Supplemental Data

NMDA Receptors Mediate Olfactory Learning and Memory in *Drosophila*

Shouzhen Xia, Tomoyuki Miyashita, Tsai-Feng Fu, Wei-Yong Lin, Chia-Lin Wu, Lori Pyzocha, Inn-Ray Lin, Minoru Saitoe, Tim Tully, and Ann-Shyn Chiang

Supplemental Experimental Procedures

Fly Stocks

Flies used were from wild-type Canton-S w(CS10), the "Cantonized" EP331, EP3511, hs-GAL4 (P26), Cosmid-A, Cosmid-B, Cosmid-C, and genetic combinations among them. EP-element insertions EP331 and EP3511 were from Exelixis, Inc., hs-GAL4 from Dr. Yi Zhong, and the three cosmid stocks from Dr. Jairaj Acharya. The genetic background for all stocks was "Cantonized" by outcrossing their heterozygous virgins to w(CS10) males for six generations.

Cloning of *dNR1* and *dNR2* cDNAs

The full-length *dNR1* cDNAs were obtained with the strategy as outlined in Figure S1. The 3-day-old flies were used for total RNA extraction with Rezol C&T (PROtech Technology), and the polyA$^+$ RNA was isolate with polyATract mRNA Isolation System III (Promega). Then, complementary DNAs (cDNAs) were reversely transcribed from the purified mRNAs and used as templates for PCRs. Specific sense (NR15, see Table S1 for primer sequences) and antisense (NR13) primers were designed from the published cDNA encoding *dNR1* (Acc# X71790). The amplified PCR fragment was further subcloned into pGEM-T for sequencing. Finally, the sequenced 2993-bp PCR fragment was used as a probe for the further cloning of 3′ and 5′ ends of the full-length cDNA.

The *dNR1* 5′ and 3′ end cDNA fragments were obtained with GeneRacer Kit (Invitrogen) following the manufacturer's instructions. The *dNR1* 5′ end fragment was isolated with the RNA ligase-mediated and oligo-capping RACE methods. An RNA oligonucleotide (5′-CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGA GUAGAAA-3′) was first obtained for the 5′ end of decapped mRNA with RNA ligation. The ligated mRNA was then reversely transcribed with the *dNR1* specific primer NR15 to create RACE-ready *dNR1* cDNA with known priming sites at the 5′ and 3′ ends with ThermalScript II RT (GibCo BRL). The 5′ end fragment was amplified with a *dNR1* antisence specific primer (NR15A) and GeneRacer 5′ primer (GR5). The mRNAs tagged with the GeneRacer RNA oligo would be fully reverse transcribed and amplified with PCR. Next, the GeneRacer 5′ end nested (GR5N) and the *dNR1* antisense (NR15B, NR15C) primers were applied for additional PCR amplification. The *dNR1* 3′ end fragment was cloned with an anchor primer (GRdT18) in reverse transcription for cDNA synthesis. Then *dNR1*-sepecific primer (NR13A) and GeneRacer 3′ end primer (GR3) were applied for 3′ end first PCR amplification. The *dNR1*-sepecific (NR13B, NR13C) and GeneRacer 3′ end nested (GR3N) primers were used for 3′ end nested PCR amplification. Amplified *dNR1* 3′ and 5′ end cDNA fragments were cloned in pGEMT-easy vectors with the TA cloning Kit (Promega) and were sequenced from both directions.

The partial sequence of the *dNR2* gene has been recovered previously via homology search of the *Drosophila* DNA sequence database or cloning [S1, S2], but it failed to form functional NMDAR

