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Ventral hippocampal molecular pathways and impaired neurogenesis associated with 5-HT_{1A} and 5-HT_{1B} receptors disruption in mice

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HIGHLIGHTS

- Deletion of both 5-HT_{1A} and 5-HT_{1B} receptors induce higher emotionality in mice.
- Behavior was associated with 723 differentially expressed genes in the hippocampus
- Ingenuity analyses revealed gene changes associated with neurogenesis function
- Cell survival but not proliferation was decreased in the hippocampus of mutant-mice.

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ABSTRACT

The serotonergic system has been widely implicated in stress related psychiatric disorders such as depression and anxiety. Generation of receptor knockout mice has offered a new approach to study processes underlying anxiety. For instance, knockout mice for both 5-HT_{1A} and 5-HT_{1B} receptors (5-$HT_{1A/1B}^{-/-}$) display an anxious phenotype, associated with robust physiological and neurochemical changes related to brain serotonin function. As ventral hippocampus is a key region in the mediation and genesis of anxiety, we explored the transcriptome changes induced by the genetic inactivation of these two receptors in 5-$HT_{1A/1B}^{-/-}$ mice. Dissociation of ventral *vs.* dorsal hippocampus was confirmed by the over-expression of selective markers in both regions. 723 genes were observed up/down regulated in 5-$HT_{1A/1B}^{-/-}$ mice. Using Ingenuity, biological networks and signal transduction pathway analysis corresponding to the identified gene revealed putative dysregulation of nervous system development and function, especially genes associated with long-term potentiation and adult neurogenesis (including *Bdnf*, *Camk2a*, *Camk4*, and *Klf9*). Furthermore, immunohistochemistry experiments studying adult hippocampal neurogenesis in adult 5-$HT_{1A/1B}^{-/-}$ mice showed a decreased survival, but not proliferation of newborn cells in our model.

1. Introduction

The serotonergic system plays an important role in the control and regulation of anxiety or depression [8]. In clinic, selective serotonin reuptake inhibitors (SSRI) are widely used in the treatments of depressive and anxious disorders [1]. However, SSRI activity is limited in the first weeks of treatment by activation of 5-HT_{1A} somatodendritic and 5-HT_{1B} presynaptic receptor subtypes that control the release of serotonin. Knockout animal models for either 5-HT_{1A} [21] or 5-HT_{1B} receptors [28] have been generated to better understand their respective roles in the physiopathology and treatment of depressive/anxious disorders. Interestingly, mice lacking 5-HT_{1A}/5-HT_{1B} receptors (5-$HT_{1A/1B}^{-/-}$ mice) display higher anxiety confirmed in different behavioral tests (the open field, the elevated-plus maze and the novelty suppressed feeding paradigms) associated with a greater spontaneous firing rate of dorsal raphe nucleus 5-HT neurons, an elevated basal dialysate levels of 5-HT at serotonergic nerve terminal brain regions and an increased basal core body temperature [10].

This anxious phenotype observed in 5-$HT_{1A/1B}^{-/-}$ mice may be associated with changes in gene expression compared to their littermates in brain areas linked to emotion and anxiety regulation. For instance, ventral hippocampus is involved in the development and maintenance of anxious states [3] and has a strong connection with prefrontal cortex [35] and amygdala [22], and a higher innervations density of serotoninergic projections than the dorsal part [7]. Moreover, ventral hippocampus can not only regulate the

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dopaminergic transmission in the prefrontal cortex [20] and modulate the serotonin release induced by stress [25] or corticosterone [4], but also affect the emotion and the anxiety-like behavior in rodents in some behavior tests [3]. In addition, recent studies discovered that a long-term exposition to stress can decrease adult hippocampal neurogenesis in ventral dentate gyrus [12], but not in dorsal dentate gyrus, this latest effect being reversed by various antidepressants [12,24].

Now, researchers begin to study the complex brain function and the mechanism of psychiatric diseases on the basis of molecular biology. Thanks to DNA microarrays technologies, researchers discovered that the expression of several genes were different in the ventral and dorsal hippocampus in mice [17]. Furthermore, SSRI treatment [31] or long-term running exercise [11] can modulate the genes expression in several limbic brain regions in mice [33]. In this study, we observed how deletion of both 5-HT_{1A} and 5-HT_{1B} receptors could affect gene expression and neurogenesis in the ventral hippocampus of 5-$HT_{1A/1B}{}^{-/-}$ mice.

2. Methods

2.1. Animals

Male 5-$HT_{1A/1B}$ receptors knockout mice (5-$HT_{1A/1B}{}^{-/-}$) and their littermates (5-$HT_{1A/1B}{}^{+/+}$) on C57BL6/Sv129 background, 6–8 weeks old, were used in all experiments (see Supplementary methods, [10]). 8 animals/genotype were used for microarray. Microarray findings were confirmed using RT-qPCR on a second cohort of 8 mice/group. 6 mice/genotype were used for immunohistochemistry experiments. All procedures followed the recommendations of the European Community (86/609/EEC) and the French National Committee (87/848) for care and use of laboratory animals (permission #92-196).

2.2. Brain area microdissection

After cervical dislocation brains were quickly harvested and dorsal and ventral hippocampi were separated under the microscope and stored at −80 °C before RNA extraction (see Supplementary methods). Their exact separation was confirmed by quantification of expression of 2 tissue-specific genes in ventral (Decorin, *Dcn*) *versus* dorsal hippocampus (lactose-phlorizinhydrolase, *Lct*) respectively [17] (Supplementary methods).

2.3. RNA extraction

Tissue samples were homogenized and total RNA was extracted using TRIzol, following manufacturer's protocol (Invitrogen, France). RNA concentrations and quality were determined by NanoDrop® spectrophotometer (NanoDrop technologies, USA) and a RNA LabChip® 6000 Nano kit (Agilent Technologies, USA). Samples with a RIN < 8.0 (RNA Integrity Number) were excluded.

2.4. Microarray experiments and analysis

Gene expression profile analysis was performed using Whole Mouse Genome Oligo Microarrays 4 × 44k (G4122F, Agilent Technologies, USA). The comparison between samples was performed in an indirect way using a common reference, a pool of total RNA (Universal mouse reference RNA #740,100, Stratagene). Technical details of microarray experiments are available in Supplementary methods. Genes were considered significantly differentially expressed with the following criteria: Student's *t*-test *p*-value for genotype effect <0.05 and absolute fold-change between genotype ≥1.3.

2.5. Real-time quantitative PCR (RT-qPCR)

RT-qPCR was used for the validation of brain area microdissection and confirmation of our gene expression analysis. Total RNA (1 μg) was reverse-transcribed with Anchored-Oligo(dT)18 primers by using Transcriptor First Strand cDNA (Roche®, Synthesis Kit, USA). RT-qPCR performance was performed as previously described [10]. *Actin*, *Gapdh* and *Ppig* were used as internal controls and full description of primer sequence is available in Supplementary materials. Normalization of gene expression was performed with GeNorm VBA [34].

