Experience-induced forgetting by WT1 enables learning of sequential tasks.

Chiara Mariottini [1,2], Leonardo Munari [1], Ellen Gunzel [1,2,], Joseph M. Seco [1,], Nikos Tzavaras [1], Jens Hansen [1,2], Sarah A. Stern [4,5], Virginia Gao [4,5], Georgia E. Hodes [3], Scott J. Russo [3], Vicki Huff [6], Marc Birtwistle [1,2], Cristina M. Alberini [4], Robert D. Blitzer [1,7], and Ravi Iyengar[*1,2].

1, Department of Pharmacology and Systems Therapeutics, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

2, Systems Biology Center New York Icahn School of Medicine at Mount Sinai , New York, NY, USA.

3, Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

4, Center for Neural Science, New York University, New York, NY, USA.

5, Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

6, Department of Genetics, University of Texas M.D. Anderson Cancer Center, Houston, TX , USA.

7, Department of Psychiatry, Icahn School of Medicine at Mount Sinai , New York, NY, USA.

[*]Correspondence and requests to:

Dr. Ravi Iyengar

Dept. of Pharmacology and Systems Therapeutics, Box 1215

Icahn School of Medicine at Mount Sinai

One Gustave Levy Place

New York, NY,10029

ravi.iyengar@mssm.edu

Phone 212-659-1707

Abstract

Remembering and forgetting are important aspects of normal behavioral adaptation; however, the molecular basis of forgetting has been less studied. Using rat and mouse models we find that WT1, a transcriptional repressor that is activated in the hippocampus by LTP producing stimuli and behavioral memory, enables forgetting. Acute or tonic knockdown of WT1 did not affect short-term memory but enhanced long-term memory and enables a switch from circuit to cellular computation in the hippocampus. A control theory model predicts that WT1 could be a general repressor of memory or a regulator that preserves the ability to remember multiple sequential experiences. Using sequential training for two tasks, mice with non-functional WT1 have better memory for the first task, but show impaired memory for the second task. Taken together, our observations indicate that WT1 mediates an experience-activated forgetting process that preserves the capability of the animal to remember other new experiences.

One sentence summary

The transcription factor WT1 is a core component of an active forgetting process, and is required for normal behavioral flexibility by allowing LTM for successive experiences.

Introduction

"Without forgetting it is quite impossible to live at all" – a statement by Nietzsche succinctly summarizes homeostasis at higher levels of human behavior and cognition. Remembering and forgetting are coupled activities within a system whereby a balance between the two processes is essential for system performance and sustenance. While the mechanisms of the formation and consolidation of memories have been extensively studied in humans and model organisms from rodents to flies and worms, studies on forgetting have been relatively sparse. The study of remembering and forgetting as integrated phenomena has been largely pursued by cognitive psychologists who have emphasized the role of interference processes in the loss of memory as an active form of forgetting (*1, 2*). Active forgetting by interference is thought to affect two processes: the consolidation of a memory, and the retrieval of a consolidated memory. A study in *Drosophila* has shown that Rac is involved in forgetting by inhibiting the consolidation of labile memories (*2*). A study in rodents provided evidence that the growth and connectivities of new neurons disrupt memories and enable forgetting (*3*). Implicit in this line of reasoning about forgetting is a time delay between the learning process required for memory formation and the active processes involved in forgetting. Across scales of biological organization, the relationship between remembering and forgetting resembles the relationship between the forward and reverse reactions of a reversible biochemical reaction such as protein phosphorylation where the level of the phosphorylated state of the protein depends on the balance of protein kinase and protein phosphatase enzymatic activities. This analogy leads to a new conjecture that remembering and forgetting could be tightly coupled learned processes that proceed over similar time frames, and that the formation of memory represents a balance between these processes.

A general postulate in systems biology is that higher order physiological functions of organs arise from interactions between molecular components within the cells that make up the organ. The molecular (*4*) and cellular basis (*5*) of memories have been vast areas of studies that provide strong support for this general principle. Based on this principle we hypothesized that local processes of remembering and forgetting, scaled to the brain circuit level, optimize physiological properties to ensure stable encoding of new experiences while preserving the capacity to

remember additional experiences that could occur at any time. If this hypothesis is correct, we should be able to discover molecular components and cellular and circuit mechanisms that underlie the active forgetting process. We have used both rat and mouse models to identify molecular and cellular mechanisms that underlie early active forgetting. As the encoding of context-related experience initially takes place in the hippocampus (*6, 7*), we hypothesized that near simultaneous active forgetting also is likely to occur there. Moreover, since at the molecular level memory formation involves transcriptional regulators such as CREB family proteins (*8*) and secreted ligands such as BDNF(*9*) and IGF2 (*10, 11*), we anticipated that forgetting also might involve transcriptional regulation and secreted and diffusible ligands. We searched widely for potential forgetting-related transcriptional regulators that are activated by induction of LTP (long term potentiation) at excitatory synapses, a cellular model of learning and memory (*12*). We identified Wilm's Tumor 1 (WT1), a key protein that is critical for kidney development (*13*), as a transcriptional regulator that is activated by hippocampal LTP induction and behavioral learning in rats. The role of WT1 in forgetting was tested using antisense oligonucleotide-mediated acute knockdown of WT1 expression in rats and conditional expression of non-functional WT1 gene in mice. Our results indicate that WT1 in CA1 pyramidal neurons controls circuit-level memory encoding to enable active forgetting.

Study design

Since this study involves multiple experimental approaches that taken together could lead to misinterpretation of results, we devised experimental designs that would reduce the possibility of such errors. We conducted most key experiments in two animal models. The target of interest, the transcription factor WT1, was downregulated in rat hippocampus with antisense oligonucleotide injection. Parallel experiments were conducted in mice homozygous for a conditionally inactive Wt1 allele ($Wt1^{fl}$) under transcriptional control of the CaMKIIα promoter expressed in the forebrain. This approach ensures that the convergent results we obtained were not due to either off-target effects of antisense oligonucleotides or unveiling of an adaptive response in mice. We conducted analogous electrophysiological experiments in both animal models, and only results obtained in both systems were used for further investigation and

analysis. We also conducted multiple analogous behavioral experiments in both models. Biochemical determinants that were initially identified in rats, such as extracellular ligands under the control of WT1, were tested in electrophysiological experiments in both species. These coordinated analyses yielded a set of convergent results and the conclusions from these results are consistent across the animal models, providing confidence that our interpretation is correct.

Results

WT1 activity in excitatory CA1 neurons interferes with forgetting and future learning

Although the roles of individual transcription factors in LTP and learning and memory are well established, a broad experimental characterization of the transcription factors activated by LTP and memory formation was not available when we started this study. We utilized an unbiased approach to identify transcription regulators that were activated by strong high-frequency stimulation (Strong-HFS) of the Schaffer collaterals in rat hippocampal slices, which induces protein synthesis-dependent late-LTP (L-LTP) at synapses on pyramidal neurons in CA1 neurons (*14*). Using a commercial protein/DNA binding array, we found nearly 40 transcription factors (TFs) that were activated (Fig. 1A and Fig. S1A). One intriguing factor in this list was WT1, a transcriptional repressor that also is involved in mRNA targeting and translation (*15*). WT1 is a well-established regulator of kidney development (*13*) but is not known to play any role in normal brain function. Independent evidence for WT1 activation following L-LTP induction came from mRNA-seq experiments that identified genes whose expression levels changed 90 minutes after induction of L-LTP. Computational analysis allowed us to predict the transcription factors that control the expression of these genes (see Table S1 for complete list of DEG), and we found that WT1 was ranked second, followed by members of the CREB family (ATF2 and ATF4; Fig. S1B). WT1 binding to a DNA consensus sequence increased after induction of L-LTP, providing functional evidence for WT1 activation (Fig. 1B). In addition to this increase in WT1 activity, WT1 protein expression was increased in area CA1 30 minutes after L-LTP induction (Fig. S2A).

