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Nicotinic Acetylcholine Receptor β2 Subunits in the Medial Prefrontal Cortex Control Attention

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animals (*15*, *17*). Nevertheless, future studies should determine whether *Sxl* homologs are expressed in the germ line of non-drosophilids. Moreover, it would be of particular interest to identify downstream targets of *Sxl* in the *Drosophila* germ line and to test whether these genes have a widespread role in germline sex determination.

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Materials and Methods
SOM Text
Figs. S1 to S9
Tables S1 to S5
References

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Nicotinic Acetylcholine Receptor β2 Subunits in the Medial Prefrontal Cortex Control Attention

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More than one-third of all people are estimated to experience mild to severe cognitive impairment as they age. Acetylcholine (ACh) levels in the brain diminish with aging, and nicotinic ACh receptor (nAChR) stimulation is known to enhance cognitive performance. The prefrontal cortex (PFC) is involved in a range of cognitive functions and is thought to mediate attentional focus. We found that mice carrying nAChR β2-subunit deletions have impaired attention performance. Efficient lentiviral vector–mediated reexpression of functional β2-subunit–containing nAChRs in PFC neurons of the prelimbic area (PrL) completely restored the attentional deficit but did not affect impulsive and motivational behavior. Our findings show that β2-subunit expression in the PrL PFC is sufficient for endogenous nAChR-mediated cholinergic regulation of attentional performance.

Cortical acetylcholine (ACh) release from the basal forebrain is essential for proper sensory processing and cognition (*1*–*3*) and tunes neuronal and synaptic activity in the underlying cortical networks (*4*, *5*). Loss of cholinergic function during aging and Alzheimer's disease results in cognitive decline, notably a loss of memory and the ability to sustain attention (*6*, *7*). Interfering with the cholinergic system strongly affects cognition (*3*, *8*–*13*). Rapid changes in prefrontal cortical ACh levels at the scale of seconds are correlated with attending and detecting cues (*14*, *15*). Various types of nicotinic ACh receptor (nAChR) subunits are expressed in the prefrontal cortex (PFC) (*16*–*18*), and in particular nAChRs containing β2 subunits are thought to enhance attention (*13*). However, the causal relation between nAChR β2 subunits (henceforth β2*-nAChRs) expressed in the medial PFC (mPFC) and attention performance has not yet been demonstrated.

We first determined whether absence of nicotinic β2 subunits affects attentional behavior in the five-choice serial reaction time task (5-CSRTT), a well-established test setup that taxes various aspects of attentional control over performance (*19*). Mice lacking β2 subunits of nAChRs ($\beta2^{-/-}$) and their wild-type littermates (WT) were trained to detect and respond to a brief light stimulus randomly presented in one of five nose poke holes to receive a food pellet (*20*). $\beta2^{-/-}$ mice showed normal locomotor activity in an open field test (fig. S1), normal sensorimotor gating in a prepulse inhibition test (fig. S2), and normal acquisition in the 5-CSRTT (fig. S3). After complete acquisition of the 5-CSRTT, animals were trained at the stimulus duration of 1 s (SD1) for 10 more days until they reached stable performance (fig. S4). Baseline 5-CSRTT performance was then calculated from the 6th until the 10th session at SD1 (Fig. 1, A and B). $\beta2^{-/-}$ mice exhibited significantly more omissions than their WT littermates [$F_{(1,27)}$ = 12.45; $P < 0.01$] (Fig. 1A), whereas the level of accuracy was not significantly different [$F_{(1,27)}$ = 2.56; not significant (NS)] (Fig. 1B). We found no effect of genotype on any other measures, such as number of initiated trials [$F_{(1,27)}$ = 1.99; NS], number of premature responses [$F_{(1,27)}$ = 0.003; NS], correct response latency [$F_{(1,27)}$ = 2.03; NS], or latency to collect earned food pellets [$F_{(1,27)}$ = 0.12; NS] (table S1), suggesting that increased omissions reflected impairments in stimulus detection processes in $\beta2^{-/-}$ mice rather than motor or motivational deficits. $\beta2^{-/-}$ mice and their WT littermates did not differ in the number of food pellets earned by responding to a single cue light nor in the maximal number of responses in a progressive ratio for earning food pellets (fig. S5). In contrast to $\beta2^{-/-}$ mice,

