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RNA Editing of Serotonin 2C Receptor and Alcohol Intake

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Serotonin 2C receptor (5-HT_{2C}R) belongs to the superfamily of seven transmembrane domain receptors coupled to G proteins (GPCR). It is broadly distributed in the CNS and its expression is relatively high in the limbic system including the amygdala, nucleus accumbens (NAc), hippocampus, and hypothalamus. Based on its expression patterns and numerous pharmacological studies, 5-HT_{2C}R is thought to be involved in various brain functions including emotion, appetite, and motor behavior. Here, we review 5-HT_{2C}R and its relationship with alcohol intake with a particular focus on the involvement of 5-HT_{2C}R mRNA editing and its association with alcohol preference in mice. RNA editing is a post-transcriptional modification mechanism. In mammals, adenosine is converted to inosine by the deamination enzymes ADAR1 and ADAR2. 5-HT_{2C}R is the only GPCR subjected to RNA editing within the coding region. It has five editing sites in exon 5 that encode the second intracellular loop. Consequently, three amino acids residues (I156, N158, and I160) of the unedited receptor (INI) may be altered to differently edited isoforms, resulting in a change of receptor activity such as 5-HT potency and G-protein coupling. 5-HT_{2C}R in the NAc is involved in enhanced alcohol drinking after chronic alcohol exposure and alterations in 5-HT_{2C}R mRNA editing is important in determining the alcohol preference using different strains of mice and genetically modified mice. RNA editing of this receptor may participate in the development of alcoholism.

Keywords: 5-HT_{2C}R, RNA editing, alcohol intake, nucleus accumbens, mice

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INTRODUCTION

The serotonin 2C receptor (5-HT_{2C}R) is a member of the 5-HT receptor family, which is divided into seven groups from 5-HT_1R to 5-HT_7R (Hoyer et al., 1994). 5-HT is produced in neurons located in specific areas of the brainstem that project axons throughout the central nervous system (CNS) (Dahlstroem and Fuxe, 1964). 5-HT acts as a neurotransmitter via receptors and it is involved in the regulation of emotional control, sleep, appetite, and learning. Many studies have reported the roles of 5-HT in psychiatric disorders such as depression and schizophrenia (Mohammad-Zadeh et al., 2008). The seven 5-HT receptors are further divided into at least 14 subgroups (Barnes and Sharp, 1999). In this review article we describe the general aspects of 5-HT_{2C}R, its mRNA editing mechanism, and the relationship between 5-HT_{2C}R and alcohol intake, particularly alterations in 5-HT_{2C}R mRNA editing and alcohol drinking behavior.

5-HT$_{2C}$R

5-HT$_{2C}$R belongs to the family of seven transmembrane G protein coupled receptors (GPCRs). Although it has a similar binding activity to 5-HT in 5-HT$_{1A}$R and 5-HT$_{1B}$R, it forms a family with 5-HT$_{2A}$R and 5-HT$_{2B}$R due to the similarity of their amino acid sequences. 5-HT$_{2C}$R has 57% amino acid identity with 5-HT$_{2A}$R (Hoyer et al., 2002). The *5-HT$_{2C}$R* gene (*HTR2C*) is located on chromosome Xq24 and harbors multiple introns within its coding regions (Milatovich et al., 1992). In addition to splicing variants, *5-HT$_{2C}$R* pre-mRNA undergoes RNA editing at five sites (Fitzgerald et al., 1999; Werry et al., 2008; Chagraoui et al., 2016).

