

SUPPORTING INFORMATION

Global transcriptomic effects of environmentally relevant concentrations of the neonicotinoids clothianidin, imidacloprid, and thiamethoxam in honey bees (*Apis mellifera*)

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Materials and methods

RNA isolation, reverse transcription, and quantitative PCR

The brains of frozen bees were removed in total by opening the cranium using a scalpel and forceps. Total RNA of three bee brains were pooled in each replicate and isolated using RNeasy mini kit from Qiagen according to the manufacturer's instructions. For each exposure concentration, five biological replicates were isolated. The quantity and the quality of the isolated RNA was analysed using Experion™ Automated Electrophoresis System (Biorad) according to the manufacturer's instructions. RNA samples were diluted to 100 ng/mL with RNase/DNase free water and stored at -80°C until further use. To validate RNA-sequencing data, 1000 ng RNA were reverse transcribed as described before.²¹ qPCR of selected target genes based on SYBR green fluorescence (SYBR green PCR master mix; Roche) was performed as previously.^{16,21} Primer sequences were taken from literature or self-designed using NCBI primer BLAST tool and Geneious v. 11.0.4⁴² (Table S2).

Illumina RNA sequencing experiment

Library preparation

The quality of the isolated RNA was determined with a Qubit® (1.0) Fluorometer (Life Technologies, California, USA) and Bioanalyzer 2100 (Agilent, Waldbronn, Germany). Only those samples with a 260 nm/280 nm ratio between 1.8–2.1 and a 28S/18S ratio within 1.5–2 were further processed. The TruSeq RNA Sample Prep Kit v2 (Illumina, Inc, California, USA) was used in the succeeding steps. Briefly, total RNA samples (100-1000 ng) were ribo-depleted using Ribo Zero Gold (Epicentre®, USA) and then fragmented. Fragmented samples were reverse transcribed to cDNA, end-repaired and polyadenylated before ligation of TruSeq adapters. Fragments containing TruSeq adapters on both ends were selectively enriched with PCR. The quality and quantity of the enriched libraries were validated using Qubit® (1.0) Fluorometer and the Caliper GX LabChip® GX (Caliper Life Sciences, Inc., USA). The libraries were normalized to 10 nM in Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20.

Cluster Generation and Sequencing

The TruSeq SR Cluster Kit HS4000 (Illumina, Inc, California, USA) was used for cluster generation using 2 nM of pooled normalized libraries on the cBOT. Sequencing was performed on the Illumina HiSeq 4000 single end 125 bp using the TruSeq SBS Kit HS4000 (Illumina, Inc, California, USA) .

Tables

Table S1: Concentration of the three neonicotinoids used in the present study.

Compound	Concentration (ng/bee)	Concentration (ng/mL sucrose solution)
Clothianidin	0.3 and 3	3 and 30
Imidacloprid	0.3 and 3	3 and 30
Thiamethoxam	0.1 and 1	1 and 10

Table S2: Sequences of qPCR primers

Transcript	Sequence 5`>3`	Accession number	Source (References)
<i>ribosomal protein s5</i>	AATTATTTGGTCGCTGGAATTG	XM_394577	4
	TAACGTCCAGCAGAATGTGGTA		
<i>actin</i>	TGCCAACACTGTCCTTTCTG	AB023025	5
	AGAATTGACCCACCAATCCA		
<i>efl-α</i>	AATTTCTGGTTGGCATGGAG	NM_001014993	
	CGGAGAGCCTTGTCTGTAGG		
<i>achrα1</i>	GAAATACGTGGCGATGGTGC	NM_001098220	1
	GTGGTATCGTACGGCTCGG		
<i>achrα2</i>	CCGAACCTCTACGTACCGAGC	NM_001011625	
	TCGAACGTCTATCTCGCACG		
<i>alpha-glucosidase hbg3</i>	TACCTGGCTTCGTGTCAAC	NM_001011608	6
	ATCTTCGGTTTCCCTAGAGAATG		
<i>biphosphate-nucleotidase</i>	CCTTTGAGCACAACGATCAGC	XM_006567837.2	This study
	TGGCCACTTCTGTATCTGCT		
<i>CAAT/enhancer binding protein beta</i>	TCTCGACCAGTTCCAGCAAC	XM_003250952.3	
	GACGAGAGAAAGGCGCGATA		
<i>cyp9e2</i>	TCACGCGTTCAGCTTCTTCT	XM_006562301.2	
	CGTCGATCTCCTCCTGAAGC		
<i>Mrjp-1</i>	CACAGCCCAAGATGGAATTT	NM_001328480	5
	AAGAGGACGCCACTCTTTGA		
<i>Mrjp-2</i>	GGAAAGGGAGGGCTAGTGTC	NM_001011580	
	TCGATCGTCATTTTGGCATA		
<i>Mrjp-3</i>	ATTGCCGTAAACGCCACTAC	NM_001011601.1	
	CAATCGATGGAAGGAATCGT		
<i>vitellogenin</i>	GCAGAATACATGGACGGTGT	NM_001011578	7
	GAACAGTCTTCGGAAGCTTG		

Table S3: Number of up- and down-regulated unigenes.

Exposures	Concentration	Up-regulated	Down-regulated	Total
Solvent control	0.1% DMSO	6	2	8
Clothianidin	0.3 ng/bee	4	14	18
	3 ng /bee	10	234	244
Imidacloprid	0.3 ng/bee	7	19	26
	3 ng/bee	36	77	113
Thiamethoxam	0.1 ng/bee	4	2	6
	1 ng/bee	4	21	25

Table S4: List of differentially expressed unigenes of *A. mellifera* treated with 0.3 ng/bee and 3 ng/bee clothianidin (CLO_0_3 and CLO_3), 0.3 ng/bee and 3 ng/bee imidacloprid (IMD_0_3 and IMD_3) and 0.1 ng/bee and 1 ng/bee thiamethoxam (TMX_0_1 and TMX_1), respectively, or the solvent control (0.1% DMSO). Unigenes that were expressed in < 50% of the replicates of each exposure are in *italic* and were not used for further analyses. logFC = log fold change, FDR = corrected *p*-value of false discovery rate, Function = annotated function, where NA = no annotation.

Unigene_ID	Treatment	logFC	FDR	Function
DN179898_c0_g1	CLO_0_3	7.230	7.6E-05	Atypical protein kinase C-like
DN64336_c0_g1	CLO_0_3	4.704	1.7E-04	Opsin-1
DN97433_c0_g1	CLO_0_3	3.017	0.024	Uncharacterized LOC413386
DN62390_c0_g1	CLO_0_3	2.395	0.047	Uncharacterized LOC102672266
DN177206_c0_g1	CLO_0_3	-5.273	7.6E-05	Down syndrome cell adhesion molecule homolog
DN96981_c0_g1	CLO_0_3	-11.136	0.003	Glucose dehydrogenase [FAD, quinone]
DN76764_c3_g1	CLO_0_3	-2.032	0.003	Cysteine-rich venom protein 1
DN40944_c0_g1	CLO_0_3	-6.975	0.009	Uncharacterized LOC105736543
DN72015_c0_g1	CLO_0_3	-3.579	0.010	Peroxidase
DN54048_c0_g1	CLO_0_3	-4.750	0.016	Protein takeout
DN42667_c0_g1	CLO_0_3	-3.306	0.020	Clavesin-1
DN63481_c0_g1	CLO_0_3	-6.912	0.030	Uncharacterized LOC408608
DN48039_c0_g1	CLO_0_3	-2.689	0.030	Chaoptin
DN58089_c0_g1	CLO_0_3	-2.435	0.030	Uncharacterized LOC100576603
DN74981_c2_g1	CLO_0_3	-2.274	0.030	Scavenger receptor class B member 1
DN57559_c1_g1	CLO_0_3	-3.868	0.039	Bruchpilot (brp)
DN14115_c0_g1	CLO_0_3	-3.131	0.039	Uncharacterized LOC110119361
DN72237_c0_g1	CLO_0_3	-2.140	0.039	NA
DN36139_c1_g1	CLO_3	4.707	0.003	Tether containing UBX domain for GLUT4
DN75605_c5_g1	CLO_3	5.114	0.003	Hymenoptaecin
DN45341_c0_g1	CLO_3	7.484	0.004	3'(2'),5'-bisphosphate nucleotidase 1
DN75605_c5_g3	CLO_3	4.696	0.007	Hymenoptaecin
DN63314_c0_g1	CLO_3	2.158	0.012	Apidaecins type 14
<i>DN47644_c0_g1</i>	<i>CLO_3</i>	<i>7.748</i>	<i>0.014</i>	<i>F-box/SPRY domain-containing protein 1</i>
DN63943_c0_g1	CLO_3	2.122	0.022	Cytochrome P450 4C1
DN166285_c0_g1	CLO_3	2.286	0.033	Protein still life, isoform SIF type 1
DN93945_c0_g1	CLO_3	3.679	0.034	Uncharacterized LOC551079
DN76848_c0_g1	CLO_3	2.415	0.039	Uncharacterized LOC100577522
DN76643_c11_g2	CLO_3	-2.793	1.6E-07	Protein TAR1
DN76643_c11_g3	CLO_3	-2.824	5.4E-07	Putative Uncharacterized protein ART2
DN76643_c11_g5	CLO_3	-2.768	5.4E-07	Uncharacterized LOC100865248
DN73859_c8_g1	CLO_3	-2.569	8.0E-07	Uncharacterized LOC100866091
DN73859_c8_g3	CLO_3	-2.544	9.8E-06	Uncharacterized LOC105736981
DN73859_c8_g2	CLO_3	-2.426	9.8E-06	18S ribosomal RNA gene
DN74807_c2_g1	CLO_3	-3.037	1.5E-04	NA
DN67690_c1_g2	CLO_3	-2.429	2.1E-04	Gustatory receptor 10 (Gr10)
DN75650_c2_g2	CLO_3	-2.627	0.001	Scutellata interNAI transcribed spacer 2
DN61790_c0_g1	CLO_3	-2.228	0.001	Transcription factor AP-1
DN154310_c0_g1	CLO_3	-5.533	0.001	Ras-related protein Rab-3