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mice lacking α7 subunits of nAChRs ($\alpha7^{-/-}$) exhibited similar levels of omission [$F_{(1,35)}$ = 0.10; NS] and accuracy [$F_{(1,35)}$ = 0.05; NS] as their WT littermates [but see supporting online material (SOM) text; Fig. 1, C and D; and table S3]. To further characterize attentional deficits, we compared performance in a variable stimulus procedure, in which stimulus durations were randomly decreased to 0.5 and 0.25 s (fig. S6 and table S2). $\beta2^{-/-}$ mice made significantly more omissions than WT mice at every stimulus duration (fig. S6A and table S2) but had similar accuracy and motivation to earn food rewards (fig. S6B and fig. S5), whereas no difference was observed between $\alpha7^{-/-}$ and WT animals (fig S6, C and D, and table S4).

To further understand the specific role of β2-containing nAChRs in mediating the effects of endogenous ACh on cognition (*21*), we selectively reexpressed the β2 subunit (*22*) in the prelimbic area (PrL) of the mPFC of $\beta2^{-/-}$ mice. The mPFC is critically involved in attentional performance (*23*). We reexpressed β2 subunits in combination with enhanced green fluorescent protein (eGFP) by injection of the β2-eGFP bi-cistronic vector (*24*, *25*) into the PrL PFC of $\beta2^{-/-}$ mice (KO^{VEC}). As a control, we used a lentiviral vector expressing eGFP only in $\beta2^{-/-}$ mice (KO^{eGFP}) and WT littermates (WT^{eGFP}). Coronal sections showing the site of lentivirus injection revealed that viral reexpression was selective to the PrL of the mPFC (Fig. 2A). The efficacy of this in vivo reexpression strategy was demonstrated by confocal analysis showing that eGFP colocalized with a neuronal marker (NeuN) in KO^{VEC} mice (Fig. 2A) and indicates efficient transduction of β2-eGFP vectors in PrL neurons. β2 subunits do not form functional nAChRs by themselves but require nAChR α subunits to coassemble into functional receptors (*26*). Therefore, in KO^{VEC} mice not all eGFP-expressing neurons will have β2*-nAChRs. Only in neurons that express nAChR α subunits will lentivirus-mediated expression of β2 subunits result in functional nAChRs containing β2 subunits. We thus made whole-cell recordings from eGFP-expressing neurons in the three groups and tested their response to ACh (Fig. 2, B to D). In WT^{eGFP} mice, locally applied ACh (1 mM) induced inward currents with slow kinetics, characteristic of β2*-nAChRs (Fig. 2D). These currents were strongly reduced by the antagonist of β2*-nAChRs, dihydro-β-erythroidine [DHβE 1 μM; Student's *t* test $t_{(7)} = -3.15$; $P < 0.05$ (Fig. 2, D and E)]. KO^{eGFP} neurons never showed slow inward currents in response to ACh application (Fig. 2D). Neurons in the mPFC of KO^{VEC} mice

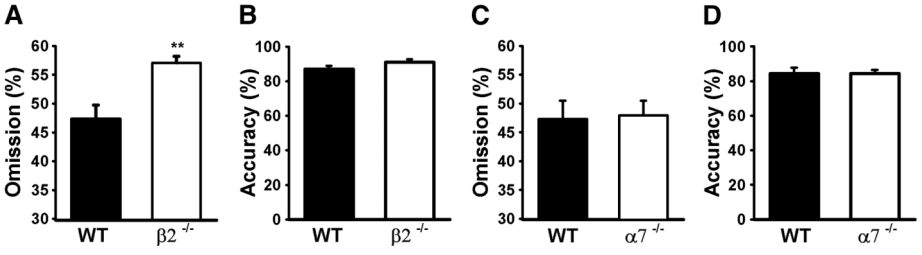

Fig. 1. β2-nAChR subunit is necessary for normal performance in 5-CSRTT. (**A** and **B**) Percentage omission (A) and accuracy (B) of WT (n = 15, black) and $\beta2^{-/-}$ mice (n = 14, white) during baseline training (SD1). $^{**}P < 0.01$, Newman-Keuls post hoc test. (**C** and **D**) Percentage omission (C) and accuracy (D) of WT (n = 12, black) and $\alpha7^{-/-}$ mice (n = 25, white) during SD1. Data in all figures are shown as mean ± SEM.