The distribution of 5-HT$_{2C}$R in the brain indicates its role in a variety of functions. Radioautography by tritium labeling, immunohistochemistry and *in situ* hybridization have revealed that 5-HT$_{2C}$R is widely expressed in the CNS but not in the peripheral nervous system (Werry et al., 2008). In the CNS, it is more broadly expressed than 5-HT$_{2A}$R and 5-HT$_{2B}$R. It is strongly expressed in the choroid plexus along the cerebral ventricle (Sanders-Bush and Breeding, 1988), followed by the prefrontal cortex, basal ganglia (caudate nucleus and substantia nigra), and limbic system including the anterior olfactory nucleus, lateral habenular nucleus, hippocampus, amygdala, cingulate cortex, nucleus accumbens, ventral tegmental area (VTA) and hypothalamus in rats (Clemett et al., 2000; Li et al., 2004). In humans, 5-HT$_{2C}$R was reported to be expressed in the cerebral cortex, cerebellum, and substantia nigra (Pasqualetti et al., 1999). Regarding its intracellular localization, 5-HT$_{2C}$R is mainly present in the post-synaptic membrane, but in some brain regions it is expressed in the presynaptic membrane (Becamel et al., 2004). Its distribution in specific brain regions and pharmacological studies using agonists and antagonists of 5-HT$_{2C}$R revealed it has a role in emotion and hypothalamic function. If the physiological functions of this receptor are disturbed, various diseases such as anxiety, depression, addiction, obesity, and epilepsy may develop (Di Giovanni and De Deurwaerdere, 2016; Palacios et al., 2017). Analyses using *HTR2C* gene knockout mice also supports this idea (Tecott et al., 1995; Chou-Green et al., 2003). 5-HT$_{2C}$R couples with Gq/11, Gα12/13, and Gαi and regulates pathways at the second messenger level via inositol-3-phosphate, Ca^{2+}, cAMP, and arachidonic acid. In addition, the activation of cGMP, ERK1/2 and protein kinase C were also reported to act as second messengers (Berg et al., 1994; Werry et al., 2008). These findings support the idea that a disturbance in one or more of these pathways may cause the development of diseases related to 5-HT$_{2C}$R. Therefore, drug design studies have targeted this receptor. However, this is not simple because 5-HT$_{2C}$R has constitutive activity in the absence of ligand binding and amino acid changes occur due to mRNA editing and alternative splicing. It was recently reported that the truncated isoform of 5-HT$_{2C}$R generated by alternative slicing heterodimerizes with full length 5-HT$_{2C}$R intracellularly to decrease receptor signaling (Martin et al., 2013; Zhang et al., 2016; Stamm et al., 2017). It was reported that 5-HT2CR also dimerizes with other receptors, which impacts subsequent signaling (Schellekens et al., 2013, 2015).

RNA EDITING of 5-HT$_{2C}$R mRNA

A variety of gene products occurs by post-transcriptional modification even in the same genome. Most well-known alternative splicing occurs in more than 70% of mammalian genes (Maas et al., 2006). Another modification is mRNA editing. In vertebrates, adenosine of RNA is deaminated to inosine by adenosine deaminase enzymes acting on RNA (ADAR). Inosine is regarded as guanosine when RNA is transcribed because of its structural similarity. ADARs specifically catalyze double strand RNAs. To date, ADAR1, ADAR2, and ADAR3 have been identified, whereas target RNAs of ADAR3 have not been found (Nishikura, 2010). RNA is an energetically unstable molecule, thus it is considered that RNA is edited in order to respond rapidly to a change in the surrounding environment (Tohda, 2014). Most RNA editing occurs in 3′ or 5′ untranslated regions and this regulates gene expression. Less than 30 genes undergo mRNA editing within coding regions (Nishikura, 2010). Human ENCODE RNA-seq data indicate that only 123 editing sites are present in protein-coding sequences (Park et al., 2012). In these cases, a different isoform can be produced after RNA editing. The majority of genes that undergo mRNA editing within exons are ion channels or receptors of neurotransmitters. Among them, two neurotransmitter receptors in the CNS, GluR2/GluA2, a subunit of the AMPA type glutamatergic receptor and 5-HT$_{2C}$R, a GPCR, have been intensively analyzed. GluR2/GluA2, which regulates Ca^{2+} influx into the cell, undergoes editing at two sites. Glutamine (Q) is substituted to arginine (R) by ADAR2 at the Q/R site and arginine is substituted to glycine (G) by ADAR1 and ADAR2 at the R/G site. Usually the Q/R site of GluR2 is edited 100% to inhibit the Ca^{2+} influx; however, when the editing frequency is decreased, the permeability of Ca^{2+} into the cell is increased causing neuronal cell death. A decrease of RNA editing at the Q/R site of GluR2 in motor neurons in the anterior horn of the spinal cord was suggested to cause amyotrophic lateral sclerosis (Kwak et al., 2010).