DN74797_c5_g1	CLO_3	-2.514	0.001	Zinc finger protein sens
DN49347_c0_g1	CLO_3	-10.511	0.002	NA
DN55290_c0_g1	CLO_3	-8.357	0.002	Meteorin-like protein-like
DN2457_c0_g1	CLO_3	-3.081	0.002	Major royal jelly protein 3
DN71219_c1_g1	CLO_3	-3.455	0.002	RNA-binding protein 38
DN74132_c0_g1	CLO_3	-2.653	0.002	Nesprin-1
DN74675_c0_g1	CLO_3	-4.177	0.002	Uncharacterized LOC100577822
DN69034_c0_g1	CLO_3	-9.514	0.003	NA
DN74612_c5_g1	CLO_3	-2.952	0.003	NA
DN74421_c2_g1	CLO_3	-4.757	0.003	Transcriptional regulator Myc-B
DN38621_c0_g1	CLO_3	-3.221	0.003	Xyloside xylosyltransferase 1
DN69692_c0_g1	CLO_3	-2.401	0.003	Protein cueball
DN49793_c0_g1	CLO_3	-9.098	0.003	Uncharacterized LOC105681316
DN67466_c0_g1	CLO_3	-4.943	0.003	Protein lethal(2)essential for life
DN72857_c0_g2	CLO_3	-3.559	0.003	Uncharacterized LOC102656532
DN75651_c0_g1	CLO_3	-3.286	0.003	NA
DN59262_c6_g1	CLO_3	-5.670	0.003	Protein lethal(2)essential for life
DN76250_c1_g5	CLO_3	-4.751	0.003	Sonic hedgehog protein A
DN74437_c0_g1	CLO_3	-4.260	0.003	Protein lethal(2)essential for life
DN75117_c0_g1	CLO_3	-2.900	0.003	Uncharacterized LOC102654214
DN74437_c0_g2	CLO_3	-4.011	0.004	Protein lethal(2)essential for life-like
DN67466_c0_g2	CLO_3	-4.686	0.004	Protein lethal(2)essential for life-like
DN72100_c1_g1	CLO_3	-2.768	0.004	Asparagine synthetase [glutamine-hydrolyzing]
DN76643_c11_g1	CLO_3	-2.816	0.004	NA
DN76452_c9_g1	CLO_3	-4.063	0.005	Uncharacterized LOC102655090
DN57965_c0_g1	CLO_3	-4.009	0.005	NA
DN24592_c0_g1	CLO_3	-3.861	0.005	CCAAT/enhancer-binding protein beta
DN71658_c1_g2	CLO_3	-4.392	0.005	Protein lethal(2)essential for life-like
DN70322_c0_g1	CLO_3	-3.201	0.006	Uncharacterized LOC100576417
DN76162_c2_g1	CLO_3	-2.147	0.006	ABC transporter G family member 23
DN60149_c0_g1	CLO_3	-5.168	0.006	NA
DN60288_c3_g1	CLO_3	-2.525	0.006	RNA-binding protein 24-like
DN69034_c0_g2	CLO_3	-7.907	0.006	NA
DN71940_c0_g1	CLO_3	-6.991	0.007	Protein lethal(2)essential for life-like
DN70306_c0_g1	CLO_3	-4.576	0.007	A disintegrin and metalloproteinase with thrombospondin motifs 8
DN75947_c3_g1	CLO_3	-2.958	0.007	NA
DN76024_c0_g2	CLO_3	-2.450	0.007	NA
DN66320_c1_g1	CLO_3	-2.979	0.007	Transcription factor kayak, isoforms A/B/F
DN50865_c0_g1	CLO_3	-2.420	0.007	RNA-binding protein 24-like
DN71658_c1_g1	CLO_3	-4.323	0.007	Protein lethal(2)essential for life-like
DN69092_c4_g1	CLO_3	-2.811	0.007	Uncharacterized LOC107965107
DN73697_c5_g1	CLO_3	-2.454	0.008	NA
DN63481_c0_g1	CLO_3	-6.253	0.008	Uncharacterized LOC408608
DN64957_c0_g1	CLO_3	-6.017	0.008	Dual oxidase maturation factor 1
DN72415_c5_g1	CLO_3	-2.320	0.008	NA
DN74226_c0_g1	CLO_3	-3.048	0.008	PAB-dependent poly(A)-specific ribonuclease subunit PAN3

DN32911_c0_g1	CLO_3	-5.620	0.009	Sperm flagellar protein 2-like
DN52835_c0_g1	CLO_3	-3.352	0.009	Major royal jelly protein 1-like
DN63082_c1_g1	CLO_3	-2.421	0.009	Max-binding protein MNT
DN63590_c0_g1	CLO_3	-2.446	0.009	NA
DN75678_c2_g1	CLO_3	-2.127	0.009	Growth factor receptor-bound protein 14
DN71656_c0_g1	CLO_3	-2.627	0.009	Protein hairy
DN74938_c1_g1	CLO_3	-2.247	0.009	Homeobox protein extradenticle
DN32778_c0_g1	CLO_3	-5.556	0.010	Pyruvate dehydrogenase (acetyl-transferring) kinase, mitochondrial-like
DN75210_c1_g1	CLO_3	-3.854	0.010	MFS-type transporter SLC18B1-like
DN61692_c0_g1	CLO_3	-2.910	0.010	NA
DN70889_c0_g1	CLO_3	-2.432	0.010	Uncharacterized LOC102653944
DN72448_c4_g1	CLO_3	-2.521	0.010	NA
DN71058_c0_g1	CLO_3	-3.254	0.010	ABC transporter G family member 20
DN71508_c0_g1	CLO_3	-3.151	0.010	DNA-binding protein D-ETS-4
DN74009_c0_g1	CLO_3	-3.069	0.010	Uncharacterized LOC102655655
DN75281_c1_g1	CLO_3	-2.685	0.011	NA
DN52825_c0_g1	CLO_3	-3.323	0.011	Insulin-like growth factor 2 mRNA-binding protein 1
DN24643_c0_g1	CLO_3	-5.819	0.011	NA
DN58883_c0_g1	CLO_3	-2.006	0.011	Ataxin-2 homolog
DN75210_c1_g2	CLO_3	-3.672	0.011	Protein takeout
DN67284_c0_g1	CLO_3	-2.880	0.011	NA
DN58433_c0_g1	CLO_3	-2.405	0.011	Uncharacterized LOC102654214
DN71360_c2_g1	CLO_3	-3.312	0.012	Protein lethal(2)essential for life
DN63878_c0_g1	CLO_3	-2.244	0.012	Uncharacterized LOC100576972
DN59700_c0_g1	CLO_3	-3.833	0.012	Uncharacterized LOC107964135
DN38040_c0_g1	CLO_3	-7.649	0.012	<i>cAMP-dependent protein kinase type I regulatory subunit</i>
DN74976_c2_g1	CLO_3	-2.392	0.012	NA
DN75739_c1_g1	CLO_3	-2.178	0.012	Protein mab-21
DN68524_c0_g1	CLO_3	-2.400	0.012	Transcription factor kayak
DN76190_c1_g2	CLO_3	-2.374	0.012	Uncharacterized LOC408853
DN73949_c3_g1	CLO_3	-3.626	0.012	Flavin-containing monooxygenase FMO GS-OX-like 4
DN60379_c0_g1	CLO_3	-4.488	0.012	NA
DN74653_c3_g1	CLO_3	-2.155	0.012	UPF0676 protein C1494.01-like
DN51191_c0_g1	CLO_3	-4.650	0.013	Uncharacterized LOC726251
DN7651_c0_g1	CLO_3	-2.646	0.013	Major royal jelly protein 3 (mrjp3) gene
DN68729_c3_g1	CLO_3	-2.540	0.013	Globin
DN75916_c0_g1	CLO_3	-2.182	0.013	Protein mab-21
DN53561_c0_g1	CLO_3	-6.319	0.013	Stress-activated protein kinase JNK
DN74226_c0_g2	CLO_3	-2.023	0.013	Proton-coupled amino acid transporter-like protein pathetic
DN91543_c0_g1	CLO_3	-5.163	0.014	NA
DN72015_c0_g1	CLO_3	-3.887	0.014	Peroxidase
DN75607_c6_g1	CLO_3	-3.425	0.014	Glutamate receptor 3
DN70384_c0_g2	CLO_3	-3.328	0.014	Uncharacterized LOC102653888
DN72143_c6_g4	CLO_3	-2.271	0.014	Mediator of RNA polymerase II transcription subunit 25

DN69856_c0_g1	CLO_3	-2.148	0.014	Xaa-Pro aminopeptidase 1-like
DN74081_c2_g3	CLO_3	-3.445	0.014	Uncharacterized LOC108002632
DN4374_c0_g1	CLO_3	-2.563	0.014	Uncharacterized LOC100577920
DN76210_c0_g3	CLO_3	-2.373	0.014	Cytochrome b5 reductase 4
DN76894_c4_g1	CLO_3	-2.203	0.014	Extra macrochaetae (Emc)
DN75864_c1_g1	CLO_3	-2.458	0.014	Dual oxidase
DN70988_c0_g1	CLO_3	-2.434	0.014	Ras-specific guanine nucleotide-releasing factor RalGPS1
DN165376_c0_g1	CLO_3	-5.702	0.014	Rho guanine nucleotide exchange factor 18
DN72857_c0_g1	CLO_3	-3.707	0.014	Uncharacterized LOC100577265
DN68466_c0_g1	CLO_3	-2.436	0.014	NA
DN108589_c0_g1	CLO_3	-2.173	0.014	Transcription factor kayak
DN60926_c0_g1	CLO_3	-5.392	0.014	NA
DN60692_c0_g1	CLO_3	-4.210	0.015	DNA repair protein RAD51 homolog 4
DN68606_c3_g2	CLO_3	-3.135	0.015	Alpha-crystallin B chain
DN68606_c3_g1	CLO_3	-3.114	0.015	Protein lethal(2)essential for life
DN73362_c6_g1	CLO_3	-2.850	0.015	Uncharacterized LOC107964253
DN141693_c0_g1	CLO_3	-2.571	0.015	Carnica major royal jelly protein 3 (mrjp3) gene
DN63031_c0_g1	CLO_3	-2.371	0.015	Platelet binding protein GspB
DN76195_c2_g1	CLO_3	-2.001	0.015	Glutathione-specific gamma-glutamylcyclotransferase 1
DN68410_c0_g1	CLO_3	-3.230	0.015	Uncharacterized LOC105735373
DN65217_c0_g1	CLO_3	-4.732	0.015	A disintegrin and metalloproteinase with thrombospondin motifs 7
DN39725_c0_g1	CLO_3	-2.831	0.015	Heat shock 70 kDa protein cognate 4
DN69198_c0_g1	CLO_3	-3.090	0.015	Carboxypeptidase B
DN65314_c0_g1	CLO_3	-2.129	0.015	Homeobox protein homothorax
DN66120_c1_g1	CLO_3	-2.644	0.016	NA
DN76783_c8_g1	CLO_3	-2.357	0.016	Alpha-1,6-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase
DN66787_c1_g1	CLO_3	-4.110	0.016	Uncharacterized LOC102655249
DN75371_c1_g1	CLO_3	-2.758	0.016	Glucose dehydrogenase [FAD, quinone]
DN73510_c1_g1	CLO_3	-2.658	0.016	Uncharacterized LOC102653857
DN75539_c1_g1	CLO_3	-2.153	0.016	Uncharacterized LOC100576878
DN64644_c0_g1	CLO_3	-2.712	0.017	NA
DN73250_c1_g1	CLO_3	-3.140	0.017	Cilia- and flagella-associated protein 20
DN65694_c0_g1	CLO_3	-2.487	0.017	Uncharacterized LOC102653664
DN72604_c1_g1	CLO_3	-2.312	0.017	NA
DN59461_c0_g1	CLO_3	-3.472	0.018	Probable phospholipid-transporting ATPase IA
DN50985_c0_g1	CLO_3	-2.055	0.018	Uncharacterized LOC107964613
DN62655_c0_g1	CLO_3	-3.128	0.019	NA
DN30501_c0_g2	CLO_3	-3.017	0.019	Protein lethal(2)essential for life
DN76394_c4_g1	CLO_3	-3.009	0.019	Protein PRRC1-A-like
DN75044_c0_g1	CLO_3	-2.474	0.019	Protein yellow
DN95401_c0_g1	CLO_3	-2.307	0.019	Transcription factor kayak
DN70440_c0_g1	CLO_3	-2.290	0.019	NA
DN75539_c1_g2	CLO_3	-2.148	0.019	Uncharacterized LOC100576878
DN62791_c2_g1	CLO_3	-3.038	0.019	Uncharacterized LOC107965177
DN76630_c5_g1	CLO_3	-2.423	0.020	Tribbles homolog 2

