

Table S1. The sample size of bees in the study split into the different treatments and variables

		Abdominal contractions (bees)	Aggression (bees)	Ovary activation (bees)	Egg laying (cages)	Fat-body lipids (bees)	Gene expression (bees)
Worker – single treatment (6d)	C	-	30	30	-	30	6
	FH	39	30	30	-	30	6
	PH	30	30	30	-	30	6
	HY	-	30	30	-	30	6
	AN	30	30	30	-	30	6
Worker – single treatment (10d)	C	-	-	-	10	-	-
	FH	-	-	-	10	-	-
	PH	-	-	-	10	-	-
	HY	-	-	-	10	-	-
	AN	-	-	-	10	-	-
Workers – multiple treatments (7d)	C	-	-	42	-	7	7
	FH	-	-	45	-	7	6
	PH	-	-	45	-	7	-
	HY	-	-	45	-	7	-
	AN	-	-	48	-	7	5
Virgin queens	C	-	-	20	20	14	6/4*
	FH	10	-	20	20	14	5/3*
	PH	12	-	19	20	14	-
	HY	-	-	20	20	14	-
	AN	11	-	21	21	13	6/5*
Mated queens	C	-	-	17	10	14	6/6*
	FH	10	-	19	11	14	6/6*
	PH	10	-	18	11	14	-
	HY	-	-	18	10	14	-
	AN	8	-	20	11	14	6/6*

*The first number applies to all genes whereas the second number applies to the gene PHGP, where four samples omitted due to a technical problem.

Table S2. primers sequences and accession numbers of *Bombus impatiens* used in the study.

Gene	Accession no.	F primer	R primers
<i>Phospholipase A2 (HKG)</i>	LOC100747217	CATTCCGCAAGTGGTAGGT	GGTCACACCGAAACCAGATT
<i>Arginine kinase (HKG)</i>	LOC100741837	GTTGGTAGGGCAGAAGTCA	AGGTCTACCGTCGTCTGGTG
<i>sima</i>	LOC100746032	GGAAAAGTCTCGTGATGCTGC	AGATGCCTGTTCCGATGGC
<i>tango</i>	LOC100740860	GAAGCACCTGATTCTGGAGGC	TGTAATTCAAAACAGGCGCCAC
<i>fatiga</i>	LOC100747684	CGGACCGATAGTCAGTCGTG	GCACCGTGAACACCTTAGGA
<i>Vitellogenin</i>	LOC10074717	CAGCCGCCAACATATGATAACCT	CCCTCCGTTCGAAGTGATAA
<i>FOXO</i>	LOC100745007	CAACAACAATCGAAACAGG	CTCCCCATCAATTGTCCCATC
<i>PHGP</i>	LOC100745584	ACTGTACGATGAGTATGCTGAC	TTCGGTACCTCCTGGTTCCCT

Table S3. The time to enter anesthesia, the recovery time from anesthesia and the number of abdominal contractions exhibited by bees in 20 minutes from the initiation of movement. Data are presented as means \pm S.E.M

	Workers	Virgin queens	Mated queens
Time to enter anesthesia (seconds)			
Full hypercapnia	14.3 \pm 0.3	17.8 \pm 0.7	18.7 \pm 0.4
Partial hypercapnia	34.7 \pm 1	29.8 \pm 2.2	31.2 \pm 1
Anoxia	29.9 \pm 0.9	21.5 \pm 1.2	22.3 \pm 1.3
Effect of treatment	ANOVA Mixed model, $F_{2,158}=134.3$, $p<0.0001$ followed by Tukey HSD $p<0.0001$ for all groups		
Effect of caste	ANOVA Mixed model, $F_{2,158}=5.18$, $p=0.006$ followed by Tukey HSD $p=0.004$ for workers vs virgin queens		
The recovery time from anesthesia (minutes)			
Full hypercapnia	29.7 \pm 1	33.9 \pm 1.9	38.4 \pm 0.7
Partial hypercapnia	7.07 \pm 0.6	31.5 \pm 1.4	28.8 \pm 0.8
Anoxia	4.33 \pm 0.4	21.6 \pm 1.1	24.6 \pm 2.2
Effect of treatment	ANOVA Mixed model, $F_{2,158}=72.6$, $p<0.0001$ followed by Tukey HSD $p<0.0001$ for all groups		
Effect of caste	ANOVA Mixed model, $F_{2,158}=50.1$, $p<0.0001$ followed by Tukey HSD $p<0.001$ for workers vs. queens		
Abdominal contractions number in 20 minutes			
Full hypercapnia	1215 \pm 116	1190 \pm 200	594 \pm 161
Partial hypercapnia	1264 \pm 162	1384 \pm 183	1764 \pm 73
Anoxia	526 \pm 42	955 \pm 198	475 \pm 170
Effect of treatment	ANOVA Mixed model, $F_{2,158}=14.8$, $p<0.0001$ followed by Tukey HSD $p<0.03$ for all groups		
Effect of caste	ANOVA Mixed model, $F_{2,158}=1.16$, $p=0.31$		