Reversal of Synapse Degeneration by Restoring Wnt Signaling in the Adult Hippocampus

Graphical Abstract

Highlights
- Wnt signaling is required for synapse integrity in the adult hippocampus
- Dkk1 induces synapse loss and deficits in synaptic plasticity and long-term memory
- Dkk1 disassembles synapses by activating the Gsk3 and Rock pathways
- Synapse loss and memory defects are reversible by reactivation of the Wnt pathway

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In Brief
Deficiency in Wnt signaling has been implicated in Alzheimer’s disease. Marzo et al. elucidate the impact of the Wnt antagonist Dkk1 in the adult hippocampus, showing synapse loss and defects in synaptic plasticity and long-term memory. They also reveal that cessation of Dkk1 expression induces synapse regeneration and recovery of long-term memory.
Reversal of Synapse Degeneration by Restoring Wnt Signaling in the Adult Hippocampus

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SUMMARY

Synapse degeneration occurs early in neurodegenerative diseases and correlates strongly with cognitive decline in Alzheimer’s disease (AD). The molecular mechanisms that trigger synapse vulnerability and those that promote synapse regeneration after substantial synaptic failure remain poorly understood. Increasing evidence suggests a link between a deficiency in Wnt signaling and AD. The secreted Wnt antagonist Dickkopf-1 (Dkk1), which is elevated in AD, contributes to amyloid-β-mediated synaptic failure. However, the impact of Dkk1 at the circuit level and the mechanism by which synapses disassemble have not yet been explored. Using a transgenic mouse model that inducibly expresses Dkk1 in the hippocampus, we demonstrate that Dkk1 triggers synapse loss, impairs long-term potentiation, enhances long-term depression, and induces learning and memory deficits. We decipher the mechanism involved in synapse loss induced by Dkk1 as it can be prevented by combined inhibition of the Gsk3 and RhoA-Rock pathways. Notably, after loss of synaptic connectivity, reactivation of the Wnt pathway by cessation of Dkk1 expression completely restores synapse number, synaptic plasticity, and long-term memory. These findings demonstrate the remarkable capacity of adult neurons to regenerate functional circuits and highlight Wnt signaling as a targetable pathway for neuronal circuit recovery after synapse degeneration.

INTRODUCTION

Synapse loss and dysfunction are an early occurrence in several neurodegenerative conditions, including Alzheimer’s disease (AD). Synapse vulnerability strongly correlates with cognitive decline before detectable neuronal death [1, 2] and might contribute to the subsequent neuronal degeneration. Surprisingly, little is known about the molecular mechanisms that trigger synapse vulnerability in neurodegenerative diseases and even less about how this process can be prevented or reversed.

Increasing evidence suggests that deficient canonical Wnt signaling contributes to AD pathogenesis. Wnts are secreted proteins that modulate several aspects of brain development and function, including synapse formation, synaptic transmission, experience-mediated synaptic remodeling, and adult neurogenesis [3–7]. Genome-wide association studies (GWASs) have revealed a link between genetic variants of the Wnt co-receptor LRP6, which are associated with decreased canonical Wnt signaling activity, and late onset AD [8, 9]. Loss of function of LRP6 in hippocampal neurons results in synaptic defects, cell death, and exacerbation of amyloid deposition in a mouse model of AD [10]. Importantly, the secreted protein Dickkopf-1 (Dkk1), which blocks canonical Wnt-Gsk3 signaling by sequestering the LRP6 receptor [11, 12], is elevated in post-mortem brains from AD patients and in AD animal models [13–15]. In addition, oligomers of amyloid-β (Aβ), the main component of amyloid plaques in AD, induce Dkk1 expression in cultured neurons and in brain slices [13, 16, 17]. Dkk1 disassembles excitatory synapses in a similar manner to Aβ in cultured hippocampal neurons [17]. Importantly, blockade of Dkk1 with neutralizing antibodies protects synapses from Aβ-mediated disassembly [17]. Collectively, these results suggest that Dkk1-mediated deficiency of Wnt signaling could contribute to synapse vulnerability. However, the impact of Dkk1 on hippocampal circuits, which are severely affected in AD, and its mechanism of action have not been explored.

Restoration of synaptic function after substantial synapse loss is crucial for the treatment of neurodegenerative diseases, as diagnosis is often obtained after significant damage has occurred. Although some downstream targets of Aβ have been identified [18–21], only a limited number of studies has shown the ability of these molecules to fully restore function after significant synapse degeneration [18, 20]. Thus, the identity of the signaling pathways that could restore synapse function remains poorly understood.

Here, we demonstrate a critical role for Wnt signaling in synapse stability and synaptic plasticity in the adult hippocampus. Using a transgenic mouse model that allows inducible expression of Dkk1, we investigated the contribution of deficient Wnt signaling to synapse function in the adult hippocampus without compromising embryonic and postnatal development. Inducible Dkk1 expression triggers disassembly of excitatory
The hippocampus plays a role in emotional and cognitive functions, such as anxiety, learning, and memory [32, 33]. We investigated the impact of Dkk1 expression in these processes. The exploratory activity and anxiety level, evaluated through an open-field and elevated plus maze, were identical in iDkk1 mice and controls (Figures S3A and S3B). In addition, no defects were observed in the swimming speed and traveled distance in a Morris water maze (MWM) (Figure S3C), demonstrating that Dkk1 expression does not affect motor function and hippocampal-dependent emotional behaviors.

