Supporting Information Appendix For

Modulation of behavioral phase changes in the migratory locust by the catecholamine metabolic pathway

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Summary

Supporting Methods

RNA preparation

In order to eliminate the heterogeneity of different samples, total RNA was extracted from head tissues that molts after 2-3 days following the protocol of RNAeasy mini kit (Qiagen).

Isolation of gregarious nymphs

The fourth-stadium gregarious nymphs on the second day after ecdysis were separately reared in the solitary rearing cages with food. After 0, 1, 4, 8, 16, 32 hours of treatments, head tissues of nymphs were dissected and put into the liquid nitrogen immediately for qPCR experiments.

Crowding of solitary nymphs

Two fourth-stadium solitary locusts were introduced into the solitary rearing cage which contains 20 gregarious nymphs of the same developmental stage with food. Then, after stayed with the stimulus group for 0, 1, 4, 8, 16, 32 hours, head tissues of nymphs were dissected and put into the liquid nitrogen immediately for qPCR experiments.

Quantification of dopamine and serotonin in brain tissue extracts

Dopamine and serotonin in the extracts of 10 brains (without optic lobes) were quantified with reverse-phase high performance liquid chromatography (HPLC) and mass spectrometry (MS) analysis. The brain tissues of the fourth-stadium gregarious and solitary nymphs were immediately dissected and stored in liquid nitrogen. 15 brains were homogenized using mortals and pestle that were pre-colded by liquid nitrogen, transferred to 1.5ml Eppendorf tubes, and lyzed in 200μl ice-cold 0.1 M perchloric acid on ice for 10min. The homogenate were centrifuged at 14000g for 10min at 4℃. The supernatant were filtered by 0.45 um filter, transferred to a second Eppendorf tube and stored at -80°C until MS analysis. 20μl supernatant were automatically loaded into Inlet Systems (Agilent Technologies 1200 Series RRLC) with a C18 reversed phase column (Eclipse Plus C18, 3.0 × 100 mm, 1.8 μm). An Agilent 6460 mass selective detector with an Agilent Jet Stream (AJS) (G1958-65138)

ion source (Agilent Technologies, Palo Alto, CA, USA) was used. Instrumental settings for the Agilent6460 mass selective detector included: gas temperature:350℃, gas flow:4l/min, sheath gas temperature: 350, sheath gas flow: 12 l/min, nebulizer pressure: 344.8 kPa, capillary voltage: 3500V, nozzle voltage: 0V in negative mode and 500V in positive mode. Nitrogen gas was used as the drying and collision gas for all LC–MS/MS instruments. Data analysis was performed using Agilent Mass Hunter B.02.01 software. Dopamine and serotonin were quantified by reference to external standards.

Pharmacological injection

1) AMPT injection

The α-methyl-DL-tyrosine methyl ester hydrochloride (AMPT; Sigma-Aldrich), a competitive inhibitor of tyrosine hydroxylase, was previously used to deplete dopamine stores in insects (1). AMPT solution (80μg/μl) was directly injected into the thoracic hemocoels of gregarious nymphs via the abdomen by using a microsyringe ventrally between the second and third abdominal segments, with syringe needle' s tip in the thoracic hemocoel. We injected the volume 2μl to the third stadium and 4μl to the fourth stadium of gregarious nymphs successively at 48h intervals for 3 consecutive times. Control group received the same volume of ringer's solution as that of AMPT solution. Then 20 gregarious nymphs were put into the plastic box and cultured together (10cm×10cm×10cm).

2) Injection of reserpine

Reserpine, a specific inhibitor of vesicle amine transporter in synapse (2) was dissolved in dimethysulfoxide (DMSO; Sigma-Aldrich) to give concentration of 80μg/μl. 1μl of 80μg/μl reserpine or DMSO control was injected into thoracic hemocoels (same as AMPT injection) of the fourth-stadium gregarious nymphs. Then, 20 gregarious animals were returned into the plastic box (10cm×10cm×10cm) and allowed to recover for 48h before behavioral assay.

3) Injections of dopamine receptor antagonists

Four different dopamine receptor antagonists, chlorpromazine (CPZ), SCH23390, raclopride and apokyn hydrate (Sigma-Aldrich), were adiministered individually to

monitor their effects on the behavior of gregarious nymphs. Chlorpromazine can cover all pharmacological types of dopamine receptor (3). SCH23390 is the selective antagonist of D1 dopamine receptor with minimal effects on D2 receptor. It can successfully block the action of dopamine receptor in several insects including locusts (4). Raclopride acts as an antagonist on D2 dopamine receptors in invertebrates (3). Apokyn hydrate is the non-selective dopamine receptor antagonist and widely used in vertebrates (5). 60ug of chlorpromazine (20μg/μl), 80μg of SCH23390 (20μg/μl), 80μg of raclopride (10μg/μl) and 80ug of apokyn hydrate (10μg/μl) were injected into thorax hemocoels of gregarious nymphs，respectively. Control group received the same volume of ringer's solution as that of antagonist solution. Then gregarious nymphs stayed in the rearing cage for 1h before behavioral assay.

