HHS Public Access

Author manuscript

Hippocampus. Author manuscript; available in PMC 2016 May 01.

Published in final edited form as:

Hippocampus. 2015 May ; 25(5): 670–678. doi:10.1002/hipo.22402.

The canonical Notch pathway effector RBP-J regulates neuronal plasticity and expression of GABA transporters in hippocampal networks

Shuxi Liu[1,2,6,*], **Yue Wang**[5,*], **Paul F. Worley**[2,3], **Mark P. Mattson**[3,5], and **Nicholas Gaiano**[1,2,3,4]

[1]Neuroregeneration Program, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205

[2]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

[3]Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205

[4]Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

[5]Laboratory of Neurosciences, NIA/NIH, Baltimore, MD 21224

Abstract

Activation of the Notch pathway in neurons is essential for learning and memory in various species from invertebrates to mammals. However, it remains unclear how Notch signaling regulates neuronal plasticity, and whether the transcriptional regulator and canonical pathway effector RBP-J plays a role. Here we report that conditional disruption of RBP-J in the postnatal hippocampus leads to defects in long-term potentiation (LTP), long-term depression (LTD), and in learning and memory. Using gene expression profiling and chromatin immunoprecipitation, we identified two GABA transporters, GAT2 and BGT1, as putative Notch/RBP-J pathway targets, which may function downstream of RBP-J to limit the accumulation of GABA in the Schaffer collateral pathway. Our results reveal an essential role for canonical Notch/RBP-J signaling in hippocampal synaptic plasticity and suggest that role, at least in part, is mediated by the regulation of GABAergic signaling.

INTRODUCTION

Among the most conserved signaling cascades in animals, the Notch pathway is well known to regulate neural progenitor maintenance and differentiation (Louvi and Artavanis-Tsakonas, 2006; Pierfelice et al., 2011). The Notch receptors (Notch1–4 in mammals), are transmembrane proteins expressed on the surface of signal receiving cells. The binding of ligands (members of the Jagged and Delta-like families) to Notch receptors triggers two

Correspondence to either: Shuxi Liu, Ph.D., shuxi.liu@nih.hhs.gov, Mark Mattson, Ph.D., Mark.mattson@nih.gov.

[6]Present address: Receptor Biology Section, NINDS/NIH, Bethesda, MD 20892.

[*]Contributed equally to this work.

The authors declare no competing financial interests.

proteolytic cleavage events, the second of which releases the intracellular domain of Notch (NICD) which translocates to the nucleus where it binds to RBP-J (which acts as a repressor in the absence of NICD) and Mastermind-like (Maml) proteins, thereby creating a transcriptional activator complex. Canonical Notch targets are expected to contain the RBP-J consensus binding site GTGGGAA (Tun et al., 1994;). So-called “non-canonical Notch signaling” has also been described (Andersen et al., 2012), and while the molecular cascade(s) involved in such putative signaling remain poorly understood, it is generally defined as not involving RBP-J.

There is increasing evidence, in both vertebrates and invertebrates, that Notch signaling plays important roles in neurons, and can regulate axonal path finding, synaptic plasticity, long-term memory, and animal behavior (Ables et al., 2011; Pierfelice et al., 2011; Yoon et al., 2012). How Notch signaling influences these processes is uncertain, although some reports have suggested an active role, having shown that the pathway is stimulated in neuronal circuits engaged in information processing, (Alberi et al., 2011; Lieber et al., 2011), and that Notch1 activation requires the immediate early plasticity gene *Arc/Arg3.1* (Alberi et al., 2011).

In neural progenitors, the Notch pathway utilizes RBP-J to regulate the expression of target genes, in particular the Hes/Hey family of transcriptional regulators (Iso et al., 2003). However, it is unclear whether the effects of Notch on neuronal plasticity are mediated by RBP-J, and which downstream targets (canonical or otherwise) are involved. Work by Sato et al. reported that loss of RBP-J does not adversely affect memory formation or neuronal health (Sato et al, 2012). However, that work was limited to aged mice (12–18 months), performed a limited battery of behavioral assays, and did not examine synaptic plasticity directly.

Here we present evidence that conditional disruption of RBP-J in postnatal neurons impairs numerous aspects of synaptic plasticity and animal behavior. In addition, we have identified the GABA transporters, GAT2 and BGT1, as putative Notch pathway targets in that context, and present evidence that GABAergic neurotransmission plays a role in the Notch/RBP-J-mediated regulation of neuronal plasticity, learning and memory.

MATERIALS AND METHODS

Animals and ISH

All mice were maintained in accordance with the Institutional Animal Care and Use Committees at Johns Hopkins University School of Medicine, and the National Institute on Aging Intramural Research Program. RBP-J conditional knockout (cKO) and non-mutant littermate control mice were obtained by crossing RBP-J flox/flox mice to the CaMKII-Cre (T29-1) mouse line (Tsien et al., 1996). Brother-sister mating was used to generate mice for experimental analyses, and control mice were non-mutant siblings. Maximal electroconvulsive shock (MECS) was performed as described with modification (Ma et al., 2009). Four shocks were delivered at one-hour intervals and the animals were sacrificed 30 minutes after the fourth shock. Three animals were used for each condition. The CA1 region was isolated and used for microarray and quantitative RT-PCR analyses.

In situ RNA hybridization (ISH) was performed on 20 μm coronal brain sections with digoxigenin-labeled RNA probe for RBP-J (Wilkenson, 1992). Images were collected using a Zeiss Axioskop with an Axiocam and were processed using Adobe Photoshop.

Behavioral experiments

All animals were housed individually and were habituated to daily handling for seven days before behavioral testing. The mice underwent a series of behavior assays, some of which are stressful and may induce anxiety or aggressive behavior. Therefore, in order to reduce the influence from one behavioral test to the next, we housed the animals individually. We ordered the behavioral experiments from least to most stressful as follows: open field, elevated plus maze, rotarod, Y maze, novel object recognition, social interaction, fear conditioning and Morris water maze. Standard protocols for the novel object recognition test (Bevins and Besheer, 2006), social interaction test (Kaidanovich-Beilin et al., 2011), and the Morris water maze (Vorhees and Williams, 2006) were used to characterize the behavior of RBP-J cKO mice as compared to controls. Statistical analyses were performed using one- and two-way ANOVA, and Student's t-test).

Microarray Analysis, Chromatin immunoprecipitation (ChIP) and quantitative PCR

Total RNA isolated from region CA1 of the hippocampus was subjected to microarray analysis (Affymetrix, Microarray Core Facility, High Throughput Center, Johns Hopkins University. ChIP assays were done as described previously (Grego-Bessa et al., 2007) using cultured cortical neurons (14 days in culture). Quantitative PCR was performed on the IP eluate with primers designed to amplify regions upstream of transcriptional start sites with RBP-J binding sites (−3647 bp in BGT1, and −183 bp in GAT2). Primer sequences are available upon request. Antibodies used were mouse anti-RBP-J (Cat. No. K0043, Institute of Immunology, Japan) and rabbit anti-RBP-J (Cat. No. ab25949, Abcam).