Immunohistochemical analysis in area CA1 of both rat and mouse hippocampus showed that WT1 protein is predominantly localized within the nuclei of pyramidal neurons (Fig. 1C and 1D respectively) with a weaker immunoreactivity within the proximal apical dendrites (Fig. 1D), while WT1 immunoreactivity was not detected in GFAP-positive astrocytes (Fig. 1C).

We determined the role of WT1 in hippocampal plasticity using an acute ablation model. WT1 antisense oligonucleotides (WT1 ODNs) were injected *in vivo* bilaterally into the dorsal hippocampus (2 injections per side, each 2 nmol, separated by 2 h) and significantly decreased WT1 protein levels in area CA1 compared to controls injected with scrambled ODNs (Fig. S3A). We hypothesized that if WT1 is a regulator of learning and memory, then its ablation would interfere with the retention of hippocampus-dependent forms of memory (see behavioral protocol in Fig. 1E, top panel). Animals injected with either WT1 ODNs or scrambled ODNs did not exhibit any difference in memory retention 1 hr after training in a novel object placement test (NOPT) (Fig. 1E, bottom left panel). However, we saw significantly enhanced memory in rats injected with WT1 ODNs for both NOPT and contextual fear conditioning (CFC) when tested 24 hr after training (Fig. 1E, bottom left and right panel). These convergent findings in two different behavioral tests suggest that WT1 is a regulatory transcription factor that is activated during memory formation, but suppresses memory. Rats injected with WT1 ODNs or Scrambled ODNs-injected rats did not differ in locomotor activity (Fig. S3B).

A single bilateral injection of WT1 ODNs (2 nmol/side) also enhanced the performance of rats trained in inhibitory avoidance (IA; another hippocampus-dependent memory task) and tested 24 hr after training (Fig. S3C, bottom left panel), although the effects were variable; a single bilateral injection of a higher dose of WT1 ODNs (4 nmol/side) produced the same significant increase in memory retention measured 24 hr after training (Fig. S3C, bottom right panel); however this higher dose tended to induce seizure activity in the animals, suggesting that loss of WT1 increases excitability and neuronal activity in the hippocampus. Such seizures were not observed when control scrambled ODNs (4 nmol/side) were injected into each side of the hippocampus (Fig. S3C). Hence, for behavioral experiments, we avoided single high doses of the WT1 ODNs and greatly reduced the number of animals that showed seizures (Fig. S3D). Any animal that exhibited seizure activity was excluded from the study.

Since Strong-HFS increased the expression and transcriptional activity of WT1 (Fig. 1A and 1B, and Fig. S1A and S2A), we asked whether behavioral training would produce similar effects. We trained rats in CFC and found that WT1 binding to DNA was increased in dorsal hippocampus after training (Fig. 1F), as was WT1 expression after training in CFC or inhibitory avoidance (Fig. S2B and S2C).

To evaluate the role of WT1 on synaptic plasticity and memory across different species, we generated a cohort of experimental mice by crossing *Wt1*$^{fl/fl}$ animals (*16*) with *Camk2a-Cre* transgenic mice, in which expression of Cre-recombinase is under the transcriptional control of a 8.5kb region 5' of the *Camk2a* gene (*17*) (Jackson lab: http://jaxmice.jax.org/strain/005359.html). Expression of this transgene recombinase in *Wt1*$^{fl/fl}$ animals results in the forebrain expression of an in-frame internal *Wt1* deletion allele encoding a truncated WT1 protein that lacks zinc fingers 2 and 3 (Fig. S4A), which are thought to be essential for WT1 DNA and RNA binding activity (*18*) (*19*). *Wt1*$^{fl/fl}$*; Camk2a-Cre* mice (referred from now on as *Wt1Δ* mice) are viable, of normal size and weight, with no gross differences in hippocampal morphology compared to wild-type littermates (referred from now on as *Control* mice; Fig. S4B). The transgenic mice also were similar to littermate controls with respect to protein levels in their urine and blood chemistry (metabolic enzyme and electrolyte panel; Fig. S4C and S4D). Animals from the two groups showed similar open field activity and pain responses (Fig. S5A and S5B). However, *Wt1Δ* mice had a significant tendency towards an anxious phenotype when scored in an elevated plus maze (Fig. S5C).

In agreement with the WT1 acute knockdown rat model, we saw that mice lacking functional WT1 in the hippocampus showed enhanced LTM for NOPT when tested 24 hr after training (Fig. 1G). This enhancement was specific to LTM, since it was not seen when NOPT was tested 4 hr after training (Fig. 1G). The *Wt1Δ* mice also showed enhanced LTM for CFC, tested 24 h after training (Fig. 1H)

The effects of WT1 on LTM prompted us to determine if there was any effect of WT1 conditional knockout on extinction of conditioned fear. We found that compared to control littermates, *Wt1Δ* mice were deficient in extinguishing the fear memory (Fig. 1I), indicating that the ability of the hippocampus to flexibly encode and retain memory requires functional WT1.

WT1 imposes a requirement for circuit-level computation in synaptic plasticity

Contextual memory is encoded by persistent forms of synaptic plasticity in the hippocampus, particularly LTP (*20, 21*); therefore, we hypothesized that WT1 knockdown would affect the ability to induce or maintain hippocampal LTP. Single intrahippocampal injection of WT1 ODNs significantly decreased WT1 expression (Fig. S6A) but did not alter basal synaptic transmission compared to slices from control rats injected with scrambled ODNs (Fig. S6B), nor did it affect the induction or maintenance of LTP induced by Strong-HFS (Fig. S6C). However, a role for WT1 in synaptic plasticity was revealed by stimulation with a weak high-frequency stimulation (Weak-HFS) protocol, which produced only decremental synaptic potentiation in control slices but stable LTP in slices from animals injected with WT1 ODNs (Fig. 2A). To test whether an effect of WT1 depletion on interneurons might contribute to the observed enhancement of LTP by facilitating induction (*22*), we stimulated slices with Weak-HFS in the presence of the GABA-A receptor antagonist bicuculline. Under these conditions, slices from WT1-depleted hippocampi still showed enhanced LTP, indicating that WT1 likely regulates synaptic plasticity through direct effects on pyramidal neurons (Fig. 2B). While a hallmark of L-LTP is the requirement for *de novo* protein synthesis, translation inhibitors only modestly inhibited the persistent LTP that was induced in WT1-depleted hippocampus (Fig. S6D), suggesting that WT1 knockdown itself might increase the expression of important plasticity-related proteins.

Acute WT1 knockdown also enhanced long-term depression (LTD), induced by synaptic stimulation or by direct activation of metabotropic glutamate receptors (Fig. S6E and S6F), suggesting that WT1 is a general regulator of synaptic plasticity in rats.

In agreement with the results seen with acute WT1 knockdown in rats, hippocampal slices obtained from *Wt1Δ* mice showed sustained LTP following Weak-HFS, while slices from control littermates produced only transient potentiation (Fig. 2C). When compared to their control littermates, *Wt1Δ* mice showed increased basal Schaffer collateral – CA1 synaptic efficiency with no difference in paired-pulse ratio (Fig. 2D), indicating that WT1 regulates synaptic efficiency through a postsynaptic mechanism. In contrast to acute knockdown of WT1 in rats,

Wt1Δ mice expressed normal LTD (data not shown). Due to this difference in the two animal models, we did not examine LTD in further experiments.

We hypothesized that WT1 knockdown might enhance LTP by increasing pyramidal cell excitability, since postsynaptic spiking during stimulation facilitates LTP induction (*23*). To test this hypothesis, whole-cell recordings were obtained from pyramidal neurons in area CA1 of rat hippocampus. In neurons from WT1-depleted hippocampi, weak depolarizing currents (20-50 pA) were more likely to evoke action potentials than in neurons from scrambled ODNs-injected hippocampi (Fig. 2E), indicating that WT1 knockdown increases excitability. Notably, in response to relatively strong depolarizations (70-100 pA), neurons in WT1-depleted slices fired *fewer* action potentials than those treated with scrambled ODNs (mean number of spikes = 2.1 ± 0.173 and 1.375 ± 0.125, respectively; two-tailed t-test, $p < 0.05$. See traces). No significant differences were observed in the amplitude, frequency or inter-event interval in both spontaneous and mEPSCs (Fig. S7A and S7B). These results suggest that WT1 serves to dampen the postsynaptic response to a weak stimulus, while preserving the normal dynamic range of the response to superthreshold stimuli.