4) Dopamine injection

Dopamine hydrochloride (dopamine hydrocloride, Sigma-Aldrich) was dissolved in ringer's solution. 2μl of 80μg/μl solution was injected into thorax hemocoels of solitary nymphs. Then, animals stayed for 1h in their rearing cages alone before behavior assay. Control animals received the corresponding volume of ringer's solution.

5) Injections of dopamine receptor agonists

S (+)-apomorphine was chosen to activate the action of dopamine receptor and investigate its roles in the behavior of solitary nymphs. S (+)-apomorphine is a non selective dopamine receptor agonist with high affinity for invertebrate dopamine receptor (6). 2μl apomorphine (10μg/μl) was injected into thorax hemocoels of the fourth-stadium solitary nymphs. Control animals received the same volume of ringer's solution. Then animals stayed for 1h in their rearing cages alone or 1h of crowding with 30 gregarious nymphs in cages (10cm×10cm×10cm) before behavior assay.

Long-term injection was also conducted to examine the chronic effects of apomorphine on the behavior of solitary nymphs. We gave 1 μl apomorphine (10ug/ul) to the third-stadium solitary animals and 2 μl to the fourth-stadium nymphs at 48h time intervals. Control animals received the same volume of ringer's solution. The

injected nymphs then live in crowding with 30 nymphs in cages (10cm×10cm×10cm) before behavior assay. Behavior assay was performed 24 h after the last injection.

6) AMTP injection

Systematic injection of α-methyltryptophan(AMTP, Sigma-Aldrich), a competitive inhibitor of 5HT synthesis, was previously used to deplete 5HT stores in the desert locust (7). AMTP solution (80μg/μl) was directly injected into thoracic hemocoels of gregarious nymphs via the abdomen by using a microsyringe ventrally between the second and third abdominal segments, with syringe needle's tip in thoracic hemocoels. We injected 2μl to the third-stadium gregarious nymphs and 4μl to the fourth-stadium nymphs successively at 48h intervals for 3 consecutive times. Control group received the same volume of ringer's solution as that of AMTP. Then, 20 gregarious nymphs were put into the plastic box and cultured together in cages (10cm×10cm×10cm).

7) Injections of serotonin receptor antagonists

Two different selective serotonin receptor antagonists, ketanserin and methiothpin (Sigma-Aldrich), were used to cover the range of possible pharmacological types of serotonin receptor and investigate their effects on the behavior of gregarious nymphs. Ketanserin and methiothpin are the selective antagonists of serotonin receptor (7). The antagonist solution contained 20 μg/μl of ketanserin and 20μg/μl of methiothpin in ringer's solution. 2μl of antagonist solution containing 20 μg/μl of ketanserin and 20μg/μl of methiothpin was injected into gregarious nymphs. Then, nymphs were reared in cages for 1h before behavior assay.

8) Injection of serotonin

The fourth-stadium solitary nymphs were injected with 160ug serotonin (40μg/μl) or ringer saline control. Then, animals were reared alone in cages for 1h before their behavior was assayed.

9) Injections of serotonin receptor agonist

5HT receptor agonist cocktail containing α-methylserotonin and 5-carboxamidotryptamine (5CT) that were successfully used in the desert locust (7) was used to activate the serotonergic pathways. Solitary nymphs were injected with

4μl of α-methylserotonin (5μg/μl) and 5CT (5μg/μl) using the method as described above. Control insect received the same volume of ringer's solution. Then animals were reared alone in cages for 1h recovery before their behavior was assayed. Control animals received the same volume of control solution.

10) Injection of *5*-HTP

5-hydroxytryptophan (5-HTP), an intermediate product in serotonin metabolic pathway, is the rate-limiting factor for serotonin synthesis (7). Solitary nymphs were injected with 100μg 5-HTP (10μg/μl) or ringer's saline control, and animals were exposed 1h of crowding with 30 gregarious nymphs in cages (10cm×10cm×10cm) before behavior assay.

Long-term injection was also conducted to investigate the chronic impacts of 5-HTP on the behavior of solitary nymphs. We gave 5μl 5-HTP (10μg/μl) to the third-stadium solitary nymph and 10μl to the fourth-stadium nymphs at 48h intervals. Control animals received the same volume of ringer's solution. The injected nymphs then live in crowding with 30 nymphs in the cages (10cm×10cm×10cm) before behavior assay. Behavior assay was performed 24 h after the last injection.

RNA interference (RNAi)

Primers were fused with T7 promoter sequence (underlined). We used sense primer 5′GGATCCTAATACGACTCACTATAGGTTCGCCATCAAGAAGTCCTAC3' and antisense primer 5′GGATCCTAATACGACTCACTATAGG TGAGGTGGTTGCA GTTGTCC 3' for *pale* clone and dsRNA preparation. For *ebony* clone and dsRNA preparation, the sense primer is 5′ GGATCCTAATACGACTCACTATAGG GATTATTCTCGCCTCTACCG 3' and the anti-sense primer is 5′GGATCCTAATA CGACTCACTATAGGTCAACTTGGCATTTACAGGA3'.The amplification product of *ebony* is 513bp. For clone and dsRNA preparation of vesicle monoamine transporter1 (*vat1*), the sense primer is 5′GATCCTAATACGACTCACTATAGGAT GGCAGTTTCAGCAAGA 3' and the antisense primer is 5′ GGATCCTAATACGAC TCACTATAGGCTCCACCTCCGATTGACT3'. Resulting products of *pale*, *ebony* and *vat1* excluding the fused T7 promoter is 733bp, 513bp and 432bp respectively. Green fluorescence protein (GFP) was used as RNAi control. The sense primer 5'

