

The synergistic effect of thiamethoxam and synapsin dsRNA targets  
neurotransmission to induce mortality in *Aphis gossypii*

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## Supplementary Materials

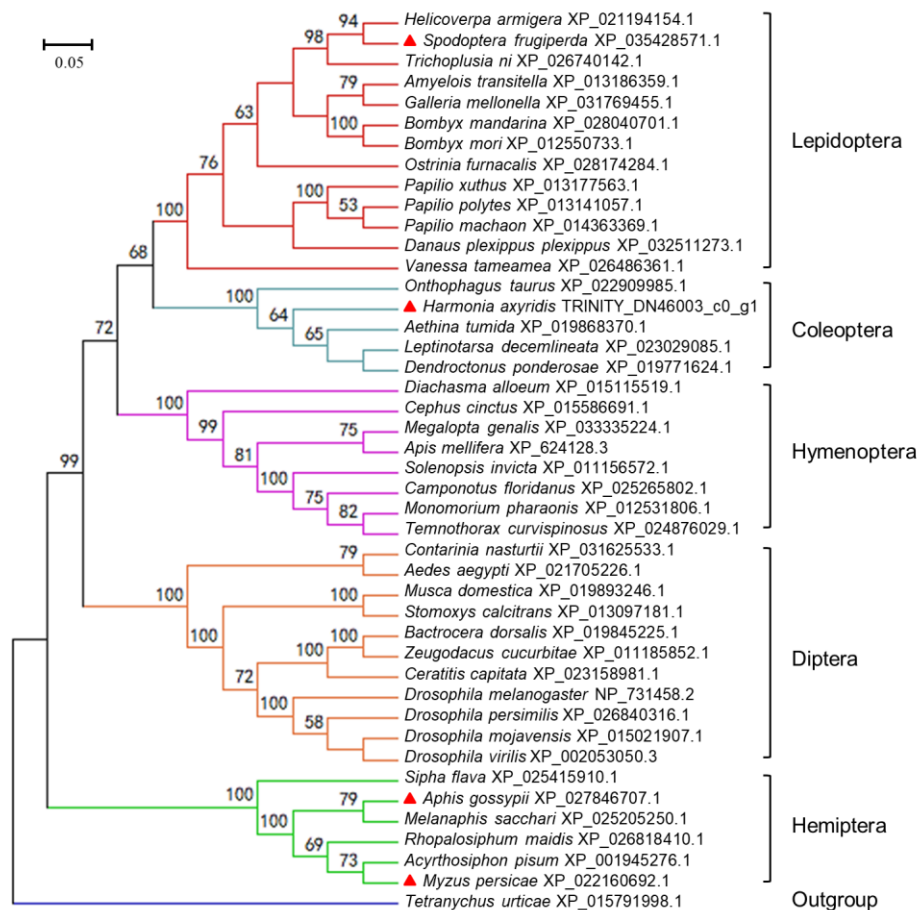


Figure S1. Phylogenetic analyses of *synapsin* in diverse insect species. Based on coding sequence alignment of synapsins from diverse insects, an unrooted NJ tree was generated. GenBank accession numbers and gene IDs from the transcriptome database were indicated.

