. Moreover, several neurobiological changes that have been implicated in antidepressant-like actions such as increased hippocampal neurogenesis (Dranovsky & Hen, 2006, Liu *et al.*, 2006) and synaptic plasticity (Bachis *et al.*, 2008, Dranovsky & Hen, 2006) are usually detected after chronic, but not single drug administration.

In conclusion, despite these limitations, the present paper confirmed that inhibition of NO formation produces antidepressant-like effects in animals and suggests that this effect could involve changes in the expression of genes related to oxidative stress, neuroplastic and neurogenic processes.

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Conflict of interest

The author states there is no conflict of interest on present study.

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Tables

Table 1: Number of genes and proteins with expression changes into each comparison set.

Experimental groups	Total genes evaluated		Total protein spots evaluated	
VEH + NAI	26,406		340	
7-NI + NAI	26,256		296	
VEH + FST	19,270		616	
7-NI + FST	19,072		446	
IMI + FST	24,936		496	
Compared groups	**N genes (%)**	**Total**	**N protein (%)**	**Total**
VEH + NAI X VEH + FST	↑ 157 (10.68) ↑ 1,312 (89.32)	1,469	↑ 33 (50.77) ↑ 32 (49.23)	65
VEH + FST X 7-NI + FST	↑ 123 (10.24) ↓ 1,078 (89.76)	1,201	↑ 10 (9.34) ↓ 97 (90.66)	107
VEH + FST X IMI + FST	↑ 173 (10.25) ↓ 1,514 (89.75)	1,687	↑ 43 (47.26) ↓ 48 (52.74)	91

Table 2: Biological processes enriched by genes differentially expressed between experimental groups.

GeneGo Pathway	**N Genes**	***P***
VEH + FST X VEH + NAI		
Oxidative phosphorylation	29	2.68^{-11}
Apoptosis and survival controlled by HTR1A signaling	20	2.60^{-8}
Cytoskeleton remodeling controlled by Rho GTPases	11	3.45^{-7}
Cytoskeleton remodeling on neurophilaments	11	7.90^{-7}
VEH + FST X 7-NI + FST		
Oxidative phosphorylation	22	2.28^{-08}
Beta-adrenergic receptors transactivation	10	4.01^{-07}
Cell adhesion	17	2.05^{-06}
Cytoskeleton remodeling FAK signaling	12	3.97^{-06}
Transcription induced by CREB pathway	9	1.24^{-05}
VEH + FST X IMI + FST		
Oxidative phosphorylation	36	2.28^{-8}
Apoptosis and survival signaling by HTR_{1A}	18	1.28^{-7}
Cytoskeleton remodeling	22	3.78^{-6}
Transcription control by heterochromatin protein 1 family	11	5.25^{-6}
Neurophysiological process signaling by neuronal HTR_{1A}	14	6.02^{-6}
VEH + NAI X 7-NI + NAI		
Cytoskeleton remodeling	41	4.63^{-14}
Cytoskeleton remodeling associated with TGF and WNT	41	1.30^{-12}
Cell adhesion	36	7.37^{-11}
Cytoskeleton remodeling modulated by PKA	20	1.52^{-09}
Cytoskeleton remodeling modulated by integrin outside signaling	20	1.15^{-07}

Fig 1.

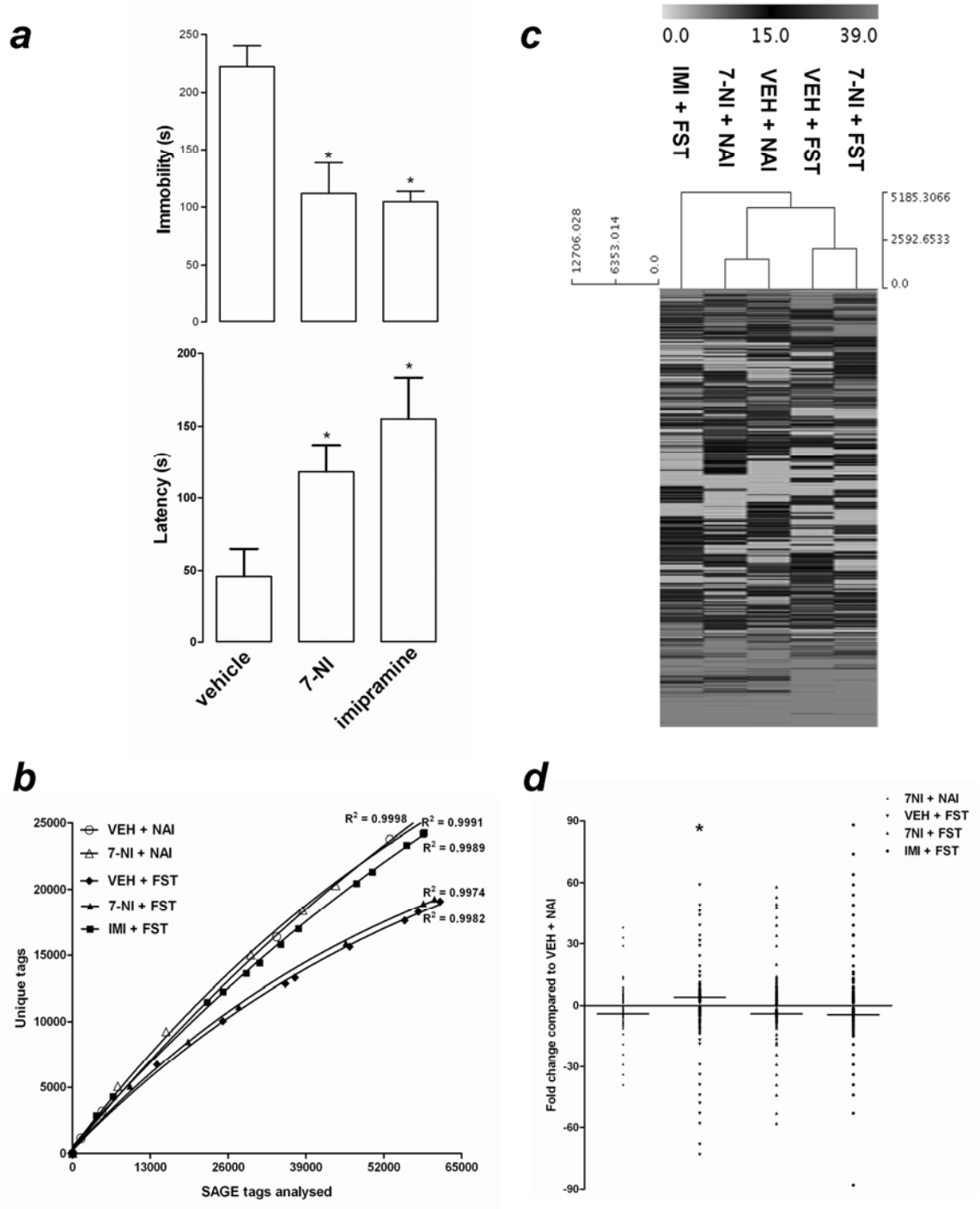

a
Immobility (s)
Latency (s)
vehicle
7-NI
imipramine
c
0.0
15.0
39.0
IMI + FST
7-NI + NAI
VEH + NAI
VEH + FST
7-NI + FST
12706.028
6353.014
0.0
5185.3066
2592.6533
0.0
b
VEH + NAI
7-NI + NAI
VEH + FST
7-NI + FST
IMI + FST
R² = 0.9998
R² = 0.9991
R² = 0.9989
R² = 0.9974
R² = 0.9982
Unique tags
SAGE tags analysed
d
7NI + NAI
VEH + FST
7NI + FST
IMI + FST
Fold change compared to VEH + NAI

Fig 2.

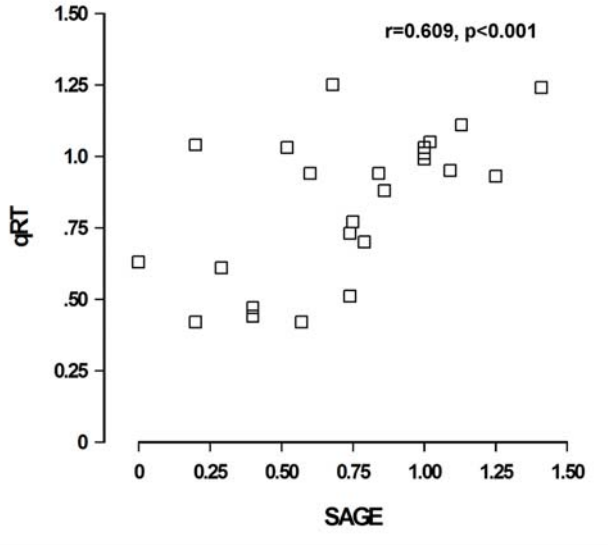
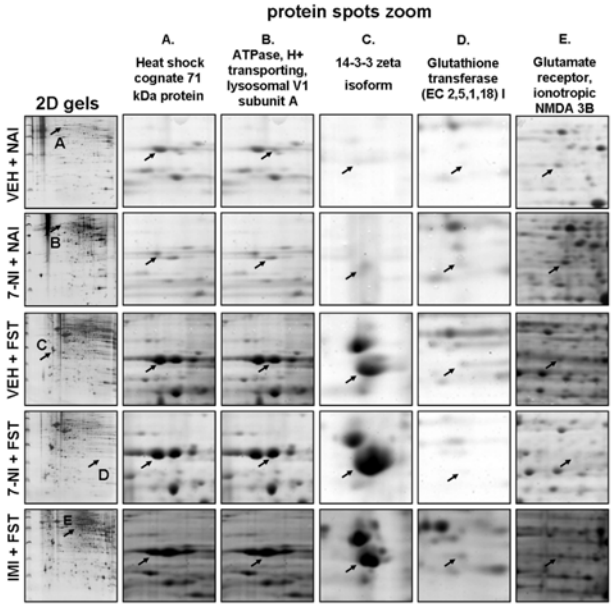

r=0.609, p<0.001
qRT
SAGE
1.50
1.25
1.0
.75
.50
0.25
0
0
0.25
0.50
0.75
1.00
1.25
1.50

