Wnt2 Expression and Signaling Is Increased by Different Classes of Antidepressant Treatments

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Background: Despite recent interest in glycogen synthase kinase-3β (GSK-3β) as a target for the treatment of mood disorders, there has been very little work related to these illnesses on the upstream signaling molecules that regulate this kinase as well as downstream targets.

Methods: With a focused microarray approach we examined the influence of different classes of antidepressants on Wnt signaling that controls GSK-3β activity as well as the transcription factors that contribute to the actions of GSK-3β.

Results: The results demonstrate that Wnt2 is a common target of different classes of antidepressants and also show differential regulation of Wnt-GSK-3β signaling genes. Increased expression and function of Wnt2 was confirmed by secondary measures. Moreover, with a viral vector approach we demonstrate that increased expression of Wnt2 in the hippocampus is sufficient to produce antidepressant-like behavioral actions in well-established models of depression and treatment response.

Conclusions: These findings demonstrate that Wnt2 expression and signaling is a common target of antidepressants and that increased Wnt2 is sufficient to produce antidepressant effects.

Key Words: Antidepressants, depression, Wnt signaling

Major depression is caused by a combination of genetic and environmental factors and is one of the top three most widespread and debilitating illnesses worldwide (1). Currently, available antidepressants require weeks or months to produce a therapeutic response, and only one-third of patients experience improvement with the first drug prescribed (1). Moreover, the mechanisms underlying the therapeutic efficacy of these agents remain largely unclear. Over the past decade, intracellular pathways, including the glycogen-synthase kinase-3 (GSK-3) system, have been implicated in the adaptive responses underlying mood disorder medications (2–4). Lithium, which is used for the treatment of bipolar disorder as well as depression, inhibits the activity of GSK-3β (5). In addition, selective inhibitors of GSK-3 have antidepressant efficacy in behavioral models of depression, providing further evidence that this system is a viable target for depression (6,7).

Activation of Wnt signaling is one of the primary pathways leading to inhibition of GSK-3β activity (8). Wnts are secreted glycoproteins that signal through the frizzled (Fz) receptors (9). In the mouse genome, 19 Wnts and 13 Fzs have been identified and can activate any of the three major intracellular signaling pathways: the canonical or Wnt/β-catenin, planar cell polarity (PCP), and Wnt/calcium cascades (10). Wnt signaling is crucial for normal embryonic development as well as neuroplasticity in the adult brain (11–13). Wnt-pathways have been implicated in various physiological functions, such as cell fate determination, cell and tissue polarity (14), synaptogenesis, dendritic morphogenesis (15), and axon remodeling (16,17). Moreover, abnormal Wnt signaling has been implicated in various disorders, ranging from cancer and neurodegeneration to psychiatric illnesses (18–22).

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To identify the common signaling targets of different classes of antidepressants, we employed focused microarrays (300 base pair probes) containing most of the known genes in the Wnt-Fz signaling pathway as well as different classes of neurotrophic factors, growth factors, neuroplasticity-related signaling cascades, and transcription factors (23,24). The different classes of antidepressants tested include selective serotonin (5-HT) reuptake inhibitors (SSRIs) (citalopram or fluoxetine), a selective norepinephrine (NE) reuptake inhibitor (SNRI) (atomoxetine), a dual 5-HT/NE reuptake inhibitor (venlafaxine), and chronic electroconvulsive shock (ECS). The results demonstrate that selected Wnt signaling-related genes are regulated by different classes of antidepressants. Notably, we identify Wnt2 as a common target and determine the functional consequences of altered Wnt2 expression with a viral expression approach.

Methods and Materials

Animals

Male Sprague–Dawley rats (160–180 g for antidepressant treatment; 250–300 g for adeno-associated virus [AAV] studies) (Charles River Laboratories, Wilmington, Massachusetts) were housed, two or three/cage, under standard illumination parameters (12-hour light/dark cycle) and were given free access to food and water. All procedures were in strict accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Yale University Animal Care and Use Committee.

Antidepressant Administration

Rats were administered (IP) the following agents twice daily for 5 days for subchronic treatment or for 3 weeks for chronic treatment: vehicle (distilled water), citalopram (15 mg/kg), fluoxetine (5 mg/kg), venlafaxine (15 mg/kg), or atomoxetine (3 mg/kg). Bilateral ECS was administered via moistened pads on spring-loaded ear clip electrodes with a pulse generator (ECT Unit 57,800–001; Ugo Basile, Comerio, Italy) (frequency, 100 pulses/sec; pulse width, .5 msec; shock duration, .5 sec; current, 55 mA) as previously described (25).

