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Functional relevance of serotonin 2C receptor mRNA editing in antidepressant- and anxiety-like behaviors

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Abstract

Serotonin 2C receptors (5-HT_{2C}R) have been shown to undergo post-transcriptional RNA editing. This modification affects the affinity, coupling and constitutive activity of the receptor. *In vivo*, manipulations such as stress or antidepressant administration dramatically modify the pattern of 5-HT_{2C}R mRNA editing, suggesting that this phenomenon might be involved in the pathophysiology of stress-related disorders. Indeed, alterations of 5-HT_{2C}R mRNA editing have been observed in depressed patients. Thus, the recent development of mice expressing either the non-edited (5-HT_{2C}R-INI) or the fully-edited form of 5-HT_{2C}R (5-HT_{2C}R - VGV) provides a novel opportunity to investigate the relevance of this phenomenon in the context of stress-related disorders. We observed that both 5-HT_{2C}R-INI and 5-HT_{2C}R-VGV mice exhibit exaggerated anxiety-like behaviors in the elevated plus maze paradigm. This phenotype was observed when the INI or VGV mutations were present in mice on a BALB/c background, as well as non-significant trends in the same direction in mice on a C57BL/6J background. In animal models of antidepressant-like activity, the absence of editing of 5-HT_{2C}R mRNA (5-HT_{2C}R-INI) induced an increase in the time spent immobile in the forced-swim test (FST) and tail suspension test (TST). Complete editing of 5-HT2C receptor mRNA (5-HT_{2C}R-VGV) induced antidepressant-like behavior in the FST and TST, as reflected by a significant decrease in time spent immobile. These phenotypes were unrelated to alterations in locomotor activity in both 5-HT_{2C}R-INI and -VGV. In the TST, these phenotypes were accompanied by a decrease and an increase in response to desipramine in 5-HT_{2C}R-INI and -VGV, respectively. These data constitute the first *in vivo* demonstration of a role for 5-HT_{2C}R mRNA editing in anxiety- and depression-related behaviors.

Keywords

RNA editing; 5-HT_{2C} receptor; anxiety; antidepressant; transgenic mice

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1. Introduction

Human and rodent serotonin 2C receptors (5-HT_{2C}Rs) undergo post-transcriptional RNA editing. Five closely spaced adenosines, located within a sequence encoding the second intracellular loop, are converted to inosine to alter the coding potential of three triplet codons (Burns et al., 1997). Through the action of specific deaminase enzymes (ADAR), this post-transcriptional modification alters agonist binding affinity, G-protein coupling and constitutive activity of the receptor (Werry et al., 2008). Thus, editing of 5-HT_{2C}Rs represents a functional adaptation of the serotoninergic system and consequently may play a role in the etiology of stress-related disorders.

Dysregulation in 5-HT_{2C}R mRNA editing has been observed in the frontal cortex of depressed suicide victims (Gurevich et al., 2002; Niswender et al., 2001). Specifically, in suicide victims with a history of major depression, C′ site editing was significantly increased while D site editing was significantly decreased, and these changes could be reversed by fluoxetine treatment (Niswender et. al., 2001). Preclinically, C57BL/6J and BALB/c, two inbred mouse strains, exhibit differences in basal editing rates of 5-HT_{2C}R in the prefrontal cortex (Englander et al., 2005). Further, exposure to certain types of stress during development or adulthood increases 5-HT_{2C}R mRNA editing in the prefrontal cortex of BALB/c mice, and this alteration can be counteracted by antidepressant treatment (Bhansali et al., 2007). These data confirm a potential role of 5-HT_{2C}R mRNA editing in the susceptibility to stress and antidepressant response. Of interest, BALB/c mice demonstrate higher levels of baseline anxiety-like behaviors compared to C57BL/6J mice (Griebel, et. al., 2000; Lepicard et. al., 2000), which may be a reflection of differences in 5-HT_{2C}R mRNA editing seen between the two strains.

Recently, mice expressing either the non-edited (5-HT_{2C}R-INI) or the completely-edited form (5-HT_{2C}R –VGV) of the 5-HT_{2C} receptor were generated (Kawahara et al., 2008). Characterizing these mice in behavioral paradigms that evaluate anxiety- and antidepressant-like behavior will enable us to evaluate the functional significance of receptor mRNA editing in the context of anxiety disorders and depression.