Fig 3.


protein spots zoom
2D gels
A.
Heat shock cognate 71 kDa protein
B.
ATPase, H+ transporting, lysosomal V1 subunit A
C.
14-3-3 zeta isoform
D.
Glutathione transferase (EC 2,5,1,18) I
E.
Glutamate receptor, ionotropic NMDA 3B
VEH + NAI
7-NI + NAI
VEH + FST
7-NI + FST
IMI + FST
A
B
C
D
E

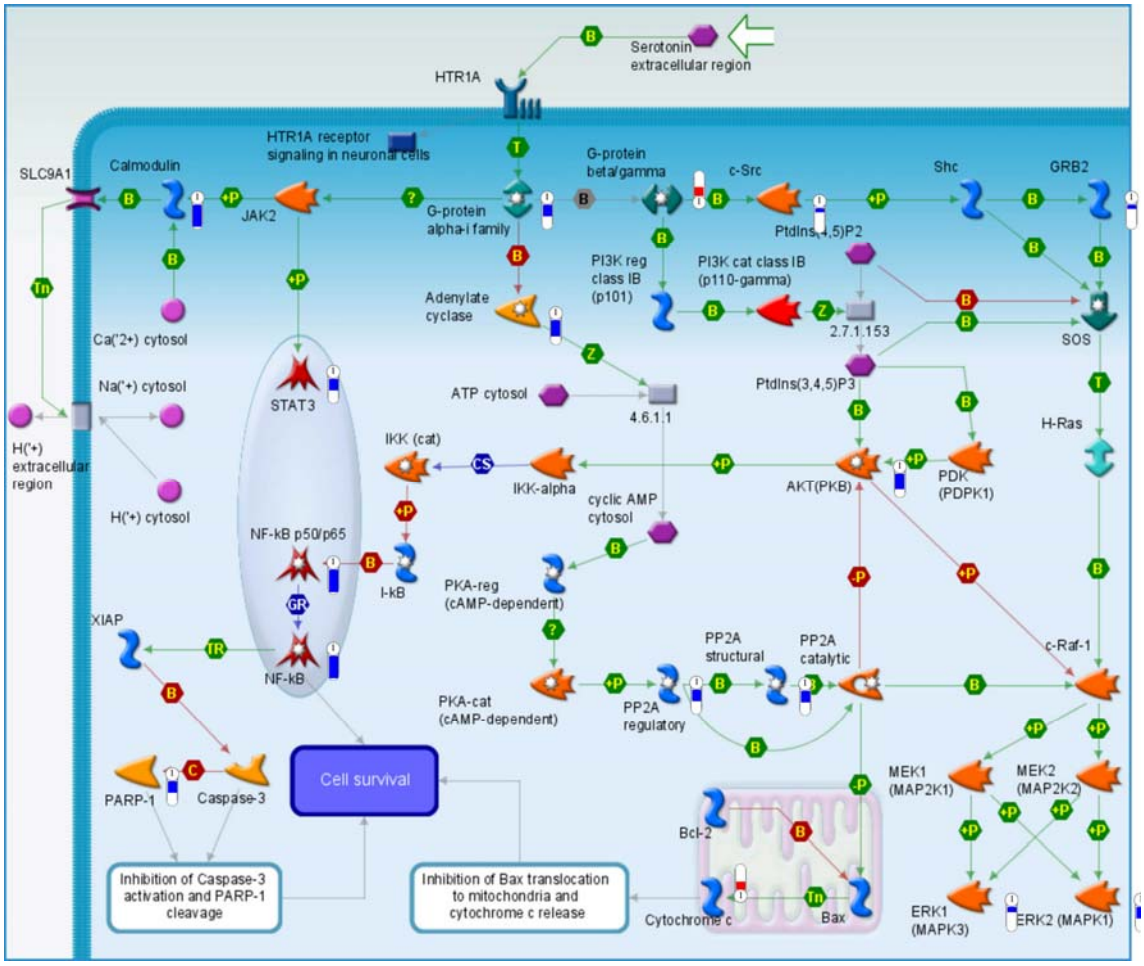
Supplement S1: Genes differentially expressed between the VEH + FST and VEH + NAI groups associated to oxidative phosphorylation, according to GeneGo analyses.

Gen tag	Symbol	Metabolic pathway	95%ci	FC
AATGGCTAAC	Cycs	Cytochrome c, somatic	[1.27; 3.40[38]]	**6.91**
TTAATATTTA	Ndufa6	NADH dehydrogenase 1 alpha subcomplex, 6 (B14) (predicted)	[1.08; 24]	**3.95**
TTAATAAATG	Cox4i1	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	[1.17; 4.26]	**2.19**
AATAAAAGTT	Atp5a1	ATP synthase subunit alpha, mitochondrial	[0; 2.57]	**1.81**
CAGAGTCGCT	Uqcrh	Cytochrome b-c1 complex subunit 6, mitochondrial	[-3.35; -1.17]	**-1.93**
ATGGCATCGT	Ndufab1	NADH dehydrogenase 1, alpha/beta subcomplex, 1 (predicted)	[-3.35; -1.22]	**-1.98**
GAGGGCTTCC	Atp5d	ATP synthase subunit delta, mitochondrial	[-3.35; -1.33]	**-2.07**
CGGGATCTGC	Atp5o	ATP synthase subunit O, mitochondrial	[-3; -1.44]	**-2.1**
GTGACAACTG	Cox8a	Cytochrome c oxidase polypeptide 8A, mitochondrial	[-2.85; -1.78]	**-2.26**
TGGGCACCTG	Atp5e	ATP synthase subunit epsilon, mitochondrial	[-3.35; -1.56]	**-2.27**
GCATACGGCG	Atp5i	ATP synthase subunit e, mitochondrial	[-4.88; -1.17]	**-2.3**
TTCTGGCTGC	Uqcrc1	Cytochrome b-c1 complex subunit 1, mitochondrial	[-4.88; -1.17]	**-2.3**
CCAGTCCTGG	Atp5g1	ATP synthase lipid-binding protein, mitochondrial	[-5.25; -1.86]	**-2.99**
AGGAGTTGCT	Ndufs8	NADH dehydrogenase Fe-S protein 8 (predicted)	[-32.33; -1.38]	**-5.07**
GGAAAGCTGG	Atp5b	ATP synthase subunit beta, mitochondrial	[0; 2.57]	**-9.92**
TTCTCAGCAG	Atp5c1	ATP synthase subunit gamma, mitochondrial	[0; 2.57]	**-9.92**
GTTGAGTCTG	Atp5j	ATP synthase-coupling factor 6, mitochondrial	[0; 2.57]	**-9.92**
GGCATAATTA	Ndufa3	similar to NADH-ubiquinone oxidoreductase B9 subunit	[0; 2.57]	**-9.92**
CGCTCGACCT	Ndufb4	NADH dehydrogenase 1 beta subcomplex, 4, 15kDa	[0; 2.57]	**-9.92**
TGGCTACTAA	Ndufb6	NADH dehydrogenase 1 beta subcomplex, 6 (predicted)	[0; 2.57]	**-9.92**
TTCCCTTCCT	Ndufb8	NADH dehydrogenase 1 beta subcomplex 8 (predicted)	[0; 2.57]	**-9.92**
CTTGCAAGTG	Ndufb9	NADH dehydrogenase 1 beta subcomplex, 9 (predicted)	[0; 2.57]	**-9.92**
TGGGCTGGTG	Ndufs7	NADH dehydrogenase Fe-S protein 7	[0; 2.57]	**-9.92**
AGAACTGCAG	Sdhb	Succinate dehydrogenase iron-sulfur subunit, mitochondrial	[0; 2.57]	**-9.92**
TAGACACAGC	Ndufa11	NADH dehydrogenase 1 alpha subcomplex subunit 11	[0; 1.7]	**-14.88**
TTACTGACTT	Ndufa5	NADH dehydrogenase 1 alpha subcomplex subunit 5	[0; 1.7]	**-14.88**
GCCCGGCCGG	Ndufc2	NADH dehydrogenase 1, subcomplex unknown, 2	[0; 1.7]	**-14.88**
ACTCAGCAAT	Ndufs5	NADH dehydrogenase Fe-S protein 5b, 15kDa	[0; 1.7]	**-14.88**
AGCGCCCAGA	Cox6a2	Cytochrome c oxidase polypeptide 6A2, mitochondrial	[0; 0.69]	**-34.72**
GGCCGCCCCA	Cox6a1	Cytochrome c oxidase polypeptide 6A1, mitochondrial	[0; 0.59]	**-39.68**

ci = confidence interval; FC = fold change.

Supplement S2

Biological pathways signaled by HTR_{1A} and responsible for controlling cell apoptosis and survival, according to GeneGo bank. Columns indexed by number 1 indicate the fold change rate for the genes differently expressed between VEH + NAI and VEH + FST. Red columns indicate increase of expression, and blue columns indicate increase of expression compared to the group VEH + NAI.