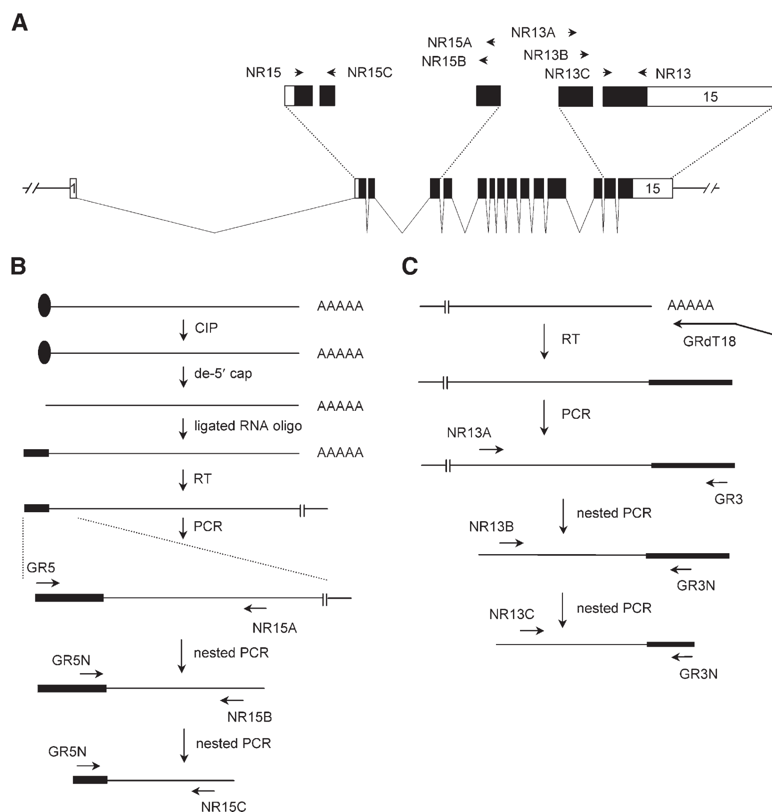

Figure S1. Schematic Diagram of the *dNR1* Cloning Strategy

(A) The known cDNA (X71790 [S9]) is shown with exons denoted as boxes and primers denoted as arrows indicating the direction of PCR amplification.

(B) RACE-PCR of the 5′ end of the cDNA was carried out beginning from the ligated GeneRacer oligo mRNA, which then was reverse transcribed with the *dNR1* specific NR15 primer to create RACE-ready cDNA. The cDNA was amplified with PCRs by using the GeneRace 5′ primers (GR5 or GR5N) and the reverse *dNR1* primers (NR15A-C). The final PCR product was sequenced to identify the 5′ end cDNA fragment.

(C) The 3′-RACE was initiated with the first strand cDNA as the template, which was transcribed with the GeneRacer-d(T) primer (GRdT18). The GeneRacer 3′ primers (GR3 and GR3N) and the reverse *dNR1* primers (NR13A-C) were used for the initial and nested PCR amplifications.

Table S1. Primer Sequences for Cloning of *dNR1* and *dNR2*

Primers	Sequences
GR5	5′-CGACTGGAGCACGAGGACACTGA-3′
GR5N	5′-GGACACTGACATGGACTGAAGGAGTA-3′
GR3	5′-GCTGTCAACGATACGCTACGTAACG-3′
GR3N	5′-CGCTACGTAACGGCATGACAGTG-3′
GRdT18	5′-GCTGTCAACGATACGCTACGTAACGGCATGACAGTG$(T)_{18}$-3′
NR15	5′-ATGGCTATGGCTGAGTTTGT-3′
NR15A	5′-CCGCGAGGAGATGCCAATGACGGGAATTG-3′
NR15B	5′-ATCCGCTTGTATAACTCACGGCCGCCGG-3′
NR15C	5′-CGAAACGACTACGGCGTACACCCGATTCT-3′
NR13	5′-TTAGACGACCAGATGCGACA-3′
NR13A	5′-AAGCGCCTGGACATTGCCCGACATGCGG-3′
NR13B	5′-CGGACAAGTGGCGGGGCACCATAGAGAA-3′
NR13C	5′-CGAAAGACTATTCGTGCCTCGCTGGCGA-3′
NR25	5′-ACGATGACTTTGTGCAGGCT-3′
NR25A	5′-CACCCGGGCCTCACTGTTAACAAGCTCC-3′
NR25B	5′-GTCGCTTGTGCGGGTGACCACGATCGAG-3′
NR25C	5′-CCTGCATCTCAGCCACACGCTCCCGCAC-3′
NR23	5′-GAGATGATGGTGGTTCAGC-3′
NR23A	5′-GCCACCGAGATCCTGAAGAAGCACC-3′
NR23B	5′-AAGGTCAAGCACGAGCTGGACATGT-3′
NR23C	5′-GACAAGCATGGCATCAAGGCGCCGC-3′

channel even when coexpressed along with *dNR1* in *Xenopus* oocytes (data not shown). This prompted us to fully clone the gene. The *dNR2* cDNAs were isolated with a similar strategy (Figure S2) by using the primers specific to *dNR2* (Table S1). Briefly, the 3-day-old flies were used for total RNA extraction and isolation of the polyA$^+$ RNA. Complementary DNAs (cDNAs), reversely transcribed from the purified mRNAs, were used as templates for PCRs. Specific sense primer (NR25) and antisense primer (NR23) were designed, according to the cDNA encoding the putative *Drosophila* NMDA receptor subunit (EG:80H7.7). Then, the amplified PCR fragment was subcloned into pGEM-T for sequencing. Finally, the sequenced 2351-bp PCR fragment was used as a probe for the further cloning of 3′ and 5′ ends of the full-length cDNA with the GeneRacer Kit.