Neuronal cell culture

Neuronal cultures were prepared from the cortex or hippocampus of E17.5 mouse embryos as described (Alberi et al, 2011). Neurons were pharmacologically manipulated after 14 days *in vitro* (DIV). The pFUGW lentiviral vector (provided by Richard Huganir) was used to express Cre recombinase in neuronal cultures to disrupt RBP-J. Hippocampal neurons, derived from embryos homozygous for the floxed RBP-J allele, were infected one week after plating, and treated with bicuculline (Tocris, 50 μM for 2–6 hours) seven days after infection. RNA for analysis was obtained using the RNeasy Kit (Qiagen).

Hippocampal slice electrophysiology

Transverse hippocampal slices (350 m) were prepared from RBP-J cKO and control mice, and equilibrated for 1 hour in artificial cerebrospinal fluid prior to electrophysiological recordings. Slices were maintained at 30–32C during recording, and kept in a holding chamber up to 6 hours. Field excitatory postsynaptic potentials (fEPSPs) were recorded in CA1 stratum radiatum as described previously (Wang et al., 2004). LTP was induced by high frequency stimulation (HFS, 100 Hz, 1 sec), with 50 M picrotoxin to block $GABA_A$ activity. LTD was induced with 900 pulses at 1 Hz with a stimulation intensity that evoked

about 30–40% of the maximum of fEPSP. In some experiments 100 pulses at 10 Hz was used (without picrotoxin). Plots were normalized to the initial slope of the fEPSPs; each data point represents the averaged values for 1 min (three consecutive sweeps with an interval of 20 sec). Values are mean ± SEM. Data were collected using an Axopatch 200B amplifier (Molecular Devices); signals were filtered at 2 kHz, digitized at 10 kHz and analyzed using pCLAMP 9 software (Molecular Devices).

RESULTS

Postnatal RBP-J disruption impairs synaptic transmission and plasticity

Based upon our prior work on Notch1 function in neuronal plasticity (Alberi et al, 2011), we tested the role of the canonical Notch effector RBP-J in that context, by generating a conditional deletion of RBP-J. This was done using the “floxed” allele (Han et al., 2002), and a CamKII-Cre line that deletes in many excitatory neurons in the hippocampus and elsewhere (T29-1, Tsien et al, 1996). As seen by in situ hybridization, four weeks after birth, RBP-J transcripts were largely absent from the CA1 region, where deletion was expected, but not from the dentate gyrus, where deletion was not expected (Fig. 1A).

We used electrophysiological methods to evaluate the role of RBP-J in synaptic transmission and plasticity of the Schaffer collateral pathway in hippocampal slices. First, in contrast to our prior findings with Notch1 cKO slices (Alberi et al. 2011), we observed reduced basal synaptic transmission (I/O curves) in RBP-J cKO mice (10 slices, 5 mice each, $p<0.01$) (Fig. 1B). In addition, we found that paired-pulse facilitation (PPF) was significantly decreased in RBP-J cKO mice with inter-pulse intervals (IPI) of 50 ms and 100 ms (Fig. 1C). Thus, loss of RBP-J function in neurons leads to reduced basal synaptic transmission and altered presynaptic neurotransmission.

We next evaluated long-term potentiation (LTP) and long-term depression (LTD) in the Schaffer collateral pathway in RBP-J cKO hippocampal slices. While LTP was induced by high frequency stimulation (100 Hz, 1s) in RBP-J mutants, the magnitude was significantly reduced compared to slices from control mice (Fig. 1D). More strikingly, LTD was fully inhibited in RBP-J mutants compared to controls (Fig. 1E). These changes in synaptic plasticity in the absence of RBP-J function were consistent with our previous Notch1 results (Alberi et al, 2011), although the effect of RBP-J disruption on LTD was more severe than that of Notch1 disruption, suggesting that other Notch receptors, signaling through RBP-J, might play a role in LTD.

RBP-J is required for hippocampal-dependent learning and memory

We used learning and motor performance assays to examine the role of RBP-J in behavior. For many assays, including the rotarod test, open field test, elevated plus maze, Y maze and fear conditioning, we did not observe significant differences between RBP-J cKO and control animals (Table 1). However, performance differences were seen using the novel object recognition, social interaction, and the Morris water maze assays, supporting a role for RBP-J in learning and memory.

In the novel object recognition test, while control mice spent twice as much time exploring a novel object as they did a familiar (previously encountered) object, RBP-J cKO mice spent equal amounts of time exploring both, indicating they did not remember the 'familiar' object (Fig. 2A). Similarly, in the social interaction test, control mice could distinguish between mice they were newly exposed to, and those they had interacted with previously (indicated by more time spent sniffing and interacting with the 'unfamiliar' subject), while RBP-J cKO mice could not (Fig. 2B).

Next we tested spatial learning and memory of RBP-J cKO mice using the Morris water maze. RBP-J cKO mice and controls performed similarly with respect to target acquisition, reverse learning, and time in the target quadrant 24 hours after training (Fig. 2C–E). However, RBP-J cKO mice exhibited a significant defect (35% reduction) in finding the target quadrant when probe trials were performed 10 days after training (Fig. 2F). Collectively, these behavioral results indicate that deletion of RBP-J in postnatal neurons impairs hippocampus-dependent learning, and long-term spatial memory retention, while leaving short-term spatial learning and memory intact.

GABA transporters GAT2 and BGT1 are potential Notch/RBP-J targets in neurons

To gain insight into how Notch/RBP-J signaling influences synaptic plasticity, learning, and memory, it will be important to identify the relevant target genes. With this in mind, we first used microarray analysis to screen for Notch1-dependent activity-induced gene expression in CA1 of the hippocampus. We compared gene expression changes after maximal electro-convulsive shock (MECS), in CA1 of Notch1 cKO (see Alberi et al., 2011) and control mice (Supporting information). In Notch1 cKO animals, 280 genes exhibited reduced induction in response to MECS, and thus were potential targets of neuronal Notch1 signaling. We list 20 genes that were down-regulated the most in Notch1 cKO mice upon MECS (Table 2) and 20 genes that were up-regulated the most upon MECS (Table 3). The expression of the majority of these genes was not different in wild type and Notch1 cKO mice under basal (no MECS) conditions, suggesting their expression is regulated by neuronal activity.

During canonical signaling, the intracellular domain of Notch binds to RBP-J, which is localized to target genes containing one or more RBP-J binding sites. To identify genes that might be direct pathway targets, we scanned for RBP-J sites in the regulatory regions of the 280 potential Notch1-responsive genes found by microarray. Some of the RBP-J consensus binding sites in theses genes are shown in Tables 2 and 3. Several candidate genes with RBP-J sites were then tested for RBP-J binding, using chromatin immunoprecipitation (ChIP), and those encoding the GABA transporters Slc6a12 (BGT1) and Slc6a13 (GAT2) were found to be heavily enriched by ChIP (220-fold and 15-fold, compared to the input respectively, Fig. 3A,B).