Supplement S3: Genes differentially expressed between VEH + FST and VEH + NAI groups associated with apoptosis and survival controlled by 5-HTR_{1A} signaling, according to GeneGo analyses.

Gen tag	Symbol	Metabolic pathway	95%ci	FC
TCACACATAT	Gng10	guanine nucleotide binding protein (G protein), gamma 10	[1.44; 3.40^{38}]	**7.9**
AATGGCTAAC	Cycs	Cytochrome c, somatic	[1.27; 3.4^{38}]	**6.91**
ATTTGAAATA	Gnai2	Guanine nucleotide-binding protein G(i), alpha-2 subunit	[1.38; 10.11]	**3.36**
GTCTGCTCCC	Ppp2r5b	protein phosphatase 2, regulatory subunit B (B56), beta isoform	[-4.88; -1.27]	**-2.45**
CCCGTTCTCC	Calm3	Calmodulin	[-8.09; -1.38]	**-3.04**
GCCCAGGTGT	Src	Proto-oncogene tyrosine-protein kinase Src	[-10.11; -1.08]	**-3.04**
AAGCCTTGCT	Grb2	Growth factor receptor-bound protein 2	[-11.5; -1.17]	**-3.29**
TAACGCCCTT	Mapk3	Mitogen-activated protein kinase 3	[-11.5; -1.5]	**-3.65**
ACCTTTGGCC	Gnao1	Guanine nucleotide-binding protein G(o) subunit alpha	[0; 2.57]	**-9.92**
TATGAGAATG	Mapk1	Mitogen-activated protein kinase 1	[0; 2.57]	**-9.92**
CTGCCGCCTC	Parp1	Poly [ADP-ribose] polymerase 1	[0; 2.57]	**-9.92**
TTTTTTTGTG	Ppp2r1a	protein phosphatase 2, regulatory subunit A, alpha isoform	[-6.69; -1.38]	**-9.92**
AAGTCTTTCT	Ppp2r5c	protein phosphatase 2, regulatory subunit B' gamma isoform	[0; 2.57]	**-9.92**
GCTCCCTGGA	Ppp2r5d	protein phosphatase 2, regulatory subunit B (B56), delta isoform	[0; 2.57]	**-9.92**
CCTGCAGTTT	Stat3	Signal transducer and activator of transcription 3	[0; 2.57]	**-9.92**
GGCAATATCC	Adcy3	Adenylate cyclase type 3	[0; 1.7]	**-14.88**
GGGTGCAGCG	Akt2	RAC-beta serine/threonine-protein kinase	[0; 1.7]	**-14.88**
GGATTGGGGC	Calm1	Calmodulin	[0; 1.22]	**-19.84**
CTGAGCAGTG	Rela	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	[0; 1.22]	**-19.84**

ci = confidence interval; FC = fold change.

Supplement S4: Genes differently expressed between the VEH + NAI and VEH + FST groups associated to regulation of action cytoskeleton by Rho GTPase and Cytoskeleton remodeling, according to GeneGo analyses.

Gen tag	Symbol	Metabolic pathway	95%ci	FC
Regulation of action cytoskeleton by Rho GTPases				
AAGAAAAGTG	Actr3	Actin-related protein 3	[1.17; 4.88]	**2.33**
GAAGCAGGAC	Cfl1	Cofilin-1	[-1.86; -1.17]	**-1.47**
GGCTGGGGGC	Pfn1	Profilin-1	[-2.7; -1.08]	**-1.7**
CCCTGAGTCC	Actb	Actin, cytoplasmic 1	[-3.55; -1.22]	**-2.08**
GTGGTGAGGA	Arpc1a	Actin-related protein 2/3 complex subunit 1A	[-6.69; -1.04]	**-2.53**
AGGACAGCAA	Myl6	similar to myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	[-10.11; -1.63]	**-3.72**
AGGACAGCAA	Myl6b	similar to myosin light chain 1 slow a	[-10.11; -1.63]	**-3.72**
TGGGTAGGTG	Cdc42	Cell division control protein 42 homolog	[0; 2.57]	**-9.92**
GAAGAGAATC	Limk1	LIM domain kinase 1	[0; 2.57]	**-9.92**
CTCCAGACAC	Myh14	myosin, heavy polypeptide 14	[0; 2.57]	**-9.92**
AGGCAGACTA	Limk2	LIM domain kinase 2	[0; 1.7]	**-14.88**
GCCTCTCAAT	Arpc5	Actin-related protein 2/3 complex subunit 5	[0; 1.22]	**-19.84**
Cytoskeleton remodeling				
CCCTGAGTCC	Actb	Actin, cytoplasmic 1	[-3.55; -1.22]	**-2.08**
CCAGCTCTTG	Dctn2	Dynactin subunit 2	[-15.67; -1.08]	**-3.38**
GGCGGGGGCC	Plec1	Plectin-1	[-11.5; -1.33]	**-3.55**
TCCTCAGCAC	Capzb	F-actin-capping protein subunit beta	[-32.33; -1.22]	**-4.56**
CACTGTCTGT	Tubg1	Tubulin gamma-1 chain	[-99; -1.08]	**-6.08**
TGCTTGGATC	Actr10	ARP10 actin-related protein 10 homolog (S. cerevisiae)	[0; 2.57]	**-9.92**
GCTTGCTGCC	Tuba1a	Tubulin alpha-1A chain	[0; 2.57]	**-9.92**
CCCTCGCCCG	Tubb2c	Tubulin beta-2C chain	[0; 2.57]	**-9.92**
GACTCCGTCC	Tubb4	tubulin, beta 4	[0; 2.57]	**-9.92**
GGGATTTGTG	Tubb5	Tubulin beta-5 chain	[0; 1.7]	**-14.88**
ATGCTTCTAC	Ina	Alpha-internexin	[0; 1.04]	**-24.8**

ci = confidence interval; FC = fold change.

Supplement S5: Genes differently expressed between the VEH + FST and 7-NI + FST groups associated to oxidative phosphorylation, according to GeneGo analyses.

Gen tag	Symbol	Metabolic pathway	95%ci	FC
GCAAGAGACT	Ndufb5	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5 (predicted)	[1.04; 99]	5.91
ATCAAGGTGG	Cox8a	Cytochrome c oxidase polypeptide 8A, mitochondrial	[1.04; 13.29]	3.28
TGGGCACCTG	Atp5e	ATP synthase subunit epsilon, mitochondrial	[-1.94; -1.04]	-1.42
TTGATTTTTT	Ndufa4	similar to NADH-ubiquinone oxidoreductase MLRQ subunit	[-3.55; -1.04]	-1.9
TTCTGGCTGC	Uqcrc1	Cytochrome b-c1 complex subunit 1, mitochondrial	[-4; -1.04]	-1.95
GTTGAGTCTG	Atp5j2	ATP synthase, H+ transporting, mitochondrial F0 comp. sub. f, isof. 2	[0; 2.45]	-2.24
ATGGCATCGT	Ndufab1	NADH dehydrogenase 1, alpha/beta subcomplex, 1	[-4; -1.38]	-2.27
TGATTTCTGT	Atp5a1	ATP synthase subunit alpha, mitochondrial	[0; 2.45]	-9.9
GGAAAGCTGG	Atp5b	ATP synthase subunit beta, mitochondrial	[0; 2.45]	-9.9
TTCTCAGCAG	Atp5c1	ATP synthase subunit gamma, mitochondrial	[0; 2.45]	-9.9
GTCCAAGATA	Atp5j	ATP synthase-coupling factor 6, mitochondrial	[0; 1.7]	-9.9
GGCATAATTA	Ndufa3	NADH-ubiquinone oxidoreductase B9 subunit	[0; 2.57]	-9.9
CCATCATCTG	Ndufb8	NADH dehydrogenase 1 beta subcomplex 8	[0; 2.45]	-9.9
GCCCGGCCGG	Ndufb9	NADH dehydrogenase 1 beta subcomplex, 9	[0; 2.45]	-9.9
AGAACTGCAG	Sdhb	Succinate dehydrogenase iron-sulfur subunit, mitochondrial	[0; 2.45]	-9.9
TATGAGGAAG	Sdhc	succinate dehydrogenase complex, subunit C, integral membrane protein	[0; 2.45]	-9.9
GCCGAGTGTA	Atp5l	similar to CG6105-PA	[-4; -1.33]	-14.88
TAGACACAGC	Ndufa11	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 11	[0; 1.7]	-14.88
TTACTGACTT	Ndufa5	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5	[0; 1.7]	-14.88
ATGGGGAGGC	Ndufb11	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 11 (predicted)	[0; 1.7]	-14.88
AGGAGTTGCT	Ndufs5	NADH dehydrogenase (ubiquinone) Fe-S protein 5b, 15kDa	[0; 1.7]	-14.88
TATCCAAGTC	Uqcrc2	Cytochrome b-c1 complex subunit 2, mitochondrial	[0; 1.22]	-19.84