Next, we investigated short-term memory using the discrete trial version of the spontaneous alternation T-maze test (30-s delay) [34]. This task depends on the animals’ natural tendency to alternate and enter the previously unvisited arm. Both control and iDkk1 mice alternated between the two arms above chance (Figure S3D), indicating that short-term memory is unaffected in iDkk1 mice. We then evaluated hippocampus-dependent spatial reference learning and long-term memory using the MWM test [35–37]. Mice were first trained on the cued version of the task (platform marked by a visible flag). No difference in the time required to reach the visible platform was observed between control and iDkk1 mice (Figure 1E), demonstrating that iDkk1 mice have no visual and procedural skills defects. Subsequently, mice were trained over 5 days to locate an invisible platform. The platform was removed during two probe tests (before the 4th day and 24 hr after the 5th day of training). iDkk1 mice took twice as long as controls to find the hidden platform on the 3rd and 4th days of training (Figure 1E), demonstrating an inability to remember the location of the platform. Similarly, during the first probe test (probe I), iDkk1 mice spent less time in the target quadrant and crossed the virtual platform location significantly fewer times than controls (Figures 1F and 1G), demonstrating impaired reference memory acquisition. Interestingly, after two further training days, iDkk1 mice reached the same performance level as control mice (probe II; Figures 1F and 1G), suggesting that additional training can overcome this memory deficit, as shown in some AD mouse models [38–40]. Thus, deficient Wnt signaling in the adult hippocampus leads to deficits in spatial memory acquisition.

To extend our study of memory-related hippocampal function, we used a single-trial contextual fear-conditioning paradigm [41,
Figure 1. Expression of Dkk1 in Adult Hippocampus Induces Defects in Long-Term Memory

(A) Top: schematic of doxycycline-induced Dkk1 expression. Mice carrying the rtTA gene under the CaMKIIa promoter are crossed with tetO-Dkk1 mice to generate double transgenic animals (iDkk1). Bottom: schematic representation of doxycycline feeding schedule in adult mice (see Figure S1).

(B) RT-PCR for Dkk1 in hippocampus of adult iDkk1 and control mice with or without doxycycline administration (Doxy).

(C) In situ hybridization for Dkk1 mRNAs in adult hippocampus of iDkk1 and control mice. The scale bar represents 250 μm.

(D) Images and quantification of NeuN-positive CA1 neurons in control and iDkk1 mice fed with doxycycline for 14 days. Quantification of NeuN-positive CA1 neurons is also shown after 3.5 months of diet containing doxycycline (ANOVA; four mice per genotype per condition). The scale bar represents 50 μm (see also Figure S2).

(E) Escape latency in the Morris water maze (MWM) (*p < 0.05; repeated-measures ANOVA; 11 control and 12 iDkk1 mice; see also Figure S3).

(F) Number of platform crossings in the MWM during probe I (left) or during probe II (right; *p < 0.05; Student’s t test).

(G) Time spent in each quadrant during probe I (left) and during probe II (right; *p < 0.05; Student’s t test).

(H) Percentage of freezing time evaluated 24 hr after the foot shock (**p < 0.01; ANOVA; eight control and seven iDkk1 mice).

Data are represented as mean ± SEM.
Deficient Wnt Signaling Impairs Basal Synaptic Transmission and Synaptic Plasticity

Changes in long-term memory have been correlated with changes in long-term synaptic plasticity (i.e., LTP and LTD). We therefore investigated the ability of iDkk1 mice to express LTP at Schaffer collateral (SC)-CA1 synapses. A theta-burst stimulation (TBS) protocol was chosen as it mimics hippocampal activity during spatial learning. TBS induced a 40% potentiation in control mice, whereas in iDkk1 mice it failed to potentiate these synapses (Figure 2A), demonstrating that Wnt blockade in the adult hippocampus results in the absence of TBS-induced LTP.

This defect could be due to a decreased connectivity, as a minimal number of synapses is required to promote LTP induction as defined as cooperativity. Analyses of input-output curves at the SC-CA1 synapses revealed a defect at the strongest intensities of stimulation in iDkk1 mice, as the field excitatory postsynaptic potential (fEPSP) slope reached only half the magnitude of control animals (Figure 2B). Thus, CA1 synaptic connectivity is affected by Dkk1 expression.