The role of the CA1 region in memory processing involves the circuit-level integration of information arriving from the entorhinal cortex via two major inputs: (1) the direct temporoammonic pathway, in which entorhinal neurons of the perforant path synapse on distal apical dendrites of CA1 pyramidal neurons, and (2) an indirect input, in which entorhinal activity provides phase-delayed information to proximal apical dendrites in CA1 through a series of three synapses: perforant path→dentate gyrus, mossy fibers→CA3, and Schaffer collaterals→CA1. The CA1 pyramidal neuron functions as a coincident detector, integrating the temporally segregated streams of information from cortical activity (*24*). This coincidence detection function can be studied in hippocampal slices where the two inputs are activated independently (Fig. 2F, left panel) (*25*). We reasoned that the anti-plasticity effect of WT1 could impose a need for convergent activity of both inputs to induce LTP at the Schaffer collateral (SC) - CA1 synapse, and that WT1 depletion might allow SC stimulation alone to fully induce LTP without the added information provided by the TA input (Fig. 2F, right panel). We tested this hypothesis by inducing LTP at the SC→CA1 synapses with theta-burst stimulation (TBS) at both the temporoammonic and SC inputs, with SC stimulation phase-delayed relative to

temporoammonic. In hippocampi from control rats injected with scrambled ODNs, the induction of LTP required activation of both inputs (Fig. 2G, left and right panel). However, the TA input became dispensable in WT1-depleted hippocampus, so that SC stimulation alone was as effective in inducing LTP as dual pathway stimulation (Fig. 2G, middle and right panel). Thus, the normal level of WT1 imposes a requirement for circuit-level computation in the CA1 neuron, leading to LTP. In contrast, in WT1-depleted hippocampus circuit-level computation no longer is necessary: SC→CA1 activity can induce long lasting LTP without confirmatory input from TA→CA1. Combined with our findings of increased pyramidal cell excitability and altered spike encoding of depolarization in WT1-depleted hippocampus, this result indicates that WT1 activity regulates the computational properties of CA1 pyramidal cells, by controlling the likelihood of inducing LTP based on activity in only one input to CA1.

WT1 utilizes diffusible cytokines as downstream effectors

One of the best characterized genes modulated by WT1 transcriptional activity is insulin-related growth factor 2 (*Igf2*) (*26*), which we have shown to be a memory and LTP enhancer (*10*, *11*). Using quantitative real time RT-PCR, we confirmed that acute WT1 knockdown leads to a significant increase in IGF2 mRNA expression in dorsal hippocampus (Fig. 3A). To identify additional genes that could mediate the ability of WT1 depletion to enhance hippocampal synaptic plasticity and memory, we used mRNA-seq to determine which mRNAs were modulated in rat hippocampus by acute injection of WT1 ODNs. We found that among the many (~100) differentially expressed genes (Table S2) were some that have been previously implicated in synaptic plasticity and memory (e.g. *Egr1*, also known as *Zif268*). Interestingly, we found that ~30 of the differentially expressed genes coded for secreted diffusible molecules (Fig. 3B, top panel). These were, in large part, well-characterized immune system and stress response-related genes (Fig. 3B, bottom panel), suggesting that WT1 could utilize cytokines to control synaptic function and memory formation.

Based on the genes whose expression increased upon acute WT1 knockdown, and which therefore are normally repressed by WT1, we examined several potential mediators for the enhanced synaptic plasticity seen in WT1-depleted hippocampus. We found that a specific

antibody against the IGF2 receptor (IGF2-R Ab) interfered with the ability of WT1 knockdown to enhance LTP induction (Fig. 3C), consistent with the hypothesis that loss of WT1-mediated repression of *Igf2* modulates, at least in part, the enhanced plasticity of the WT1 acute knockdown model. We then investigated both *Ccl2* and *Il1β*, which were among the most highly up-regulated genes following WT1 depletion, since previous findings implicated both cytokines in synaptic plasticity and memory (*27-30*). CCL2 has excitatory and pro-plasticity effects (*27*), and we found that a CCL2 receptor antagonist (CCL2-RA, aka RS-102895) reduced the ability of WT1 depletion to enhance LTP, induced either by weak stimulation of SC alone (Weak HFS; Fig. 3C) or by dual stimulation of the SC and TA inputs (TBS; Fig. 3D). No significant effect was observed when slices injected with scrambled ODNs were incubated with CCL2 receptor antagonist and exposed to dual stimulation of the SC and TA inputs, suggesting that CCL2 is not required for L-LTP induced by dual pathway activation (Fig. S8A). In contrast to CCL2, most studies have shown IL1β to exert an *inhibitory* effect on LTP and memory (*29, 30*) (but see also(*31*)). Recombinant IL1β (rat rIL1b) accordingly showed a trend to reduce the LTP enhancement in slices from WT1 ODNs-treated rats (Fig. 3E). Interestingly, another gene that is up-regulated by WT1 depletion encodes IL1-RN, which is an endogenous inhibitor of the IL1 receptor (Table S2). In control rat hippocampal slices, recombinant IL1-RN (rat rIL1-RN) enhanced LTP induced by weak HFS to the SC input, but it had no effect in acutely WT1-depleted hippocampus (Fig. 3E). These results suggest that in the WT1 acute knockdown rat model, the inhibitory effects of IL1β on LTP are, at least in part, neutralized by a simultaneous increase in the expression of IL1-RN (*28*).

Extending these findings to the mouse model, we found that bath application of the antibody against the IGF2 receptor (IGF2-R Ab) or the CCL2 receptor antagonist (CCL2-RA) inhibited LTP in slices from *Wt1Δ* animals (Fig. 3F).

WT1 controls active forgetting required for sequential learning from different experiences

What could be the value of WT1-mediated forgetting in the hippocampus? One possibility is that active forgetting serves to prevent excessive encoding of low-salience events in LTM, consistent with the observation that WT1 depletion enables relatively weak input from the trisynaptic

pathway (CA3→CA1 alone in slice experiments) to induce LTP (Fig. 2G). However the consequences of changing WT1 levels are not intuitively obvious. To obtain experimentally testable predictions we developed a toy control theory-based model of an information processing and response system. In the model we postulated that experience activates two parallel pathways: a pro-memory pathway that includes transcription factors like CREB and EGR1, and a pro-forgetting pathway that includes WT1. Together the pathways control the activity level of effectors to regulate balance between remembering and forgetting. The total number of effectors could either be in excess of that needed to encode multiple experiences, or they could limit the encoding capacity of the corticohippocampal circuit (Fig. 4A). We used this model to run simulations to sudy the effect of varying the activity of the pro-forgetting pathway for a fixed stimulus. The results of the simulations are shown in (Fig. S9A). The model predicts that if the encoding capacity of the corticohippocampal circuit is limited, then over-encoding of the first experience could interfere with the ability to encode subsequent experiences. Alternatively if effectors were not limiting then reducing WT1 levels could enhance the ability to remember both experiences (this is shown schematically in the bar graphs in Fig. 4A and in the simulation results in Fig. S9). Since WT1 level controls the activity dynamics of the pro-forgetting pathway, we asked how WT1 depletion would *interfere* with normal learning of two sequential behavioral experiences. We first trained mice in NOPT, which does not yield LTM in wild-type animals; the second experience was CFC, which normally does induce LTM (Fig. 4B). *Wt1Δ* mice showed significant LTM for NOPT, while *Controls* did not (Fig. 4C). LTM for NOPT was similarly enhanced in *Wt1Δ* mice when testing was delayed until 48 hr after training (Fig. S10A). When we tested for sequential learning we found that in *Wt1Δ* mice the first NOPT experience reduced LTM for the subsequent CFC test: these mice showed lower levels of freezing compared to control littermates when tested 24 hr after CFC training (Fig. 4D). We probed the duration of this proactive interference effect by extending the interval between NOPT and CFC to 10 days (Fig. 4E). Consistent with previous experiments, *Wt1Δ* mice showed enhanced 24-hr retention for NOPT (Fig. 4F). When CFC was delayed, these mice showed no deficit in LTM for the subsequent CFC training (Fig. 4G), suggesting that a homeostatic process restores the number of available effectors over time. Taken together, these data indicate that WT1 is critical for an active forgetting process that prevents anterograde interference between successive learning experiences.