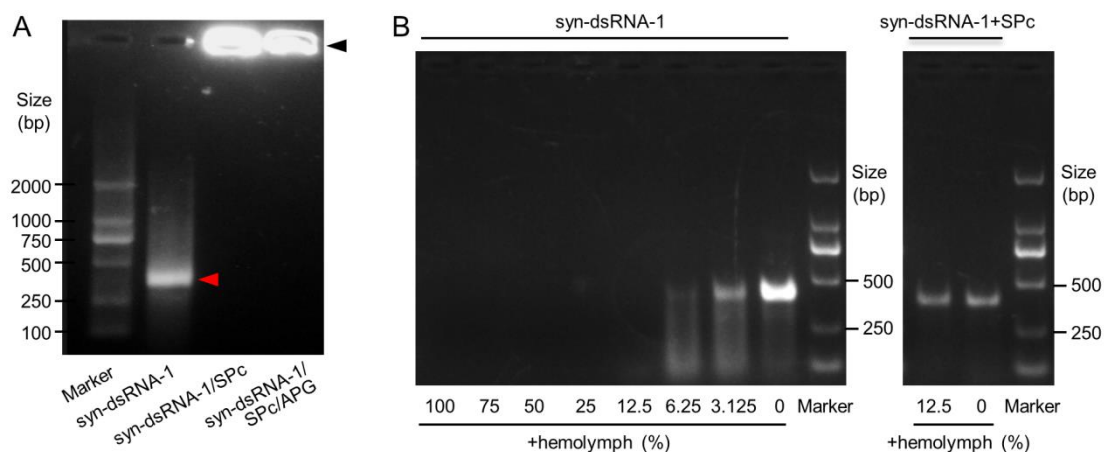


Figure S2. SPc protected syn-dsRNA from degradation. (A) The electrophoretic result showed the binding of syn-dsRNA to the nanocarrier SPc. The 313 bp syn-dsRNA-1 moved in the agarose gel (red arrowhead) during electrophoresis. After mixed with the nanocarrier SPc, dsRNA was stuck in the agarose gel (black arrowhead, lane 3) because of the neutralization of charges by SPc. The non-ionic surfactant APG did not affect the binding of syn-dsRNA-1 and SPc (black arrowhead, lane 4). (B) SPc protected dsRNA from degradation by aphid hemolymphs. Without SPc, syn-dsRNA was gradually degraded by high concentrations of aphid hemolymphs (left panel). In 12.5% and more hemolymphs, syn-dsRNA was completely degraded. By adding SPc (+SPc) with 1:1 mass ratio, syn-dsRNA was not, or mostly not degraded by 12.5% hemolymphs (right panel), indicating a protection of dsRNA by SPc.

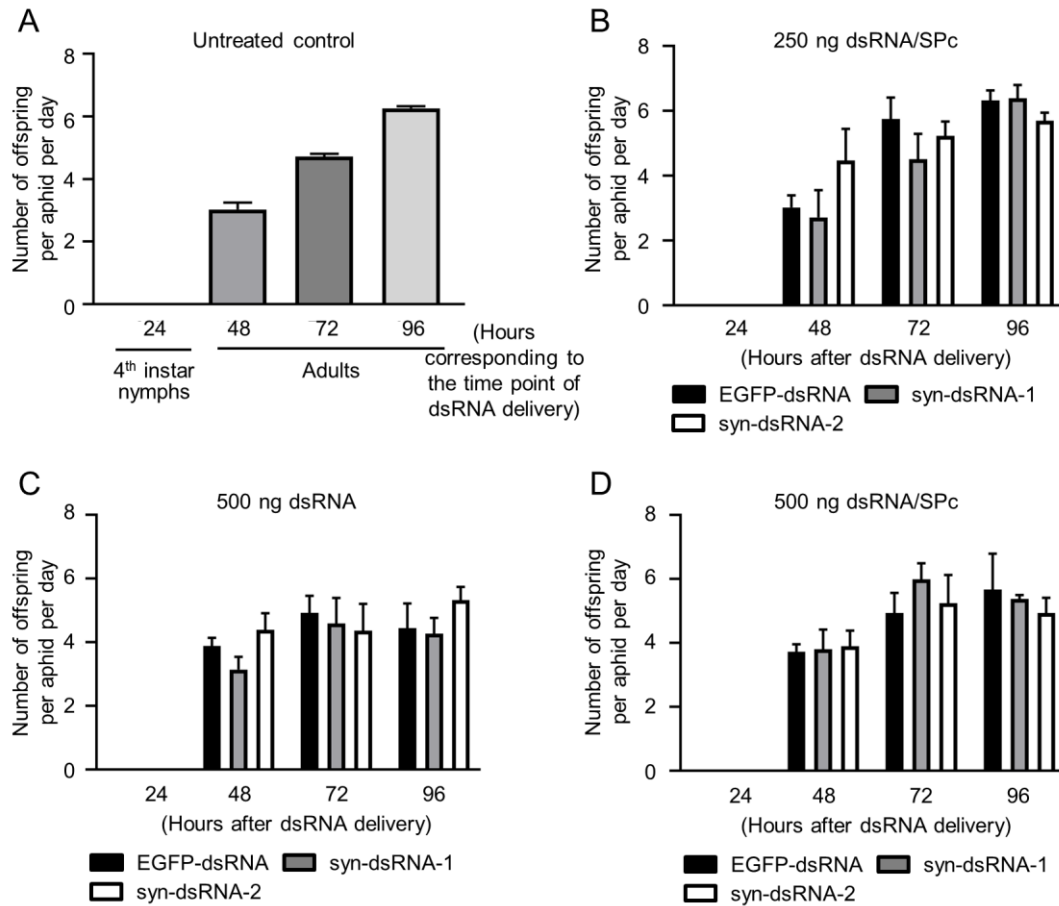


Figure S3. The offspring of survivors after syn-dsRNA delivery were comparable to that of untreated control and EGFP-dsRNA control. (A) Each adult aphid laid 3 to 6 nymphs per day. (B) The survivors treated by 250 ng syn-dsRNA showed normal number of offspring. (C, D) The survivors treated by 500 ng syn-dsRNA without (C) or with SPc (D) showed normal fertility.