LTD is crucial to synaptic function, and its modulation by Wnt signaling remains unknown. To examine the impact of Dkk1 on LTD, we used a protocol that effectively induces LTD in adult mice with a strong low-frequency stimulation (LFS) consisting of two trains of 900 pulses at 2 Hz. With this protocol, we observed a 20%–30% depression at the SC-CA1 synapses in both control and iDkk1 animals (Figure S4). We therefore decided to use a sub-threshold LFS (weak LFS) protocol, which has been shown to unmask enhanced LTD after exposure to Aβ. We decided to use a sub-threshold LFS (weak LFS) protocol, which has been shown to unmask enhanced LTD after exposure to Aβ.
and the co-receptors LRP6, resulting in the inhibition of Gsk3β-mediated phosphorylation and stabilization of β-catenin, which translocates to the nucleus and activates transcription [51] (Figure S5). In contrast, in the presence of Dkk1, binding of Wnts to Fz/LRP6 is blocked, resulting in enhanced Gsk3β-mediated β-catenin degradation by the proteasome pathway (Figure S5) [12, 51]. Thus, Dkk1 effectively blocks the function of several Wnts that signal through the LRP6 receptor. To investigate the impact of Dkk1 expression on canonical Wnt signaling, we evaluated β-catenin levels. Indeed, expression of Dkk1 resulted in fewer β-catenin puncta in the CA1 stratum radiatum of iDkk1 mice (Figures 5A and 5B), indicating that Dkk1 blocks the canonical Wnt-β-catenin pathway. Co-localization with the synaptic marker vGlut1 showed that most β-catenin puncta were extrasynaptic, indicating that the loss of β-catenin induced by Dkk1 was not due to synapse loss. These results suggest that Dkk1 expression blocks canonical Wnt signaling in the adult hippocampus.

Next, we evaluated whether Dkk1-mediated synaptic loss is due to blockade of canonical Wnt signaling. We used the specific Gsk3 inhibitor BIO (6-bromoindirubin-3′-oxime), which activates the Wnt pathway downstream of Dkk1 [52, 53]. Using a concentration of BIO, which does not affect synapse number on its own (Figures 5C and 5D), we found that this Gsk3 inhibitor partially prevented Dkk1-mediated synapse loss (Figures 5C and 5E). Given the partial protection by both Gsk3β inhibition and Rock inhibition on Dkk1-mediated synapse degeneration, we examined the combined effect of Gsk3β and Rock inhibitors and found complete blockade of Dkk1-induced synapse loss (Figures 5C and 5F). These results demonstrate a novel role for RhoA-Rock pathway in Dkk1 function and suggest that Dkk1 promotes synapse disassembly by blocking canonical Wnt signaling and activating the RhoA-Rock pathway.

**Synaptic Loss, Plasticity Defects, and Behavioral Impairment Are Reversible**

Diagnosis of neurodegenerative diseases is often made after substantial loss of synaptic connectivity has occurred. Thus, understanding the reversible nature of synaptic degeneration is crucial for developing therapies for the treatment of cognitive impairments in neurodegenerative diseases. We therefore examined whether Dkk1-mediated synapse loss and network dysfunction are reversible. We performed in vivo on-off experiments (Figure 6A), in which Dkk1 expression was induced for 2 weeks with doxycycline (On Doxy), followed by withdrawal of doxycycline for a further 2 weeks (Off Doxy). RT-PCR revealed that Dkk1 was expressed during the “on” period, but not after the “off” period, confirming that Dkk1 expression is tightly regulated by doxycycline (Figure 6B). Remarkably, the number of excitatory synapses fully recovered to control levels after doxycycline withdrawal (Figures 6C and 6D). These results...
demonstrate that, even after significant degeneration, the number of synaptic connections can be restored when Dkk1 expression is turned off in the adult hippocampus.

We then evaluated whether defects in basal transmission, long-term plasticity, and long-term memory could be reversed in iDkk1 mice. We found that cessation of Dkk1 expression resulted in full recovery of basal synaptic transmission as indicated by the overlapping input-output curves from control and iDkk1 mice (Figure 6E). Notably, TBS fully induced LTP in iDkk1 mice by the overlapping input-output curves from control and iDkk1 mice. We found that cessation of Dkk1 expression reversed LTD in both control and iDkk1 mice (Figure 6F). Moreover, weak LFS induced short-term depression without inducing LTD in both control and iDkk1 mice (Figure 6G). Finally, using the contextual fear-conditioning test, we found that turning off Dkk1 expression in iDkk1 mice completely recovers their ability to form long-term memory, as the percentage of freezing time was similar to control mice (Figure 6H). Taken together, these studies show the remarkable capacity of the adult hippocampus to regenerate synapses that integrate into functional neuronal circuits. They also demonstrate that synapse degeneration can be reversed in the adult mouse brain by modulating Wnt signaling.

**DISCUSSION**

Here, we report that deficiency in Wnt signaling by inducibly expressing the specific Wnt antagonist Dkk1 in the adult hippocampus triggers the loss of excitatory synapses in CA1 neurons, impairs synaptic plasticity, and alters hippocampal-dependent function. These defects occur in the absence of cell death and require the combined activation of Gsk3β and Rock. Notably, Dkk1-induced synaptic defects are fully reversed upon cessation of Dkk1 expression. Our findings demonstrate that iDkk1 mice provide a unique model system to study the in vivo impact of deficient Wnt signaling on synapse vulnerability and to elucidate the molecular mechanisms that contribute to synapse regeneration after substantial synapse loss and dysfunction.