2. Materials and Methods

2.1 Animals

5-HT_{2C}R-VGV and 5-HT_{2C}R-INI expressing mice were generated and backcrossed for at least 10 generations into both C57BL/6J and BALB/c genetic backgrounds (Kawahara et al., 2008). Mice were matched for both age (3 to 8 months) as well as sex (both M and F) across groups. There was no effect of sex on behaviors observed. Mice were group-housed with food and water available *ad libitum*. All animals were housed in a temperature- and humidity-controlled animal care facility with a 12 hr light/dark cycle (lights on a 6:00 A.M.). All procedures were approved by the Institutional Care and Use Committee of the Wistar Institute.

2.2 Elevated-plus maze (EPM)

Animals are placed in the center of a maze that consists of two perpendicular, intersecting runways, each 7.6 cm wide and 60 cm long. One runway has 15-cm-tall black walls (closed), while the other has no walls except for a 1-cm-tall lip at the edge (open). The runways are positioned 30 cm above the floor on a pedestal. Behavior of mice is scored by an observer blinded to genotype. Mice are scored on several anxiety-related variables such as time spent and numbers of entries in open arms, as well as latency to enter open arms. In addition, ethologically-relevant behaviors such as stretch-and-attend posture, rearings, grooming, and head dips were quantified in both open and closed arms.

2.3 Locomotor activity

Animals were placed in a new cage for 30 minutes after injection with desipramine or vehicle, and the distance traveled was measured using video tracking, performed by the Noldus Ethovision program (Noldus Information Technology, Leesburg, VA).

2.4 Forced Swim Test (FST)

Mice were placed individually into plexiglass cylinders (23 cm tall and 14 cm in diameter) filled with water (22–24 degrees C) to a depth of 15 cm. All test sessions were recorded by a video camera positioned directly above the cylinders. Total time spent immobile (making only slight movements necessary to remain afloat vs. active escape-oriented behaviors such as swimming or climbing) during the last four minutes of the six-minute test period was quantified by a blinded observer.

2.5 Tail Suspension Test (TST)

Mice were individually suspended by the tail from a metal rod (35 cm above the floor) using adhesive tape. Mice demonstrated several escape-oriented behaviors interspersed with temporally increasing bouts of immobility. Duration of immobility during the entire six-minute test period was scored from videotapes by a blinded observer.

2.6 Drugs

Desipramine (DMI) was obtained from Sigma (St. Louis, MO) and was dissolved in 0.9% saline immediately prior to use. Drugs were administered intraperitoneally by injection.

2.7 Statistics

All data were analyzed using Student's *t*-test (EZM) or a two-way analysis of variance (ANOVA), followed by Bonferonni post-hoc tests (locomotor activity, FST and TST, body weights). All statistical analyses are summarized in Tables 1 and 2. The level of significance was set at $p < 0.05$.

3. Results

BALB/c and C57BL/6J mice exhibit different 5-HT_{2C}R editing profiles (Englander et al., 2005). These mice vary in baseline response in anxiety- and stress-related paradigms as well as their response to antidepressant drugs (Griebel, et. al., 2000; Lepicard et. al., 2000). To determine if 5-HT_{2C}R editing contributes to these differences, we assessed anxiety-related behavior using the elevated plus maze (EPM) in both 5-HT_{2C}R-INI and 5-HT_{2C}R-VGV mice in these two genetic backgrounds. As shown in Figure 1, mice expressing only the non-edited form of 5-HT_{2C}Rs (5-HT_{2C}R-INI mice) spent significantly less time in the open arms (Figure 1B), displayed significantly fewer entries into open arms (Figure 1C), as well as significantly fewer head-dips (Figure 1D) and rearings (Figure 1E); the direction of change in all of these behaviors is associated with increased anxiety-like behavior (all statistical analyses can be found in Table 1). Further, latency to enter the open arms was also significantly increased in 5-HT_{2C}R-INI mice (Figure 1A), an additional change associated with increased anxiety-like behavior. Of interest, BALB/c animals harboring the totally edited form of 5-HT_{2C}Rs (5-HT_{2C}R-VGV mice) also showed significantly increased latency to enter the open arms (Figure 1F), significantly decreased entries into and time spent in the open arms (Figures 1 G and H), as well as significantly decreased head dips and rearings (Figures 1 I and J) compared to their wild-type counterparts, again showing increased anxiety-like behavior.