Regarding 5-HT$_{2C}$R, adenosine to inosine editing can occur at five sites (A–E) in exon 5, which encodes a second intracellular loop. The A and B sites are catalyzed by ADAR1 and the D site is catalyzed by ADAR2 (**Figure 1A**). The C and E sites are edited by ADAR1 and 2. The second intracellular loop is an important region for coupling to G proteins, which affects downstream signaling cascades. The presence or absence of editing at each of the five sites results in changes in three amino acid sequences at 156 (isoleucine, I), 158 (asparagine, N), and 160 (isoleucine, I) (**Figure 1B**). When mRNA editing occurs at A and B sites of the 156 non-edited isoform, isoleucine may change to valine (V) or methionine (M). At C and E sites, 158 asparagine may change to aspartic acid (D), serine (S), or glycine (G). At the D site, 160 isoleucine can be substituted to valine (V). If editing happens at all sites, a VGV type isoform is generated. Thus, from the non-edited INI isoform, 24 isoforms can be produced theoretically (**Figure 1C**) (Wang et al., 2000; Werry et al., 2008). 5-HT$_{2C}$R has its own constitutive activity in the absence of ligand binding. The unedited isoform INI has the highest constitutive activity, which is downregulated in edited isoforms (Herrick-Davis et al., 1999; Niswender et al., 1999). Moreover, the sensitivity and binding