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Microarray Analysis of Gene Expression

Array analysis was performed as previously described (23,26) with minor modifications. Briefly, analysis was performed on a focused array containing approximately 3000 targets (300 base pair open-reading frame polymerase chain reaction [PCR] products) for growth factor signaling, transcription factors, kinases and genes implicated in neuropsychiatric disorders, and drug action. Total RNA (5 μg) was extracted from hippocampus with the phenol-free total RNA isolation kit, RNAqueous (Ambion, Austin, Texas) and was reverse-transcribed into complementary DNA with oligo-dT primers containing a nucleic acid capture sequence unique for Cy3 or Cy5. Arrays were hybridized with a 2-step protocol where vehicle and antidepressant-treated samples were hybridized overnight, followed by stringent washing and then poststaining with fluorescent dendrimers (Genisphere, Hatfield, Pennsylvania). Slides were subsequently subjected to washes, then scanned, and analyzed with GenePix Pro 6.0 software (Molecular Devices, Sunnyvale, California).

Statistical Analysis of Microarray Data

Microarray image files were subjected to statistical analysis as previously described with minor modifications (23,24). Files created by GenePix analysis were imported into expression analysis software GeneSpring GX 7.3.1 (Silicon Genetics, Redwood City, California) for additional visualization and data-mining. In a two-step process, raw values were initially normalized/spot to control channel values, followed by per-chip normalization to positive control genes (β-tubulin and cyclophilin). Gene regulation was determined by taking the log ratio of the median experimental channel signal to the median control channel signal. Up- and downregulated genes were defined as having an average expression ratio of > 1.3 or $< .7$, respectively. Lists of regulated genes (antidepressant drug treatment vs. vehicle-treated) were attained by unpaired *t* test. Statistical significance was considered $p < .05$ following adjustment with Benjamini and Hochberg False Discovery Rate multiple testing correction.

Quantitative Real-Time Reverse Transcription-PCR

A sensitive Sybr Green-based protocol that can reliably detect twofold differences in gene expression was used (23). Complementary DNA was synthesized from 3 μg of total RNA, isolated from the same tissue sample used in the microarray analysis. All the PCR data were normalized to the housekeeping gene, cyclophilin. A primer set was designed with the Primer3 software (http://frodo.wi.mit.edu/primer3/).

In Situ Hybridization Analysis

In situ hybridization was conducted on coronal brain section 14μ thick by hybridization with the 35S-labeled Wnt2 riboprobe as previously described (23,25). Wnt2 intensity was measured by analysis of the number of grains in 20 cells/area for a total of 100 cells from five different regions of dentate gyrus for each animal. For nonspecific binding, grains were counted in 20 cells/adjacent area of molecular layer to each analyzed area of dentate gyrus for a total 100 cells. An overall average for cells and for animals was calculated.

Immunoblotting

Briefly, hippocampus was homogenized in ice cold lysis buffer, and lysates were centrifuged at 20,000 rpm for 10 min. Supernatants were resolved in sodium dodecyl sulphate–polyacrylamide gels, and transferred to nitrocellulose. Blots were probed with antibodies to phospho-Ser9-GSK-3β (cat. no. 9336) and total GSK-3β (cat. No. 9332) (Cell Signaling Technology, Beverly, Massachusetts). Immunoblots were developed with horseradish peroxidase-conjugated goat anti-mouse or goat anti-rabbit IgG (Bio-Rad), followed by detection with enhanced chemiluminescence.

Construction of AAV-Control and AAV-Wnt2 Plasmids

The plasmid p CMV-Wnt2 was generated by first ligating the Wnt2 CDS into pGEM-T Easy vector with pGEM Easy vector systems (Promega, Madison, Wisconsin) to generate pGEM-Wnt2 plasmid. The pGEM-Wnt2 was digested with *SacII*, filled with *C* and *G* by DNA polymerase to get the blunt end. *SmaI*-digested pCMV-MCS and *SpeI*-digested Wnt2 insert were ligated with Rapid DNA ligation kit (Roche, Basel, Switzerland) to generate pCMV-Wnt2. To generate the AAV-Wnt2 plasmid, pAAV-MCS vector, cut with *NsiI*, *ClaI*, and *NdeI*, was ligated to pCMV-Wnt2, cut with *NdeI* and *NsiI*. The pAAV-Wnt2 plasmid was designed to coexpress DSRed and Wnt2 under the control of two independent CMV promoters. Control plasmid (AAV-DSRed) expresses only DSRed.

Viral Production and Purification

The virus was generated with a triple-transfection, helper-free method and purified with a modified version of a published protocol (27). Briefly, human embryonic kidney-293 cells were cultured in five 150 × 25 mm cell culture dishes and transfected with pAAV-DSRed/Wnt2, pHelper and pAAV-RC plasmids (Stratagene, La Jolla, California) with a standard calcium phosphate method, and subsequent purification was done as described previously (27).

