NCS-1 in the Dentate Gyrus Promotes Exploration, Synaptic Plasticity and Rapid Acquisition of Spatial Memory

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SUMMARY

The molecular underpinnings of exploration and its link to learning and memory remain poorly understood. Here we show inducible modest overexpression of neuronal calcium sensor 1 (Ncs1) selectively in the adult murine dentate gyrus (DG) promotes a specific form of exploratory behavior. The mice also display a selective facilitation of long-term potentiation (LTP) in the medial perforant path and a selective enhancement in rapid-acquisition spatial memory, phenotypes reversed by direct application of a cell-permeant peptide (DNIP) designed to interfere with NCS-1 binding to the dopamine type-2 receptor (D2R). Moreover, the DNIP and the D2R-selective antagonist, L-742-626 attenuated DG LTP and spatial memory in control mice. These data demonstrate a role for NCS-1 and D2R in DG plasticity and provide novel insight for understanding how the DG contributes to the origin of exploration and spatial memory acquisition.
INTRODUCTION

The remarkable plasticity of the tri-synaptic hippocampal network is suggested to underlie several neural processes including new acquisition of spatial memory (Goodrich-Hunsaker et al., 2008; Kandel, 2006; Nakazawa et al., 2004; Scoville and Milner, 1957; Squire et al., 2004) and curiosity-driven exploration (Lever et al., 2006). While the three best studied anatomical hippocampal subregions, CA1, CA3 and DG (the dentate gyrus) must act cooperatively to produce a functional hippocampus, strong evidence demonstrates each subregion performs its own specific, specialized operations. For example, region CA1 is involved in the temporal pattern association and temporal pattern completion aspects of memory formation (Klausberger and Somogyi, 2008), while subregion CA3 supports spatial pattern association and spatial pattern completion (Kesner, 2007b). The DG, on the other hand, orthogonalizes sensory information cumulated from the entorhinal cortex (EC) and together with area CA3, underlies spatial pattern separation (Kesner et al., 2004). Detection of spatial novelty depends on both areas CA3 and DG (Kesner et al., 2004) and the perirhinal cortex (Kumaran and Maguire, 2007). Because novelty detection is a prerequisite for novelty exploration, plasticity mechanisms within these regions are ideally suited to serve a role in the generation of curiosity-driven behavior, a possibility we explore here. Enhancement of plasticity via genetic manipulation in the forebrain improves long-term spatial memory formation (Malleret et al., 2001), but analogous experiments have not been described for more anatomically restricted regions.
within the hippocampus. Here we report the emergence of specific increases in exploration, facilitated plasticity and enhanced rapid acquisition of spatial memory following inducible modest overexpression of neuronal calcium sensor 1 (\textit{Ncs1}) selectively in the adult DG of a transgenic mouseline (DGNCS-1 mice).

NCS-1 plays a critical role in several forms of neuromuscular physiology and short-term neuroplasticity (for review see (Braunewell, 2005; Burgoyne, 2007; Burgoyne et al., 2004; Burgoyne and Weiss, 2001; Hilfiker, 2003) including axonal development in \textit{Lymnaea stagnalis} (Hui et al., 2007), Kv4-current modulation in the mouse myocardium (Guo et al., 2002), P/Q-type calcium channel activity-dependent facilitation in rat Calyx of Held (Tsujimoto et al., 2002), neurotransmitter release in \textit{Xenopus} and \textit{Drosophila} (Pongs et al., 1993; Wang et al., 2001) and long-term depression (LTD) in the perirhinal cortex (Jo et al., 2008). In mammals, \textit{Ncs1} is highly expressed in the DG and dynamically regulated during in vivo perforant path long-term potentiation (LTP) (Genin et al., 2001), but whether the increase in \textit{Ncs1} mRNA is a result of LTP or a factor in its induction or maintenance is unknown. Here we show DGNCS-1 mice have a lower threshold and higher ceiling for LTP in the corticohippocampal medial perforant path (MPP).

This is exciting because LTP is widely considered a molecular model for mammalian learning and memory in mammals and \textit{Ncs1} is already known to control memory in \textit{C. elegans} (Gomez et al., 2001), an invertebrate system. Though the neural circuits important for NCS-1-mediated memory in \textit{C. elegans} were established, molecular mechanisms remain elusive and no role for NCS-1
in learning and memory in mammals has been reported. An enhancement in rapid acquisition of spatial memory in DGNCS-1 mice is the first demonstration of a role for NCS-1 in learning and memory in higher organisms, where the nervous system is more complex (Lein et al., 2007) and physiological correlates exist (Bliss and Collingridge, 1993).

NCS-1 binds dopamine-type 2 receptors (D2R), regulates D2R phosphorylation through an interaction with G-protein-coupled receptor kinase 2 (GRK2) and controls D2R surface expression in kidney 293 cells (Kabbani et al., 2002). However, there is no evidence for an in vivo role for NCS-1 in D2R regulation. Here we show increased surface expression of D2R selectively in the DG molecular layer of DGNCS-1 mice, where dopaminergic modulation of synaptic plasticity may be critical to memory formation (Korz and Frey, 2007; Kovacs et al., 1979; Manahan-Vaughan and Kulla, 2003). For example, pharmacological antagonism of D2R reduces basolateral amygdala-DG LTP (Abe et al., 2008) and pharmacological activation of D2R improves working memory (Wilkerson and Levin, 1999) and alleviates scopolamine-induced passive-avoidance amnesia (Sigala et al., 1997). Yet, pharmacological antagonism of D2R can impair water maze learning if administered immediately post-training (Setlow and McGaugh, 2000), and how D2R is regulated during memory acquisition is unclear. Many of these studies employed systemic injections that affect the entire CNS, whereas the function of D2R is likely anatomically distinct. In DGNCS-1 mice, changes to D2R surface expression in the DG MPP may directly facilitate plasticity and enhance spatial memory.
because these effects were blocked by a small cell-permeant D2R/NCS-1 Interfering Peptide (DNIP) designed to compete with NCS-1 binding to D2R.

What makes animals curious is a difficult question to examine. In essence, we can only infer curiosity based on the behavior of the animal, much as we can only infer learning and memory based on the performance of an animal in a well-designed task. Recently, rearing – environmental exploration supported on hind legs – has emerged as a method to examine curiosity-driven exploration in rodents (Lever et al., 2006). Rearing behaviour in mice can be induced using either stressful or non-stressful novelty; rearing in response to stressful stimuli, for example a cat or brightly lit arena, is motivated by fear, whereas rearing in safe, comfortable environments is thought to be motivated by curiosity (Lever et al., 2006). We aimed to examine specific effects on curiosity-driven exploration by comparing rearing behaviour in these two distinct types of environments and found DGNCS-1 mice demonstrate a selective enhancement in rearing in response to non-stressful novelty, which was reversed by direct infusion of the DNIP to the DG in cannulized subjects. DGNCS-1 also showed increased exploration in the hole board test and a selective increase in exploring novel environments under dimly-lit conditions.

Because human NCS1 expression is associated with psychiatric disorders (Koh et al., 2003) and D2R is a major target for anti-psychotic treatment, it is also of clinical interest to investigate what role the interaction between NCS-1 and D2R plays in the mammalian nervous system. Here we restrict our focus to the
DG and provide novel insight for how this specialized brain area controls exploration and spatial memory.
RESULTS

Generation of Mice with Inducible Ncs1 Overexpression in the DG

We generated mice with inducible overexpression of rat Ncs1 selectively in DG granular neurons using the rtTA2-M2 system (Michalon et al., 2005) (Figure 1A). Both the tetO-ncs (GenBank construct submission FJ756409) and rtTA2 (construct described in Michalon et al., 2005) lines were created on the identical background strain in order to exclude potential differences between genotypes arising from contaminating donor alleles (Armstrong et al., 2006). By using the rat gene, silent single nucleotide divergence between mouse and rat provided a simple reverse transcriptase-based discernment of exogenous vs endogenous Ncs-1 mRNA, whilst conserving the NCS-1 amino acid sequence (Figure 1B). Camk2a-rtTA2 mice (rtTA2) bred to a rtTA-responsive-teto-LacZ reporter line showed prominent rtTA2 expression in the dorsal DG granule cells of the hippocampal formation (Figure 1C). Rat Ncs1 expression was doxycyline (dox)-dependent and only occurred in double transgenic mice (DGNCS-1) positive for both the rtTA2 and tetO-ncs transgenes (Figure 1D). Quantitative westerns of microdissected brain tissue showed a 40 % increase in NCS-1 selectively in the DG (Figure 1E). Similarly, immunofluorescence analysis of confocal z-stacks from the MPP demonstrated a 40 % increase in NCS-1 reactivity (Figure 1G-I). Immunohistochemistry on frozen sagittal sections also demonstrated a subtle increase in NCS-1 levels selectively in the DG (Figure 1J). Because the rat and NCS-1 proteins are identical, post-translational modifications and sub-cellular
targeting of the exogenous protein should follow that of the endogenous protein. Throughout all experimental analyses, the other two transgenic genotypes (tetO-ncs and rtTA2) were not statistically different from wild-type (for example: in NCS-1 DG quantification: $p=0.16, 0.26$; in rearing in the safe environment: $p=0.16, 0.55$; in time spent in the target quadrant of the Morris water maze: $p=0.84, p=0.47$) and the three genotypes were pooled for comparison against DGNCS-1 mice (Figure S1A-B).

**Ncs1 Overexpression Enhances Curiosity-Driven Rearing**

Prior to any experimentation, subjects were handled for a minimum of 10 min per day for 10 consecutive mornings. Experiments were conducted on DGNCS-1 and littermate control subjects on dox for at least 6 days. This excluded any effects due to minor weight loss (see Figure S1D). Moreover, all experiments were conducted during the same circadian window to reduced phasic variation (Cain et al., 2008; Valentinuzzi et al., 2008).