DN46214_c0_g1	CLO_3	-3.571	0.021	hbg3 gene for alpha-glucosidase
DN65709_c2_g1	CLO_3	-3.452	0.022	MFS-type transporter SLC18B1-like
DN66453_c0_g1	CLO_3	-4.701	0.022	Uncharacterized LOC102656775
DN67936_c0_g1	CLO_3	-3.016	0.022	Trypsin-1
DN70862_c2_g1	CLO_3	-2.681	0.022	BAG domain-containing protein Samui
DN75539_c1_g3	CLO_3	-2.128	0.022	Uncharacterized LOC100576878
DN8732_c0_g1	CLO_3	-4.029	0.022	NA
DN74205_c0_g1	CLO_3	-2.460	0.023	Uncharacterized LOC724756
DN57437_c0_g1	CLO_3	-2.227	0.024	NA
DN68015_c0_g1	CLO_3	-2.601	0.025	CCAAT/enhancer-binding protein
DN48609_c0_g1	CLO_3	-2.690	0.025	Heat shock protein 83
DN58930_c0_g1	CLO_3	-2.733	0.025	Heat shock protein 83
DN62787_c2_g1	CLO_3	-3.259	0.025	NA
DN45240_c0_g1	CLO_3	-3.005	0.026	NA
DN73859_c6_g1	CLO_3	-2.572	0.026	NA
DN58787_c0_g1	CLO_3	-2.433	0.026	Uncharacterized LOC100577920
DN66827_c0_g1	CLO_3	-2.284	0.026	Uncharacterized LOC102655792
DN76190_c1_g1	CLO_3	-2.165	0.026	Uncharacterized LOC408853
DN64837_c0_g1	CLO_3	-2.083	0.026	G-box-binding factor-like
DN69403_c1_g2	CLO_3	-2.172	0.026	Tctex1 domain-containing protein 2
DN50910_c0_g1	CLO_3	-2.231	0.026	Breast cancer metastasis-suppressor 1-like protein-A
DN60138_c0_g1	CLO_3	-2.135	0.027	Histone H3
DN132114_c0_g1	CLO_3	-2.243	0.027	Uncharacterized LOC107964762
DN69727_c0_g2	CLO_3	-2.486	0.027	titin
DN73049_c0_g1	CLO_3	-3.813	0.027	NA
DN65519_c0_g1	CLO_3	-3.527	0.027	Bardet-Biedl syndrome 7 protein
DN64520_c0_g1	CLO_3	-2.720	0.027	Heat shock 70 kDa protein cognate 4
DN71469_c3_g1	CLO_3	-2.560	0.027	Nuclear factor interleukin-3-regulated protein
DN64088_c2_g1	CLO_3	-2.149	0.027	Lipopolysaccharide-induced tumor necrosis factor-alpha factor homolog
DN67295_c0_g2	CLO_3	-2.003	0.027	Mitochondrial folate transporter/carrier
DN60489_c0_g1	CLO_3	-2.221	0.028	Uncharacterized LOC100577920
DN55112_c1_g1	CLO_3	-2.481	0.028	NA
DN47412_c0_g1	CLO_3	-2.411	0.028	NA
DN60457_c0_g1	CLO_3	-2.763	0.028	Heat shock 70 kDa protein cognate 4
DN72143_c6_g1	CLO_3	-2.524	0.029	NA
DN74940_c0_g1	CLO_3	-2.469	0.029	Heat shock 70 kDa protein cognate 4
DN48415_c0_g1	CLO_3	-2.055	0.029	Uncharacterized LOC107964762
DN62308_c0_g1	CLO_3	-2.070	0.029	Uncharacterized LOC726980
DN141512_c0_g1	CLO_3	-3.004	0.029	PAB-dependent poly(A)-specific ribonuclease subunit PAN3
DN75037_c1_g4	CLO_3	-2.064	0.029	Phospholipase ABHD3
DN63568_c2_g1	CLO_3	-2.331	0.030	Uncharacterized LOC100577920
DN73959_c0_g1	CLO_3	-2.172	0.031	G protein-coupled receptor kinase 2
DN76918_c3_g1	CLO_3	-2.091	0.031	Glutamate receptor ionotropic, kainate 2
DN61300_c0_g1	CLO_3	-2.076	0.031	Uncharacterized LOC102656532
DN69105_c0_g1	CLO_3	-2.729	0.032	NA
DN109934_c0_g1	CLO_3	-3.539	0.033	DNA (cytosine-5)-methyltransferase PliMCI

DN69286_c0_g1	CLO_3	-3.544	0.033	Uncharacterized LOC107965060
DN36517_c0_g1	CLO_3	-2.752	0.033	Acyl-CoA Delta(11) desaturase
DN70964_c0_g1	CLO_3	-2.811	0.033	26S proteasome non-ATPase regulatory subunit 2
DN76380_c2_g1	CLO_3	-2.168	0.035	78 kDa glucose-regulated protein
DN76652_c1_g1	CLO_3	-3.081	0.036	NA
DN68737_c0_g2	CLO_3	-2.070	0.036	Uncharacterized LOC409690
DN75451_c6_g5	CLO_3	-2.767	0.036	NA
DN74254_c0_g1	CLO_3	-2.306	0.037	Large neutral amino acids transporter small subunit 2
DN73483_c1_g1	CLO_3	-2.858	0.037	Uncharacterized LOC102654285
DN66614_c0_g1	CLO_3	-2.191	0.037	Histone H2BNA3
DN60011_c1_g1	CLO_3	-2.008	0.038	NA
DN17303_c0_g1	CLO_3	-2.420	0.038	TATA-binding protein-associated factor 172
DN74380_c1_g2	CLO_3	-2.165	0.039	Uncharacterized LOC102655564
DN72426_c2_g1	CLO_3	-2.503	0.040	Uncharacterized LOC725233
DN17286_c0_g1	CLO_3	-2.272	0.040	RNA-binding protein 24-like
DN47377_c0_g1	CLO_3	-5.950	0.042	Uncharacterized LOC107993966
DN59387_c0_g1	CLO_3	-4.377	0.042	NA
DN61846_c0_g1	CLO_3	-2.481	0.042	Tubulin beta chain
DN76185_c0_g1	CLO_3	-2.502	0.042	BAG domain-containing protein Samui
DN75593_c2_g1	CLO_3	-2.275	0.042	Carboxylesterase clade I, member 1
DN45930_c0_g1	CLO_3	-2.092	0.042	NA
DN135199_c0_g1	CLO_3	-2.467	0.042	RNA-binding protein 24-like
DN76521_c0_g1	CLO_3	-2.225	0.042	Uncharacterized LOC100577920
DN64929_c0_g1	CLO_3	-3.350	0.043	NA
DN76377_c3_g1	CLO_3	-2.144	0.043	DNA ligase 1-like
DN13432_c0_g1	CLO_3	-3.508	0.044	CCAAT/enhancer-binding protein
DN75883_c0_g1	CLO_3	-2.188	0.045	NA
DN46520_c0_g1	CLO_3	-4.109	0.046	NA
DN68702_c0_g1	CLO_3	-2.858	0.046	Uncharacterized LOC105735102
DN35293_c0_g1	CLO_3	-2.749	0.047	NA
DN52509_c0_g1	CLO_3	-2.370	0.047	Heat shock protein 83
DN55726_c0_g1	CLO_3	-2.257	0.047	NA
DN62705_c0_g1	CLO_3	-2.033	0.047	Max-binding protein MNT
DN72999_c0_g2	CLO_3	-3.546	0.048	Ras guanine nucleotide exchange factor P
DN69883_c0_g1	CLO_3	-3.282	0.048	UBX domain-containing protein 11-like
DN73388_c0_g1	CLO_3	-3.209	0.048	Flocculation protein FLO11
DN57956_c0_g1	CLO_3	-2.443	0.048	Silk fibroin 1 mRNA
DN64830_c0_g1	CLO_3	-2.484	0.049	NA
DN68915_c0_g2	CLO_3	-2.445	0.049	NA
DN72732_c16_g2	CLO_3	-2.144	0.049	Major royal jelly protein 3
DN64990_c20_g3	CLO_3	-2.095	0.049	Major royal jelly protein 2
DN55064_c0_g1	CLO_3	-2.384	0.049	Heat shock protein 83
DN75605_c5_g1	IMD_0_3	5.409	0.002	Hymenoptaecin
DN36548_c0_g1	IMD_0_3	6.625	0.004	Sodium/potassium-transporting ATPase subunit beta-2-like
DN64336_c0_g1	IMD_0_3	3.215	0.009	Rhodopsin
DN75355_c1_g1	IMD_0_3	3.833	0.016	Probable serine/threonine-protein kinase DDB