Stereotaxic Surgery and Infusions

Rats were anesthetized with xylazine (6 mg/kg IM, Lloyd Laboratories, Shenandoar, Iowa) and ketamine (80 mg/kg IM, Fort Dodge Animal Health, Overland Park, Kansas). Bilateral viral injections were performed with coordinates −4.3 mm (anterior/posterior), −2.0 mm (lateral), and −4.2 mm (dorsal/ventral) relative to the bregma (28). A total of 2 μl of purified virus was delivered at a rate of .1 μl/min followed by 5 min of rest. Needles were removed and the scalp incision was closed with wound clips. After behavioral testing, animals were perfused with phosphate-buffered saline. One-half of the brain was quickly frozen, and 14-μ sections were cut with cryostat to perform in situ hybridization for Wnt2. The other half of the brain was kept overnight in 4% paraformaldehyde and then transferred to 30% sucrose. The 40-μ sections were cut with a microtome for DSRed visualization.

Learned Helplessness Paradigm

The learned helplessness (LH) procedure was performed in custom-built, 2-chambered shuttle boxes (Med Associates, Georgia, Vermont) as previously described (29,30). Inescapable shock (IES) consisted of 60 unsignaled, randomized foot shocks at an intensity of .8 mA. For active avoidance testing, 24 hours after the IES, animals were exposed to 30 escape trials with an FR1 schedule in which a single crossing terminated the footshock. Numbers of escape were automatically scored. Results are expressed as number of escape failures observed in the 30-trial period. Two sets of animals were used for behavioral testing. Animal groups used for the LH paradigm were different from the group used to perform the other behavioral tests.

Sucrose Preference Test

All behavioral testing was performed during the light cycle as previously described (29,31). Animals were habituated to a sucrose solution (1%; Sigma, St. Louis, Missouri) for 48 hours. The sucrose preference test (SPT) was performed after a 4-hour water deprivation on the test day. For 1 hour, the rats were presented with two identical bottles, one filled with the sucrose solution and the other with water. Sucrose and water consumption were determined by measuring the change in volume of fluid.

Novelty Suppressed Feeding

Novelty suppressed feeding (NSF) was conducted as previously described (29). Animals were food-deprived for 12 hours and on the test day were placed in an open field (76.5 cm × 76.5 cm × 40 cm, Plexiglas) with eight pellets of food in the center. The animals were given 8 min to approach the food and eat. The test was stopped as soon as the animal took the first bite. The latency to eat was recorded in seconds. As a control, food consumption, after the 12-hour food deprivation, in the home cage was quantified.

Forced Swim Test

The forced swim test (FST) was a 2-day paradigm in which rats were placed for 15 min in a clear cylinder with water (24 ± 1°C, 45-cm depth). Twenty-four hours after the forced swim, on the test day, rats were placed again for 10 min in a clear cylinder with water (24 ± 1°C, 45-cm depth). The sessions were recorded from the side, and the latency to immobility and the immobility time during the first 5 min was calculated by a blind observer. Latency to become immobile and immobility times were scored as previously described (29,31,32).

Locomotor Activity

Ambulatory locomotor activity was assessed in clear plastic boxes fitted to automated activity meters (MED Associates, St. Albans, Vermont) consisting of two parallel rows of photosensors (16 sensors/row, 2.5 cm apart). Locomotor activity was recorded in 5-min bins for a total of 30 min by using Med PC software.

Results

Influence of Different Classes of Antidepressants on Wnt Signaling

To identify the changes in hippocampal gene expression in response to different antidepressants, we performed microarray analysis with a focused array that included multiple classes of signaling molecules. The expression of several genes involved in the Wnt signaling pathway was regulated by chronic antidepressant administration. As seen in Figure 1A, Wnt2 was upregulated by chronic administration of each of these antidepressants tested (21 days). The results also show that Wnt2 expression was increased by chronic ECS (10 days). In addition, Wnt7b was also upregulated, although it was increased by only atomoxetine and ECS (Figure 1A).