Table S1. Volcano plot data for energy production related genes.

Table S2. Volcano plot data for neural function related genes.

Table S3. The primer sequences for qPCR.

Gene	Primer name	Primer sequence (5'-3')
<i>beta-actin</i>	act-F	GGGAGTCATGGTTGGTATGG
	act-R	TCCATATCGTCCCAGTTGGT
<i>EGFP</i>	EGFP-F	CCTAGTCCCCACAAACGAGT

	EGFP-R	CAAGCCGTCGAACATACTGG
<i>synapsin</i>	syn-F	GCAGGTTTCAGGTATCACCCA
	syn-R	TAGATTTCGCTTTTCGCCGTT
<i>Epidermal growth factor receptor</i>	EPS15-F	TGGTCGTATTTTGTGCGCGTC
<i>substrate 15-like 1 (Eps15-like 1)</i>	EPS15-R	AAGTAGCCATCCTGAACGC
<i>Intersectin-1/ Dynamin associated</i>	Dap160-F	GTCACATTACGACTTTGCCTTT
<i>protein 160 (Dap160)</i>	Dap160-R	R:CGGTGTTCTACCCCTGCTTA
<i>Acetylcholinesterase</i>	Ace-F	CTGCAGAAATGCACGTTGGT
	Ace-R	TCTTTCAAGGCTTGGGCGAT
<i>Neurologin-1</i>	Nlg1-F	TCCGAAAGTATCCCGTGCTG
	Nlg1-R	GCGTGACGACAACCAATTCC
<i>dynamin</i>	dyn-F	ACCTGAGCAAGGACGACAAC
	dyn-R	TGTACACGCTGCGGTATTTG
<i>stoned-B</i>	stnB-F	GCCTTCACACGAAGCCAAAG
	stnB-R	CGCATTGGAGTAGGACTGGG
<i>Synaptotagmin</i>	Syt-F	AAGTCAATCTGGTGGTGACGG
	Syt-R	TGTCGGACCAGTGCCGTAAT
<i>Endophilin-A</i>	EndoA-F	ACGTCTGGAGCAAACCATCA
	EndoA-R	GTTGCGTGGTATTTGCCGT
<i>octopamine receptor beta-2R</i>	oct-F	TCAAGCCGCTCAAGTATCC
	oct-R	CCGTAGAAGATGGGCACGAA
<i>Acetylcholine receptor subunit</i>	AChR $\alpha$ -F	GACAGACACTGCACACGAGA
<i>alpha-like 1 (AChR<math>\alpha</math>-like 1)</i>	AChR $\alpha$ -R	TTGTATGGTTTCGTTCCGGCA
<i>V-Type proton ATPase 21 kDa</i>	V-Type-F	GGTTGGAGCCGCTTTAGGAA
<i>proteolipid subunit</i>	V-Type-R	GCTTCGCAGAAGATGACGGA

Table S4. The primer sequences for amplification of dsRNA.

Gene	Primer name	Primer sequence (5'-3')
<i>EGFP</i>	T7-EGFP-F	<u>TAATACGACTCACTATAGGG</u> CACAAGTTCAGCGTGTCGG
	T7-EGFP-R	<u>TAATACGACTCACTATAGGG</u> GTTACCTTGATGCCGTTT
<i>synapsin</i>	T7-syn-1-F	<u>TAATACGACTCACTATAGGG</u> CGGCGATGTTGGAAGAAACC
	T7-syn-1-R	<u>TAATACGACTCACTATAGGG</u> CGTCTAGCCGGAATAGCTGG
<i>synapsin</i>	T7-syn-2-F	<u>TAATACGACTCACTATAGGG</u> GCAAACGGTTCTTTGGCGAT
	T7-syn-2-R	<u>TAATACGACTCACTATAGGG</u> CCCGACTTTGAGGACCACTG

Note: The underlined sequences were T7 promoter sequences.

Table S5. Toxicity of thiamethoxam to adults of *A. gossypii* at 48 h.

<b>Insecticide</b>	<b>Regression equation (y=)</b>	<b>LC50 (mg/L)</b>	<b>95% confidence interval</b>	<b>Correlation coefficient</b>
Thiamethoxam	0.439X+0.1655	5.249	3.771-7.296	0.9452

Video S1. Behavior of the EGFP-dsRNA treated melon aphid.

Video S2. Behavior of the syn-dsRNA treated melon aphid.