In the adult hippocampus, Dkk1 expression blocks Wnt signaling without affecting cell viability or the stem cell niche. Previous studies have shown that Dkk1 can promote cell death in models of AD, epilepsy, and ischemia [13, 29, 60, 61] and affect adult neurogenesis by modulating the generation of immature neurons in the adult DG [30]. However, we found no evidence of increased cell death or an effect on the number of newborn neurons in the adult hippocampus of iDkk1 mice. This could be attributed to low levels of Dkk1 expression after 2 weeks induction of this protein. Given the direct effect of Wnts on synapses [62–64], our results suggest that Dkk1 induces synaptic vulnerability by directly targeting synapses.

Figure 4. Dkk1 Does Not Affect Inhibitory Synapses in the Hippocampus in iDkk1 Mice

(A) Confocal images of adult CA1 stratum radiatum show the presence of inhibitory synapses identified by co-localized presynaptic vGat and postsynaptic gephyrin puncta (white arrows). The scale bars represent 2 μm. Quantification is shown on the side (Kruskal-Wallis test; three mice per genotype).

(B) Representative mIPSC traces recorded at 0 mV from CA1 cells in acute hippocampal slices and quantification of mIPSC frequency and amplitude. Numbers inside bars indicate the number of cells recorded from at least seven mice per genotype (Mann-Whitney test for frequency; Student’s t test for amplitude).

Data are represented as mean ± SEM.
influence the stability of the synapse by modulating different
targets, such as β-catenin and microtubules in the case of Gsk3β or
the actin cytoskeleton through the Rock pathway. Alternatively,
both pathways could interact as recently reported for the role
of Wnts in cell migration [66]. Future studies will elucidate the
downstream events by which these two pathways contribute
to Dkk1-mediated synapse vulnerability.

Induced Dkk1 expression affects long-term plasticity and
memory. iDkk1 mice exhibit impaired hippocampus-dependent
function as demonstrated by defects in contextual fear memory
[16, 67]. Memory deficits have been associated with
defects in long-term plasticity in the hippocampus [68–70].
Consistently, iDkk1 mice exhibit a significant impairment in
LTP, a defect that could be due to the loss of 40% of excitatory
synapses [47] and/or to the impaired ability of remaining synap-
ses to respond to LTP induction. We also demonstrate a novel
function for Wnt signaling in LTD. Previous studies showed
that Gsk3β activation suppresses LTP [71] and enhances LTD
[72], suggesting a role for Gsk3β downstream of Dkk1-mediated
synaptic dysfunction.

Understanding the molecular pathways that promote the
regeneration of synapses that integrate into networks is crucial
for developing effective therapies to promote functional recov-
er. Here, we report that synapse loss, defects in synaptic
plasticity, and memory deficits can be fully restored in iDkk1
mice after cessation of Dkk1 expression. Our findings demon-
strate the remarkable capacity of adult neurons to regenerate
functional circuits after substantial synapse loss and highlights
that Wnt signaling is a targetable pathway in neurodegenerative
diseases.

**EXPERIMENTAL PROCEDURES**

**Animals**

Experiments were performed according to the Animals Scientific procedures
Act UK (1986). Double transgenic mice (iDkk1) were obtained as described in
[22]. Adult (3–6 months old) iDkk1 and control mice (tetO-Dkk1, CaMKIIα-rtTA2,
or wild-type littermates) were fed with pellets containing 6 mg/kg doxycycline
(Datesand Group) ad libitum for 2 weeks, unless otherwise indicated. For the

![](image-url)
on-off experiment, 2 weeks of doxycycline feeding was followed by 2 weeks of feeding with the original diet. Males were used for electrophysiological and behavioral experiments, whereas both genders were used for cellular biology experiments. See the Supplemental Experimental Procedures for more details.

**Hippocampal Culture, Cell Transfection, and Drug Treatment**

Hippocampal cultures were prepared from embryonic day 18 (E18) embryos of Sprague-Dawley rats as described previously [73] and maintained for 21 days in vitro (DIVs). Purified recombinant Dkk1 (200 ng/mL; PeproTech) was applied to cells for 2 hr in the presence or absence of the Gsk3 inhibitor BIO (200 nM; BioVision Technologies) and ROCK inhibitor Y27632 (10 μM; Selleckchem). See the Supplemental Experimental Procedures for further details.

**Immunofluorescence Staining**

Brain slices from control and iDkk1 mice were incubated in blocking solution (10% donkey serum and 0.02% v/v Triton X-100 in PBS) for 4 hr at room temperature (RT). Primary antibodies were incubated overnight at 4°C. Secondary antibodies conjugated with Alexa 488, 568, or 647 (1:600; Invitrogen) were incubated at RT for 2 hr. In some experiments, brain sections were incubated with Hoescht for 5 min. Samples were washed in PBS and mounted in Fluoromount-G (SouthernBiotech).