The identification of *Gat2* and *Bgt1* as Notch1-dependent activity-induced neuronal genes, with RBP-J bound to their transcriptional regulatory regions, indicated that the genes encoding these GABA transporters might be direct Notch/RBP-J targets. Neither GAT2 nor BGT1 had significantly altered basal expression after disruption of Notch1 (in CA1) or RBP-J (in cultured neurons) (Fig. 3C,E). Interestingly, consistent with RBP-J being a repressor in the absence of pathway stimulation, we observed a 3-fold increase in BGT1

expression in the RBP-J cKO animals, although that increase did not achieve statistical significance.

To evaluate the importance of NICD1/RBP-J signaling on BGT1 and GAT2 expression in response to neuronal activity, we used maximal electroconvulsive shock (MECS) in vivo, and treatment with the $GABA_A$ receptor blocker bicuculline on cortical neurons in vitro. While expression ratio of MECS:Controls in non-mutant animals revealed a 3-fold increase, no increase was seen in Notch1 cKO animals after MECS (Fig 3D). Although the GAT2 expression ratio comparison (MECS:no MECS) did not reach statistical significance, direct comparison of expression values revealed a significant 2.2-fold increase in GAT2 expression in controls in response to MECS ($p<0.045$), while no increase was seen in Notch1 cKO animals. Consistent with these in vivo results, in response to bicuculine-induced activity in neuronal cultures, expression of both GAT2 and BGT1 increased in control cultures, but not RBP-J cKO cultures (Fig. 3F). Thus, Notch/RBP-J signaling appears to directly and positively regulate neuronal expression of the GABA transporters BGT1 and GAT2 in response to activity.

To test the whether GABA receptors are expressed in hippocampal CA1 neurons, we carried out GAT2 in situ hybridization. GAT2 mRNA was detected by the antisense probe in hippocampal CA1, CA3 and dentate gyrus (Fig. 3G), but not by the sense probe (Fig. 3H, J). In the RBP-J cKO the expression of GAT2 was significantly reduced in CA1 whereas the expression in DG remained intact (Fig.3I). Together, the in vivo and in vitro data both suggest GAT2 as a transcriptional target of canonical Notch/RBP-J pathway in CA1 neurons.

GABA inhibition is enhanced in RBP-J cKO mice

Based upon our evidence that GAT2 and BGT1 are Notch/RBP-J targets genes in neurons, we reasoned that increased extracellular GABA could explain the reduction in excitatory basal transmission observed in RBP-J cKO mice (Fig. 1B). Thus, we blocked NMDA and AMPA receptors pharmacologically, and measured inhibitory postsynaptic currents (IPSC) at CA1 synapses in hippocampal slices. Consistent with increased extracellular GABA in RBP-J cKO slices, while holding the membrane potential at −70 mV, we observed a 20% elevation in IPSC amplitude at CA1 pyramidal synapses as compared to controls (Fig. 4A). This IPSC elevation could be mimicked by inhibiting GABA reuptake in control slices, with the selective GAT2 antagonist SNAP-5114 (Borden et al., 1994; Madsen et al., 2009) (Fig. 4B,C). GAT2 blockade in RBP-J cKO slices did not change the IPSC amplitude, consistent with the idea that GAT2 function is compromised in RBP-J cKO mice.

Based on the data presented, our working model is that activity-induced Notch/RBP-J signaling modulates synaptic transmission and plasticity by increasing GABA transporter expression to reduce extracellular GABA. As such, the disruption in synaptic function seen in RBP-J cKO animals could be explained, at least in part, by decreased GABA transporter expression and the resulting increased extracellular GABA. In further support of this model, the disrupted LTD observed in RBP-J cKO hippocampal slices (see Fig. 1E), can be restored by addition of picrotoxin to inhibit $GABA_A$ receptors (Fig. 4D).

DISCUSSION

Our findings indicate that canonical Notch signaling is required for hippocampal plasticity, learning and memory. In our model, deletion of RBP-J occurs 3–4 weeks after birth (Tsien et al., 1996), when the hippocampal neuronal circuitry has largely been established, thus avoiding potential confounds associated with loss of Notch pathway function during neural development. Conditional disruption of either Notch1 (Alberi et al., 2011), or the canonical effector RBP-J (present study), resulted in similar changes in synaptic plasticity, although more severe effects were observed in RBP-J cKO mice. While LTD at CA1 synapses was reduced in slices from Notch1 cKO mice, it was absent in slices from RBP-J cKO mice. In addition, while PPF appeared normal in Notch1 cKO mice, was decreased in RBP-J cKO mice. The latter suggests that Notch receptors other than, or in addition to, Notch1 may mediate a presynaptic function for the pathway, or that Notch-independent RBP-J function plays a role.

Using gene expression profiling of hippocampal tissue from Notch1 cKO mice and non-mutant siblings, and ChIP analysis from cultured hippocampal neurons, we identified the GABA transporters GAT2 and BGT1 as putative activity-dependent targets of Notch1/RBP-J signaling in neurons. Consistent with this possibility, disruption of RBP-J in the hippocampus led to reduced activity-dependent expression of GAT2 and BGT1, increased IPSCs in CA1 pyramidal neurons, and perturbed synaptic plasticity. In addition, pharmacological inhibition of GAT2 elevated IPSCs in CA1 neurons in slices from WT mice to a level comparable to that of RBP-J cKO mice, and LTD was restored to a normal level at CA1 synapses in slices from RBP-J cKO mice, when the slices were treated with the GABA receptor antagonist picrotoxin. Thus, disruption of Notch/RBP-J signaling leads both to reduced activity-dependent expression of GABA transporters, and to functional deficits consistent with reduced GABA transporter activity.

Previous studies have found the GABA transporters GAT1, GAT2 and BGT1 expressed in adult mouse brain (Liu et al., 1993; Evans et al., 1996), with GAT2 and BGT1 in neurons and glial cells (Borden et al., 1995; Conti et al., 1999). In addition, BGT1 is expressed in pyramidal cells of the rat hippocampus, with expression in the CA3 region upregulated by neuronal injury (Zhu and Ong, 2004b). With respect to cellular localization, GABA transporters are present in presynaptic and extrasynaptic membranes (reviewed in Brickley and Mody, 2012), and when expressed in cultured hippocampal neurons, BGT1 and GAT2 are localized in high amounts in the dendrites (Ahn et al., 1996).

Considering that GAT2 and BGT1 are not exclusively expressed in pyramidal neurons in the hippocampus, their altered expression in RBP-J cKO mice could be a secondary effect of inactivation of Notch signaling. However, because the T29-1 CaMKII-Cre transgenic line used to generate the Notch1 cKO and RBP-J cKO animals used in our work (Alberi et al., 2011 and present study) expresses Cre recombinase in CA1 pyramidal cells (Tsien et al., 1996), it seems unlikely that the effects we have observed are due to cell non-autonomous alterations in GABA transporter function. In any event, our results show that the basal expression levels of BGT1 and GAT2 are very low, and that their expression is increased considerably, in a Notch signaling-dependent manner, in response to neuronal activity.

We observed that the IPSC was elevated at CA1 synapses in RBP-J cKO mice, consistent with the possibility that GABA levels were elevated as the result of reduced expression of GABA transporters. The increased GABA inhibition in RBP-J neurons was eliminated by blockade of $GABA_A$ receptors. Thus we deduced that the reduced excitatory neurotransmission in the RBP-J cKO mice was due to increased GABA inhibition. However, the role of reduced GAT2 and/or BGT1 levels in the abnormalities in LTP and LTD, and learning and memory processes in RBP-J cKO mice remains to be determined.

