

Supplementary data

Table S1 Amino acid identities (in %) between NMDAR subunits in *D. punctata* and other insects. DpNR1 was compared with NR1 in other species, and DpNR2A-1 was compared with NR2 in other species.

	DpNR1B	<i>D. melanogaster</i>	<i>A. mellifera</i>	<i>A. aegypti</i>
DpNR1A	65.6	65.4	75.4	68.2
DpNR1B	---	55.6	62.7	58.8
DpNR2A-1	---	67.3%	75.6%	71.0%

Table S2 Degenerate primer and RACE primer sequences for cloning *DpNR1A*, *DpNR1B* and *DpNR2* cDNAs.

Name	Symbol	Degenerate/RACE primer sequences
NMDA receptor subunit 1 variant A	NR1A	F 5'- CTRTCGCCCGATGGTCARTTYGG -3' R 5'- CAGTATGAAYACYCCTGCCATRT -3' Fn 5'- ACGTATTTCAACATYGGYGGHGT -3' Rn 5'- CCAAATTGGCCATCCGGCGACAA -3' 3'Race 5'- GGATTCGCCATGATCATTGTGGCA -3' 5'Race 5'- GGATCCATTGAAATCGCTGTGGA -3'
NMDA receptor subunit 1 variant B	NR1B	F 5'- CTRTCGCCCGATGGTCARTTYGG -3' R 5'- CAGTATGAAYACYCCTGCCATRT -3' Fn 5'- ATCCACAGCTCCGACACDGAYGG -3' Rn 5'- GTACCTTCTCCTATGCCACTATT -3' 3'Race 5'- TAAATGATGCGAGACTGCGTA -3' 5'Race 5'- CCACACATCAGCTTGATGGGA -3'
NMDA receptor subunit 2	NR2	F 5'- ATACCGGTCATCKCVTGGAAAYGC -3' R 5'- AACATCCAAGARGCYGTRTCRAA -3' Fn 5'- CTCGGAGAGGGAAGCAGTTGTGG -3' Rn 5'- TCGAGCAGTCGYTTRTTRAACAT -3' 3'Race 5'- CCAGAACCGATCCACTGTAGCA -3' 5'Race 5'- CTCTCCGCTCAAGGCCGGAATT -3'

Table S3 Oligonucleotide sequences for primers used in q-RT-PCR for reference and target genes. Efficiencies and R² values are indicated.

Reference genes	F-primer	R-primer	Amplicon size (bp)	Efficiency (%)	R ²
<i>Tubulin</i>	5'-AAATTACCAACGCTTGCTTTGAA-3'	5'-TGGCGAGGATCGCATTTT-3'	58	95.1	0.993
<i>EF1α</i>	5'-TCGTCTTCCTCTGCAGGATGTCT-3'	5'-GGGTGCAAATGTCACAACCATACC-3'	109	99.2	0.994
<i>Armadillo</i>	5'-GCTACTGCACCACTCACAGAATTATT-3'	5'-CTGCAGCATACTGTTGCAACA-3'	64	94.5	0.980
Target genes	F-primer	R-primer	Amplicon size (bp)	Efficiency (%)	R ²
<i>DpNR1A</i>	5'- ATCGAGAAGCGGAAAACACT -3'	5'- GTTGCTGGATCATTGACACC -3'	80	90.2	0.984
<i>DpNR1B</i>	5'- GCACACTTTGGGACTCAAGA -3'	5'- CCTCCAGCAACTAGCATGAA -3'	54	100	0.983
<i>DpNR2</i>	5'- AAGAACCAGAACCGATCCAC -3'	5'- GGCCACTAGGAAATCCAAAA -3'	105	91.7	0.974
<i>DpVg</i>	5'-AAAGGTGTCCTCAGCCAGC-3'	5'-TCCTCCATCTCGGATTGGGA-3'	105	95.1	0.998

Table S4 Nucleotide sequences of primers used in making the dsRNA constructs.

Gene	F-primer (5'-3')	R-primer (5'-3')
<i>DpNR2</i>	TAATACGACTCACTATAGGGAGAACAGGATATGGTATCGCCTTTAGC	TAATACGACTCACTATAGGGAGATCTTGAGCTTCGAAAACACTGCAC
<i>DpNR12</i>	TAATACGACTCACTATAGGGAGATAACTGGGACAGTCCACAC	TAATACGACTCACTATAGGGAGATCAGGTGAGAGTCCAACATGG
<i>pJET</i>	TAATACGACTCACTATAGGGAGATTGCGCTCACTGCCAATTGC	TAATACGACTCACTATAGGGAGA CTGGCCTTTTGCTCACATGTT

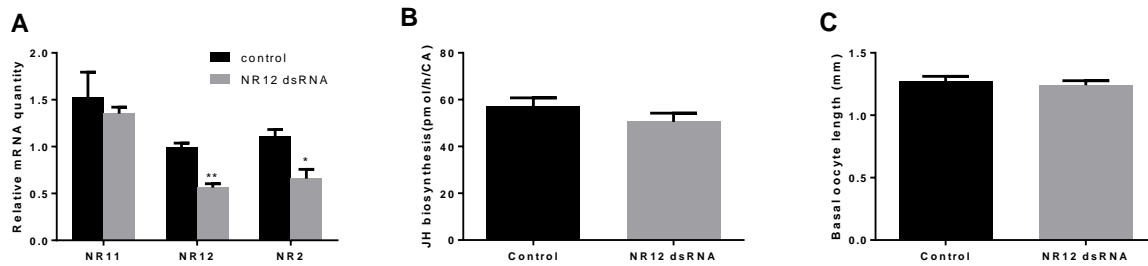


Fig. S1. The effect of *DpNR1B* dsRNA treatment on JH biosynthesis and basal oocyte growth, and the interactions among these genes in mated female *D. punctata*. (A) Relative quantity of *DippuNR1A*, *DippuNR1B* and *DippuNR2* mRNA levels in brain between control and dsRNA treated animals. mRNA levels were normalized against levels of *Tubulin* and *EF1a* mRNA (Marchal et al., 2013b). The data represent the average of 3 biologically independent pools (5 animals each pool), run in triplicate. (B) JH biosynthesis by CA from control and dsRNA-treated animals. (C) Basal oocyte length in control and dsRNA-treated animals. Values represent mean \pm SEM. Levels of significance to the control are indicated with the asterisk symbol: *P < 0.05, **P < 0.01

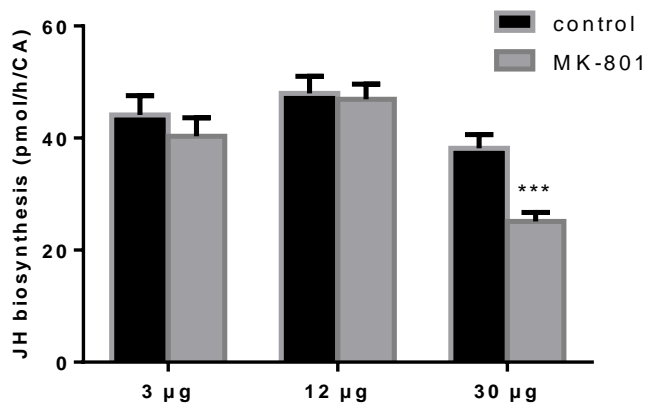


Fig. S2. JH biosynthesis of CA in day 4 mated female *D. punctata* injected with different doses of MK-801 on days 0, 1 and 3. Control was injected with ddH₂O. Values represent mean \pm SEM (n \geq 17). Levels of significance to the control are indicated with the asterisk symbol: ***P < 0.001