Hippocampal neurons were fixed in 4% paraformaldehyde (PFA) in PBS for 20 min at RT, permeabilized for 5 min in 0.02% v/v Triton X-100 in PBS, and blocked in 5% BSA for 1 hr. Primary antibodies and secondary antibodies were each incubated for 1 hr at RT. Samples were washed in PBS and mounted in FluorSave Reagent (Millipore). See the Supplemental Experimental Procedures for more details.

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**Figure 6. Synapse Loss, Long-Term Plasticity, and Memory Defects Are Reversible**

(A) Schematic representation of doxycycline feeding schedule in adult mice.

(B) RT-PCR for Dkk1 in mice fed with doxycycline for 2 weeks (On Doxy) or after subsequent 2 weeks without doxycycline (Off Doxy).

(C) Images of hippocampal CA1 show excitatory synapses (co-localized vGlut1 and PSD95 puncta; white arrows). The scale bar represents 2 μm.

(D) Quantification of excitatory synapses (*p < 0.05; Kruskal-Wallis test; six mice per genotype).

(E) Input-output curves show no difference between control and iDkk1 mice after doxycycline withdrawal (nine slices from five controls and 11 slices from seven iDkk1 mice; repeated-measures ANOVA).

(F) TBS-induced LTP in control and iDkk1 mice after doxycycline withdrawal (seven slices from six controls and nine slices from six iDkk1 mice; repeated-measures ANOVA).

(G) Weak LFS failed to induce LTD at the SC-CA1 synapses of control or iDkk1 mice after doxycycline withdrawal (nine slices from seven controls and eight slices from five iDkk1 mice; repeated-measures ANOVA).

(H) Percentage of freezing time evaluated 24 hr after the foot shock (15 control and 14 iDkk1 mice).

Data are represented as mean ± SEM.

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For evaluation of synaptic puncta, stacks of eight equidistant planes (0.2 μm; 76 × 76 nm/pixel) from hippocampal slices and cultured neurons were acquired on an Olympus FV1000 confocal microscope using a 60 × 1.35 numerical aperture (NA) oil objective. Four to seven fields were taken per brain slice, and three to four slices were analyzed per mouse. For hippocampal neurons, eight to ten image stacks of EGFP-transfected cells were taken per condition. Analysis was performed in Velocity software (PerkinElmer). See the Supplemental Experimental Procedures for more details.

**Electrophysiology**

For field potential recordings, parallel bipolar stimulation electrodes were placed in the stratum radiatum of the CA1 region and Schaeffer collateral fibers were stimulated with 0.1 ms duration constant-current paired-pulses (pulse interval 50 ms) delivered to the pathway at intervals of 10 s. Stimulus current was adjusted at the beginning of each recording to give a response approximately 50% of the maximum fEPSPs slope, after recording an input-output curve. fEPSPs were monitored using low-resistance glass pipettes (1–2 MΩ), filled with 4 mM NaCl in ACSF. Slices were subjected to a 15–20 min period of pre-LTP/pre-LTD baseline measurement every 10 s. Provided that the control response did not change by more than 5% during this 15–20 min period, LTP or LTD was induced. LTP was induced by a TBS protocol, which involved delivering two TBSs at an interval of 10 s, and each TBS was composed of five trains of stimuli at intervals of 200 ms, where each train contained four stimuli at 100 Hz. Two protocols of LFS consisting of two trains of 900 pulses delivered at 2 Hz with a 2.5 min gap (strong LFS) or one train of 900 pulses delivered at 2 Hz (weak LFS) were used to induce a LTD. Stimulus intensity for the TBS and LFS was the same as baseline recordings. Paired-pulse fEPSPs (20 Hz) were recorded at intervals of 10 s for at least 50 min after delivery of the TBS or LFS, and the slope of each fEPSP was measured. fEPSP-PPR was calculated as the ratio of the slope of the second to the first fEPSP. Recordings were made using an Axopatch 200B amplifier, filtered (1 kHz) and digitized (10 kHz), and then analyzed using WinEDR software or WinWCP software (freely available at http://spider.science.strath.ac.uk/sipbs/software_ses.htm). For these experiments and patch-clamp recordings, see the Supplemental Experimental Procedures for further information.

**Behavioral Studies**

For all behavioral tests, adult male mice were handled daily for approximately 2 min, at least 4 days before the beginning of the test. Throughout experimentation and data analysis, the experimenter was blind to genotype. MWM, contextual fear conditioning, T-maze spontaneous alternation, open field, and elevated plus maze tasks are described in the Supplemental Experimental Procedures.

**Statistical Analyses**

For behavioral analyses, each mouse group consisted of at least seven animals. For immunofluorescence, data were generated from three or more independent experiments, each with one to four mice per genotype. All results were expressed as mean ± SEM. Statistical significance was calculated on the basis of a Student’s t test, one-way ANOVA, or ANOVA for repeated measures when samples were normally distributed, followed by Scheffe or Bonferroni posteriori comparisons. Mann-Whitney or Kruskal-Wallis tests were used for non-normally distributed data followed by Dunn-Sidak posteriori comparisons (*p < 0.05, **p ≤ 0.001, ***p ≤ 0.01).