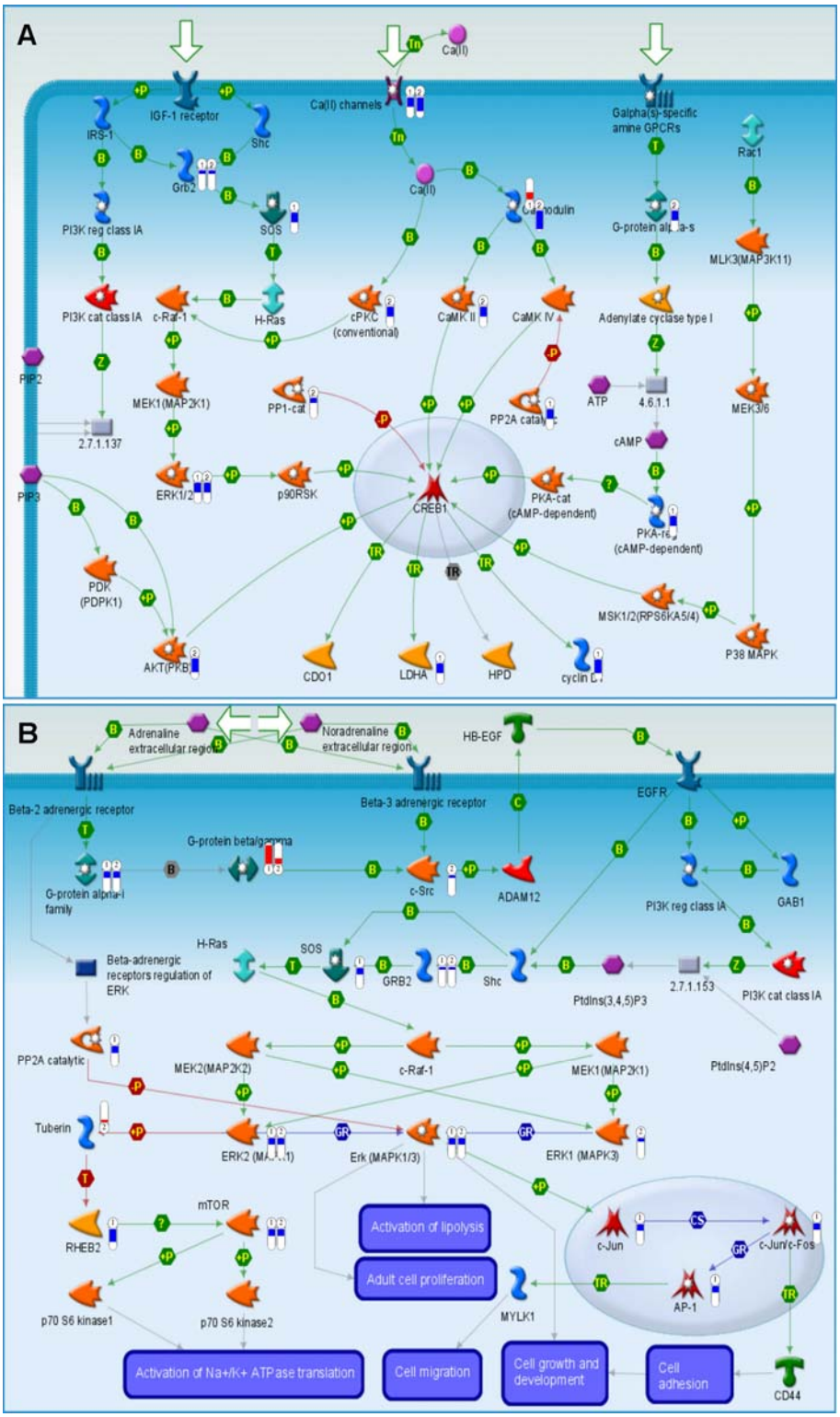
ci = confidence interval; FC = fold change.

Supplement S6: Genes differentially expressed between the VEH + FST and 7-NI + FST associated to cytoskeleton remodeling signaling by FAK and cell adhesion, according to GeneGo analyses.

Gen tag	Symbol	Metabolic pathway	95%ci	FC
Cytoskeleton remodeling signaling by FAK				
CACTTATAAA	Calm1	Calmodulin	[1.04; 99]	**5.91**
CTCAGAGGTC	Pak1	Serine/threonine-protein kinase PAK 1	[-4; -1.17]	**-2.1**
AAGCCTTGCT	Grb2	Growth factor receptor-bound protein 2	[-8.09; -1.04]	**-2.64**
ACGTTTCTTC	Plcb1	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-1	[-24; -1.08]	**-4.06**
TGGAAACCAC	Cdc42	Cell division control protein 42 homolog	[0; 2.45]	**-9.9**
TATGAGAATG	Mapk1	Mitogen-activated protein kinase 1	[0; 2.45]	**-9.9**
TTCTAGCAGA	Sos2	Son of sevenless homolog 2 (Drosophila)	[0; 2.45]	**-9.9**
TGGTTTCATT	Traf3	Tnf receptor-associated factor 3 (predicted)	[0; 2.45]	**-9.9**
AAGGCTGAAA	Plcb4	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-4	[0; 2.45]	**-9.9**
GTTTTGATTC	Ptk2	Focal adhesion kinase 1	[0; 2.45]	**-9.9**
CTGCCTTAAT	Ccnd3	G1/S-specific cyclin-D3	[0; 1.22]	**-19.84**
GGCTCCAGTC	Bcar1	Breast cancer anti-estrogen resistance protein 1	[0; 0.79]	**-30.0**
Cell adhesion				
GCTGTGGCCA	Gnb4	Guanine nucleotide-binding protein subunit beta-4	[1.22; 3.4038]	**29.3**
GCTTTATTGT	Actb	Actin, cytoplasmic 1	[1.38; 5.25]	**2.58**
CTCAGAGGTC	Pak1	Serine/threonine-protein kinase PAK 1	[-4; -1.17]	**-2.1**
AAGCCTTGCT	Grb2	Growth factor receptor-bound protein 2	[-8.09; -1.04]	**-2.64**
GTGGTGAGGA	Arpc1a	Actin-related protein 2/3 complex subunit 1A	[-9; -1.22]	**-3.05**
TGCCTTGTCC	Actn4	Alpha-actinin-4	[0; 2.45]	**-9.9**
TGGGTAGGTG	Cdc42	Cell division control protein 42 homolog	[0; 2.45]	**-9.9**
ACCTTTGGCC	Gnao1	Guanine nucleotide-binding protein G(o) subunit alpha 1	[0; 2.45]	**-9.9**
TTCTAGCAGA	Sos2	Son of sevenless homolog 2 (Drosophila)	[0; 2.45]	**-9.9**
GAAGGTTTTA	Jun	Transcription factor AP-1	[0; 2.45]	**-9.9**
GAAGAGAATC	Limk1	LIM domain kinase 1	[0; 2.45]	**-9.9**
TATGAGAATG	Mapk1	Mitogen-activated protein kinase 1	[0; 2.45]	**-9.9**
GTTTTGATTC	Ptk2	Focal adhesion kinase 1	[0; 2.45]	**-9.9**
GCGTCGCTGA	RGD1564327	Integrin alpha 8	[0; 2.45]	**-9.9**
TTCAGCAGCA	Gnb1	Guanine nucleotide-binding protein subunit beta-4	[0; 1.7]	**-14.88**
TGTTCACTAA	Serpine2	Glia-derived nexin	[0; 1.22]	**-19.84**
GGCTCCAGTC	Bcar1	Breast cancer anti-estrogen resistance protein 1	[0; 0.79]	**-30.0**

ci = confidence interval; FC = fold change.

Supplement S7. Biological pathways associated to gene transcription regulated by the CREB pathway (A) and beta-adrenergic receptors transactivation pathway (B) according to GeneGo. Columns indexed by number 1 indicate the fold change rate for the genes differently expressed between VEH + FST and 7-NI + FST. Red columns indicate increase of expression, and blue columns indicate increase of expression compared to the group 7-NI + FST. Columns indexed by number 2 indicate the fold change rate for the genes differently expressed between VEH + NAI and VEH + FST. Red columns indicate increase of expression, and blue columns indicate increase of expression compared to the group VEH + NAI.


A
IGF-1 receptor
IRS-1
Shc
Grb2
SOS
PI3K reg class IA
PI3K cat class IA
H-Ras
c-Raf-1
MEK1(MAP2K1)
ERK1/2
p90RSK
PIP2
PIP3
2.7.1.137
PDK (PDPK1)
AKT(PKB)
Ca(II) channels
Ca(II)
cPKC (conventional)
CaMK II
CaMK IV
PP1-cat
CREB1
PKA-cat (cAMP-dependent)
CDO1
LDHA
HPD
Galpha(s)-specific amine GPCRs
G-protein alpha-s
Adenylate cyclase type I
ATP
4.6.1.1
cAMP
Rac1
MLK3(MAP3K11)
MEK3/6
MSK1/2(RPS6KA5/4)
P38 MAPK
B
Adrenaline extracellular region
Noradrenaline extracellular region
Beta-2 adrenergic receptor
Beta-3 adrenergic receptor
G-protein alpha-i family
c-Src
ADAM12
HB-EGF
EGFR
PI3K reg class IA
GAB1
Beta-adrenergic receptors regulation of ERK
GRB2
PtdIns(3,4,5)P3
2.7.1.153
PI3K cat class IA
PtdIns(4,5)P2
PP2A catalytic
MEK2(MAP2K2)
c-Raf-1
MEK1(MAP2K1)
Tuberin
ERK1 (MAPK3)
Erk (MAPK1/3)
mTOR
RHEB2
p70 S6 kinase1
p70 S6 kinase2
Activation of lipolysis
Adult cell proliferation
MYLK1
c-Jun
c-Jun/c-Fos
AP-1
CD44
Activation of Na+/k+ ATPase translation
Cell migration
Cell growth and development
Cell adhesion

Supplement S8: Genes differentially expressed between the groups VEH + FST and 7-NI + FST associated to transcriptional CREB pathway and pathway signaling by β-adrenergic receptor, according to GeneGo analyses.