Our findings that neuronal activity drives expression of GAT2 and BGT2 in a Notch- and RBP-J-dependent manner, have implications not only for normal synaptic plasticity and associated behaviors, but also for pathological conditions that involve excitotoxic damage to neurons. Increased GABA transporter expression would be expected to reduce synaptic GABA levels, and could thereby contribute to enhanced excitability in conditions such as epilepsy, traumatic brain injury, and stroke. Indeed, it was reported that Notch signaling promotes neuronal degeneration in a mouse model of ischemic stroke (Arumugam et al., 2006). Furthermore, in zebrafish, disruption of Mind bomb, an E3 ubiquitin ligase required for Notch signaling, leads to perturbed GABAergic signaling and seizure activity (Hortopan et al., 2010). It will be of considerable interest to determine if induction of GABA transporter genes by activation of the Notch/RBP-J pathway plays a role in neurological disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors thank Dr. Lavinia Alberi for input during the course of this project. Funding for this work came from Institute for Cell Engineering at Johns Hopkins University (N.G), a Young Investigator Award from the Brain and Behavior Research Foundation (N.G), the James S. McDonnell Foundation (N.G.), the National Institute of Mental Health (P.W.), and the Intramural Research Program of the National Institute on Aging, National Institutes of Health.

REFERENCES

Ables JL, Breunig JJ, Eisch AJ, Rakic P. Not(ch) just development: Notch signalling in the adult brain. Nat Rev Neurosci. 2011; 12:269–283. [PubMed: 21505516]

Ahn J, Mundigl O, Muth TR, Rudnick G, Caplan MJ. Polarized expression of GABA transporters in Madin-Darby canine kidney cells and cultured hippocampal neurons. J Biol Chem. 1996; 271:6917–6924. [PubMed: 8636119]

Alberi L, Liu S, Wang Y, Badie R, Smith-Hicks C, Wu J, Pierfelice TJ, Abazyan B, Mattson MP, Kuhl D, Pletnikov M, Worley PF, Gaiano N. Activity-induced Notch signaling in neurons requires Arc/Arg3.1 and is essential for synaptic plasticity in hippocampal networks. Neuron. 2011; 69:437–444. [PubMed: 21315255]

Andersen P, Uosaki H, Shenje LT, Kwon C. Non-canonical Notch signaling: emerging role and mechanism. Trends Cell Biol. 2012; 22:257–265. [PubMed: 22397947]

Arumugam TV, Chan SL, Jo DG, Yilmaz G, Tang SC, Cheng A, Gleichmann M, Okun E, Dixit VD, Chigurupati S, Mughal MR, Ouyang X, Miele L, Magnus T, Poosala S, Granger DN, Mattson MP. Gamma secretase-mediated Notch signaling worsens brain damage and functional outcome in ischemic stroke. Nat Med. 2006; 12:621–623. [PubMed: 16680150]

Bevins RA, Besheer J. Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. Nat Protoc. 2006; 1:1306–1311. [PubMed: 17406415]

Borden LA, Smith KE, Vaysse PJ, Gustafson EL, Weinshank RL, Branchek TA. Re-evaluation of GABA transport in neuronal and glial cell cultures: correlation of pharmacology and mRNA localization. Receptors Channels. 1995; 3:129–146. [PubMed: 8581400]

Brickley SG, Mody I. Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. Neuron. 2012; 73:23–34. [PubMed: 22243744]

Conti F, Zuccarello LV, Barbaresi P, Minelli A, Brecha NC, Melone M. Neuronal, glial, and epithelial localization of gamma-aminobutyric acid transporter 2, a high-affinity gamma-aminobutyric acid plasma membrane transporter, in the cerebral cortex and neighboring structures. J Comp Neurol. 1999; 409:482–494. [PubMed: 10379832]

Evans JE, Frostholm A, Rotter A. Embryonic and postnatal expression of four gamma-aminobutyric acid transporter mRNAs in the mouse brain and leptomeninges. J Comp Neurol. 1996; 376:431–446. [PubMed: 8956109]

Grego-Bessa J, Luna-Zurita L, del Monte G, Bolos V, Melgar P, Arandilla A, Garratt AN, Zang H, Mukouyama YS, Chen H, Shou W, Ballestar E, Esteller M, Rojas A, Perez-Pomares JM, de la Pompa JL. Notch signaling is essential for ventricular chamber development. Dev Cell. 2007; 12:415–429. [PubMed: 17336907]

Han H, Tanigaki K, Yamamoto N, Kuroda K, Yoshimoto M, Nakahata T, Ikuta K, Honjo T. Inducible gene knockout of transcription factor recombination signal binding protein-J reveals its essential role in T versus B lineage decision. Int Immunol. 2002; 14:637–645. [PubMed: 12039915]

Hortopan GA, Dinday MT, Baraban SC. Spontaneous Seizures and Altered Gene Expression in GABA Signaling Pathways in a mind bomb Mutant Zebrafish. J Neurosci. 2010; 30:13718–13728. [PubMed: 20943912]

Iso T, Kedes L, Hamamori Y. HES and HERP families: multiple effectors of the Notch signaling pathway. J Cell Physiol. 2003; 194:237–255. [PubMed: 12548545]

Kaidanovich-Beilin O, Lipina T, Vukobradovic I, Roder J, Woodgett JR. Assessment of social interaction behaviors. J Vis Exp. 2011 Feb 25.(48):pii: 2473. 2011.

Lieber T, Kidd S, Struhl G. DSL-Notch signaling in the Drosophila brain in response to olfactory stimulation. Neuron. 2011; 69:468–481. [PubMed: 21315258]

Liu QR, Lopez-Corcuera B, Mandiyan S, Nelson H, Nelson N. Molecular characterization of four pharmacologically distinct gamma-aminobutyric acid transporters in mouse brain. J Biol Chem. 1993; 268:2106–2112. [PubMed: 8420981]

Louvi A, Artavanis-Tsakonas S. Notch signalling in vertebrate neural development. Nat Rev Neurosci. 2006; 7:93–102. [PubMed: 16429119]

Madsen KK, Clausen RP, Larsson OM, Krogsgaard-Larsen P, Schousboe A, White HS. Synaptic and extrasynaptic GABA transporters as targets for anti-epileptic drugs. J Neurochem. 2009; 109(Suppl 1):139–144. [PubMed: 19393020]

Pierfelice T, Alberi L, Gaiano N. Notch in the vertebrate nervous system: an old dog with new tricks. Neuron. 2011; 69:840–855. [PubMed: 21382546]

Sato C, Turkoz M, Dearborn JT, Wozniak DF, Kopan R, Hass MR. Loss of RBPj in postnatal excitatory neurons does not cause neurodegeneration or memory impairments in aged mice. PLoS One. 2012; 7(10):e48180. 2012. [PubMed: 23110206]

Tsien JZ, Chen DF, Gerber D, Tom C, Mercer EH, Anderson DJ, Mayford M, Kandel ER, Tonegawa S. Subregion- and cell type-restricted gene knockout in mouse brain. Cell. 1996; 87:1317–1326. [PubMed: 8980237]

