



Figure S1:

For direct tissue peptide profiling, distinct parts of the brain/subesophageal ganglion of *Nasonia* were dissected, which are marked in grey. AL, antennal lobe; aMe, accessory medulla; PI, *pars intercerebralis*; IPC, laterale protocerebellum; SEG, subesophageal ganglion (ventral part).

Fig. S2: Annotated or cloned *Nasonia vitripennis* neuropeptide preprohormones

The putative signal peptides (predicted by SignalP) are boxed, the putative active neuropeptides or protein hormones (based on similarity to neuropeptides or protein hormones identified in other insects and the peptides found with mass spectrometry) are marked in yellow. Green indicates putative basic cleavage sites; glycines used for amidation are shown in blue. Dots indicate missing N- or C-termini. Underlined cysteine residues are proposed to be involved in cystine bridges. It is not certain if the identified ITG-like and NVP-like sequences are neuropeptide precursors, although both have orthologues in the honeybee (9).

>AKH (NV_13000); **cloned cDNA** (see Fig. S3)

MNCRSLALGLCLCVVLVQTRAAEGQLNFSTGWGKRSSHLLQPARASSSSSS
SSSSSSTSAAAAAASSGIGRPRQQADLDYFLQRYRRLRKIEAQLANSQV

>ACP (NV_30201)

MGRRLSIGLAAAAILFSCMLHFALAQVTFSKGWGPGKRSALYETDCSRVNYK
SLALLFHTLLAEVKHLMACDHQATVNYLQSVRQ

>Allatostatin A (NV_13011)

MSSSNLSGTAMSLTIFCVLSILGGTSVAMDEQPSASSSSGVSSSSSSSHGPHFS
PLVDPREQIVGGKRAYTYRSEYKRLPIYQFGLGKRWVDDKRSQPFSGFLGKR
TRPYSFGLGKRSSYSEDDDSRYGLDLSYLIQPSDLYEQLAQRDALENYLQQQ
QAIKRGGFNFGKGRDAEMNEGMMREDGLHEKVPVKHSRDKYLFGLGKR
FYEPATMQDDEDEEMLEDA

>Allatostatin C (NV_30011); **cloned cDNA** (see Fig. S4)

MMSLRIAVCTAVALVVLVDWSTALPAGDKEALMNGLNMMGDEEGAVER
DLLNYLVGRRFVKRLRNQADVDDLQRRKNYWRQCAFNAVSCFGK

>Allatostatin CC (NV_30012); **cloned cDNA** (see Fig. S5)

MTSSGCRTFALATALTILGTVSGSPRGHALAKRSADANVIEYDPDYEYQRVM
PKRAALLLDRLVALQKAVENDDVGKENSNSYVRSLPNFSDMPDSQRMQLS
NEKTMDLQRRGQAKGRVYWRCYFNAVTCFKRK

>Bursicon-alpha (NV_13010)

MAHYRCKEYFFITNYFNISLGWFILFINGLSAIVHIDECEVTPVIHVLPQYTGCV
KPIPSFAKGRCSSYLQVSGSKIWQMERSCMCCQESGEREASVSLFCPKTKAG
DRKFRKVITKAPLDCMCRPCTSIEERIVIPQEIAGLSNNGPLSNSAHFRNTFKLT

>Bursicon-beta (NV_03820)

MRLIVSLLIAMFLKKIVLANALVNENCEKLDSEIRITKDEYSETGKLFRTCSDEI
QVSKCEGYCDSQVQPSIVTSTGFTKECCSCREEFLEERTVYLNNCYDSNGKQF
SLLDNSNMAIKIREPTNCKCIKCGFL

>CCAP (NV_04678)

MPDGSIKEQVLKLVLSLLIIESVEIARADDGLEIVAMKRPFCNAFTGCGRKRD
PSYASGLQLPIPLYRALLRLSSLRNGAAAEVRMRQRPLGLIGPENLVQLGPQA
PRLMAAPVVVPSLY

CCHamide-1 (scaffold176)

MRGNPELVAPRNVYCWAGILILISSFAGCAAGSCLSYGHSCWGAHGGKRSGV
VRPVDSLMPDLSLKHPAAGQRVVSPSFGERWLLSRLVNRQLMQVPLRGRM
LTQPLGDELPEVTS¹LAKHRCVFAFLYTYYPAS

CCHamide-2 (scaffold218)