Heterologous coexpression of *dNR1* and *dNR2* in *Xenopus* Oocytes and S2 cells

The *dNR1* and *dNR2-2* cDNAs were subcloned into pGEMHE vector, and sense cRNAs were transcribed in vitro with T7 polymerase. Oocytes were injected with 50 nl of the in vitro-transcribed cRNAs [S3, S4], and recordings were carried out with the holding potential of –70 mV 2–3 days after injection at room temperature with two electrode voltage clamp technique (Axoclamp 2B, Axon Instruments). External Mg^{2+}-free recording solution contained 10 mM HEPES (pH 7.2), 115 mM NaCl, 2.5 mM KCl, 2 mM $BaCl_2$, and 200 μM niflumic acid, and 0.1–10 mM $MgCl_2$ was added for Mg^{2+}-containing recording solution.

The Schneider 2 (S2) cell line was purchased from Invitrogen. The cells were grown in Schneider's *Drosophila* medium (Invitrogen) supplemented with 10% fetal bovine serum and 100 units/ml penicillin-streptavidin at 25°C without CO_2. Then, they were transfected transiently with the *dNR1*, *dNR2-2*, and EGFP cDNA by lipofectamine 2000 (Invitrogen). Whole-cell recordings of S2 cells were performed at room temperature 40 hr after transfection in HL-3 solution consisting of 70 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 5 mM trehalose, 115 mM sucrose, and 5 mM HEPES (pH 7.2) with or without 20 mM $MgCl_2$. The pipette recording solution contained 140 mM KCl, 0.01

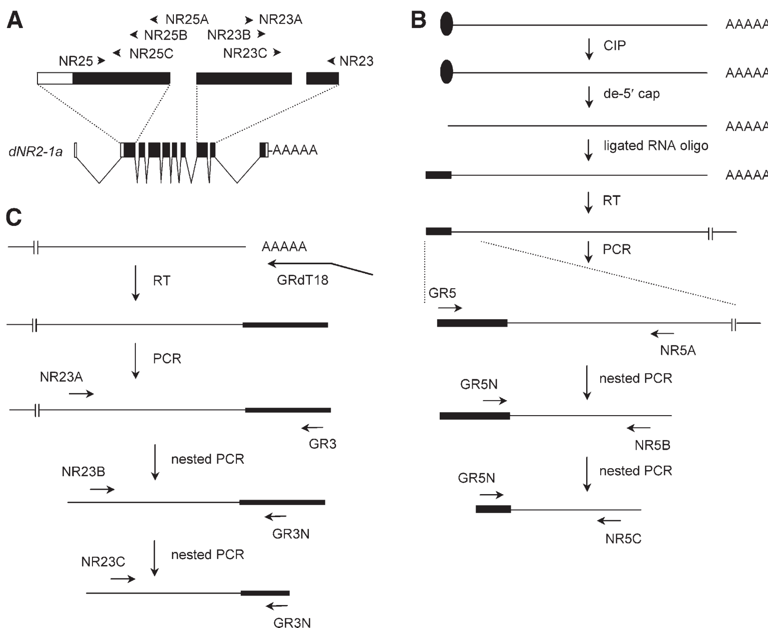

Figure S2. Cloning Strategy of *dNR2*

(A) The known partial cDNA (EG:80H7.7 [S10]) is shown with exons denoted as boxes and primers denoted as arrows indicating the direction of PCR amplification.

(B) The 5′-RACE was carried out beginning from the ligated GeneRacer oligo mRNA, which then was reverse transcribed with the *dNR2* specific NR25 primer to create RACE-ready cDNA. The cDNA was amplified with PCRs by using the GeneRace 5′ primers (GR5 or GR5N) and the reverse *dNR2* primers (NR25A-C). The final PCR product was sequenced to identify the 5′ end cDNA fragment.

(C) 3′-RACE was initiated with the first strand cDNA as the template, which was transcribed with the GeneRacer-d(T) primer (GRdT18). The GeneRacer 3′ primers (GR3 and GR3N) and the reverse *dNR2* primers (NR23A-C) were used for the initial and nested PCR amplifications.