Discussion

This study identifies transcriptional repression by WT1 as a key process that controls memory and capacity to learn by actively enabling forgetting. At the cellular and circuit levels, several forms of signaling determine the organization and persistence of memory. Among these, the roles of synaptic transmission, membrane excitability, and intracellular signaling are well known. Less recognized is the importance of neuronally released local diffusible signals that act as paracrine and autocrine signals, which are better known for organizing the connectivity of the developing nervous system. In the adult hippocampus, activated neurons release diffusible signals, such as growth factors and cytokines that contribute to synaptic plasticity and memory formation. Most of these proteins, such as IGF2, CCL2, BDNF, and Wnts (*32-35*), constitute one component of a distributed set of pro-memory pathways. In an experience-activated neuronal circuit, released pro-memory diffusible signals could increase the number of neurons that encode a memory for that experience. For example, factors released by activated neurons within the circuit would facilitate plasticity in nearby neurons. This signaling represents an associative mechanism for encoding a complex experience under conditions where at least some of the neurons in the circuit have been activated. Such associativity need not be limited to interactions between neurons, pro-memory factors could enhance LTP at weakly stimulated synapses on a neuron, increasing that neuron's contribution to the engram.

Associativity over long intervals (*36*) is advantageous for linking together experiences that are causally related despite the time that has elapsed, such as the earliest locations in a traveled path that culminates in reward or punishment. However, prolonged opportunities for associative interactions also risk exhausting the available encoding capacity of a circuit due to excessive plasticity. This study indicates that WT1 and transcriptional repression are critical for preventing such over-encoding, since loss of WT1 allows a relatively weak experience to produce LTM of an experience that interferes with normal encoding for a subsequent experience that occurs two days later. Importantly, WT1 is rapidly activated by experience (contextual learning or synaptic stimulation). Thus, WT1 is a key molecule in an *active pro-forgetting* process that limits

encoding of LTM. Since WT1 regulates the expression of many genes, it is likely that the pro-forgetting process is mediated by multiple mechanisms, one of which is WT1-mediated repression of released pro-plasticity factors such as IGF2, IL1RN, and CCL2. In addition, experience-driven increases in WT1 may recruit intracellular signaling cascades that antagonize the consolidation of STM to LTM.

Accurate encoding and retrieval of LTM in the corticohippocampal circuit relies on coordinated activity in two major inputs, both originating in the entorhinal cortex but activating hippocampal CA1 neurons either directly, or through the trisynaptic pathway. At the level of CA1 neuron, a nonlinear response to synaptic input might underlie the synergistic effect of activating both pathways (*37*). In our study, depletion of WT1 eliminated the requirement for dual input to CA1 neurons. This shift in computational capability from the circuit to cell level reduces learning capacity for multiple experiences. If one considers the organism as a system that needs to maintain the capability to properly encode a stream of external events without prior knowledge of how often new events may occur, then preserving capability to remember future events even while stably encoding a current event, represents an essential adaptation for survival. If so, Nietzsche's statement is not only philosophy but also basic biology.

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Author Contributions

C.M. conducted most of the biochemical experiments, the surgeries in rats and the behavior experiments in rats. L.M. conducted all the behavior experiments in mice. E.G. and J.M.S performed field recordings experiments while N.T. did patch clamp experiments. J.H. analyzed mRNA seq data and did enrichment analysis. S.A.S and V.G. helped with inhibitory avoidance experiments and contextual fear conditioning experiments in rats. G.E.H. and S.J.R. provided behavior equipments for mice experiments. V.H. provided the *Wt1*$^{fl/fl}$ animals. M.B. developed and ran the memory homeostasis model. C.M., L.M., C.M.A., R.D.B. and R.I. designed the experiments and computational models. C.M., R.D.B. and R.I. wrote the manuscript. All authors assisted in editing the manuscript.

Figure Legends

Fig. 1: Wilm's Tumor 1 (WT1) activation in excitatory CA1 neurons inhibits learning. A, Protein-DNA binding assay comparing rat CA1 extracts from control tissue (left) with extracts obtained from stimulated tissue collected 30 minutes after stimulation with Strong-HFS (right). WT1 is circled in red; numbers in parentheses indicate two different DNA probes with WT1 consensus sites. **B**, Electromobility shift assay (EMSA) showed that *in vitro* WT1 binding to a DNA consensus sequence (WT1/DNA complex) was increased 10 and 30 minutes after stimulation with Strong-HFS (Stim 10, 30) compared to control unstimulated CA1 (C). The specificity of DNA-protein binding was verified by incubation with excess unlabeled cold probe (CP). **C**, Immunostaining of rat CA1 showed that WT1 is expressed in pyramidal neurons and not GFAP (glial fibrillary acidic protein) positive astrocytes. Scale bar=50 µm. **D**, Immunostaining of mouse CA1 regions showed WT1 expression mainly in cell bodies but also in proximal dendrites stained with β-tubulin antibody. Scale bar=50 µm. **E**, Protocol for behavioral experiments in the WT1 acute knockdown rat model (top). Arrows indicate bilateral injections (2 nmol/side) of either WT1 ODNs or scrambled ODNs. Injections of WT1 ODNs did not affect memory retention in a novel object placement task (NOPT) 1 hr after training, since both groups showed similar exploration times of the new location (measured as recognition index; see Methods). In contrast, at 24 hr WT1 ODNs-injected animals better memory than controls

(bottom left; ** p<0.01). Similarly, injections of WT1 ODNs increased freezing time in rats trained in contextual fear conditioning (CFC) and tested 24 h after training (bottom right; ** p<0.01). **F**, Electromobility shift assay (EMSA) showed increased WT1 binding to DNA after CFC training. Nuclear proteins from dorsal hippocampi were extracted from shocked rats (S) at indicated times after training and compared to nuclear extracts obtained from rats that were exposed to the same context but that did not receive a footshock (C). The specificity of DNA-protein binding was verified by incubation with excess of unlabeled cold probe (CP). **G**, Compared to control littermates (*Control*), conditional expression of truncated WT1 in transgenic mice (*Wt1*Δ mice) did not affect the amount of time animals spent exploring the new location in NOPT when tested 4 hrs after training; conversely *Wt1*Δ animals spent significantly more time exploring the new location when tested 24 hr after training (* p<0.05). **H**, *Wt1*Δ animals showed enhanced freezing 24 hr after training in CFC (** p<0.01). **I**, *Wt1*Δ mice exhibited a lower rate of extinction than their control littermates, expressed as mean ± s.e.m. % freezing on Day 1 (bottom left panel) or as the % freezing on Day 5/Day 1 (bottom right panel; * p<0.05).