...GCSAFGHSCYGGHGKR¹SYSESVDL²DLARNKEGTQQNDYDLSRSNAESVAL
VPSQGYNRQEQRSIHSIHERPNTNSWSL³FIRKLVNYFKFL

>Corazonin (NV_09278)

MIRGLTIALVTVALVSLATCQTFQYSRGWTNGKRAETSPLASILDYRAAMAG
AGGRMNDLQMARCANLLKWKMFLLSSGDNEEVCRVPC¹EFLDVL²RQCLIRQ
DKASPNDLSDFRRPSAPALETSSSLY

>Diuretic hormone (DH44/CRF-like) (NV_13001)

...GKRIGSLSVVNSVDVLRERVLLELARRKAMENQQQLGENQYVFKSVGKR...

>Diuretic hormone (DH31-like) (NV_02135)

MQREIALSWLLATEAALAINRVEALPFISRLWNQFQEDYPEAAQELLDRIE
NFAVYAQPDNAKRGLDLGLNRGFSGSQAAKHLMGLAAANYAGGPGRRRRS
EQA

>EH (NV_08338)

MVLSKRLLVLLMSYLVFLTVA¹AAHPSVGTCFRNCSQSKKFLGIYFDVQLCD
EYCVKFKGKMTPDVEDPDSLAPFITNLNEVQ

>ETH (NV_01589)

MKRLVNTFISRNYILSAIFIVAVLAIENKIVAADEP¹PAFFLKI²AKNIPRIGRSEPY
DEYAIKNSNVKDDIPWHKGEISKRRVGFSPESNTYAWQHFLAIEGPPELWRT
LAGYSHDPLYKTDDFNELWSRDKR¹TNNPEA

>ILP-B (NV_03688)

MSNMSTSRLSGLVTLTFAILLLLQLVQAQSDVYQYEQKRQGASKYCGQQLS
NALQLVCHGRYNPMFKKSVGQGMEMDDYPFNYDDSY¹PFRSRAVANAMMG
RFGAGRFRRRDSRGVHDECCCLKSCTMNEMRSYCAI²PQ

>ILP-C (NV_30146)

MYPSDSDR¹TTSTTRRSIGSRCWWLCLLLVLLVLSLELTRHQAEANNIHRRFYR
RAVRLCSRSLSDALYLLCKDRGYNEPFTSSETEVRHTTG²PGLVEECCYNSCS
EQMEQYCKPRNKA

>Inotocin (NV_08469)

MSKVIIVLTTLVALSYGCLITNCPRGGKRGDPTFLL¹ENIARECPACG²REEQGRC
FGPHCCSPSMGCLIGTPETLRCR³KESLYSRPCVAGFAMCQGN⁴SGRCAANGIC
CSQESCFIDSACKLVDETGNDRKIGAEFGAFLENAGTNEHIL

>ITG-like putative neuropeptide (NV_06225)

MLLAVLALMNCGADAWGGLFNRFSP¹EMLSNMGYGSHGGYLGRSSAFLQH²G
SSFSDNGLDDGADEPCYERRCNTNEDCPGSVCMNPEGEWPEGRCMFIYGLK
QGELCRRDNDCETGLMCAEVAGSDSLSCQPPVTSNKQYSETCSMSSECDISR

GLCCQLQRRHRQTPRKMC SYFKDPLVCIGPVAADQVKHV VQYTSGEKRITG
KNNRLYKRGFA

>Ion transporting peptide ITP (NV_07921)

MASVVGVL CARCSETIMIVFLSLLPLPSVDPDHDVLADKRSFMDIQCKGVYDK
ALFARLDRI CEDCYNLFREPQLHTLCRKNCF TSDYFKGCLDVLLLODEM EW I
QTSIKQLHGADPGV

>Myosuppressin (NV_09202)

MLKYSSSRSNVAMLTLLCLCAVLATLCGEAHGMPPAQCSPGLLDEVPPRIRK
VCAALSTIYELGSAMENYINDKVLREDIPLPDSGVKRQVDHVFLRFGRRR

>Neuroparsin (NV_03041)

MASDTLYIFCMATLILLRQVLNELAGGIKPAGGTATPPQRPQHRRTAPGLDSS
DQKNDVLSADRFLGHPTI ERAVQRSECEGCGANCKQCKYGSAYSLWCGLDE
CLKGPGEMCGGVRNKYGECDGMYCRCNKCTGCSIDTLECFSGFCPILEQQ
MQLRHQDHSMHGLQLDK