Tun T, Hamaguchi Y, Matsunami N, Furukawa T, Honjo T, Kawaichi M. Recognition sequence of a highly conserved DNA binding protein RBP-J kappa. Nucleic Acids Res. 1994; 22:965–971. [PubMed: 8152928]

Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc. 2006; 1:848–858. [PubMed: 17406317]

Wang Y, Chan SL, Miele L, Yao PJ, Mackes J, Ingram DK, Mattson MP, Furukawa K. Involvement of Notch signaling in hippocampal synaptic plasticity. Proc Natl Acad Sci USA. 2004; 101:9458–9462. [PubMed: 15190179]

Yoon KJ, Lee HR, Jo YS, An K, Jung SY, Jeong MW, Kwon SK, Kim NS, Jeong HW, Ahn SH, Kim KT, Lee K, Kim E, Kim JH, Choi JS, Kaang BK, Kong YY. Mind bomb-1 is an essential modulator of long-term memory and synaptic plasticity via the Notch signaling pathway. Mol Brain. 2012; 5:40. [PubMed: 23111145]

Zhao JW, Du JL, Li JS, Yang XL. Expression of GABA transporters on bullfrog retinal Muller cells. Glia. 2000; 31:104–117. [PubMed: 10878597]

Zhu XM, Ong WY. Changes in GABA transporters in the rat hippocampus after kainate-induced neuronal injury: decrease in GAT-1 and GAT-3 but upregulation of betaine/GABA transporter BGT-1. J Neurosci Res. 2004b; 77:402–409. [PubMed: 15248296]

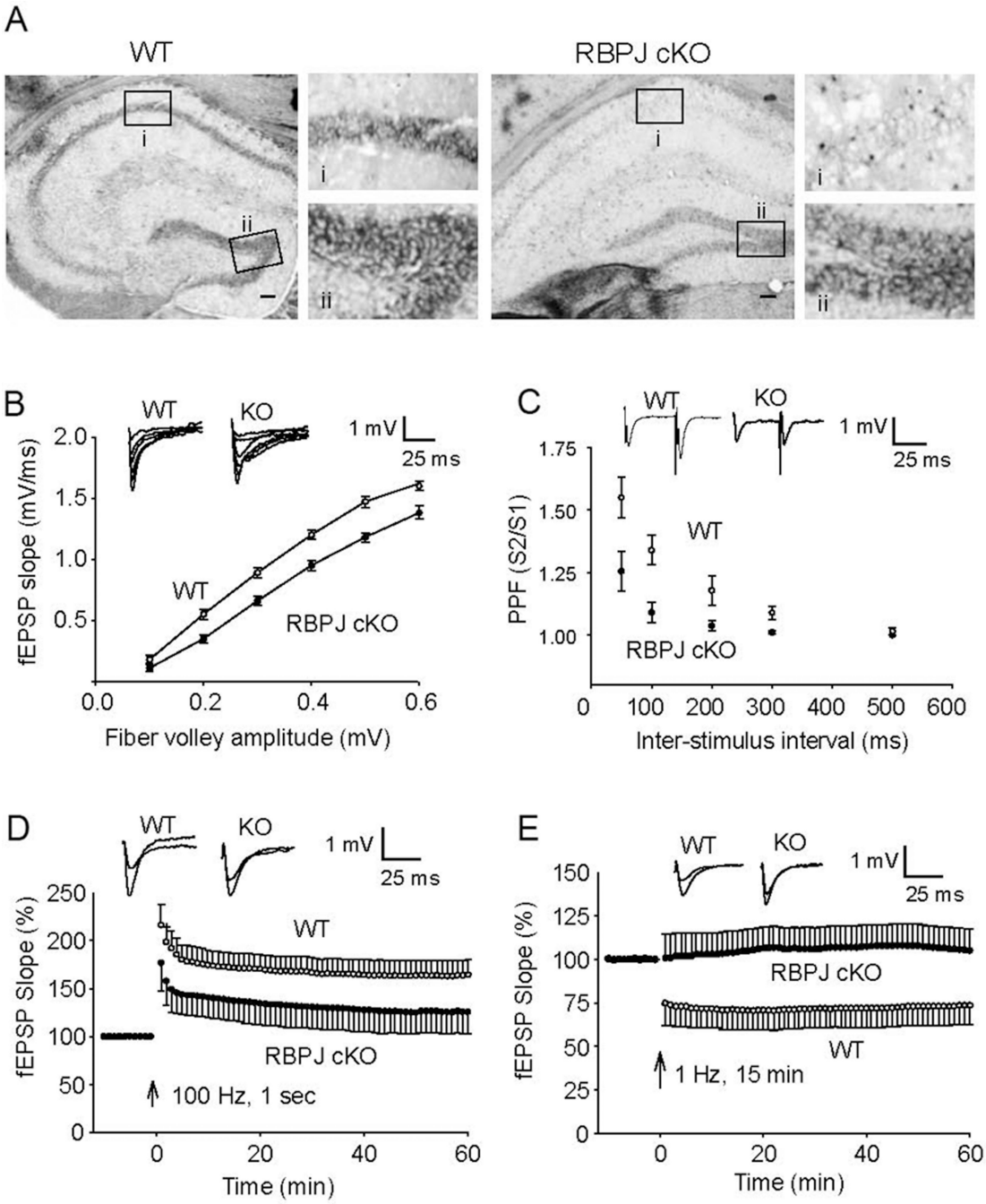

Figure 1. Synaptic transmission and plasticity are impaired in hippocampal neurons lacking RBP-J

(A) In situ hybridization in adult brain sections reveals that RBP-J mRNA is detected in CA1 region (i) of wild type (WT) mice but is depleted in RBP-J cKO mice. As expected, RBP-J expression in the dentate gyrus (ii) is not affected in RBP-J cKO mice. Scale bars, 100m. (B) Input/output curve of fEPSP in CA1 of RBP-J cKO slices are decreased compared to WT slices ($p<0.01$; one-way ANOVA, DF between groups = 1, residual DF = 8.). (C) Paired-pulse facilitation (PPF) is reduced at CA1 synapses in hippocampal slices from RBP-J cKO mice compared to WT ($p<0.01$ for 50 ms and 100 ms; one-way ANOVA,

DF between groups = 1, residual DF = 8.). (D) LTP is reduced at CA1 synapses in hippocampal slices from RBP-J cKO mice compared to WT ($p<0.01$, t test). (E) LTD is abolished at CA1 synapses in slices from RBP-J mice compared to WT mice ($p<0.01$, t test). For (B) through (E), values are the mean and SEM of recordings from 10 slices from five WT mice and 10 slices from five RBP-J cKO mice.