In the C57BL/6J background, we observed behavioral changes in the same direction as the BALB/c mice in both 5-HT_{2C}R-INI (Figure 2A–E) and 5-HT_{2C}R-VGV (Figure 2F–J) mice,

but these alterations were less marked, failing to reach significance except in the case of the ethologically-relevant behaviors (rearings and head-dips), in which there were significant decreases (Figures 2D, I and J). It is important to note that wildtype animals of this genetic background exhibited increased latency to enter the open arms, as well as fewer entries into open arms, less time spent in open arms, and fewer rearings and head dips compared to their wildtype BALB/c counterparts (Figures 1 and 2), indicative of increased anxiety-like behaviors at baseline in the C57BL/6J strain.

As the differences in anxiety-like behavior between transgenic lines were more pronounced in mice on the BALB/c background, we focused our investigation of the role of 5-HT_{2C}R mRNA editing on antidepressant response in the BALB/c strain. Saline-treated 5-HT_{2C}R-INI mice showed a significant increase in the time spent immobile (a pro-depressant effect) in the FST (Figure 3A) but not in the TST (Figure 3B). In contrast, we observed that 5-HT_{2C}R-VGV mice displayed a robust and significant decrease in time spent immobile in both the FST (Figure 3D) and TST Figure 3E), an antidepressant-like phenotype. Desipramine (DMI) decreased immobility in both transgenic lines in both the FST (Figures 3A and D) and TST (Figures 3B and E), the expected antidepressant-like effect. However, the effects of DMI were attenuated in 5-HT_{2C}R-INI mice and potentiated in 5-HT_{2C}R-VGV mice in the TST paradigm (Figure 3B, 3E).

As has been previously reported (Kawahara et al., 2008), the 5-HT_{2C}R-VGV mice used in behavioral experiments weighed significantly less than their wildtype littermates, while body weights of 5-HT_{2C}R-INI mice were comparable to wildtype controls (Table 2). However, we did not observe any significant alterations of locomotor activity in either transgenic line that might confound interpretation of results in the FST or TST (Figure 3C, 3F).

4. Discussion

This study is the first to examine the effects of 5-HT_{2C}R mRNA editing on behaviors related to psychiatric disease. Our results in the EPM indicate that both complete editing of 5-HT_{2C}R mRNA (5-HT_{2C}R –VGV) and lack of editing of the 5-HT_{2C}R mRNA (5-HT_{2C}R –INI) induced an anxiogenic-like effect in the BALB/c strain (Figure 1), and a trend toward increased anxiety-like behavior in the C57BL/6J strain (Figure 2). In the BALB/c strain, complete editing of 5-HT_{2C}R mRNA (5-HT_{2C}R –VGV) elicited an antidepressant-like phenotype in both the FST and TST, and accentuated the effects of DMI in the TST (Figures 3D and E). In contrast, BALB/c mice expressing only the un-edited form of 5-HT_{2C}R mRNA (5-HT_{2C}R –INI) showed a pro-depressant effect in the FST only and an attenuation of the effect of DMI in the TST (Figures 3A and B). The lack of effect in the TST at baseline in the 5-HT_{2C}R –INI mice may be due to a ceiling effect.

We observed a significantly lower average body weight in the 5-HT_{2C}R –VGV mice (Table 2), as has been reported previously (Kawahara et al., 2008). However, this difference in body weight does not appear to affect overall locomotor activity in these mice, which was not significantly different from wildtype (Figure 3F). Although there is a non-significant trend towards reduced locomotor activity in the 5-HT_{2C}R –VGV mice, this does not confound the results of the FST and TST experiments, as these mice showed a *decrease* in immobility in these tests, which is opposite in direction to any potential decrease in general activity levels. Decreased body mass and adiposity might affect behavior specifically in the FST in that leaner mice might be forced to swim more due to an inability to float. However, the 5-HT_{2C}R –VGV mice showed an identical phenotype in the TST, an independent measure of antidepressant response that does not involve swimming or floating behavior and thus should not be affected by body weight or adiposity. Of note, the 5-HT_{2C}R –INI mice did not show alterations in locomotor activity (Figure 3C) or body weight (Table 2) as compared to their wildtype

littermates, removing either of these confounds from interpretation of their behavioral phenotype.