>NPF (NV_13002)

MRVSLAMMHLTYLTLTVLALGSVSVQSEPEPMARPTRPKVFESPEELRQYLD
LVKEYYSLSGKARYGKRAEPYAMQQGYEHPLNFLRMLLAFSRQKQELVDKD
ERSKLQIMQPYDRVARYYEIME

>NVP-like putative neuropeptide (NV_30009)

MLSDWLWLLAFGAMLVAVQSLPAGLSTSSGSSSADETKKTEQTMRPKVKR
AQEMLMFGNQNRQQAATAESNNAANNYSPPAEKR T LSNGLDDVSSALSDV
EQQQQQQAAIMANSNNHPKALPSGYMMDQPGYAAAREELREPRYKRELD
LDPEDLITFLTYDNERRNRQNRWNYGNEEYEGADDDSNMIGLDDDEDPRGSS
WLDSQQYGQPQAQHHYLSGEPLLSSELAALARNRPSASSGYDQYLGQQ
YGTAGQQYETAPQVQYGTQPQYGLPYLQQHASYYSPKRFMVARKRSQNYDS
YGGRSGLLLNSRGYPSYQHRLLY

>orcokinin (NV-30010)

...MQEWKSPKQYHYSGGNLDHIGGNLLRKKVVKIPNEYFTETTTTSPVET
TTSDLKADRR LIGVAELKSQAEERQTRNARNDYDSPDYGHGQKFTGHRKRPDS
THRLINKGFSETIIPSDIIEYTNQKRNFDEIDRSFGSGFSKRNFNDEIDRSFGSGF
NKKRNFNDEIDRTGFSGFNKRNFDEIDRSFGSGFNKRNFDEIDRSFGSGFNKRNF
DEIDRSFGSGFNKRNFDEIDRTGFSGFNKRNFDEIDRSFGSGFNKRNFDEIDRS
FGSGFNKRNFDEIDRSVPGFAKR SIASSRSDRIFCISIIYHAQDI

>PDF (NV_15000)

MRSWVSHLIRAFFVFGAILCASASMEDTSHMIMNNPYGRSLDAELITRLLLAP
QRLCHPKR NSELINSLSLPKNMNNA GK

>PTTH (NV_30191)

MKLQTFVLVLLTVQHRAKGLQLESYSDDFSDVELPTDERDDCS DGT CYAEKR II
LPEKIVEHRQLMPRFATKLQSVNLAESFPQPQWRPVCGCVTQHKL VNLGEGH
YPRYITTARCKSKTVANRFYQCKYYDYRVHVLVKRGLNSIPKNADEL D VRD
VEELPLPESLHANWQLFALSVSVA CV AVERV

>Pyrokinin (NV_30144)

MELARLINGARTKAILCALLVIVLMANRVAGQYDGRGSDMVEGPRVERMHP
ETSGGCVGAHCLTQNSEGPVGAMWFGPRLGRRRRSDKFTPKKIEALSEMLGS
PNWNLV TIPGGEDKRQETTFTPRLGRELENAISVYDLVRGLVSSVDDQNGKD
RDQQAPPPMFPRLGRITLLTPRLEHELRLNLLRKLQMQ

RYamide (NV_30121)

MISSSRKIRRVSDYLKLDKLVWLWISGIFLTLVSSQDNFYASGRFGKRKYALS
MSQIPLCSKFDRESEDRSAGNSLKDSSLFSSARFGRSEDRNTGNSLRDSSSFFPA
RYGRSEDRSTGNSLRDSSSFFPARFGRSEDRSTGNSLKDSSSFFSPARYGRSEDR
SSGNSLKESSFFSPGRYGRSEGHKNPKELPKFFEIKPRVDQFFIGSRYGKRSL
MLEPQPPLEALHNQRFEAAIDYLDRIKQNLAEAEIEDETRDASRDDELVEAI
YPNDYTGLSKI

>sNPF (NV_13003)

..LIDESPNSEKSANLRELYRMLVQRSVDDSGSLRDLAEHLQPRAA
ERSPSLRLRFGRSYPKYPVSRTHA

>SIFamide (NV_30008)

MSAARFALVLMVVLAVAILNVDAAYRKPPFNCSIFGKRANSVSDYDYASRA
MDAICEIAKTNCNAYYGPQDSN

>Tachykinin (NV_03478)