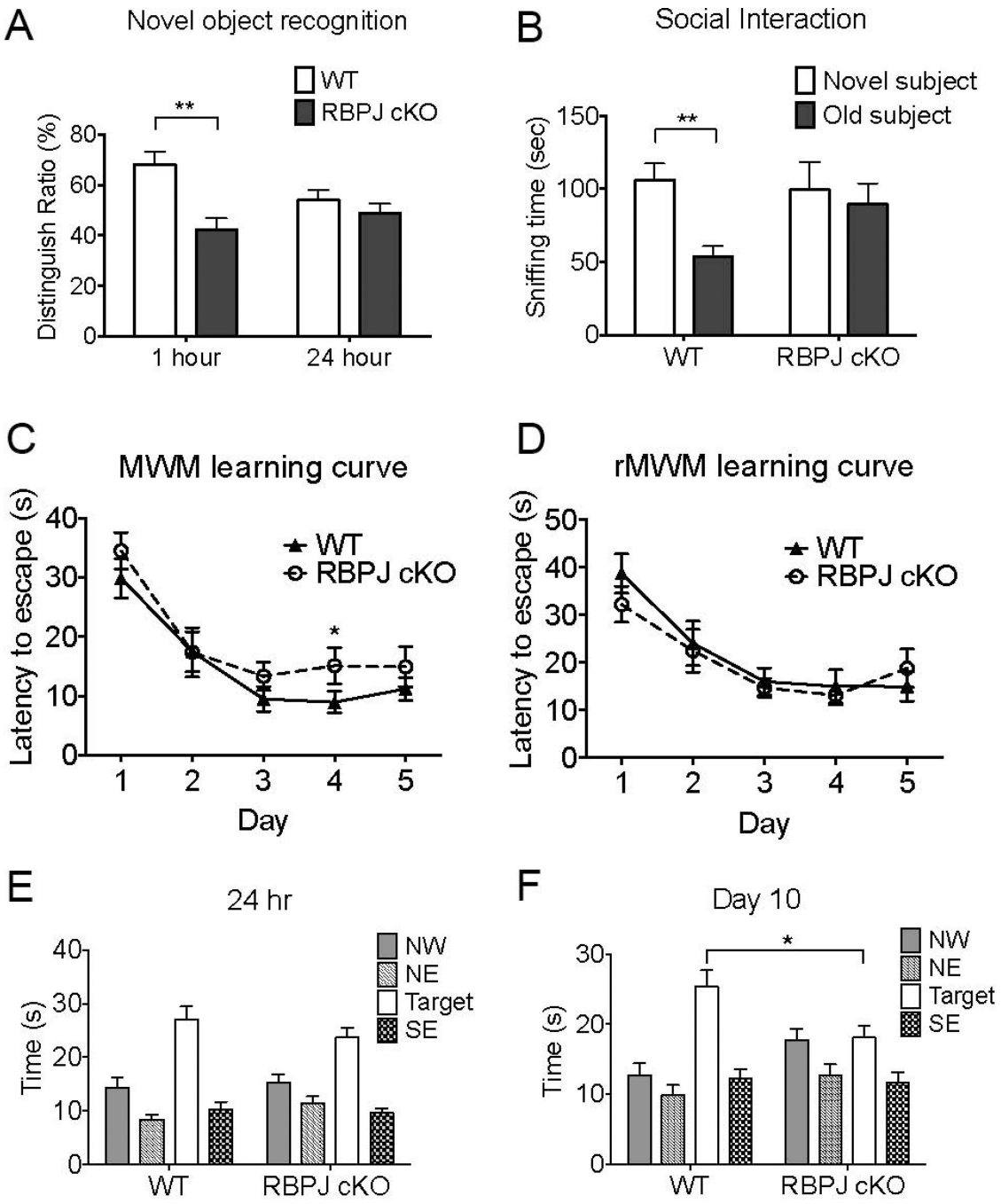

Figure 2. Hippocampus dependent memory is impaired in RBP-J cKO mice

(A–B) RBP-J cKO animals showed clear deficits in novel object recognition (A, WT n=9, RBP-J cKO n=8, $p<0.01$) and social interaction (B, WT n=14, RBP-J cKO n=11, $p<0.01$). (C–D) RBP-J cKO mice had a slight deficit in the Morris water maze (MWM) learning trials (C, WT n=12, RBP-J KO n=8, $p<0.05$) but not in reversal learning trials (D). (E–F) Mutant mice behaved normally in the MWM probe trial at 24 hours ($p<<0.01$ for both WT and RBP-J cKO for target quadrant selection over each of the other quadrants), but showed deficits in consolidated spatial memory during the probe trial at 10 days. At 10 days, RBP-J

cKO mice did not select the target preferentially over the other three quadrants (in particular NW), and target selection by RBP-J cKO animals was significantly reduced as compared to controls (WT n=14, RBP-J cKO n=12, *p<0.05). Values are shown as the mean and SEM. Comparison are all done with student's t test.

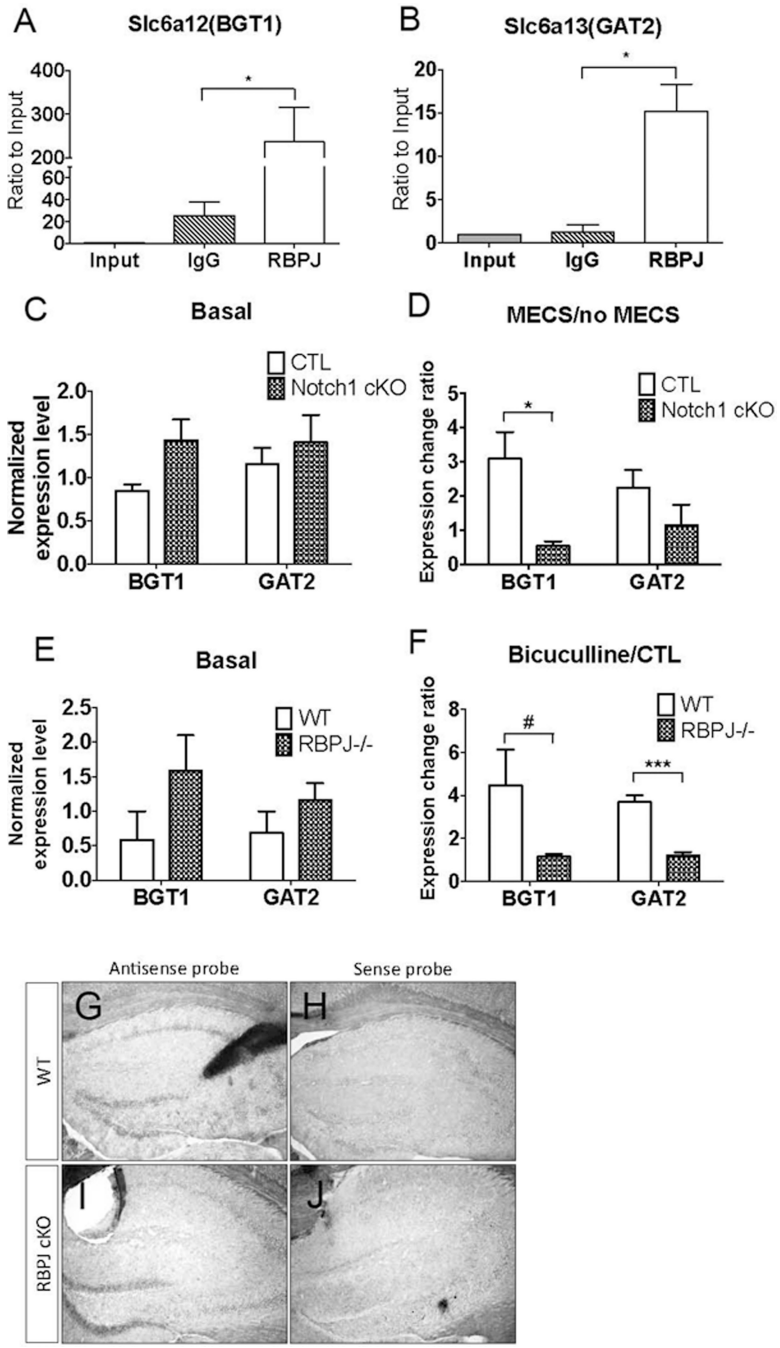

Figure 3. Identification of BGT1 and GAT2 as targets of canonical Notch signaling