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures and five figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.07.024.

**AUTHOR CONTRIBUTIONS**

P.C.S. conceived the project. All authors contributed to the design of experiments, interpretation of the data, and writing of the manuscript. S.P. performed the initial characterization of the Dkk1 induction in the adult hippocampus. D.L., A.M., and M.P. performed behavioral experiments; D.L., S.G., A.M., and F.M. performed cell biology experiments; and A.M. and M.S.-R. performed the electrophysiological recordings. F.C. contributed to design and analysis of behavioral assays. P.C.S. and A.G. provided funding and supervised the project.

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**REFERENCES**

1. Shankar, G.M., and Walsh, D.M. (2009). Alzheimer’s disease: synaptic dysfunction and Abeta. Mol. Neurodegener. 4, 48.

2. Arendt, T. (2009). Synaptic degeneration in Alzheimer’s disease. Acta Neuropathol. 118, 167–179.

3. Budnik, V., and Salinas, P.C. (2011). Wnt signaling during synaptic developmental and plasticity. Curr. Opin. Neurobiol. 21, 151–159.

4. Inestrosa, N.C., and Arenas, E. (2010). Emerging roles of Wnts in the adult nervous system. Nat. Rev. Neurosci. 11, 77–86.

5. Gogolla, N., Galimberti, I., Deguchi, Y., and Caroni, P. (2009). Wnt signaling mediates experience-related regulation of synapse numbers and mossy fiber connectivities in the adult hippocampus. Neuron 62, 510–525.

6. Kuwabara, T., Hsieh, J., Muotri, A., Yeo, G., Warashina, M., Lie, D.C., Moore, L., Nakashima, K., Asashima, M., and Gage, F.H. (2009). Wnt-mediated activation of NeuroD1 and retro-elements during adult neurogenesis. Nat. Neurosci. 12, 1097–1105.

7. Ciani, L., Marzo, A., Boyle, K., Stamatakou, E., Lopes, D.M., Anane, D., McLeod, F., Rosso, S.B., Gibb, A., and Salinas, P.C. (2015). Wnt signalling tunes neurotransmitter release by directly targeting Synaptotagmin-1. Nat. Commun. 6, 8302.

8. De Ferrari, G.V., Papassotiropoulos, A., Biechele, T., Warrant De-Vrieze, F., Avila, M.E., Major, M.B., Myers, A., Sáez, K., Hernández, J.P., Zhao, A., et al. (2007). Common genetic variation within the low-density lipoprotein receptor-related protein 6 gene alternative splice variant is associated with Alzheimer’s disease. Proc. Natl. Acad. Sci. USA 104, 9434–9439.

9. Alarcón, M.A., Medina, M.A., Hu, Q., Avila, M.E., Bustos, B.I., Pérez-Palma, E., Peralta, A., Salazar, P., Ugaré, G.D., Reyes, A.E., et al. (2013). A novel functional low-density lipoprotein receptor-related protein 6 gene alternative splice variant is associated with Alzheimer’s disease. Neurobiol. Aging 34, 1709.e9–1709.e18.

10. Liu, C.C., Tsai, C.W., Deak, F., Rogers, J., Penuliar, M., Sung, Y.M., Maher, J.N., Fu, Y., Li, X., Xu, H., et al. (2014). Deficiency in LRP6-mediated Wnt signaling contributes to synaptic abnormalities and amyloid pathology in Alzheimer’s disease. Neuron 84, 63–77.

11. Mao, B., Wu, W., Li, Y., Hoppe, D., Stannek, P., Glinka, A., and Niehrs, C. (2001). LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. Nature 411, 321–325.

12. Niehrs, C. (2006). Function and biological roles of the Dickkopf family of Wnt modulators. Oncogene 25, 7469–7481.

13. Caricasole, A., Copani, A., Caraci, F., Aronica, E., Rozemuller, A.J., Caruso, A., Storto, M., Gaviraghi, G., Terstappen, G.C., and Nicoletti, F.
(2004). Induction of Dickkopf-1, a negative modulator of the Wnt pathway, is associated with neuronal degeneration in Alzheimer’s brain. J. Neurosci. 24, 6021–6027.

14. Rosi, M.C., Luccarini, I., Grossi, C., Fiorentini, A., Spillantini, M.G., Prisco, A., Scali, C., Giani Friddo, M., Caricasole, A., Terstappen, G.C., and Casamenti, F. (2010). Increased Dickkopf-1 expression in transgenic mouse models of neurodegenerative disease. J. Neurochem. 112, 1539–1551.

15. Bayod, S., Felice, P., Andrés, P., Rosa, P., Camins, A., Pallas, M., and Canudas, A.M. (2015). Downregulation of canonical Wnt signaling in hippocampus of SAMP8 mice. Neurobiol. Aging 36, 720–729.