GATCCTAATACGACTCACTATAGGCACAAGTTCAGCGTGTCCG3'and antisense primer 5' GGATCCTAATACGACTCACTATAGGGTTCACCTTGATGC CGTTC 3' were used for GFP dsRNA preparation. The final product of GFP excluding the fused T7 promoter is 420bp. The sense primer and antisense primer of *henna* for RNAi are 5' GGATCCTAATACGACTCACTATAGG ACTCGCCAAAT GCCTCAA3' and 5'GGATCCTAATACGACTCACTATAGGACACGGAAAGCCA ACCCT 3', respectively, and the length of the amplified product is 534bp. The sense primer and antisense primer of *dopamine receptor D1* for RNAi are 5'GGATCCTAATACGACTCACTATAGGCTCCGTGGGTAAATGAAGACT3'and 5'GGATCCTAATACGACTCACTATAGGATAACACTCACACTTGCCGAT3', the length of the amplified product is 785bp. PCR products were purified using QIAquick PCR purification kit (Qiagen, Valencia, United States). RNA was prepared using the Promega RiboMax T7 system (Promega, Wisconsin, United States) and annealed by incubation at 70°C for 10 min followed by slow cooling to room temperature. All RNA products were extracted with phenol/chloroform and precipitated with ethanol. The integrity of dsRNA was confirmed using 1% agarose gels and the dsRNA products were diluted with nuclease-free Ringer solution to the final concentration of 6μg/μl. Needles were baked to remove RNase. 2μl dsRNA of target gene or GFP control was injected into the thoracic hemocoels of the third-stadium gregarious nymphs at 48h intervals for 3 consecutive times. dsRNA-injected nymphs were raised in crowding form with the population size of 20 in cages (10cm×10cm×10cm). Behavior and body color were analyzed in the fourth stadium. The efficiency of RNAi in target genes was validated by quantitative PCR.

References

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7. Anstey ML, Rogers SM, Ott SR, Burrows M, Simpson SJ (2009) Serotonin mediates behavioral gregarization underlying swarm formation in desert locusts. *Science* 323:627-630.