(A–B) The promoter regions of BGT1 (Slc6a12) and GAT2 (Slc6a13) were immunoprecipitated from the genomic DNA of cultured mouse cortical neurons using anti-RBP-J antibodies (n=3, $p<0.05$). (C) Basal expression levels of BGT1 and GAT2 were not affected in Notch1 cKO mouse CA1 region (for BGT1, CTL, n=3, Notch1 cKO, n=3; for GAT2, CTL, n=6, Notch1 cKO, n=4) (D) Maximal electroconvulsive shock (MECS) induced expression of BGT1 by 3.1 fold (MECS, n=3, no MECS, n=3; *$p<0.05$), in CA1 of CTL animals, but not in Notch1 cKO animals. (MECS, n=3, no MECS, n=3). A similar

trend was observed for GAT2, and direct comparison of individual expression levels revealed MECS led to a 2.2-fold increase in CTL animals ($p<0.44$), but no such increase in Notch1 cKO animals. (E) Basal expression levels of BGT1 and GAT2 were not significantly affected by disruption of RBP-J in cultured mouse cortical neurons (WT n=2; RBP-$J^{-/-}$, n=3). (F) Bicuculline induced expression of BGT1 (ratio as compared to untreated control cultures) by 4.5 fold ($p<0.06$) and GAT2 by 3.7 fold ($p<0.05$) in WT mouse cortical neurons, but not in neurons lacking RBP-J. Values are shown as the mean and SEM. (G–J) In situ hybridization reveals that GAT2 mRNA is detected by antisense probe in both CA1 and DG regions of adult WT mouse brain, and that its level is reduced in CA1 of RBP-J cKO mouse brain. The GAT2 sense probe showed no signal in CA1 or DG, demonstrating specificity of the antisense probe hybridization.

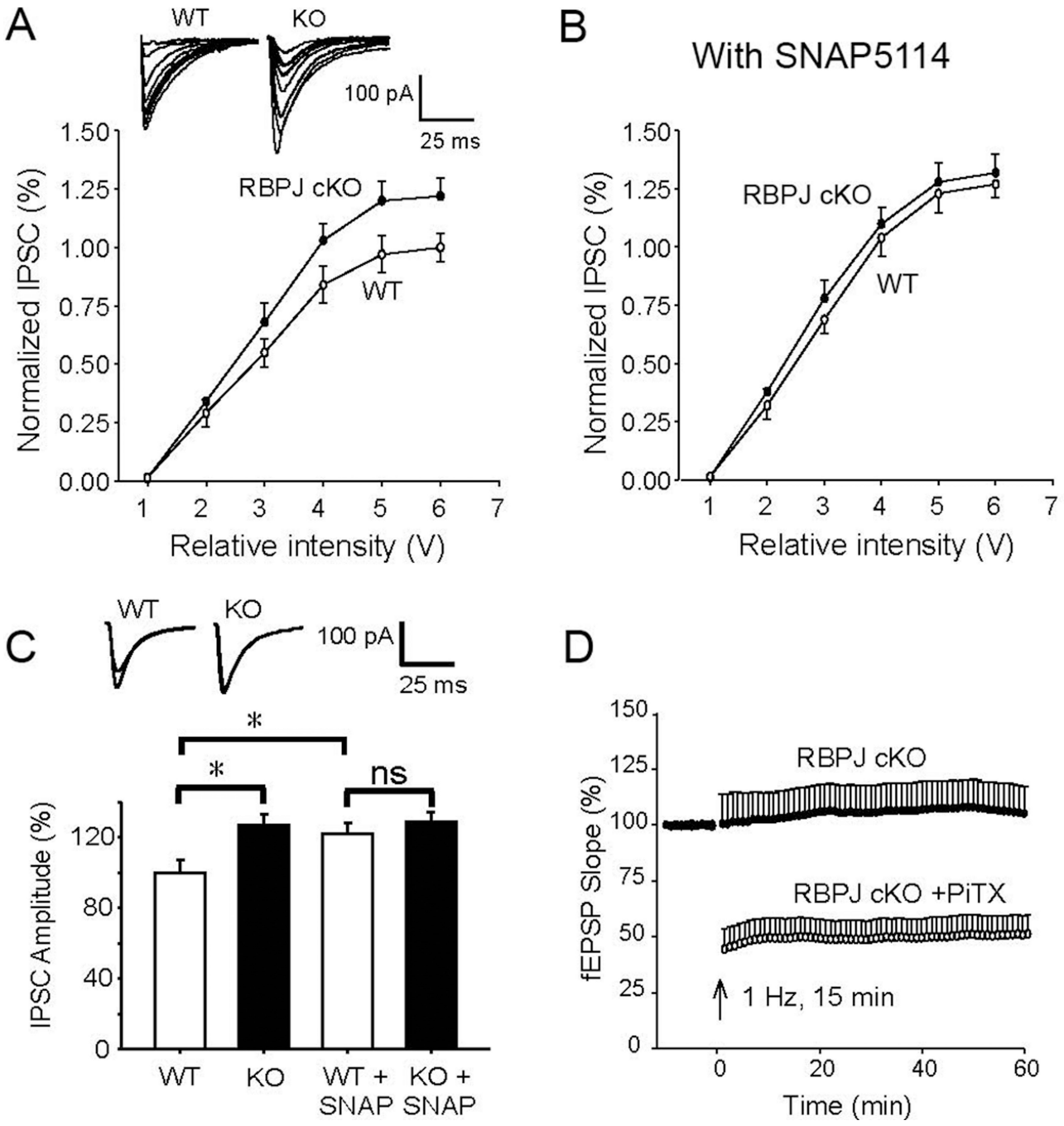

Figure 4. Changes in GABAergic signaling may influence synaptic plasticity changes associated with RBP-J disruption

(A) Comparison of inhibitory postsynaptic currents (IPSCs) intensity as a function of stimulation intensity recorded from CA1 neurons in slices (recordings were made at −70 mV, original traces are shown above) reveals increased IPSCs in RBP-K cKO animals as compared to controls ($p<0.05$; one-way ANOVA, DF between groups = 1, residual DF = 10). (B) Same comparison as in (A), but in the presence of the GAT2 transporter antagonist SNAP5114, no difference was observed between WT and RBP-J cKO slices. For both (A) and (B) 8–10 slices from 4 WT and 4 RBP-J cKO mice were examined. (C) Bar chart

representation of the data shown in (A, B), including comparison of WT slices with and without SNAP5114 (*p<0.05; ns: not significant, t test). Values in (A–C) are the mean and SEM. (D) GABA receptor blockade (treatment with 50 μM picrotoxin) restores LTD at CA1 synapses in hippocampal slices from RBP-J cKO mice (n=6 each with and without PiTX).