The hippocampus is suggested to be important for curiosity-driven rearing behavior (Lever et al., 2006), but there are no direct measurements of curiosity in rodents since behaviors driven by curiosity can also be driven by fear. Thus we compared rearing behavior in two separate environments using two separate cohorts of animals to infer specific effects on curiosity-driven exploration. In a dimly-lit, non-stressful environment, DGNCS-1 subjects spent nearly twice as much time rearing compared to their littermate controls (Figure 2A). DGNCS-1 mice also spent less time inactive (still) in this environment (Figure 2C),
suggested more time spent exploring. We know the increase in rearing and
decrease in activity is not a general effect on locomotion because there were no
differences between the genotypes for any of the standard measures when we
examined a second cohort of animals in an overhead-lit, stressful environment
(Figure 2B,D), where rearing is escape-oriented. Thus the increase in rearing is a
specific effect and likely the result of a more curious animal.

To further explore this possibility, we designed an experiment similar to
the radial arms maze in which the latency and number of exploratory events for
novel environments in either dim or bright lighting could be easily determined.
We termed this experiment the “New Frontier Exploration Test” (for more detail
see the full experimental procedures in the supplementary section). In the dimly-
lit version of this task, DGNCS-1 mice demonstrated a nearly 2-fold increase in
the number of frontiers visited and a nearly 2-fold decrease in the latency to
explore 1 or all 4 new frontiers (Figure 2E,G). Yet, no genotypic differences in
either measure were observed when bright overhead lighting was used (Figure
2F,H).

DGNCS-1 subjects also demonstrated lower latencies to explore the holes
of the hole board test (Figure 2I) and spent longer exploring the very first hole
they encountered, a behavior repeated for each time they encountered a new
hole (Figure 2J).

Littermate control and DGNCS-1 subjects demonstrated similar anxiety as
measured by the light/dark box (Figure 2K,L) indicating that this behavior does
not confound the exploration tasks.
**Ncs1 Overexpression in the DG Enhances Synaptic Plasticity**

Elevation of DG NCS-1 did not affect basal synaptic transmission along the MPP (Figure S2A,B), the major excitatory innervation of the hippocampus and the primary source of sensory information relayed from the EC. Synaptic responses possessed double exponential decay (not shown) as well as short-term paired-pulse depression (Figure S2C), confirming electrode placement along the medial and not lateral perforant path (Bramham and Sarvey, 1996; Hanse and Gustafsson, 1992; McNaughton, 1980). Because the Ncs1 homolog, Freq, affects calcium-dependent neurotransmitter release in *Drosophila* and *Xenopus* (Pongs et al., 1993; Wang et al., 2001), we measured paired pulse-depression (PPD), a short-term form of pre-synaptic plasticity known to be sensitive to calcium, but found no differences (Figure S2C), corroborating a lack of Ncs1 overexpression in EC pre-synaptic terminals. When we stimulated to induce LTP along the MPP by applying an above-threshold stimulus (4 x 100 Hz trains), DGNCS-1 slices demonstrated more robust plasticity than their littermate controls (Figure 3A). Bath application of the NMDAR antagonist D-APV abolished LTP in DGNCS-1 mice (Figure 3A).

There is a strong frequency dependence for the induction of LTP in the DG, whereby tetanic stimulation with lower frequencies of the β and γ-range (10-75 Hz) typically fail to elicit LTP (Rick and Milgram, 1996). This high-threshold for plasticity is believed to be critical for sparse encoding of the external world and underlie learning and memory functions of the dentate (Barry et al., 2006; Coulter
and Carlson, 2007; Marr, 1971; McNaughton, 1987). To investigate if not only the level, but also the threshold of LTP was altered, we delivered a below-threshold stimulus using 4 tetanic trains at half the previous frequency. Strikingly, DGNCS-1 slices showed strong LTP even with this weak stimulation, while control slices showed very modest short-term potentiation and failed to elicit LTP (Figure 3B). DGNCS-1 slices stimulated with the below-threshold stimulus attained similar levels of LTP as compared to control slices given a strong stimulus (compare white bar in Figure 3A to black bar in Figure 3B). Maximal potentiation was also greater in DGNCS-1 mice following repetitive weak stimulation (Figure 3C). Thus, by overexpressing Ncs1 in the DG, we created a mouse with a selective enhancement in EC-DG NMDAR-dependent plasticity.

Importantly, DGNCS-1 slices and littermate control slices had equivalent LTP in area CA1 (Figure 3D), further demonstrating the regional selectivity of the plasticity enhancement.

**Ncs1 Overexpression Leads to Faster and Longer-Lasting Displaced Object Recognition Learning and Memory**

Due to the role of the DG in conjunctive encoding (O'Reilly and McClelland, 1994) and pattern separation (Bakker et al., 2008; Kesner, 2007a; Leutgeb et al., 2007; McHugh et al., 2007), it is possible that modulating NMDAR-dependent plasticity at this synapse will alter representations of the external environment. If such an alteration led to a distortion of environmental cues, or impairment in pattern completion, it should be discernable in space-dependent, cognitive tasks.
Thus, we examined the performance of the DGNCS-1 mice in two spatial learning and memory paradigms.

We first chose the object recognition task since it provides an elegant method to investigate both spatial and non-spatial one-trial, non-aversive memory. Object recognition is an ethological paradigm that captures latent learning in the absence of explicit rewards. During training, DGNCS-1 and littermate control subjects spent equivalent time investigating the objects and no differences between genotypes were observed in ambulation, anxiety, grooming or risk-assessing behaviors (Figure S3). Yet, as previously observed, DGNCS-1 subjects spent more time rearing (Figure S3). In a standard short-term memory paradigm (15 minutes habituation/training; see Figure S4), both DGNCS-1 and littermate control mice demonstrated a strong preference for the displaced and novel objects, suggesting the change in DG NMDAR-dependent plasticity did not disrupt spatial learning, at least not in this task (Figure 4A).

This raised the exciting possibility that facilitated DG synaptic plasticity could lead to superior neural-encoding of space and enhance learning. Therefore, we shortened the time given for subjects to become familiar with objects and object locations and again tested short-term memory. Remarkably, only the DGNCS-1 group showed an ability to discriminate between displaced and stationary objects in this harder version of the task (Figure 4B). Therefore, enhancing NMDAR-dependent plasticity at this synapse may promote the rapid acquisition of spatial memory, although testing this directly would be a major challenge.
It is well established that the DG is involved in short-term memory (Kesner, 2007a), while the involvement of the DG in long-term memory has been less explored. We therefore trained the subjects in a third paradigm for which control animals demonstrate short-term memory (Figure S5), but not long-term memory. Remarkably, DGNCS-1 animals were still able to discriminate between displaced and stationary objects even after a 24 hour interval (Figure 4C). Therefore, DG plasticity may promote the rapid acquisition of short and long-term spatial memory.

**Ncs1 Overexpression Enhances Water Maze Learning**

To investigate if the spatial memory enhancements in the DGNCS-1 mice would translate to another hippocampus-dependent task, we turned to the Morris water maze. In a stringent paradigm using ample distal spatial cues and a large platform in a small pool, subjects were trained with a single trial and then tested for retention of the platform location in a probe (no platform) trial 24 hours later. This procedure was repeated three times using three different platform locations and the performance of each subject was averaged over all three sessions (see Figure S4 for experimental design). Not surprisingly, control animals failed to demonstrate a preference for the target quadrant during the probe trial (Figure 4D). DGNCS-1 animals, however, spent nearly 50 % more time searching in the target quadrant than they did in any of the other three quadrants, suggesting they learned and recalled the platform location. Though DGNCS-1 animals did not have a faster latency to the counter location (set as twice the platform area)
DG NCS-1 underlies exploration and spatial memory

(Figure 4E), they did pass though it more frequently (Figure 4F) and spent more time searching that area of the maze (Figure 4G).

We also assessed learning and memory in the water maze by training the same subjects with four trials on a single day using a 45 min inter-trial interval. In this task, control and DGNCS-1 subjects had similar latencies to the platform by the 4th trial (Figure 4H); however, only DGNCS-1 subjects showed a statistically significant decrease in latency to reach the platform on trial 2 compared to trial 1. When tested in a probe trial 24 hours later, no differences were observed between the genotypes in any of the standard measures of memory performance (Figure 4I-K). Thus, if control subjects receive sufficient training, they can perform at the level of DGNCS-1 mice.

**NCS-1 Controls D2R Surface Expression in Hippocampus Neurons**

NCS-1 and D2R associate in vitro and colocalize in monkey and rat striatum (Kabbani et al., 2002), but whether NCS-1 and D2R interact in mouse hippocampus is unknown. Co-labeled mouse brain sections for surface D2R under non-permeabilizing conditions and for NCS-1 after permeabilization showed overlap (Figure 5A-F). NCS-1 and D2R also co-immunoprecipitated from mouse hippocampus lysates (Figure 5G).

To investigate the function of NCS-1/D2R interaction, we designed the DNIP, a cell-permeant NCS-1/D2R interfering peptide (Figure 5H), by coupling the HIV-1 TAT protein transduction domain sequence to the minimal region of D2R that binds NCS-1 (Kabbani et al., 2002). Application of the DNIP decreased surface levels of D2R in acute dissociated rat hippocampus cultures (Figure 5I)
and in the perforant path of mouse hippocampus slices (Figure S6A-C), suggesting endogenous NCS-1 may serve to facilitate D2R surface expression. Indeed, D2R desensitization in HEK 293 cells is attenuated by NCS-1 (Kabbani et al., 2002). While whole surface expression of D2Rs in the DG and areas CA1-3 of DGNCS-1 brains was comparable to that of littermate control animals (Figure 5Q,R), immunofluorescence analysis of D2R surface expression showed an enhancement in surface D2R restricted to the molecular layer of the DG, suggesting the receptors are properly targeted (Figure 5K-P).