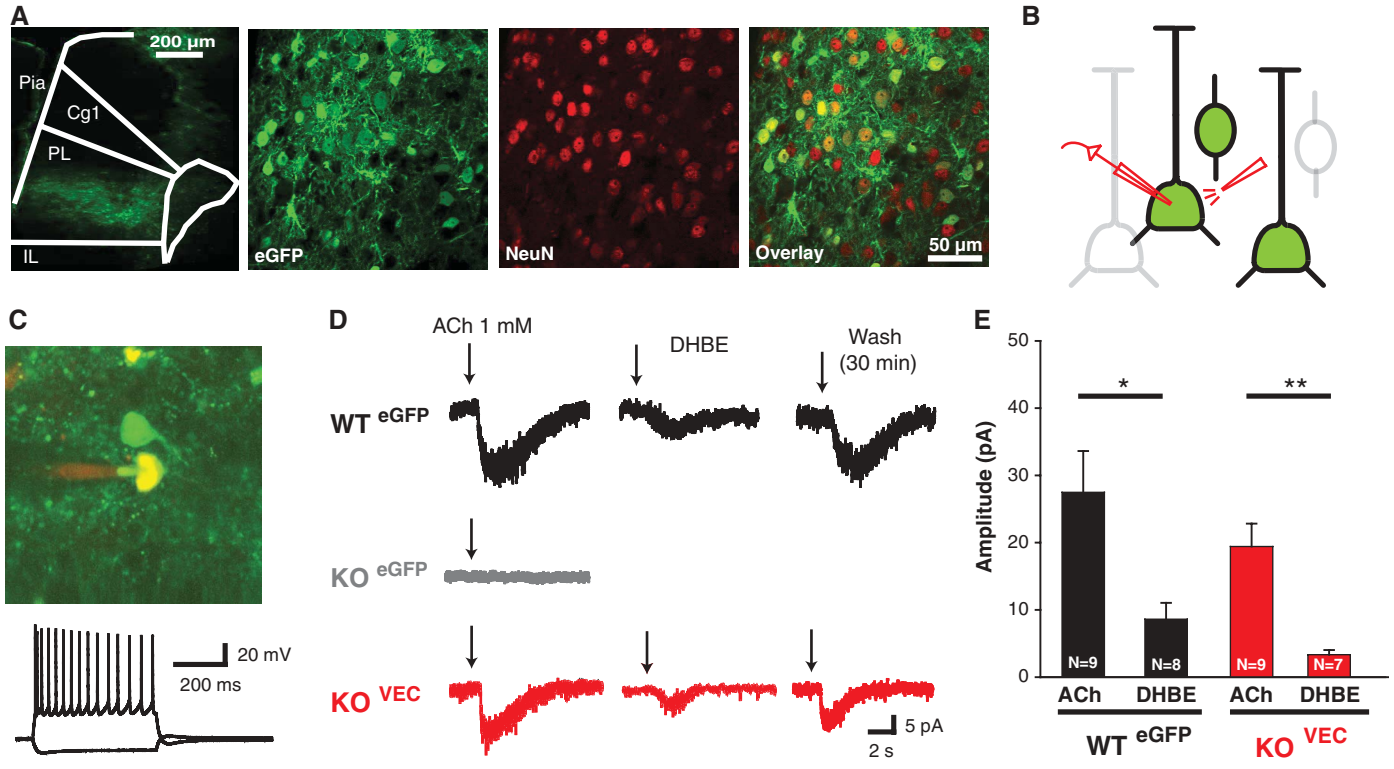

Fig. 2. Lentiviral restoration of functional β2*-nAChRs in the mPFC. (**A**) Coronal section (1.9 mm from bregma) showing the injection site in the prelimbic mPFC (left) and confocal images of acute coronal sections showing neuronal eGFP (green) expression (red, NeuN) in KO^{VEC} mice and merged image (right). (**B**) Experimental setup. (**C**) Patched eGFP-positive neuron. (**D**) Current traces recorded from WT^{eGFP} (n = 9, black), KO^{eGFP} (n = 9, gray), and KO^{VEC} (n = 9, red) neurons. ACh was locally applied (1 mM, 100 ms) in control (left), in the presence of β2-containing nAChRs antagonist, DHβE 1 μM (middle), or after 30 min washout (right). (**E**) Summary of ACh-induced inward currents for WT^{eGFP} (black) and KO^{VEC} (red). nAChR current amplitudes of WT^{eGFP} and KO^{VEC} neurons were not statistically different.