				G0271402-like
DN156066_c0_g1	IMD_0_3	3.329	0.020	Synaptotagmin-14
DN160035_c0_g1	IMD_0_3	7.376	0.028	Uncharacterized LOC408988
DN75605_c5_g3	IMD_0_3	4.250	0.036	Hymenoptaecin
DN39352_c0_g1	IMD_0_3	-5.730	1.6E-09	Uncharacterized LOC413055
DN74754_c11_g2	IMD_0_3	-2.044	1.6E-09	Alpha-amylase 4N
DN46214_c0_g1	IMD_0_3	-4.473	3.7E-07	hbg3 gene for alpha-glucosidase
DN75806_c0_g2	IMD_0_3	-2.134	1.2E-06	Calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1
DN174389_c0_g1	IMD_0_3	-2.054	7.2E-06	hbg3 gene for alpha-glucosidase
DN74754_c11_g4	IMD_0_3	-2.277	3.6E-05	Alpha-amylase-related protein
DN74309_c3_g1	IMD_0_3	-2.043	1.7E-04	hbg3 gene for alpha-glucosidase
DN73289_c4_g1	IMD_0_3	-2.035	2.8E-04	hbg3 gene for alpha-glucosidase
DN73289_c4_g2	IMD_0_3	-2.055	0.001	Alpha-glucosidase (Hbg3)
DN55553_c0_g1	IMD_0_3	-2.163	0.002	Uncharacterized protein KIAA0513
DN24192_c0_g1	IMD_0_3	-8.303	0.005	Protein turtle
DN75688_c1_g1	IMD_0_3	-2.144	0.005	Uncharacterized LOC105735502
DN75210_c1_g2	IMD_0_3	-2.155	0.012	Protein takeout
DN75627_c4_g1	IMD_0_3	-2.592	0.016	Uncharacterized LOC107964352
DN2457_c0_g1	IMD_0_3	-2.070	0.020	Major royal jelly protein 3
DN46728_c0_g1	IMD_0_3	-2.152	0.022	Ubiquitin carboxyl-terminal hydrolase 47
DN2445_c0_g1	IMD_0_3	-5.205	0.038	RNA-binding protein 4.1-like
DN68624_c0_g1	IMD_0_3	-3.194	0.041	NA
DN71456_c0_g1	IMD_0_3	-2.152	0.041	NA
DN178528_c0_g1	IMD_3	5.183	0.043	5-aminolevulinate synthase, erythroid-specific, mitochondrial
DN61093_c0_g1	IMD_3	7.482	0.001	Alpha-(1,3)-fucosyltransferase C
DN71316_c0_g1	IMD_3	2.610	0.022	Apyrase
DN179898_c0_g1	IMD_3	7.146	2.3E-05	Atypical protein kinase C
DN71115_c1_g1	IMD_3	5.828	0.004	Calexitin-2
DN73281_c0_g1	IMD_3	2.305	0.027	Centrosomal protein of 131 kDa
DN63059_c1_g1	IMD_3	2.410	0.042	Cytochrome b561 domain-containing protein 2-like
DN76503_c0_g1	IMD_3	2.117	0.015	DNA helicase MCM8
DN51818_c0_g1	IMD_3	7.587	0.003	Doublesex (Dsx)
DN53473_c0_g1	IMD_3	2.151	0.030	Ecdysone-induced protein 75 (E75)
DN119505_c0_g1	IMD_3	5.444	0.014	Esterase FE4-like
DN46161_c0_g1	IMD_3	6.873	0.004	hbg3 gene for alpha-glucosidase
DN75605_c5_g3	IMD_3	3.975	0.008	Hymenoptaecin
DN75605_c5_g1	IMD_3	4.127	0.009	Hymenoptaecin
DN68800_c1_g1	IMD_3	2.023	0.009	Inositol-3-phosphate synthase 1-B
DN78421_c0_g1	IMD_3	2.008	0.004	KH domain-containing protein akap-1
DN73732_c0_g2	IMD_3	2.916	0.003	NA
DN132577_c0_g1	IMD_3	4.035	0.026	Na(+)/H(+) exchange regulatory cofactor NHE-RF1
DN160358_c0_g1	IMD_3	2.277	0.040	Paired amphipathic helix protein Sin3a
DN81495_c0_g1	IMD_3	2.497	0.031	Probable G-protein coupled receptor Mth-like 5
DN164890_c0_g1	IMD_3	5.418	1.4E-06	Protein FAM117B
DN64825_c0_g1	IMD_3	2.675	0.029	Sex determination locus

DN76848_c0_g1	IMD_3	2.221	0.031	Uncharacterized LOC100577522
DN61430_c0_g2	IMD_3	3.019	0.011	Uncharacterized LOC102653727
DN72525_c0_g1	IMD_3	2.374	0.042	Uncharacterized LOC102655536
DN68175_c0_g1	IMD_3	4.096	4.9E-04	Uncharacterized LOC102655574
DN151760_c0_g1	IMD_3	2.685	0.044	Uncharacterized LOC105737494
DN76633_c3_g2	IMD_3	2.693	0.014	Uncharacterized LOC107965915
DN160035_c0_g1	IMD_3	7.286	0.008	Uncharacterized LOC408988
DN128102_c0_g1	IMD_3	4.673	1.3E-04	Uncharacterized LOC409502
DN66546_c1_g1	IMD_3	8.443	4.5E-05	Uncharacterized LOC410363
DN52341_c0_g1	IMD_3	4.134	0.022	Uncharacterized LOC410363
DN31074_c0_g1	IMD_3	2.866	0.046	Uncharacterized LOC726101
DN169391_c0_g1	IMD_3	8.030	0.001	V-type proton ATPase 16 kDa proteolipid sub-unit
DN172616_c0_g1	IMD_3	5.831	2.4E-05	WD repeat-containing protein 82
DN147012_c0_g1	IMD_3	6.318	0.023	Zinc finger Ran-binding domain-containing protein 2-like
DN36517_c0_g1	IMD_3	-2.362	0.023	Acyl-CoA Delta(11) desaturase
DN69631_c0_g1	IMD_3	-2.001	4.7E-05	alpha-(1,3)-fucosyltransferase C-like
DN74754_c11_g2	IMD_3	-2.061	4.5E-04	Alpha-amylase 4N
DN74754_c11_g4	IMD_3	-2.434	3.1E-04	Alpha-amylase-related protein
DN75389_c2_g1	IMD_3	-2.456	0.026	Alpha-tocopherol transfer protein-like
DN6381_c0_g1	IMD_3	-3.816	1.8E-05	Ankyrin repeat and SOCS box protein 18
DN74212_c0_g1	IMD_3	-2.031	0.050	Arrestin homolog
DN71224_c0_g1	IMD_3	-2.313	0.006	Bestrophin-1
DN59252_c0_g1	IMD_3	-2.579	0.004	Bestrophin-4
DN75806_c0_g2	IMD_3	-2.341	1.2E-06	Calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1
DN69674_c0_g1	IMD_3	-2.476	6.6E-05	cAMP-responsive element-binding protein-like 2
DN24592_c0_g1	IMD_3	-2.352	0.030	CCAAT/enhancer-binding protein beta
DN48039_c0_g1	IMD_3	-2.577	0.012	Chaoptin
DN72072_c1_g1	IMD_3	-2.276	4.7E-04	Circadian locomotor output cycles protein kaput-like
DN74861_c4_g1	IMD_3	-2.046	0.012	Ecdysone-induced protein 75 (E75)
DN72173_c1_g1	IMD_3	-2.111	0.030	Ecdysone-induced protein 75 (E75)
DN60530_c0_g1	IMD_3	-2.483	0.010	Esterase FE4-like
DN76573_c2_g4	IMD_3	-2.483	0.010	Four and a half LIM domains protein 2
DN72121_c3_g1	IMD_3	-2.210	0.045	Four and a half LIM domains protein 2
DN67985_c1_g2	IMD_3	-2.034	0.001	Furin-like protease 1
DN76168_c2_g1	IMD_3	-5.832	2.7E-04	General odorant-binding protein 69a
DN75371_c1_g1	IMD_3	-2.108	0.025	Glucose dehydrogenase [FAD, quinone]
DN29366_c0_g1	IMD_3	-3.516	0.001	Glucose oxidase
DN51391_c0_g1	IMD_3	-6.441	5.0E-05	Glutamate decarboxylase
DN18223_c0_g1	IMD_3	-2.070	0.016	Glutamate receptor ionotropic, kainate 2
DN74309_c3_g1	IMD_3	-2.214	1.8E-08	hbg3 gene for alpha-glucosidase
DN46214_c0_g1	IMD_3	-4.423	2.9E-07	hbg3 gene for alpha-glucosidase
DN65436_c0_g1	IMD_3	-2.140	4.6E-05	hbg3 gene for alpha-glucosidase
DN72213_c0_g1	IMD_3	-2.066	0.002	hbg3 gene for alpha-glucosidase
DN73289_c4_g1	IMD_3	-2.173	0.004	hbg3 gene for alpha-glucosidase
DN65447_c0_g1	IMD_3	-2.022	0.016	Hexokinase type 2

DN52447_c0_g1	IMD_3	-2.297	0.016	Homeobox protein araucan
DN56343_c0_g1	IMD_3	-2.695	0.043	Inositol oxygenase
DN2458_c0_g1	IMD_3	-4.584	0.009	Luciferin 4-monooxygenase
DN62886_c0_g1	IMD_3	-2.495	0.030	Membrane alanyl aminopeptidase
DN72909_c3_g1	IMD_3	-2.079	0.043	Microtubule-associated protein futsch-like
DN62542_c0_g1	IMD_3	-2.053	0.010	Mitochondrial 10-formyltetrahydrofolate dehydrogenase
DN76608_c0_g1	IMD_3	-2.000	0.022	Monocarboxylate transporter 12
DN63045_c0_g1	IMD_3	-2.776	0.006	NA
DN68624_c0_g1	IMD_3	-3.300	0.010	NA
DN76643_c11_g1	IMD_3	-2.156	0.012	NA
DN61034_c0_g1	IMD_3	-2.138	0.032	NA
DN68143_c0_g1	IMD_3	-2.729	0.029	NADH-ubiquinone oxidoreductase chain 2
DN75782_c2_g1	IMD_3	-2.120	0.050	Neuropilin and tolloid-like protein 1
DN70556_c0_g1	IMD_3	-3.500	0.002	Odorant binding protein 6 (Obp6)
DN59234_c1_g1	IMD_3	-2.421	0.002	Protein 60A
DN106298_c0_g1	IMD_3	-2.510	0.030	Protein couch potato-like
DN63379_c0_g1	IMD_3	-2.368	1.3E-04	Protein dead ringer
DN90120_c0_g1	IMD_3	-2.091	0.043	Protein kinase C and casein kinase substrate in neurons protein 1
DN62657_c0_g1	IMD_3	-3.233	0.003	Protein SCA1
DN154310_c0_g1	IMD_3	-4.161	0.001	Ras-related protein Rab-3
DN74981_c2_g1	IMD_3	-2.053	0.031	Scavenger receptor class B member 1
DN74234_c0_g1	IMD_3	-2.043	0.004	Sodium/hydrogen exchanger 3
DN29706_c0_g1	IMD_3	-8.068	0.002	<i>Solute carrier family 25 member 46</i>
DN54922_c0_g1	IMD_3	-2.408	0.005	TGF-beta receptor type-1
DN48935_c0_g1	IMD_3	-2.609	0.003	Transcription factor Ken 2-like
DN74049_c1_g1	IMD_3	-2.483	0.001	Transcription factor Sox-2
DN46728_c0_g1	IMD_3	-2.052	0.013	Ubiquitin carboxyl-terminal hydrolase 47
DN71129_c0_g1	IMD_3	-2.014	0.002	Uncharacterized LOC100578747
DN37716_c0_g1	IMD_3	-4.372	4.6E-05	Uncharacterized LOC100578769
DN62006_c0_g1	IMD_3	-2.390	0.001	Uncharacterized LOC100578835
DN70393_c3_g1	IMD_3	-2.308	9.0E-05	Uncharacterized LOC102653672
DN60620_c0_g1	IMD_3	-2.146	0.016	Uncharacterized LOC102654485
DN71063_c6_g1	IMD_3	-2.377	5.0E-05	Uncharacterized LOC102654697
DN65579_c0_g1	IMD_3	-2.067	0.035	Uncharacterized LOC102655457
DN36048_c0_g1	IMD_3	-2.282	0.001	Uncharacterized LOC102655706
DN47520_c0_g1	IMD_3	-2.122	0.002	Uncharacterized LOC102655966
DN75903_c2_g2	IMD_3	-2.124	0.036	Uncharacterized LOC102655966
DN67284_c0_g1	IMD_3	-2.480	0.035	Uncharacterized LOC102676782
DN73771_c0_g1	IMD_3	-2.008	0.001	Uncharacterized LOC105737287
DN57433_c0_g1	IMD_3	-2.085	0.015	Uncharacterized LOC107964278
DN50985_c0_g1	IMD_3	-2.153	0.006	Uncharacterized LOC107964613
DN61179_c0_g1	IMD_3	-2.230	0.002	Uncharacterized LOC108003476
DN59783_c0_g1	IMD_3	-2.417	0.003	Uncharacterized LOC408897
DN41830_c0_g1	IMD_3	-2.298	0.007	Uncharacterized LOC408898
DN75048_c6_g1	IMD_3	-2.013	0.021	Uncharacterized LOC408957
DN56983_c0_g1	IMD_3	-3.040	0.007	Uncharacterized LOC552366