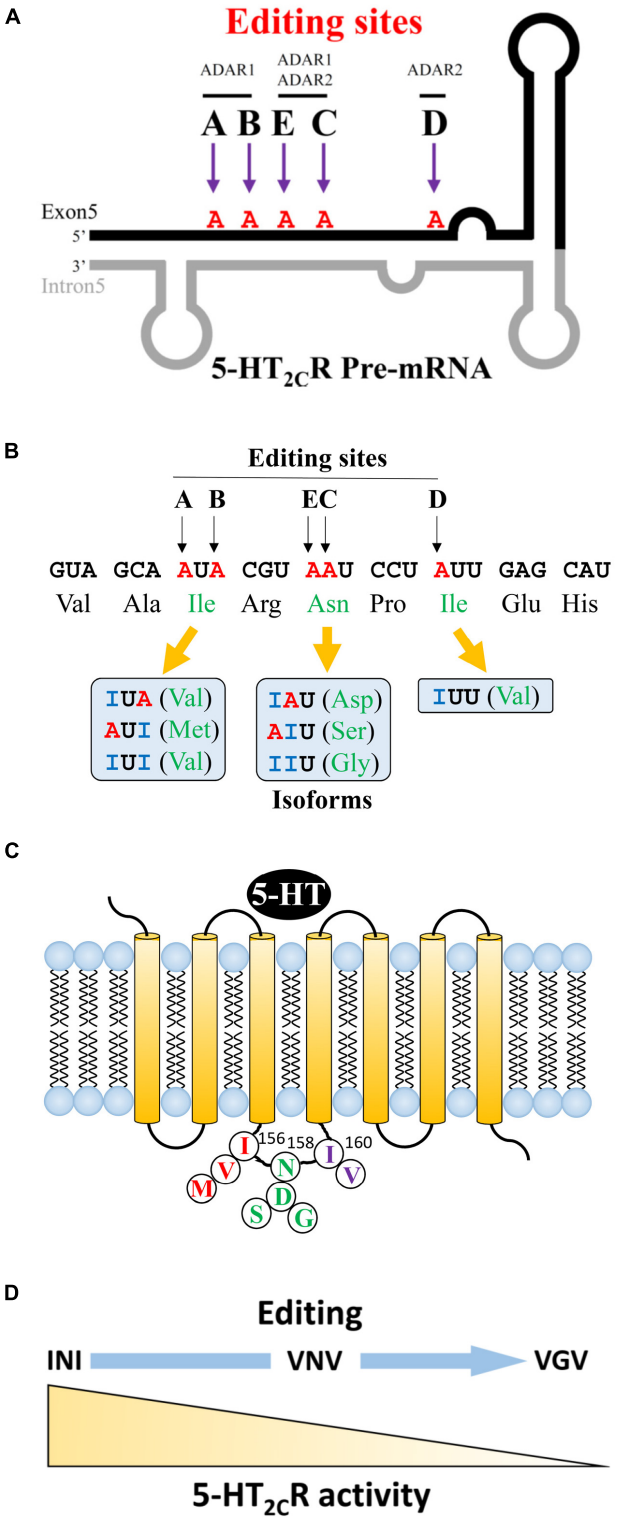

FIGURE 1 | Editing sites at 5-HT$_{2C}$R pre-mRNA and various amino acid sequences. **(A)** Five sites (A to E) in exon 5 of 5-HT$_{2C}$R mRNA. Adenosine to Inosine (A to I) RNA editing occurs in the region where exon 5 makes a double strand with intron 5. Editing enzyme ADAR1 acts on A and B sites, whereas ADAR2 acts on D sites. E and C sites are edited by both ADAR1 and ADAR2. *(Continued)*

FIGURE 1 | Continued
(B) The structure of 5-HT$_{2C}$R has seven transmembrane regions. Three amino acids (156, 158, 160) in the second intracellular loop may be edited by RNA editing. **(C)** The variation of edited isoforms. From A and B editing sites, non-edited isoleucine (Ile) can be edited to valine (Val) or methionine (Met). Asparagine (An) in E and C sites may be edited to aspartic acid (Asp), serine (Ser), or glycine (Gly). Amino acid isoleucine at the D site may be edited to valine (Val). **(D)** 5-HT$_{2C}$R activity is reduced in relation to an increase in RNA editing.

affinity to 5-HT varies dependent on the specific isoform (Burns et al., 1997; Fitzgerald et al., 1999; Berg et al., 2001; Gurevich et al., 2002). We previously examined the activity of each isoform *in vitro* by measuring the constitutive activity and inositol phosphate productivity after 5-HT stimulation. We observed that the non-edited INI isoform had the highest activities and that the all-edited VGV isoform had the lowest activities (**Figure 1D**) (Watanabe et al., 2014). Regarding the amount of each isoform, differences among species and brain regions were reported. The most abundant isoform in the whole brain was the VSV isoform in humans and the VNV isoform in rats. In humans, VSV is the major isoform in the thalamus, hypothalamus, and amygdala; however, ISV and VSI are the major isoforms in the cerebellum and hippocampus, respectively (Wang et al., 2000). Recently, it was reported that VSV was the predominant isoform in many regions except INI in the cerebellum of the porcine brain (Larsen et al., 2016). We examined the isoforms in three mouse strains: VNV was the most abundant in the nucleus accumbens (NAc), dorsal raphe nucleus (DRN) and amygdala (Hackler et al., 2006). The second most common isoform was VSV in the NAc and VNI in the DRN suggesting regional differences (Wang et al., 2000).

Taken together, these findings suggest that 5-HT$_{2C}$R editing may be involved in emotional and psychiatric disorders. Patients with these disorders might have an altered frequency of 5-HT$_{2C}$R RNA editing. Indeed, many studies have reported that 5-HT$_{2C}$R RNA editing was altered in patients with major depression or in suicide victims (Niswender et al., 2001; Gurevich et al., 2002; Iwamoto and Kato, 2003; Dracheva et al., 2008; Simmons et al., 2010; Di Narzo et al., 2014; Weissmann et al., 2016). In schizophrenia patients, 5-HT$_{2C}$R RNA editing was reduced in the frontal cortex (Sodhi et al., 2001). Anxiety and stress conditions also may be related to 5-HT$_{2C}$R RNA editing (Englander et al., 2005; Mombereau et al., 2010; Bombail et al., 2014). In addition to mental distress, editing changes in 5-HT$_{2C}$R mRNA were reported to be involved in obesity, and spinal cord and peripheral nerve injury (Morabito et al., 2010; Schellekens et al., 2012; Uchida et al., 2017).