16. Killick, R., Ribe, E.M., Al-Shawi, R., Malik, B., Hooper, C., Fernandes, C., Dobson, R., Nolan, P.M., Lourdusamy, A., Fumey, S., et al. (2011). Clusterin regulates β-amyloid toxicity via Dickkopf-1-driven induction of the wnt-PCP-JNK pathway. Mol. Psychiatry 16, 89–99.

17. Curic, S.A., Dickins, E.M., and Salinas, P.C. (2012). The secreted Wnt antagonist Dickkopf-1 is required for amyloid β-mediated synaptic loss. J. Neurosci. 32, 3492–3498.

18. Cisse, M., Halabisky, B., Harris, J., Devidze, N., Dubal, D.B., Sun, B., Orr, B., et al. (2014). Bie, B., Wu, J., Yang, H., Xu, J.J., Brown, D.L., and Naguib, M. (2014). Intranasal administration of nerve growth factor antagonist Dickkopf-1 is required for amyloid β-mediated synaptic loss. J. Neurosci. Biobehav. Protoc. 14, 88–98.

19. Rosi, M.C., Luccarini, I., Grossi, C., Fiorentini, A., Spillantini, M.G., Prisco, A., Scali, C., Giani Friddo, M., Caricasole, A., Terstappen, G.C., and Casamenti, F. (2010). Increased Dickkopf-1 expression in transgenic mouse models of neurodegenerative disease. J. Neurochem. 112, 1539–1551.

20. Nagahara, A.H., Merrill, D.A., Coppola, G., Csillag, S., Hall, E., and Cattaneo, A. (2005). Intranasal administration of nerve growth factor (NGF) rescues recognition memory deficits in AD11 anti-NGF transgenic mice. Proc. Natl. Acad. Sci. USA 102, 3811–3816.

21. Bayod, S., Felice, P., Andrés, P., Rosa, P., Camins, A., Pallas, M., and Canudas, A.M. (2015). Downregulation of canonical Wnt signaling in hippocampus of SAMP8 mice. Neurobiol. Aging 36, 720–729.

22. Killick, R., Ribe, E.M., Al-Shawi, R., Malik, B., Hooper, C., Fernandes, C., Dobson, R., Nolan, P.M., Lourdusamy, A., Fumey, S., et al. (2011). Clusterin regulates β-amyloid toxicity via Dickkopf-1-driven induction of the wnt-PCP-JNK pathway. Mol. Psychiatry 16, 89–99.

23. Curic, S.A., Dickins, E.M., and Salinas, P.C. (2012). The secreted Wnt antagonist Dickkopf-1 is required for amyloid β-mediated synaptic loss. J. Neurosci. 32, 3492–3498.

24. Cisse, M., Halabisky, B., Harris, J., Devidze, N., Dubal, D.B., Sun, B., Orr, B., et al. (2014). Bie, B., Wu, J., Yang, H., Xu, J.J., Brown, D.L., and Naguib, M. (2014). Intranasal administration of nerve growth factor antagonist Dickkopf-1 is required for amyloid β-mediated synaptic loss. J. Neurosci. Biobehav. Protoc. 14, 88–98.

25. Rosi, M.C., Luccarini, I., Grossi, C., Fiorentini, A., Spillantini, M.G., Prisco, A., Scali, C., Giani Friddo, M., Caricasole, A., Terstappen, G.C., and Casamenti, F. (2010). Increased Dickkopf-1 expression in transgenic mouse models of neurodegenerative disease. J. Neurochem. 112, 1539–1551.

26. Bayod, S., Felice, P., Andrés, P., Rosa, P., Camins, A., Pallas, M., and Canudas, A.M. (2015). Downregulation of canonical Wnt signaling in hippocampus of SAMP8 mice. Neurobiol. Aging 36, 720–729.

27. Killick, R., Ribe, E.M., Al-Shawi, R., Malik, B., Hooper, C., Fernandes, C., Dobson, R., Nolan, P.M., Lourdusamy, A., Fumey, S., et al. (2011). Clusterin regulates β-amyloid toxicity via Dickkopf-1-driven induction of the wnt-PCP-JNK pathway. Mol. Psychiatry 16, 89–99.

28. Rosi, M.C., Luccarini, I., Grossi, C., Fiorentini, A., Spillantini, M.G., Prisco, A., Scali, C., Giani Friddo, M., Caricasole, A., Terstappen, G.C., and Casamenti, F. (2010). Increased Dickkopf-1 expression in transgenic mouse models of neurodegenerative disease. J. Neurochem. 112, 1539–1551.

29. Bayod, S., Felice, P., Andrés, P., Rosa, P., Camins, A., Pallas, M., and Canudas, A.M. (2015). Downregulation of canonical Wnt signaling in hippocampus of SAMP8 mice. Neurobiol. Aging 36, 720–729.