2.6. Functional analysis

Selected 723 genes were overlaid on the global molecular network of Ingenuity Pathway Analysis (Ingenuity® Systems, www.ingenuity.com, see Supplementary methods) allowing for generation of gene networks based on their connectivity. Their score takes into account the relative numbers of network eligible molecules, of molecules analyzed and the total number of molecules in Ingenuity's knowledge base. Disease links are generated on the literature-based association with illness by IPA.

2.7. Immunohistochemistry

Cell survival: Mice were administered with BrdU (150 mg/kg twice a day during 3 days) 4 weeks before sacrifice. We then proceeded as described [5]. BrdU-positive cells were counted under Olympus BX51 microscope ($n = 6$/genotype).

Cell proliferation: For Ki67 immunohistochemistry, floating sections were washed in PBS buffer containing 0.3% Triton X-100, blocked and incubated with Ki67 primary antibody overnight at 4 °C (1/100, Vector Labs, Burlingame, USA). The next day, sections were washed in PBS and incubated with fluorescent-label-coupled secondary antibodies for 2 h at room temperature.

2.8. Statistical analysis

Two-tailed Student's *t*-test analyses were performed on data obtained from the RT-qPCR of relative transcripts levels and on immunostaining levels using StatView 5.0 software (SAS Institute, Cary, NC, USA) and expressed as mean ± SEM. *p*-Values <0.05 were considered statistically significant. A Pearson correlation coefficient was used to measure the similarity of gene expression profiles between microarrays and RT-qPCR.

3. Results

3.1. Dissociation of ventral versus dorsal hippocampus by qPCR

The accuracy of dissociation of dorsal and ventral hippocampus was confirmed by the differential expression of two gene markers. *Dcn* expression was over 14 times greater in the ventral compared to dorsal hippocampus ($p < 0.0001$, Supplementary Fig. 1B), while *Lct* expression was selectively expressed in the dorsal compared to ventral hippocampus ($p < 0.0001$, Supplementary Fig. 1C).

3.2. Differentially expressed genes in the ventral hippocampus of 5-$HT_{1A/1B}{}^{-/-}$

924 probe sets corresponding to 723 genes transcripts were differentially expressed in the ventral hippocampus of 5-$HT_{1A/1B}{}^{-/-}$ mice compared to 5-$HT_{1A/1B}{}^{+/+}$ mice (33 genes up-regulated, 690 down-regulated with criteria absolute Fold-Change > 1.3, a mean log 2 intensity level > 9.0 and *p*-value <0.05 were significantly

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Table 1
Summary of genes differentially expressed in 5-HT$_{1A/1B}^{-/-}$ mice compared to 5-HT$_{1A/1B}^{+/+}$ mice.

GeneBank ID	Gene name	Gene symbol	Fold change
NM_011888	Chemokine (C–C motif) ligand 19	**Ccl19**	35.9 (1.00E−6)
AK035950	RIKEN full-length enriched library cDNA	Clone: 9630020E20	5.81 (1.24E−3)
AK170208	DEP domain containing MTOR-interacting protein	Deptor	3.18 (1.24E−3)
AK040896	RIKEN full-length enriched library cDNA	Clone: A530041D01	2.36 (3.00E−5)
NM_172609	Translocase of outer mitochondrial membrane 22 homolog (yeast)	Tomm22	2.23 (5.00E−5)
NM_145457	Polyadenylate binding protein-interacting protein 1	Paip1	2.2 (1.18E−2)
NM_025311	DNA segment, Chr 14, ERATO Doi 449, expressed	D14Ertd449e	1.74 (7.00E−5)
NM_152894	Processing of precursor 1, ribonuclease P/MRP family (*S. cerevisiae*)	Pop1	1.62 (1.20E−4)
NM_025938	Ribonuclease P 14 subunit (human)	Rpp14	1.59 (9.00E−4)
NM_007749	Cytochrome c oxidase, subunit VIIc	Cox7c	1.59 (4.93E−3)
NM_027219	CDC42 effector protein (Rho GTPase binding) 1	Cdc42ep1	1.58 (8.00E−4)
NM_080728	Myosin, heavy polypeptide 7, cardiac muscle, beta	Myh7	1.49 (1.79E−2)
NM_009242	Secreted acidic cysteine rich glycoprotein	Sparc	1.48 (7.60E−4)
AK162552	Dynactin 4	Dctn4	1.47 (9.20E−3)
NM_016972	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	Slc7a8	1.46 (1.13E−2)
NM_026174	Ectonucleoside triphosphate diphosphohydrolase 4	Entpd4	1.46 (2.00E−5)
NM_010127	POU domain, class 6, transcription factor 1	Pou6f1	1.45 (4.09E−2)
NM_183358	Growth arrest and DNA-damage-inducible, gamma interacting protein 1	Gadd45gip1	1.44 (2.20E−4)
NM_023537	RAB3B, member RAS oncogene family	Rab3b	1.39 (1.73E−3)
AK044807	Ubiquitin-conjugating enzyme E2E 2 (UBC4/5 homolog, yeast)	Ube2e2	1.38 (8.12E−3)
NM_145600	Zinc finger protein 330	Zfp330	1.38 (1.90E−2)
NM_010330	Embigin	**Emb**	−2.22 (1.00E−6)
AK090125	RIKEN full-length enriched library cDNA	Clone: G431002D05	−2.26 (5.00E−5)
NM_172586	Zinc finger protein 322A	Zfp322a	−2.27 (1.00E−6)
NM_013497	CAMP responsive element binding protein 3	**Creb3**	−2.35 (1.00E−6)
NM_175654	Histone cluster 1, H4d	Hist1h4d	−2.36 (1.00E−6)
NM_007598	CAP, adenylate cyclase-associated protein 1 (yeast)	**Cap1**	−2.38 (1.00E−6)
NM_021365	X-linked lymphocyte-regulated 4b	Xlr4b	−2.4 (1.90E−4)
NM_146230	Acetyl-coenzyme A acyltransferase 1B	Acaa1b	−2.58 (1.00E−6)
BC050890	Fanconi anemia, complementation group G	**Fancg**	−2.61 (1.04E−2)
NM_133362	Erythroid differentiation regulator 1	Erdr1	−2.67 (1.00E−5)
NM_026127	RIKEN cDNA 4833420G17 gene	4833420G17Rik	−2.68 (1.00E−6)
BF467941	Histone cluster 1, H4i	Hist1h4i	−2.75 (2.20E−4)
NM_001024712	Predicted gene 3696	ENSMUSG00000072735	−2.82 (2.00E−4)
NM_026035	Mitochondrial ribosomal protein L55	Mrpl55	−2.83 (1.59E−2)
NM_011740	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	Ywhaz	−2.89 (1.00E−6)
NM_013917	Pituitary tumor-transforming gene 1	**Pttg1**	−3.04 (1.00E−6)
NM_008509	Lipoprotein lipase	Lpl	−3.18 (2.00E−5)
NM_029865	Occludin/ELL domain containing 1	Ocel1	−3.4 (1.00E−5)
BC022221	Ubiquitin specific peptidase 53	**Usp53**	−3.74 (1.00E−6)
BC020150	RIKEN cDNA 4930520O04 gene	4930520O04Rik	−6.25 (1.00E−6)