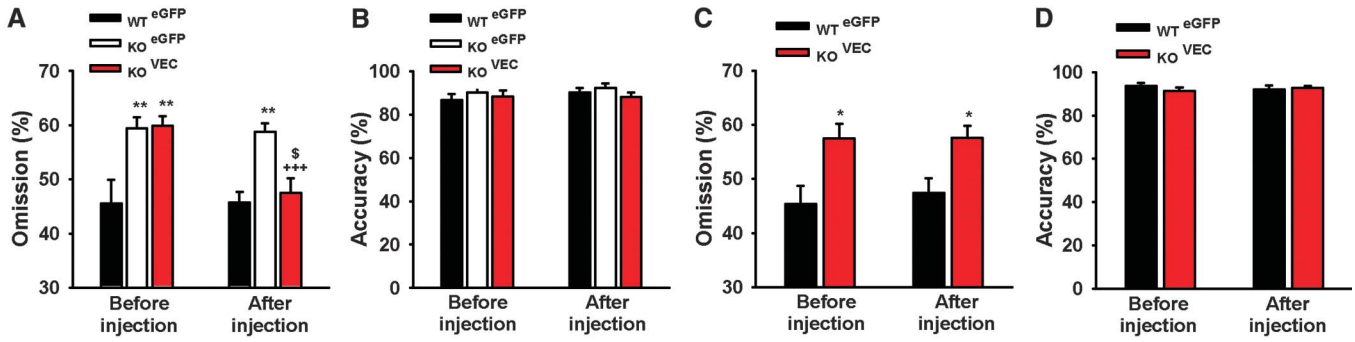

Fig. 3. Targeted reexpression of β2-nACR subunits in PrL mPFC restores performance. (**A** and **B**) Percentage omission (A) and accuracy (B) (SD1) before and after viral injection for WT^{eGFP} (n = 11), KO^{eGFP} (n = 11), and KO^{VEC} mice (n = 11). $^{**}P < 0.01$, compared with WT^{eGFP}; $\$P < 0.05$, compared with KO^{eGFP}; $^{+++}P < 0.001$, before and after virus injection, Newman-Keuls post hoc test. (**C** and **D**) Reexpression of β2-nACR subunits in the anterior cingulate cortex did not restore attention performance. Percentage omission (C) and accuracy (D) (SD1) before and after viral injection for WT^{eGFP} (n = 11) and KO^{VEC} mice (n = 4). $^{*}P < 0.05$, compared with WT^{eGFP}.

showed slow inward currents reminiscent of functional β2*-nAChR responses [n = 9 of 17 EGFP-positive neurons (Fig. 2D)]. These currents were strongly reduced by DHβE [$t_{(6)} = -5.02$; $P < 0.01$ (Fig. 2, D and E)], showing the successful reexpression of functional β2*-nAChRs in KO^{VEC} mice.

We addressed the question of whether β2*-nAChRs specifically in the PrL mPFC would be sufficient for optimal attentional performance. We therefore tested whether impaired performance of $\beta2^{-/-}$ mice was rescued by targeted reexpression of the β2 subunit in the PrL mPFC. Preliminary analysis before viral expression showed comparable findings between the independent batches of mice, with a significant increase in omissions in $\beta2^{-/-}$ mice (fig. S7). One week after virus introduction, WT^{eGFP}, KO^{eGFP}, and KO^{VEC} mice were retrained in the 5-CSRTT procedure by using SD1 for 14 days before the effects of lentiviral intervention were assessed.