Gene tag	Symbol	Description	95%ci	FC
Transcriptional CREB pathway				
CACTTATAAA	Calm1	Calmodulin 1	[1.04; 99]	**5.91**
AAGCCTTGCT	Grb2	Growth factor receptor-bound protein 2	[-8.09; -1.04]	**-2.64**
AGAAAGAAAA	Cacnb4	Calcium channel. Voltage-dependent. beta 4 subunit	[0; 2.45]	**-9.9**
CAGTTGGCAG	Ldha	L-lactate dehydrogenase A chain	[0; 2.45]	**-9.9**
TATGAGAATG	Mapk1	Mitogen-activated protein kinase 1	[0; 2.45]	**-9.9**
GATTATAGAG	Ppp2cb	Serine/threonine-protein phosphatase 2A catalytic subunit beta isoform	[0; 2.45]	**-9.9**
TCAGGACAGT	Prkar1b	cAMP-dependent protein kinase type I-beta regulatory subunit	[0; 2.45]	**-9.9**
TTCTAGCAGA	Sos2	Son of sevenless homolog 2 (Drosophila)	[0; 2.45]	**-9.9**
GTCCAGGAAA	Ccn1	G1/S-specific cyclin-D1	[0; 1.7]	**-14.88**
Pathway signaling by β-adrenergic receptor				
GCTGTGGCCA	Gnb4	Guanine nucleotide-binding protein subunit beta-4	[1.22; 3.40^{38}]	**29.3**
AAGCCTTGCT	Grb2	Growth factor receptor-bound protein 2	[-8.09; -1.04]	**-2.64**
ATGCTGAACC	Frap1	FKBP12-rapamycin complex-associated protein	[0; 2.45]	**-9.9**
ACCTTTGGCC	Gnao1	Guanine nucleotide-binding protein G(o) subunit alpha 1	[0; 2.45]	**-9.9**
GAAGGTTTTA	Jun	Transcription factor AP-1	[0; 2.45]	**-9.9**
TATGAGAATG	Mapk1	Mitogen-activated protein kinase 1	[0; 2.45]	**-9.9**
GATTATAGAG	Ppp2cb	Serine/threonine-protein phosphatase 2A catalytic subunit beta isoform	[0; 2.45]	**-9.9**
TTCTAGCAGA	Sos2	Son of sevenless homolog 2 (Drosophila)	[0; 2.45]	**-9.9**
TTCAGCAGCA	Gnb1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	[0; 1.7]	**-14.88**
GTGGGGAAAG	Rheb	GTP-binding protein Rheb	[0; 1.22]	**-19.84**

ci = confidence interval; FC = fold change.

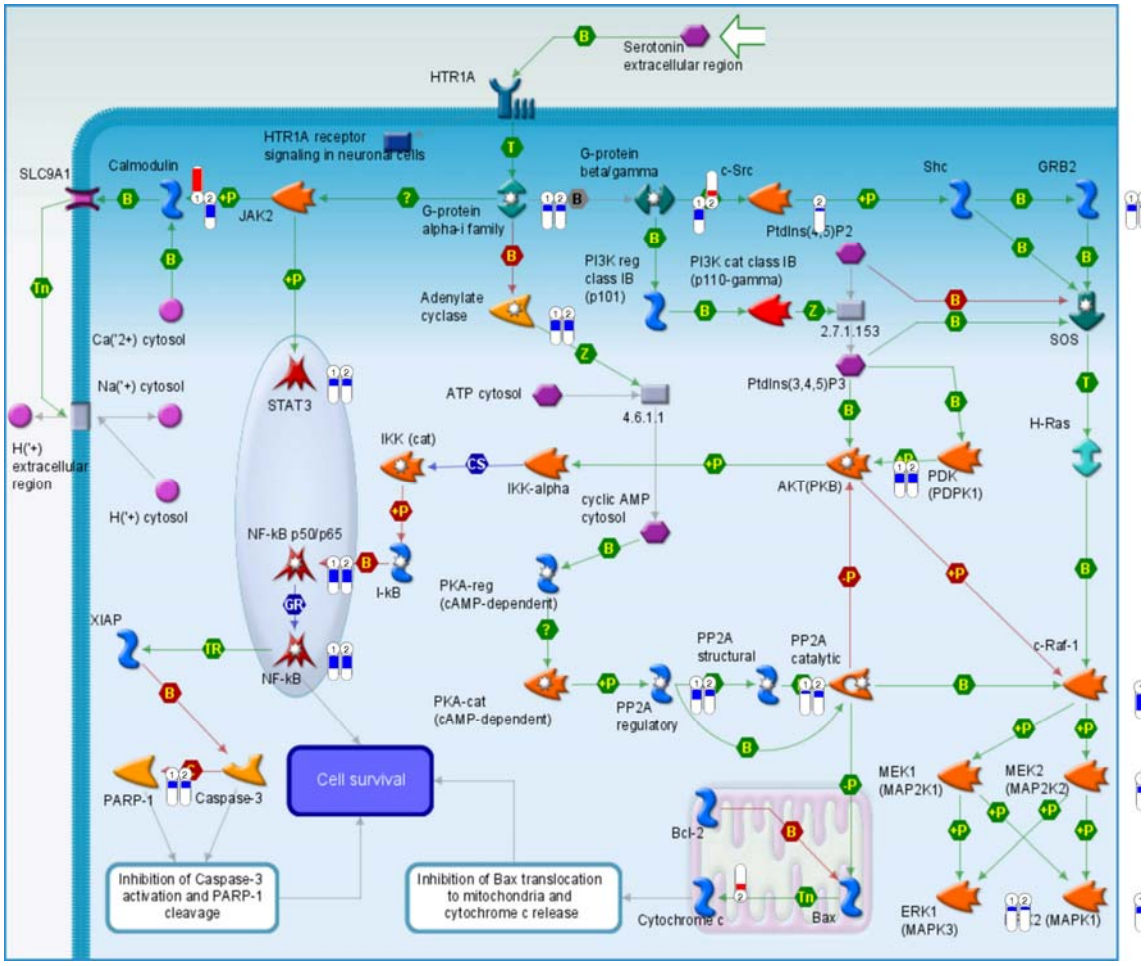
Supplement S9: Genes differentially expressed between the groups VEH + FST and IMI + FST associated with oxidative phosphorylation, according to GeneGo analyses.

Gene tag	Symbol	Description	95%ci	FC
ACCTCTCGAT	Coxa8	Cytochrome c oxidase polypeptide 8A, mitochondrial	[2.57; 3.40^{38}]	**59.25**
CTGGAAGCTG	Cox4i1	Cytochrome c oxidase subunit 4 isoform 1. mitochondrial	[1.86; 3.40^{38}]	**44.44**
CTTAGTGTTC	LOC679739	NADH dehydrogenase (ubiquinone) Fe-S protein 6	[1.38; 3.40^{38}]	**34.56**
GCAAGAGACT	Ndufb5	NADH dehydrogenase (ubiquinone) 1 beta subcomplex. 5	[1.08; 99]	**5.97**
TATCCAAGTC	Uqcr	Similar to ubiquinol-cytochrome c reductase subunit	[1.5; 6.14]	**2.89**
AATAAAAGTT	Atp5A1	ATP synthase subunit alpha. mitochondrial	[1.27; 4]	**2.21**
ATGGCATCGT	Ndufab1	NADH dehydrogenase (ubiquinone) 1. alpha/beta subcomplex. 1	[-2.45; -0.96]	**-1.52**
GGGCAACCAG	Uqcrq	Cytochrome b-c1 complex subunit 8	[-2.57; -0.96]	**-1.56**
GCATACGGCG	Atp5i	ATP synthase subunit e. Mitochondrial	[-3.55; -0.96]	**-1.79**
CAGAGTCGCT	Uqcrh	Cytochrome b-c1 complex subunit 6. mitochondrial	[-3.17; -1.08]	**-1.83**
TACCATCTTT	Sdhc	succinate dehydrogenase complex. subunit C. integral membrane protein	[-5.25; -0.96]	**-2.13**
GAAATATGTG	Atp5g3	ATP synthase lipid-binding protein. mitochondrial	[-4.26; -1.44]	**-2.43**
TACTAGAAAA	Ndufs2	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2. mitochondrial	[-8.09; -1.08]	**-2.81**
GGGAGCTGTG	Ndufs7	NADH dehydrogenase (ubiquinone) Fe-S protein 7	[-15.67; -1.08]	**-3.35**
GGCCGCCCCA	Cox6a1	Cytochrome c oxidase polypeptide 6A1. mitochondrial	[-24; -1.04]	**-4.02**
CCAGTCCTGG	Atp5q1	ATP synthase lipid-binding protein. mitochondrial	[-9; -2.57]	**-4.56**
GAAAGTAGGT	Cox7b	Cytochrome c oxidase polypeptide 7B. mitochondrial	[-3.40^{38}; -1.27]	**-7.03**
TGACCTGTGA	Atp5b	ATP synthase subunit beta. Mitochondrial	[0; 2.45]	**-9.92**
TTCTCAGCAG	Atp5c1	ATP synthase subunit gamma. mitochondrial	[0; 2.45]	**-9.92**
ACCTGGCAGG	Atp5	ATP synthase subunit delta. mitochondrial	[0; 2.45]	**-9.92**
GTTGAGTCTG	Atp5j	ATP synthase-coupling factor 6. mitochondrial	[0; 2.45]	**-9.92**
CGCTCGACCT	Ndufb4	NADH dehydrogenase (ubiquinone) 1 beta subcomplex. 4. 15kDa	[0; 2.45]	**-9.92**
GCTGGCTAGT	Cox5b	Cytochrome c oxidase subunit 5B. mitochondrial	[0; 2.45]	**-9.92**
CCATCATCTG	Nbufb8	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 8	[0; 2.45]	**-9.92**
AACAAGGAGT	Sdha	Succinate dehydrogenase [ubiquinone] flavoprotein subunit. mitochondrial	[0; 2.45]	**-9.92**
AACAGTGCGG	Ndufc2	NADH dehydrogenase (ubiquinone) 1. subcomplex unknown. 2	[0; 2.45]	**-9.92**
GGCATAATTA	Ndufa3	NADH-ubiquinone oxidoreductase B9 subunit (Complex I-B9)	[0; 2.45]	**-9.92**
AACAGTGCGG	Nbufb9	NADH dehydrogenase (ubiquinone) 1 beta subcomplex. 9	[0; 2.45]	**-9.92**
AGAACTGCAG	Sdhb	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit. mitochondrial	[0; 2.45]	**-9.92**
TAGACACAGC	Ndufa10	NADH dehydrogenase 1 alpha subcomplex 10-like protein	[0; 1.17]	**-14.8**
AACCCAAGTT	Atp5o	ATP synthase subunit O. Mitochondrial	[0; 1.63]	**-14.8**
TTACTGACTT	Ndufa5	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5	[0; 1.63]	**-14.8**
ACTCAGCAAT	Ndufs5	NADH dehydrogenase (ubiquinone) Fe-S protein 5b. 15kDa	[0; 1.63]	**-14.8**
TATCCAAGTC	Uqcrc2	Cytochrome b-c1 complex subunit 2. mitochondrial	[0; 1.17]	**-19.8**
AGGGTGCCAT	Ndufa11	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 11	[0; 1.63]	**-19.8**
AGCGCCCAGA	Cox6a2	Cytochrome c oxidase polypeptide 6A2. mitochondrial	[0; 0.67]	**-34.7**

