Methods

Wild radish experiments.

We used *R. raphanistrum* seeds from a second generation of untreated greenhouse-grown plants. We germinated about 10 seeds from each of 13 maternal families in a greenhouse. At the four-leaf stage, each plant was randomly assigned to one of the two treatments (3–5 plants per treatment per family). The caterpillar herbivory treatment was maintained throughout the growth of the plant. Effects of herbivory on seed set are reported elsewhere[19]. Seeds from 8 of the original 13 families were chosen for the transgenerational experiment because they spanned the range of tolerance to herbivory[19], and 2–4 seeds from each of 58 maternal plants within 8 grandmaternal families (a total of 126 plants) were grown in a greenhouse and inoculated with a single newly hatched *P. rapae* larva. Caterpillars were not caged. After four days, the caterpillars were weighed.

Effects of grandmaternal family (fixed), maternal environment (control or herbivory; fixed), maternal family (nested within grandmaternal family by treatment interaction; random) and seed mass (covariate) on caterpillar growth were analysed using a mixed-model analysis of variance (ANOVA). *F*-ratios for grandmaternal family and treatment effects were calculated with maternal family nested in grandmaternal family by treatment interaction mean-square and degrees of freedom in the denominator.

For phytochemical analysis, glucosinolates were analysed using modified procedures for determination of trimethylsilyl glucosinolate derivatives with capillary gas chromatography and flame ionization detection[20]. Seed nitrogen and carbon were determined from a separate set of seeds using dynamic flash-combustion and gas-chromatographic separation and a thermal-conductivity detection system (Division of Agriculture and Nature Resources, University of California, Davis).

Daphnia experiments.

We used a *Daphnia cucullata* clone isolated from Thaler lake, Germany. The experiments were conducted in the laboratory under constant conditions at 20 °C and fluorescent light in a synthetic medium. The F_0 generation had been synchronized by always raising the third brood offspring born within 12 h, starting from a single *Daphnia*. *Daphnia* were fed *ad libitum* daily with *Scenedesumus acutus* ($1.5\ mg\ C\ l^{-1}$).

Animals with freshly deposited eggs were placed into 0.75 l medium containing a 125-μm net cage, enclosing either 10 fourth-instar larvae of *Chaoborus flavicans* or 4 *Leptodora kindtii*, or no predators for controls. The net cages prevented direct contact between predators and prey.

Predation experiments with *Chaoborus* were conducted for 0.5 h with each prey morph separately in 100 ml medium with 10 prey organisms and a single predator. Predation trials with *Leptodora* were conducted with 10 prey organisms of each morph together in 0.5 l in 24-h experiments. In each experiment, animals of the same age and body size class (0.6–0.8 mm, from the eye to the base of the tail spine) but of different morphology were compared.

We induced helmet formation in *Daphnia* by using *Chaoborus* kairomones. We placed four *Chaoborus* larvae in net cages in 1.5-l beakers and changed the water ever day. Chaoborids were fed daily with 10–15 prey (*D. cucullata* and *Ceriodaphnia* sp.). The F_0 generation had been born and raised in these beakers in either the control or kairomone treatment. We used three beakers per treatment as replicates, which did not differ and were pooled for analysis.

Daphnids were measured with a digital image-analysis system (SIS, Münster, Germany). To compensate for small changes in body length within an age class, we calculated the relative helmet length (helmet length/body length × 100). Relative helmet length is a good predictor of the defensive effect within an age class. The relative values were arcsin-transformed for analysis. In the induction treatment, differences between control and kairomone treatment were compared using *t*-tests. Effects of induced *Daphnia* phenotypes on predation were analysed by using a Mann–Whitney-*U*-test for *Chaoborus* and with a paired Wilcoxon test for related samples for *Leptodora*. To test for transgenerational effects, we compared the treatments within each brood and age class (Table 2, Fig. 3) with ANOVA and Bonferroni adjustments.

