Supplemental Materials for Shafer *et al.* "Widespread receptivity to neuropeptide PDF…

N-D-07-01431

Statistical analysis 1. On the effects of 10mM forskolin on l-vLNs (Data from figures 1C-G).

Data were analyzed through one-way repeated measures ANOVA with the fixed factor Drug [Vehicle, FSK, ddFSK] and within-subjects factor time (56 measurements from 0 to 275 seconds). A Tukey post-hoc comparison of marginal means was performed on the levels of the fixed factor Drug.

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	11.673	55	.212	314.040	.000
	Greenhouse-Geisser	11.673	2.679	4.358	314.040	.000
	Huynh-Feldt	11.673	3.015	3.872	314.040	.000
	Lower-bound	11.673	1.000	11.673	314.040	.000
time * Drug	Sphericity Assumed	12.855	110	.117	172.924	.000
	Greenhouse-Geisser	12.855	5.358	2.399	172.924	.000
	Huynh-Feldt	12.855	6.030	2.132	172.924	.000
	Lower-bound	12.855	2.000	6.427	172.924	.000
Error(time)	Sphericity Assumed	1.561	2310	.001		
	Greenhouse-Geisser	1.561	112.508	.014		
	Huynh-Feldt	1.561	126.620	.012		
	Lower-bound	1.561	42.000	.037		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1985.247	1	1985.247	36565.922	.000
Drug	33.240	2	16.620	306.120	.000
Error	2.280	42	.054		

Tukey Multiple Comparisons

	N	Subset	
Drug	1	2	1
FSK	14	.7262	
Vehicle	14		.9701
ddFSK	17		.9776
Sig.		1.000	.792

There is a strongly significant within-subjects effect of time ($p<0.001$). There is also a strongly significant ($p<0.001$) effect of the drug treatment. A Tukey HSD post-hoc comparison of the treatment means reveals that the vehicle and ddFSK treatments comprise a statistically homogenous subset that is distinct from the FSK treatment group.

Statistical analysis 2. On the effects of 10^{-5}M PDF on the l-vLNs and s-vLNs (data from figure 2)

Data were analyzed by two-way repeated measures ANOVA with fixed factors cell-type [l-vLNs or s-vLNs] and peptide [PDF, Vehicle] using all time measurements as within-subjects factors.

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	6.412	55	.117	53.338	.000
	Greenhouse-Geisser	6.412	1.978	3.242	53.338	.000
	Huynh-Feldt	6.412	2.258	2.839	53.338	.000
	Lower-bound	6.412	1.000	6.412	53.338	.000
time * Cell	Sphericity Assumed	1.141	55	.021	9.490	.000
	Greenhouse-Geisser	1.141	1.978	.577	9.490	.000
	Huynh-Feldt	1.141	2.258	.505	9.490	.000
	Lower-bound	1.141	1.000	1.141	9.490	.004
time * peptide	Sphericity Assumed	1.846	55	.034	15.356	.000
	Greenhouse-Geisser	1.846	1.978	.933	15.356	.000
	Huynh-Feldt	1.846	2.258	.817	15.356	.000
	Lower-bound	1.846	1.000	1.846	15.356	.000
time * Cell *	Sphericity Assumed	1.110	55	.020	9.232	.000
peptide	Greenhouse-Geisser	1.110	1.978	.561	9.232	.000
	Huynh-Feldt	1.110	2.258	.491	9.232	.000
	Lower-bound	1.110	1.000	1.110	9.232	.004
Error(time)	Sphericity Assumed	4.448	2035	.002		
	Greenhouse-Geisser	4.448	73.178	.061		
	Huynh-Feldt	4.448	83.556	.053		
	Lower-bound	4.448	37.000	.120		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1941.465	1	1941.465	11797.583	.000
Cell	3.250	1	3.250	19.749	.000
Peptide	3.145	1	3.145	19.108	.000
Cell * Peptide	1.222	1	1.222	7.424	.010
Error	6.089	37	.165		

There is a strongly significant within-subjects effect of time ($p<0.001$). There is also a strongly significant ($p<0.001$) effect of both main effects factors (Peptide and Cell type). Additionally, there was a significant interaction ($p=0.01$) indicating that the different cell types responded differently to the peptide treatments.

Statistical analysis 3. On the effects of various concentrations of PDF on the s-vLNs (data from figure 3A)

Data were analyzed through one-way repeated measures ANOVA with the fixed factor PDF Concentration [Vehicle, 10^{-9} PDF, 10^{-8} PDF, 10^{-7} PDF, 10^{-6} PDF, 10^{-5} PDF].

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	42.526	98	.434	273.412	.000
	Greenhouse-Geisser	42.526	2.752	15.450	273.412	.000
	Huynh-Feldt	42.526	3.096	13.734	273.412	.000
	Lower-bound	42.526	1.000	42.526	273.412	.000
time * PDFConcentration	Sphericity Assumed	11.170	490	.023	14.364	.000
	Greenhouse-Geisser	11.170	13.762	.812	14.364	.000
	Huynh-Feldt	11.170	15.482	.722	14.364	.000
	Lower-bound	11.170	5.000	2.234	14.364	.000
Error(time)	Sphericity Assumed	10.421	6566	.002		
	Greenhouse-Geisser	10.421	184.416	.057		
	Huynh-Feldt	10.421	207.453	.050		
	Lower-bound	10.421	67.000	.156		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	5063.635	1	5063.635	22961.759	.000
PDFConcentration	44.129	5	8.826	40.022	.000
Error	14.775	67	.221		

There is a strongly significant within-subjects effect of time ($p<0.001$). There is also a strongly significant ($p<0.001$) effect of the PDF concentration treatment suggesting that FRET values are responsive to PDF concentrations.

Statistical analysis 4. On the effects of peptide washout following the addition of 10^{-6}M PDF on the s-vLNs (data from figures 3C and D).

Data were analyzed with a set of two one-way repeated measures ANOVAs followed by Tukey post-hoc comparisons. The analyses were delimited into pre-wash sets of treatments and post-wash sets of treatments. Each set (pre-wash and post-wash) were treated separately.

Prewash

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	7.077	27	.262	87.363	.000
	Greenhouse-Geisser	7.077	2.666	2.654	87.363	.000
	Huynh-Feldt	7.077	3.649	1.939	87.363	.000
	Lower-bound	7.077	1.000	7.077	87.363	.000
time * Treatment	Sphericity Assumed	3.340	54	.062	20.615	.000
	Greenhouse-Geisser	3.340	5.332	.626	20.615	.000
	Huynh-Feldt	3.340	7.298	.458	20.615	.000
	Lower-bound	3.340	2.000	1.670	20.615	.000
Error(time)	Sphericity Assumed	1.296	432	.003		
	Greenhouse-Geisser	1.296	42.658	.030		
	Huynh-Feldt	1.296	58.386	.022		
	Lower-bound	1.296	16.000	.081		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	360.231	1	360.231	2317.727	.000
Treatment	6.785	2	3.392	21.827	.000
Error	2.487	16	.155		

Tukey Multiple Comparisons

	N	Subset	
Treatment	1	2	1
MockWashout	5	.7256	
Washout	9	.8136	
Vehicle	5		1.0251
Sig.		.139	1.000

There is a strongly significant within-subjects effect of time ($p<0.001$). There is also a strongly significant ($p<0.001$) effect of the treatment. A Tukey post-hoc comparison reveals that the

Mock-washout and Washout treatments (heretofore only exposed to PDF) form a homogenous subset of marginal means.