Top 20 genes for which expression is significantly up-regulated or down-regulated in 5-HT$_{1A/1B}^{-/-}$ *versus* 5-HT$_{1A/1B}^{+/+}$ mice (n = 8 mice/genotype), sorted by fold-change (p-value). Genes confirmed by RT-qPCR are shown in bold.

differentially expressed, see Supplementary Table 1). Top 20 up- and down-regulated genes are shown in Table 1. Expression changes were confirmed on 9 genes (*Ccl19*, *Creb3*, *Cap1*, *Fancg*, *Usp53*, *Pttg1*, *Bdnf*, *Mapk1*, *Emb*) using RT-qPCR, with a high array/qPCR concordance (r = 0.823, p < 0.01) and similar directionality of expression for 8 out of 9 genes (Fig. 1).

3.3. Functional analysis suggests impairment of genes involved in neurogenesis

Using IPA analysis, 25 networks were significantly associated with gene changes, with at least 15 genes identified (Table 2). Network including genes involved in the development and function of the nervous system was the second most associated to our dataset (score = 41, Table 2 and Fig. 2A), an association observed in other gene networks including different lists of genes (Networks #11, #16, #17 and #20, see Supplementary Table 2), thus making this biological function the most associated one linked to our dataset. Interestingly, 6 genes of this specific network were involved with the neurogenesis, an association also strongly observed in the whole dataset (50 genes, p < 0.01, Table 2). Among all steps of neurogenesis, genes were mostly linked (although not exclusively) to differentiation of neurons (*Atp2b2*, *Bdnf*, *Eya1*, *Hdac1*, *Id4*, *Klf9*, *Map2k1*, *Mapk1*, *Mib1*, *Ptprz1*, *Rb1*, *Rhoa*, *Rtn4*, *Smarca1*, *Tcf4*, *Tgfb2*, *Thra*, *Ttc3*, *Znf536*, p < 4E−20), and 3 genes were also related to survival of neurons (*Tgfb2*, *Bdnf*, *Rb1*, p < 8E−6).

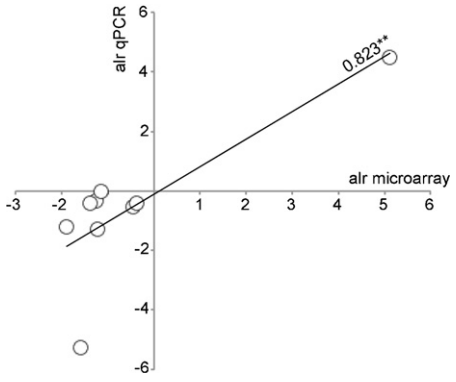

Fig. 1. qPCR validation of differential gene expression in 5-HT$_{1A/1B}^{-/-}$ mice compared to 5-HT$_{1A/1B}^{+/+}$ mice. Microarray results were confirmed by RT-qPCR in another cohort of animals with a high array/qPCR concordance (r = 0.823, p = 0.006) and similar directionality of expression for 8 out of 9 genes (n = 8 mice/genotype).

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Table 2
Top 5 IPA findings associated with 723 differentially expressed genes in 5-HT$_{1A/1B}^{-/-}$ mice compared to 5-HT$_{1A/1B}^{+/+}$ mice.

Top 5 networks	Score	Focus molecules
1. RNA post-transcriptional modification, gene expression, cell-to-cell signaling and interaction	46	30
2. Nervous system development and function, cellular assembly and organization, cell-to-cell signaling and interaction	41	28
3. Gene expression, cellular assembly and organization, cell cycle	41	29
4. Cellular development, hematopoiesis, infectious disease	37	26
5. Infectious disease, cellular development, skeletal and muscular system development and function	36	26
Top diseases and disorders	# Molecules	*p*-Value
Neurological disease	219	5.56E−09–1.34E−02
Bipolar disorder	58	6.92E−03
Huntington's disease	57	1.46E−08
Genetic disorder	341	1.46E−08–1.34E−02
Skeletal and muscular disorders	171	1.46E−08–1.15E−02
Gastrointestinal disease	208	2.05E−06–1.43E−02
Inflammatory disease	174	2.05E−06–1.15E−02
Top physiological system development and function	# Molecules	*p*-Value
Nervous system development and function	111	2.66E−06–1.36E−02
Neurogenesis	50	2.01E−02
Guidance of neurites	32	8.08E−03
Neurological process of neurons	29	2.09E−03
Neurotransmission	24	2.74E−03
Connective tissue development and function	49	1.99E−05–1.47E−02
Organismal development	122	3.32E−05–1.43E−02
Tissue development	122	3.46E−05–1.43E−02
Development of nervous tissue	33	3.15E−04
Development of brain	27	2.46E−04
Embryonic development	83	8.78E−05–1.36E−02
Top canonical pathways	−log(*p*-value)	Ratio
Synaptic long term potentiation	7.03	0.149 (17/114)
Protein kinase A signaling	7.01	0.0945 (31/328)
Calcium signaling	5.61	0.0966 (20/207)
PPARα/RXRα activation	5.21	0.120 (19/186)
Ephrin receptor signaling	5.07	0.095 (19/200)
Others pathways		
CREB signaling in neurons	3.51	0.0792 (16/202)
cAMP-mediated signaling	3.36	0.0822 (18/219)
Synaptic long-term depression	2.83	0.0816 (12/147)

Results show top biological networks, physiological system development and function, diseases and disorders and canonical pathways obtained by Ingenuity Pathway Analysis (IPA). Network scores represent negative log-values of right-tailed Fisher's Exact Tests for network consistence. Pathways and disease *p*-values represent significance of over-representation of candidate genes within respective gene groups. *p*-Value ranges indicate values for various disease sub-classifications.

Among the disease and disorders most associated with our dataset, genetic and neurological diseases, including bipolar disorder (58 genes, $p < 0.001$) and Huntington disease (57 genes, $p < 0.0001$), we found psychological and mood disorders (90 and 65 genes, respectively, $p < 0.001$).

Notably, canonical pathways include genes involved in hippocampus neurotransmission such as synaptic long-term potentiation ($p < 1E{-}7$), protein kinase A, which is under the control of 5-HT$_{1A}$ or 5-HT$_{1B}$ receptors ($p < 1E{-}7$) and calcium signaling ($p < 1E{-}5$) (Table 2).