DN27455_c0_g1	TMX_0_1	4.234	0.003	Basic-leucine zipper transcription factor A
DN64336_c0_g1	TMX_0_1	3.851	0.007	Rhodopsin
DN45341_c0_g1	TMX_0_1	7.589	0.012	3'(2'),5'-bisphosphate nucleotidase 1
DN46161_c0_g1	TMX_0_1	7.001	0.047	hbg3 gene for alpha-glucosidase
DN151538_c0_g1	TMX_0_1	-5.521	0.012	NA
DN75688_c1_g1	TMX_0_1	-1.982	0.016	Uncharacterized LOC105735502
DN45341_c0_g1	TMX_1	7.549	0.004	3'(2'),5'-bisphosphate nucleotidase 1
DN53641_c0_g1	TMX_1	3.129	0.044	Junctophilin-1-like
DN64336_c0_g1	TMX_1	4.135	0.001	Opsin-1
DN139979_c0_g1	TMX_1	7.079	0.044	Protein turtle
DN74754_c11_g2	TMX_1	-2.213	0.001	Alpha-amylase 4N
DN74754_c11_g4	TMX_1	-2.287	0.002	Alpha-amylase-related protein
DN6381_c0_g1	TMX_1	-3.005	0.014	Ankyrin repeat and SOCS box protein 18
DN24592_c0_g1	TMX_1	-2.853	0.035	CCAAT/enhancer-binding protein beta
DN76168_c2_g1	TMX_1	-4.616	0.004	General odorant-binding protein 69a
DN70556_c0_g1	TMX_1	-3.219	0.028	General odorant-binding protein 72
DN75371_c1_g1	TMX_1	-2.455	0.049	Glucose dehydrogenase [FAD, quinone]
DN51391_c0_g1	TMX_1	-5.755	0.001	Glutamate decarboxylase
DN46214_c0_g1	TMX_1	-4.010	0.033	hbg3 gene for alpha-glucosidase
DN29323_c0_g1	TMX_1	-2.329	0.032	Homeobox protein B-H2-like
DN69788_c0_g1	TMX_1	-2.280	0.004	NA
DN74807_c2_g1	TMX_1	-2.596	0.004	NA
DN51171_c0_g1	TMX_1	-2.877	0.014	NA
DN60901_c0_g1	TMX_1	-2.615	0.024	NA
DN75651_c0_g1	TMX_1	-2.551	0.026	NA
DN72237_c0_g1	TMX_1	-2.036	0.044	NA
DN54048_c0_g1	TMX_1	-3.854	0.021	Protein takeout
DN74981_c2_g1	TMX_1	-2.473	0.006	Scavenger receptor class B member 1
DN76565_c0_g1	TMX_1	-2.699	0.001	Uncharacterized LOC102654995
DN75688_c1_g1	TMX_1	-2.885	0.001	Uncharacterized LOC105735502
DN75734_c2_g1	TMX_1	-2.076	0.020	Uncharacterized LOC412763
DN63314_c0_g1	DMSO	2.224	0.038	Apidaecins type 14
DN51049_c1_g1	DMSO	8.334	2.2E-04	Ecdysone-induced protein 75 (E75)
DN75809_c3_g2	DMSO	2.168	0.008	Histone H2A.Z-specific chaperone CHZ1-like
DN75605_c5_g3	DMSO	4.520	0.038	Hymenoptaecin
DN76711_c7_g1	DMSO	5.985	0.008	NA
DN56359_c0_g1	DMSO	10.516	1.4E-04	Neurofilament heavy polypeptide
DN2445_c0_g1	DMSO	-6.746	0.001	RNA-binding protein 4.1-like
DN46214_c0_g1	DMSO	-4.094	0.039	hbg3 gene for alpha-glucosidase

Table S5: Enriched GO terms of biological processes (BP) that were significantly overrepresented before multiple testing (Fisher's exact test; $p < 0.01$) of differentially expressed unigenes of *A. mellifera* treated with the solvent control (0.1% DMSO).

Category	GO term	p-value	Ontology
GO:0042742	defense response to bacterium	0.0005	BP
GO:0009617	response to bacterium	0.0006	BP
GO:0045087	innate immune response	0.0007	BP
GO:0098542	defense response to other organism	0.0012	BP
GO:0006955	immune response	0.0020	BP
GO:0051707	response to other organism	0.0027	BP
GO:0006952	defense response	0.0034	BP
GO:0043207	response to external biotic stimulus	0.0039	BP
GO:0009607	response to biotic stimulus	0.0041	BP
GO:0002376	immune system process	0.0086	BP

Table S6: Enriched GO terms of biological processes (BP), molecular functions (MF) and cellular components (CC) that were significantly overrepresented before multiple testing (Fisher's exact test; $p < 0.01$) of differentially expressed unigenes of *A. mellifera* treated with 0.3 ng/bee clothianidin.

Category	GO term	p-value	Ontology
GO:0019262	N-acetylneuraminate catabolic process	0.002	BP
GO:0006054	N-acetylneuraminate metabolic process	0.003	BP
GO:0022610	biological adhesion	0.004	BP
GO:0042595	behavioral response to starvation	0.005	BP
GO:0007601	visual perception	0.005	BP
GO:0050953	sensory perception of light stimulus	0.005	BP
GO:0007306	eggshell chorion assembly	0.006	BP
GO:0044706	multi-multicellular organism process	0.006	BP
GO:0010899	regulation of phosphatidylcholine catabolic process	0.006	BP
GO:0015920	lipopolysaccharide transport	0.006	BP
GO:0032490	detection of molecule of bacterial origin	0.006	BP
GO:0032497	detection of lipopolysaccharide	0.006	BP
GO:0043654	recognition of apoptotic cell	0.006	BP
GO:0060696	regulation of phospholipid catabolic process	0.006	BP
GO:0044406	adhesion of symbiont to host	0.007	BP
GO:0032770	positive regulation of monooxygenase activity	0.007	BP
GO:0051000	positive regulation of nitric-oxide synthase activity	0.007	BP
GO:0035376	sterol import	0.007	BP
GO:0035382	sterol transmembrane transport	0.007	BP
GO:0070508	cholesterol import	0.007	BP
GO:0008037	cell recognition	0.007	BP
GO:0034384	high-density lipoprotein particle clearance	0.007	BP
GO:0006910	phagocytosis, recognition	0.008	BP

GO:0035461	vitamin transmembrane transport	0.008	BP
GO:0051606	detection of stimulus	0.008	BP
GO:0048842	positive regulation of axon extension involved in axon guidance	0.008	BP
GO:0008343	adult feeding behavior	0.008	BP
GO:0008364	pupal chitin-based cuticle development	0.008	BP
GO:0010886	positive regulation of cholesterol storage	0.008	BP
GO:0044703	multi-organism reproductive process	0.008	BP
GO:0046693	sperm storage	0.009	BP
GO:0044712	single-organism catabolic process	0.009	BP
GO:0009595	detection of biotic stimulus	0.010	BP
GO:0098581	detection of external biotic stimulus	0.010	BP
GO:0006707	cholesterol catabolic process	0.010	BP
GO:0016127	sterol catabolic process	0.010	BP
GO:0046692	sperm competition	0.010	BP
GO:0018298	protein-chromophore linkage	0.010	BP
GO:0010866	regulation of triglyceride biosynthetic process	0.010	BP
GO:0010867	positive regulation of triglyceride biosynthetic process	0.010	BP
GO:0008747	N-acetylneuraminate lyase activity	0.001	MF
GO:0016833	oxo-acid-lyase activity	0.003	MF
GO:0008035	high-density lipoprotein particle binding	0.006	MF
GO:0070506	high-density lipoprotein particle receptor activity	0.006	MF
GO:0034186	apolipoprotein A-I binding	0.007	MF
GO:0001875	lipopolysaccharide receptor activity	0.007	MF
GO:0030169	low-density lipoprotein particle binding	0.009	MF
GO:0042600	chorion	0.001	CC
GO:0030312	external encapsulating structure	0.008	CC

Table S7: Enriched GO terms of biological processes (BP), molecular functions (MF) and cellular components (CC) that were significantly overrepresented before multiple testing (Fisher's exact test; $p < 0.01$) of differentially expressed unigenes of *A. mellifera* treated with 3 ng/bee clothianidin.