MTSPAAARLRATTMLLTLLTLGVSAQHSMTTIRGQTDRDSEIILETKAPQQL
PPQLAYVREPEELDGYQALDSTKHLREPSSSYLERLLLTEELDKRASMRGFQG
MRGKKSVDPTSSFLAGYSSPEELQYYEAYEHEKRAPMGFQGMRGKKSAD
LEEDAYYKRAPMGFQGMRGKKSAAIEEVLEELEKRAMMGGFQGMRGKKSPE
PSVWNKRAPMGFQGMRGKSSFLDELDELEKRALLGFHGMRGKKNVVAEL
AYEPADM DGYVEKRPMMMGFHGMRGKRSASFYGVGERYEKRSPYRFFGTR
GKKNPRWEMRGKFGVGRGKWSLAPRMPYDELLRH

| | | |
|--|---|-----|
| | CACATCTCGGCTCCACATAAGTAGCACACGCAGCTTCCTTCCTCGCCCCGAGACGCACC | -1 |
| ATG | AAC TGC AGG AGC CTG CTC GCG CTG GGA CTC TGT CTG TGC GTC GTC CTG | 51 |
| M | N C R S L L A L G L C L C V V L | 17 |
| CCG | CAG ACT CGG GCC GCC GAG GGT CAG TTG AAC TTC TCC ACC GGC TGG GGC | 102 |
| V | Q T R A A E G Q L N F S T G W G | 34 |
| AAG | CGA AGC AGT CAT CTG CTC CAG CCG GCG CGC GCT TCG TCA TCC TCG TCC | 153 |
| K | R S S H L L Q P A R A S S S S S | 51 |
| TCG | TCC TCG TCC TCG TCC TCA ACG TCG GCG GCG GCG GCG GCG GCT TCC | 204 |
| S | S S S S S S T S A A A A A A S | 68 |
| TCG | GGA ATC GGC AGG CCT CGA CAG CAA GCC GAT CTC GAC TAC TTT CTG CAG | 255 |
| S | G I G R P R Q Q A D L D Y F L Q | 85 |
| CGC | TAC TAT CGC CGC CTG CGC AAG ATC GAA GCC CAG CGA CTG GCG AAT TCT | 282 |
| R | Y Y R R L R K I E A Q R L A N S | 102 |
| CAG | GTG TAA | |
| Q | V * | |
| ATGGCGCTGATGGAAGCTCGTATAGTTAGAACAGAAGATTTGTATTTGTTTGTATTATACCGTATG | | 333 |
| AAGCTTATTTTAATAATAAAATTAATATTCGATTGC (A) _N | | 370 |

Figure S3:

cDNA and deduced amino acid sequence of the AKH preprohormone from *Nasonia vitripennis* (GenBank accession number HM461995). Nucleotides are numbered from 5' – to 3' –end. Amino acid residues are numbered from the first start (ATG) codon in the open reading frame. The signal peptide is highlighted in yellow, the immature AKH peptide in blue and the dibasic cleavage site in red. The stop codon is indicated by an asterisk. Exon-intron boundaries are highlighted in grey. The putative polyadenylation signal in the 3'-noncoding region is underlined.

CTCTCTCTCTCTCTCTCT -135

CTCTGGCTTCTGCTCTTACCGTTTCGCTGTCGTCGGCCCTACCATCGCCGATTATCCGCCGCTAACT -68

CGAGCCACCTGTCAGATTGGAAATTTCCAGCCGCAATTACACACAAACTCACACCGAATCAGCAAA -1

| | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ATG | ATG | TCT | TTA | AGA | ATT | GCC | GTT | TGC | ACA | GCA | GTA | GCG | CTC | GTA | GTG | CTC | 51 |
| M | M | S | L | R | I | A | V | C | T | A | V | A | L | V | V | L | 17 |
| GTC | GAC | TGG | TCG | ACG | GCA | CTT | CCA | GCT | GGG | GAT | AAG | GAG | GCG | CTC | ATG | AAC | 102 |
| V | D | W | S | T | A | L | P | A | G | D | K | E | A | L | M | N | 34 |
| GGG | CTT | AAT | ATG | ATG | GGT | GAC | GAG | GAA | GGG | GCA | GCA | GTA | GAG | AGG | GAC | CTG | 153 |
| G | L | N | M | M | G | D | E | E | G | A | A | V | E | R | D | L | 51 |
| CTC | AAC | TAC | CTC | GTA | GGC | AGA | AGG | TTC | GTT | AAA | CGC | CTA | CGC | AAC | CAA | GCC | 204 |
| L | N | Y | L | V | G | R | R | F | V | K | R | L | R | N | Q | A | 68 |
| GAC | GTC | GAC | GAT | CTG | CAG | CGC | AAG | CGA | AAC | TAC | TGG | AGG | CAG | TGC | GCT | TTT | 255 |
| D | V | D | D | L | Q | R | K | R | N | Y | W | R | Q | C | A | F | 85 |
| AAC | GCC | GTC | TCC | TGC | TTC | GGC | AAG | TAG | | | | | | | | | 282 |
| N | A | V | S | C | F | G | K | * | | | | | | | | | 93 |