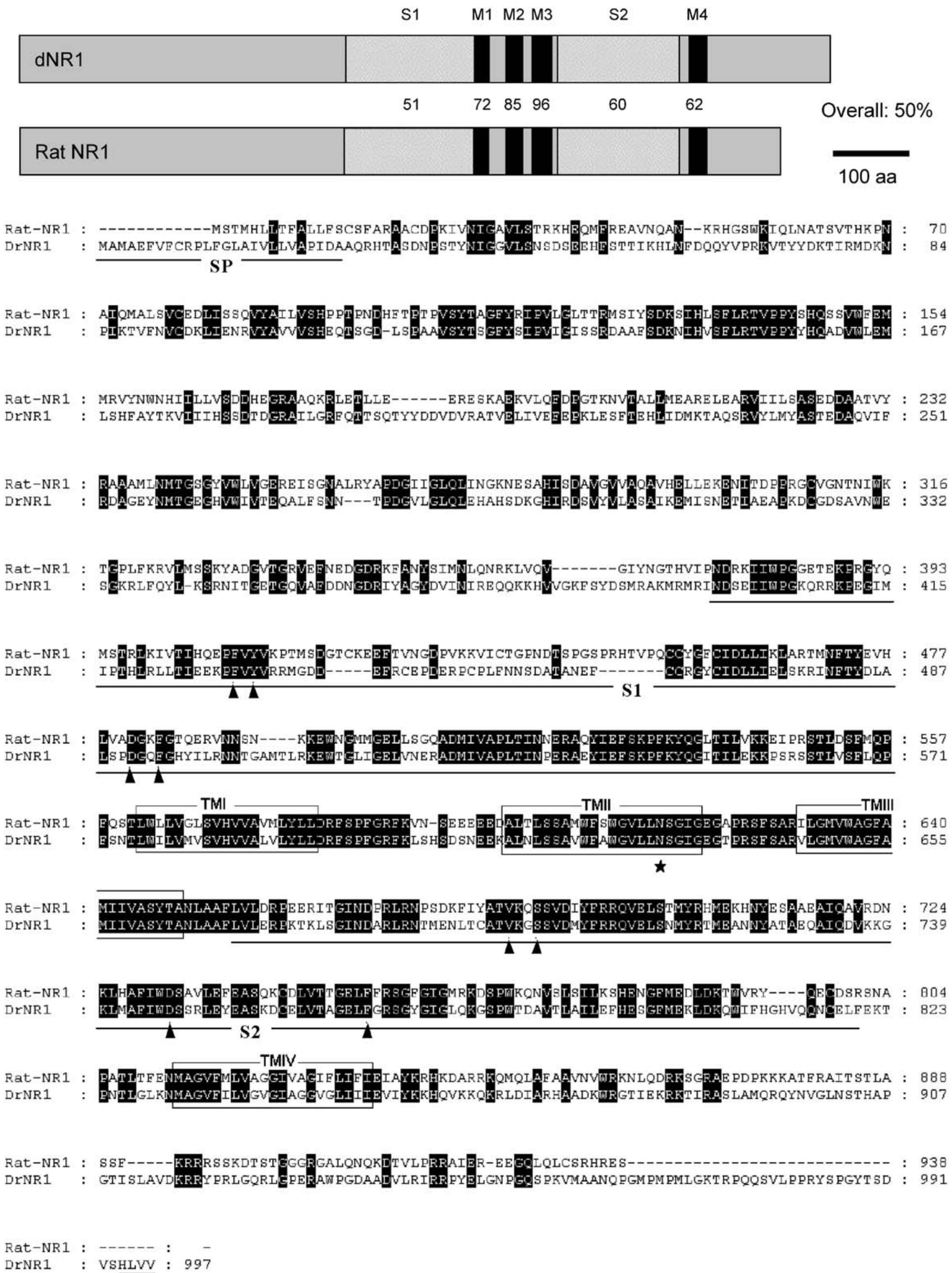

Figure S3. Predicted Domain Structure and Amino Acid Sequence of dNR1

(Top) Protein domains in dNR1 and rat NR1 receptors with the percent amino acid identity between the homologs indicated. Abbreviations are as follows: M1–4, transmembrane domains 1–4; S1 and S2, ligand binding domains 1 and 2. (Bottom) Putative amino acid sequence of dNR1 and its alignment with rat NR1. The dNR1 sequence is numbered beginning from the first predicted methionine. The open boxes indicate transmembrane domains. The underlined regions indicate signal peptide (SP) and the two ligand binding domains (S1–S2) with high homology to bacterial amino acid binding proteins. The conserved residues for glycine binding are indicated with arrow heads. The conserved asparagine found in the TMII domain of NMDA receptors is indicated with an asterisk. The predicted type II PDZ domain binding motif is indicated by a bar.