Fig. 2: WT1 regulates synaptic function, L-LTP, and excitability in CA1 pyramidal neurons, and integration of cortical inputs to the hippocampus. A, Antisense-mediated knockdown of WT1 increased the ability of weak-HFS (delivered at arrow) to induce LTP. Representative fEPSPs show superimposed traces recorded during baseline and 60 min post-HFS. Calibrations: 0.5 mV/10 ms (left panel). The increase in fEPSP slope above baseline during the final 10 minutes was greater in slices from hippocampi injected with WT1 ODNs compared with controls injected with scrambled ODNs (right panel; mean fEPSP ± s.e.m.; two-way ANOVA, * p<0.05). **B**, WT1 ODNs mediated LTP enhancement was not mediated by effects on inhibitory interneurons, since it was intact in the presence of bicuculline (10 μM). Representative fEPSPs show superimposed traces recorded during baseline and 60 min post-HFS. Calibrations: 0.5 mV/10 ms. fEPSP slope over the final 10 minutes of recording showed that treatment with WT1 ODNs significantly enhanced L-LTP (right panel; mean fEPSP ± s.e.m.; two-way ANOVA, * p<0.05). **C**, *Wt1*Δ mice showed enhanced LTP compared to control littermates (*Control*). Representative fEPSPs show superimposed traces recorded during baseline and 60

min post-HFS. Calibrations: 0.5 mV/10 ms. Summary of the final 10 minutes of recording showed that LTP was significantly enhanced in WT1 transgenic mice compared to their control littermates (right panel; mean fEPSP ± s.e.m; two-way ANOVA, * $p<0.05$). **D**, *Wt1*Δ mice showed increased basal synaptic efficiency (left panel; linear regression t-test, *** $p<0.001$) but did not affect paired-pulse ratio (right panel; two-way ANOVA, $p>0.05$). **E,** WT1 depletion increased excitability of CA1 pyramidal neurons. Whole-cell patch recordings were obtained in current clamp mode, and the number of spikes evoked by a series of depolarizing current steps was counted. Inset on the right shows the probability of evoking at least one spike in response to a weak (20-50 pA) or a stronger (60-90 pA) current step in neurons from WT1-depleted (red) or control (gray) hippocampi (n=4 for WT1 ODNs, n=5 for scrambled ODNs; two-tailed Chi-square test, ** $p< 0.01$). Resting membrane potential and input resistance measured -63.75 ± 3.15 mV and 105.8 ± 21.76 MΩ in the WT1 ODNs group, and -60.80 ± 2.85 mV and 109.3 ± 20.04 MΩ in the Scrambled ODNs group. Inset on the left shows representative traces in cells from WT1 ODNs-injected (red) or Scrambled ODNs-injected (gray) hippocampi. Calibration: 50 mV/100 ms. **F**, Model of WT1 depletion effect on corticohippocampal input to CA1. Left: Normally, activation of both the direct temporoammonic pathway (blue) and the trisynaptic pathway (green) are required for LTP induction at the Schaffer collateral (SC) → CA1 synapse. Right panel: In WT1-deficient hippocampus, enhanced basal efficiency of SC → CA1 signaling and / or CA1 excitability enable trisynaptic pathway activity alone to induce LTP. EC= entorhinal cortex; DG = dentate gyrus. **G**, TBS of the Schaffer collaterals (SC) induced stable LTP in slices from rats injected with Scrambled ODNs only when combined with phase-delayed TBS at the temporoammonic (TA) pathway (left and right panels). Conversely, in slices from WT1 ODNs-injected hippocampi, the same TBS of SC alone induced LTP, which did not differ from that induced by dual-pathway TBS (center and right panels). Representative fEPSPs show superimposed traces recorded during baseline and 60 min post-TBS. Calibrations: 0.5 mV/10 ms. Statistical analysis by two-way ANOVA, * $p<0.05$).

Fig. 3: **Similar effectors mediate WT1 action in rats and mice. A,** Quantitative real time PCR showed that WT1 acute knockdown significantly increases IGF2 mRNA expression (* $p<0.05$). **B**, Top: Heatmap of secreted molecules whose mRNA expression was regulated by WT1 acute

knockdown as determined by mRNA-seq. Numbers above each gene indicate the rank position of that gene based on the log_2-fold change. For the complete list of all the differentially expressed genes after WT1 acute knockdown see Table S2. Bottom: List of the top 10 biological processes most significantly affected by WT1 acute knockdown as identified by mRNA-seq. **C**, WT1 ODNs-mediated LTP enhancement was blocked by bath application of a specific antibody directed against the IGF2 receptor (IGF2-R Ab, 5 μg/ml) or by an antagonist of the CCL2 receptor (CCL2-RA, RS102895, 10 μM). Representative fEPSPs show superimposed traces recorded during baseline and 60 min post-HFS. Calibrations: 0.5 mV/10 ms (left panel). Summary of the final 10 minutes of recording showed that LTP in WT1 ODNs injected slices was significantly reduced by bath application of IGF2-R Ab or CCL2-RA (right panel; mean fEPSP ± s.e.m; two-way ANOVA, ** $p<0.01$ for IGF2-R Ab; * $p<0.05$ for CCL2-RA). For ease of comparison data for the scrambled ODNs and the WT1 ODNs group, in both the time course and bar graph, are the same that have been shown in Fig. 2A. **D**, Induction of LTP by dual-pathway stimulation requires CCL2 receptor activation. In the presence of the CCL2 receptor antagonist RS102895 (CCL2-RA; 10 μM), combined TBS of the SC and TA pathway failed to induce LTP. Representative fEPSPs show superimposed traces recorded during baseline and 60 min post-TBS. Calibrations: 0.5 mV/10 ms (left panel). Summary of the final 10 minutes of recording showed that in WT1 ODNs injected slices LTP induced by dual pathway stimulation was significantly reduced by bath application of CCL2-RA (right panel; mean fEPSP ± s.e.m; two-way ANOVA; * $p<0.05$). **E**, Bath application of rat recombinant IL1β (rIL1β, 100 ng/ml) or of rat recombinant IL-1 RN (rIL-1 RN, 1 μg/ml) did not significantly affect the magnitude of LTP induced by WT1 knock-down; conversely bath application of rIL-1 RN significantly enhanced LTP induced by Weak-HFS in control tissue. Representative fEPSPs show superimposed traces recorded during baseline and 60 min post-HFS. Calibrations: 0.5 mV / 10 ms (left panel). The arrow indicates time of Weak-HFS (left panel). Summary graph indicates mean fEPSP ± s.e.m. for the final 10 minutes of the recording (right panel; two-way ANOVA, **$p< 0.01$ for Control vs Control+ rIL1-RN; $p>0.05$ for WT1 ODNs vs WT1 ODNs + rIL1β and WT1 ODNs vs WT1 ODNs + rIL1-RN). For ease of comparison data for the WT1 ODNs group in both the time course and the bar graph are the same that have been shown in Fig. 2A. **F**, The enhanced LTP induced by Weak-HFS in *Wt1*Δ slices was abolished by bath application of a specific antibody against the IGF2 receptor (IGF2-R Ab, 5 μg/ml) or an antagonist for the CCL2

receptor (CCL2-RA, RS102895, 10 μM). Representative fEPSPs show superimposed traces recorded during baseline and 60 min post-HFS. Calibrations: 0.5 mV/10 ms. (left panel). Summary of the final 10 minutes of recording showed that LTP in hippocampal slices from *Wt1*Δ mice was significantly reduced by bath application of IGF2-R Ab or CCL2-RA (right panel; mean fEPSP ± s.e.m; two-way ANOVA, * $p<0.05$). For ease of comparison data for the *Wt1*Δ group in the bar graph are the same as in Fig. 2C.

Figure 4: WT1 mediated forgetting. A, Schematic representation of WT1-mediated regulation of forgetting. An initial experience such as task 1 (NOPT) activates both pro-memory and pro-forgetting pathways. When the pro-forgetting pathways are inhibited such as by ablation of WT1 there is prolonged memory for task 1. Such retention of task 1 memory may or may not interfere with the ability to remember a task 2 (CFC) based on the availability of effectors (limiting vs in excess). **B**, Summary scheme for sequential training. **C**, *Wt1*Δ animals showed increased time spent exploring the new location when first trained in NOPT and tested 24 hr after training; * $p<0.05$. **D**, *Wt1*Δ mice subsequently trained on CFC spent less time freezing when tested 24 hr later; ** $p<0.01$). **E**, Summary scheme for long-interval sequential training. **F**, *Wt1*Δ animals showed increased time spent exploring the new location when first trained in NOPT and tested 24 hr after training; * $p<0.05$). **G**, *Wt1*Δ mice spent comparable amount of time freezing when CFC training was performed 10 days after NOPT training; $p>0.05$.