Category	GO term	p-value	Ontology
GO:0042026	protein refolding	7.9E-10	BP
GO:0010998	regulation of translational initiation by eIF2 alpha phosphorylation	1.3E-09	BP
GO:0043558	regulation of translational initiation in response to stress	3.4E-09	BP
GO:0043555	regulation of translation in response to stress	5.4E-09	BP
GO:0006457	protein folding	4.3E-07	BP
GO:0061077	chaperone-mediated protein folding	3.2E-06	BP
GO:0045214	sarcomere organization	3.5E-06	BP
GO:0006446	regulation of translational initiation	6.8E-06	BP
GO:0009408	response to heat	7.4E-06	BP
GO:0031032	actomyosin structure organization	2.1E-04	BP
GO:0030029	actin filament-based process	2.9E-04	BP
GO:0055090	acylglycerol homeostasis	4.0E-04	BP
GO:0070328	triglyceride homeostasis	4.0E-04	BP
GO:0001935	endothelial cell proliferation	4.7E-04	BP

GO:0030036	actin cytoskeleton organization	5.0E-04	BP
GO:0009266	response to temperature stimulus	0.001	BP
GO:0022008	neurogenesis	0.001	BP
GO:0042063	gliogenesis	0.001	BP
GO:0006950	response to stress	0.001	BP
GO:0042744	hydrogen peroxide catabolic process	0.002	BP
GO:0007275	multicellular organismal development	0.002	BP
GO:0042743	hydrogen peroxide metabolic process	0.002	BP
GO:0033559	unsaturated fatty acid metabolic process	0.003	BP
GO:0045579	positive regulation of B cell differentiation	0.003	BP
GO:0051180	vitamin transport	0.004	BP
GO:0032496	response to lipopolysaccharide	0.005	BP
GO:0050896	response to stimulus	0.005	BP
GO:0016310	phosphorylation	0.005	BP
GO:0045621	positive regulation of lymphocyte differentiation	0.006	BP
GO:0007306	eggshell chorion assembly	0.006	BP
GO:0045577	regulation of B cell differentiation	0.006	BP
GO:0010899	regulation of phosphatidylcholine catabolic process	0.006	BP
GO:0015920	lipopolysaccharide transport	0.006	BP
GO:0032490	detection of molecule of bacterial origin	0.006	BP
GO:0032497	detection of lipopolysaccharide	0.006	BP
GO:0043654	recognition of apoptotic cell	0.006	BP
GO:0060696	regulation of phospholipid catabolic process	0.006	BP
GO:0055088	lipid homeostasis	0.006	BP
GO:0010608	posttranscriptional regulation of gene expression	0.006	BP
GO:0050673	epithelial cell proliferation	0.006	BP
GO:0002237	response to molecule of bacterial origin	0.007	BP
GO:0001676	long-chain fatty acid metabolic process	0.007	BP
GO:0007480	imaginal disc-derived leg morphogenesis	0.007	BP
GO:0007339	binding of sperm to zona pellucida	0.007	BP
GO:0035036	sperm-egg recognition	0.007	BP
GO:0043207	response to external biotic stimulus	0.007	BP
GO:0050994	regulation of lipid catabolic process	0.007	BP
GO:0035376	sterol import	0.007	BP
GO:0035382	sterol transmembrane transport	0.007	BP
GO:0070508	cholesterol import	0.007	BP
GO:0032770	positive regulation of monooxygenase activity	0.007	BP
GO:0051000	positive regulation of nitric-oxide synthase activity	0.007	BP
GO:0044406	adhesion of symbiont to host	0.007	BP
GO:0006417	regulation of translation	0.008	BP
GO:0006468	protein phosphorylation	0.008	BP
GO:0042742	defense response to bacterium	0.008	BP
GO:1903052	positive regulation of proteolysis involved in cellular protein catabolic process	0.009	BP
GO:0019369	arachidonic acid metabolic process	0.009	BP
GO:0008593	regulation of Notch signaling pathway	0.009	BP
GO:0009607	response to biotic stimulus	0.009	BP
GO:0035461	vitamin transmembrane transport	0.009	BP

GO:0006910	phagocytosis, recognition	0.009	BP
GO:0034384	high-density lipoprotein particle clearance	0.009	BP
GO:0008283	cell proliferation	0.009	BP
GO:0035120	post-embryonic appendage morphogenesis	0.009	BP
GO:0051082	unfolded protein binding	1.4E-11	MF
GO:0046982	protein heterodimerization activity	1.2E-05	MF
GO:0046983	protein dimerization activity	1.3E-04	MF
GO:0005212	structural constituent of eye lens	0.001	MF
GO:0035326	enhancer binding	0.003	MF
GO:0005515	protein binding	0.003	MF
GO:0001071	nucleic acid binding transcription factor activity	0.004	MF
GO:0003700	transcription factor activity, sequence-specific DNA binding	0.004	MF
GO:0020037	heme binding	0.005	MF
GO:0016705	oxidoreductase activity	0.005	MF
GO:0008134	transcription factor binding	0.006	MF
GO:0008035	high-density lipoprotein particle binding	0.006	MF
GO:0070506	high-density lipoprotein particle receptor activity	0.006	MF
GO:0000981	RNA polymerase II transcription factor activity	0.006	MF
GO:0046906	tetrapyrrole binding	0.006	MF
GO:0001540	beta-amyloid binding	0.007	MF
GO:0003677	DNA binding	0.007	MF
GO:0001875	lipopolysaccharide receptor activity	0.007	MF
GO:0034186	apolipoprotein A-I binding	0.007	MF
GO:0000786	nucleosome	1.8E-08	CC
GO:1990104	DNA bending complex	1.8E-08	CC
GO:0044815	DNA packaging complex	1.6E-07	CC
GO:0032993	protein-DNA complex	1.3E-06	CC
GO:0030018	Z disc	1.1E-05	CC
GO:0044449	contractile fiber part	2.9E-04	CC
GO:0005576	extracellular region	0.009	CC

Table S8: Enriched GO terms of biological processes (BP) and molecular functions (MF) that were significantly overrepresented before multiple testing (Fisher's exact test; $p < 0.01$) of differentially expressed unigenes of *A. mellifera* treated with 0.3 ng/bee imidacloprid.

Category	GO term	p-value	Ontology
GO:0060179	male mating behavior	0.002	BP
GO:0051704	multi-organism process	0.002	BP
GO:0042595	behavioral response to starvation	0.002	BP
GO:0033057	multicellular organismal reproductive behavior	0.003	BP
GO:0044705	multi-organism reproductive behavior	0.004	BP
GO:0008343	adult feeding behavior	0.004	BP
GO:0009605	response to external stimulus	0.005	BP
GO:0042742	defense response to bacterium	0.005	BP
GO:0019098	reproductive behavior	0.005	BP
GO:0009617	response to bacterium	0.006	BP
GO:0044706	multi-multicellular organism process	0.006	BP

GO:0045087	innate immune response	0.007	BP
GO:0032504	multicellular organism reproduction	0.008	BP
GO:0044703	multi-organism reproductive process	0.009	BP
GO:0004556	alpha-amylase activity	9.0E-06	MF
GO:0016160	amylase activity	9.0E-06	MF
GO:0048101	calcium- and calmodulin-regulated 3',5'-cyclic-GMP phosphodiesterase activity	0.001	MF
GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds	0.003	MF
GO:0016798	hydrolase activity, acting on glycosyl bonds	0.003	MF
GO:0047555	3',5'-cyclic-GMP phosphodiesterase activity	0.007	MF
GO:0004115	3',5'-cyclic-AMP phosphodiesterase activity	0.008	MF

Table S9: Enriched GO terms of biological processes (BP), molecular functions (MF) and cellular components (CC) that were significantly overrepresented before multiple testing (Fisher's exact test; $p < 0.01$) of differentially expressed unigenes of *A. mellifera* treated with 3 ng/bee imidacloprid.

Category	GO term	p-value	Ontology
GO:0007474	imaginal disc-derived wing vein specification	0.0044	BP
GO:2000312	regulation of kainate selective glutamate receptor activity	0.0093	BP
GO:0004556	alpha-amylase activity	0.0001	MF
GO:0016160	amylase activity	0.0001	MF
GO:0008145	phenylalkylamine binding	0.0039	MF
GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds	0.0053	MF
GO:0005549	odorant binding	0.0061	MF
GO:0048101	calcium- and calmodulin-regulated 3',5'-cyclic-GMP phosphodiesterase activity	0.0064	MF
GO:0016798	hydrolase activity, acting on glycosyl bonds	0.0078	MF
GO:0005550	pheromone binding	0.0083	MF
GO:0097467	type III terminal bouton	0.0040	CC
GO:0098839	postsynaptic density membrane	0.0099	CC

Table S10: Enriched GO terms of biological processes (BP) and molecular functions (MF) that were significantly overrepresented before multiple testing (Fisher's exact test; $p < 0.01$) of differentially expressed unigenes of *A. mellifera* treated with 0.1 ng/bee of thiamethoxam.

Category	GO term	p-value	Ontology
GO:0050427	3'-phosphoadenosine 5'-phosphosulfate metabolic process	0.002	BP
GO:0034035	purine ribonucleoside bisphosphate metabolic process	0.002	BP
GO:0018298	protein-chromophore linkage	0.003	BP
GO:0046854	phosphatidylinositol phosphorylation	0.004	BP
GO:0033865	nucleoside bisphosphate metabolic process	0.005	BP
GO:0033875	ribonucleoside bisphosphate metabolic process	0.005	BP
GO:0034032	purine nucleoside bisphosphate metabolic process	0.005	BP
GO:0046834	lipid phosphorylation	0.005	BP
GO:0008441	3'(2'),5'-bisphosphate nucleotidase activity	0.000	MF
GO:0008252	nucleotidase activity	0.002	MF

GO:0009881	photoreceptor activity	0.003	MF
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Table S11: Enriched GO terms of biological processes (BP), molecular functions (MF) and cellular components (CC) that were significantly overrepresented before multiple testing (Fisher's exact test; $p < 0.01$) of differentially expressed unigenes of *A. mellifera* treated with 1 ng/bee thiamethoxam.