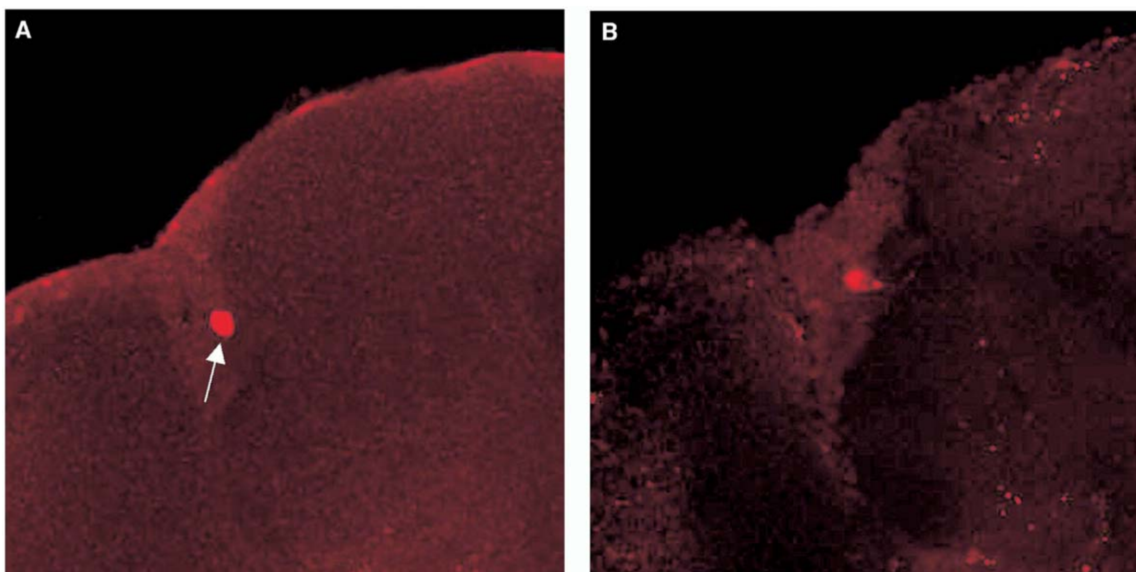

Figure S4. The Anti-*dNR1* Antibody Shows Specific Immunostaining for dNR1

(A) Immunolabeling of dNR1 in the brain of the wild-type adults. The DAL neurons (arrow) show strong, and the surrounding protocerebrum region shows weak but detectable, immunoreactivity.

(B) Reduction of dNR1 immunoreactivity in DAL neurons and the surrounding brain region in P26/+, EP331/+ flies 15 hr after heat-shock treatment. In both cases, only a single optical slice through cell body of DAL neuron is shown. The reduction of immunoreactivity in DAL neurons and other brain regions by genetic disruption of dNR1 expression indicates specific labeling for dNR1. The immunoreactivity remaining after heat shock may represent residual dNR1 or nonspecific labeling.

mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM EGTA, 1 mM Na_2ATP, 0.1 mM cAMP, 0.1 mM GTP, and 5 mM HEPES (pH 7.3). Currents were recorded from S2 cells at –70 mV with an Axoclamp 2B patch clamp amplifier (Axon Instruments). Data analysis was performed with Igor software (WaveMetrics, Lake Oswego, Oregon). Data were presented as means $\pm$ SEM ($n = 8$).

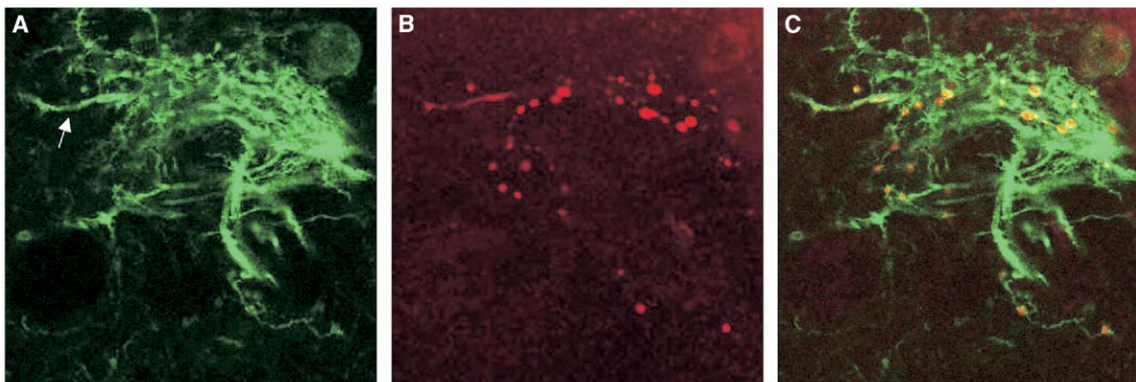

Figure S5. Immunopositive Puncta Are Synapse-like and Distributed Along Fibers of DAL Neurons in the Superior Medial Protocerebrum

(A) Expression of GFP in fibers of DAL neurons (arrow) with the C133 (radish [S11]) GAL4 driver and UAS-mCD8:GFP transgene in transheterozygous flies.