3.4. Disruption of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors affects cell survival, but not proliferation of hippocampal neurogenesis in adult mice

Cell survival was decreased by ≈40% in the adult hippocampus of 5-HT$_{1A/1B}^{-/-}$ compared to their control littermates, with no observed differences along the dorso-ventral axis ($F_{(1,10)} = 5.41$, $p < 0.05$, Fig. 2B). No significant change in hippocampus cell proliferation between genotypes was observed ($F_{(1,10)} = 0.09$, $p > 0.7$, Fig. 2C).

4. Discussion

Large-scale genes' expression analysis in the ventral hippocampus allowed to describe the genetic mechanisms, induced by the disruption of both 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors. It might account, at least in part, for the development and maintenance of this anxious phenotype. We report here robust, qPCR-confirmed transcriptional changes, affecting the expression of 723 genes in the ventral hippocampus of 5-HT$_{1A/1B}^{-/-}$ mice compared to wild-type littermates. We previously describe these double mutants as a mouse model of an anxiety/depressive-like state induced by genetic deletion of both receptors and chronic paroxetine treatment reversed the anxious phenotype [10].

Main IPA findings obtained from these differentially expressed genes confirmed their association with (1) the nervous system development and function and (2) neurological diseases. Unsurprisingly, our dataset was associated with Huntington's disease and bipolar disorder, both pathologies sharing biological pathways, and for which anxiety has been mentioned as a high comorbidity in humans [6,14], as well as in transgenic mouse models of both diseases [16,19]. In 5-HT$_{1A/1B}^{-/-}$ mice, among the 111 differentially expressed genes associated with nervous system development and function, 24 were associated with

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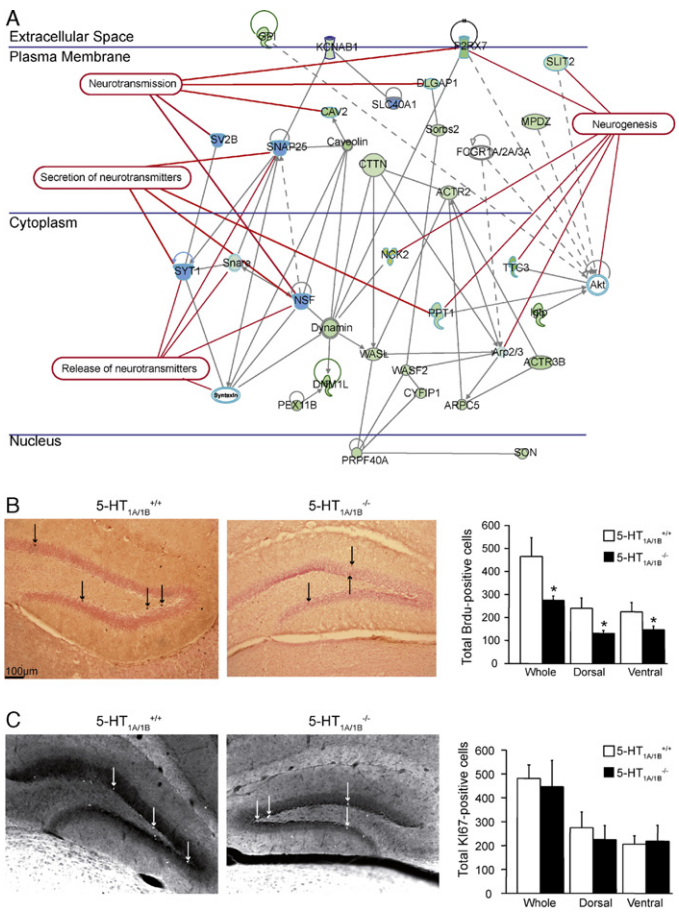

Fig. 2. Altered neurogenesis in 5-$HT_{1A/1B}^{-/-}$ mice. (A) The top genes and molecules interaction network built on gene selected was significantly connected to neurogenesis, release/secretions of neurotransmitters, and neurotransmission. (B) Representative illustrations of BrdU immunoreactivity (10× magnification) in the dentate gyrus of 5-$HT_{1A/1B}^{+/+}$ and 5-$HT_{1A/1B}^{-/-}$ mice showing a decrease in cell survival in the hippocampus of 5-$HT_{1A/1B}^{-/-}$ ($n = 6$/genotype); $^*p < 0.05$. (C) Representative illustrations of Ki67 immunoreactivity (10× magnification) in the dentate gyrus of 5-$HT_{1A/1B}^{+/+}$ and 5-$HT_{1A/1B}^{-/-}$ mice showing a lack of change in cell proliferation within hippocampus between genotype ($n = 6$/genotype).

neurotransmission and 50 were associated with neurogenesis, according to IPA categorization. While we previously related altered serotonergic neurotransmission in this mouse model [10], we show here that cell survival (including potentially new-born neurons), but not proliferation of hippocampal newborn cells, was altered in this model. Neurogenesis includes several stages and we found downregulated genes in the adult hippocampus associated with all stages: cellular proliferation (4), neuron migration into the granule cell layer (9), maturation (4) and differentiation (19) into granular neurons and cell survival (3). Thus, both microarrays and immunochemistry suggest that adult hippocampal neurogenesis might be affected in 5-$HT_{1A/1B}^{-/-}$ mice.

Some studies looked at the effects of 5-HT_{1A} or 5-HT_{1B} genetic or pharmacological manipulation, and more precisely their effects on adult hippocampal neurogenesis. Using constitutive knockout models, 5-HT_{1A} disruption failed to alter cell proliferation in the adult hippocampus [27], as observed here, while yet no studies have been performed in 5-$HT_{1B}^{-/-}$ mice. Pharmacological stimulation of 5-HT_{1A} receptors increased cell proliferation, while their blockade induced opposite effects [2,15,23,27]. Indeed, pharmacological blockade of 5-HT_{1A} receptors may only exert its effects on other stages of neurogenesis [15]. 5-HT_{1B} receptor blockade had no effect on cell proliferation within the hippocampus, but pharmacological dissection of auto- *vs.* heteroreceptors suggests that activation of these latter may participate in cell proliferation [2]. Thus, taken together with our data, these studies suggest that the impact of global 5-HT_{1B} receptor deletion on cell proliferation may be undetectable and that studies selectively disrupting pre- *vs.* post-synaptic 5-HT_{1B} receptors would be of great interest.

We observed a decrease in cell survival within the dentate gyrus in both dorsal and ventral regions of the 5-$HT_{1A/1B}^{-/-}$ mouse hippocampus. While no studies have looked at effects of genetic disruption of these receptors on cell survival, pharmacological studies may provide some hints on the role exerted by 5-HT_{1A} receptors on this step. Indeed, activation of 5-HT_{1A} receptor agonist in rodents increased cell survival in a time-dependent manner [15,32], while its blockade had opposite effects [15], reinforcing the present results. Unfortunately, yet no study observed the effects of chronic pharmacological blockade or stimulation of 5-HT_{1B} receptors on cell survival in the hippocampus.