ci = confidence interval; FC = fold change.

Supplement S10

Biological pathways linked to apoptosis and survival signaled by HTR_{1A} according to GeneGo. Red columns indicate increase of expression, and blue columns indicate increase of expression compared to the group IMI + FST. Columns indexed by number 2 indicate the fold change rate for the genes differently expressed between VEH + NAI and VEH + FST. Red columns indicate increase of expression, and blue columns indicate increase of expression compared to the group VEH + NAI.


Serotonin extracellular region
HTR1A
HTR1A receptor signaling in neuronal cells
SLC9A1
Calmodulin
JAK2
G-protein alpha-i family
G-protein beta/gamma
c-Src
Shc
GRB2
PtdIns(4,5)P2
PI3K reg class IB (p101)
PI3K cat class IB (p110-gamma)
Adenylate cyclase
2.7.1.153
SOS
Ca(2+) cytosol
Na(+) cytosol
H(+) extracellular region
H(+) cytosol
STAT3
ATP cytosol
4.6.1.1
PtdIns(3,4,5)P3
H-Ras
IKK (cat)
IKK-alpha
AKT(PKB)
PDK (PDPK1)
cyclic AMP cytosol
NF-kB p50/p65
I-kB
PKA-reg (cAMP-dependent)
XIAP
NF-kB
PP2A structural
PP2A catalytic
c-Raf-1
PKA-cat (cAMP-dependent)
PP2A regulatory
MEK1 (MAP2K1)
MEK2 (MAP2K2)
PARP-1
Caspase-3
Cell survival
Bcl-2
Cytochrome c
Bax
ERK1 (MAPK3)
2 (MAPK1)
Inhibition of Caspase-3 activation and PARP-1 cleavage
Inhibition of Bax translocation to mitochondria and cytochrome c release

Supplement S11: Genes differentially expressed between the groups VEH + FST and IMI + FST associated to apoptosis and survival HTR_{1A} and neurophysiological process $5HTR_{1A}$ signaling in neuronal cells according to GeneGo analyses.

Gene tag	Symbol	Description	95%ci	FC
Apoptosis and survival HTR_{1A} signaling				
ACGAACCTGG	Calm3	Calmodulin	[1.38; 3.4038]	**34.56**
TTTTTTTGTG	Ppp2r1a	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65)	[-5.67; -1.22]	**-2.57**
TAACGCCCTT	Mapk3	Mitogen-activated protein kinase 3	[-24; -2.13]	**-6.03**
TGCTTTGTAG	Gnao1	Guanine nucleotide-binding protein G(o) subunit alpha 1	[0; 2.45]	**-9.92**
ATGTTTAAGG	Grb2	Growth factor receptor-bound protein 2	[0; 2.45]	**-9.92**
CCTTCATCAA	Mak2k2	Dual specificity mitogen-activated protein kinase kinase 2	[0; 2.45]	**-9.92**
TATGAGAATG	Mapk1	Mitogen-activated protein kinase 1	[0; 2.45]	**-9.92**
CTGCCGCCTC	Parp1	Poly [ADP-ribose] polymerase 1	[0; 2.45]	**-9.92**
AAGTCTTTCT	Ppp2r5	protein phosphatase 2, regulatory subunit B' gamma isoform	[0; 2.45]	**-9.92**
GCATCCTGCT	Stat3	Signal transducer and activator of transcription 3	[0; 2.45]	**-9.92**
TGTCTCATTG	Adcy2	Adenylate cyclase 2	[0; 1.63]	**-14.8**
GGCAATATCC	Adcy3	Adenylate cyclase type 3	[0; 1.63]	**-14.8**
GGGTGCAGCG	Akt2	RAC-beta serine/threonine-protein kinase	[0; 1.63]	**-14.8**
TTCAGCAGCA	Gnb1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	[0; 1.63]	**-14.8**
ACAGTTTACC	Ppp2r4	protein phosphatase 2A, regulatory subunit B (PR 53) (predicted)	[0; 1.63]	**-14.8**
ACAGTCCCCA	Calm1	Calmodulin	[0; 1.17]	**-19.8**
CTGAGCAGTG	Rela	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	[0; 1.17]	**-19.8**
GAGTCCTGGC	Raf	RAF proto-oncogene serine/threonine-protein kinase	[0; 1]	**-24.8**
Neurophysiological process HTR_{1A} signaling in neuronal cells				
TTTTTCAATC	Map2	Microtubule-associated protein 2	[1.5; 32.33]	**5.47**
AGGTATTGTA	Gria3	Glutamate receptor 3	[1.04; 24]	**3.98**
GCTGGCAGCC	Ppp1ca	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	[-2.57; -0.96]	**-1.56**
GACACTGGAT	Ppp1r1a	Protein phosphatase 1 regulatory subunit 1A	[-15.67; -2.33]	**-5.63**
TAACGCCCTT	Mapk3	Mitogen-activated protein kinase 3	[-24; -2.13]	**-6.03**
TGCTTTGTAG	Gnao1	Guanine nucleotide-binding protein G(o) subunit alpha 1	[0; 2.45]	**-9.92**
TTGTGATGAC	Kcnj5	G protein-activated inward rectifier potassium channel 4	[0; 2.45]	**-9.92**
CCTTCATCAA	Map2k2	Dual specificity mitogen-activated protein kinase kinase 2	[0; 2.45]	**-9.92**
TATGAGAATG	Mapk1	Mitogen-activated protein kinase 1	[0; 2.45]	**-9.92**
AAGGCTGAAA	Plc	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-4	[0; 2.45]	**-9.92**
TGTCTCATTG	Adcy2	Adenylate cyclase type 2	[0; 1.63]	**-14.8**
GGCAATATCC	Adcy3	Adenylate cyclase type 3	[0; 1.63]	**-14.8**
TTCAGCAGCA	Gnb1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	[0; 1.63]	**-14.8**
GAGTCCTGGC	Raf1	RAF proto-oncogene serine/threonine-protein kinase	[0; 1]	**-24.8**

ci = confidence interval; FC = fold change.

