Characterization of a set of abdominal neuroendocrine cells that regulate stress physiology using colocalized diuretic peptides in *Drosophila*

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Supplementary Figures 1 – 7 (Fig. S1- Fig. S7)
Figure S1: LK and DH44 expression in the larval Drosophila CNS. (A) Lk-GAL4 drives expression in five pairs of neurons in the brain; however, four of these pairs do not display any LK-immunoreactivity [20]. (B) These four pairs of neurons display ITP-immunoreactivity. (C) Three pairs of neurons in the subesophageal ganglion express Lk in larval Drosophila. (D) Seven pairs of neurons in the larval ventral nerve cord (VNC) express Lk. (E) A schematic of LK-expressing neurons in the larval brain and VNC of Drosophila. Neurons displaying LK-immunoreactivity are labelled in red and neurons displaying ITP-immunoreactivity are labelled in black. (F) DH44-GAL4 driven GFP and DH44-immunoreactivity is present in three pairs of median neurosecretory cells in the larval brain. (G) DH44 is expressed in several neurons, with strong expression seen in seven pairs of neurosecretory cells in the larval VNC. In both F and G, there are some neurons that contain GFP but do not contain DH44-immunoreactivity.
Figure S2: LK expression in adult Drosophila brain. LK-GAL4 drives weak GFP expression in four pairs on neurons in the adult brain. The location of these cells is indicated by white boxes. Scale bar: 50 µm.
Figure S3: LK and DH44 are co-expressed in neurons of the ventral nerve cord but not in the brain of larval Drosophila. (A) DH44-GAL4 driven GFP is not co-localized with LK-immunoreactivity in the larval brain. (B) DH44-GAL4 driven GFP is co-localized with LK-immunoreactivity in all seven pairs of abdominal LK neurons (ABLKS) in the ventral nerve cord (VNC) (C) Lk-GAL4 driven GFP is not co-localized with DH44-immunoreactivity in the larval brain. (D) Lk-GAL4 driven GFP is co-localized with DH44-immunoreactivity in ABLKS in the larval VNC.
Figure S4: Knockdown of DH44 using Actin5c-GAL4. Actin5C-GAL4 driven DH44 knockdown results in a complete abolishment of DH44-immunoreactivity in the six neurons in pars intercerebralis.

Figure S5: Number of LK-immunoreactive neurons in the VNC following knockdown of DH44 and Lk using DH44-GAL4. Lk knockdown but not DH44 knockdown causes a significant decrease in the number of LK-immunoreactive neurons that could be detected in the adult VNC. (**** p < 0.0001, as assessed by One-way ANOVA).
Figure S6: Knockdown of DH44 using DH44-GAL4 impacts stress resistance and feeding in Drosophila. DH44-GAL4 driven DH44 knock down results in a significant increase in survival compared to control flies under (A) desiccation (compared to the GAL4 control), (B) starvation and (C) ionic stress (artificial food supplemented with 4% NaCl). Data are presented in survival curves and the error bars represent standard error (**** p < 0.0001, as assessed by Log-rank (Mantel-Cox) test) (D) Lk knock down results in a delayed recovery from chill coma. (* p < 0.05, as assessed by Log-rank (Mantel-Cox) test) (E) There is no significant difference (One-way ANOVA) in feeding as measured by capillary feeding (CAFE) assay between Lk knock down and control flies. Results are presented as cumulative food intake over four days. (F) There is no significant difference in wet weight, dry weight and water content of DH44-knockdown and control flies. Legend for B-F is the same as the one in A.
Figure S7: Knockdown of Lk in ABLKs with DH44-GAL4 does not influence LK-stimulated Malpighian tubule secretion \textit{ex vivo}. (A) Secretion rates of $10^{-9}$ M LK stimulated MTs isolated from DH44 > w$^{1118}$ ($n = 14$) or DH44 > Lk RNAi flies ($n = 25$). (B) Secretion rates of $10^{-10}$ M LK stimulated MTs isolated from DH44 > w$^{1118}$ ($n = 10$) or DH44 > Lk RNAi flies ($n = 12$). For both A and B, secretion rates were measured at 10 min intervals for 30 min before and after the addition of peptide (indicated with an arrow). (C, D) Change (%) in secretion determined by comparing the secretion rate over the first 30 min to the maximum secretion rate following peptide application. The legend and sample size for C and D are the same as the one in A and B, respectively. Asterisk indicates significantly different secretion rate compared to basal secretion rate (secretion rate prior to the addition of peptide). (NS = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Mann-Whitney U test).