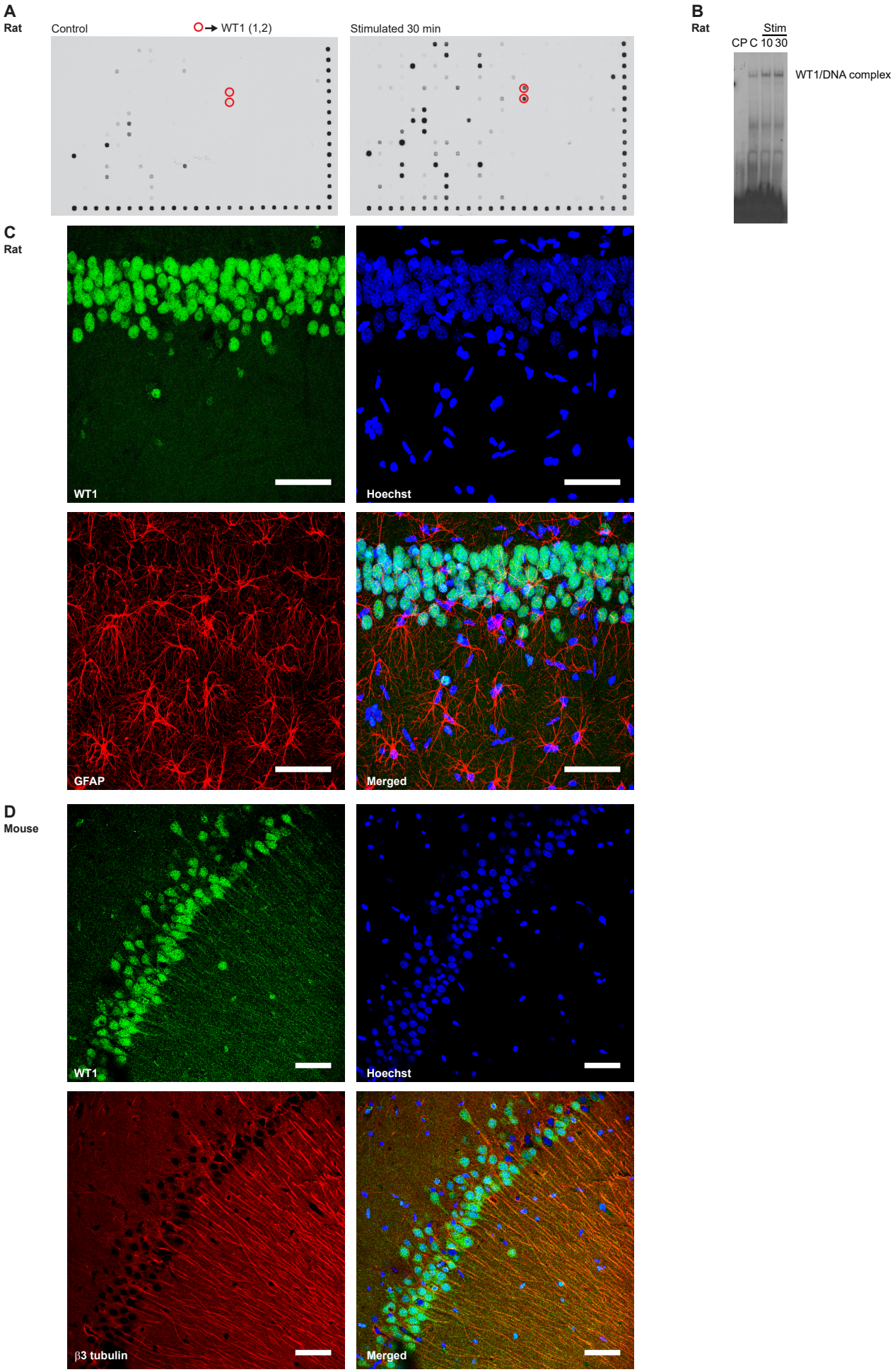
A
Rat
Control
WT1 (1,2)
Stimulated 30 min
B
Rat
Stim
CP C 10 30
WT1/DNA complex
C
Rat
WT1
Hoechst
GFAP
Merged
D
Mouse
WT1
Hoechst
β3 tubulin
Merged

Fig. 1, part I

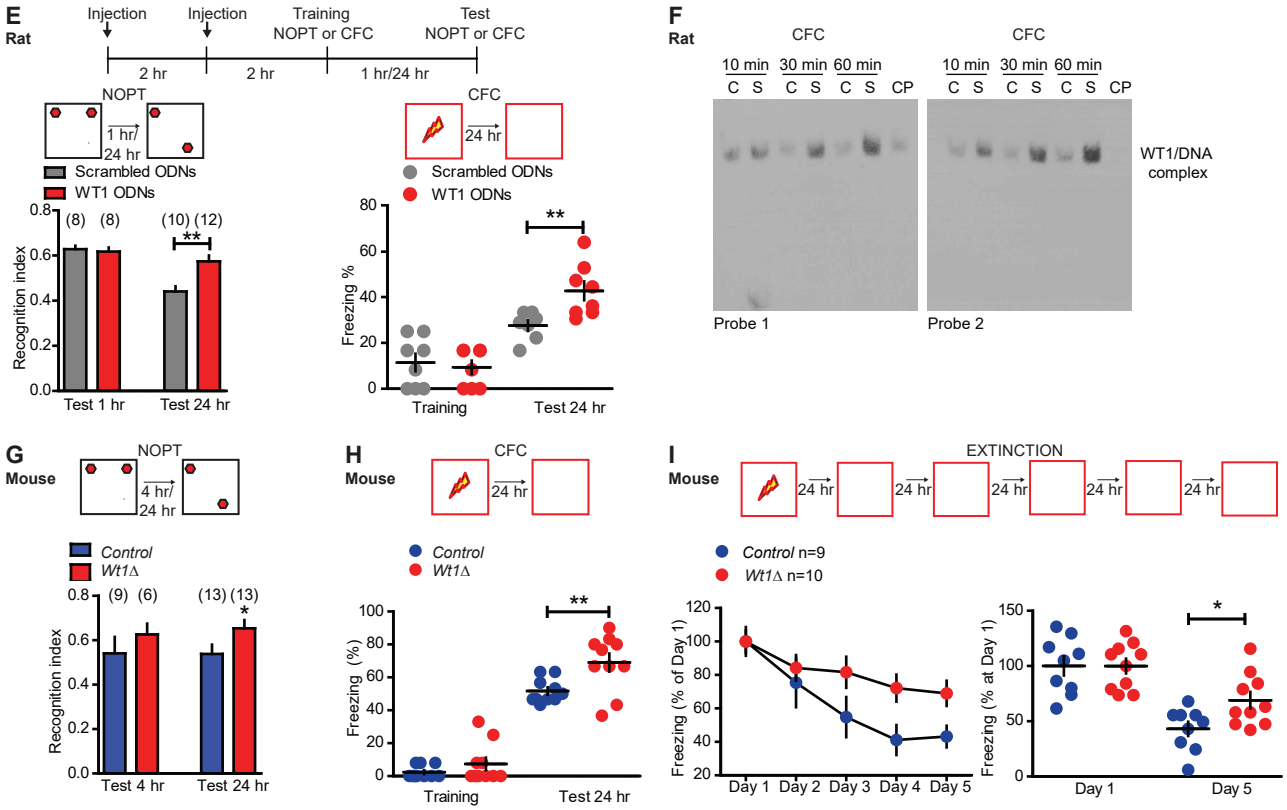
E
Rat
Injection
Injection
Training
NOPT or CFC
Test
NOPT or CFC
2 hr
2 hr
1 hr/24 hr
NOPT
1 hr/
24 hr
Scrambled ODNs
WT1 ODNs
Recognition index
(8) (8)
(10) (12)
**
Test 1 hr
Test 24 hr
CFC
24 hr
Scrambled ODNs
WT1 ODNs
Freezing %
**
Training
Test 24 hr
F
Rat
CFC
CFC
10 min 30 min 60 min
C S C S C S CP
WT1/DNA
complex
Probe 1
Probe 2
G
Mouse
NOPT
4 hr/
24 hr
Control
Wt1Δ
Recognition index
(9) (6)
(13) (13)
*
Test 4 hr
Test 24 hr
H
Mouse
CFC
24 hr
Control
Wt1Δ
Freezing (%)
**
Training
Test 24 hr
I
Mouse
EXTINCTION
24 hr
Control n=9
Wt1Δ n=10
Freezing (% of Day 1)
Day 1 Day 2 Day 3 Day 4 Day 5
Freezing (% at Day 1)
*
Day 1
Day 5