Wnt ligands bind to and signal through Fz receptors and can activate three different signaling pathways, including the Wnt/β-catenin cascade. We found evidence that 1 of the 10 Fz subtypes, Fz9, is upregulated by both atomoxetine and venlafaxine but not by the other three treatments (Figure 1A). We also found that Fz-related protein (sFRP3 or Frzb) is upregulated by citalopram, fluoxetine, and venlafaxine but not by atomoxetine or ECS (Figure 1A). The sFRPs were initially found as Wnt scavengers that bind to Wnts and prevent Wnt-Fz activation, but recent

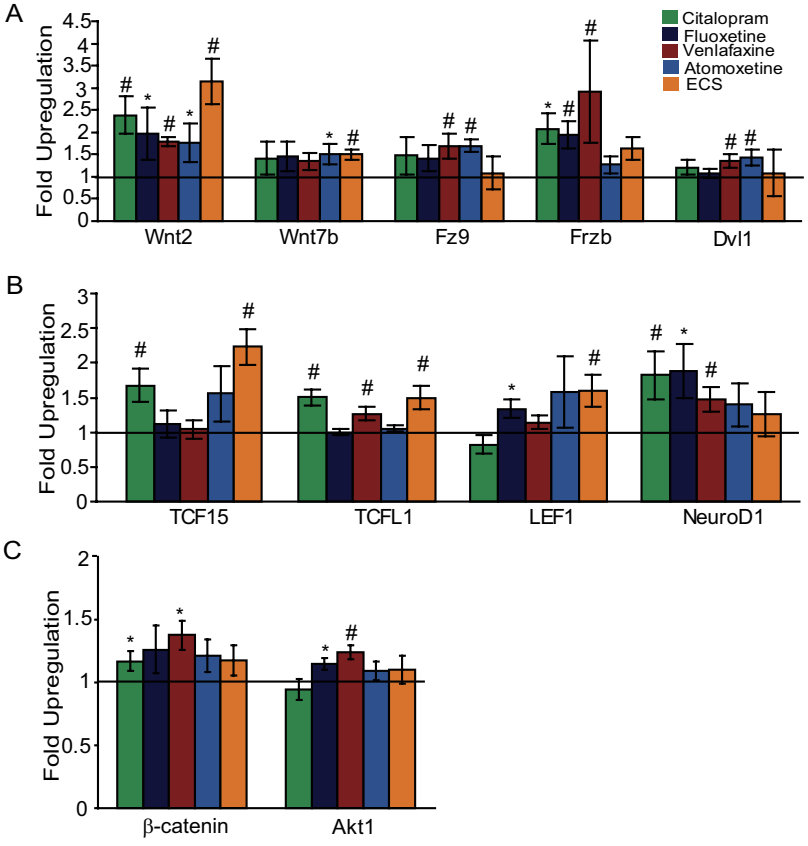

Figure 1. Microarray analyses of Wnt signaling components in hippocampus in response to chronic antidepressant administration. Rats were administered vehicle, citalopram (15 mg/kg), fluoxetine (5 mg/kg), venlafaxine (15 mg/kg), or atomoxetine (3 mg/kg) twice daily for 3 weeks. Electroconvulsive shock (ECS) was administered once daily for 10 days. Gene expression in the hippocampus was determined by microarray analysis with a focused microarray. **(A)** Wnt/Fz/Dvl subtype expression. **(B)** T-cell factor (TCF)/lymphoid enhancer-binding factor (LEF) subtype and NeuroD1 expression **(C)** β-catenin/Akt1 expression. The results are expressed as the mean ratio of fold change in gene expression and are the mean ± SEM of $n = 6$ (vehicle and drugs) or $n = 4$ (sham and ECS). Horizontal line at ratio of 1 indicates no regulation. *$p <$ .05 (Student t test; $p < .05$; no False Discovery Rate adjustment). #$p < .05$ (Student t test on samples after adjustment for False Discovery Rate; $p < .05$).

studies have reported direct binding of sFRPs to Fzs resulting in activation (33). We also examined Dvl, a cytoplasmic scaffolding protein involved in Wnt signaling. Dvl1 was significantly upregulated by venlaflaxine and atomoxetine but not the other treatments tested (Figure 1A). There were no significant effects on expression levels of Dvl2 and Dvl3 (not shown).

Wnt signaling leads to inhibition of GSK-3β activity. In the absence of Wnt stimulation, GSK-3β phosphorylates β-catenin and targets it for ubiquitination and proteosomal degradation. Activation of canonical Wnt signaling thereby leads to β-catenin stabilization. We found significant upregulation of β-catenin messenger RNA (mRNA) in response to citalopram and venlafaxine but not the other antidepressants tested (Figure 1C). We also found that expression of Akt1, another kinase that phosphorylates and inactivates GSK-3β, is increased by venlafaxine (Figure 1C).

The β-catenin can translocate to the nucleus and regulate gene expression via the lymphoid enhancer-binding factor (LEF)/T-cell factor (TCF) family of transcription factors. Levels of TCFL1, TCF15, and LEF1 were significantly upregulated by the different antidepressant treatments (Figure 1B). There are multiple genes that are regulated by LEF/TCF transcription factors, but recent studies have demonstrated that a crucial target for the Wnt pathway in adult neurogenesis was to activate the transcription factor, NeuroD1 (34,35). In the current study, we found that expression of NeuroD1 was significantly increased by citalopram, fluoxetine, and venlafaxine (Figure 1B).