(B) dNR1-immunopositive puncta and fibers (red) in the same region described above.

(C) Merged image of (A) and (B), revealing all the dNR1-positive puncta distributed along fibers of DAL neurons. This observation suggests (but does not prove) that much of dNR1 is localized to synapses.

Table S2. Amino Acid Identity between dNR2 Variants and dNR1 and Its Homologues in Rat and *C. elegans*

	dNR1	NR1	NR2A	NR2B	NR2C	NR2D	NR3B	dNR2-1	dNR2-2	dNR2-3
dNR2-1	28%	24%	31%	33%	32%	32%	27%	100%	97%	97%
dNR2-2	27%	24%	30%	32%	31%	32%	27%	97%	100%	95%
dNR2-3	27%	24%	30%	32%	31%	32%	27%	97%	95%	100%

Table S3. Amino Acid Identity and Similarity at Ligand Binding and Pore-Forming Domains between dNR2 Variants and Its Rat Homologues and *C. elegans*

Domain	dNR1	NR1	NR2A	NR2B	NR2C	NR2D	NR3B	NMR-2
S1	41/58	42/56	45/58	50/73	38/62	48/72	49/67	55/73
S2	26/47	28/52	38/58	37/57	36/58	37/57	30/55	41/63
TM2	N.S.	N.S.	36/58	36/58	36/62	41/65	N.S.	50/65

N.S., no significant homology.

Q-PCR for Detection of Antisense Message

The total RNA was extracted with NucleoSpin RNA II kits (MACHEREY-NAGEL, Germany) and then used for synthesizing first-strand cDNA with Omiscript RT kit (Qiagen) according to the manufacturer's protocols. RNA expression levels were quantified by Q-PCR (Applied Biosystems 7000). Q-PCR and quantitative measurements were performed with the SYBR-Green PCR-Master Mix (Applied Biosystems). The results were normalized to the relative amount of actin (*Act57B*). Primers used (forward and reverse, respectively) were 5′-GGGAAG GCTGAAAAAATGATAGAGAGAG-3′ and 5′- AGCAAATTTAGATCGA GATCGAAGCAC-3′ for anti-*dNR1* mRNA, and 5′-TGCCGTCTTCC CCTCAATCG-3′ and 5′-CGTACGAGTCCTTCTGGCCCATAC-3′ for *Act57B*. All reactions were done in parallel by using six independent RNA isolations for each group.

Antibody Production and Western Blotting

The rabbit anti-*dNR1* antiserum was raised against a peptide corresponding to 81 amino acids in the cytoplasmic region of *dNR1*. The sequence of the peptide was the following: NH_2-GQFGHYILR NNTGAMTLRKEWTGLIGELVNERADMIVAPLTINPERAEYIEFSKPFK YQGITILEKKPSRSSTLVSFLQPF-COOH. The mouse monoclonal anti-*dNR2* antibody was raised against a peptide corresponding to 114 amino acids in the transmembrane region of *dNR2*. The sequence of the peptide was the following: NH_2EFHEFSGLNDSR LVHPFSHKPSFKFGTIPYSHTDSTIDGVAAVLNGNLDSFITDGTVLDY LVAQDEDCRLMTVGSWYAMTGYGLAFSRNSKYVQMFNTGTCRPG KQEHKSSDPL-COOH.

For Western blotting, the head of adult flies was homogenized with lysis buffer (25 mM HEPES [pH 7.5], 100 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1% SDS, 0.2% Triton X-100, 0.2% NP-40, 1 mM EDTA, 1 mM EGTA, protease inhibitor cocktail tablet [Roach]) centrifuge at 14,000 rpm at 4°C for 50 min, and then the supernatant was saved. Lysate proteins were electrophoresed on a 6% SDS-PAGE and then elctroblotted onto PVDF membranes. Immobilized proteins were probes with rabbit ployclonal anti-*dNR1* or mouse monoclonal anti-*dNR2* antibodies, or a mouse monoclonal anti-actin antibody (Developmental Studies Hybridoma Bank) as loading controls, and the membrane was incubated with HRP-conjugated goat-anti-rabbit IgG secondary antibody. The positive signal was visualized with Qentix Western signal enhancer and SS West Pico Substrate detection (Pierce). Quantification of Western result was performed by digital image analysis with a Hewlett-Packard scanner and Totallab analyzer software (Nonlinear Dynamics, Biodynamics) from experiments that were repeated nine times for homozygous EP331/EP331 and EP3511/EP3511 flies or four times for EP331/+, hs-GAL4/+ flies. The level of dNR1 was normalized to the actin control.