5-HT_{2C}R-null mice were the first genetically-altered mice studied to investigate the role of this receptor in psychiatric disorders. Mice lacking the 5-HT_{2C}R exhibit a dramatic hyperphagia and late-onset obesity (Tecott et al., 1995), but their phenotypes in anxiety- and depression-like paradigms are somewhat ambiguous. 5-HT_{2C}R-null mice have decreases in anxiety-like behavior (Heisler et al., 2007) but also increased obsessive compulsive-like behaviors (Chou-Green et al., 2003). These mice display no baseline difference in depression-related behaviors, but their response to antidepressants is potentiated (Cremers et al., 2004). Here, the results of the EPM indicate that complete editing of 5-HT_{2C}R mRNA induces an anxiogenic-like effect. In the FST and TST, this manipulation elicits an antidepressant-like phenotype and accentuates the effects of desipramine. 5-HT_{2C}R-VGV mice have 5-HT_{2C}R mRNA and protein levels comparable to wild-type mice, but 5-HT_{2C}R binding and 5-HT_{2C}R-mediated neurotransmission are increased in these animals (Kawahara et al., 2008). This observed gain of function might contribute to the behavioral alterations seen here in 5-HT_{2C}R-VGV mice. Indeed, pharmacological studies demonstrate that activation of 5-HT_{2C}Rs induce anxiety-like behaviors in rodents and humans (Griebel, 1995; Millan, 2005). Further, selective 5-HT_{2C}R agonists, such as WAY 163909, have been shown to exert antidepressant-like effects in a range of rodent assays (Cryan and Lucki, 2000; Rosenzweig-Lipson et al., 2007). The present data are consistent with genetic ablation studies where the loss of 5-HT_{2C}Rs induced an anxiolytic-like effect. Thus, complete editing of 5-HT_{2C}R mRNA likely alters basal anxiety-like behaviors and antidepressant-like behavior via an increase in 5-HT_{2C}R function.

Further emphasizing the importance of mRNA editing for behavioral phenotypes, 5-HT_{2C}R-VGV mice share some behavioral alterations with the recently-developed mice overexpressing the RNA editing enzyme ADAR2 (Singh et al., 2007; Singh et al., 2009). Indeed, ADAR2 transgenic animals displayed an increase in basal anxiety-like behaviors, analogous to 5-HT_{2C}R-VGV mice. However, ADAR2 transgenic mice exhibit an increase in immobility in the FST, whereas we observed decreased immobility in the 5-HT_{2C}R-VGV mice. One reason for this discrepancy could be that ADAR2 transgenic mice also show alterations in editing of other mRNAs, such as AMPA glutamate receptor B subunit, α3 subunit of the $GABA_A$ receptor, ADAR2 itself and microRNAs (Higuchi et al., 1993; Nishikura, 2006; Ohlson et al., 2007; Palladino et al., 2000), some of which could contribute to behavioral phenotypes. Moreover, ADAR2 regulates the editing of only the C and D sites (not A and B), suggesting that the overexpression of ADAR2 might result in partially-edited 5-HT_{2C}R mRNA rather than the fully-edited VGV form expressed in the 5-HT_{2C}R-VGV mice (Wang et al., 2004; Yang et al., 2004).

In the present study, we showed that absence of editing in 5-HT_{2C}R-INI mice induced anxiety-like behaviors in the EPM and a pro-depressive phenotype in the FST. At the molecular level, INI receptor isoforms are spontaneously internalized in an agonist-independent manner through a GRK/βarrestin-dependent mechanism (Marion et al., 2004), suggesting that this genetic manipulation might induce a decrease in 5-HT_{2C}R function. However, these assertions can only be confirmed using appropriate *in vivo* studies. Although our data suggest that the absence of editing affects anxiety- and depression-like behavior in mice, further investigation of the functional consequences of lack of mRNA editing on receptor expression, affinity, and activity is necessary.