The DNIP also prevented quinpirole from attenuating the forskolin-induced cellular cyclic adenosine monophosphate (cAMP) response (Figure 5J), proving the active peptide can functionally inhibit D2R. Importantly, the DNIP did not affect surface expression of D1R (Figure S6D-F, Figure S7A) or D3R (Figure S6G-I). The DNIP also failed to block D1R-mediated cAMP response and did not prevent pharmacological antagonism of these receptors (Figure S7B), demonstrated it is not a non-specific, global regulator of receptor surface expression.

**D2R and G-protein Signaling Mechanisms May Underlie the Enhanced LTP in DGNCS-1 Slices**

We investigated if the LTP enhancement in the *Ncs1* overexpressing mice required the interaction of NCS-1 and D2R by recording LTP in the presence of the DNIP. The DNIP and srDNIP did not affect basal transmission (Table S1), short-term plasticity (Table S1), or LTP in littermate controls following the below-
threshold stimulus (Figure 6A). In DGNCS-1 slices, however, the DNIP blocked
LTP following the below-threshold stimulus (Figure 6B), suggesting the
interaction of NCS-1 and D2R is critical for facilitation of LTP in DGNCS-1 slices,
and may also underlie the promotion of specific forms of exploration and
enhancements in spatial memory acquisition. The DNIP also attenuated above-
threshold LTP in control slices (Figure 2C), demonstrating an endogenous role
for the NCS-1/D2R interaction in long-term synaptic plasticity.

To further investigate if D2Rs and downstream signaling pathways of D2R
underlie enhanced LTP in DGNCS-1 slices, we applied L-741,626 at a
concentration selective for D2Rs (50 nM). Here we found the D2R antagonist
attenuated and normalized the extent of LTP (Figure 6D), suggesting D2R is
important for DG LTP in wild-type slices and is also critical for the plasticity
enhancement in DGNCS-1 slices. The Gβγ blocker, gallein also attenuated LTP in
littermate controls and normalized LTP in DGNCS-1 animals (Figure 6E).

Because mGluR5-mediated LTD in adjacent brain areas involves NCS-1
(Jo et al., 2008), we also applied the mGluR5 antagonist, MPEP to DGNCS-1 and
littermate control slices. Yet, we still observed a statistical genotypic difference
(Figure 6F), indicating the mGluR5 receptor subtype is not involved in this
particular form of NCS-1-dependent plasticity. Similarly, inhibitory modulation is
not a potential contributor to the enhanced plasticity in DGNCS-1 slices since
LTP was prevented by removal of the GABA_A blocker, bicuculline from the
perfusion aCSF (Figure S8). Moreover, the contribution of NMDAR and AMPAR
to baseline field responses were comparable in DGNCS-1 and littermate control
slices (Figure S9) and surface expression of GluR2 was comparable between the groups in areas CA1-3 and the DG (Figure 5Q,R). The finding that baseline NMDAR transmission is not affected in DGNCS-1 slices demonstrates that although NMDAR-dependent mechanisms are required to initial LTP in DGNCS-1 slices, baseline NMDAR levels do not mediate the plasticity enhancement.

**NCS-1 and D2R Underlie Curiosity-Driven Behavior and Spatial Memory**

We explored if the interaction of NCS-1 and DNIP was important for curiosity-driven exploration in DGNCS-1 and littermate subjects by direct infusion of the DNIP to the DG. Representative cannulae tracks and dorsal view of infusion sites are shown in Figure 7B. The DNIP and srDNIP peptides conjugated to fluorescent probes directly infused into the dentate showed a degree of diffusion sufficient to cover approximately one third of the DG and less than 5% of area CA1 (Figure 7G). High power 3D confocal z-stack imaging of individual granule neurons demonstrated the peptides penetrate into cells (Figure 7H). DGNCS-1 and control subjects infused 3 to 5 hours prior to examination demonstrated equivalent rearing behavior in a safe environment (Figure 7A). There were no differences in any of the other standard measures (not shown). Because the DNIP normalized rearing behavior in this type of environment, NCS-1 interaction with D2R is likely important for the promotion of exploratory rearing in DGNCS-1 subjects.
Infusion of the DNIP also blocked both the short and long-term spatial memory enhancements (Figure 7C,D), but the same dose did not affect the performance of littermate control animals in the standard version of the task (Figure 7E). To investigate if endogenous levels of NCS-1 and D2R cooperate to mediate the rapid acquisition of spatial memory, we infused a higher (5x) dose of the peptide into the DG of control mice and again examined displaced object learning and memory. Here, the DNIP prevented spatial memory formation (Figure 7E), indicating the NCS-1/D2R interaction is required in this task. Blockade of D2R by bilateral infusion of L-741,626 into the DG also prevented displaced object discrimination in littermate control animals on dox (Figure 7F), demonstrating the importance of D2R in spatial memory acquisition. Importantly, novel-object recognition (which involves other brain areas) was unaffected by either DNIP or L-741-626, suggesting the relationship between NCS-1 and D2R in the DG is not critical for enhancing single-modality learning and memory (see Figure 8A).
DISCUSSION

To understand the molecular underpinnings of exploration and its link to learning and memory we must understand the molecular underpinnings of plasticity in brain regions that underlie these behaviors. Here we identify the importance of the DG in a specific form of exploration. Other behaviors seem to have nearly direct anatomical correlates as well: Fear learning and fear responses are largely mediated by the amygdala (Fanselow and LeDoux, 1999; Phelps and LeDoux, 2005), acquisition of new declarative memories is dependent on an intact hippocampus (Scoville and Milner, 1957; Squire et al., 2004) and future planning is strongly associated with prefrontal cortex activity (Hunt, 2008; Tanji and Hoshi, 2001). However there are also excellent examples of cognitive functions that are apparently highly dispersed, such as visual processing (Celesia and DeMarco, 1994; Tamraz, 1994), or share anatomical regions of overlapping function, such as facial recognition (Brown and Aggleton, 2001; Law et al., 2005; Squire et al., 2007). Here we demonstrate a potential link between DG NMDAR-dependent long-term plasticity and curiosity-driven exploratory rearing behavior in a mouse line that also demonstrates enhanced rapid acquisition of spatial memory. We identify the calcium sensor, NCS-1, as a new regulator of D2R surface expression in the DG molecular layer and provide evidence to suggest changes in D2R expression or modification underlie the promotion of a specific form of exploration, facilitated LTP and enhanced memory in the DGNCS-1 mouseline. The DNIP, engineered to compete for NCS-1/D2R binding, reversed all DGNCS-
1 electrophysiological and behavioral phenotypes and selectively blocked spatial learning in control subjects. Pharmacological blockade of D2Rs by L-741,626 normalized the enhanced LTP in DGNCS-1 slices, attenuated control plasticity and prevented rapid acquitting of spatial memory. Our data demonstrate roles for NCS-1 and D2R in DG plasticity and provide novel insight for understanding the molecular mechanisms of exploration and its link to spatial memory acquisition.

**Selection of Model**

NCS-1 was a promising protein to study because of its expressional regulation during in vivo LTP (Genin et al., 2001) and involvement in learning and memory in *C. elegans* (Gomez et al., 2001). We selected an inducible transgenic system to genetically perturb *Ncs1* expression in adult mice because NCS-1 is critical in the development of the nervous system and muscle (Blasiole et al., 2005; Coukell et al., 2004; Hui et al., 2007; Nakamura et al., 2006). Constitutive overexpression, or null or conditional mutations may have caused developmental abnormalities, obscuring interpretation of the data. It was also our aim to isolate and study a subregion of the hippocampus in order to better understand its function. As such, we help unveil the importance of the DG in neurobehavioral outcomes of DGNCS-1 mice.

**The Role of the DG in Rapid Acquisition of New Spatial Memory**

Sparse encoding is a central feature for pattern separation processes in the DG (Bakker et al., 2008; Kesner, 2007a; Leutgeb et al., 2007; McHugh et al., 2007).
and is believed to be critical for accurate spatial mapping during learning and memory (Coulter and Carlson, 2007; Marr, 1971). Sparse encoding is only possible because DG granule neurons form synaptic contacts with a relatively modest number of CA3 pyramidal neurons. Thus, it is important that the number of DG neurons stimulated during encoding of the environment does not change such that inputs into CA3 become non-specific or inadequately orthogonalized, a situation that could result in catastrophic interference (Hetherington, 1990). A lack of memory deficits in the DGNCS-1 animals suggests sparse encoding in the DG can be preserved when the threshold for plasticity is reduced and the ceiling of plasticity is increased.

**The Role of the DG in Spatial Novelty vs Object Novelty**

DGNCS-1 mice display enhanced displaced object recognition, but normal novel object recognition. This might suggest NCS-1 and D2R function specifically in the DG to mediate multimodal information storage (Figure 8A). One major difference between novel object discrimination and displaced object discrimination is the number of sensory cues required to differentiate old from new (i.e. produce a mismatch). To recognize a novel spatial change, the many distal cues of the surrounding environment must be used, requiring integration of multiple sources of sensory information (multimodal input). On the other hand, to recognize a new object, any single cue (shade, shape, texture etc...) can be employed to generate a mismatch and signal novelty. Since DG LTP is enhanced in these animals, and could underlie the enhancements in memory (though we do not
attempt to prove this directly), it may be reasonable to also predict DG LTP does not underlie novel object recognition. Instead, other regions of the hippocampus are likely more important. Direct perforant path projections from the EC to areas CA3 or CA1 of the hippocampus may sufficiently encode single-modality, non-spatial information without undergoing conjunctive encoding or pattern separation processes via the DG (see model in Figure 8A).