Postwash

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	1.058	98	.011	3.030	.000
	Greenhouse-Geisser	1.058	2.175	.486	3.030	.057
	Huynh-Feldt	1.058	2.845	.372	3.030	.041
	Lower-bound	1.058	1.000	1.058	3.030	.101
time * Treatment	Sphericity Assumed	2.315	196	.012	3.315	.000
	Greenhouse-Geisser	2.315	4.351	.532	3.315	.019
	Huynh-Feldt	2.315	5.690	.407	3.315	.010
	Lower-bound	2.315	2.000	1.157	3.315	.062
Error(time)	Sphericity Assumed	5.586	1568	.004		
	Greenhouse-Geisser	5.586	34.807	.160		
	Huynh-Feldt	5.586	45.522	.123		
	Lower-bound	5.586	16.000	.349		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1052.779	1	1052.779	620.641	.000
Treatment	32.509	2	16.255	9.583	.002
Error	27.140	16	1.696		

Tukey Multiple Comparisons

	N	Subset	
Treatment	1	2	1
MockWash	5	.5761	
Washout	9		.8451
Vehicle	5		.9102
Sig.		1.000	.676

There is a strongly significant within-subjects effect of time ($p<0.001$). There is also a strongly significant ($p=0.002$) effect of the treatment. A Tukey post-hoc comparison reveals that the Mock-washout and Washout treatments (previously only exposed to PDF) are no longer statistically homogenous. Instead, the washout treatment (exposed to PDF and then washed) is now statistically homogenous with the vehicle treatment.

Statistical analysis 5. On the effects of a suite of neuropeptides at 10^{-6}M on the s-vLNs (data from figure 3E).

Data were analyzed through one-way repeated measures ANOVA with the fixed factor Peptide [DH31, DH44, sNPF, CT, IPNa, PACAP38, PDF, Vehicle, VIP, MTYa, AstC, DMS] and within-subjects factor time. A Tukey post-hoc comparison of marginal means was performed on the levels of the fixed factor Peptide.

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	32.436	98	.331	319.584	.000
	Greenhouse-Geisser	32.436	3.066	10.578	319.584	.000
	Huynh-Feldt	32.436	3.432	9.452	319.584	.000
	Lower-bound	32.436	1.000	32.436	319.584	.000
time * Peptide	Sphericity Assumed	17.502	1078	.016	15.676	.000
	Greenhouse-Geisser	17.502	33.729	.519	15.676	.000
	Huynh-Feldt	17.502	37.749	.464	15.676	.000
	Lower-bound	17.502	11.000	1.591	15.676	.000
Error(time)	Sphericity Assumed	12.585	12152	.001		
	Greenhouse-Geisser	12.585	380.214	.033		
	Huynh-Feldt	12.585	425.532	.030		
	Lower-bound	12.585	124.000	.101		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	10827.710	1	10827.710	43177.426	.000
Peptide	51.333	11	4.667	18.609	.000
Error	31.096	124	.251		

Tukey Multiple Comparisons

Peptide	N	Subset	
		1	2
PDF	13	.7727	
DH31	13	.7934	
sNPF	12		.9004
DH44	12		.9068
CT	10		.9173
PACAP38	12		.9221
IPNa	8		.9224
Vehicle	11		.9408
VIP	11		.9430
MTYa	9		.9535
AstC	14		.9541
DMS	11		.9627
Sig.		.998	.150

There is a strongly significant within-subjects effect of time ($p<0.001$). There is also a strongly significant ($p<0.001$) effect of the peptide treatment. A Tukey HSD post-hoc comparison of the treatment means reveals that only the DH31 and PDF marginal means are statistically distinct from the vehicle controls. Both statistically distinct means form a single homogenous subset with FRET loss levels greater than controls.

Statistical analysis 6. On the effects of a suite of neuropeptides at 10^{-6}M on the l-vLNs (data from Figure 3F).

Data were analyzed through one-way repeated measures ANOVA with the fixed factor Peptide [DH31, DH44, sNPF, CT, IPNa, PACAP38, PDF, Vehicle, VIP, MTYa, AstC, DMS] and within-subjects factor time. A Tukey post-hoc comparison of marginal means was performed on the levels of the fixed factor Peptide.

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	18.841	98	.192	390.710	.000
	Greenhouse-Geisser	18.841	3.226	5.840	390.710	.000
	Huynh-Feldt	18.841	3.769	4.998	390.710	.000
	Lower-bound	18.841	1.000	18.841	390.710	.000
time * Peptide	Sphericity Assumed	16.974	1078	.016	32.001	.000
	Greenhouse-Geisser	16.974	35.490	.478	32.001	.000
	Huynh-Feldt	16.974	41.464	.409	32.001	.000
	Lower-bound	16.974	11.000	1.543	32.001	.000
Error(time)	Sphericity Assumed	4.340	8820	.000		
	Greenhouse-Geisser	4.340	290.370	.015		
	Huynh-Feldt	4.340	339.247	.013		
	Lower-bound	4.340	90.000	.048		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	7566.269	1	7566.269	110753.345	.000
Peptide	47.141	11	4.286	62.730	.000
Error	6.148	90	.068		

Tukey Multiple Comparisons

	N	Subset				
Peptide	1	A	B	C	D	E
DH31	7	.6885				
DH44	5		.8957			
sNPF	6		.9133	.9133		
CT	6		.9134	.9134		
IPNa	6		.9222	.9222	.9222	
PACAP38	10		.9333	.9333	.9333	
PDF	14		.9388	.9388	.9388	
Vehicle	14		.9388	.9388	.9388	
VIP	6			.9570	.9570	.9570
MTYa	9				.9590	.9590
AstC	12				.9634	.9634
DMS	7					.9990
Sig.		1.000	.080	.070	.111	.097

There is a strongly significant within-subjects effect of time ($p<0.001$). There is also a strongly significant ($p<0.001$) effect of the peptide treatment. A Tukey HSD post-hoc comparison of the treatment means reveals that only the DH31 and DMS marginal means are statistically distinct from the vehicle controls. The DH31 produces a higher FRET loss while the DMS produces a lower FRET loss than controls.

Statistical analysis 7. On the effects of 10^{-5}M PDF on s-vLNs in w^{1118} and han^{5304} flies (data from figure 4).

Data were analyzed through two-way repeated measures ANOVA with the fixed factors genotype [w^{1118}, han^{5304}] and Drug [Vehicle, FSK, PDF] followed by a Tukey post-hoc comparison of the three levels of Drug.