Fig. 1, part II

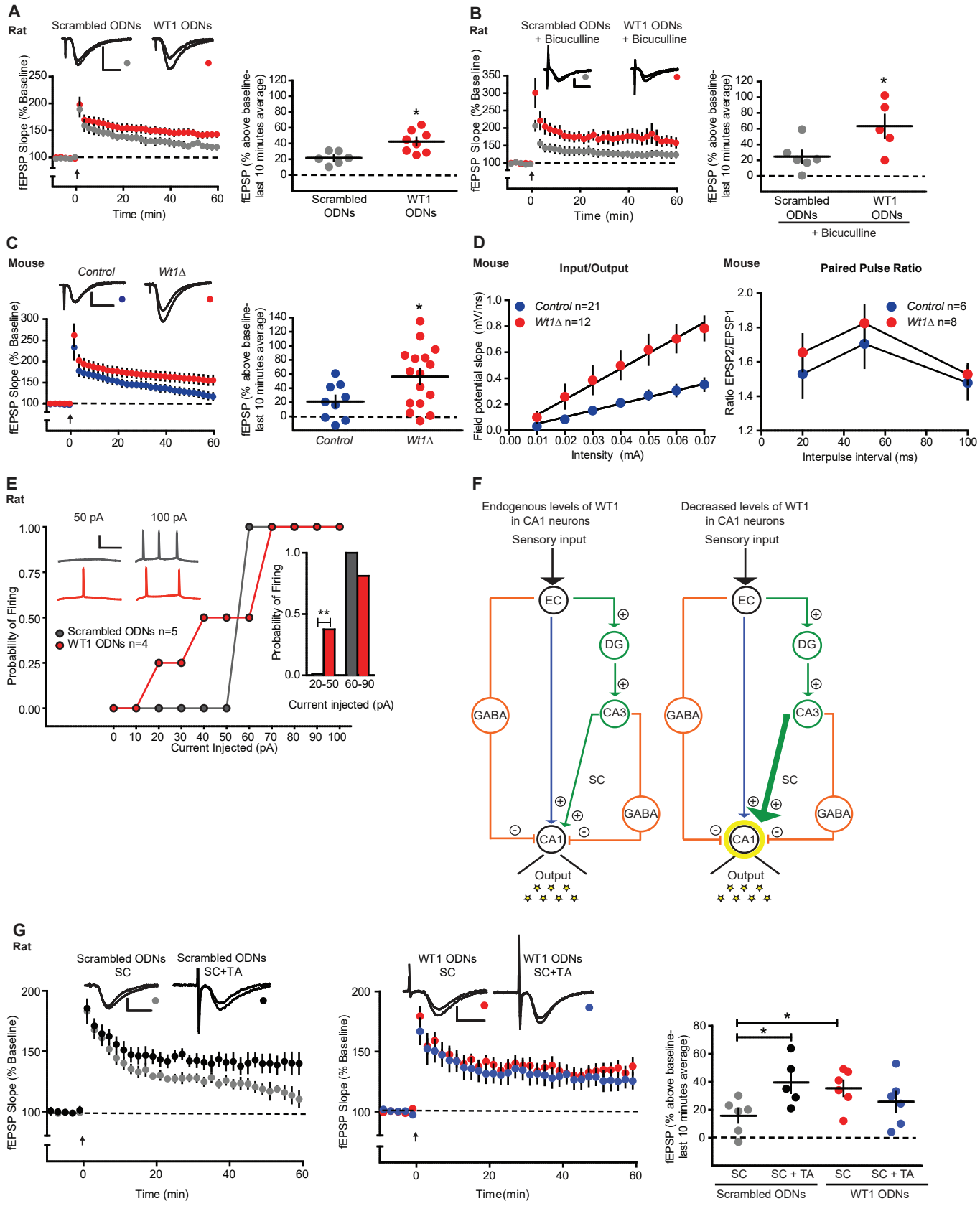
A
Rat
Scrambled ODNs
WT1 ODNs
fEPSP Slope (% Baseline)
Time (min)
fEPSP (% above baseline-last 10 minutes average)
Scrambled ODNs
WT1 ODNs
B
Rat
Scrambled ODNs + Bicuculline
WT1 ODNs + Bicuculline
fEPSP Slope (% Baseline)
Time (min)
fEPSP (% above baseline-last 10 minutes average)
Scrambled ODNs
WT1 ODNs
+ Bicuculline
C
Mouse
Control
Wt1Δ
fEPSP Slope (% Baseline)
fEPSP (% above baseline-last 10 minutes average)
Control
Wt1Δ
D
Mouse
Input/Output
Control n=21
Wt1Δ n=12
Field potential slope (mV/ms)
Intensity (mA)
Mouse
Paired Pulse Ratio
Control n=6
Wt1Δ n=8
Ratio EPSP2/EPSP1
Interpulse interval (ms)
E
Rat
50 pA
100 pA
Probability of Firing
Scrambled ODNs n=5
WT1 ODNs n=4
Current Injected (pA)
20-50
60-90
Current injected (pA)
F
Endogenous levels of WT1 in CA1 neurons
Decreased levels of WT1 in CA1 neurons
Sensory input
EC
DG
CA3
CA1
GABA
SC
Output
G
Rat
Scrambled ODNs SC
Scrambled ODNs SC+TA
WT1 ODNs SC
WT1 ODNs SC+TA
fEPSP Slope (% Baseline)
Time (min)
fEPSP (% above baseline-last 10 minutes average)
SC
SC + TA
Scrambled ODNs
WT1 ODNs