The Role of the DG in Exploration and the Link to Memory

An isolated enhancement in curiosity-driven rearing by selective alteration of the DG is exciting because while the medial hypothalamus, amygdala and nucleus accumbens are all known to be critical for defensive rearing behavior (Lever et al., 2006; Sandner et al., 1987; Silveira and Graeff, 1992), anatomical regions specifically responsible for curiosity-driven rearing have proved more difficult to identify (Lever et al., 2006).

Since novelty-detection is required for novelty-exploration and the DG is important for novelty-detection (Kesner et al., 2004), it is ideally suited to play a role in generating curiosity. Indeed, the DG sends projections to the nucleus accumbens, a region responsible for initiating rearing (Lever et al., 2006), via two major paths. The first is through the tri-synaptic hippocampal loop and the other through the EC and subiculum. Either, or both, of these pathways could be critical to initiating curiosity-driven rearing. We observe promotion of a specific form of exploratory behaviour in a mouseline with selective manipulation to the DG, but other subregions of the hippocampus may also be important, particularly
if signaling from the DG to the nucleus accumbens is relayed – and therefore potentially modified – through the remainder of the hippocampal circuit.

Though facilitated DG NMDAR-dependent plasticity in DGNCS-1 animals may underlie the enhanced learning and memory in this mouse line, it is worth entertaining the hypothesis that increased curiosity may also contribute. Rearing in novel environments allows animals to make use of a superior vantage point, including longer sight and alternative air currents (Barry et al., 2006; Hetherington, 1990; Lever et al., 2006). A mutant model of fragile X syndrome demonstrates reduced rearing (Mineur et al., 2002) and dopamine dysfunction has recently been implicated to underlie fragile X-syndrome (Weinshenker and Warren, 2008). DGNCS-1 subjects behave as if they incorporate a richer sensorium, which could be due to increased rearing, facilitated plasticity, or both. Though we do not test this directly, we agree with the prediction that a richer sensorium can ultimately translate into a more detailed environmental map (Barry et al., 2006), which in turn could enhance spatial memory acquisition and the potential for novelty-detection. In Figure 8A, we provide a model for how NCS-1 and “curiosity” originating in the DG might help establish spatial maps and memory.

Several studies have found stereotypy and increased locomotion in rodent models of schizophrenia, such as following MK801 administration (Tiedtke et al., 1990). However, hyperactivity is more frequently found for horizontal locomotion (Clapcote et al., 2007; Kellendonk et al., 2006). Moreover, because hyperactivity persists in brightly lit environments typical for locomotion experimentation,
increased rearing in the DGNCS-1 animals does not likely signify a psychological endophenotype. We feel this result supports the selectivity of induced \textit{Ncs1} expression since NCS-1 and D2R are both implicated in schizophrenia as well as other psychiatric disorders.

**Clinical Considerations**

It is important to identify promising molecular targets that could be used to enhance cognition in human diseases. We feel NCS-1 and D2R are such targets. Moreover, while in this study we focused on the DG, these findings have broad implications for other diseases of the nervous system since NCS-1 and D2R are co-expressed in many cell types and are implicated in important psychological pathologies including schizophrenia, bi-polar disorder and addiction (Berke and Hyman, 2000; Joyce et al., 1993; Kabbani and Levenson, 2006; Koh et al., 2003; Souza et al., 2006).

There is good reason to predict the DNIP would most readily reduce D2R surface expression in areas of the brain that show high levels of D2R expression, such as the striatum. Because the striatum is implicated in neuroendophenotypes of psychiatric disorders, including schizophrenia (Kellendonk et al., 2006) and addiction (Gerdeman et al., 2003), it would be interesting to test the effects of the DNIP in relevant rodent models.

In summary, we show NCS-1 and D2R combine to regulate curiosity-driven exploratory behavior, NMDAR-dependent perforant path plasticity and rapid acquisition of short and long-term spatial memory. These results offer novel
insights into behavioral, cellular and molecular mechanisms governing the origin of exploration, the formation of memory and underscore the importance of their relationship.
EXPERIMENTAL PROCEDURES

For full experimental procedures, see the accompanying supplementary section online at http://www.cell.com/neuron.
ACKNOWLEDGEMENTS

We are indebted to Shenna A. Josselyn, Roderick McInnes, Robert Gerlai and Karine Nader for excellent advice on the manuscript. This work was funded by a grant through the CIHR (MOP-13239 and CTP-79858). B.J.S. holds a CIHR doctoral research award. J.G. holds a NARSAD Young Investigator Award. A.N. holds a SLRI Summer Student Fellowship. J.C.R. holds a Tier One CRC Chair in Learning and Memory.
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prefrontal cortex of schizophrenic and bipolar patients. Proc Natl Acad Sci U S A 100, 313-317.


FIGURE LEGENDS

Figure 1. Generation of DGNCS-1 Mice

(A) Schematic representation of the rtTA2 system and transgenic constructs used to generate the tetO-ncs, rtTA2 and DGNCS-1 lines.

(B) Representative sequencing chromatograms of endogenous mus Ncs1 mRNA amplicon (upper chromatogram) compared to rat Ncs1 mRNA amplicon from a DGNCS-1 animal on dox (lower chromatogram). Asterisks indicate silent single nucleotide divergence.

(C) β-gal staining of horizontal adult mouse brain sections from rtTA2/LacZ and littermate control mice.

(D) Selective amplification of rat Ncs1 RNA from hippocampal extracts of DGNCS-1 mice on dox. No expression was observed in littermate controls or in DGNCS-1 hippocampii off dox.

(E) Hippocampal subregion CA1-3 and DG lysates blotted for NCS-1 and β-tubulin (Tub).

(F) NCS-1 normalized to Tub from various isolated brain regions of DGNCS-1 and littermate control mice on dox (n = 6).

(G) Quantitative z-stack immunofluorescence of NCS-1 in the medial perforant path of DGNCS-1 animals compared to littermate controls.

(H,I) NCS-1 immunofluorescence images from DG MPP. Representative layer of maximum fluorescent intensity in (H) control and (I) DGNCS-1 slices with intensity plots shown below (n = 4). Scale bar = 18 µm.
(J) Immunohistochemical staining of NCS-1 demonstrates a subtle increase in staining selectively in the MPP of the DG.

(Hip = hippocampus) (**p < 0.01) Error bars, SEM.

**Figure 2. Elevation of NCS-1 in the Adult DG Selectively Promotes Curiosity-Driven Rearing**

(A) Exploratory rearing in a dimly-lit, safe novel environment was increased in DGNCS-1 mice compared to their littermate controls. Data presented are the average of two 5 min sessions (DGNCS-1, n = 8; Control, n = 16).

(B) Rearing in a brightly-lit, stressful environment remained unchanged between the genotypes, indicating fear-motivated rearing was unaffected. Separate cohorts of mice were used for each of the environment types (DGNCS-1, n = 12; Control, n = 11)

(C, D) Time spent on other behaviors in the (C) safe and (D) fearful environment. (DGNCS-1, n = 8; Control, n = 16).

(E-H) New Frontier Exploration Test. Under dimly-lit conditions, the DGNCS-1 group had (E) a lower latency to reach any or all novel environments and (G) more total novel environment visit events. (F, H) Under bright lights no genotypic difference was observed. (DGNCS-1, n = 8; Control, n = 11)

(I, J) In the hole board test, (I) DGNCS-1 mice spent more total time exploring holes. (J) DGNCS-1 mice spent more time exploring a hole during primary encounter of the first and all subsequent holes. (DGNCS-1, n = 9; Control, n = 10)
(K,L) DGNCS-1 and littermate control subjects (K) spent equivalent time in each chamber of the light/dark box and (L) demonstrated equivalent latencies to move into the brightly-lit chamber. (DGNCS-1, n = 9; Control, n = 10)

(* p < 0.05, **p < 0.01) Error bars, SEM.

**Figure 3. Modest Elevation of NCS-1 Enhances Long-Term Synaptic Plasticity**

(A) Modest overexpression of Ncs1 in the DG enhanced MPP LTP following above-threshold tetanic stimulation (4 x 100 Hz). LTP in DGNCS-1 slices is blocked by the NMDAR antagonist D-APV. (DGNCS-1, n = 11 slices from n = 8 mice; Control, n = 20 slices from n = 11 mice; DGNCS-1 + D-APV, n = 2 slices from n = 2 mice).

(B) Only slices from DGNCS-1 mice produced robust LTP following below-threshold tetanic stimulation (4 x 50 Hz). (DGNCS-1, n = 10 slices from n = 9 mice; Control, n = 6 slices from n = 5 mice).

(C) DGNCS-1 slices show higher maximum potentiation following eight sets of weak tetanic stimulation (2 x 50 Hz each), as compared to controls. Bar graph shows maximum potentiation during 5 min period following the final stimulus (DGNCS-1, n = 9 slices from n = 4 mice; Control, n = 3 slices from n = 1 mouse).

(D) No genotypic difference was observed in LTP along the Schaffer Collateral CA1 synapse. (DGNCS-1, n = 6 slices from n = 3 mice; Control, n = 5 slices from n = 4 mice)
Inset sample traces (scale bar of 0.5 mV and 10 ms) show superimposition of baseline and the periods used to generate bar graphs. Bar graph shows LTP at 60 min. (*p < 0.05, ** p < 0.01) Error bars, SEM.

**Figure 4. Elevation of DG NCS-1 Enhances Rapid Acquisition of Spatial Memory**

(A) In a standard version of the object recognition task, both DGNCS-1 and littermate control animals demonstrated a preference for the displaced and novel objects when tested following 2 min intervals (DGNCS-1, n = 8; Control, n = 16).