| | |
|---|------|
| GCTGCGGCTGATGAATTAGATCGCTTCGCGGATGACTATGTGGGCCAGACGACGCGAATTATTAAT | 349 |
| CTTAATAATATATTACGGCCCATTCGGAGCGACGTGATGACGCAGCGCTATAATACTGAATAGAA | 416 |
| CAAACAATTTGCTTCTGTGAACAGAAAAACAAGCTTCGTTAACTAACTGTCATTCGATGTATA | 483 |
| CCTCAGATATTCGATTTATTCATCGGACGCACACGCTTATGTCTATATGAATTTTAATTGTTAGAT | 550 |
| GATAGAGAGATGGTTCGGTATACCACGCGCCAACAGTATTTTTTATTTTTCTCTCTTTTTCTCTCGATG | 617 |
| ATATTGTGTGTTTTGTATACTTATAGCTTAGTTGGGAGTTGTCTTGGGATTATGTATGGTTCGATGGCT | 684 |
| TCTCATGCGGGTACTTGCATGCACAATTACCGGAAGTTAGCTTTTGTGGTAATTTTCGTTTATACC | 751 |
| GTTATCGTGGCGAATTTTTTTTTAATTTTATCTCGCGCAACTTACTGTACATATGCTAGTTGAGCT | 818 |
| GGAAGAGATCAAGATAGAATAGATAATTATAGAAATTTTCGGCGATTTCGTTTTGTAATTTGAATTTTA | 885 |
| TATTTAGATTTGACTTTACGATATCGACTTTACGACCGAGACGATTCTCCTATTAGTTTCATGTTTTT | 952 |
| TAAGTTGATTTTCGCGAGTAAGCATTTTTTCGGCACTAAAAATTTACCAGTATCCTATG(A) _N | 1009 |

Figure S4:

cDNA and deduced amino acid sequence of the allatostatin C preprohormone from *Nasonia vitripennis* (GenBank accession number HM461994). Nucleotides are numbered from 5' – to 3' –end. Amino acid residues are numbered from the first start (ATG) codon in the open reading frame. The signal peptide is highlighted in yellow and the immature allatostatin C peptide in blue. The basic amino acid residues used for cleavage are shown in red. The stop codon is indicated by an asterisk. Exon-intron boundaries are highlighted in grey. An in frame stop codon in the 5'-untranslated region is underlined.