Secondary Confirmation of Wnt2 Gene Expression and Function

To further validate the findings obtained by microarrays, we performed quantitative reverse transcription (RT)-PCR. As seen in Figure 2, Wnt2 was upregulated by all four chemical antidepressants tested, confirming the microarray findings, and our previous findings with ECS (25). To determine the effect of subchronic antidepressant treatment on gene expression, drugs were administered with the same dose and regimen as used for chronic treatment but for only 5 days. For these and the following studies of GSK-3, we chose two representative antidepressants, fluoxetine and venlafaxine. No significant change in the average mRNA fold change with subchronic (5 days) treatment of either fluoxetine (.92 ± .09) or venlafaxine (1.01 ± .08) was found,

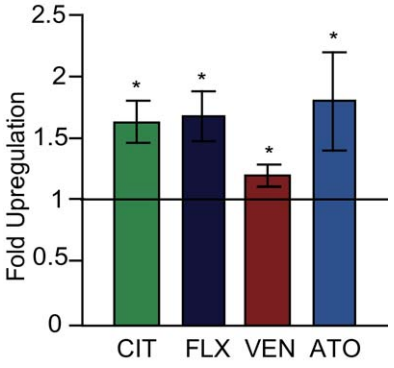

Figure 2. Confirmation of microarray results by reverse-transcription polymerase chain reaction (RT-PCR). Quantitative RT-PCR analysis of Wnt2 expression by chronic (3 weeks) chemical antidepressant administration in hippocampus. Data are normalized to cyclophilin and are the mean ratio (antidepressant/vehicle) of fold change ± SEM of five individual animals. Horizontal line at ratio of 1 indicates no regulation. *$p < .05$; compared with vehicle-treated control (Student *t* test). CIT, citalopram; FLX, fluoxetine; VEN, venlafaxine; ATO, atomoxetine.

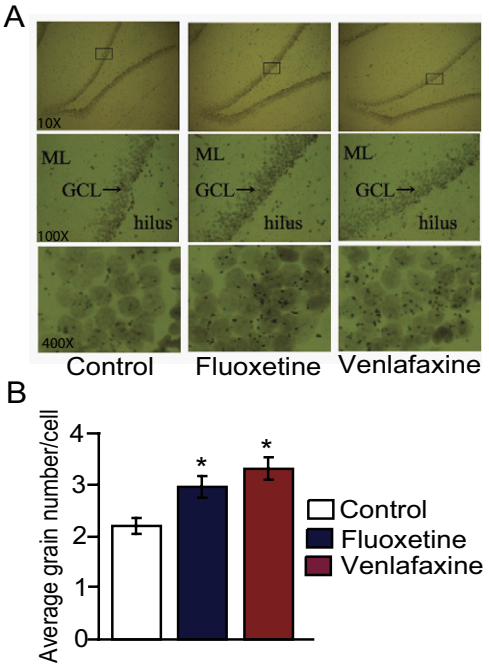

Figure 3. Chronic antidepressant administration increases Wnt2 messenger RNA (mRNA) in dentate gyrus (DG) of the hippocampus. The expression of Wnt2 mRNA in DG was determined by in situ hybridization analysis. **(A)** Representative images of emulsion-dipped slides at different magnifications: upper; 10×, middle; 100×, lower; 400×. **(B)** Quantitation of Wnt2 mRNA in the DG. Results are presented as the mean average number of grains ± SEM of six replicates. *$p < .05$, compared with vehicle-treated control (Student *t* test). ML, molecular layer; GCL, granular cell layer.

indicating that the induction of Wnt2 expression requires chronic treatment.

Because Wnt2 was upregulated by chronic treatment of all antidepressants tested, we also performed in situ hybridization and emulsion autoradiography to determine the expression in subregions of the hippocampus. Figure 3 shows representative photomicrographs of silver grain distribution for Wnt2 mRNA in dentate gyrus of rat hippocampus. There was a significant increase in the density of the Wnt2 mRNA in the dentate gyrus of antidepressant-treated rats (Figure 3).

Activation of canonical Wnt signaling results in the destabilization of protein complex composed of Axin, adenomatous polyposis coli, β-catenin and GSK-3β, resulting in an increase in β-catenin levels. Some studies show that the activation of the PI3-kinase/Akt pathway results in an increase in Akt1 catalytic activity, which in turn phosphorylates GSK-3β at Ser-9 in a Dvl1-mediated mechanism resulting in the inhibition of its kinase activity (36,37). Because we found an increase in both β-catenin and Akt1 in response to some of the antidepressant treatments and to determine whether the regulation of Wnt signaling by chronic antidepressant treatment affects GSK-3 phosphorylation, levels of phospho-Ser9-GSK-3β were determined by immunoblot analysis. As seen in Figure 4, chronic administration of fluoxetine or venlafaxine significantly increased the ratio of phospho-Ser9-GSK-3β/GSK-3β (lower band), whereas the GSK-3β protein levels were unaffected (Figure 4). There was a similar trend for induction of phospho-GSK-3α (upper band), although this effect was not significant (data not shown).