At the end of these 14 days, WT^{eGFP} and KO^{eGFP} animals performed at the same levels as they showed before virus injection, but KO^{VEC} mice performed significantly better than before injection (Fig. 3). The percentage of omission of the three groups of mice was differentially affected by lentivector injection [group effect $F_{(2,30)}$ = 10.73 and $P < 0.001$; injection time effect $F_{(1,30)}$ = 6.12, $P < 0.05$; group × injection time interaction $F_{(2,30)}$ = 9.29; $P < 0.001$] (Fig. 3A). Although KO^{eGFP} mice made more omissions than WT^{eGFP} at each time point (WT^{eGFP} versus KO^{eGFP}, $P < 0.01$), both groups exhibited the same percentage of omissions before and after virus injections (NS, eGFP) and hence were not affected by eGFP expression. Reexpression of β2 subunits in the mPFC (KO^{VEC}) significantly decreased the percentage of omissions (KO^{VEC} before versus KO^{VEC} after, $P < 0.001$). Moreover, the rescue in KO^{VEC} mice was complete, and these mice reached the same number of omissions as WT^{eGFP} mice (WT^{eGFP} versus KO^{VEC}, NS) and made significantly fewer omissions than KO^{eGFP} mice (KO^{eGFP} versus KO^{VEC}, $P < 0.05$). This rescue effect was selective for omissions because β2 reexpression had no significant effect on accuracy [group effect $F_{(2,30)}$ = 1.92, NS; injection effect $F_{(1,30)}$ = 2.42, NS; group × injection interaction $F_{(2,30)}$ = 2.36, NS] (Fig. 3B) or any other measures (table S5). This rescue effect was also observed during a variable stimulus procedure (fig. S9), as well as during a variable intertrial interval procedure in which the stimulus presentations were temporally unpredictable (fig. S10), further supporting the conclusion that β2-subunit restoration in the PrL is sufficient for proper attention performance. A similar rescue effect of β2 reexpression in KO^{VEC} mice was observed in an independent group of animals (fig. S11). After these behavioral experiments, the mice were killed, and neuronal expression of eGFP and functional β2*-nAChRs in the PrL was confirmed. β2-subunit reexpression in the anterior cingulate had no effect on omission or accuracy (Fig. 3, C and D), in line with the finding that cholinergic projections to the anterior cingulate cortex are not involved in 5-CSRTT performance (*27*).

Our findings show that expression of β2*-nAChRs is necessary for optimal attentional performance in mice and that restoring expression of β2*-nAChRs in the mPFC PrL area is sufficient for optimal performance. Nicotinic AChRs containing β2 subunits are located on cell bodies of neurons as well as on thalamocortical afferents in the PrL PFC (*16*, *17*). The latter have been suggested to be involved in attention and processing of sensory stimuli (*17*). The present study reveals that restoration of β2*-nAChR receptors, specifically in the PrL area of the mPFC, is sufficient to restore the attentional deficit of $\beta2^{-/-}$ mice to WT levels. Attentional control therefore appears to be mediated by endogenous ACh acting on β2*-nAChR receptors expressed by neurons located within the PrL mPFC, although a role for β2*-nAChRs on thalamic projections cannot be entirely excluded on the basis of the present results. Nevertheless, the nAChR system in the PrL mPFC is a principal factor in attentional control. Consistent with this, rapid changes of ACh levels in mPFC are correlated with cue attending and detection (*14*), an effect mainly due to mPFC β2*-nAChRs stimulation (*28*). Our findings have implications relevant for understanding the neurobiology of attention and suggest agonists or positive allosteric modulators at these mPFC β2*-nAChRs within the PrL PFC as potential targets for the development of more effective treatments for cognitive impairments.

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Materials and Methods
SOM Text
Figs. S1 to S11
Tables S1 to S5
Reference (*29*)

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Schema-Dependent Gene Activation and Memory Encoding in Neocortex

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When new learning occurs against the background of established prior knowledge, relevant new information can be assimilated into a schema and thereby expand the knowledge base. An animal model of this important component of memory consolidation reveals that systems memory consolidation can be very fast. In experiments with rats, we found that the hippocampal-dependent learning of new paired associates is associated with a striking up-regulation of immediate early genes in the prelimbic region of the medial prefrontal cortex, and that pharmacological interventions targeted at that area can prevent both new learning and the recall of remotely and even recently consolidated information. These findings challenge the concept of distinct fast (hippocampal) and slow (cortical) learning systems, and shed new light on the neural mechanisms of memory assimilation into schemas.