CGCGGATCCGAACACTGCGTTTGCTGGC -470

TTTGATGAACATTGCGCCGGCCGACTACCGCTGGGGCTAACTTGCCCGTCGCTCGCGCGGCAACGCA -403

CACATACTCTCCTTTTGGCGCCGCATCAGCCCTAGCACCCAACCTGTTGCCGTCACTCCACCGCCGC -336

AGGACCTCCAGAGTAGCGCGCACCGATATACGCCATATACGCCGGGATTCGCACAAGTTTGCAATTT -269

TTTTTCCAAACTGCAGTATGGGTGAAAATTGATTTCGTGCGGTTCGACTGGAAAGAGTTTTTAAATGCGT -202

AGAGCGGTGAAACATTGCGAGGATTGACGCTTGC GCGAGGAGAAAAGATCGGAGAGAGGAAAAAGGCG -135

GGCCTCTAGTCGGAGCTTAGTCAGGTGCGCGCGCCGCTACGCACAGAGCTTCACGCAAGGCTTCG -68

CTCGAAAAGCAGCGAGTGGATTGTTTCGCCGTTGTTACCGTCAACCTTGTTGACTTGATCTTCGGG -1

| | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ATG | ACG | AGC | TCT | GGC | TGC | CGT | ACC | TTC | CTG | GCG | CTC | GCA | ACG | GCG | CTC | ACG | 51 |
| M | T | S | S | G | C | R | T | F | L | A | L | A | T | A | L | T | 17 |
| ATC | CTC | GGC | ACC | GTC | AGC | GGC | TCG | CCG | CGC | GGA | CAC | GCG | CTC | GCC | AAG | AGA | 102 |
| I | L | G | T | V | S | G | S | P | R | G | H | A | L | A | K | R | 34 |
| AGC | GCC | GAT | GCT | AAC | GTT | ATC | GAG | TAT | CCG | GAT | TAC | GAG | TAT | CAG | AGA | GTT | 153 |
| S | A | D | A | N | V | I | E | Y | P | D | Y | E | Y | Q | R | V | 51 |
| ATG | CCA | AAA | AGA | GCA | GCA | CTT | CTA | CTA | GAT | CGA | TTG | TTA | GTG | GCT | TTG | CAA | 204 |
| M | P | K | R | A | A | L | L | L | D | R | L | L | V | A | L | Q | 68 |
| AAA | GCA | GTT | GAA | AAC | GAT | GAT | GTA | GGA | AAA | GAA | AAC | TCT | AAC | TCA | TAC | GTC | 255 |
| K | A | V | E | N | D | D | V | G | K | E | N | S | N | S | Y | V | 85 |
| AGG | TCA | CTA | CCG | AAC | TTC | TCA | GAT | ATG | CCT | GAT | TCG | CAG | AGG | ATG | CAA | CTT | 306 |
| R | S | L | P | N | F | S | D | M | P | D | S | Q | R | M | Q | L | 102 |
| TCT | AAC | GAA | AAA | ACG | ATG | GAT | CTT | CAA | CGG | CGA | GGC | CAA | GCA | AAA | GGT | CGC | 357 |
| S | N | E | K | T | M | D | L | Q | R | R | G | Q | A | K | G | R | 117 |
| GTT | TAT | TGG | CGT | TGT | TAC | TTT | AAT | GCT | GTG | ACA | TGC | TTT | AAA | AGG | AAG | TGA | 408 |
| V | Y | W | R | C | Y | F | N | A | V | T | C | F | K | R | K | * | 133 |

TTATCCATTAGAAAAGACGAGGCATGGTGGTCTTTACTTTAAAAATTGTTTTACTTCTTTCACTCAG 481

TGTGGCTATAGTATTTTTCATACCATTATACGGAAGAATGGAGTTGCTAAAACAATTATCATAATAT 548

CAGTAAATTTGAGAATATATTTTAATTTTATGTTTATATATGTAATGATTTCCATAAAAAATA 615

CACGAAATTATATTTAAGTCATGCTGTGAATAGATCC (A)_N 652

Figure S5:

cDNA and deduced amino acid sequence of the allatostatin CC preprohormone from *Nasonia vitripennis* (GenBank accession number GU937435). Nucleotides are numbered from 5' – to 3' –end. Amino acid residues are numbered from the first start (ATG) codon in the open reading frame. The signal peptide is highlighted in yellow and the immature allatostatin CC peptide in blue. The basic amino acid residues used for cleavage are shown in red. The stop codon is indicated by an asterisk. Exon-intron boundaries are highlighted in grey.