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	39.231	98	.400	211.924	.000
	Greenhouse-Geisser	39.231	2.611	15.026	211.924	.000
	Huynh-Feldt	39.231	2.962	13.246	211.924	.000
	Lower-bound	39.231	1.000	39.231	211.924	.000
time * Genotype	Sphericity Assumed	1.913	98	.020	10.335	.000
	Greenhouse-Geisser	1.913	2.611	.733	10.335	.000
	Huynh-Feldt	1.913	2.962	.646	10.335	.000
	Lower-bound	1.913	1.000	1.913	10.335	.002
time * Drug	Sphericity Assumed	13.423	196	.068	36.256	.000
	Greenhouse-Geisser	13.423	5.222	2.571	36.256	.000
	Huynh-Feldt	13.423	5.923	2.266	36.256	.000
	Lower-bound	13.423	2.000	6.712	36.256	.000
time * Genotype * Drug	Sphericity Assumed	2.692	196	.014	7.271	.000
	Greenhouse-Geisser	2.692	5.222	.516	7.271	.000
	Huynh-Feldt	2.692	5.923	.454	7.271	.000
	Lower-bound	2.692	2.000	1.346	7.271	.001
Error(time)	Sphericity Assumed	11.292	5978	.002		
	Greenhouse-Geisser	11.292	159.262	.071		
	Huynh-Feldt	11.292	180.660	.063		
	Lower-bound	11.292	61.000	.185		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	4400.847	1	4400.847	21419.645	.000
Genotype	8.321	1	8.321	40.501	.000
Drug	68.555	2	34.278	166.835	.000
Genotype * Drug	12.883	2	6.441	31.351	.000
Error	12.533	61	.205		

Tukey Multiple Comparisons

Drug	N	Subset		
	1	2	3	1
FSK	21	.6946		
PDF	25		.8258	
Veh	21			.9556
Sig.		1.000	1.000	1.000

There is a strongly significant within-subjects effect of time ($p<0.001$). There is also a strongly significant ($p<0.001$) effect both main effects factors (Drug and Genotype) in addition to a significant interaction suggesting that the genotypes respond differently to the drug treatments. A Tukey post-hoc comparison reveals that there are no homogenous subsets of marginal means for the Drug treatment levels; each level produces its own distinct effect.

Statistical analysis 8. On the effects of DH31 on the l-vLNs and s-vLNs in w^{1118} and han^{5304} flies (data from Figure 5).

Data were analyzed using repeated measures two-way ANOVA with main effects factors genotype [Han^{5304}, w^{1118}] and cell-type [l-vLNs, s-vLNs].

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	53.048	98	.541	124.398	.000
	Greenhouse-Geisser	53.048	3.014	17.600	124.398	.000
	Huynh-Feldt	53.048	3.440	15.423	124.398	.000
	Lower-bound	53.048	1.000	53.048	124.398	.000
time * Genotype	Sphericity Assumed	1.549	98	.016	3.632	.000
	Greenhouse-Geisser	1.549	3.014	.514	3.632	.014
	Huynh-Feldt	1.549	3.440	.450	3.632	.011
	Lower-bound	1.549	1.000	1.549	3.632	.063
time * Cell	Sphericity Assumed	.605	98	.006	1.419	.004
	Greenhouse-Geisser	.605	3.014	.201	1.419	.239
	Huynh-Feldt	.605	3.440	.176	1.419	.235
	Lower-bound	.605	1.000	.605	1.419	.239
time * Genotype * Cell	Sphericity Assumed	1.799	98	.018	4.218	.000
	Greenhouse-Geisser	1.799	3.014	.597	4.218	.007
	Huynh-Feldt	1.799	3.440	.523	4.218	.005
	Lower-bound	1.799	1.000	1.799	4.218	.045
Error(time)	Sphericity Assumed	20.469	4704	.004		
	Greenhouse-Geisser	20.469	144.679	.141		
	Huynh-Feldt	20.469	165.103	.124		
	Lower-bound	20.469	48.000	.426		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	3623.110	1	3623.110	4790.274	.000
Genotype	.246	1	.246	.325	.571
Cell	.021	1	.021	.028	.869
Genotype * Cell	2.890	1	2.890	3.820	.056
Error	36.305	48	.756		

There is a strongly significant within-subjects effect of time ($p<0.001$). There is no significant effect of either main effects factor (genotype or cell-type) but a marginally significant interaction between the two ($p=0.056$).

Statistical analysis 9. On the effects of PDFr overexpression on the responses of l-vLNs and s-vLNs to 10^{-5}M PDF (data from figure 6).

Data were analyzed by two-way repeated measures ANOVA with fixed factors cell-type [l-vLNs, s-vLNs] and PDFr expression [control, over-expression].

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	154.575	98	1.577	237.744	.000
	Greenhouse-Geisser	154.575	2.562	60.342	237.744	.000
	Huynh-Feldt	154.575	2.743	56.343	237.744	.000
	Lower-bound	154.575	1.000	154.575	237.744	.000
time * Cell	Sphericity Assumed	2.245	98	.023	3.453	.000
	Greenhouse-Geisser	2.245	2.562	.876	3.453	.023
	Huynh-Feldt	2.245	2.743	.818	3.453	.020
	Lower-bound	2.245	1.000	2.245	3.453	.067
time * PDFr	Sphericity Assumed	15.379	98	.157	23.653	.000
	Greenhouse-Geisser	15.379	2.562	6.003	23.653	.000
	Huynh-Feldt	15.379	2.743	5.606	23.653	.000
	Lower-bound	15.379	1.000	15.379	23.653	.000
time * Cell * PDFr	Sphericity Assumed	5.689	98	.058	8.750	.000
	Greenhouse-Geisser	5.689	2.562	2.221	8.750	.000
	Huynh-Feldt	5.689	2.743	2.074	8.750	.000
	Lower-bound	5.689	1.000	5.689	8.750	.004
Error(time)	Sphericity Assumed	54.615	8232	.007		
	Greenhouse-Geisser	54.615	215.177	.254		
	Huynh-Feldt	54.615	230.451	.237		
	Lower-bound	54.615	84.000	.650		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	4413.371	1	4413.371	8245.491	.000
Cell	8.589	1	8.589	16.047	.000
PDFr	61.820	1	61.820	115.497	.000
Cell * PDFr	18.758	1	18.758	35.045	.000
Error	44.961	84	.535		

There is a strongly significant within-subjects effect of time ($p<0.001$). There is also a strongly significant ($p<0.001$) effect of both main effects factors (Peptide and PDFr over-expression state). Additionally, there was a significant interaction ($p<0.001$) indicating that the different cell types responded differently to the state of PDFr overexpression.

Statistical analysis 10. On the effects of 10^{-6}M PDF on various clock neuron classes (data from figure 7).

Data were analyzed through two-way repeated measures ANOVA with the fixed factors peptide treatment [Vehicle, PDF] and Cell [5th s-vLN, dLN, DN1a, DN1p, DN2, DN3]. A Tukey post-hoc comparison of the cell types was then performed.