Fig.S1

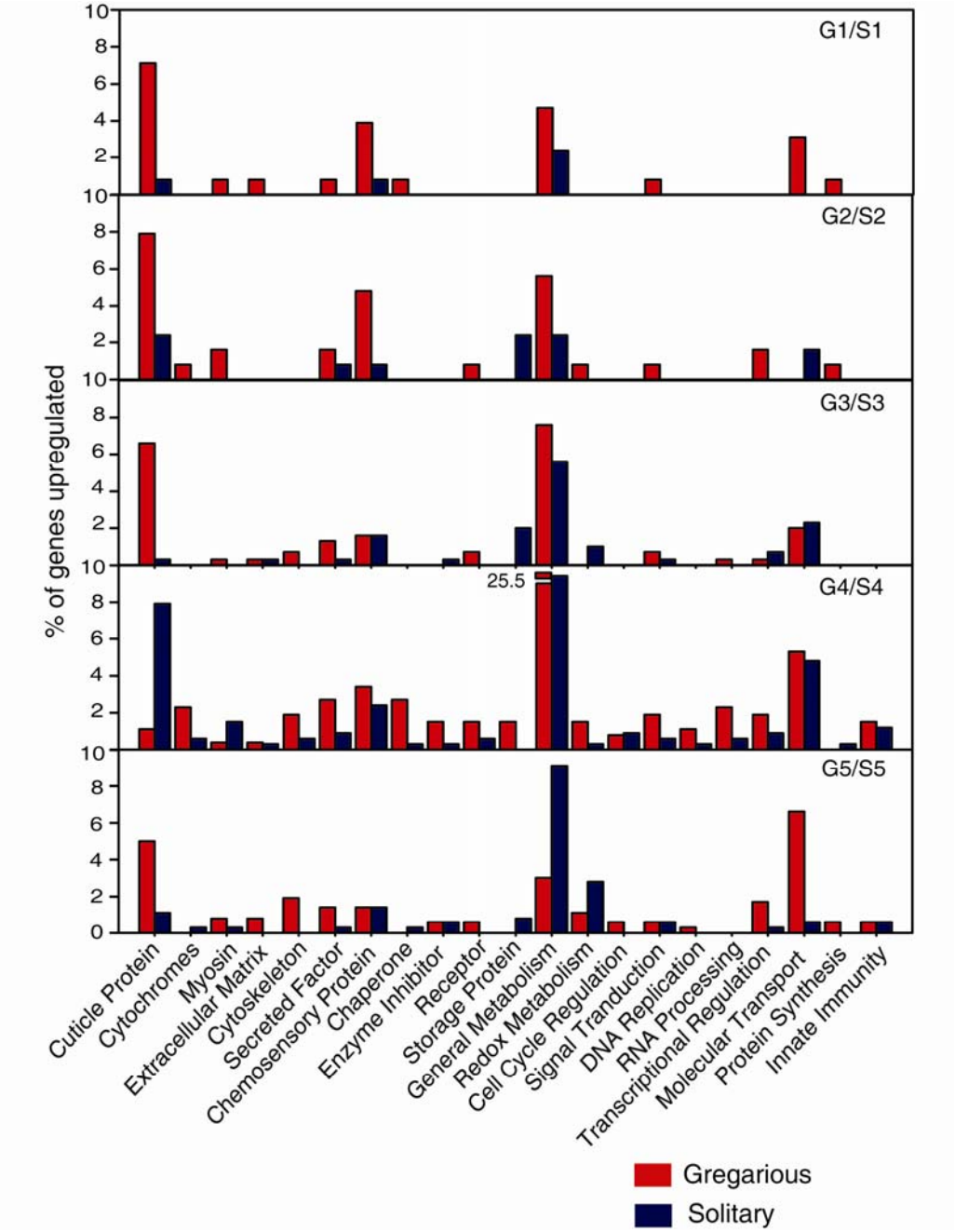

Fig.S1. Functional classification of differentially expressed genes in pair wised comparison between two phases of locust nymphs. The classification is based on gene ontology data and a homology search using BLAST data in NCBI and functional domains in InterPro. S, solitary phase. G, gregarious phase. Numbers 1-5, larval stadia.

Fig.S2

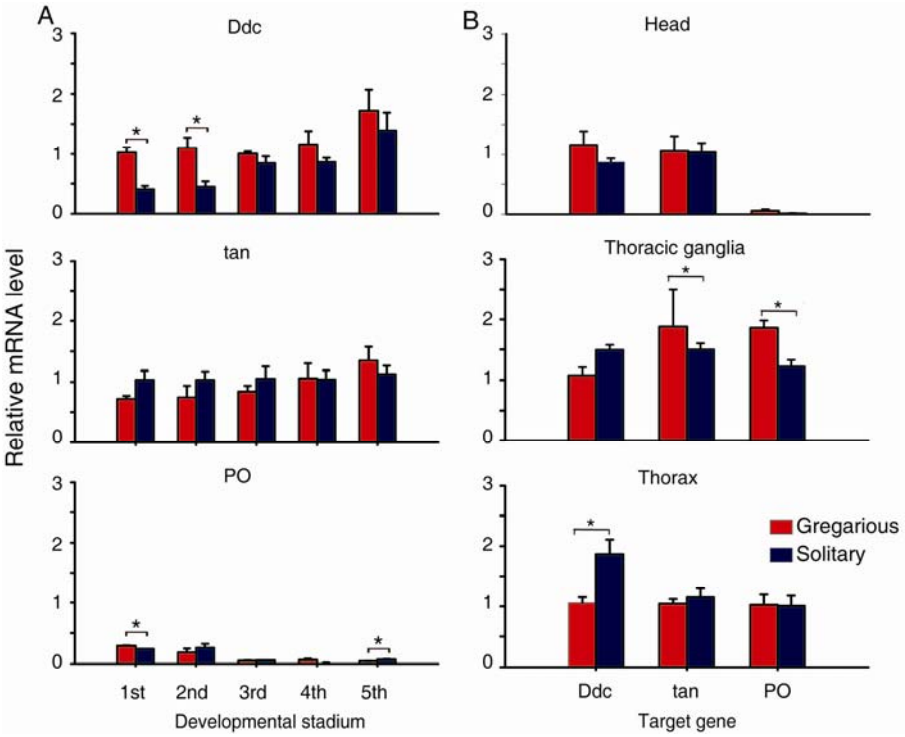

Fig.S2. The temporal and spatial expression of genes involved in dopamine metabolism analyzed by qPCR. (*A*) The temporal and spatial expression of genes in the head part of solitary and gregarious nymphs analyzed by qPCR. (*B*) The spatial expression of genes involved in dopamine metabolism in the head, thorax and thoracic ganglia of the fourth-stadium solitary and gregarious nymphs analyzed by qPCR. Asterisk indicates statistically significant change (mean + SE) (*$p < 0.05$, Student's t-test). Abbreviations: Ddc, Dopa decarboxylase; PO, Phenoloxidase.