Recently, the gut microbiota was reported to influence 5-HT$_{2C}$R mRNA editing levels during development in mouse brain (van de Wouw et al., 2019). Early life stress related to maternal separation induced increased depression-like behavior and 5-HT$_{2C}$R RNA editing during mouse adulthood (Bhansali et al., 2007). Moreover, alterations in editing might occur trans-generationally. It was reported that chronic unpredictable stress in pre-reproductive female rats affected the 5-HT$_{2C}$R RNA editing in two generations of offspring (Zaidan et al., 2018). Maternal treatment with a serotonin-specific reuptake

inhibitor (SSRI), fluoxetine, after stress reversed the effect of these editing changes in the prefrontal cortex and amygdala of new born offspring (Zaidan and Gaisler-Salomon, 2015). Collectively, environmental conditions that affect the editing of 5-HT$_{2C}$R mRNA with its receptor function might be therapeutic targets of disease.

ALCOHOL DRINKING BEHAVIOR AND 5-HT$_{2C}$R

It is generally thought that alcohol is consumed for its positive reinforcing effects and that chronic exposure to alcohol results in adaptations with abnormal drinking patterns. The mesolimbic dopaminergic projections from the VTA to the NAc in the midbrain have been implicated in playing an essential role in the brain reward system (Engel and Jerlhag, 2014). Dopaminergic dysfunction in the NAc caused by chronic alcohol consumption is involved in alcoholism (Heinz, 2002). One of the modulating factors of this VTA-NAc dopaminergic system is 5-HT from neurons of the DRN (Yoshimoto and McBride, 1992). 5-HT stimulates the alcohol-induced excitation of VTA neurons (Brodie et al., 1995). Chronic alcohol exposure affects serotonergic synaptic transmission and causes adaptive changes in its receptors. 5-HT$_{2C}$R appears to undergo such adaptive changes (Pandey et al., 1995; Lovinger, 1997). Treatment of the NAc with a 5-HT$_{2C}$R antagonist inhibited alcohol-induced behavioral sensitization in mice (Andrade et al., 2011). We previously reported that among 5-HT receptors, 5-HT$_{2C}$R in the NAc was involved in increased alcohol drinking behavior of C57BL/6J mice after chronic alcohol exposure (Yoshimoto et al., 2012). We developed a chronic alcohol exposure animal model via the inhalation of vapored ethanol. After chronic exposure to alcohol, mice had a higher alcohol intake compared with control animals, whereas their water consumption was similar to that of the control group. These mice had an enhanced expression of 5-HT$_{2C}$R at the mRNA and protein levels in the NAc. The expression of 5-HT$_7$R mRNA in the NAc was also increased; however, only systemic treatment with a specific 5-HT$_{2C}$R antagonist or intra NAc treatment inhibited the enhanced alcohol intake after chronic alcohol exposure (Yoshimoto et al., 2012).