Category	GO-term	p-value	Ontology
GO:0005975	carbohydrate metabolic process	0.001	BP
GO:0007608	sensory perception of smell	0.002	BP
GO:0007600	sensory perception	0.002	BP
GO:0007606	sensory perception of chemical stimulus	0.003	BP
GO:0042595	behavioral response to starvation	0.004	BP
GO:0003008	system process	0.004	BP
GO:0008343	adult feeding behavior	0.006	BP
GO:0050427	3'-phosphoadenosine 5'-phosphosulfate metabolic process	0.006	BP
GO:0044706	multi-multicellular organism process	0.007	BP
GO:0032501	multicellular organismal process	0.007	BP
GO:0010899	regulation of phosphatidylcholine catabolic process	0.007	BP
GO:0015920	lipopolysaccharide transport	0.007	BP
GO:0032490	detection of molecule of bacterial origin	0.007	BP
GO:0032497	detection of lipopolysaccharide	0.007	BP
GO:0043654	recognition of apoptotic cell	0.007	BP
GO:0060696	regulation of phospholipid catabolic process	0.007	BP
GO:0044406	adhesion of symbiont to host	0.008	BP
GO:0032770	positive regulation of monooxygenase activity	0.008	BP
GO:0051000	positive regulation of nitric-oxide synthase activity	0.008	BP
GO:0035376	sterol import	0.008	BP
GO:0035382	sterol transmembrane transport	0.008	BP
GO:0070508	cholesterol import	0.008	BP
GO:0008364	pupal chitin-based cuticle development	0.008	BP
GO:0034384	high-density lipoprotein particle clearance	0.008	BP
GO:0006910	phagocytosis, recognition	0.008	BP
GO:0034035	purine ribonucleoside bisphosphate metabolic process	0.009	BP
GO:0035461	vitamin transmembrane transport	0.009	BP
GO:0046693	sperm storage	0.009	BP
GO:0010886	positive regulation of cholesterol storage	0.009	BP
GO:0044703	multi-organism reproductive process	0.009	BP
GO:0051606	detection of stimulus	0.010	BP
GO:0046692	sperm competition	0.010	BP
GO:0004556	alpha-amylase activity	5.92E-06	MF
GO:0016160	amylase activity	5.92E-06	MF
GO:0005549	odorant binding	3.14E-04	MF
GO:0008145	phenylalkylamine binding	0.001	MF
GO:0008441	3'(2'),5'-bisphosphate nucleotidase activity	0.002	MF
GO:0005550	pheromone binding	0.002	MF
GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds	0.003	MF

GO:0016798	hydrolase activity, acting on glycosyl bonds	0.004	MF
GO:0043176	amine binding	0.004	MF
GO:0008035	high-density lipoprotein particle binding	0.007	MF
GO:0070506	high-density lipoprotein particle receptor activity	0.007	MF
GO:0034186	apolipoprotein A-I binding	0.008	MF
GO:0001875	lipopolysaccharide receptor activity	0.008	MF
GO:0008252	nucleotidase activity	0.010	MF
GO:0030169	low-density lipoprotein particle binding	0.010	MF
GO:0005576	extracellular region	1.49E-05	CC
GO:0030115	S-layer	0.002	CC
GO:0005618	cell wall	0.006	CC
GO:0030312	external encapsulating structure	0.007	CC

Table S12: Transcriptional alterations in the de novo assembly of eight selected genes (and one housekeeping gene) that have previously been shown to be affected by the exposure to low and high concentrations of clothianidin (CLO_0_3 and CLO_3), imidacloprid (IMD_0_3 and IMD_3) and thiamethoxam (TMX_0_1 and TMX_1) in the previous study, respectively.²¹

Function	unigene ID	Treatment	logFC	PValue	FDR
<i>acetylcholine receptor subunit alpha 1 (nAChRa1)</i>	DN75460_c2_g1	CLO_0_3	-0.424	0.255	0.745
	DN75460_c2_g1	CLO_3	-1.902	0.000	0.011
	DN75460_c2_g1	IMD_0_3	-0.203	0.367	0.824
	DN75460_c2_g1	IMD_3	-1.031	0.000	0.004
	DN75460_c2_g1	TMX_0_1	-0.566	0.040	1.000
	DN75460_c2_g1	TMX_1	-1.136	0.000	0.053
	DN75460_c2_g1	DMSO	-0.197	0.505	1.000
<i>acetylcholine receptor subunit alpha 2 (nAChRa2)</i>	DN75496_c1_g1	CLO_0_3	-0.359	0.195	0.704
	DN75496_c1_g1	CLO_3	-0.059	0.853	0.976
	DN75496_c1_g1	IMD_0_3	-0.334	0.203	0.722
	DN75496_c1_g1	IMD_3	-0.425	0.136	0.447
	DN75496_c1_g1	TMX_0_1	-0.289	0.233	1.000
	DN75496_c1_g1	TMX_1	-0.379	0.182	0.865
	DN75496_c1_g1	DMSO	0.388	0.156	1.000
<i>apidaecin</i>	DN71685_c0_g1	CLO_0_3	-0.134	0.532	0.880
	DN71685_c0_g1	CLO_3	-0.371	0.140	0.655
	DN71685_c0_g1	IMD_0_3	0.003	0.995	1.000
	DN71685_c0_g1	IMD_3	-0.089	0.665	0.881
	DN71685_c0_g1	TMX_0_1	-0.111	0.581	1.000
	DN71685_c0_g1	TMX_1	-0.148	0.490	1.000
	DN71685_c0_g1	DMSO	-0.056	0.785	1.000
<i>catalase</i>	DN76717_c2_g1	CLO_0_3	-0.319	0.232	0.730
	DN76717_c2_g1	CLO_3	-0.960	0.013	0.263
	DN76717_c2_g1	IMD_0_3	-0.069	0.741	0.956
	DN76717_c2_g1	IMD_3	-0.064	0.791	0.934
	DN76717_c2_g1	TMX_0_1	-0.241	0.283	1.000

	DN76717_c2_g1	TMX_1	-0.185	0.446	1.000
	DN76717_c2_g1	DMSO	-0.469	0.093	1.000
<i>cyclic AMP-responsive element-binding protein (creb)</i>	DN71165_c0_g1	CLO_0_3	-0.265	0.337	0.791
	DN71165_c0_g1	CLO_3	-1.093	0.004	0.140
	DN71165_c0_g1	IMD_0_3	-0.204	0.354	0.817
	DN71165_c0_g1	IMD_3	-0.412	0.101	0.390
	DN71165_c0_g1	TMX_0_1	-0.164	0.469	1.000
	DN71165_c0_g1	TMX_1	-0.490	0.073	0.699
	DN71165_c0_g1	DMSO	-0.231	0.357	1.000
<i>defensin-1</i>	DN58511_c1_g2	CLO_0_3	-0.681	0.040	0.540
	DN58511_c1_g2	CLO_3	-0.541	0.099	0.602
	DN58511_c1_g2	IMD_0_3	-0.908	0.002	0.217
	DN58511_c1_g2	IMD_3	-0.554	0.131	0.439
	DN58511_c1_g2	TMX_0_1	-0.482	0.167	1.000
	DN58511_c1_g2	TMX_1	-0.632	0.134	0.808
	DN58511_c1_g2	DMSO	-0.943	0.037	1.000
<i>cAMP-dependent protein kinase catalytic subunit (pka)</i>	DN64126_c0_g1	CLO_0_3	0.107	0.719	0.942
	DN64126_c0_g1	CLO_3	0.075	0.823	0.970
	DN64126_c0_g1	IMD_0_3	0.050	0.876	0.982
	DN64126_c0_g1	IMD_3	0.095	0.770	0.927
	DN64126_c0_g1	TMX_0_1	0.093	0.762	1.000
	DN64126_c0_g1	TMX_1	0.101	0.754	1.000
	DN64126_c0_g1	DMSO	0.357	0.241	1.000
<i>vitellogenin</i>	DN76398_c0_g1	CLO_0_3	-0.034	0.955	0.997
	DN76398_c0_g1	CLO_3	0.288	0.659	0.923
	DN76398_c0_g1	IMD_0_3	0.016	0.978	1.000
	DN76398_c0_g1	IMD_3	-0.291	0.672	0.884
	DN76398_c0_g1	TMX_0_1	0.513	0.417	1.000
	DN76398_c0_g1	TMX_1	0.252	0.683	1.000
	DN76398_c0_g1	DMSO	1.523	0.016	1.000
<i>60S ribosomal protein L32</i>	DN65230_c0_g2	CLO_0_3	-0.414	0.075	0.600
(housekeeping genes)	DN65230_c0_g2	CLO_3	-0.752	0.021	0.332
	DN65230_c0_g2	IMD_0_3	-0.238	0.297	0.783
	DN65230_c0_g2	IMD_3	-0.479	0.079	0.346
	DN65230_c0_g2	TMX_0_1	-0.059	0.804	1.000
	DN65230_c0_g2	TMX_1	0.158	0.513	1.000
	DN65230_c0_g2	DMSO	-0.486	0.137	1.000

Figures

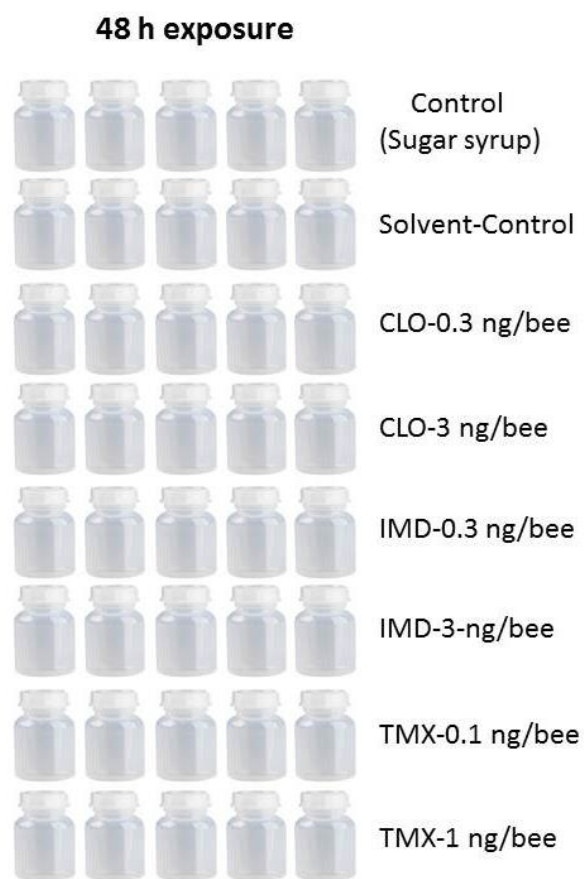


Figure S1: Experimental design of laboratory exposure experiment. Each treatment concentration consists of five bottles with ten bees each. The bees were exposed for 48 h to sucrose solution, solvent control (0.1% DMSO) or the three neonicotinoids dissolved in 0.1% DMSO. Brains of three bees per bottle were pooled to one RNA sample.