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	70.453	98	.719	244.829	.000
	Greenhouse-Geisser	70.453	3.442	20.471	244.829	.000
	Huynh-Feldt	70.453	3.766	18.706	244.829	.000
	Lower-bound	70.453	1.000	70.453	244.829	.000
time * Cell	Sphericity Assumed	5.350	490	.011	3.718	.000
	Greenhouse-Geisser	5.350	17.208	.311	3.718	.000
	Huynh-Feldt	5.350	18.831	.284	3.718	.000
	Lower-bound	5.350	5.000	1.070	3.718	.003
time * Peptide	Sphericity Assumed	17.175	98	.175	59.685	.000
	Greenhouse-Geisser	17.175	3.442	4.990	59.685	.000
	Huynh-Feldt	17.175	3.766	4.560	59.685	.000
	Lower-bound	17.175	1.000	17.175	59.685	.000
time * Cell *	Sphericity Assumed	2.352	490	.005	1.635	.000
Peptide	Greenhouse-Geisser	2.352	17.208	.137	1.635	.051
	Huynh-Feldt	2.352	18.831	.125	1.635	.044
	Lower-bound	2.352	5.000	.470	1.635	.154
Error(time)	Sphericity Assumed	46.330	15778	.003		
	Greenhouse-Geisser	46.330	554.108	.084		
	Huynh-Feldt	46.330	606.370	.076		
	Lower-bound	46.330	161.000	.288		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	9908.492	1	9908.492	17172.368	.000
Cell	12.740	5	2.548	4.416	.001
Peptide	81.426	1	81.426	141.118	.000
Cell * Peptide	7.291	5	1.458	2.527	.031
Error	92.897	161	.577		

Tukey Post-Hoc Comparison

Cell	N	Subset		
		1	2	3
DN2	16	.23321		
DN1a	22	.27193	.27193	
LNd	52	.28577	.28577	
DN3	22	.30234	.30234	.30234
DN1p	45		.32148	.32148
5thSmall	16			.36234
Sig.		.054	.321	.137

There is a strongly significant within-subjects effect of time ($p<0.001$). There is also a strongly significant ($p<0.001$) effect of both main effects factors (peptide treatment and cell-type). Additionally, there was a significant interaction ($p=0.031$) indicating that the different cell types responded differently to the peptide treatments. Finally, a Tukey post-hoc comparison revealed that there were homogenous subsets of cell types relating to their degree of responsiveness to PDF.

Statistical analysis 11. On the effects of 10^{-6}M PDF on the dorsal projections of s-vLNs (data from figure S3).

Data were analyzed using repeated measures single-factor ANOVA with main effects factor Peptide treatment [Vehicle, PDF].

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	7.292	98	.074	19.702	.000
	Greenhouse-Geisser	7.292	2.448	2.979	19.702	.000
	Huynh-Feldt	7.292	4.049	1.801	19.702	.000
	Lower-bound	7.292	1.000	7.292	19.702	.002
time * Peptide	Sphericity Assumed	4.436	98	.045	11.985	.000
	Greenhouse-Geisser	4.436	2.448	1.812	11.985	.000
	Huynh-Feldt	4.436	4.049	1.095	11.985	.000
	Lower-bound	4.436	1.000	4.436	11.985	.009
Error(time)	Sphericity Assumed	2.961	784	.004		
	Greenhouse-Geisser	2.961	19.585	.151		
	Huynh-Feldt	2.961	32.396	.091		
	Lower-bound	2.961	8.000	.370		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	667.399	1	667.399	1793.904	.000
Peptide	10.603	1	10.603	28.501	.001
Error	2.976	8	.372		

There is a strongly significant within-subjects effect of time ($p<0.001$). There is also a strongly significant ($p=0.001$) effect of the peptide treatment with mean FRET loss values of the PDF treatment higher than those of vehicle.

Statistical analysis 12. On the effects of 10^{-5}M PDF on the l-vLNs and s-vLNs observed with confocal imaging (data from figure 8).

Data were analyzed through two-way repeated measures ANOVA with the fixed factors Peptide [Vehicle, PDF] and cell-type [l-vLNs, s-vLNs].

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	13.036	98	.133	128.112	.000
	Greenhouse-Geisser	13.036	2.120	6.150	128.112	.000
	Huynh-Feldt	13.036	2.336	5.582	128.112	.000
	Lower-bound	13.036	1.000	13.036	128.112	.000
time * Cell	Sphericity Assumed	3.923	98	.040	38.552	.000
	Greenhouse-Geisser	3.923	2.120	1.851	38.552	.000
	Huynh-Feldt	3.923	2.336	1.680	38.552	.000
	Lower-bound	3.923	1.000	3.923	38.552	.000
time * Peptide	Sphericity Assumed	4.049	98	.041	39.789	.000
	Greenhouse-Geisser	4.049	2.120	1.910	39.789	.000
	Huynh-Feldt	4.049	2.336	1.734	39.789	.000
	Lower-bound	4.049	1.000	4.049	39.789	.000
time * Cell *	Sphericity Assumed	3.606	98	.037	35.433	.000
Peptide	Greenhouse-Geisser	3.606	2.120	1.701	35.433	.000
	Huynh-Feldt	3.606	2.336	1.544	35.433	.000
	Lower-bound	3.606	1.000	3.606	35.433	.000
Error(time)	Sphericity Assumed	5.393	5194	.001		
	Greenhouse-Geisser	5.393	112.352	.048		
	Huynh-Feldt	5.393	123.782	.044		
	Lower-bound	5.393	53.000	.102		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	4532.719	1	4532.719	45666.595	.000
Cell	10.831	1	10.831	109.119	.000
Peptide	11.375	1	11.375	114.606	.000
Cell * Peptide	11.135	1	11.135	112.187	.000
Error	5.261	53	.099		

There is a strongly significant within-subjects effect of time ($p<0.001$). There is also a strongly significant ($p<0.001$) effect of both main effects factors (peptide and cell-type). Additionally, there was a highly significant interaction ($p<0.001$) indicating that the different cell-types responded differently to the drug treatments.

Statistical analysis 13. On the effects of 10^{-5}M PDF on DN2s using confocal imaging (data from figure 8).

Data were analyzed through one-way repeated measures ANOVA with the fixed factor Peptide [Vehicle, PDF].

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
time	Sphericity Assumed	5.172	98	.053	30.607	.000
	Greenhouse-Geisser	5.172	2.897	1.786	30.607	.000
	Huynh-Feldt	5.172	3.761	1.375	30.607	.000
	Lower-bound	5.172	1.000	5.172	30.607	.000
time * peptide	Sphericity Assumed	1.195	98	.012	7.069	.000
	Greenhouse-Geisser	1.195	2.897	.412	7.069	.001
	Huynh-Feldt	1.195	3.761	.318	7.069	.000
	Lower-bound	1.195	1.000	1.195	7.069	.017
Error(time)	Sphericity Assumed	2.873	1666	.002		
	Greenhouse-Geisser	2.873	49.243	.058		
	Huynh-Feldt	2.873	63.929	.045		
	Lower-bound	2.873	17.000	.169		

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1469.297	1	1469.297	4278.251	.000
peptide	5.227	1	5.227	15.219	.001
Error	5.838	17	.343		

There is a strongly significant within-subjects effect of time ($p<0.001$). There is also a strongly significant ($p=0.001$) effect the peptide indicating that PDF treatments had statistically higher levels of FRET loss than vehicle treatments.