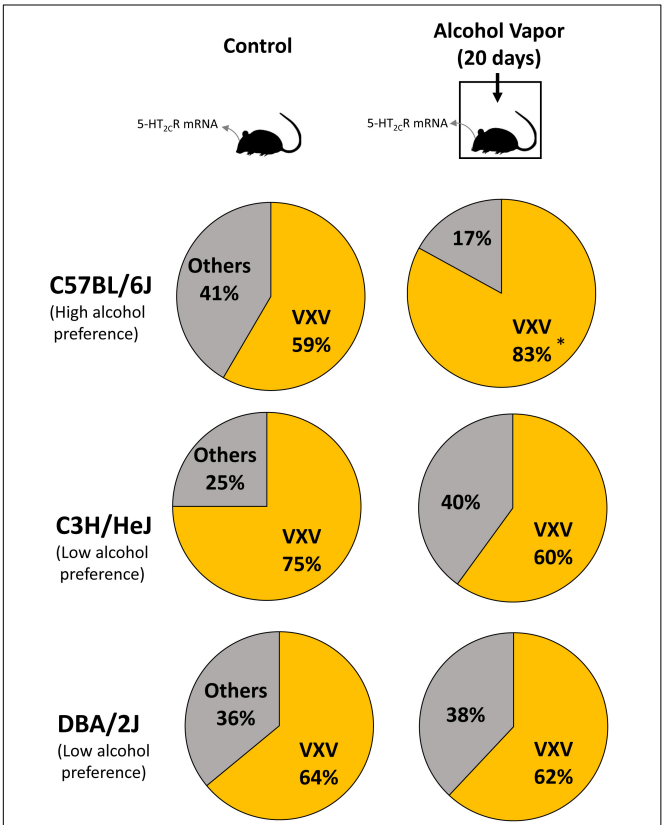

FIGURE 2 | Mice were intermittently exposed to alcohol by inhalation for a period of 20 days. Frequencies of 5-HT$_{2C}$R mRNA editing in control **(left)** and chronic alcohol exposed mice **(right)** were measured by the cloning–sequencing analysis of RT-PCR products from the NAc. Pie charts of the ratio of edited isoforms, V-X-V (at least two amino acids are edited to valine) in three strains of mice after chronic alcohol exposure. C57BL/6J mice, but not C3H/HeJ and DBA/2J mice, had increased VXV type isoforms after alcohol intake. Statistical analyses were performed using Fisher's exact test. Two-sided tests were used to calculate *P*-values. *$P < 0.01$.

Previous studies reported differences in alcohol preference among mouse inbred strains. C57BL/6J, but not C3H/HeJ and DBA/2J mice, drank more alcohol after alcohol exposure compared with controls (Yoshimoto and Komura, 1989). The expression of 5-HT$_{2C}$R mRNA was increased in the NAc of C57BL/6J mice but in C3H/HeJ or DBA/2J mice. As 5-HT$_{2C}$R is subjected to pre mRNA editing, we examined the editing frequency of 5-HT$_{2C}$R mRNA. In C57BL/6J mice, edited isoforms of 5-HT$_{2C}$R were increased in the NAc but not the hippocampus. Particularly, VXV isoforms such as VGV, VNV, VSV, and VDV in which the first (156) and third (160) of three replaceable amino acids were edited to valine, were increased in C57BL/6J mice after chronic alcohol exposure; however, these increases were not observed in C3H/HeJ or DBA/2J mice (**Figure 2** and **Table 1**). The editing enzymes ADAR1 and ADAR2 were increased in the NAc of C57BL/6J mice after chronic

TABLE 1 | Frequencies of 5-HT$_{2C}$R isoforms in C57BL/6J mice.

Isoforms	NAc of C57BL/6J			
	Control		Alcohol	
I-N-I	3%		3%	
I-S-I	0%		0%	
I-N-V	5%		0%	
I-G-V	0%		0%	
V-G-I	2%		2%	
V-N-I	21%		7%	
V-S-I	10%		5%	
V-D-I	0%		0%	
V-G-V	2%	V-X-V	2%	V-X-V
V-N-V	40%	59%	62% $P < 0.05$	83%
V-S-V	16%		10%	$P < 0.01$
V-D-V	2%		9%	

Statistical analyses were performed by Fisher's exact test. Two-sided tests were used to calculate P-values. Reproduced partly with permission by Oxford University Press (Watanabe et al., 2014).

alcohol exposure but not in C3H/HeJ or DBA/2J mice. Taken together, C57BL/6J mice showed enhanced alcohol intake after chronic alcohol exposure related to the increased RNA editing of 5-HT$_{2C}$R; however, this was not observed in C3H/HeJ or DBA/2J mice that did not show enhanced alcohol intake. From this result, alterations in the RNA editing of 5-HT$_{2C}$R may underlie alcohol preference. Next, we examined mice that exclusively expressed the non-edited INI isoform of 5-HT$_{2C}$R (Kawahara et al., 2008) and compared them with wild type littermates on the C57BL/6J background. ADARs recognize double-stranded RNA. Exon 5 of the 5-HT$_{2C}$R mRNA consists of an imperfect double-stranded RNA with intron 5. Intron 5 was deleted in INI knock-in mice to prevent editing by ADARs at five sites in exon 5. INI mice had a similar phenotype of food intake, water intake and weight gain as wild type mice. We examined alcohol consumption in INI and wild type mice after chronic alcohol exposure and observed that wild type mice had an increase in alcohol intake; however, INI mice had a similar level of alcohol intake to the controls even on the C57BL/6J background. This result indicates that the editing of 5-HT$_{2C}$R mRNA underlies the increase in alcohol consumption after chronic alcohol exposure in mice. The importance of RNA editing in alcohol preference was confirmed using non-changing RNA editing (INI) mice. The constitutive activity of 5-HT$_{2C}$R inhibits accumbal dopamine release (De Deurwaerdere et al., 2004; Di Matteo et al., 2004). 5-HT$_{2C}$R in the NAc is expressed in GABAergic neurons as well as in the VTA, DRN, and medial prefrontal cortex (Bubar et al., 2011; Spoida et al., 2014; Nocjar et al., 2015; Aoki et al., 2016). It was reported that the GABA neuronal system was also involved in alcohol reward and dependence (Koob et al., 1998). Increased edited isoforms of 5-HT$_{2C}$R with low signaling induced by chronic alcohol exposure may enhance dopamine release by modulating GABAergic neurons in the NAc. Consequently, mice may develop increased alcohol consumption. Although the mesolimbic dopamine system is modulated by 5-HT$_{2C}$R (Di Giovanni and De Deurwaerdere, 2016; De Deurwaerdere and Di Giovanni, 2017) and its RNA editing seems to affect the addiction to drugs, few studies have investigated the relationship between them. Cocaine administration to the rat cerebral cortex for 7 days did not alter the RNA editing of 5-HT$_{2C}$R in the cerebral cortex, hippocampus or midbrain (Iwamoto and Kato, 2002). Nicotine withdrawal reduced editing at the E site in the hippocampus of rats (Zaniewska et al., 2015). Further studies are necessary to reveal the role of RNA editing of 5-HT$_{2C}$R in drug addiction in the future.