Previous studies have demonstrated that BALB/c and C57BL/6J exhibit dramatically different 5-HT_{2C}R mRNA editing profiles (Englander et al., 2005). In addition, BALB/c are generally thought to be more anxious-like than C57BL/6J (Griebel et al., 2000; Lepicard et al., 2000)

but see (Trullas and Skolnick, 1993). Thus, our study was designed to evaluate whether these differences in levels of mRNA editing between the strains might be involved in determining their differential behavioral phenotypes. In the present studies, we observed a more pronounced phenotype in the BALB/c background than in C57BL/6J animals. We hypothesize that this is due to the higher baseline level of anxiety-like behaviors in the C57BL/6J that we observed. This baseline difference in anxiety-like behavior is surprising given that most of the literature finds BALB/c mice to be the more anxious-like strain, as mentioned above. Further, it may appear counterintuitive that both genetic manipulations induced a more marked effect in one strain than the other. Indeed, we would anticipate that the VGV manipulation might alter behavior more significantly in the BALB/c strain, which normally exhibits less editing, and that the INI manipulation might induce more significant alterations in the C57BL/6J strain, which exhibits a higher level of editing. Further studies are required to clarify this point and to understand how editing of 5-HT_{2C}R mRNA might participate in the behavioral characteristics of these two strains. It is clear that other genetic factors contribute to the differential phenotypes of these two strains, as differences in behavior are seen between the two strains even when levels of editing are experimentally controlled, as they are in this study.

Thus, the present studies provide the first *in* vivo demonstration of the involvement of mRNA editing of 5-HT_{2C}R mRNA in anxiety- and depression-like behaviors, taking advantage of the recently generated 5-HT_{2C}R-INI and 5-HT_{2C}R-VGV mice. These animals offer a new opportunity to investigate the relevance of 5-HT_{2C}R mRNA editing in the context of neuropsychiatric disorders.

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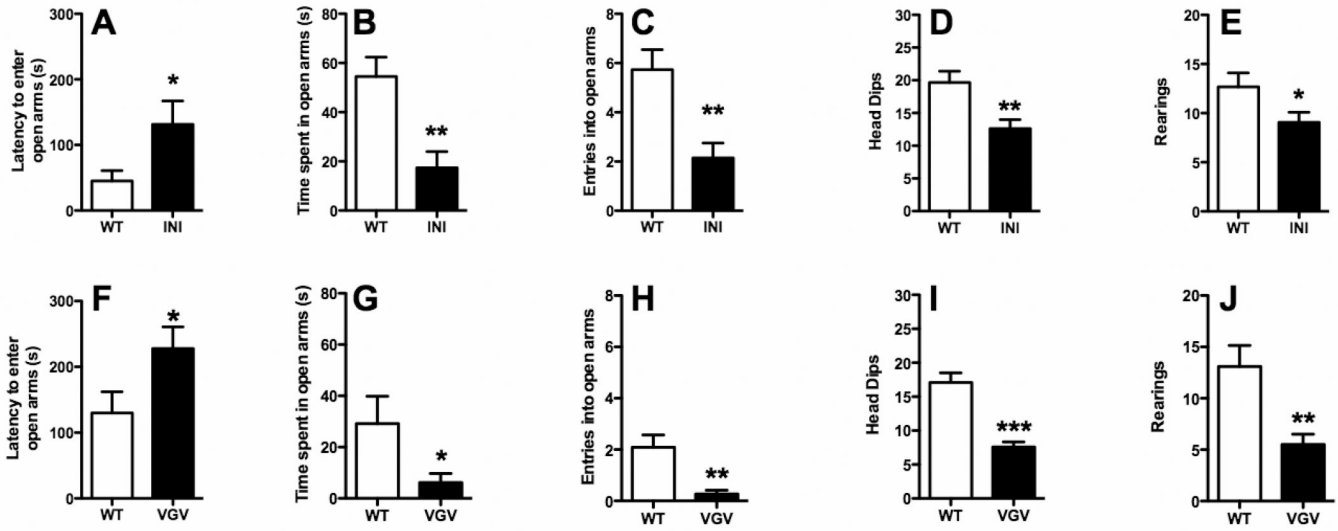

Figure 1. Alterations in 5-HT$_{2C}$R mRNA editing in BALB/c background result in increased anxiety-like behavior in the elevated plus maze

Both 5-HT$_{2C}$R-INI (A–E) and 5-HT$_{2C}$R-VGV mice (F–J) exhibit increases in anxiety-like behaviors, affecting latency to enter in open arms (A, F), time spent in open arms (B, G), the number of entries into open arms(C, H), head-dips (D, I) and rearings (E, J). Values are means +/−SEM. * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ for significant difference from wild-type animals. Full statistical analysis can be found in Table 1.