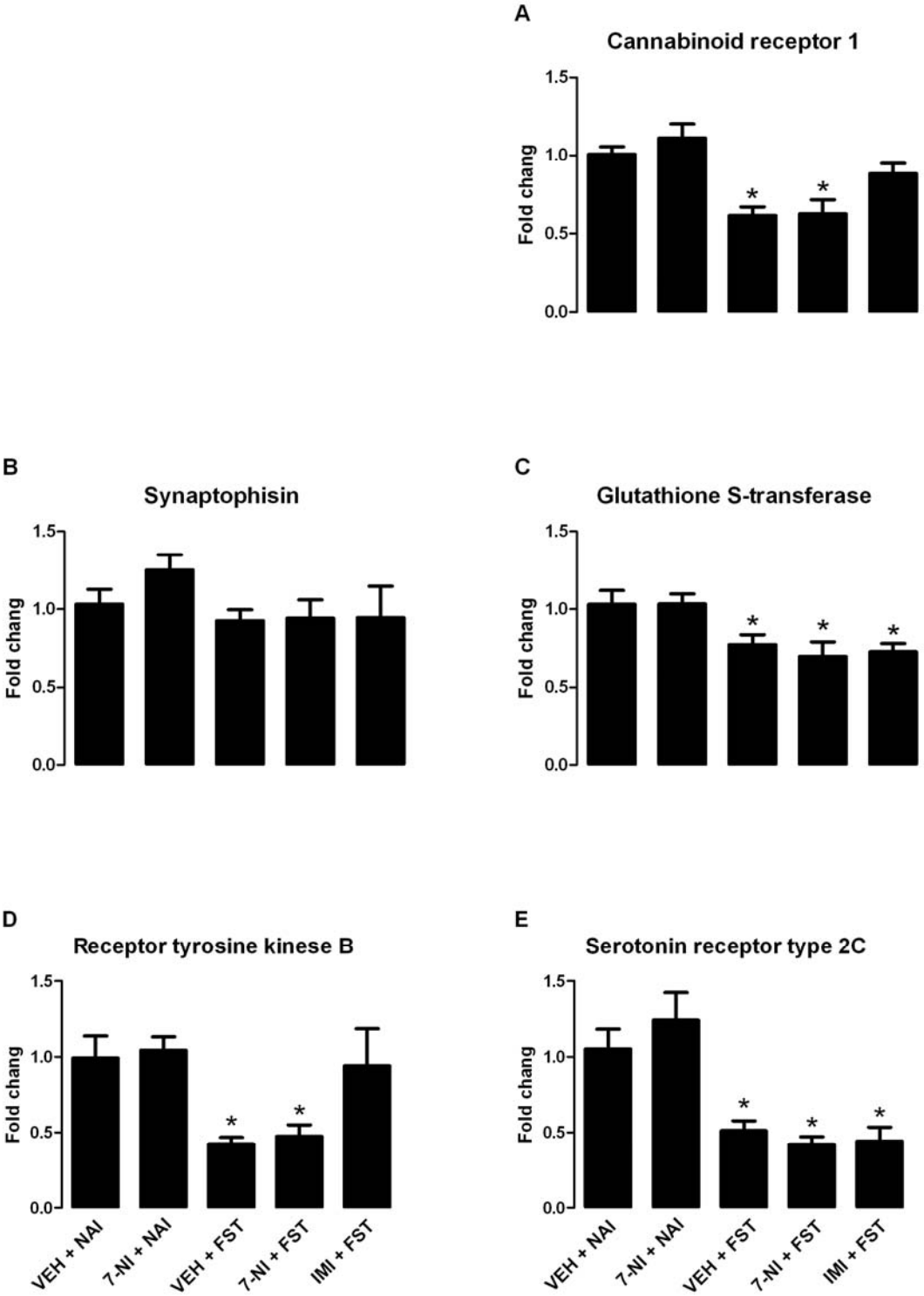
Supplement S12: Genes differentially expressed between the VEH + FST and IMI + FST groups associated to cytoskeleton remodeling and transcription control by heterochromatin protein 1 (HP1) family according to GeneGo analyses.

Gene tag	Symbol	Description	95%ci	FC
Cytoskeleton remodeling				
TAGTGATCTG	Actg1	Actin, cytoplasmic 2	[1.86; 3.40]	**44.44**
GCCTCCAAGA	Plat	Tissue-type plasminogen activator	[1.17; 3.40]	**29.62**
GAGCTGCTCA	My10	Myosin-10	[1.38; 32.33]	**4.98**
CTGCCAACTT	Cfl1	Cofilin-1	[-1.94; -1.27]	**-1.57**
CTCAGAGGTC	Pak1	Serine/threonine-protein kinase PAK 1	[-3; -0.92]	**-1.64**
CCCTGAGTCC	Actb	Actin, cytoplasmic 1	[-3.35; -1.17]	**-1.96**
GGGCGGAGCT	Myl6	similar to myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	[-6.69; -1.27]	**-2.76**
AGGACAGCAA	Myl6b	similar to myosin light chain 1 slow a	[-10.11; -1.63]	**-2.76**
GTGGTGAGGA	Arpc1a	Actin-related protein 2/3 complex subunit 1A	[-9; -1.17]	**-3.01**
TGCAGGCTGG	Csnk2k	casein kinase II, alpha 2, polypeptide (predicted)	[0; 1.17]	**-5.53**
TAACGCCCTT	Mapk3	Mitogen-activated protein kinase 3	[-24; -2.13]	**-6.03**
GTGCATCCCG	Csnk2b	Casein kinase II subunit beta	[-3.40; -1.5]	**-8.04**
TGGGTAGGTG	Cdc42	Cell division control protein 42 homolog	[0; 2.45]	**-9.92**
CAGGCCAGCC	Eif4ebp1	Eukaryotic translation initiation factor 4E-binding protein 1	[0; 2.45]	**-9.92**
ATGTTTAAGG	Grb2	Growth factor receptor-bound protein 2	[0; 2.45]	**-9.92**
CCGTTCTGGA	LOC679869	similar to transcription factor 7-like 2, T-cell specific, HMG-box	[0; 2.45]	**-9.92**
CCTTCATCAA	Map2k2	Dual specificity mitogen-activated protein kinase kinase 2	[0; 2.45]	**-9.92**
TATGAGAATG	Mapk1	Mitogen-activated protein kinase 1	[0; 2.45]	**-9.92**
GAAGATGGAG	Ncl	Nucleolin	[0; 2.45]	**-9.92**
GTTTTGATTC	Ptk2	Focal adhesion kinase 1	[0; 2.45]	**-9.92**
TGCAGGCTGG	Col1a1	Collagen alpha-1(I) chain	[0; 1.17]	**-19.8**
GAGTCCTGGC	Raf1	RAF proto-oncogene serine/threonine-protein kinase	[0; 1]	**-24.8**
Transcription control by heterochromatin protein 1 (HP1) family				
TTATGCAGTG	Ywhag	14-3-3 protein epsilon	[2.85; 3.40[38]]	**64.19**
ATGGGTGGTT	Ywhah	14-3-3 protein ETA	[1; 1.7]	**1.33**
TTTGTGACTG	Ctbp1	C-terminal-binding protein 1	[-5.25; -0.96]	**-2.13**
CCGTTCTGGA	loc679869	similar to transcription factor 7-like 2, T-cell specific, HMG-box	[0; 2.45]	**-9.92**
GTCTGTGGAG	Mef2d	Myocyte-specific enhancer factor 2D	[0; 2.45]	**-9.92**
CTTGTGCACA	Trim28	Transcription intermediary factor 1-beta	[0; 2.45]	**-9.92**
ACCGTTCTAC	Hdac6	histone deacetylase 6	[0; 1.63]	**-14.88**
ATGATCCGGT	Ywhaz	14-3-3 protein zeta/delta	[0; 1.63]	**-14.88**
CCCAAAGACA	H3f3	Histone H3,3	[0; 1.17]	**-19.8**
TATAATGGCA	Trim24	Ab2-427	[0; 0.79]	**-29.7**

ci = confidence interval; FC = fold change.

Supplement S13

Effect 7-nitroindazole, imipramine or vehicle on hipocampal gene expression at rats (n=4–8/group) submitted to the forced swimming test. The black bars represent means±SEM of fold change for qRT, *P<0.05, Duncan test. VEH + NAI = animals treated with vehicle (N=4; saline:DMSO, 1:1, 2mL/Kg, for 14 days), 7-NI + NAI = animals treated with 7-NI (N=4; 60mg/Kg by 14 days), VEH + FST = animals treated with vehicle (N=4; saline:DMSO, 1:1, 2mL/Kg for 14 days) and submitted to FST, 7-NI + FST = animals treated with 7-NI (N=3; 60mg/Kg for 14 day) and submitted to FST, IMI + FST = animals treated with imipramine (15mg/Kg for 14 days) and submitted to FST.


A
Cannabinoid receptor 1
Fold chang
1.5
1.0
0.5
0.0
*
*
B
Synaptophisin
Fold chang
1.5
1.0
0.5
0.0
C
Glutathione S-transferase
Fold chang
1.5
1.0
0.5
0.0
*
*
*
D
Receptor tyrosine kinese B
Fold chang
1.5
1.0
0.5
0.0
*
*
VEH + NAI
7-NI + NAI
VEH + FST
7-NI + FST
IMI + FST
E
Serotonin receptor type 2C
Fold chang
1.5
1.0
0.5
0.0
*
*
*
VEH + NAI
7-NI + NAI
VEH + FST
7-NI + FST
IMI + FST

Supplement 14: Proteins differentially expressed between the VEH + NAI and VEH + FST groups.

GI	Description	%	MS	FC
49481	beta tubulin	11	272	-5,14
54855	triosephosphate isomerase	8	118	2,79
111707	glutathione transferase (EC 2,5,1,18) I	10	49	-∞
123647	Heat shock cognate 71 kDa protein	6	132	-10,16
203055	ATP synthase alpha subunit precursor (EC 3,6,1,3)	13	396	-∞
203658	Cu-Zn superoxide dismutase (EC 1,15,1,1)	32	362	2,22
1170100	Glutathione S-transferase P; AltName: Full=GST class-pi	20	176	3,37
1373363	Platelet-activating factor acetylhydrolase isoform Ib beta subunit	15	169	-∞
1374715	ATP synthase beta subunit	18	253	-10,57
1915913	Ulip2 protein	4	63	8,27
6981112	isovaleryl Coenzyme A dehydrogenase	4	80	2,94
8918488	adenylate kinase isozyme 1	30	335	4,48
12853094	unnamed protein product	7	165	-∞
13385854	peptidylprolyl isomerase D	10	187	5,68
40538860	aconitase 2, mitochondrial	8	339	2,58
54400730	chaperonin containing TCP1, subunit 2 (beta)	6	270	-3,21
62653546	similar to glyceraldehyde-3-phosphate dehydrogenase	8	161	-∞
157819953	ATPase, H+ transporting, lysosomal V1 subunit A	9	220	-22,9
1184663	vacuolar adenosine triphosphatase subunit E	10	110	-∞
123230374	dynamin 1	8	390	2,28
13489067	N-ethylmaleimide-sensitive factor	4	202	-4,58
16758446	isocitrate dehydrogenase 3 (NAD+) alpha	12	236	-∞
260033	Macrophage migration inhibitory factor 13kda protein	42	70	-∞
487851	Dynamin	9	492	-4,28
54855	triosephosphate isomerase	5	72	2,79
57657	pyruvate dehydrogenase E1 alpha form 1 subunit	10	201	4,29
62653546	similar to glyceraldehyde-3-phosphate dehydrogenase	8	162	11,4
6754816	septin 2	3	71	-2,83

GI = NCBI gene identification, % = protein covery; FC = fold change on VEH + NAI compared to VEH + FST. MS = Mascot score. -∞ = protein with expression detectable only on VEH + FST group; +∞ = protein with expression detectable only on 7NI + FST group.