(B) In a minimal habituation/training version of the task, only the DGNCS-1 subjects showed discrimination between the displaced and stationary objects (DGNCS-1, n = 7; Control, n = 9).

(C) Only DGNCS-1 subjects discriminated between displaced and stationary objects when assessed 24 hours after an intermediate level of habituation/training (DGNCS-1, n = 9; Control, n = 15).

(D-G) Average of 3 probe trials, each 24 hours after a single-trial water maze experiment. DGNCS-1 mice (D) spent more time in the target quadrant, (E) had a similar latency to the counter, (F) passed more often through the counter and (G) spent more time in the counter location.

(H) Latency to reach the hidden platform for DGNCS-1 and littermate control animals for 4 trials, 45 min apart. Maximal performance was achieved on trial 2 for the DGNCS-1 groups and on trial 4 for the control group.
(I-K) Probe trial 24 hours after (H). No differences between the groups were found in (I) latency to, (J) passes through, or (K) time spent in the counter location. (DGNCS-1, n = 17; Control, n = 33). See Figure S4 for detailed schematic of experimental protocols and apparatus. S = stationary object; D = displaced object; F = familiar object; N = novel object; HAB = habituation phase; DO = displaced object phase; NO = novel object phase. (n.s. = not significant between trial, ***p < 0.001 between trial, †††p < 0.001 within genotype, *p < 0.05 between genotype, **p < 0.01 between genotype) Error bars, SEM.

**Figure 5. NCS-1 Controls D2R Surface Expression in Mouse Hippocampus Neurons**

(A) Confocal image of surface D2R immunostaining in the mouse DG.

(B) Corresponding image of NCS-1 immunostaining from the same section shown in (A).

(C) Overlay of (A) and (B), with co-distributed regions appearing in yellow. Scale bar = 240 µm.

(D-F) Higher power confocal stacks within MPP of DG molecular layer, shown as isometric projection, for each of D2R, NCS-1, and overlay, respectively. Scale bar = 18 µm.

(G) Co-immunoprecipitation of NCS-1 and D2R from mouse hippocampal lysates. p.l. = pre-cleared lysate, c.l. = crude lysate.

(H) DNIP (upper sequence) and srDNIP (lower sequence) fused to TAT.
(I) Colorimetric measurements of D2R surface expression in hippocampal cultures isolated from E17-19 fetal Wistar rats. Cultures were incubated in either 2 µM DNIP or srDNIP for 30 min (n = 3).

(J) cAMP quantification in hippocampal cultures following 30 min incubation of DNIP or srDNIP (n = 3).

(K,L) Representative surface D2R staining in the dentate of (A) control and (B) DGNCS-1 subjects. Scale bar = 45 µm.

(M-P) Quantification of confocal z-stack immunofluorescence from DGNCS-1 and control genotypes in (M) DG granule cell layer, (N) DG molecular layer, (O) hilus and (P) CA1 Schaffer collateral (SchC) molecular layer. (n = 4)

(Q,R) Biotinylated surface protein immunoblots of GluR2, D2R and N-Cadherin as the loading control.

Forsk = forskolin, Quin = quinpirole (*p < 0.05) Error bars, SEM.

**Figure 6. Antagonism of D2R and G\(_{\beta\gamma}\) Signaling Normalizes LTP in DGNCS-1 Slices**

(A) Neither the DNIP nor srDNIP (10 µM each) had an effect on plasticity following a weak tetanic stimulus (4 x 50 Hz) in control slices. (DNIP: n = 4 slices from n = 3 mice; srDNIP: n = 3 slices from n = 3 mice)

(B) LTP (4 x 50 Hz) in DGNCS-1 slices was attenuated by application of the DNIP. (srDNIP: n = 3 slices from n = 3 mice; DNIP: n = 4 slices from n = 4 mice).
(C) LTP in control slices induced by the above threshold stimulus (4 x 100 Hz) was attenuated with the DNIP. (srDNIP: n = 4 slices from n = 4 mice; DNIP: n = 7 slices from n = 4 mice)

(D) LTP (4 x 100 Hz) in DGNCS-1 slices was normalized to the level of controls with application of 50 nM of the D2R-selective antagonist, L-741,626. (Control: n = 11 slices from n = 6 mice; DGNCS-1: n = 6 slices from n = 4 mice)

(E) LTP (4 x 100 Hz) in DGNCS-1 slices was normalized to the level of controls with application of 10 µM of the G-protein beta/gamma inhibitor, gallein. (Control: n = 6 slices from n = 3 mice; DGNCS-1: n = 6 slices from n = 4 mice)

(F) In the presence of 1 µM of the mGluR5 antagonist MPEP, LTP (4 x 100 Hz) in DGNCS-1 slices was still greater than LTP in littermate control slices. (Control: n = 7 slices from n = 4 mice; DGNCS-1: n = 6 slices from n = 3 mice)

(*p < 0.05, ***p < 0.001) Error bars, SEM.

Figure 7. D2R is Important for Exploratory Rearing and Spatial Memory in DGNCS-1 and Control Subjects.

(A) Pre-infusion of a low dose of the DNIP directly into the DG of cannulized subjects attenuated curiosity-driven rearing behavior compared to sham-treated or srDNIP-infused DGNCS-1 subjects. Only a high dose (5x) of the DNIP reduced rearing behavior in control subjects. L-741,626 reduced rearing behavior in control animals. (DGNCS-1 + srDNIP/sham: n = 9; DGNCS-1 + DNIP: n = 7; Control + DNIP_{low dose}: n = 7; Control + DNIP_{high dose}: n = 2; Control sham: n = 5; Control + L-741,626: n = 4)
(B) Representative coronal section and dorsal view of a cannulized brain after removal of cannulae, showing infusion tracks and surgical placement.

(C-D) The DNIP blocked both the (C) minimal short- and (D) long-term spatial memory (displaced object discrimination) enhancement in DGNCS-1. Non-spatial memory (novel object discrimination) remained intact (n = 7). srDNIP-infused (n = 3) or sham-treated DGNCS-1 subjects (n = 6) demonstrated similar rearing to untreated DGNCS-1 subjects.

(E) The same dose of peptide had no effect on littermate controls in the standard version of the short-term memory task (n = 7). A higher (5 x) dose of the DNIP abolished spatial learning in control mice, whilst preserving novel-object recognition (n = 2).

(F) L-741,626 selectively abolished displaced object discrimination when infused bilaterally in the DG of control subjects (n = 4). Sham-treated controls still demonstrated discrimination (n = 5).

(G) Fluorescently conjugated srDNIP (blue) and DNIP (red) infused into the dentate 3 h prior to sacrifice for processing and imaging. Picogreen was applied to label nuclei. Overlay shown in lower right panel. Scale bar = 500 µm.

(H) High power 3D z-stack confocal imaging of the srDNIP in neurons of the DG. Scale bar = 35 µm.

S = stationary object; D = displaced object; F = familiar object; N = novel object. (*p < 0.05; **p < 0.01, ***p < 0.001) Error bars, SEM.
Figure 8. Models for DG Regulation of Curiosity and Multimodal Memory Formation and NCS-1/D2R Regulation of Synaptic Plasticity

(A) Multimodal information from the sensorium is integrated in the entorhinal cortex and projected (blue arrows) to the DG where pattern separation occurs. Here NCS-1 (red arrow) lowers the LTP induction threshold, as depicted by the variable resistor (jagged blue arrows), and enhances EC-DG LTP to promote rapid acquisition of space-dependent memory. Single-modality information processing (green arrows) may occur via direct projections from the EC to areas CA1 or CA3. Circuitry responsible for exploratory or curiosity-driven behavior (purple arrows) arises in the DG and projects through the EC and/or hippocampal tri-synaptic loop to the nucleus accumbens. Bi-directional signaling between the nucleus accumbens and ventral tegmental area results in rearing behavior. More rearing, in turn, contributes to a richer sensorium, potentially completing a positive-feedback loop. Circuitry responsible for fear-driven rearing (orange arrows) arises in the amygdala, a system not affected in our model. IF = information, DG = dentate gyrus, CA = cornu ammonis, EC = entorhinal cortex, Sub = subiculum, NA = nucleus accumbens, A = amygdala, VTA = ventral tegmental area. Arrow thickness approximates relative strength of synaptic contact. Arrow number approximates relative amount of innervating projections.