Fig. 2

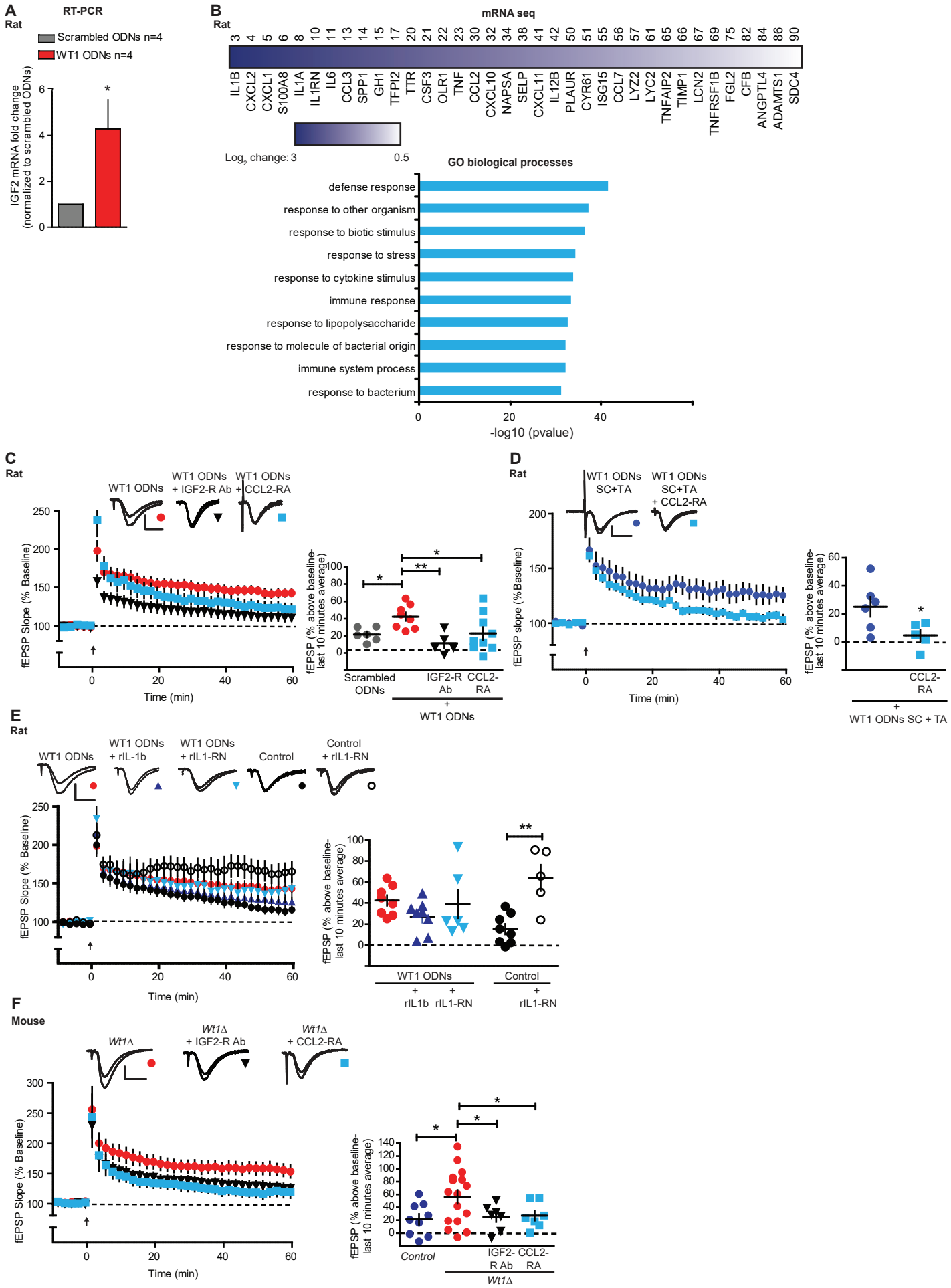
A
Rat
RT-PCR
Scrambled ODNs n=4
WT1 ODNs n=4
IGF2 mRNA fold change (normalized to scrambled ODNs)
B
Rat
mRNA seq
IL1B CXCL2 CXCL1 S100A8 IL1A IL1RN IL6 CCL3 SPP1 GH1 TFPI2 TTR CSF3 OLR1 TNF CCL2 CXCL10 NAPSA SELP CXCL11 IL12B PLAUR CYR61 ISG15 CCL7 LYZ2 LYC2 TNFAIP2 TIMP1 LCN2 TNFRSF1B FGL2 CFB ANGPTL4 ADAMTS1 SDC4
Log2 change: 3
0.5
GO biological processes
defense response
response to other organism
response to biotic stimulus
response to stress
response to cytokine stimulus
immune response
response to lipopolysaccharide
response to molecule of bacterial origin
immune system process
response to bacterium
-log10 (pvalue)
C
Rat
WT1 ODNs
WT1 ODNs + IGF2-R Ab
WT1 ODNs + CCL2-RA
fEPSP Slope (% Baseline)
Time (min)
fEPSP (% above baseline- last 10 minutes average)
Scrambled ODNs
IGF2-R Ab
CCL2-RA
+ WT1 ODNs
D
Rat
WT1 ODNs SC+TA
WT1 ODNs SC+TA + CCL2-RA
fEPSP slope (%Baseline)
Time (min)
fEPSP (% above baseline- last 10 minutes average)
CCL2-RA
+ WT1 ODNs SC + TA
E
Rat
WT1 ODNs
WT1 ODNs + rIL-1b
WT1 ODNs + rIL1-RN
Control
Control + rIL1-RN
fEPSP Slope (% Baseline)
Time (min)
fEPSP (% above baseline- last 10 minutes average)
WT1 ODNs
Control
+ rIL1b
+ rIL1-RN
+ rIL1-RN
F
Mouse
Wt1Δ
Wt1Δ + IGF2-R Ab
Wt1Δ + CCL2-RA
fEPSP Slope (% Baseline)
Time (min)
fEPSP (% above baseline- last 10 minutes average)
Control
IGF2-R Ab
CCL2-RA
Wt1Δ

Fig. 3

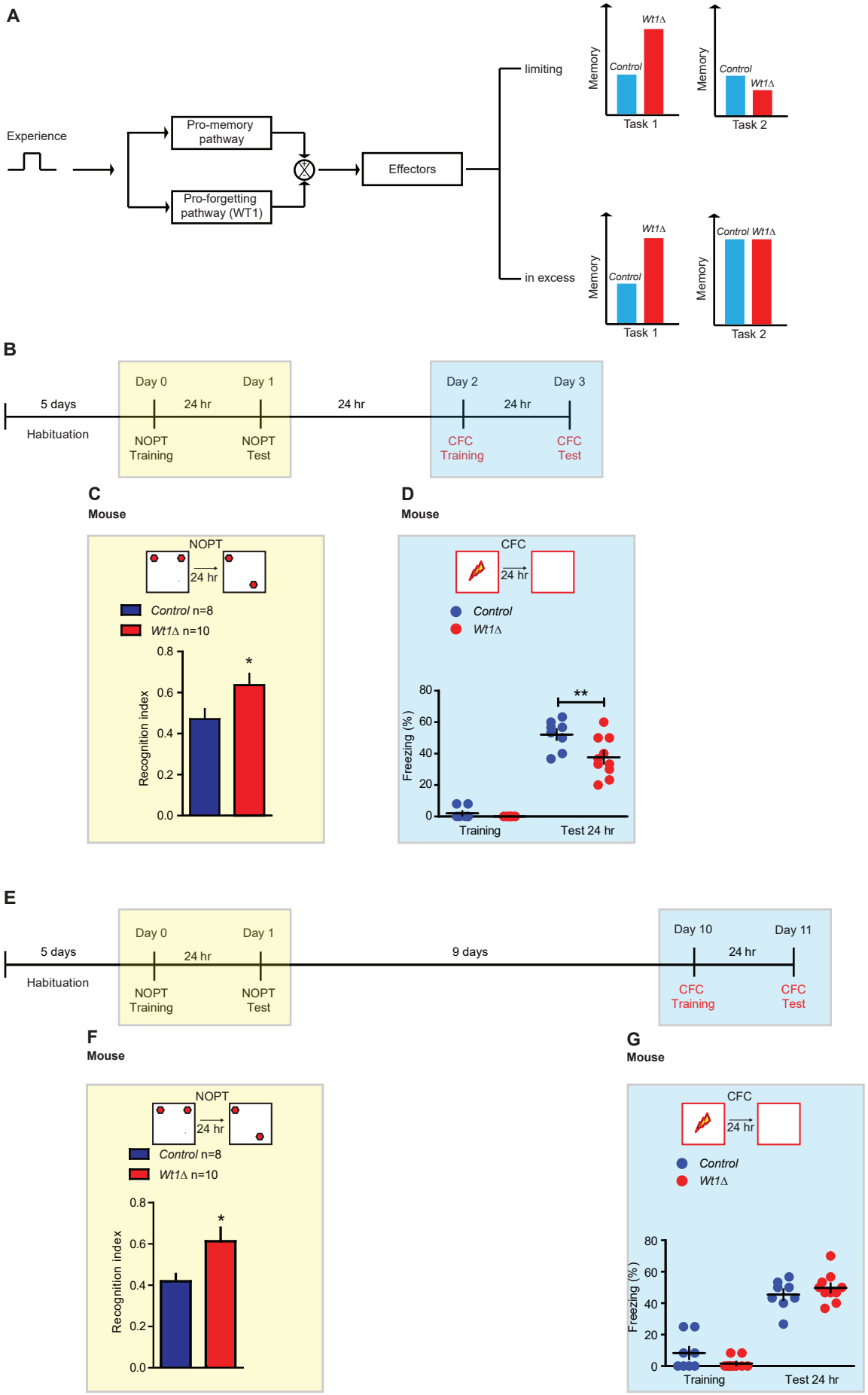
A
Experience
Pro-memory pathway
Pro-forgetting pathway (WT1)
Effectors
limiting
in excess
Memory
Control
Wt1Δ
Task 1
Task 2
B
5 days
Habituation
Day 0
Day 1
24 hr
NOPT Training
NOPT Test
Day 2
Day 3
CFC Training
CFC Test
C
Mouse
NOPT
Control n=8
Wt1Δ n=10
Recognition index
D
Mouse
CFC
Freezing (%)
Training
Test 24 hr
E
9 days
Day 10
Day 11
F
Mouse
G
Mouse

Fig. 4