Fig.S3

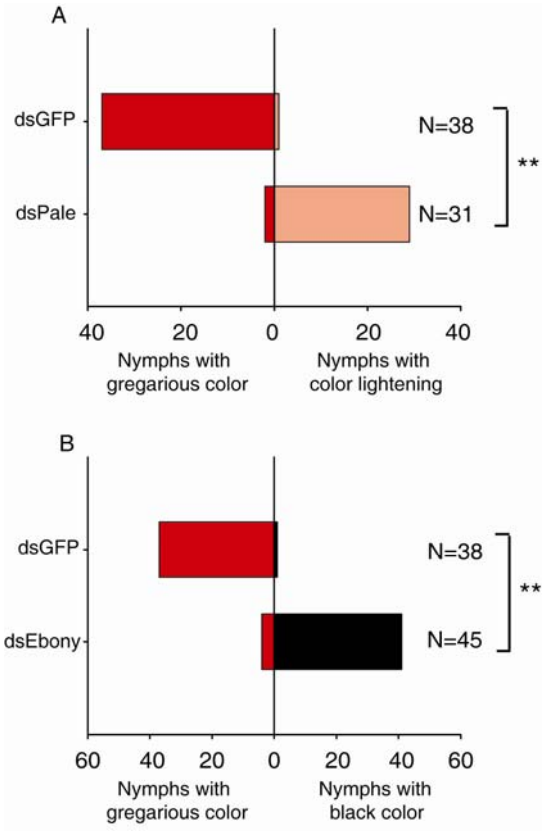

Fig.S3. The number of nymphs showing color changes after dsRNA injection. Gregarious nymphs show lightening (A) and black (B) color changes after being injected with dsPale (A) and dsEbony (B), respectively (*Mann-Whitney U test*, $p<0.001$, relative to dsGFP-injected groups).

Fig.S4

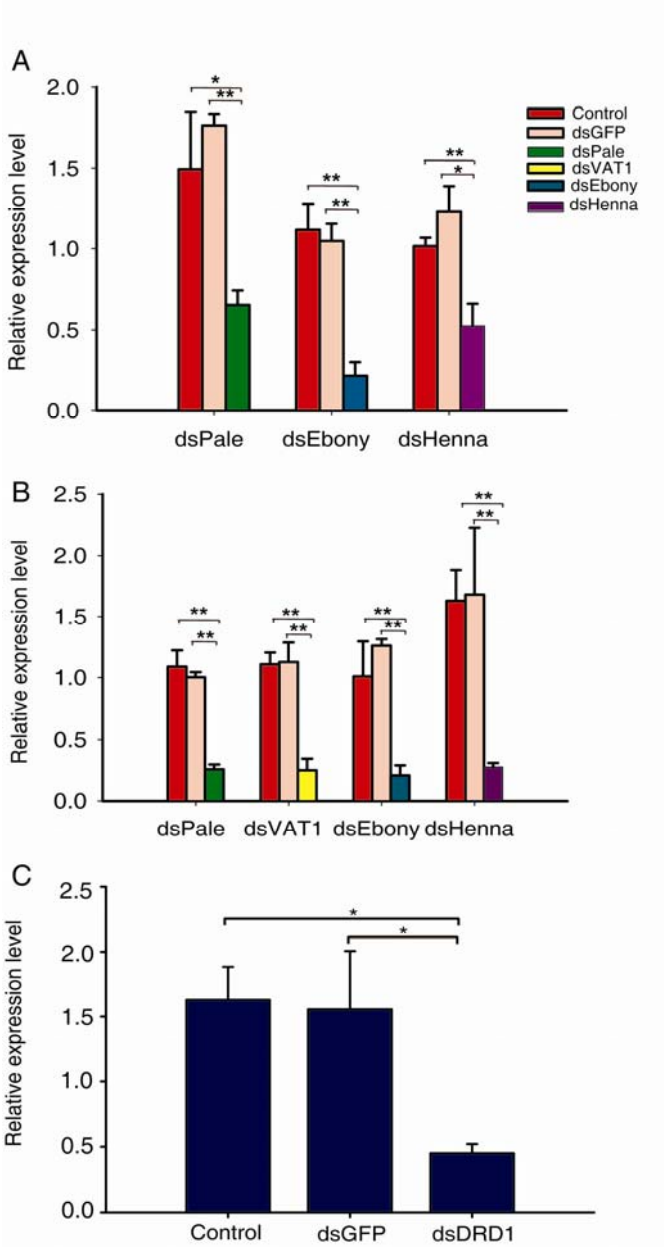

Fig.S4. The injection of dsRNA reduces expression level of target genes in catecholamine metabolic pathway in the fourth-stadium gregarious nymphs.

(*A*) *pale, ebony* and *henna* reduce the expression levels in the thorax part. (*B*) *pale, ebony*, *henna, VAT1*, and (*C*) *DRD1* reduced the expression levels in the head part. Asterisks show statistically significant differences between means (mean + SE) *(*$p < 0.05$, **$p < 0.01$, Student's t-test). Abbreviations: RNAi, RNA interference; dsRNA, double-stranded RNA; *VAT1*, vesicle amine transporter 1; *DRD1*, dopamine receptor D1.

Fig.S5

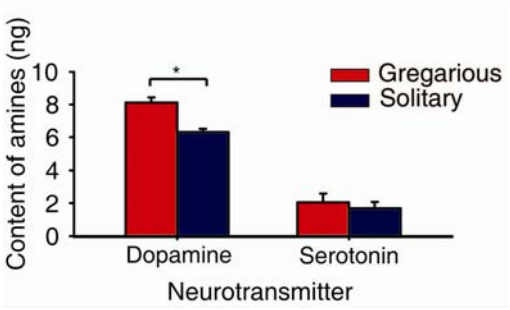

Fig.S5. The amount of dopamine and serotonin in the brain tissues of the fourth-stadium solitary or gregarious nymphs. Asterisk shows statistically significant differences between means (mean + SE) ($p < 0.05$, Student's t-test).