Immunohistochemistry

Whole-mount immunolabeling of 2–3-day-old adult brains was performed as previously described [S5, S6]. Briefly, dissected brains were fixed in 4% paraformaldehyde in PBS on ice for 2 hr, transferred to 4% paraformaldehyde in PBS with 2% Triton X-100 in a room temperature vacuum for 1 hr, and left overnight in the same solution at 4°C. Fixed tissue then was blocked for 2 days at 4°C in PBS containing 2% Triton X-100 and 10% normal goat serum (NGS) and then successively incubated for 2 days each (with washing in between) at 4°C in PBS containing 1% Triton X-100, 0.25% NGS, and (1) the rabbit anti-*dNR1* polyclonal Ab (1:500 dilution) or mouse anti-*dNR2* monoclonal Ab (1:2 dilution) or both, (2) a biotinylated goat anti-rabbit IgG (diluted 1:200) and/or a biotinylated anti-mouse IgG (diluted 1:200), and (3) a streptavidin Cy5-conjugate (1 ng/ml) in PBS containing 1% Triton X-100. Next, the brain was cleared and mounted in FocusClear (Pacgen, Vancouver, Canada) and imaged with a Zeiss LSM 510 confocal microscope. Amira 3.0 was used for 3D visualization and volume modeling.

Heat-Shock Regimen

For heat-shock induction, flies were collected within 1 day of eclosion, placed in glass bottles in groups of about 600, and kept at 18°C and 70% relative humidity. The next afternoon, 19 hr before training, groups of about 100 flies were trapped in foam-stoppered glass vials containing a strip of filter paper to absorb extra moisture. The vials then were submerged in a 30°C water bath until the bottom of the foam stopper (inside the vials) was below the surface of the water, thereby insuring that the flies could not escape heat shock. After the vials remained submerged for 1 hr, the flies were trans-

Table S4. Sensorimotor Controls for Olfactory Conditioning

	Shock Reactivity		Odor Avoidance			
Genotype (Heat Shock)	t60 V	20 V	OCT (10^0)	OCT (10^{-2})	MCH (10^0)	MCH (10^{-2})
+/+	78 ± 3	34 ± 5	74 ± 3	26 ± 7	76 ± 3	29 ± 5
EP331/EP331	76 ± 3	30 ± 4	67 ± 4	23 ± 4	79 ± 3	30 ± 5
+/+	81 ± 2	38 ± 5	72 ± 3	35 ± 5	70 ± 3	35 ± 4
EP3511/EP3511	83 ± 2	38 ± 5	68 ± 4	28 ± 4	71 ± 4	30 ± 6
+/+ (−HS)	84 ± 2	40 ± 7	78 ± 3	37 ± 7	78 ± 4	33 ± 7
+/+ (+HS)	75 ± 3	31 ± 5	81 ± 3	29 ± 7	84 ± 2	28 ± 6
EP331/hs-GAL4 (−HS)	77 ± 3	34 ± 7	80 ± 4	34 ± 6	73 ± 4	25 ± 8
EP331/hs-GAL4 (+HS)	80 ± 3	32 ± 6	74 ± 4	36 ± 7	76 ± 4	22 ± 6

Disruption of *dNR1* with hypomorphic mutations or the acute induction of anti-*dNR1* message does not affect the "task-relevant" sensorimotor responses (shock reactivity and olfactory acuity) required for proper learning performance in olfactory conditioning. The flies' task-relevant abilities to sense and escape from the electric shock (shock reactivity) and odors (olfactory acuity) were quantified in the T-maze. Sensorimotor responses to footshock and both odors (OCT and MCH) were evaluated for homozygous EP331, EP3511, and transheterozygous EP331/+,hs-GAL4/+ before (−HS) or after (+HS) heat shock. No significant differences were detected among genotypes before or after heat shock or within genotypes before and after heat shock.

ferred to standard food vials and moved back 18°C and 70% relative humidity for a 1 hr recovery. Next, the whole cycle of 1 hr heat shock at 30°C and 1 hr recovery at 18°C was repeated three more times. Finally, after 10 more hr at 18°C and 70% relative humidity, the flies were transferred to the environmental room (at 25°C and 70% relative humidity), where training occurs, for a 1–3 hr incubation. Training begins immediately after the incubation period.