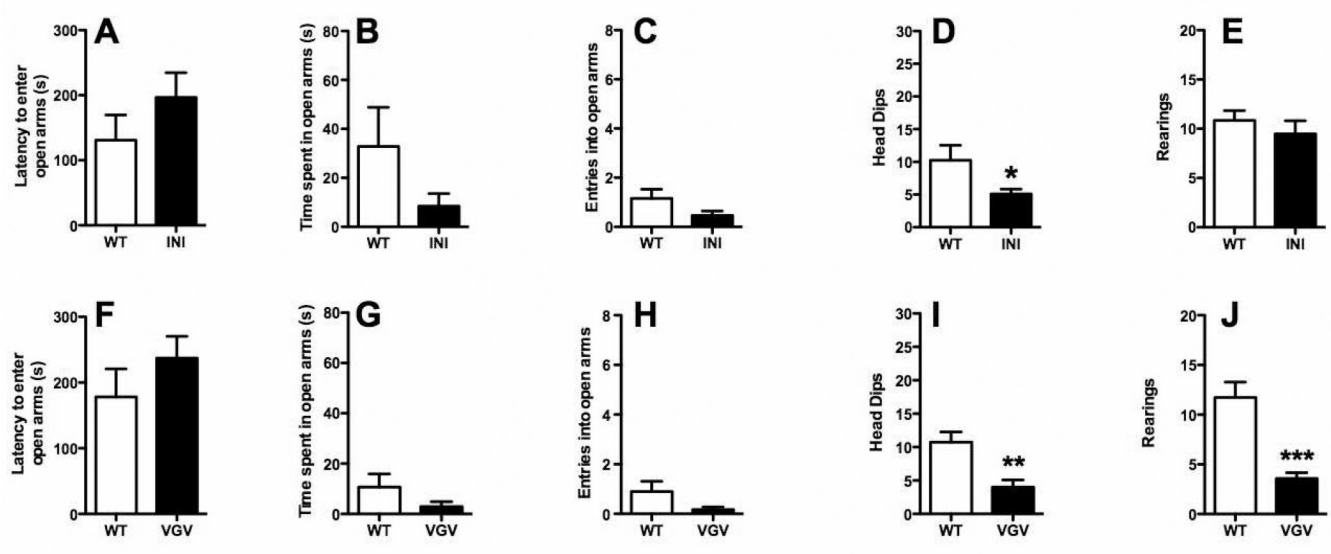

Figure 2. The anxiety-related phenotype of 5HT$_{2C}$R-INI and 5-HT$_{2C}$R-VGV mice is attenuated in C57BL/6J genetic background

Both 5-HT$_{2C}$R-INI (A–E) and 5-HT$_{2C}$R-VGV mice (F–J) exhibit increases in anxiety-like behaviors, with significant decreases in head dips in both transgenic lines (D, I) and significantly decreased rearings in VGV mice (J) as compared to wildtype animals. Latency to enter open arms (A, F), time spent in open arms (B, G), and entries into open arms (C, H) were not significantly changed in INI or VGV mice as compared to wildtype animals. Values are means +/−SEM. * $p < 0.05$, ** $p < 0.001$, and *** $p < 0.001$ for significant difference from wild-type animals. Full statistical analysis can be found in Table 1.