At the single gene level, interesting genes were changed in our dataset and are known to be involved in multiple stages of neurogenesis. For instance, *Klf9* mutation (downregulated in adult 5-$HT_{1A/1B}^{-/-}$ mice) decreases both maturation in early phase and differentiation of adult-born neurons in hippocampus, putatively conferring to Klf9$^{-/-}$ mice a greater anxious phenotype [30]. Similarly, BDNF (downregulated in 5-$HT_{1A/1B}^{-/-}$ mice) has been involved in all steps of neurogenesis [29,36]. Targeted BDNF downregulation in mice has been shown to induce higher anxious/depressed states [26,31], however not consistently reported [1], and a trend for a reduction in *Htr1a* and *Htr1b* expression in the hippocampus [18]. In addition, these heterozygous mutants BDNF$^{+/-}$ mice have increased basal extracellular 5-HT levels in the hippocampus and decreased 5-HT reuptake capacity [9].

5. Summary and limitations

Serotonin is known as a potent regulator of adult hippocampal neurogenesis and cell survival in the hippocampus of adult mice [2,15,27] and our microarray data support an overall effect of 5-HT_{1A} and 5-HT_{1B} receptors on neurogenesis, partially confirmed by immunohistochemistry. However, the specific role of the different serotonin receptor subtypes still needs to be dissected, as they may have antagonistic action depending on their localization, *i.e.*, somatodendritic *versus* at nerve terminals [2,15]. In this line of thoughts, we observed no serotonin-related changes in gene expression that could compensate the disruption of htr1a and htr1b genes in our model. Especially we observed no change in expression of mRNA coding for 5-HT_{2A} and 5-HT_{2C} receptors, which selective and non-selective chronic blockade has been shown to increase cell proliferation [13,32].

Moreover, while we observed alteration in neurogenesis-related genes and cell survival specifically in the ventral hippocampus, gene changes related to cell survival in the dorsal hippocampus may completely differ from what has been observed in the ventral hippocampus, as molecular heterogeneity exists along the dorsal–ventral axis of the hippocampus [17].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neulet.2012.05.046.

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-- SUPPLEMENTARY INFORMATION --

Ventral hippocampal molecular pathways and impaired neurogenesis associated with 5-HT_{1A} and 5-HT_{1B} receptors disruption in mice

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CONTENTS **Page #**

Supplementary Methods

Animals

5-HT_{1A} receptor knockout mice (5-$HT_{1A}^{-/-}$) generated on the C57BL/6 background [4] were crossed with homozygous 5-HT_{1B} receptor knockout mice (5-$HT_{1B}^{-/-}$) generated on the 129Sv background [5]. These F1 heterozygous 5-$HT_{1A}^{+/-}$ and 5-$HT_{1B}^{+/-}$ mice were then bred to generate double 5-$HT_{1A/1B}^{-/-}$ mice F2. All the animals were genotyped by PCR. Male animals (6 to 8 weeks old) weighing 25-30g were used. Procedures involving animals and their care were conducted in conformity with the institutional guidelines, in compliance with international laws and policies (Council directive # 87-848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions # 92-196 to AM.G).

Brain area microdissection

After cervical dislocation brains were quickly harvested. Brains were placed in a mouse brain tissue blocker (World Precision Instruments, USA) where 3 consecutive rostro-caudal 1mm sections were collected [2] and transferred into RNA stabilization solution (Applied Biosystems, France). The dorsal hippocampus was isolated from the first section, while the ventral hippocampus was dissected from the third section (Supplementary Figure 1A) under the microscope and then stored at -80°C before RNA extraction. The accuracy of dissociation of dorsal and ventral hippocampus was confirmed by the differential expression of two gene markers of these sub-regions, *Dcn* and *Lct* (Supplementary Figure 1B-C).

Microarray experiments and analysis

Gene expression profile analysis was performed using Whole Mouse Genome Oligo Microarrays 4x44k (G4122F, Agilent technologies, USA). The comparison between samples was performed in an indirect way using a common reference, a pool of total RNA (Universal mouse reference RNA #740,100, Stratagene). 1 µg of RNA was labeled using the LILPAK PLUS kit, Cy5-CTP for the reference RNA, Cy3-CTP for all samples and internal standards came from Two-color the RNA Spike-in Kit (Agilent Technologies, USA). The labeled targets were purified using the RNeasy Mini Kit (Qiagen, France) and their quality and quantity were confirmed. 825ng of Cy3- and Cy5-labeled targets were

mixed according to Agilent instructions and incubated on the microarrays slides at 65°C for 17 hours. After washing, slides were scanned using the DNA Array Scanner at 5µm resolution. Photomultiplier tube voltages were automatically adjusted to balance the distributions of the red and green intensities and to optimize the dynamics of image quantification. The data were extracted from these images using the Agilent Feature Extraction v.9.1 software, normalized (See Supplementary Information and [1]) and processed with the MAnGO software [3]. Gene expression was considered significantly detectable with a log2 intensity value threshold above 9.0. Genes were considered significantly differentially expressed with the following criteria: Student's t-test p-value for genotype effect <0.05 and absolute fold-change between genotype≥1.3.

Microarray Normalization Protocol

As the different oligonucleotide arrays are manufactured in a very reproducible manner, the reference signals were stable and homogenous, therefore allowing replacement of intensities values for each spot by their mean values to obtain a unique mean and ideal reference, whatever the conditions. For each of the studied conditions, assuming that the same quantity of labeled RNA should be hybridized, we globally adjusted the overall fluorescence signal between the different arrays of the same condition. Thus intensities of each spot were adjusted using the following formula:

$G_{ij}\ normalized = G_{ij} * \langle G \rangle / \langle G_j \rangle$

Where G_{ij} is the intensity of the spot, $\langle G \rangle$ is the mean intensity of all the arrays for a same condition, and $\langle G_j \rangle$ is the mean intensity of all spots for one array. Backgrounds were estimated in a global manner using the "morphological closing followed opening" method [1]; such strategy needs values that are low enough to be considered as negligible. Thus, they were not subtracted from the raw signal measurements. Not found, saturated, and bad spots were discarded from the subsequent analysis.

Data were then processed with the MAnGO software [3]. A Student's t-test was computed to measure the significance associated with each expression difference. Detectable expression of genes was measured using Mean log2 of intensity which is defined as the median of pixel to pixel geometric means of the “red” and “green” intensities. When this value was ≥9, the signal homogeneity was improved significantly [6]. Genes were considered significantly differentially expressed with the following

criteria: Student's t-test p-value for genotype effect <0.05 and absolute fold-change between genotype≥1.3.

Functional analysis using Ingenuity Knowledge Database

Selected genes were overlaid on the global molecular network developed from information contained in the Ingenuity Pathway knowledge base (www.ingenuity.com). This network is composed of >3.5 million literature-based biological links between genes and bioactive molecules, and sub-networks are built on genes of interest based on their connectivity within this global network. Gene networks were limited to 35 nodes. The score for a network takes into account the relative numbers of network eligible molecules, of molecules analyzed and the total number of molecules in Ingenuity's knowledge base. These scores are based on the hypergeometric distribution and represent the negative log of the right-tailed Fisher's Exact Test p-value. Disease links are based on literature-based association with illness.