Supplemental Figures

Supplemental Figure S1

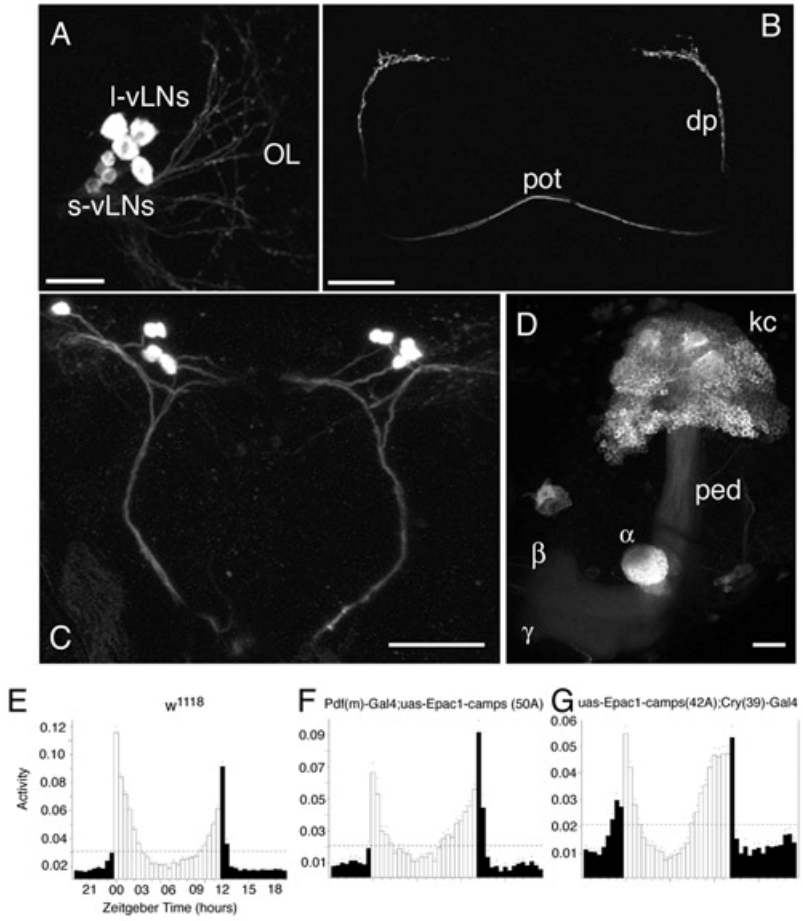

Figure S1. *Drosophila* neurons are not affected morphologically by Gal4/uas-driven Epac1-camps expression. A. Confocal z-series reconstruction of vLNs in a living *uasEpac1-camps(42A)/y;BMRJ-Gal4/+* fly. The number and morphology of the l-vLNs and s-vLNs are normal, as are the l-vLN projections over the optic lobe (OL). Scale bar = 25uM. B. Confocal z-series reconstruction of the posterior brain of the same genotype reveals normal dorsal projections (dp) and posterior optic tract (pot). Scale Bar = 50uM. C. Confocal z-series reconstruction of the corazonin neurons in a living *uas-Epac1-camps50A/+;Crz-Gal4/+* brain. The number and morphology of Epac1-camps-expressing neurons are normal. D. The morphology of the mushroom bodies is normal in living *uas-Epac1-camps(50A)/+;30Y-Gal4/+ brains*. The Kenyon cells (kc) pedunculus (ped) α-, β-, and γ-lobes are labeled. E. Average daily activity of 32 w^{1118} (wild-type) male flies during one week under a 12:12 LD cycle. F. Average daily activity of 32 *Pdf(m)-Gal4;uas-Epac1-camps(50A)* male flies during one week under a 12:12 LD cycle. G. Average daily activity of 32 *uas-Epac1-camps*(*42A*);*Cry(39)-Gal4* male flies during one week of a 12:12 LD cycle.

Supplemental Figure S2

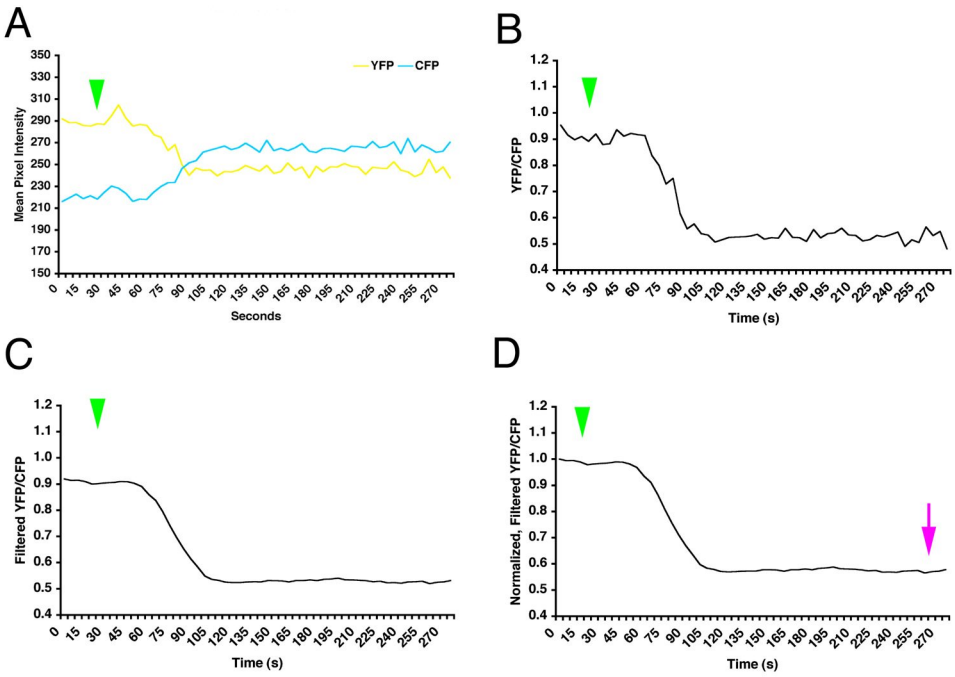

Figure S2. Quantification, filtering, and normalization of Epac1-camps FRET in a single 10µM forskolin-treated l-vLN. A. Time-course of CFP Donor and YFP FRET emission intensity values for the l-LNv shown in figure 1C. Green triangles represent the start of the drop-wise addition of 10µM forskolin in all graphs. B. Time-course of YFP/CFP ratio from A. C. The data from B after application of a 7-point moving average filter. D. The data from C after normalization to the first time-point. Averages of population responses were obtained from the filtered and normalized data. The magenta arrow indicates the lowest YFP/CFP value used to determine the maximum FRET loss (see Figure 3B).