Fig.S6

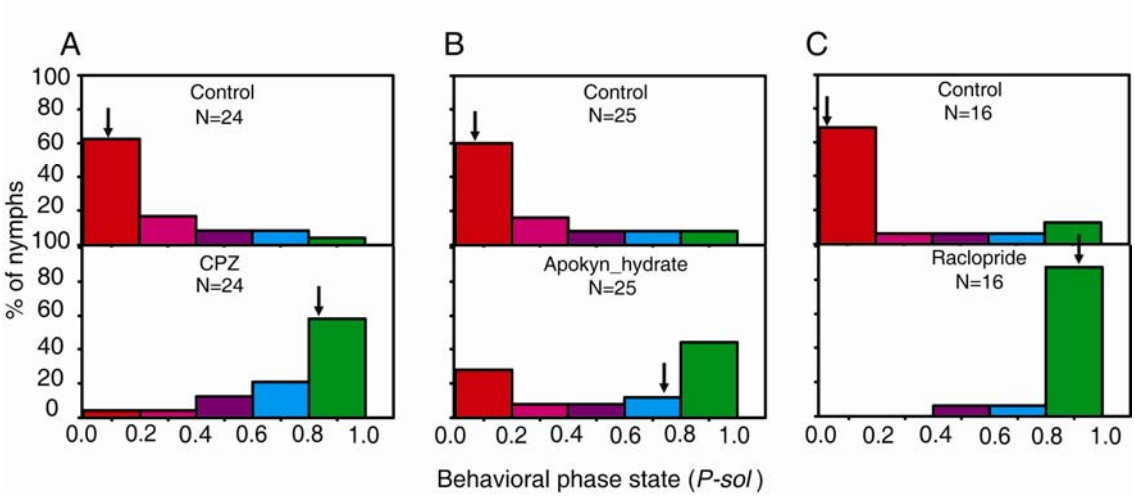

Fig.S6. Drug interferences in dopamine and serotonin signaling induce the behavioral solitarization in gregarious nymphs. The fourth-stadium gregarious nymphs injected with antagonists of dopamine receptor (*A*) CPZ (60μg), (*B*) raclopride (80μg) and (*C*) apokyn hydrate (80μg) at a 1h interval show behavioral solitarization. Saline controls are shown in the upper column (*Mann-Whitney U test*, $p<0.01$, relative to control groups). The arrows denote median *P-sol* values of the population. Abbreviation: CPZ, Chlorpromazine.

Fig. S7

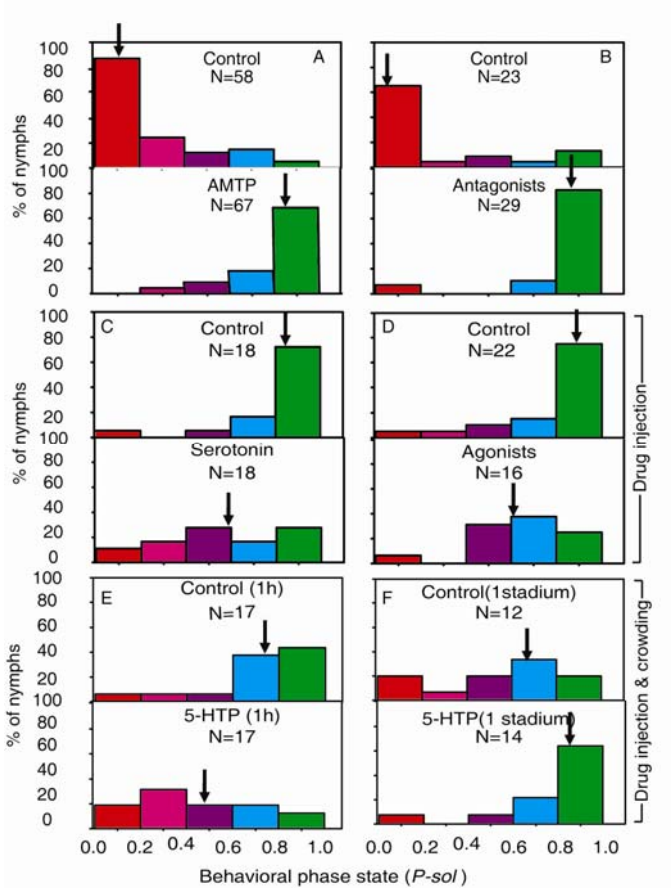

Fig.S7. Behavioral responses of gregarious and solitary nymphs to serotonin biosynthesis and receptor signaling. (*A*) The third-stadium gregarious nymphs injected with AMTP (80μg/μl), an inhibitor of serotonin synthesis, exhibit behavioral solitarization. (*B*) Behavior of fourth-stadium gregarious nymphs injected with the mixture of antagonists (ketanserin (40μg) and methiothepin (40μg)) at a 1h interval. Direct injection of (C) serotonin (80μg/μl) or (*D*) a mixture of serotonin receptor agonists (α-methylserotonin, 5μg/μl and 5-carboxmidotryptamine, 5μg/μl) also partially induced gregarization of solitary nymphs. (*E*) The fourth-stadium solitary nymphs injected with 5-HTP (100μg/μl) and exposed to 1h of crowding showed gregarious behavior. (*F*) The third-stadium solitary nymphs injected with 5-HTP (100μg/μl) and exposed to one stadium of crowding. All statistical analyses were conducted with *Mann-Whitney U test* relative to control groups ($p<0.05$).