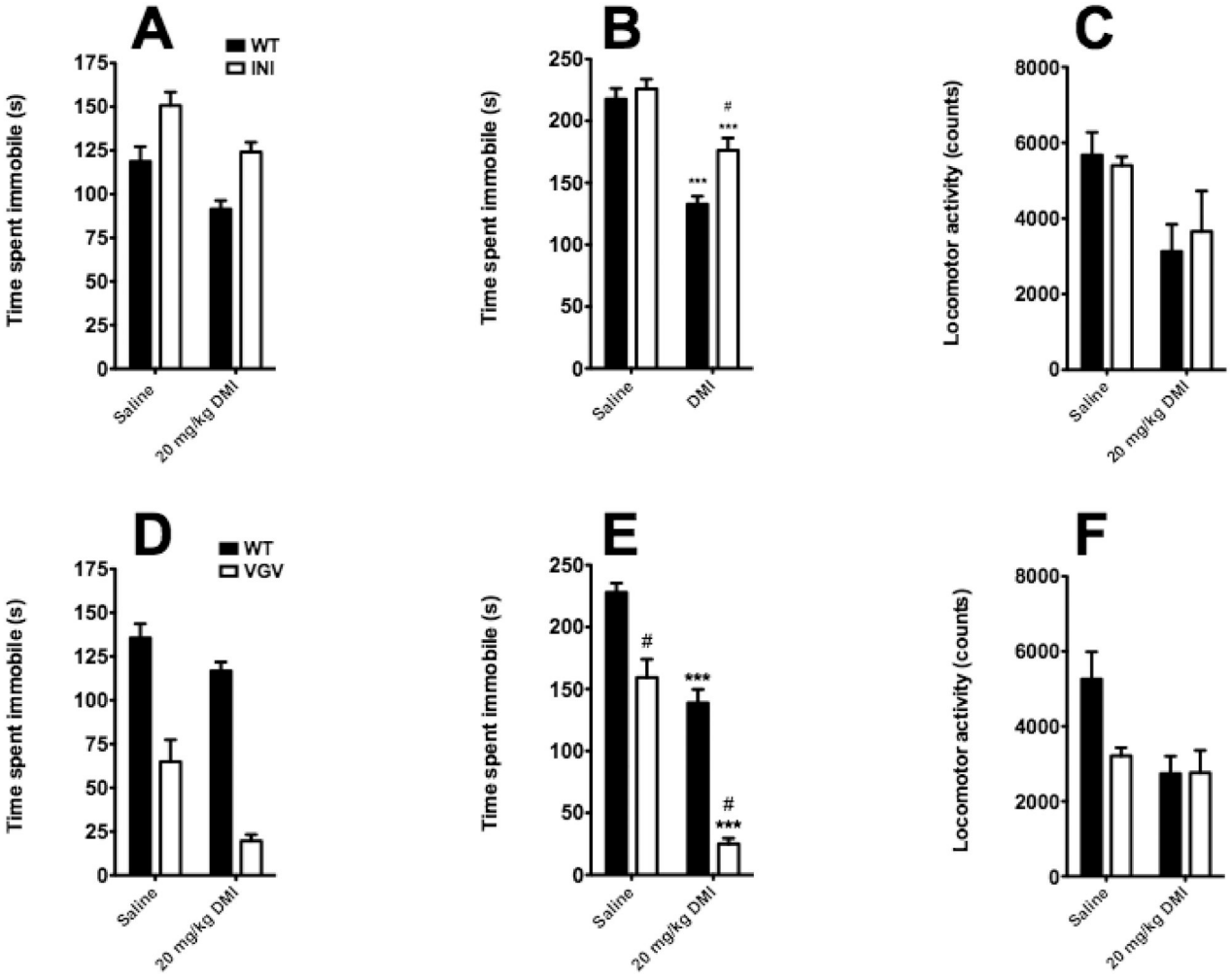

Figure 3. Impact of alterations of 5-HT$_{2C}$R mRNA editing on despair behavior and antidepressant response in FST, TST and locomotor activity

5-HT$_{2C}$R-INI mice exhibit a pro-depressant phenotype in the FST (A) but not in the TST (B). Conversely, 5-HT$_{2C}$R-VGV mice exhibit an antidepressant-like phenotype in both the FST (D) and TST (E). Both lines showed significant decreases in immobility in both tests in response to DMI (A, B, D, and E), and the effect of DMI was potentiated in 5-HT$_{2C}$R-VGV in the TST but not the FST. DMI significantly decreased locomotor activity in both lines (C and F). There was no significant difference in locomotor activity in either line after vehicle injection (C and F), although there was a trend toward a decrease in the VGV mice ($p = 0.09$)(F). Values are means +/−SEM. * $p < 0.05$ and *** $p < 0.001$ for significant difference from respective saline-treated animals. # $p < 0.05$ for significant difference from respective wild-type animals. Full statistical analysis can be found in Table 1.