Supplemental Figure S3

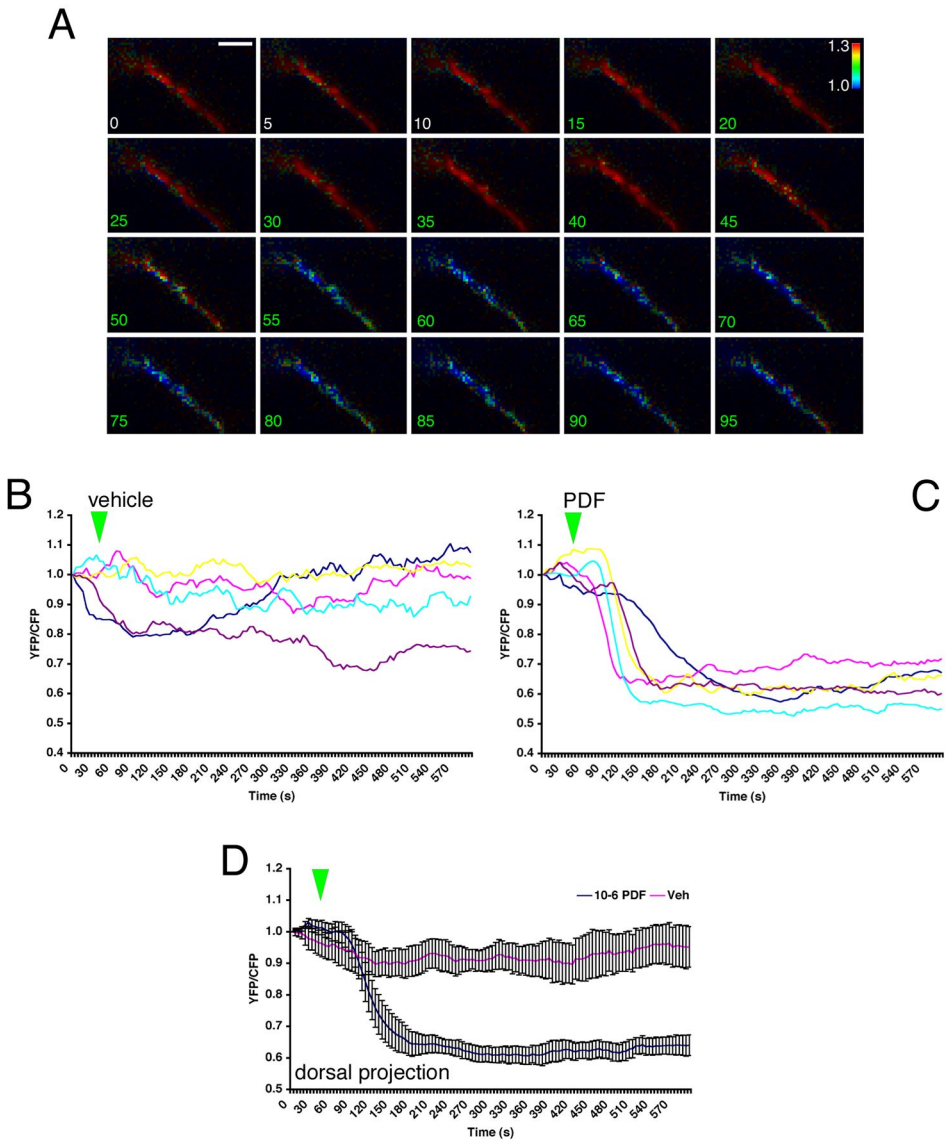

Figure S3. A. Pseudo-colored time-course of Epac1-camps FRET in the dorsal projections of the s-vLNs in a *Pdf(m)-Gal4;uas-Epac1-camps(50A)* brain treated with 10^{-6}M PDF. The time-point of each image is indicated on each frame in seconds and green times indicated the presence of PDF. The look-up table values un-normalized YFP/CFP values. Scale bar = 10µm. B. Epac1-camps FRET plots for 5 s-vLN dorsal projections from 5 vehicle treated *Pdf(m)-Gal4;uas-Epac1-camps(50A)* brains. The green triangle indicates the start of bath-application. C. Epac1-camps FRET plots for 5 s-vLN dorsal projections from 5 *Pdf(m)-Gal4;uas-Epac1-camps(50A)* brains treated with 10^{-6}M PDF. D. Average Epac1-camps FRET plots response of the s-vLN dorsal projections of PDF (blue) and vehicle (magenta) treated *Pdf(m)-*

Gal4;uas-Epac1-camps(50A) brains. A single-factor repeated measures ANOVA revealed that the effect of PDF on the dorsal projections was highly significant (p=0.01).