Table S1. Behavioral variables in the best-fit logistic regression model obtained from five stadia of locust nymphs. This table indicates significance levels of the individual parameter for the behavioral discrimination of the locust nymphs in corresponding stadia. Attraction index (AI, AI stands for the extent of the tested animal attracted by stimulus group. Total duration in stimulus area, in the opposite of the stimulus area and in the middle area was weighted by 1, -1 and 0, respectively. AI = 1 × total duration in stimulus area + (-1) × total duration in the opposite of stimulus area + 0 × total duration in middle area). Abbreviations: AI, attraction index; EFISA, entry frequency in the stimulus area; FOM, frequency of movement; TDCW, total duration in area close to the wall; TDMV, total duration of movement; TDM, total distance moved; G, gregarious phase; S, solitary phase.

	Number of Nymphs for Behavioral Model		Model							Model if Term Removed		
Stadium	**G**	**S**	**Parameters in equation**	**β**	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(β)**	**Model Log Likelihood**	**Change in -2 Log Likelihood**	**Sig. of the Change**
1st	60	60	TDM	0.027	0.009	0.459	1	0.002	1.027	-74.201	10.743	0.001
			FOM	-0.316	0.095	11.065	1	0.001	0.729	-101.234	64.808	<0.000
			Constant	-0.928	0.451	4.240	1	0.039	0.395			
2nd	60	60	TDMV	-0.052	0.013	14.794	1	<0.000	0.950	-73.949	35.077	<0.000
			TDCW	-0.008	0.003	8.774	1	0.003	0.992	-61.483	10.145	0.001
			Constant	1.415	0.318	19.804	1	<0.000	4.118			
3rd	66	60	FOM	-0.099	0.020	25.068	1	<0.000	0.905	-89.558	12.010	<0.000
			TDCW	0.007	0.003	5.226	1	0.022	1.007	56.749	6.363	0.011
			EFISA	0.208	0.083	6.303	1	0.012	1.232	56.410	5.713	0.017
			Constant	1.192	0.339	12.391	1	<0.000	3.295			
4th	100	100	TDM	-0.016	0.005	11.319	1	0.001	0.984	-68.549	13.431	<0.000
			TDMV	-0.014	0.006	4.561	1	0.033	0.986	-64.458	5.248	0.022
			AI	-0.005	0.001	12.162	1	<0.000	0.995	-69.114	14.560	<0.000
			Constant	2.428	0.377	41.393	1	<0.000	11.332			
5th	100	100	TDMV	-0.077	0.023	11.041	1	0.001	0.926	-62.67	33.026	<0.000
			TDCW	-0.012	0.004	8.584	1	0.003	0.988	-51.208	10.043	0.002
			AI	-0.008	0.002	18.881	1	<0.000	0.992	-60.244	28.115	<0.000
			Constant	2.731	0.477	32.747	1	<0.000	15.347			

Table S2. The amount of differentially expressed genes in the pare-wised comparison between solitary and gregarious phase**.** S, solitary phase. G, gregarious phase. Numbers 1-5, larval stadia.

Pare wise comparison	Up regulated (gregarious)	Up regulated (solitary)	Total
G1/S1	111	16	127
G2/S2	103	23	126
G3/S3	165	139	304
G4/S4	331	263	594
G5/S5	106	259	365

Table S3. The upregulated genes in general metabolism category in the pare-wised comparison between two phases in the fourth-stadium nymphs.