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Supplementary Tables

Supplementary Table 1: Full List of 723 genes significantly affected in 5-HT$_{1A/1B}^{-/-}$ mice.

(Separate download)

This list contains the 723 genes differentially expressed in 5-HT$_{1A/1B}^{-/-}$ versus 5-HT$_{1A/1B}^{+/+}$ mice, sorted by fold change value (up-regulated genes in red and down-regulated in green) and with their associated Student T-test p-value. Genes selected to test in RT-qPCR are shown in bold.

Supplementary Table 2: 25 biological networks associated with our dataset of genes differentially expressed in 5-HT$_{1A/1B}^{-/-}$ mice.

The top biological networks over-represented using our gene list (723 genes differentially expressed following these criteria fold change>30% and $p<0.05$) were associated with 25 biological networks according to IPA analysis. Notably, nervous system development was among the most recurrent association.

Supplementary Table 3: Serotonin-related genes expression in 5-HT$_{1A/1B}^{-/-}$ compared to 5-HT$_{1A/1B}^{+/+}$ mice.

This list contains the detectable serotonin-related genes expression in 5-HT$_{1A/1B}^{-/-}$ versus 5-HT$_{1A/1B}^{+/+}$ mice, with their fold change and associated p-value. No changes were observed between genotypes.

Supplementary Table 4: List of qPCR primers sequences.