Supplemental Figure S4

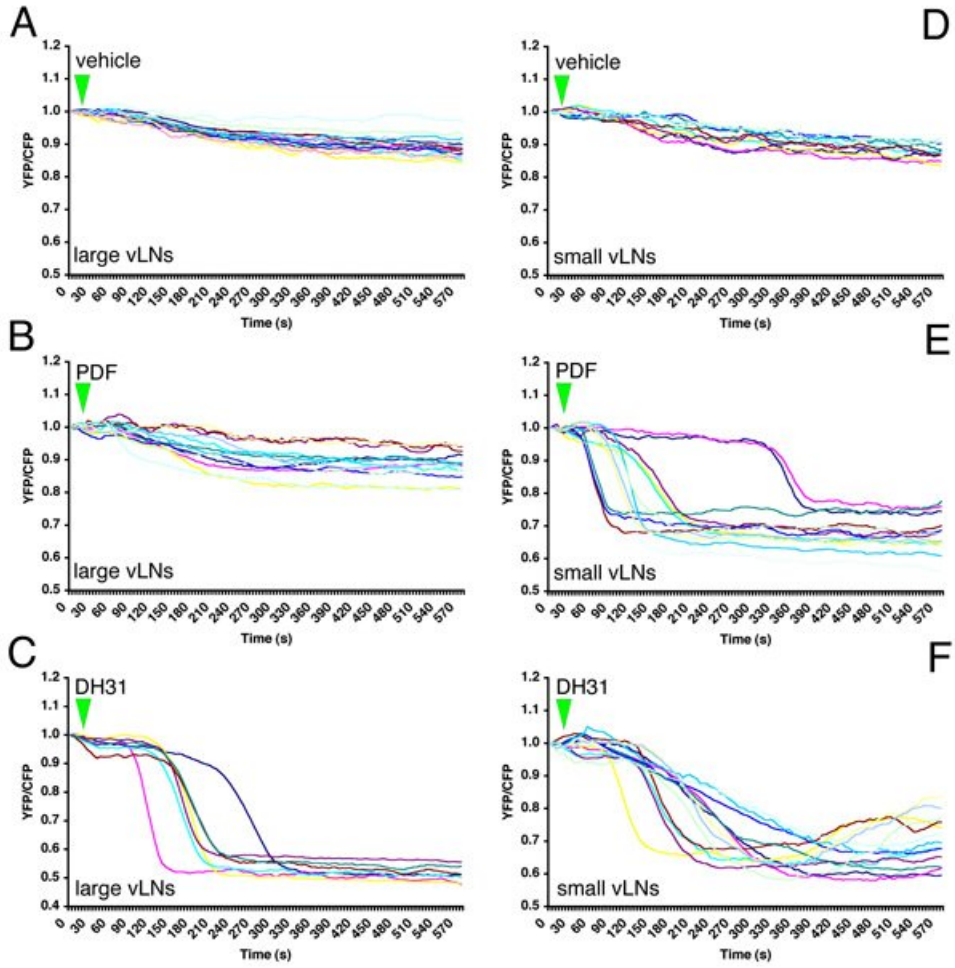

Figure S4. Both the l-vLNs and s-vLNs respond to bath-applied 10^{-6}M DH31, but only the s-vLNs respond to 10^{-6}M PDF. A-F. Individual cell plots from vehicle, DH31, and PDF treated l-vLNs and s-vLNs from figures 3E and F. A. Epac1-camps FRET time-courses for 14 l-vLNs from five brains treated with vehicle (0.1% DMSO in HL3 Saline). Green triangles indicate the start of bath-application. B. Epac1-camps FRET time-courses for 13 l-vLNs from six brains treated with 10^{-6}M PDF. C. Epac1-camps FRET time-courses for 7 l-vLNs from four brains treated with 10^{-6}M DH31. D. Epac1-camps FRET time-courses for 11 s-vLNs from five brains treated with vehicle. E. Epac1-camps FRET time-courses for 13 s-vLNs from five brains treated with 10^{-6}M PDF. F. Epac1-camps FRET time-courses for 13 s-vLNs from five brains treated with 10^{-6}M DH31.