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Genetic enhancement of learning and memory in mice

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Hebb's rule (1949) states that learning and memory are based on modifications of synaptic strength among neurons that are simultaneously active. This implies that enhanced synaptic coincidence detection would lead to better learning and memory. If the NMDA (*N*-methyl-D-aspartate) receptor, a synaptic coincidence detector[1–4], acts as a graded switch for memory formation, enhanced signal detection by NMDA receptors should enhance learning and memory. Here we show that overexpression of NMDA receptor 2B (NR2B) in the forebrains of transgenic mice leads to enhanced activation of NMDA receptors, facilitating synaptic potentiation in response to stimulation at 10–100 Hz. These mice exhibit superior ability in learning and memory in various behavioural tasks, showing that NR2B is critical in gating the age-dependent threshold for plasticity and memory formation. NMDA-receptor-dependent modifications of synaptic

efficacy, therefore, represent a unifying mechanism for associative learning and memory. Our results suggest that genetic enhancement of mental and cognitive attributes such as intelligence and memory in mammals is feasible.

The NMDA receptors are heteromeric complexes consisting of subunit 1 (NR1) and various NR2 subunits[5,6]. The NR1 subunit is essential for channel function, whereas the NR2 subunit regulates channel gating and Mg^{2+} dependency[7]. In adult forebrain regions such as the hippocampus and cortex, only NR2A and NR2B subunits are available to form the receptor complex with NR1 subunit. The recombinant NR1–NR2B complex *in vitro* shows longer excitatory postsynaptic potentials (EPSPs) than does the NRI–NR2A complex[8]. Increased incorporation of NR2B into the receptor complex *in vivo* should allow the NMDA receptors to increase the time window for detecting synaptic coincidence. NR2B expression is downregulated during the transition from juvenile to adult[9,10], correlating with the gradual shortening of the EPSP duration of the NMDA channel[11,12]. This could decrease NMDA-mediated plasticity, and perhaps explain decreased memory performance in adult animals including songbirds, monkeys and humans[13,14].

To test whether the NR2B subunit is crucial for implementing Hebb's rule and gating synaptic plasticity and memory, we overexpressed the NR2B subunit postnatally in the mouse forebrain using the CaM-kinase-II promoter[15,16] (Fig. 1a). Of seven lines produced, we report here results from two independent lines (Tg-1 and Tg-2) that we have systematically analysed. They show similar expression patterns and levels of NR2B, and nearly identical electrophysiological and behavioural phenotypes.

These transgenic animals, named *Doogie*, show normal growth and body weights and mate normally. Their open-field behaviour is indistinguishable from that of wild-type littermates, and we observed no seizure or convulsion in transgenic animals. As our northern blot analysis indicates, NR2B transgene expression is enriched in the cortex and hippocampus, with little expression in the thalamus, brainstem and cerebellum (Fig. 1b). Western blot analysis showed about twice as much NR2B protein in the cortex and hippocampus of transgenic mice as in the wild type (Fig. 1c). There is also a slight increase in NR1 protein, but no change in NR2A expression in these regions (Fig. 1c). This indicates that both the ratio of NR2B to NR2A in the receptor complex and the total number of NMDA receptors may be increased. We investigated the transgene's anatomical distribution using *in situ* hybridization and found that the transgene was highly enriched in the cortex, striatum, hippocampus and amygdala (Fig. 1d). Light microscopy showed no gross structural abnormalities in the transgenic animals (Fig. 1e, f). The shapes and architecture of dendritic spins in the hippocampus and cortex are also normal (Fig. 1g).

To evaluate the elementary properties of the NMDA receptors in single synapses, we used a single-bouton recording technique[17]. Using FM 1-43 dye to label functional synaptic sites in cultured hippocampal neurons, we positioned the tip of an iontophoretic electrode containing 150 mM glutamate next to a relatively isolated synapse (Fig. 2a) and applied glutamate to determine the functional properties of glutamate receptors located in that synapse. Glutamate-evoked responses consisted of either AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) current alone or both AMPA and NMDA currents, depending on the cell potential. The NMDA component was identified by its 'J'-shaped current–voltage relationship and long decay time (Fig. 2b). NMDA currents were isolated by clamping the cells to +40 mV to remove the voltage-dependent Mg^{2+} block. Initial experiments were carried out with 5 μM DNQX in the bath solution to block AMPA receptors, but we subsequently omitted the antagonist because NMDA current could clearly be isolated from AMPA current based on their respective time courses. All the experiments were conducted in blind fashion.