Table 1

General learning behaviors are intact in RBP-J cKO animals. The RBP-J cKO mice showed normal motor-activity (in open field), motor learning (rotarod), anxiety (elevated plus maze), short-term spatial memory (Y-maze) and fear memory (cued and contextual fear conditioning). Data is presented as the mean (± s. e. m.) value.

Behavioral tests	WT	RBP-J cKO
Open field (Total activity, beam/min)	538.71±47.41	541.82±45.53
Open field (% time outer zone/ total)	37.183 ±5.37	33.997±3.65
Elevated plus maze (% time in open arm/ total)	24.699 ±5.09	30.034 ±4.35
Rotarod (average of latency to fall, sec)	66.914 ±7.38	65.104 ±9.65
Y maze (% time in hidden arm)	36.502 ±3.08	31.378 ±2.80
Cued fear conditioning (Freezing % of time)	48.047 ±1.12	53.958 ±7.34
Contextual fear conditioning (Freezing % of time)	51.614 ±6.51	48.946 ±9.12

Table 2

Top20 genes downregulated in Notch1 cKO mice with maximal electroconvulsive shock versus wild type animals

Basal level of gene expression alteration is presented as linear fold change (Notch1 cKO no-MECS vs. WT no-MECS). Activity-dependent gene expression change is presented as linear fold change (Notch1 cKO MECS vs. WT MECS). Consensus binding sites of RBP-J are shown in base pairs from the transcription start site.

Gene Symbol	Gene AccID	Encoding protein	Fold-change (Notch1 cKO no-MECS vs. WT no-MECS)	Fold-Change (Notch1 cKO MECS vs. WT MECS)	RBP-J consensus binding sites (bp)
Lcn2	NM_008491	Lipocalin2	−1.12668	−2.52766	>±1000
Kcnj13	NM_001110227	Potassium inwardly-rectifying channel, subfamily J, member 13	−1.62993	−2.37681	>±1000
Ogn	NM_008760	Osteoglycin	−1.62952	−2.28353	N/A
Mir154	NR_029564	microRNA 154	−1.53989	−2.26671	N/A
Slc6a13	**NM_144512**	**Solute carrier family 6 (neurotransmitter transporter, GABA), member 13**	**−1.1407**	**−1.93592**	**−316**
Slc6a20a	NM_139142	Solute carrier family 6 (neurotransmitter transporter), member 20A	−1.06303	−1.86773	>±1000
Slc13a4	NM_172892	Solute carrier family 13 (sodium/sulfate symporters), member 4	−1.24126	−1.84336	>±1000
2210404J11Rik	NM_001039552	RIKEN cDNA 2210404J11 gene	−1.00588	−1.70939	−353
Slc6a12	**NM_133661**	**Solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12**	**−1.10124**	**−1.65906**	**22**
Lbp	NM_008489	Lipopolysaccharide binding protein	−1.01292	−1.63234	>±1000
Islr	NM_012043	Immunoglobulin superfamily containing leucine-rich repeat	−1.2618	−1.5728	>±1000
Slc22a8	NM_031194	Solute carrier family 22 (organic anion transporter), member 8	−1.08799	−1.55831	N/A
Mir218-1	NR_029798	microRNA 218-1	−1.0948	−1.5362	−221
Neurod4	NM_007501	Neurogenic differentiation 4	−1.0017	−1.52827	261, 405
Itih2	NM_010582	Inter-alpha trypsin inhibitor, heavy chain 2	−1.00794	−1.52741	>±1000
Anpep	NM_008486	Alanyl (membrane) aminopeptidase	−1.01986	−1.51364	57, 80
Aebp1	NM_009636	AE binding protein 1	−1.10125	−1.50587	157
Itga1	NM_001033228	Integrin alpha 1	−1.18954	−1.48838	>±1000
Ctla2a	NM_007796	Cytotoxic T lymphocyte-associated protein 2 alpha	−1.2903	−1.48513	>±1000
Ighg	X70423	Immunoglobulin heavy chain (gamma polypeptide)	−1.19948	−1.48367	>±1000

Table 3

Top20 genes up regulated in Notch1 cKO mice with maximal electroconvulsive shock versus wild type animals

Basal level of gene expression alteration is presented as linear fold change (Notch1 cKO no-MECS vs. WT no-MECS). Activity-dependent gene expression change is presented as linear fold change (Notch1 cKO MECS vs. WT MECS). Consensus binding sites of RBP-J are shown in base pairs from the transcription start site.

Gene Symbol	Gene AccID	Encoding protein	Fold-change (Notch1 cKO no-MECS vs. WT no-MECS)	Fold-Change (Notch1 cKO MECS vs. WT MECS)	RBP-J consensus binding sites (bp)
Hspa8	NM_031165	Heat shock protein 8	1.64741	1.78559	348
Morn2	NM_194269	MORN repeat containing 2	1.05556	1.59685	N/A
Ankrd57	NM_172939	Ankyrin repeat domain 57	1.18962	1.59162	N/A
Rpl5	NM_016980	Ribosomal protein L5	1.0494	1.5163	>±1000
Gzmk	NM_008196	Granzyme K	1.03068	1.50309	>±1000
Gm5070	XR_031134	Predicted gene 5070	1.07627	1.48504	N/A
Med4	NM_026119	Mediator of RNA polymerase II transcription, subunit 4 homolog (yeast)	1.07761	1.43763	>±1000
Gm5921	ENSMUST00000075898	Predicted gene 5921	1.15013	1.4217	>±1000
Mrpl48	NM_198831	Mitochondrial ribosomal protein L48	1.03269	1.4018	>±1000
Rpl10l	NM_001162933	Ribosomal protein L10-like	1.04853	1.38322	N/A
Tdrd3	ENSMUST00000022596	Tudor domain containing 3	1.09396	1.37306	N/A
Gm5210	XR_031132	Glyceraldehyde-3-phosphate dehydrogenase pseudogene	1.01459	1.36809	N/A
Chmp1b	NM_024190	Chromatin modifying protein 1B	1.03001	1.36413	−779, 667
Tmem97	NM_133706	Transmembrane protein 97	1.00046	1.35992	>±1000
Rps6	NM_009096	Ribosomal protein S6	1.09455	1.35726	>±1000
Lpl	NM_008509	Lipoprotein lipase	1.03305	1.34676	N/A
Cacybp	NM_009786	Calcyclin binding protein	1.05322	1.34247	N/A
BC061237	BC061237	cDNA sequence BC061237	1.13027	1.33128	N/A
Fcgr3	NM_010188	Fc receptor, IgG, low affinity III	1.14665	1.32268	N/A
Rab5a	NM_025887	RAB5A, member RAS oncogene family	1.03908	1.32214	