(B) NCS-1 drives D2R surface expression, a process blocked by the DNIP. Activation of D2R (blocked by L-741,626) stimulates $G_{\beta\gamma}$ downstream signaling mechanisms (blocked by gallein) that contribute to plasticity, exploration and memory.
Figure 3

A. Above Threshold

B. Below Threshold

C. Saturation

D. CA1

Graphs show the percentage of fEPSP over time for different conditions:

- Control
- DGNCS-1
- DGNCS-1 + APV

LTP and Max values are shown for each condition.
A

**Standard Short**

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Object Preference (%)

**Displaced Object**

**Novel Object**

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B

**Minimal Short**

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Object Preference (%)

**Displaced Object**

**Novel Object**

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C

**Standard Long**

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Object Preference (%)

**Displaced Object**

**Novel Object**

---

D

**Time in Quadrant (%)**

- **Target**
- **Left**
- **Right**
- **Opposite**

---

E

**Latency to Counter (s)**

**Passes (events)**

**Time in Counter (%)**

---

F

**Latency to Counter (s)**

---

G

**Latency to Counter (%)**

---

H

**n.s.**

---

I

**Latency to Counter (s)**

---

J

**Passes (events)**

---

K

**Time in Counter (%)**

---

Figure 4
Figure S5

Object Discrimination (%)

Standard 6min Short

S D F N

* **
Figure S6
Figure S7

(A) Normalized D1R Surface Expression

(B) cAMP (pmol/mL)

- Con
- SKF
- SCH
- SKF + SCH

Figure S7
Figure S8
Dentate Microdissection

Figure S10
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NCS-1 in the Dentate Gyrus Promotes Exploration, Synaptic Plasticity and Rapid Acquisition of Spatial Memory

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FULL EXPERIMENTAL PROCEDURES

Generation of Camk2a-rtTA2 and tetO-ncs Transgenic Mice

The DG-restricted rtTA2-M2 line (designated “Tg(Camk2a-rtTA2-901)SMan” on the Mouse Genome Informatics database, MGI, http://www.informatics.jax.org/) was created by random integration of the Camk2a-rtTA2 transgene as previously described (Michalon et al., 2005). Rat Ncs1 was obtained from a construct originally cloned from rat brain and subcloned into the tetO7 vector, a kind gift from Eric Kandel, using the BamH1 and Sal1 restriction sites. Linearized plasmid was purified by dialysis in microinjection buffer, diluted to 1 ng/µL and injected into oocytes of pure inbred C57BL/6 mice (Transgenic facility, Princess Margaret Hospital, Toronto). Stable oocytes were transferred to pseudo-pregnant C57BL/6 females. One founder, designated “Tg(tetO-Ncs1)JRod on the MGI database, was maintained on C57BL/6. DGNCS-1 mice were generated by breeding heterozygous tetO-ncs1 mice of either sex with a heterozygous Camk2a-rtTA2 mouse of the opposite sex (Figure S1A). Polymerase chain reaction (PCR) produced distinct amplicons for each transgenic genotype (Figure S1B). Wild-type samples were verified for DNA quality by spectrometry and control PCR (not shown). Experiments were conducted on 8-16 week old DGNCS-1 and littermate control animals on dox using balanced sex ratios.

Doxycycline Administration
Dox food was prepared fresh daily from powdered Purina Mouse Chow, water and dox (Doxycin/Doxycycline Hyclate, obtained from the Mount Sinai Hospital pharmacy in Toronto). Ingredients were homogenized, formed into pellets (~1 cm in diameter) and allowed to air dry for 3-5 hours prior to feeding. Because dox is light-sensitive, food preparation and feeding was conducted under low light conditions. Since feeding can over-power light for the control of some circadian rhythms (Pauly et al., 1975), and circadian rhythm can be a powerful modulator of behavior and learning and memory (Devan et al., 2001; Rawashdeh et al., 2007), mice were consistently fed at the start of the 12 hour dark cycle (7:00 PM). Animals were allowed to feed ad libitum for the entire dark cycle, after which all non-consumed food was removed (Figure S1C). A daily record of each mouse’s weight was kept to ensure animals were eating properly (Figure S1D). A minimum of 6 days of feeding ensued before experimentation to allow the mice to recover from a brief phase of minor weight loss.

**Vibratome Sections and LacZ**

LacZ staining was carried out as previously described (Michalon et al., 2005) and imaged with a Leica dissection microscope and color CCD camera.

**RNA Isolation, cDNA Synthesis and PCR**

After anesthetization with halothane, mice were decapitated and the head was immediately immersed in ice-cold PBS. Brain regions of interest were dissected out in the same solution, immediately frozen in liquid nitrogen and stored at -80
oC until use. RNA was extracted with Trizol Reagent (Invitrogen) as described by the manufacturer. cDNA was synthesized with MonsterScript 1st – Strand cDNA Synthesis Kit (Epicentre Biotechnologies; MS041050) and random nonomers according to the manufacturers’ instructions. cDNA was used fresh for PCR or stored at 4 oC until use. Rat RNA was amplified with forward (TCACTGAAAAGGAAGTACAGCAGTGG) and reverse (TGAAGGCCCATCGCATTCATCC) primers. Mouse RNA was amplified with forward (TCACTGAGAAAGAAGTACAGCAGTGG) and reverse (TGAAGGCCCATCGCATTCATCC) primers. PCR products were run on 1 % agarose and visualized under UV light with ethidium bromide (Sigma) or SYBR Green (Invitrogen).

**Protein Isolation, Westerns and Quantification**

After anesthetization with halothane, mice were decapitated and the head was immediately immersed in ice-cold PBS. Brains regions of interest were removed by microdissection in the same solution. To isolate hippocampus subregions, fresh hippocampii were sliced with three fused razor blades, yielding 2 slices, each ~700 µm thick. To obtain DG-enriched tissue, a circular area centered by the dentate hilus was punched out with a glass capillary affixed with thin rubber tubing to a 1 c.c. syringe. The remainder of each slice was taken as the CA1-3 enriched fraction. The ends of the hippocampus excluding the slices were used as the hippocampal fraction (Figure S10). All tissue was frozen in a microtube on dry ice and kept at – 80 oC until use. Protein factions were obtained using RIPA
buffer (Santa Cruz) according to the manufacturers’ instructions. Samples were boiled for 5 min, loaded (20 µL) on 15 % Tris-HCl Criterion Precast Gels (345-0020), transferred to polyvinylidene fluoride membrane (BioTrace; 66543), probed with the primary antibody followed by the appropriate horseradish peroxidase-conjugated secondary, reacted with Detection Reagent (GE Healthcare; RPN2106V1 and RPN2106V2), visualized on autoradiography film (HyBlot CL; E3012) and quantified with a Fluoro-S-Max chemiluminescence camera (Bio-Rad). For each anatomical region, NCS-1 protein levels were normalized to β-tubulin (detected with anti-β-tubulin (Sigma; T2200)) from the same blot. Antibodies: anti-D2R (Santa Cruz); anti-NCS-1 (ProteinTech Group); anti-rabbit IgG and anti-mouse IgG (HRP-conjugated; GE Healthcare).

**Immunohistochemistry**

Frozen 10 µM sections were fixed for 10 min in 4 % PFA, stained overnight in primary antibody and reacted with the appropriate peroxidase secondary. Colour images were generated using Image J. Black = less staining.

**Electrophysiology**

Experiments were conducted essentially as previously described (Henderson et al., 2001). Briefly, after anesthetization with halothane, mice were decapitated and the head was immediately immersed in ice cold aCSF. Brains and hippocampii were removed in the same solution and slices were prepared with a manual chopper (400 µm transverse slices). Slices were allowed to recover in
oxygenated artificial cerebral spinal fluid (aCSF; 124 mM NaCl, 3 mM KCl, 2.5 mM CaCl$_2$, 1.3 mM MgSO$_4$, 1.25 mM NaH$_2$PO$_4$, 26 mM NaHCO$_3$, 10 mM D-glucose (pH 7.4)) at room temperature for 1-2 hours and then transferred to a chamber perfused with aCSF (1 mL/min) at 30 °C. Stimulating and recording electrodes in the DG dorsal blade (suprapyramidal) were placed precisely along the MPP by way of visual observation aided with a microscope and an infrared camera. Recordings were obtained using 3 MΩ glass electrodes (containing aCSF lacking Ca$^{2+}$) and an Axopatch 1D amplifier (Axon Instruments) set to 5 kHz low-pass filtering and digitized at 20 kHz using a Digidata 1200 and pCLAMP8 software. Paired dendritic responses were evoked by stimulating with an intensity (0.05 ms pulses, 40 ms apart) that yielded field excitatory postsynaptic potentials (fEPSPs) that were 40 % of the maximum spike-free fEPSP size. Responses were evoked and collected every 20 s throughout the experiment and measured by slope (10–50 % of fEPSP rising phase). Synaptic responses showed the expected shape including double exponential decay as well as short-term PPD, confirming electrode placement along the MPP and not lateral perforant path (Bramham and Sarvey, 1996; Hanse and Gustafsson, 1992; McNaughton, 1980). All recordings were undertaken in the presence of 10 µM (-)-bicuculline methiodide (Sigma, B6889; Tocris, 2503) to block GABA$_A$ receptor-mediated inhibitory events. LTP was induced with either a weak, below-threshold (50 Hz) or strong, above-threshold (100 Hz) tetanus (0.15 ms pulses delivered in 4 trains of 0.5 s duration, 20 s apart). To recruit all synapses and achieve maximal potentiation, repeated tetani were given (0.15 ms pulses,
delivered in 2 trains of 0.5 s duration, 20 s apart, repeated every 5 min for a total of 8 repetitions). Where specified, D(-)-APV (Sigma A-169 or Tocris) was used at 50 µM in the open bath. LTP time course was plotted by normalizing to the baseline fEPSP slope (average of the 10 minute period prior to tetanus). Each point on the LTP time course plots is the mean of 6 consecutive response averages. Bar plots of LTP were calculated from responses averaged over the 55-65 min period after tetanus in Figure 3A,B. Bar plots of maximum potentiation were calculated from responses averaged over the 5 min period following the eighth stimulus (Figure 3C). Single experiment sample traces are averages from the periods indicated above.

**Behavior**

All experiments were approved by the local committee on animal care and conformed to the national guidelines (CCAC; http://www.ccac.ca). Subjects were kept in controlled environmental conditions with the lights on at 7:00 AM and lights off at 7:00 PM. To minimize any effects of circadian rhythm on behavior, all experiments were conducted between 7:30 AM and 1:30 PM. Animals were handled daily for a minimum of 10 days prior to experimentation. Experiments were performed on DGNCS-1 and littermate control animals on dox. Experimenters were blind to the genotypes of the subjects.

**Safe and Stressful Novel Environment Behavioral Examination**
Naïve, handled subjects were placed in an empty clear Plexiglas chamber (inner dimensions: 42 cm\(^3\)). For the safe environment, the room was dimly-lit and 3 identical objects were added to the arena. For the stressful environment, the conditions were identical except the chamber was brightly lit with an overhead lamp (~400 lux). Mice were observed and scored in an identical manner to those in the object recognition experiments described below.