We first determined the glutamate dose–response curve of the synaptic NMDA receptors (an index of the glutamate affinity of the NMDA receptor) and its voltage-dependent Mg^{2+} block, and found no difference between the two groups of mice (data not shown). As the recombinant NR2B subunit determines the decay phase of NMDA currents *in vitro*[8], we next measured the NMDA-channel decay time for currents evoked by a saturating dose of glutamate (100 nA of iontophoretic current, as determined from the dose–response relationship) in both transgenic and wild-type neurons. Although we found no difference in decay time at day 10 or 14 *in vitro*, the decay time of the NMDA currents from transgenic neurons at day 18 was 1.8-fold longer than that of wild-type neurons (Fig. 2c, inset and Fig. 2e; $P < 0.005$). In addition, transgenic neurons retained the juvenile-like, single-synapse peak NMDA-current amplitude over time in culture. This is in contrast to that of wild-type neurons, which decreased significantly by day 18 *in vitro* (Fig. 2d, significance between NR2B ($n = 18$) and wild type ($n = 8$), $P < 0.01$; significance between day 14 ($n = 8$) and day 18 ($n = 8$) for wild type, $P < 0.01$). This indicates that the total number of NR2B-containing NMDA receptors per single synapse is also increased in transgenic neurons at this stage. These age-dependent changes in channel decay time and peak amplitude are consistent with the *in vivo* observation that the NR2B transgene messenger RNA was detectable but low at postnatal day 14 (P14), and gradually increased to steady level about one week later (data

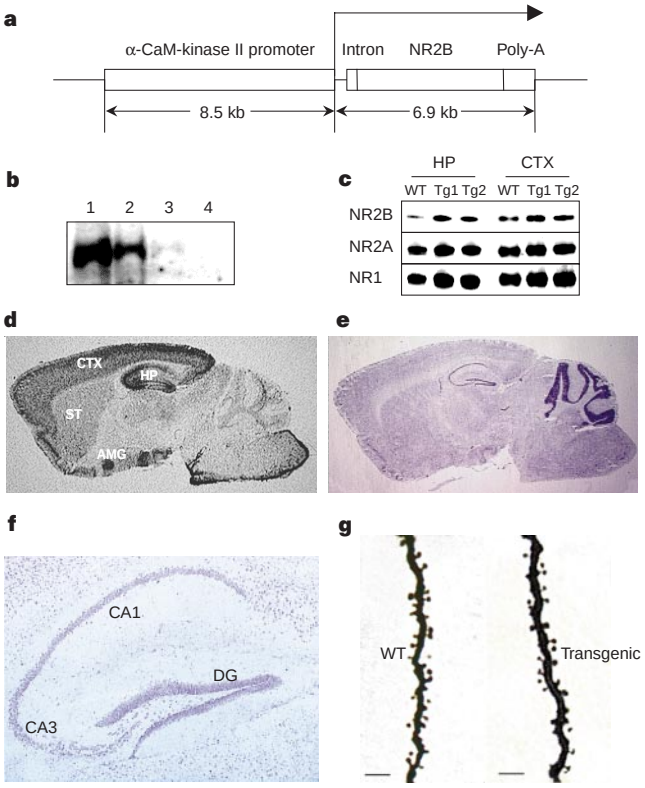

Figure 1 Construction and biochemical characterization of transgenic NR2B mice. **a**, The construct pJT–NR2B for production of NR2B transgenic mice. kb, kilobases. **b**, Expression of NR2B transgene mRNA in transgenic mice. Lane 1, cortex/striatum/amygdala; lane 2, hippocampus; lane 3, brain stem and thalamus; lane 4, cerebellum. **c**, Synaptic NMDA-receptor protein in hippocampus (HP) and cortex (CTX) in both transgenic lines (Tg-1 and Tg-2) and wild-type (WT). The same immunoblot was used for blotting with antibodies against NR1 (relative molecular mass 120K), NR2A (170K) and NR2B (180K). **d**, Forebrain-specific expression of NR2B transgene revealed by *in situ* hybridization. CTX, cortex; STM, striatum; HP, hippocampus; AMG, amygdala. **e**, Normal brain morphology in transgenic mice (Nissl staining). **f**, Higher magnification of the Nissl-stained transgenic hippocampus. DG, dentate gyrus; CA1 and CA3 are marked. **g**, Golgi staining of the dendritic spines of CA1 cells from wild-type (left) and transgenic mice (right). Scale bar, 5 μm.