Supplementary Table 2

ID	Top Functions	Score	Focus Molecules	Molecules in Network
1	RNA Post-Transcriptional Modification, Gene Expression, Cell-To-Cell Signaling and Interaction	46	30	ADCY1, ALCAM, ALG2, CASD1, CCDC88A, CD6, CPNE4, DDX3X, EEA1, EIF1AY, EIF5B,ENSA,ERK1/2,FUS,GNAI2,HNRNPU,KIF21A,LARS,MIB1, MYCBP2, NIPSNAP1, PABPC4, PDZGEF,PKC alpha/beta, PURB, Rap1, RAP2A, RAPGEF2, RAPGEF6, RBMX, Smad2/3,SREK1,SRPK2,SRSF5,TANK
2	**Nervous System Development and Function**, Cellular Assembly and Organization, Cell-To-Cell Signaling and Interaction	41	28	ACTR2, ACTR3B, Akt, Arp2/3, ARPC5, CAV2, Caveolin, CTTN, CYFIP1, DLGAP1, DNM1L, Dynamin, FCGR1A/2A/3A, GPI, Igtp,KCNAB1, MPDZ, NCK2, NSF,P2RX7, PEX11B,PPT1,PRPF40A,SLC40A1,SLIT2,SNAP25, Snare, SON, Sorbs2, SV2B, Syntaxin, SYT1, TTC3, WASF2, WASL
3	Gene Expression, Cellular Assembly and Organization, Cell Cycle	41	29	APC, BCLAF1, C18orf8, Cbp/p300, CBX3, CCAR1, CLK1, Ctbp, DIAPH2, DZIP3, ELL2, EYA1, FYCO1, GTF2H2/LOC100510744, HDAC1, HISTONE, Holo RNA polymerase II, HSPA4, KIF5B, Mi2, NCOR1, PNISR, PNN,PPIG, Profilin, RANBP2, RBM4B, RCOR1, RHOA, SMARCA5, TAF1,TIMM44, TOMM22, WWTR1, ZFP106
4	Cellular Development, Hematopoiesis, Infectious Disease	37	26	ACSL4, ARHGAP21, CTSS, DENND4A, DNAJB4, DNAJC2, DYNC1I2, DYNLT3, EDIL3, EIF2S3, EIF3A, FANCG, Gm-csf, HIVEP2, Hsp70, Hsp90, HSP, HSPA12A, IL12 (complex), INTS6, LARP1, Mediator, MHC Class II (complex), NDEL1, PLC gamma, Rac, RPS6KB1, SACS, SH3BP5L, THRA, TNPO2, YWHAE, YWHAZ, ZC3H13, ZFC3H1
5	Infectious Disease, Cellular Development, Skeletal and Muscular System Development and Function	36	26	ACAA1,Acaa1b,ANKS1B,BCL11B,CASP8AP2,Cbp,Cdc2,CREB3, CSDE1, DEK, E2F2, E2f, ENPP1, GADD45GIP1, GPD1, Hat,Hdac, NFkB (complex), PIP5K1A, PPARα-RXRα, PTP4A2, RECQL, RIOK3, RPS6KA3, Rsk, SLC27A1, SNX27, ST8SIA1, TAX1BP1, Thymidine Kinase, TNFAIP8, TNFRSF25, VSNL1, ZFAND5, ZMYND11
6	Cell Morphology, Cellular Assembly and Organization, Decreased Levels of Albumin	35	26	26s Proteasome, Alpha tubulin, ASPH, ATF2, C5orf34, CENPC1, CSNK1E, ELP2, ELP3, FBXO34, FUT8, HARS, Hist2h4 (includes others), Histone h3, Histone h4, HMG CoA synthase, Idl1, Ikb, IKK (complex), KDM1B, Pmaip1, PTEN, QKI, RBFOX1, RNF138, SRSF11, TGFB2, TGFBR1, THRAP3, TOP1, TOP2B, TRAP/Media, Ubiquitin, WSB1, YY1
7	Cell Cycle, Gene Expression, Infectious Disease	34	25	APC, ARHGDIB, CCND1,CCND2, CDC27,Cdk,CUL1,CUL3,Cyclin A,Cyclin B, Cyclin D, Cyclin E, DCUN1D1, DNA-directed DNA polymerase, DUSP7, HAUS8, LATS1,LATS2,LIMD1,Mapk,MOBKL1B,NACC1,PAPD7,POLD3, POP1, PTTG1, RAB4A, Rb,RBP1, REV3L, Rho gdi, RPP14, SPOP, STRAP, ZNF281
8	Gene Expression, Cell-To-Cell Signaling and Interaction, Embryonic Development	34	25	AHCYL2,Ahr-aryl hydrocarbon-Arnt, ANK3, ARID2,ARID1B, CENPE,CNTN1, CROCC, DHX36, Eotaxin, Ephb, Estrogen Receptor, GUCY1B3, KALRN, LIMS3L, MAPK1, MED1,NFIA,NFIB,NPR2,NRIP1, Nuclear factor 1, PARVA, PBRM1,PTPRZ1, PTS, Rxr, SMARCA2, STRBP, SWI-SNF, T3-TR-RXR, TESK1, Thyroid hormone receptor, TRIP11, VitaminD3-VDR-RXR
9	Cell-To-Cell Signaling and Interaction, Cancer, Gastrointestinal Disease	32	24	Alpha Actinin, Alpha catenin, ATP2B1, ATP2B2, BSN, Cadherin, Calcineurin A, Calcineurin protein(s), Calm1 (includes others), Calmodulin, CASK, Cytochrome c,Cytoplasmic Dynein, KCNN2, KTN1, LIN7C, MARCKSL1, PCLO, PI3K (complex), PLA2G6, Pmca, Pp2b, PPFIA2, PPFIBP1, PPP3CA, PPP3CB, RAB3B, RAB6A, Rims1, RYR3, SSX2IP, TPP2, TRPC4, UNC13A, ZFPM2
10	Cell Signaling, Molecular Transport, Nucleic Acid Metabolism	30	23	ABCA1, AKAP7, ARHGAP22, CBR1, CLIP1, CTNNβ-TCF/LEF, DYRK3, DZIP1, FOXP1, FSH, GJA1, hCG,Histone H1, HMGCR, HMGN5, IFN alpha/beta, IPO7, KIRREL, Lh, MAPRE1, MPPE1, p70S6k, Pde, PDE1A, Pka, PKIA, PP2A, PRKAC, PRKAR1A, S100A10, SMARCA1, SRD5A1, TDP2, TJP1, TSH
11	Cell-To-Cell Signaling and Interaction, **Nervous System Development and Function**, Cellular Function and Maintenance	25	20	Adaptor protein 2, Ampa Receptor, Ap1, ARNT, ATL1, Bcl9-Cbp/p300-Ctnnb1-Lef/Tcf, BDNF, CAMK4, CAMK2A, CaMKII, COL4A3BP, Creb, GABRA5, GAS1, GRI, GRIA2, GRIA3, GRIK4, HABP4, Ikk (family), Importin alpha, Importin beta, NAMPT, NCF4, NMDA Receptor, Pias, Pkc(s), RAD23B, Rar, RNMT, RTN4, Smad, TMEM184C, UBE3A, ZNF440/ZNF808
12	Cellular Assembly and Organization, Cell Death, Gene Expression	24	20	Actin,ADCY, AIFM3,AP1AR, Calpain, CAP1,CAP2, Caspase,CCNL2, CHD8, Ck2, DCLK1, DCTN4,DCTN5, DCTN6,Fibrinogen, GSR,HNRNPC, HTATSF1, LSM10, LSM12, N-cor, NR3C1, NUCKS1, PRPF39,RB1,RNA polymerase II, RNU2-1, SNAPC1, snRNP,TCF4, TPM3, Tubulin, UPF3B, ZBTB20
13	Cell Death, Lipid Metabolism, Small Molecule Biochemistry	24	20	AGL,AMPK,ATF7IP, ATPase, BLVRB,CLCN3,DLAT, F Actin, FOXN2, Growth hormone, HDL, IFN TYPE 1, IFNAR1, IgG, Immunoglobulin, INO80, Insulin, Interferon alpha, KCTD12, LPL, LUC7L2, MAP1S,MICAL2,MLH1,MYD88, MYH7, NFKB1, NOV, P38 MAPK, Pro-inflammatory Cytokine, RHOBTB3, STAT5a/b, SYT7, Trypsin,Vegf
14	Reproductive System Development and Function, Connective Tissue Disorders, Genetic Disorder	21	18	AMY2A, ANXA3, B3GALNT1, CIDEC, COL5A1, COL6A1, DIRAS3, ENPP1, EPB49, ESPL1, GARS, GATM, GSTCD, HGFAC, HNF1A, HNRPDL, IHH, KLF9, MARCH7, MBNL2, Meg3,NNMT, PDHB, PDLIM4, PDPN, PRL, progesterone, PZP, RYR3, SLC39A14, TBC1D8B, TGFB1, USO1, WNT5B, ZNF365
15	Cellular Movement, Immune Cell Trafficking, Cell-To-Cell Signaling and Interaction	21	18	ADAM23,CCL19,CD44,CD2AP,CHEMOKINE,CHGB,DDN,DEPTOR, Focal adhesion kinase, G protein alpha, GNA11, GNAQ, GNRH, Gpcr, GRM5, GRP, Gαq, HMGB1L1, Ifn gamma, IL12 (family), JAK2, Laminin, LGR4, LPAR6, LYST, Metalloprotease, Mmp, p85 (pik3r), PLC, PTK2B, SCYL3, Shc, SRC, Tlr, Tnf
16	Cell Morphology, Cellular Compromise, **Nervous System Development and Function**	18	18	AKAP9, Cofilin, DLGAP2, DUSP8, EPHA4, EPHA5, EPHA, ERBB2IP, Fcer1, Ige, Jnk, KIDINS220, Mek, Mlc, Mlcp, MLL5, MYLK, Myosin, Myosin Light Chain Kinase, NCK, PABPN1, Pak, Pkg, PP1 protein complex group, PP1-C, PPP1CB, Ppp1r2 (includes others), RNF6, ROCK1, Rock, TAOK1, TIAL1,TRAF, ZC3H4, ZDHHC2
17	**Nervous System Development and Function**, Cell-To-Cell Signaling and Interaction, Genetic Disorder	18	16	ADAM22,ADCY1,ARAP2,ATP11A, ATP11B,ATP2B2,ATP2B3,ATP9A, Ca2+, CACNA1B, CACNG7, CCL21,CSMD2, Dcc dimer, DLG4, DMXL2, FYN, JPH3, LPHN1, LRRC7, METAP2,Mg2+-ATPase, NBEA, NCKAP5, PCLO, RBM26, S100PBP, SELPLG,SLC24A2, SNX20,SNX24,VLDLR,WDR7,YTHDC1
18	Cell-To-Cell Signaling and Interaction, Tissue Development, Developmental Disorder	18	16	ABHD3,BCAN,C4orf41,CBR1,COL18A1,COL9A1,COL9A3,CUTA, ECHS1, EGFR, Egfr dimer, FBLN2, FLT4,FN1 ,GLOD4,GPCPD1, GRB2,HIC1, ID4, LIF, MCC,MCMBP,NOV,PIK3C2B,PLOD3,PUS7,RIN2,SH3BGRL,TECPR1, TESK1, TMEM47,TRAPPC8,TTC15,USP53,VANGL1
19	Cancer, Cellular Assembly and Organization, Cell-To-Cell Signaling and Interaction	17	18	14-3-3,ARHGAP12,atypical protein kinase C,BCR,CRK,DHX40, DMTF1, DOCK10, ERK, G3BP1,Gef,HNRNPR,ID4, IFRD2,Integrin, MAP2K1, MAP2K1/2, MAP3K, MARK3,Nfat (family),NRG,Pdgfr,PGM2L1, POU6F1, Ptk, Raf,Ras homolog, RASA1,RASGRF1,RB1CC1, RhoGap,Sos, TCR,THOC2, THOC7
20	Cell-To-Cell Signaling and Interaction, **Nervous System Development and Function**, Molecular Transport	17	16	AGT,APOD,ATAD2,ATP2B1,beta-estradiol, CCNI, CDCA7, Cox7c/Gm10012, CRNKL1, CTPS, D-glucose, DECR1, dihydrotestosterone, epi-androsterone, GBA2, GIGYF1, GPD1, GPD2, IGF1R, INHIBIN B, KLHL26, LIMA1, MMD, MPEG1, MYOF, NSUN2, OSBPL3, PIK3C2B, PMEPA1, RBM3, RPL19, SLC4A4, SLC7A8, SRP54, TKT
21	Post-Translational Modification, Cellular Function and Maintenance, Gene Expression	17	16	AMFR, ANAPC5, ANAPC1/LOC100286979, CMBL,CNOT6, CUL2,DDT, DSTYK, EPC2, FXYD6,HSPB11,HSPE1, HSPH1, let-7a/let-7f/let-7c (includes others), LSM12, miR-124, MORF4L1,MRPL9, RAB40B, RABGAP1L, RDH10, SAT1, SPCS3, TCEB1,Tceb2, TLE1, TP53, UBXN4, VHL, WSB1, ZCCHC11, ZCCHC24, ZER1, ZNF131, ZYG11B
22	Free Radical Scavenging, Connective Tissue Disorders, Organismal Injury and Abnormalities	16	15	ADAMTS1,Alp,ARF5,CD3,CD52,collagen,Collagen(s),EMB,Fc gamma receptor, Fgf, G protein beta gamma,HMGCS1,Ifi203,Ifn,IFN Beta,IL1, IL33, IL1RL1,LDL, LUM, MTDH,Neurotrophin,NFkB (family),ODC1, Pdgf (complex), PDGFBB, PLA2, Pld, PTPN12, Ras, Sapk, SPARC,Tgf beta,TNFAIP3,XDH
23	Developmental Disorder, Genetic Disorder, Neurological Disease	16	15	ABI2, AKIRIN2, ANKHD1, BBS1, BBS2, BBS4, BBS5, BBS7, BBS9, CCDC53, FAM21A, GRHL1,HNF4A,HOOK3,ISOC1, KIAA0196, KIAA1033, LUC7L2, NDC80,NUP62, PCM1, PCNP, PCYT1A,PEX3,PEX16, PHTF1, SEC23A, SEC23IP, SSBP1, STAU2,TRAPPC3,TRAPPC4, TRAPPC6A, TTC8, ZNF644
24	Protein Synthesis, Cell Morphology, Cellular Development	16	15	ABCE1, ACAP3, BAZ2B, CBR1, CCPG1, CDCA2, CDCA7, CSGALNACT1, DLC1, EIF6,EIF2C1,FUS,GMFG,let-7, LMNB2, MSC, MYOD1, NOV, NPEPPS, ORC3, ORC6, PRNP, RFC2,RFC4, RPL4, RPL5, RPL6, RPL34, Rpl29 (includes others), RPS16, THSD4, TNRC6C, tretinoin, UCP3, ZNF536
25	Cellular Assembly and Organization, Post-Translational Modification, Cell Cycle	16	15	CDCA5, CDK9, HCFC2, HNRNPA3, LAMTOR3, LARP7, LCN1,LCN1L1, Ncl, NOL8,PARP4, PARP8, PARP12, PARP14, PARP, PDS5A, PDS5B, REC8, RRAGA, RRAGD,SFI1, SMC1B,STAG1,STAG2, STAG3,STAT4,TANC1, TIPARP, TNF, TNIK, TNKS2, TNKS, TPP2, WAPAL, ZNF330