Behavior

Olfactory associative learning was measured by training 2–3-day-old adult flies with a Pavlovian conditioning procedure [S7]. Groups of about 100 flies received one training session, during which they were exposed sequentially to one odor (conditioned stimulus, CS+; 3-octanol or 4-methyl-cyclohexanol) paired with electric shock (US) and then a second odor (CS−; 4-methyl-cyclohexanol or 3-octanol) without US. Conditioned odor avoidance was tested immediately after training. During testing, flies were exposed simultaneously to the CS+ and CS− in a T-maze for 2 min. Then, flies were trapped in either T-maze arm, anaesthetized, and counted. From this distribution, a performance index (PI) was calculated as the number of flies avoiding the shocked odor minus that avoiding the nonshocked odor divided by the total number of flies and finally timed by 100. A 50:50 distribution (no learning) yielded a PI of zero and a 0:100 distribution away from the CS+ yielded a PI of 100.

1-day memory was evaluated after spaced or massed training, which induces strong, long-lasting memory for conditioned avoidance [S8]. Spaced training consists of ten cycles of one-session training, where a 15-min rest interval is introduced between each session. Massed training consists of ten cycles of one-session training, where one session immediately follows the previous one. Then flies were tested for memory retention of conditioned avoidance at the choice point of the T-maze after one day.

Olfactory acuity was quantified by exposing naive flies to odor (at concentrations used for Pavlovian training and 100 dilution) versus air in the T-maze during a 2-min test trial. Shock reactivity was quantified by placing grids in each arm of the T-maze and then exposing naive flies to shock versus no shock (at 20 and 60 V used for Pavlovian training) during a 2-min test trial. For both olfactory acuity and shock reactivity, PIs were calculated as above.

Supplemental References

S1. Volkner, M., Lenz-Bohme, B., Betz, H., and Schmitt, B. (2000). Novel CNS glutamate receptor subunit genes of *Drosophila melanogaster*. J. Neurochem. *75*, 1791–1799.

S2. Fu, T.F., Lin, W.Y., Liu, H.P., and Chiang, A.S. (2001). Cloning and characterization of cDNA encoding the Drosophila NMDA receptor subunit 2. Europ. Dros. Res. Conf. *17*, E9.

S3. Ultsch, A., Schuster, C.M., Laube, B., Schloss, P., Schmitt, B., and Betz, H. (1992). Glutamate receptors of *Drosophila melanogaster*: cloning of a kainate-selective subunit expressed in the central nervous system. Proc. Natl. Acad. Sci. USA *89*, 10484–10488.

S4. Schuster, C.M., Ultsch, A., Schloss, P., Cox, J.A., Schmitt, B., and Betz, H. (1991). Molecular cloning of an invertebrate glutamate receptor subunit expressed in *Drosophila* muscle. Science *254*, 112–114.

S5. Chiang, A.S., Liu, Y.C., Chiu, S.L., Hu, S.H., Huang, C.Y., and Hsieh, C.H. (2001). Three-dimensional mapping of brain neuropils in the cockroach, *Diploptera punctata*. J. Comp. Neurol. *440*, 1–11.

S6. Chiang, A.S., Lin, W.Y., Liu, H.P., Pszczolkowski, M.A., Fu, T.F., Chiu, S.L., and Holbrook, G.L. (2002). Insect NMDA receptors mediate juvenile hormone biosynthesis. Proc. Natl. Acad. Sci. USA *99*, 37–42.

S7. Tully, T., and Quinn, W.G. (1985). Classical conditioning and retention in normal and mutant *Drosophila melanogaster*. J. Comp. Physiol. [A] *157*, 263–277.

S8. Tully, T., Preat, T., Boynton, S.C., and Del Vecchio, M. (1994). Genetic dissection of consolidated memory in *Drosophila*. Cell *79*, 35–47.

S9. Ultsch, A., Schuster, C.M., Laube, B., Betz, H., and Schmitt, B. (1993). Glutamate receptors of *Drosophila melanogaster*. Primary structure of a putative NMDA receptor protein expressed in the head of the adult fly. FEBS Lett. *324*, 171–177.

S10. Madueno, E., Papagiannakis, G., Rimmington, G., Saunders, R.D., Savakis, C., Siden-Kiamos, I., Skavdis, G., Spanos, L., Trenear, J., Adam, P., et al. (1995). A physical map of the X chromosome of *Drosophila melanogaster*: cosmid contigs and sequence tagged sites. Genetics *139*, 1631–1647.

S11. Chiang, A.S., Blum, A., Barditch, J., Chen, Y.H., Chiu, S.L., Regulski, M., Armstrong, J.D., Tully, T., and Dubnau, J. (2004). radish encodes a phospholipase-A2 and defines a neural circuit involved in anesthesia-resistant memory. Curr. Biol. *14*, 263–272.