Among each of the editing sites (A–E) of 5-HT$_{2C}$R in the NAc, the editing frequency at the D site, an ADAR2 specific site, was significantly increased in C57BL/6J mice after chronic alcohol exposure. ADAR2 expression was enhanced as well as ADAR1 in the NAc (Watanabe et al., 2014). Regarding RNA editing in the NAc, GluA2 RNA editing at the Q/R site in the NAc was reduced by forced cocaine abstinence, and ADAR2 overexpression in the NAc attenuated cocaine-seeking behavior (Schmidt et al., 2015). We examined the involvement of RNA editing in alcohol drinking by deleting ADAR2 in the NAc using conditional ADAR2 knockout mice (ADAR2$^{flox/flox}$) on a C57BL/6J genetic background (Hideyama et al., 2010). Adeno-associated virus (AAV)-green fluorescent protein (GFP)/Cre into the NAc of ADAR2$^{flox/flox}$ mice was used to specifically delete the ADAR2 gene. Accumbal RNA editing frequency in the ADAR2-dependent editing sites of GluA2 Q/R, 5-HT$_{2C}$R site D and CYFIP2 K/E, was significantly reduced (Shirahase et al., 2018). In contrast to wild type mice, ADAR2 KO mice did not develop enhanced ethanol intake or ethanol preference after chronic exposure to ethanol vapor (Shirahase et al., 2018). ADAR2 mediates the RNA editing of various ion channels and receptors such as the Ca$_V$1.3 calcium ion channel, K$_V$1.1 potassium ion channel, 5-HT$_{2C}$R, GluA2, and GABA$_A$ (Bhalla et al., 2004; Bazzazi et al., 2013; Behm and Ohman, 2016). Therefore, other receptors as well as 5-HT$_{2C}$R may be involved in the alcohol drinking behavior of this model by a NAc-specific reduction of ADAR2 expression. Increased cortical expression of ADAR2 and 5-HT$_{2C}$R mRNA editing was reported in major depressive suicide victims (Simmons et al., 2010). ADAR2 is highly expressed in the brain and its degradation is regulated by E3 ubiquitin ligase WWP2 (Marcucci et al., 2011; Gallo et al., 2017). Therefore, control of the ADAR2 level in the NAc might be a target for the development of treatment for alcoholism.

CONCLUSION

We reviewed the general features of 5-HT$_{2C}$R and its mRNA editing with specific reference to alcohol preference. The accumbal expression and mRNA editing of 5-HT$_{2C}$R is involved in alcohol intake in mice and this mechanism may be also relevant to human alcoholism. The regulation of 5-HT$_{2C}$R RNA editing might be a new therapeutic strategy for alcohol addiction.

AUTHOR CONTRIBUTIONS

MT conceived the review and wrote the manuscript. YW prepared figures and table.

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