Table 1

Elevated-plus maze:				
	BALB/C			
	INI		**VGV**	
	***p*=**	***t*=**	***p*=**	***t*=**
Latency to enter in the open arm:	0.0413*	t_{25}=2.152	0.0454*	t_{22}=2.121
Time spent in the open arm:	0.0013**	t_{27}=3.579	0.0457*	t_{21}=2.124
Number of entries in open arm:	0.0016**	t_{27}=3.509	0.0015**	t_{20}=3.664
Number of Head dips:	0.0033**	t_{28}=3.208	<0.0001***	t_{21}=6.028
Number of Rearings:	0.0499*	t_{28}=2.049	0.0027**	t_{21}=3.402
	C57BL6			
	INI		**VGV**	
	***p*=**	***t*=**	***p*=**	***t*=**
Latency to enter in the open arm:	0.2367	t_{24}=1.214	0.2814	t_{22}=1.104
Time spent in the open arm:	0.1597	t_{24}=1.451	0.1486	t_{20}=1.503
Number of entries in open arm:	0.1086	t_{24}=1.666	0.0745	t_{20}=1.882
Number of Head dips:	0.0438*	t_{24}=2.128	0.0016**	t_{21}=3.629
Number of Rearings:	0.41	t_{24}=0.83	<0.0001***	t_{21}=5.085
FST:				
	INI		**VGV**	
	F	***p*=**	***F***	***p*=**
interaction	$F_{1,35}$=0.002	0.96	$F_{1,44}$=2,571	0.116
genotype	$F_{1,35}$=23.76	<0.0001***	$F_{1,44}$=103.7	<0.0001***
treatment	$F_{1,35}$=16.57	0.0003***	$F_{1,44}$=15.16	0.0003***
TST:				
	INI		**VGV**	
	F	***p*=**	***F***	***p*=**
interaction	$F_{1,45}$=4.199	0.0463*	$F_{1,49}$=4.691	0.0352*
genotype	$F_{1,45}$=9.117	0.0042**	$F_{1,49}$=77.29	<0.0001***
treatment	$F_{1,45}$=51.59	<0.0001***	$F_{1,49}$=116.3	<0.0001***
Locomotor activity:				
	INI		**VGV**	
	F	***p*=**	***F***	***p*=**
interaction	$F_{1,17}$=0.322	0.57	$F_{1,20}$=3.2856	0.084
genotype	$F_{1,17}$=0.031	0.86	$F_{1,20}$=3.14	0.09
treatment	$F_{1,17}$=9.072	0.0079**	$F_{1,20}$=6.78	0.0169*

Table 2

FST:		**Body weight (g)**	
INI		**Mean**	**SEM**
Wildtype	Saline	23.8	1.13
Wildtype	DMI	23.6	0.92
5-HT2C-INI	Saline	23.1	0.86
5-HT2C-INI	DMI	24.3	0.78
VGV			
Wildtype	Saline	23.9	0.95
Wildtype	DMI	22.8	0.79
5-HT2C-VGV	Saline	15.9[a]	0.41
5-HT2C-VGV	DMI	16.3[a]	0.51
TST:			
INI			
Wildtype	Saline	22.9	0.92
Wildtype	DMI	25.1	0.85
5-HT2C-INI	Saline	24.8	0.91
5-HT2C-INI	DMI	23.8	1.00
VGV			
Wildtype	Saline	24.9	0.74
Wildtype	DMI	26.1	1.15
5-HT2C-VGV	Saline	18.5[a]	0.55
5-HT2C-VGV	DMI	18.1[a]	1.04
Locomotor activity:			
INI			
Wildtype	Saline	24.0	0.93
Wildtype	DMI	23.5	0.99
5-HT2C-INI	Saline	25.7	1.26
5-HT2C-INI	DMI	24.8	0.83
VGV			
Wildtype	Saline	24.7	0.68
Wildtype	DMI	25.4	0.87
5-HT2C-VGV	Saline	18.5[a]	0.72
5-HT2C-VGV	DMI	18.8[a]	0.91

[a] $P < 0.0001$ for main effect of genotype (FST: $F_{1,42}=92.98$; TST: $F_{1,49}=66.68$; Locomotor activity: $F_{1,20}=64.45$). No significant main effect of genotype in INI cohorts. No significant effect of DMI or interaction in any cohort.