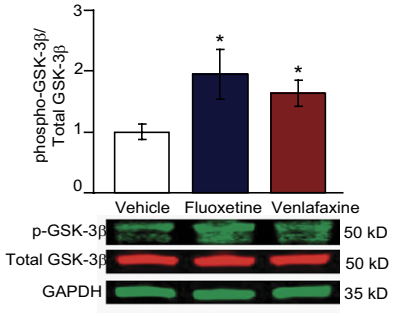

Figure 4. Chronic antidepressant administration increases glycogen synthase kinase (GSK)-3β phosphorylation. Rats were administered vehicle (distilled water, twice daily), fluoxetine (5 mg/kg, twice daily), or venlafaxine (15 mg/kg, twice daily), and whole hippocampus was subjected to western blot analysis. Optical density values were normalized to loading control (glyceraldehyde-3-phosphate dehydrogenase [GAPDH]) and expressed as the ratio of phospho-Ser9-GSK-3β/total GSK-3β. Data are expressed as a mean fold change ± SEM, compared with vehicle-treated controls ($n = 6$); *$p < .05$ (Student *t* test).

Behavioral Effects of Viral Wnt2 Expression

To determine the role of Wnt2 in behavioral models of depression/antidepressant response, an AAV vector was designed to express Wnt2 as well as the marker DS Red to allow for detection of the infected neurons. Control AAV (DS Red only) or AAV-Wnt2 was bilaterally injected into the dentate gyrus of adult rat hippocampus. Behavioral testing was done 3 weeks after viral infusion, a time point when AAV expression is optimal (Figure 5A) (27). Expression (Figure 5B) was analyzed by in situ hybridization (Figures 5B [a, b]) and DS-red staining (Figure 5B [c]). The exact cell types infected are difficult to assess, but because AAV2 selectively infects neurons, the localization of signal to the granule cell layer and hilus indicates that neurons in these regions are being targeted. Animals were tested in behavioral models known to be responsive to antidepressant administration, including LH, NSF, SPT, and FST (Figures 5C–F). Over expression of Wnt2 significantly decreased the number of escape failures in the LH paradigm, an antidepressant-like response (Figure 5C). There was also a decrease in the latency to feed in NSF, although this effect did not reach significance (Figure 5D). Animals infused with AAV-Wnt2 also displayed an increase in sucrose consumption relative to AAV control infused animals, with no change in water consumption (Figure 5E). The levels of sucrose preference (expressed as percent sucrose consumed over total fluid consumed) were 54 ± 3 and 63 ± 5 (mean ± SEM, for AAV-control and AAV-Wnt2, respectively; $p = .08$). Finally, AAV-Wnt2 had no significant effect in the FST (Figure 5F) or locomotor activity (number of beam breaks for AAV-control and AAV-Wnt2 were 580 ± 75 and 618 ± 50, respectively; mean ± SEM; $p = .33$).

Discussion

The results demonstrate that chronic antidepressant administration influences hippocampal expression of multiple components of the Wnt/β-catenin cascade, including regulation of subtypes of Wnts, Fz, β-catenin, and TCF. Notable among these signaling molecules is Wnt2, which was the only gene that was uniformly regulated by all treatments tested, including SSRI, SNRI, a dual reuptake inhibitor, and chronic ECS. Expression of some genes seemed to be more sensitive to a certain class of antidepressants. For example, FrzB, NeuroD1, and β-catenin were induced by SSRI administration, whereas Fz9 and Dvl1