| | | | | | | | | | |
|--------|--|------------------------|-----------------------------------|---------------------|--------------------|--------------------------------------|---------------------------------------|------------------|-----|
| Nv-NVP | MLSDWLWLLA-FGAMLVAVQSLPAGLSTSSGSSSSA | AETKKEQTMRPKV | KRAQ EMLMFGNQNRQ | QATAESNNA | ---NNYSPPAEKRTLSNS | --GLDDVSSALSDVE | ----- | 103 | |
| Am-NVP | MMCDWVWLLLTLCSLLMIVQSLPTNL | -----AEDTKKEQTMRPKS | KRAQ EMLMFGNQNHQ | ---- | PENNPS | ---SSYSSTA | EKRTLAAS--GLGGLKAALIEEEKPSRSNTLNNA | 102 | |
| Tc-NVP | MELRWSIRWATLASCLALSFAIPASLVEEIKTNE | LRNKVK | KRAH PQLNVGEHGREVPYYSKPTAI | KRG ANNLNK | NP | SPEQQSLSDWEQEQSLYQNPDSLADIQSSLYNAENP | ----- | 111 | |
| Nv-NVP | -----Q-----Q-QQQQAA | IMANSNNHPKAL | PSGYMMDQPGYAAAREELR | ----- | EPYK | RELDLDPEDLITFLTLTDNE | RRNRQNRNYGN | 180 | |
| Am-NVP | FYDRKNYDYGAVNELGYEIPQVWDNSPYSRYT | NEDRRKRSEKSAVASGSSTTIK | PSTTSFQSPSTQQSVQTV | KRN VPTIYQEP | RF | KRELDIDPEDVLTLLSLWENER | RKR -NWHKYMN | 221 | |
| Tc-NVP | -----FDDKTIAEYKGFHYGTNKEKLDEALENA | VLKSELYGDP | APLNQYRYYGNDQ | RRRKR RDAR | -K | IRLDSRM | KREVDLTPDEIFTILTLYENERNGYRPNW | 210 | |
| Nv-NVP | EYEGADDDSNMIGLDDDEPRGSS-WLDSQQY | GQPQQAQHHYLSGEPLLS | SELAALARNRPSASSGYDQYLGQOYGTAG | QOYETAPQVQYGT | PQYGLPYLQOHAS | YYSPE | KRF MVARK | 299 | |
| Am-NVP | EEYENVDDEDNLL--EEEDSRNIIPWMDSSVY | -----PPRHYSLDS | ---LSPSDIGIIRTHPSS | ---YEQYEN-QYG | ---QQYDTS | ---QYGSPOYGLVYPQTY | -YSAPE | KRF MISRK | 320 |
| Tc-NVP | --LEPEPSGDNLE--EEE--N--WLDAPVY | ----- | PHATGHNDLAPSY | ---LMDE | KRG -RWG | ---GFADS | --- RKKRF MAKRNDP--TRELRYLNGPN | 285 | |
| Nv-NVP | RSQNYDSYGGRSGLLLNS --RGYPSYQHRLLY | | | | | | | 329 | |
| Am-NVP | RSQAYDPYSNAAQFQLSSQSRGYP -YQHRLVY | | | | | | | 351 | |
| Tc-NVP | KND---YYTLSQLLSNQREPNVP-LYHRLVL | | | | | | | 312 | |

Figure S6:

Alignment of the NVP proteins from *N. vitripennis* (Nv-NVP), *A. mellifera* (Am-NVP) and *T. castaneum* (Tc-NVP). Amino acid residues that are common in at least two sequences are highlighted in grey. Blue letters indicate the signal peptides, putative basic cleavage sites are shown in bold. The *Nasonia* peptide identified by MS in this study is marked in green, the NVP peptide from *A. mellifera*⁹ is marked in red. In these three species, there is a moderate conservation of protein structure, but not of the NVP sequence. We could not find NVP proteins in other arthropods.

Table S1: PCR primers used to amplify *Nasonia* neuropeptide precursor cDNAs.

| Gene | accession no. | technique | Sense Primer Sequence | Antisense Primer Sequence |
|-----------------|---------------|-----------|-------------------------------|-------------------------------|
| AKH | | PCR | 5'-CACATCTCGGCTCCACATAAG | 5'-TCAGCGCCATTTACACCTGAG |
| | | 3'-RACE | 5'-GAGGGTCAGTTGAACTTCTCC | |
| | | 3'-RACE | 5'-CGAAGCAGTCATCTGCTCCAG | |
| | | 5'-RACE | | N/A |
| | | 5'-RACE | | N/A |
| Allatostatin C | | PCR | 5'-ATGATGTCTTTAAGAATTGCCGTTTG | 5'-CTACTTGCCGAAGCAGGAG |
| | | 3'-RACE | 5'-CTCGTAGGCAGAAGGTTTCGT | |
| | | 3'-RACE | 5'-AAACGCCTACGCAACCAAGC | |
| | | 5'-RACE | | 5'-CGTTCATGAGCGCCTCCTTA |
| | | 5'-RACE | | 5'-GACCAGTCGACGAGCAC |
| Allatostatin CC | GU937435 | PCR | 5'-GAGCAGCACTTCTACTAG | 5'-GCATGTCACAGCATTAAAGTAACAAC |
| | | 3'-RACE | 5'-CGCGTTTATTGGCGTTGTTACTTTA | |
| | | 3'-RACE | 5'-GTTGTTACTTTAATGCTGTGACATGC | |
| | | 5'-RACE | | N/A |
| | | 5'-RACE | | N/A |