Supplement S15: Proteins differentially expressed between the VEH + FST and 7NI + FST groups.

GI	Description	%	MS	FC
1051270	14-3-3 zeta isoform	9	178	4.94
10567816	guanine nucleotide binding protein, alpha activating polypeptide	13	335	2.45
109501183	similar to ribosomal protein L15	6	36	-∞
13489067	N-ethylmaleimide sensitive fusion protein	2	77	-2.41
1363309	phosphopyruvate hydratase (EC 4,2,1,11) - rat	7	137	-∞
16758446	isocitrate dehydrogenase 3 (NAD+) alpha	8	233	-2.13
202837	aldolase A	12	194	-∞
203033	F1-ATPase beta subunit	12	167	-∞
206671	type II cAMP-dependent protein kinase regulatory subunit	7	81	-∞
223556	tubulin alpha	17	394	136.78
241081	synuclein SYN2 [Rattus sp,]	10	153	-∞
26023949	enolase 2, gamma	13	266	-∞
306890	Chaperonin (HSP60)	2	103	6.6
42476181	malate dehydrogenase, mitochondrial	11	159	+∞
51948476	ubiquinol-cytochrome c reductase core protein I	5	152	4.65
538426	triosephosphate isomerase	11	216	+∞
56200	unnamed protein product	2	81	-2.58
57029	H(+)-transporting ATP synthase	5	96	-1.04
57429	unnamed protein product	4	106	-∞
62664759	similar to prohibitin	14	158	2.99
6756041	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein	9	178	-∞
68163565	secernin 1	7	108	-∞
7106439	tubulin, beta 5	6	154	7.29
71620	actin beta – rat	11	247	5.12
769822	glial fibrillary acidic protein	6	77	4.73
8393910	phosphatidylethanolamine binding protein	14	212	2.29
8394009	peptidylprolyl isomerase A	16	115	-∞
984553	G protein beta 1 subunit	7	90	-∞

GI = NCBI gene identification, % = protein covery; FC = fold change on 7NI + FST compared to VEH + FST. MS = Mascot score. -∞ = protein with expression detectable only on VEH + FST group; +∞ = protein with expression detectable only on 7NI + FST group.

Supplement S16: Proteins differentially expressed between the VEH + FST and IMI + FST groups.

GI	Description	%	MS	FC
56200	unnamed protein product	7	159	-2.33
56643	unnamed protein product	7	145	-∞
203055	ATP synthase alpha subunit precursor (EC 3,6,1,3)	21	621	-∞
204503	glutathione S-transferase Yb-1 subunit (EC 2,5,1,18)	22	231	-∞
204570	major beta-hemoglobin	14	174	4.33
347019	dnaK-type molecular chaperone hsp72-ps1 - rat	5	85	-∞
510108	mitochondrial long-chain enoyl-CoA hydratase/3-hydroxycyl-CoA dehydrogenase α-subunit	3	126	-2.87
538426	triosephosphate isomerase	28	323	7.19
1220484	elongation factor-1 alpha	4	96	-∞
4105605	voltage dependent anion channel	13	139	-∞
6671672	capping protein (actin filament) muscle Z-line, alpha 2	22	151	-1.82
11693172	calreticulin	8	238	12.31
12835802	unnamed protein product	2	61	-3.37
16758270	F-box protein 2	22	263	-∞
18093102	dynamin 1	2	60	-3.51
30027750	alpha-globin [Rattus sp,]	44	160	-∞
40538860	aconitase 2, mitochondrial	10	349	-∞
57977323	2',3'-cyclic nucleotide 3' phosphodiesterase	13	236	-∞
77404242	synapsin II isoform 1	6	109	-∞
109487304	similar to actin related protein 2/3 complex subunit 2	9	131	6.8
109492380	similar to Actin, cytoplasmic 2 (Gamma-actin)	11	207	-∞
149034624	glutamate receptor, ionotropic, N-methyl-D-aspartate 3B	6	31	-26.39
149064065	rCG23367	2	24	-∞
157819953	ATPase, H+ transporting, lysosomal V1 subunit A	9	200	1.85
119959830	beta-actin	16	184	-12.42
13162287	D-dopachrome tautomerase	2	107	-3.38
14043072	heterogeneous nuclear ribonucleoprotein A2/B1 isoform B1	2	42	4.18
149017236	rCG49429	18	115	-∞
16757994	pyruvate kinase, muscle	3	101	3.81
16758892	calbindin 2	4	37	-∞
18093102	dynamin 1	4	142	-4.81
187937028	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9	2	158	-∞
1944322	Unc-18 homologue	6	164	2.24
200038	neurofilament-L	6	99	+∞
202837	aldolase A	8	141	-∞
206113	phosphoglycerate kinase	7	85	-∞
206205	M2 pyruvate kinase	5	112	-∞
220838	dihydrolipoamide acetyltransferase	5	115	-2.91
25453420	glutathione S-transferase, pi	20	167	-∞
26023949	enolase 2, gamma, neuronal	11	105	-∞
3121992	Aldehyde dehydrogenase, mitochondrial ALDH1	6	80	-5.27
31982030	Rho GDP dissociation inhibitor (GDI) alpha	5	55	-∞
41350312	nascent polypeptide-associated complex alpha subunit isoform b	25	204	-∞
4507297	syntaxin binding protein 1 isoform a [Homo sapiens]	3	45	2.69
49518	N-ethylmaleimide sensitive fusion protein [Cricetulus longicaudatus]	2	46	-3.89
55741424	NADH dehydrogenase (ubiquinone) flavoprotein 1, 51kDa	22	235	-∞
55825	unnamed protein product	41	217	-∞
56200	unnamed protein product	10	203	2.8
56643	unnamed protein product	19	358	-4.92
57977323	2',3'-cyclic nucleotide 3' phosphodiesterase	7	133	-∞
6753324	chaperonin containing Tcp1, subunit 6a	2	44	-3.65
6756039	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein	7	102	-∞
6980972	glutamate oxaloacetate transaminase 2	12	233	-∞
6981146	L-lactate dehydrogenase B	6	107	-∞

GI = NCBI gene identification, % = protein covery; FC = fold change on 7NI + FST compared to VEH + FST. MS = Mascot score. -∞ = protein with expression detectable only on VEH + FST group; +∞ = protein with expression detectable only on IMI + FST group.

Legends

Fig 1

Effect of 14 days chronic treatment with 7-nitroindazole (7-NI), imipramine or vehicle on forced swimming test and hipocampal patter of gene expression. (a) Total immobility time and latency to the first immobility episode of rats (n=5–8/group) submitted to the forced swimming test. The bars represent means±SEM. *P<0.05 compared to vehicle (ANOVA). (b) Serial analyses of gene expression (SAGE) tags saturation curve (n=3–4/group). The curves represent the relation of new sequenced tags (unique tags) by the total sequenced tags at each library (SAGE tags analyzed) (B). R^2 = fitting hyperbolic curve. (c) Hierarchical clustering of SAGE libraries. (d) Differential gene expression in fold changes for SAGE libraries of the groups 7-NI + NAI, VEH + FST, 7NI + FST e FST + IMI compared to the group VEH + NAI. VEH + NAI = animals treated with vehicle (saline:DMSO, 1:1, 2mL/Kg, for 14 days), 7-NI + NAI = animals treated with 7-NI (60mg/Kg by 14 days), VEH + FST = animals treated with vehicle (saline:DMSO, 1:1, 2mL/Kg for 14 days) and submitted to FST, 7-NI + FST = animals treated with 7-NI (60mg/Kg for 14 day) and submitted to FST, IMI + FST = animals treated with imipramine (15mg/Kg for 14 days) and submitted to FST.

Fig 2

Spearman correlation to data set of cannabinoid receptor 1 (CNR1), synaptophysin (SYP), glutathione S-transferase (GST), neurotrophic tyrosine kinase receptor type 2 (NTRK2), and serotonin receptor type 2C (5-HT_{2c}) expression evaluated by SAGE and qRT methods.

Fig 3

Bidirectional protein gels of proteomic analysis of VEH + NAI, 7-NI + NAI, VEH + FST, 7NI + FST and FST + IMI groups. The arrows indicate representative proteins differently expressed. VEH + NAI = animals treated with vehicle (N=4; saline:DMSO, 1:1, 2mL/Kg, for 14 days), 7-NI + NAI = animals treated with 7-NI (N=4; 60mg/Kg by 14 days), VEH + FST = animals treated with vehicle (N=4; saline:DMSO, 1:1, 2mL/Kg for 14 days) and submitted to FST, 7-NI + FST = animals treated with 7-NI (N=3; 60mg/Kg for 14 day) and submitted to FST, IMI + FST = animals treated with imipramine (15mg/Kg for 14 days) and submitted to FST.