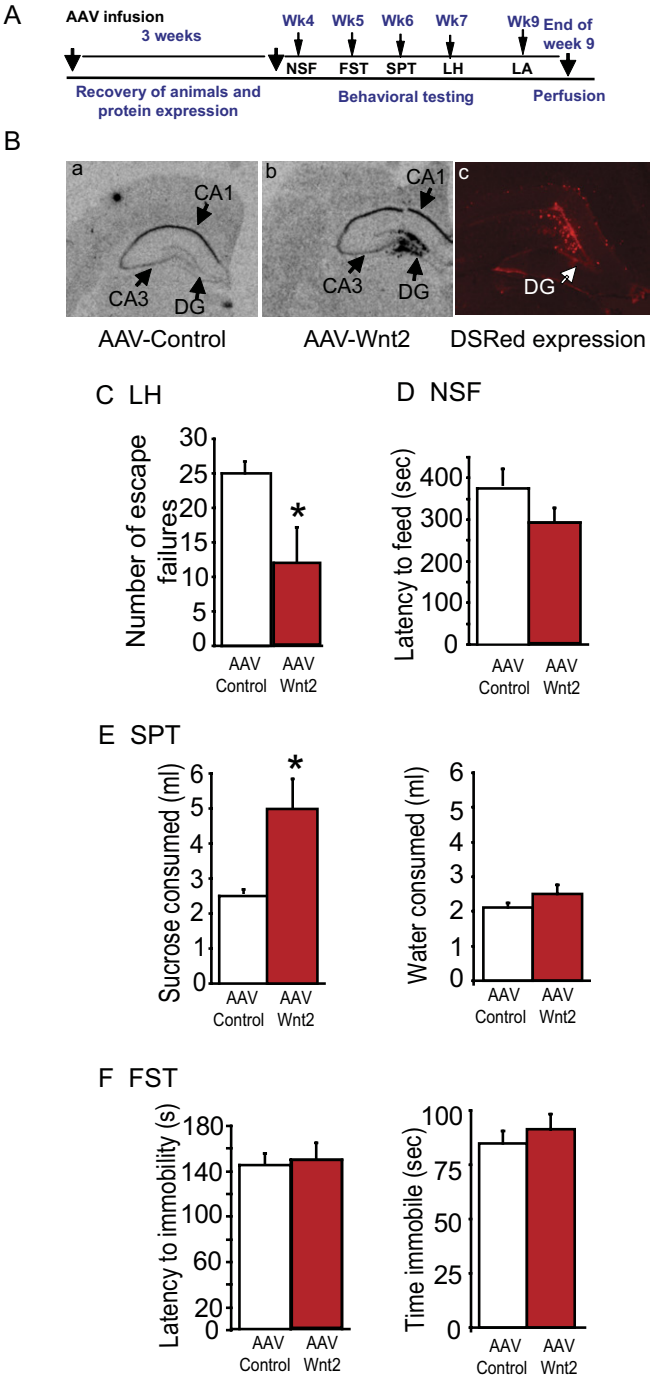

Figure 5. Influence of adeno-associated virus (AAV)-Wnt2 expression on behavioral models of depression and antidepressant response. The AAV-Wnt2 or control (AAV-DS Red) was infused into the dentate gyrus (DG) of the hippocampus, and tissue was harvested 8 weeks later. **(A)** Schematic diagram showing details of experimental timeline. **(B)** In situ hybridization analysis was conducted to determine the expression of Wnt2, and representative autoradiograms were shown for control (a) and AAV-Wnt2 (b). DS-red staining is also shown to further demonstrate localization of AAV spread (c). The influence of AAV-Wnt2 expression on different behavioral models was examined, including **(C)** escape failures in the learned helplessness (LH) paradigm; *$p = .02$; **(D)** latency to feed in the novelty suppressed feeding (NSF) test; $p = .09$; **(E)** sucrose preference test (SPT); *$p = .02$ for sucrose consumption; $p = .08$ for sucrose preference; **(F)** latency to immobility in the forced swim test (FST); $p = .39$; immobility; $p = .47$. Results are the mean ± SEM of control ($n = 8$) versus Wnt2 ($n = 7$). *$p < .05$ compared with AAV-controls (Student *t* test).

were upregulated by the SNRI and dual reuptake inhibitor. These findings suggest that certain components of Wnt signaling are differentially regulated by the 5-HT and NE neurotransmitter systems. However, there were also cases where the two SSRIs did not produce the same effects (e.g., TCF15 was induced by citalopram but not by fluoxetine). This could result from differences in the effective doses used or different actions of the two SSRIs tested. Further dose-response studies as well as additional SSRI and SNRI antidepressants will be required to determine the differential effects of these two classes of agents on selected Wnt signaling genes.

The microarray results for upregulation of Wnt2 were confirmed by RT-PCR and by in situ hybridization, the latter demonstrating increased Wnt2 expression in the dentate gyrus granule cell layer. These findings agree with a previous report that chronic ECS increases Wnt2 expression in the same hippocampal cell layer (25). The requirement for long-term (21 days) drug treatment for induction of Wnt2 was confirmed by RT-PCR, because short-term treatment (5 days) had no effect. We also found that chronic antidepressant treatment increased the phosphorylation of GSK-3β, which causes inactivation, consistent with reports that pharmacological inhibition of GSK-3β produces antidepressant-like behavioral effects (6,38–41). However, the mechanism underlying increased phosphorylation of GSK-3β is unclear. Canonical Wnt signaling results in inhibition of GSK-3β but does so by destabilization of a GSK-3β protein complex (42,43). There are reports that Wnt/Dvl1 signaling can lead to phosphorylation of GSK-3β via the PI3-kinase/Akt pathway (36,37), and further studies will be required to determine the mechanism by which antidepressants increase phospho-GSK-3β (i.e., via Wnt2/Dvl1/Akt or via another pathway such as growth factor receptor activation of PI3-kinase/Akt).