Supplementary Table 3

Gene Symbol	GeneBank Accession No.	Fold Change KO/WT	p-value
Htr1d	NM_008309	-1.03	0.85
Htr1f	NM_008310	1.07	0.70
Htr2a	NM_172812	1.03	0.82
Htr2b	NM_008311	1.05	0.76
Htr2c	NM_008312	-1.06	0.56
Htr3b	NM_020274	-1.02	0.92
Htr4	NM_008313	-1.02	0.89
Htr5a	NM_008314	-1.05	0.78
Htr5b	NM_010483	-1.13	0.12
Htr6	NM_021358	1.03	0.83
Htr7	NM_008315	-1.10	0.41
Tph1	NM_009414	-1.03	0.67
Tph2	NM_173391	-1.01	0.91
Slc6a4	NM_010484	1.03	0.87

Supplementary Table 4

Gene Symbol	GeneBank Accession No.	Forward Primer Sequence	Reverse Primer Sequence	Amplicon size(bp)
Dcn	NM_007833	AGCTTCAACAGCATCACCGTT	AGGAACATTGGCCAGACTGC	51
Lct	NM_001081078	AGCACAAGAGCGAAGAGGAC	CAAAGGGTGAGTGAGGCATT	131
Ccl19	NM_011888.2	CTGCTGGTTCTCTGGACCTT	GAAGGCTTTCACGATGTTCC	114
Bdnf	NM_007540	GTGACAGGCGTTGAGAAAGC	ATCCACCTTGGCGACTACAG	206
Mapk1	NM_001038663	TCTCCCGCACAAAAATAAGG	GCCAGAGCCTGTTCAACTTC	129
Emb	NM_010330	TGCTGCGAATGCCTTAGACT	CAACCATCCAAGGCACATTT	140
Creb3	NM_013497	TGCTCACGTGCTCTGTTAGG	CTATTCACGGGGCAACAGAT	105
Cap1	NM_007598	TGAATGTCCTCATTCCTACCG	TCCAGCGATTTCTGTCACTG	116
Fancg	BC050890	TCCCCATTCCTACCCTTTTC	ACTGCTCCTCCAGAAGGATTAAC	101
Usp53	BC022221	TCCCGGAGTCAGAACCATAG	AATTTGCTTATGGCTGTTTTCAA	122
Pttg1	NM_013917	ACGAGAAACTCCTCTTCTTCCA	AATATCTGCATCGTAACAAACAGG	100
Ppig	NM_001081086	AACTCCCAGCCTGCTTCATA	CTGGATCTGGAAGGAGTTCG	104
Actin	NM_007393	GATCTGGCACCACACCTTCT	GGGGTGTTGAAGGTCTCAAA	139
Gapdh	NM_0008084	GTGGACCTCATGGCCTACAT	TGTGAGGGAGATGCTCAGTG	125

Supplementary Figure

Supplementary Figure 1: Hippocampus microdissection and RT-qPCR confirmation of hippocampus dissection.

(**A**) Schematic representation of area microdissection according to Franklin and Paxinos atlases. (**B-C**) Differential expression of *Dcn* and *Lct* in ventral (**B**) and dorsal (**C**) hippocampus of 5-$HT_{1A/1B}^{-/-}$ and 5-$HT_{1A/1B}^{+/+}$ mice was used as a confirmation of accurate dissection. *** $p<0.0001$ using two-tail Student T-test.

SUPPLEMENTARY FIGURE 1