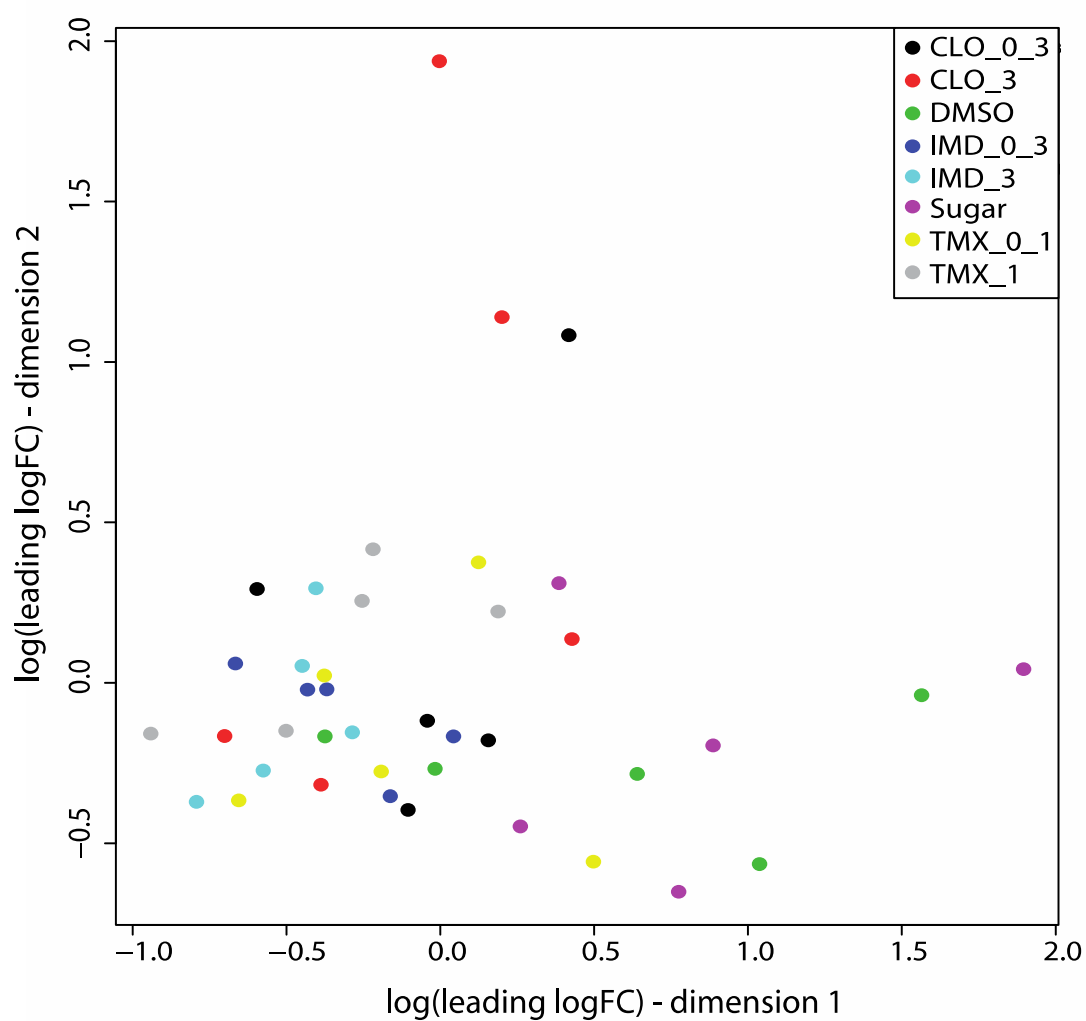


Figure S2: MDS (multidimensional scaling) plot of the normalized expression data showing relationships between the different neonicotinoid treatments, the solvent control (DMSO) and the control (sugar). Distance between samples indicates similarity.

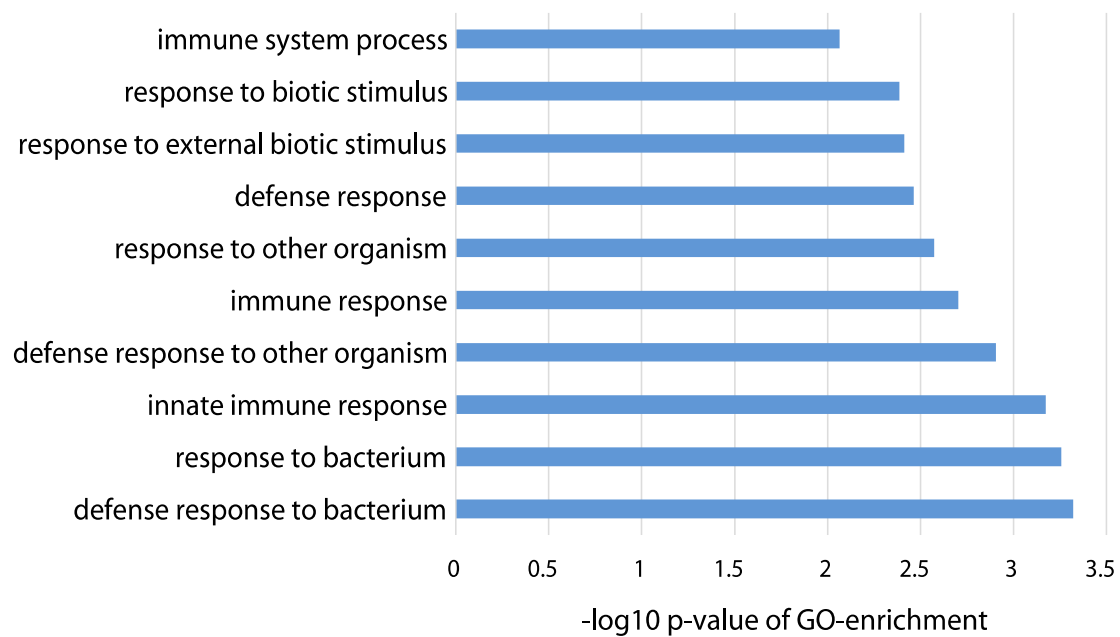
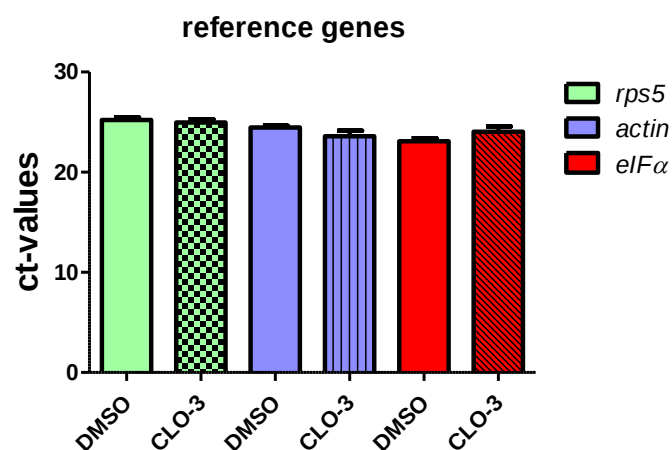


Figure S3: Enriched GO terms of biological processes that were significantly overrepresented before multiple testing (Fisher's exact test; $p < 0.01$) of differentially expressed unigenes of *A. mellifera* treated with the solvent control (0.1% DMSO in sucrose solution).

A:



B:

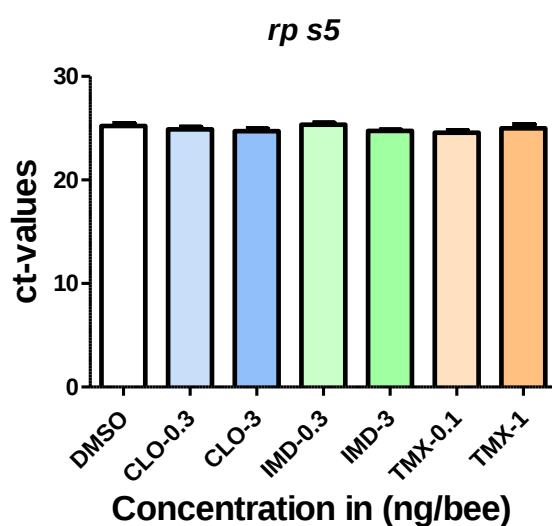


Figure S4: Validation of reference genes for quantification of gene expression in the brain of honey bees. (A) Expression of *rps5* (green), *actin* (blue) and *eIFα* (red) in the brain of bees exposed to 3 ng/bee of clothianidin or solvent control (0.1% DMSO). (B) Expression of *rps5* in the brain of bees exposed to solvent control (0.1% DMSO) (white), clothianidin (blue), imidacloprid (green) and thiamethoxam (orange). The values are given as cycle threshold values (Ct). Each bar represents the mean of three replicates with error bars indicating standard deviation.

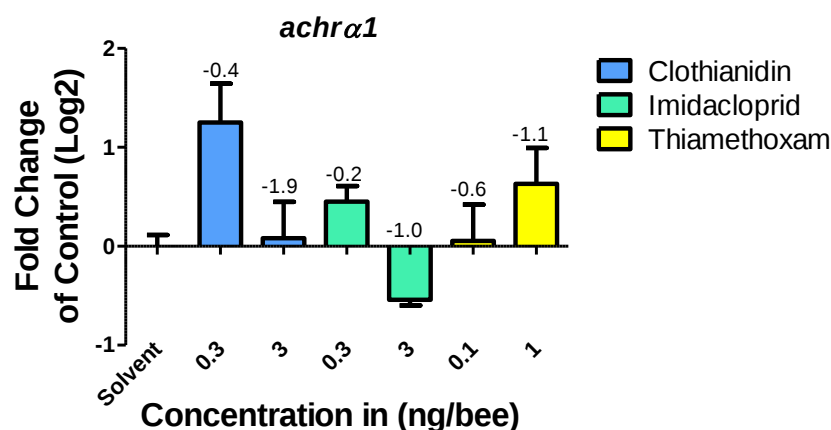


Figure S5: Abundance of transcript *achrα1* in the brain of honey bees following exposure to different concentrations of clothianidin (blue bars), imidacloprid (green bars) and thiamethoxam (yellow bars) for 48 h. Values above bars represent log₂ (fold change) of RNA sequencing. Shown are the results of five biological replicates per concentration.

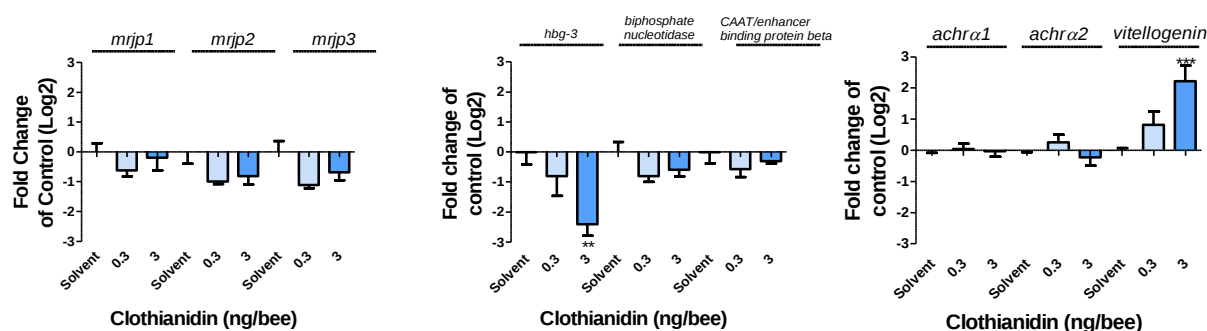


Figure S6: Abundance of transcripts *mrjp1*, *mrjp2*, *mrjp3*, *hbg-3*, *biphosphate-nucleotidase*, *CAAT/enhancer binding protein beta*, *achrα1*, *achrα2* and *vitellogenin* in the brain of honey bees following exposure to 0.3 (light blue bars) and 3 (dark blue bars) ng/bee clothianidin for 24 h. Shown are the results of five biological replicates per concentration. Significant differences with p-value of ≤ 0.05 are marked with asterisks.

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