The functional actions at the behavioral level were determined with a viral expression approach. Induction of Wnt2 expression by microinfusion of AAV-Wnt2 into the dentate gyrus granule cell layer resulted in significant antidepressant-like responses in the LH and SPT, but no significant effects were found in NSF or FST. In the current LH paradigm expression of Wnt2 before exposure to IES assesses the ability of Wnt2 to prevent the development of escape deficits. It would also be interesting to test the influence of Wnt2 in a reversal paradigm, where Wnt2 is expressed after exposure to IES. Another consideration is the possibility that Wnt2 expression alters learning, which could also contribute to the more rapid responding observed in the active avoidance test. Expression of Wnt2 significantly influenced behavior in the SPT, which is typically used as a measure of anhedonia that occurs in response to repeated stress exposure (29). Although, the paradigm used in the current study did not include repeated stress, anhedonic responses were observed in AAV-infused control animals (i.e., no preference for sucrose compared with water). This is likely due to the stress resulting from cranial surgery as well as the stress encountered in the NSF and FST that precede the SPT.

Although not significant, there was a trend for AAV-Wnt2 to produce an effect in the NSF test, which is responsive to chronic antidepressant treatment. It would be interesting in future studies to determine whether Wnt2 expression influences behavior in other models of anxiety, such as the elevated plus maze. The lack of effect of Wnt2 on the FST was surprising but could be explained by several possibilities. First, the FST is a different despair paradigm relative to LH, notably being responsive to acute antidepressants, indicating that different signaling mechanisms are involved. Second, FST/immobility could be influenced by brain regions other than or in addition to the hippocampus. Third, although expression of Wnt2 in the dorsal hippocampus was sufficient to alter LH and SPT behavior, it is possible that more widespread expression or targeting of other parts of hippocampus (e.g., ventral) would influence FST behavior. Although we have shown that antidepressant drugs decrease immobility in previous studies, a positive control was not included in the current study, and it is possible that the animals were unresponsive for an unknown reason. Finally, although the results demonstrate that Wnt2 expression can influence LH and SPT behavior, future studies will be needed to determine whether Wnt2 signaling is also necessary for the actions of antidepressant drug treatments. These studies will require the development of a Wnt2 knockdown strategy (e.g., viral expression of Wnt2 shRNA or Wnt2 conditional deletion mutant mice).

Wnt signaling pathways have been implicated in many aspects of neuronal morphology and function that could contribute to antidepressant-like actions, including axon and dendrite remodeling and development, synaptogenesis, neuroplasticity, and neurogenesis (11–13,15,17). The actions of antidepressants are thought to occur in part via regulation of similar cellular processes (e.g., increased synaptic plasticity, spine/synapse formation, neurogenesis), and it is possible that these effects occur, in part, via regulation of Wnt signaling. For example, Wnt2 signaling plays a critical role in activity-dependent formation of dendrites via intracellular β-catenin (44); Wnt7b has been shown to regulate dendritic arborization via Dvl1 (15), and interestingly, we found that Dvl1 is upregulated but only by atomoxetine and venlafaxine and not by SSRIs. Regulation of neurogenesis in adult hippocampus is reported to occur via Wnt3a (45). Unfortunately, the signal strength of Wnt3a in the current study was too low to be included in the analysis, and further work will be required to determine whether antidepressant treatment regulates the expression of Wnt3a and, alternatively, whether Wnt2 regulates neurogenesis. Recent studies demonstrate that ablation of NeuroD1 in the adult neurogenic niche decreases neurogenesis and show that NeuroD1 links Wnt signaling and TCF/LEF activity (34,35). With regard to Wnt receptors, mutation of Fz9 decreases the number of dentate gyrus granule cells and reduces visual and spatial memory processing (46). Dvl1 is the major Dvl subtype in brain, and recent studies have shown that Dvl1 accumulates at growing axon tips and is required for axon formation (47).

In summary, the results of this study provide evidence for the regulation of multiple components of Wnt/β-catenin signaling, both canonical and noncanonical pathways (PCP and Wnt/calcium cascades), showing that a complex network of molecular changes might contribute to the efficacy of antidepressant treatment. Importantly, the results demonstrate that local expression of Wnt2 in the hippocampus is sufficient to produce antidepressant-like effects in behavioral models. These studies identify specific molecular substrates within the Wnt/β-catenin pathway that could be targeted for the development of novel therapeutic medications.

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