Memory consolidation consists of two processes. Cellular consolidation is mediated by synaptic and signal transduction mechanisms that store newly encoded memory traces on-line (*1*, *2*). Systems consolidation involves a time-limited interaction between the medial temporal lobe and the neocortical areas that eventually store long-term memory traces (*3*–*5*). Studies monitoring cerebral glucose use, immediate early gene (IEG) activation, and dendritic spine formation (*6*–*9*) indicate that rapid on-line encoding of episodic-like memory in the hippocampus can be followed by temporally graded neural changes in the medial prefrontal (mPFC), orbitofrontal (Orb), and retrosplenial (RSC) cortices.

This apparent sequence of events does not preclude the possibility of simultaneous encoding or "tagging" in the hippocampus and cortex (*9*, *10*). Indeed, when systems consolidation occurs in the presence of relevant prior knowledge (*11*, *12*), the "assimilation" of new paired-associate (PA) memories into existing activated cortical schemas proceeds very rapidly (*13*), reflecting an influence of prior knowledge on the rate of consolidation (*14*). The associative encoding of such PAs requires the hippocampus (*13*, *15*, *16*), accompanied by novelty-triggered cellular consolidation (*17*), but may also involve simultaneous cortical encoding. However, if parallel cortical encoding into a schema occurs, it may be driven solely in a bottom-up manner by the hippocampus or may also reflect the influence of activated prior knowledge already stored in cortex.

Study 1 mapped IEG activation in numerous brain areas of rats during both the retrieval of original PAs and the learning of new PAs after extensive prior training of a schema over many weeks (fig. S1). Guided by the retrieval cue of different flavors of food given in the start box of an event arena on each of six daily training trials, the animals learned to recall the location of the appropriate sand well, where they were rewarded by retrieving more of that same flavored food. Once performance reached asymptote over 6 weeks (fig. S2), a critical session of retrieval and new learning was conducted.

The 21 trained animals were then divided into three groups (Fig. 1A), to which a group of seven caged control animals (group CC) was added. One group had six trials with the original set of PAs and thus had only to retrieve (group OPA, i.e., original paired-associates). Another group had four successive trials with the original PAs and was then exposed to two new PAs that we had shown (*13*) could be encoded and successfully assimilated into the existing cortical schema (group NPA, i.e., new paired-associates). The third group was exposed to six new combinations of flavor and location that constituted a set of six new PAs (group NM, i.e., new map). Although this group was subjected to much greater "novelty," it was in a manner that should not allow successful cortical assimilation (timeline in Fig. 1A). The performance during that single session reflected these different conditions (Fig. 1B; latency data in fig. S3). After a further interval of 80 min (optimized for IEG signal detection of the neural correlates of the events of trials 5 and 6), the animals were first given a cued-recall test. This showed effective memory for the new PAs in the NPA group but no learning by the NM group (Fig. 1C). Brain sections were then prepared for histochemical analysis of two plasticity-associated IEGs—*Zif268* (*Egr1*) and *Arc* (activity-regulated cytoskeleton-associated protein) (*18*, *19*). Quantitative blind analysis of entire brain regions revealed a striking learning-associated increase in IEG expression in the prelimbic region (PrL) of the mPFC that was nonmonotonic with respect to the extent of learning-associated novelty (Fig. 1, D and E). IEG expression was highest in the NPA rats for whom activated prior knowledge was relevant to new PA information.

Detailed analysis revealed three broad patterns of IEG activation (fig. S4 and tables S1 and S2). First, a group of cortical regions [PrL, anterior cingulate (ACC), and RSC] showed the same nonmonotonic pattern of higher Zif268 and Arc expression in group NPA as in groups OPA and NM, despite the latter group being exposed to greater novelty (Figs. 1E and 2A); analyses of variance (ANOVAs) based on average values from all three regions showed a significant inverted U-shaped effect (Fig. 2A). Non-mnemonic aspects such as motivation were excluded as contributing factors by analysis of latency rather than choice (see fig. S3). Second, and in contrast, area CA1 of the hippocampus (Fig. 2B) showed a large increase in Arc expression in groups NPA and NM, with a monotonic trend favoring the highest expression in group NM; Zif268 levels were unchanged (see also fig. S7). Third, certain cortical regions showed little absolute change in IEG expression across the trained groups or relative to group CC [including the primary somatosensory "barrel" cortex (Ssp); Fig. 2C]. Barrel cortex was therefore chosen as the control region for study 2.

In study 2, we sought to determine whether the significant increase in both Zif268 and Arc

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