**New Frontier Exploration Task**

Mice were allowed to climb from their home cage onto any of four platforms, each elevated 15 cm above the floor. The platforms connected the home cage to novel environments (18 cm x 30 cm), also 15 cm above the floor. A subject with two or more paws in the novel environment area was recorded as “visiting” that environment. Subjects were required to return to their home cage before they could be counted as visiting any environment twice. Mice were given 15 min to explore the arena. See Figure S5 for a schematic diagram of the test.

**Hole Board Test**

Behavioral observations were made for 5 min in a dimly-lit circular environment (r = 25 cm, 40 cm high) containing 4 circular holes (r = 1 cm) elevated 8 mm above a clean surface. The latency to explore each hole and the time spent exploring the holes was recorded with Noldus Observer software.

**Light/Dark Box**
Mice were placed in a closed black Plexiglas chamber (20 cm³) for 2 minutes, after which a trap door was raised 4 cm to allow entry into a brightly-lit transparent Plexiglas chamber (20 cm³). Latency to enter the brightly-lit chamber as well as time spent in the chambers was recorded using Noldus Observer software.

**Object Recognition.**

Experiments were conducted in a the same Plexiglas environment used for to examine rearing behavior and, as depicted in Figure S4, using objects upon which the mice were unable to climb. All protocols followed the same sequence of 5 sections: (1) habituation – (2) interval – (3) displaced object discrimination – (4) interval – (5) novel object discrimination. The length of each sequence was varied between protocols in order to investigate different forms of learning and memory (Figure S4). During the habituation and displaced object sections, all 3 objects were identical. During the 2 min intervals between sections, subjects were removed from the Plexiglas box and held away from view of the apparatus in the hand of one experimenter. During 24 hour intervals, animals were returned to their home cages. Between subjects, the entire apparatus including objects was cleaned with 70 % ethanol to remove any trace scents. All sections were completed in a low-lit room, inside a large cloth curtain (~4 m in diameter), on which were hung spatial cues. Two independent observers, visible to the subjects, both blind to the genotypes of the animals, scored behavior live using Noldus Observer software. The position of the observers and spatial cues
remained fixed throughout the experiments. The criteria used for scoring the behaviors shown in Figure S3 were as follows: Still; subject is stationary and not performing any of the other scored behaviors (includes in-between behaviors). Wall Walking; animal is walking within 5 cm of wall. Centre Walking; animal is walking beyond 5 cm from wall. Rear (rearing); animal is upright, potentially sniffing the environment. An animal with one or both paws on the Plexiglas wall or any object to which it is not attending is also counted as rearing. Risk (assessment of risk); animal is walking or stationary with their body elongated and close to the ground. The tail is pointed directly back in a rigid line. The nose is positioned outward. Head Grooming; animal is grooming their paws and head. Body Grooming; animal is grooming their body. Objects (attending to objects); animals were considered to be attending to objects whenever they were actively investigating the objects, generally by sniffing within 1 cm. Rearing on objects, or (in rare cases) standing on objects was not considered as attending behavior, unless the animal was actively investigating the object. Contact with the object was not required, as long as the subject made clear signs of investigation. Time spent wall walking vs center walking was taken as a measure for anxiety. Total time still vs walking was taken as a measure for locomotion.

Morris Water Maze

A schematic of apparatus and procedure is shown in Figure S4. The water maze consisted of a small circular pool (117 cm in diameter) filled to 20 cm below the rim with water (24.5 – 26.0 °C) rendered opaque with non-toxic white tempera
paint obtained from the local art supply store. The large platform (15 cm in
diameter) was submersed 1 cm below the water surface. During acquisition trials,
subjects were given 180 s to find the platform. In rare cases where the subject
did not find the platform within the 180s trial time frame, the experimenter guided
the subject to the platform, returned behind a curtain for 5 s and then removed
the subject from the platform (this was repeated for mice that would not stay on
the platform). During probe (no platform) trials, subjects were allowed to swim for
60 s. In a minimal training protocol, DGNCS-1 and littermate controls were given
a single trial to find a hidden platform. Memory retention of the former platform
location was tested in a probe trial following a 24 hour interval. Immediately after
all animals completed the probe trial, subjects were trained to a new platform
location, again using only 1 trial. This minimal procedure was repeated for a total
of three times. Results from all three probe trials were averaged for each animal
and used for statistical analysis. After the 3rd probe trial, subjects were trained to
a 4th platform location using 4 trials (standard training protocol) spaced by 45 min,
and given a 4th probe trial the next day.

**Immunofluorescence in DGNCS-1 Slices**

After brief anesthetization with halothane, mice were decapitated. Brains were
removed in ice-cold aCSF and sliced fresh in the same solution on a vibratome
(Series 1000 Sectioning System, Pelco 101) coronally either 300 or 400 µm
sections. Slices were immersed in 4 % paraformaldehyde (PFA) in phosphate
buffered saline (PBS, pH 7.2) for 5 min to provide light fixation without disrupting
plasma membranes. All further processing was carried out at 4 °C in PBS containing 2 % bovine serum albumin (BSA) (blocking solution). Following 1 hour blocking (or processing as described below), sections were incubated in monoclonal anti-D2R (Santa Cruz) overnight in the absence of any permeabilizing agent, washed thoroughly three times with the blocking solution, incubated with 1:100 of the appropriate fluorescent secondary (Jackson Immunoresearch) for 2 hrs, washed thoroughly three times with the blocking solution and mounted or, when required, incubated with polyclonal anti-NCS-1 (Proteintech Group, 10506-2-AP) overnight in the presence of 0.5 % Triton-X 100, washed thoroughly three times with blocking solution, incubated with 1:100 appropriate secondary (Jackson Immunoresearch) and again washed thoroughly three times with blocking solution. Finally, sections were rinsed in PBS alone and mounted on Fisher Superfrost slides in antifade reagent (Slowfade, Molecular Probes). All images were captured within 1 week of staining by sequential confocal microscopy (Leica SP2 system).

**Immunofluorescence in DNIP-Treated Slices**

Slices were prepared as described in the preceding section on electrophysiology, except immediately following sectioning, slices were added directly into an oxygenated aCSF bath containing either 10 µM of the active or scrambled DNIP. After 1 hr, slices were blocked in 1 % donkey serum, stained for surface receptors under non-permeabilizing conditions with anti-D1R (Calbiochem), anti-D2R (Santa Cruz) or anti-D3R (Santa Cruz) and the appropriate secondary
(Jackson Immunoresearch), mounted and imaged as described in the preceding section on immunofluorescence in DGNCS-1 slices.

**Co-immunoprecipitation**

Anti-NCS-1 (Proteintech Group) and pre-cleared murine hippocampal lysates were incubated overnight at 4 °C. Using protein A Sepharose CL-4B, co-IP was continued according to the manufacturers’ instructions. To the washed beads were added 30 µL Laemmli Sample Buffer (Bio-Rad) containing 5 % β-mercaptoethanol. Western blotting was performed as described above.

**Peptide Synthesis**

Peptides were synthesized by Dr. Wang at the Advanced Protein Technology Centre at the Hospital for Sick Children, Toronto. High performance liquid chromatographic analysis revealed greater than 95 % purity (data not shown). Each peptide consisted of an N-terminal HIV-1 TAT protein transduction domain coupled via a tetra-glycine spacer to either an active or scrambled cytosolic D2R sequence (Figure 5H). Active peptide comprised of the exact 9 amino acids reported in Kabbani et al. (2002) as the minimal region of D2R that can bind NCS-1. These 9 amino acids were pseudorandomized to generate the scrambled peptide. Protein-peptide blasts confirm the scrambled sequence contains no strong homology to any known protein in the mouse proteome.

**Primary Cultures of Hippocampal Neurons**
Hippocampii dissected from E17-19 fetal Wistar rats were prepared in ice-cold 
Hank’s buffered salt solution (HBSS). Tissue was mechanically dissociated 
through trituration through a fire-polished Pasteur pipette. The single cell 
suspensions were counted and plated at desired densities onto poly-D-lysine 
(100 ug/mL; Sigma) coated tissue culture plates containing Neurobasal medium 
supplemented with 10 % horse serum, 0.5 mM L-glutamine and pen/strep 
(Invitrogen). The following day, neurons were supplemented with an equal 
volume of Neurobasal/B27 medium (Invitrogen). Cultures were incubated at 37 
ºC in a 5 % CO₂ incubator in Neurobasal/B27 medium for 10-14 days before use.

**Cell-ELISA Assays**

Cell-ELISA assays (colorimetric assays) were performed essentially as 
previously described (Lee et al., 2002; Man et al., 2000). Neurons were treated 
with 2 µM active or scrambled DNIP for 30 min @ 37ºC in Neurobasal medium 
(Invitrogen). Neurons were subsequently washed in cold PBS, fixed in 2 % PFA 
for 10 min and where appropriate permeabilized with 0.5 % Triton X-100. Cells 
were incubated with monoclonal D2R antibody against the extracellular amino 
terminus (Santa Cruz) to label proteins on the cell surface under non-
permeabilized conditions or the entire receptor pool under permeabilized 
conditions. After incubation with corresponding HRP-conjugated secondary 
antibodies (Sigma), HRP substrate OPD (Sigma) was added and the reaction 
was stopped with 3 N HCl. Cell surface expression is presented as the ratio of
colorimetric readings under non-permeabilized conditions to those under permeabilized conditions.