30. Killick, R., Ribe, E.M., Al-Shawi, R., Malik, B., Hooper, C., Fernandes, C., Dobson, R., Nolan, P.M., Lourdusamy, A., Fumey, S., et al. (2011). Clusterin regulates β-amyloid toxicity via Dickkopf-1-driven induction of the wnt-PCP-JNK pathway. Mol. Psychiatry 16, 89–99.

31. Curic, S.A., Dickins, E.M., and Salinas, P.C. (2012). The secreted Wnt antagonist Dickkopf-1 is required for amyloid β-mediated synaptic loss. J. Neurosci. 32, 3492–3498.

32. Cisse, M., Halabisky, B., Harris, J., Devidze, N., Dubal, D.B., Sun, B., Orr, B., et al. (2014). Bie, B., Wu, J., Yang, H., Xu, J.J., Brown, D.L., and Naguib, M. (2014). Intranasal administration of nerve growth factor antagonist Dickkopf-1 is required for amyloid β-mediated synaptic loss. J. Neurosci. Biobehav. Protoc. 14, 88–98.

33. Rosi, M.C., Luccarini, I., Grossi, C., Fiorentini, A., Spillantini, M.G., Prisco, A., Scali, C., Giani Friddo, M., Caricasole, A., Terstappen, G.C., and Casamenti, F. (2010). Increased Dickkopf-1 expression in transgenic mouse models of neurodegenerative disease. J. Neurochem. 112, 1539–1551.

34. Bayod, S., Felice, P., Andrés, P., Rosa, P., Camins, A., Pallas, M., and Canudas, A.M. (2015). Downregulation of canonical Wnt signaling in hippocampus of SAMP8 mice. Neurobiol. Aging 36, 720–729.
53. Purro, S.A., Ciani, L., Hoyos-Flight, M., Stamatakou, E., Siomou, E., and Krause, U., Ryan, D.M., Clough, B.H., and Gregory, C.A. (2014). An unexpected role for a Wnt-inhibitor: Dickkopf-1 triggers a novel cancer survival mechanism through modulation of aldehyde-dehydrogenase-1 activity. Cell Death Dis. 5, e1093.

54. Krause, U., Ryan, D.M., Clough, B.H., and Gregory, C.A. (2014). An unexpected role for a Wnt-inhibitor: Dickkopf-1 triggers a novel cancer survival mechanism through modulation of aldehyde-dehydrogenase-1 activity. Cell Death Dis. 5, e1093.

55. Caneparo, L., Huang, Y.L., Staudt, N., Tada, M., Ahrendt, R., Kazanskaya, Endo, Y., Beauchamp, E., Woods, D., Taylor, W.G., Toretsky, J.A., Uren, Petratos, S., Li, Q.X., George, A.J., Hou, X., Kerr, M.L., Unabia, S.E., Pozueta, J., Lefort, R., Ribe, E.M., Troy, C.M., Arancio, O., and Shelanski, Sfakianos, M.K., Eisman, A., Gourley, S.L., Bradley, W.D., Scheetz, A.J., Cappuccio, I., Calderone, A., Busceti, C.L., Biagioni, F., Mastroiacovo, F., Busceti, C.L., Biagioni, F., Moyanova, S.G., Meisler, M.H., Battaglia, G., Caricasole, A., Bruno, V., and Nicoletti, F. (2009). Induction of the Wnt antagonist, Dickkopf-1, contributes to the development of neuronal death in models of brain focal ischemia. J. Cereb. Blood Flow Metab. 29, 264–276.

56. Davis, E.K., Zou, Y., and Ghosh, A. (2008). Wnts acting through canonical and noncanonical signaling pathways exert opposite effects on hippocampal synapse formation. Neural Dev. 3, 32.

57. Sharma, K., Choi, S.Y., Zhang, Y., Nieland, T.J., Long, S., Li, M., and Huganir, R.L. (2013). High-throughput genetic screen for synaptogenic factors: identification of LRP6 as critical for excitation synapse development. Cell Rep. 5, 1330–1341.

58. Liu, J., Zhang, Y., Xu, R., Du, J., Hu, Z., Yang, L., Chen, Y., Zhu, Y., and Gu, L. (2013). PI3K/Akt-dependent phosphorylation of GSK3β and activation of RhoA regulate Wnt5a-induced gastric cancer cell migration. Cell. Signal. 25, 447–456.

59. fortress, A.M., Schram, S.L., Tuscher, J.J., and Frick, K.M. (2013). Canonical Wnt signaling is necessary for object recognition memory consolidation. J. Neurosci. 33, 12619–12626.

60. Bailey, C.H., and Kandel, E.R. (1993). Structural changes accompanying memory storage. Annu. Rev. Physiol. 55, 397–428.

61. Ge, Y., Dong, Z., Bagot, R.C., Howland, J.G., Phillips, A.G., Wong, T.P., and Wang, Y.T. (2010). Hippocampal long-term depression is required for the consolidation of spatial memory. Proc. Natl. Acad. Sci. USA 107, 16697–16702.

62. Morris, R.G., Anderson, E., Lynch, G.S., and Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature 319, 774–776.