Gene_ID	Annotation	Ratio(Log2 (G/S))	Pvalue
Carbohydrate metabolism			
LM01578	Pyruvate dehydrogenase E1 component alpha subunit type II	2.13	6.77E-07
LM02028	Alpha-galactosidase A precursor	0.87	3.68E-04
LM03873	Alpha-galactosidase A precursor	0.79	3.71E-04
LM07103	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4	0.86	8.64E-06
LM09139	UDP-N-acetylhexosamine pyrophosphorylase	1.19	9.02E-06
Chitin metabolism			
LM03161	Peritrophin-1 precursor	0.84	4.37E-04
LM03419	Gasp	0.65	5.02E-04
LM03818	Peritrophin-A	0.75	7.98E-04
LM03991	Gasp	1.23	3.25E-06
Dopa metabolism			
LM01233	Tyrosine hydroxylase(Pale)	1.92	2.71E-08
LM01742	Tan	0.64	1.01E-03
LM03547	Phenylalanine-4-hydroxylase/Tryptophan 5-monooxygenase(Henna)	1.48	4.87E-07
LM06090	Ebony	1.04	1.90E-07
LM06555	Ebony	1.01	9.64E-05
LM06559	Phenylalanine-4-hydroxylase/Tryptophan 5-monooxygenase(Henna)	1.10	1.16E-06
Lipid metabolism			
LM02273	Triacylglycerol lipase	1.36	5.76E-05
LM06045	1-acyl-sn-glycerol-3-phosphate acyltransferase alpha	0.87	9.41E-04
LM07113	Elongation of very long chain fatty acids protein 1 (CGI-88)	1.62	6.77E-07
LM08075	Elongation of very long chain fatty acids 4 protein	0.93	1.52E-04
Others			
LM00470	Glutamine synthetase	0.92	8.83E-06
LM00565	Bifunctional 3'-phosphoadenosine 5'-phosphosulfate synthethase	1.09	2.73E-06
LM01578	Pyruvate dehydrogenase E1 component alpha subunit type II	2.13	6.77E-07
LM01902	CG31973-PA	1.04	1.22E-05
LM02240	IPR002057:Isopenicillin N synthetase	1.46	1.76E-06
LM03179	Lysozyme precursor (1,4-beta-N-acetylmuramidase)	0.98	4.07E-04
LM03688	CG7532-PA	2.39	0
LM03833	15-hydroxyprostaglandin dehydrogenase	0.86	3.54E-04
LM05365	Xaa-Pro dipeptidase	1.21	1.38E-06
LM06588	ENSANGP00000011013	1.25	4.87E-07
LM06873	Matrix metalloproteinase-14 precursor	2.49	0
LM07242	Thyroid peroxidase precursor (TPO)	0.83	1.76E-04
LM07798	IPR000834:Peptidase M14, carboxypeptidase A	1.00	3.53E-04
LM09139	UDP-N-acetylhexosamine pyrophosphorylase	1.19	9.02E-06

Table S4. The list of upregulated gene functional categories in the fourth-stadium gregarious nymphs analyzed by Fisher's test (p<0.05).

GO_ID	GO-Term	Pvalue
Biological Process		
GO:0042423	catecholamine biosynthetic process	2.85E-03
GO:0006584	catecholamine metabolic process	2.85E-03
GO:0018958	phenol metabolic process	2.85E-03
GO:0042398	amino acid derivative biosynthetic process	6.76E-03
Molecualr Function		
GO:0042302	structural constituent of cuticle	0.00E+00
GO:0005198	structural molecule activity	2.12E-12
GO:0005351	sugar:hydrogen ion symporter activity	1.16E-04
GO:0030246	carbohydrate binding	4.34E-04
GO:0005214	structural constituent of chitin-based cuticle	5.54E-04
GO:0051119	sugar transmembrane transporter activity	9.48E-04
GO:0016714	oxidoreductase activity	9.71E-04
GO:0008061	chitin binding	1.10E-03
GO:0015144	carbohydrate transmembrane transporter activity	1.48E-03
GO:0030247	polysaccharide binding	1.53E-03
GO:0015291	secondary active transmembrane transporter activity	1.56E-03
GO:0008643	carbohydrate transport	2.17E-03
GO:0001871	pattern binding	2.31E-03
GO:0004099	chitin deacetylase activity	2.85E-03
GO:0015293	symporter activity	4.08E-03
GO:0005355	glucose transmembrane transporter activity	5.59E-03
GO:0015145	monosaccharide transmembrane transporter activity	9.13E-03
GO:0015149	hexose transmembrane transporter activity	9.13E-03
Cellular Component		
GO:0005576	extracellular region	6.94E-04
GO:0005865	striated muscle thin filament	9.13E-03

Table S5 The list of upregulated gene functional categories in the fourth-stadium solitary nymphs analyzed by Fisher's test ($p<0.05$).

GO_ID	GO-Term	Pvalue
Biological Process		
GO:0032787	monocarboxylic acid metabolic process	3.60E-04
GO:0006090	pyruvate metabolic process	4.69E-04
GO:0044262	cellular carbohydrate metabolic process	1.42E-03
GO:0006723	cuticle hydrocarbon biosynthetic process	1.68E-03
GO:0042811	pheromone biosynthetic process	1.68E-03
GO:0042810	pheromone metabolic process	1.68E-03
GO:0006082	organic acid metabolic process	3.18E-03
GO:0019752	carboxylic acid metabolic process	3.18E-03
GO:0008652	amino acid biosynthetic process	6.50E-03
GO:0006006	glucose metabolic process	6.71E-03
GO:0006081	aldehyde metabolic process	8.77E-03
GO:0006096	glycolysis	9.43E-03
GO:0006334	nucleosome assembly	9.53E-03
Molecular Function		
GO:0016903	oxidoreductase activity, acting on the aldehyde	1.06E-04
GO:0016717	oxidoreductase activity, acting on paired donors	2.63E-04
GO:0004768	stearoyl-CoA 9-desaturase activity	2.63E-04
GO:0016620	oxidoreductase activity	7.15E-04
GO:0016491	oxidoreductase activity	9.87E-04
GO:0004478	methionine adenosyltransferase activity	1.68E-03
GO:0030955	potassium ion binding	2.10E-03
GO:0031420	alkali metal ion binding	3.26E-03
GO:0005344	oxygen transporter activity	6.58E-03
GO:0004263	chymotrypsin activity	9.53E-03
Cellular Component		
GO:0005811	lipid particle	3.62E-03
GO:0005788	endoplasmic reticulum lumen	4.74E-03
GO:0000786	nucleosome	4.90E-03