**cAMP Assays**

Primary cultures of dissociated hippocampal neurons were first washed in cold PBS. All subsequent drug dilutions were made in Neurobasal media (Invitrogen). Neurons were then incubated for 15 min with 0.5 mM 3-isobutyl-1-methylxanthine (IBMX; Sigma) and 1 μM propranolol (Sigma) containing either 2 μM of either active or scrambled DNIP. To investigate D2R function, neurons were treated with either 10 μM forskolin, 10 μM quinpirole or both for another 30 min at 37°C. To investigate D1R function, neurons were treated with either 1 μM SFK81297, 1 μM SCH23390 or both for another 30 min at 37°C. After incubation, cells were washed once with PBS before the addition of cell lysis buffer (R&D systems). Supernatants were collected and used in cAMP assays as directed by the manufacturer (R&D systems).

**Cannulization and Infusions**

DGNCS-1 subjects were anesthetized with isoflurane and transferred to a stereotaxic apparatus by gently fixing the head with ear bars and softly clamping the open snout on a stainless steel nose piece. The nose was encapsulated in a small breathing chamber through which isoflurane blended with oxygen was provided throughout surgery. The subject’s eyes were protected with Vaseline and Betadine ointment was applied topically to the head, between the ears. After
testing for analgesia, a scalpel was used to expose the skull. All membranes were pushed aside and the skull surface was cleaned with mild hydrogen peroxide. With a remote, pedal-driven drill, two holes were made in the cranium to allow for passage of cannulae. Stainless steel guide cannulae (custom built by Small Parts) were positioned in the brain at coordinates -2.0 mm from bregma, +/- 1.5 mm from midline and -1.2 mm from the dura. Cannulae were affixed to the skull with dental cement. Once cement cured, the skin was loosely sutured with Supramid (SC2080). Subjects were administered 60 µL dilute temgesic (1/10 in PBS) 10-15 min prior to recovering from the operation under a heat lamp. DNIP peptide (low dose, 25 ng/µL) was infused 3-5 hours prior to analysis and L-741,626 (5 µM) was infused 45-75 min prior to analysis using injection cannulae protruding 0.5 mm below the termination of the guide cannulae (to -1.7 mm from the dura) with a Harvard Precision Pump at 0.1 µL/min for 5 min, for a total of 0.5 µL per hemisphere. A further 5 min were allowed for diffusion before removing the injection cannulae. At least 8 days were interspaced between surgery and infusions. Sham-treated controls represent animals temporally implanted with the complete guide cannulae and treated exactly the same as subjects who retained their cannulae.

**Antibody List**

Anti-NCS-1 (Rabbit; Proteintech Group)
Anti-D2R (Mouse; Santa Cruz)
Anti-D1R (Rabbit; Calbiochem)
Anti-D3R (Mouse; Santa Cruz)
Anti-GluR2 (Rabbit; Millipore)
Anti-N-Cadherin (Goat; Santa Cruz)

**Statistical Analysis**

Statistical group comparisons were carried out with a two-way ANOVA Tukey honest significant difference (HSD) for comparisons of equal $n$, or Tukey HSD for unequal $n$ (Spjotvoll/Stoline test) where required using Statistica software.

Differences were considered statistically significant at $p < 0.05$. 
SUPPLEMENTARY REFERENCES


SUPPLEMENTARY FIGURE LEGENDS

**Figure S1. Breeding, Experimental Design and Mass Measurements**

(A) Breeding scheme to generate and study DGNCS-1 mice.

(B) Corresponding PCR for each of the 4 possible resulting genotypes.

(C) Daily experimental scheme.

(D) Daily mouse mass measurements taken every evening from the first evening of dox administration until return to routine diet. Each cohort is a group of DGNCS-1 and littermate control mice that underwent a doxing regime simultaneously. Mice from every cohort were used for any combination of biochemical, eletrophysiological or behavioral experiments. A transient decrease in weight lasting about 6 days was observed. Subjects were not tested during this time. Colored lines represent individual mice. White circles are the mean. Error bars, SEM.

**Figure S2. Baseline Synaptic Transmission and Short-Term Depression**

DGNCS-1 slices demonstrate comparable MPP-dentate input/output curves plotted either as a function of (A) stimulation intensity or (B) fiber volley size.

(DGNCS-1, n = 8 slices from n = 8 mice; Control, n = 16 slices, n = 16 mice).

(C) DGNCS-1 mice show comparable PPD, a short-term form of plasticity (DGNCS-1, n = 6 slices from n = 6 mice; Control, n = 6 slices from n = 6 mice). Error bars, SEM.
**Figure S3. Object Recognition Baseline Behavior**

Behavior of DGNCS-1 and littermate control subjects in the habituation phase of the standard short object recognition task separated into three 5 min bins (1 | 2 | 3). Overexpression of Ncs1 in the DG did not affect the duration of time spent still, walking along the walls or in the centre, assessing the environment for risk, grooming the head or body, or attending to the objects, but did affect the duration of time spent rearing. See full experimental methods for detailed description of each behavior (*p< 0.05) Error bars, SEM.

**Figure S4. Learning and Memory Tasks**

Three object recognition and two water maze protocols were used to investigate short and long-term spatial and non-spatial memory. HAB = habituation phase; DO = displaced object phase; NO = novel object phase; HC = Home cage. See full experimental procedures for detailed descriptions.

**Figure S5. Short-term Object Discrimination in Control Animals**

Control animals given a 6 min habituation period demonstrate short-term object discrimination. (n = 7) S = stationary object; D = displaced object; F = familiar object; N = novel object. *p < 0.05, **p < 0.01 Error bars, SEM

**Figure S6. The DNIP Selectively Decreases D2R Surface Expression in the DG Perforant Path**
(A-C) Surface D2R staining in the DG molecular layer of (A) scrambled DNIP and (B) active DNIP treated slices. (C) Mean confocal z-stack fluorescence +/- SEM (scramble, n = 5; active, n = 7).

(D-F) Surface D1R staining in the dentate perforant path of (D) scrambled DNIP and (E) active DNIP treated slices. (F) Mean confocal z-stack fluorescence +/- SEM (scrambled, n = 5; active, n = 4).

(G-I) Surface D3R staining in the dentate perforant path of (G) scrambled DNIP and (H) active DNIP treated slices. (I) Mean confocal z-stack fluorescence +/- SEM (scrambled, n = 5; active, n = 5). Pico = Picogreen nuclear stain. D/P = dopamine receptor fluorescence normalized to Picogreen nuclear stain. Scale bar = 150 µm. (*p<0.05, **p < 0.01) Error bars, SEM.

Figure S7. The DNIP does not affect D1R Surface Expression or Function

(A) Colorimetric measurements of D1R surface expression in hippocampal cultures isolated from E17-19 fetal Wistar rats. Cultures were incubated in either 2 µM active or scrambled DNIP for 30 min (n = 3).

(B) cAMP quantification in hippocampal cultures following 30 min incubation of active or scrambled DNIP. SFK = SFK81297, SCH = SCH23390 (n = 3). (**p < 0.01 compared to control, ***p < 0.01 compared to control, ##p < 0.01 compared to SFK treated neurons, ###p < 0.001 compared to SFK treated neurons) Error bars, SEM.
Figure S8. Enhanced LTP in DGNCS-1 is not Mediated by a Change in Inhibition

No LTP was induced when an above-threshold train (4 x 100 Hz) was given in the absence of the GABA\textsubscript{A} blocker, bicuculline.

Figure S9. NMDA and AMPA Component of Synaptic Responses

(A-F) Pharmacology was used to isolate NMDA and AMPA component of the synaptic response in the dentate of NCS-1 overexpressors. aCSF with lower Mg\textsuperscript{2+} (0.325 mM) was used to enhance NMDA receptor activation. The time-course plots (A-C) show the integral (proportional to synaptic current) over various portions of the fEPSP.

(A) The initial fast component of the fEPSP area (0-3 ms, largely due to AMPA receptor activation, see insert diagram) plotted over time.

(B) The entire fEPSP area (0-25 ms, see insert) plotted over time.

(C) Integral of the fEPSP late component (5-25 ms, contribution nearly equal for both NMDA and AMPA receptors, see insert, diagram) plotted over time.

(D) Upper portion show sample synaptic responses from a control slice at various time points (superimposed): Start of experiment (black trace, -5 to 0 minute range), after addition of D-APV to block NMDA receptors (dark gray, 5 to 10 minutes) and after further addition of CNQX to block AMPA receptors (light gray, 15 to 20 minutes).

Lower portion shows subtracted traces to reveal the AMPA (black) and NMDA (blue) component of the response.
(E) As in (D) except that traces are taken from a representative DGNCS-1 slice. NMDA component trace shown in red. Scale bar for D and E traces is shown at lower right.

(F) The percentage contribution of the NMDA fEPSP area (5-25 ms) as a function of total NMDA + AMPA (0-3 ms) area did not differ between Control and DGNCS-1 slices.

(G) Comparison of the average AMPA:NMDA fEPSP area. Above data (A-G) is from n=10 slices from N=5 animals (Control) and n=8 slices from N=4 animals (DGNCS1).

**Figure S10. Microdissection Technique to Isolate DG and CA1-3-specific Hippocampus Subregional Fractions**

Freshly removed hippocampii were sliced with 2 fused safety blades. A glass capillary tube was centered about the hilus and pressed into each resulting sections (~700 um thick) in ice-cold PBS. The remainder of each slice was taken as the CA1-3 fraction. Fractions were processed immediately or frozen on dry ice and kept at -80 °C until use.

**Table S1. Baseline Synaptic Responses and Short-Term Depression**

Shown is the average fEPSP slope from MPP-dentate and Schaffer Collateral-CA1 slice recordings. There were no genotypic differences in basal synaptic transmission, nor paired-pulse ratio in any groups. Mean +/- SEM.