Supplemental Figure S5

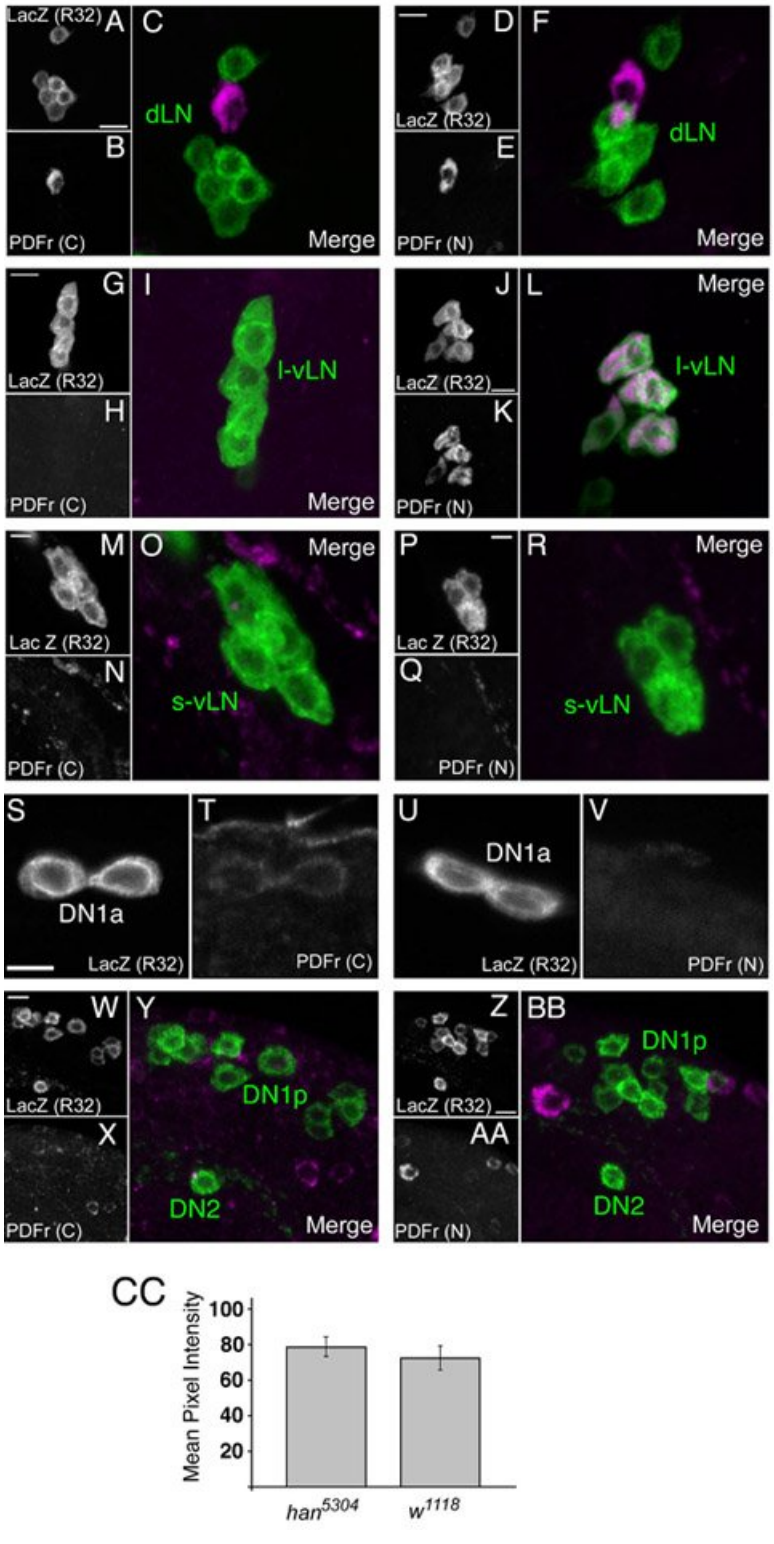

Figure S5. A comparison of anti-PDFr immunosignals in the clock neurons of P{*R32*-lacZ} flies. A-C. Anti-PDFr(C) immunostaining in the dLNs. A. A z-series of anti-β-gal staining in the region containing the dLNs (scale bar = 10uM). B. Anti-PDFr(C) signals in the same z-series. C. Merged image of (A) and (B) with β-gal in green and anti-PDF(C) in magenta. There is no clear overlap of anti-PDFr and anti-β-gal immunosignals. D-E. Anti PDFr(N) immunostaining in the dLNs. D. A z-series of anti- β-gal staining containing the dLNs (scale bar = 10uM). E. Anti-PDFr(N) in the same series. C. Merged image (D) and (E) with β-gal in green and anti-PDF(N) in magenta. There is no clear overlap of anti-PDFr and anti- β-gal immunosignals. Note the similarities in size and location of the PDFr-positive cells in (C) and (F). G-I. Anti-PDFr(C) immunostaining in the l-vLNs. G. A z-series of anti-β-gal staining containing the l-vLNs (scale bar = 10uM). H. Anti-PDFr(C) in the same z-series. D. Merged image of G and H with β-gal in green and anti-PDF(C) in magenta. There are no cell bodies immunoreactive to anti-PDFr(C) in this z-series. J-L. Anti-PDFr(N) immunostaining in the l-vLNs. J. A z-series of anti-LacZ staining containing the l-vLNs (scale bar = 10uM). K. Anti-PDFr(N) in the same z-series. L. Merged images of (J) and (K) reveal clear co-localization of PDFr immunosignals and R32- β-gal (c.f. Hyun *et al.* 2005). M-O. Anti-PDFr(C) immunostaining in the s-vLNs. M. A z-series of anti-β-gal staining containing the s-vLNs (scale bar = 5uM). N. Anti-PDFr(C) signals in the same z-series. O. Merged image of (M) and (N) with β-gal in green and anti-PDF(C) in magenta. PDFr(C)-positive puncta a visible near the s-vLN cell bodies, but there is no co-localization. P-R. Anti-PDFr(N) immunostaining in the s-vLNs. P. A z-series of anti-β-gal staining containing the s-vLNs (scale bar = 5uM). Q. Anti-PDFr(N) signals in the same z-series. R. Merged image of (P) and (Q) with β-gal in green and anti-PDF(N) in magenta. PDFr(N)-positive puncta a visible near the s-vLN cell bodies, but there is no co-localization. S-T. Anti-PDFr(C) immunostaining in $DN1_a$s. S. A single optical section of β-gal-labeled $DN1_a$s. T. The same optical section scanned for PDFr(C) immunoreactivity reveals weak PDFr(C) immunosignals (Mertens *et al.* 2005; scale bar = 5uM). U-V. Anti PDFr(N) immunostaining in $DN1_a$s. U. A single optical section of β-gal-labeled $DN1_a$s. V. The same optical section scanned for PDFr(N) immunoreactivity reveals no PDFr(N) immunosignals. W-Y. Anti-PDFr(C) immunostaining in $DN1_p$s and the DN2s. W. A z-series containing β-gal-labeled DN1ps and a DN2. X. The same z-series scanned for anti-PDFr(c). Y. A merged image of E and F reveals PDFr(C)-positive soma and puncta with no clear overlap in immunosignals (Mertens *et al.* 2005). Z-BB. Anti-PDFr(N) immunostaining in $DN1_p$s and the DN2s. Z. A z-series containing β-gal-labeled $DN1_p$s and a DN2. AA. The same z-series scanned for anti-PDFr(N). BB. A merged image of (H) and (I) reveals PDFr(N)-positive soma and puncta with no clear overlap in immunosignals. CC. A quantification of anti-PDFr(N) immunosignals in the l-vLNs of han^{5304} and w^{1118} flies reveals no significant difference in staining intensity.

Supplemental Figure S6

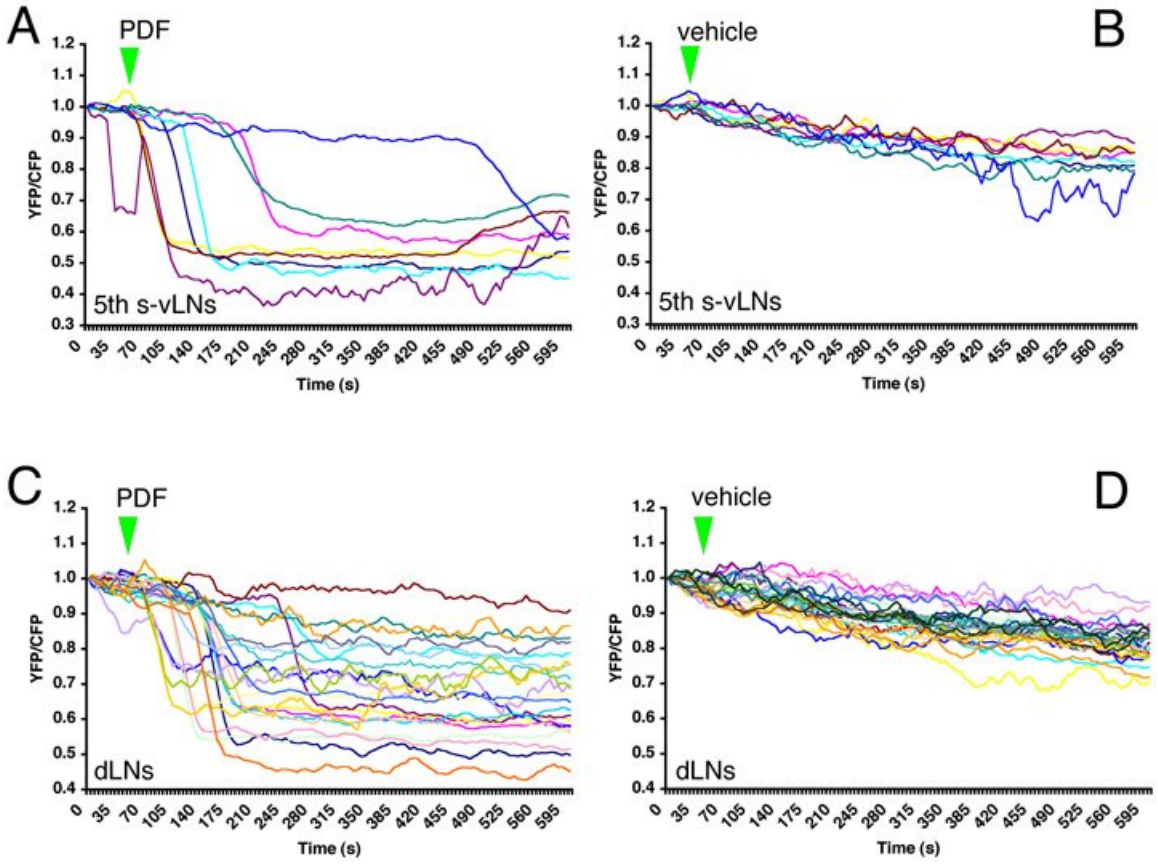

Figure S6. The 5th s-vLN and the dLNs respond to bath-applied PDF with cAMP increases. A. Epac1-camps FRET time-courses for eight 5th s-vLNs from eight *uas-Epac1-camps(42A)/y;Cry(39)-Gal4/Pdf-Gal80* brains treated with 10^{-5}M PDF. Green triangles indicate the time of bath application. B. Epac1-camps FRET time-courses for eight 5th s-vLNs from eight *uas-Epac1-camps(42A/y);Cry(39)-Gal4/Pdf-Gal80* brains treated with vehicle (0.1% DMSO in HL3 saline). C. Epac1-camps FRET time-courses for 24 dLNs from eight *uas-Epac1-camps(42A)/y;Cry(39)-Gal4* brains treated with 10^{-5}M PDF. Note the presence of neurons that did not respond to PDF. Green triangles indicate the time of bath application. D. Epac1-camps FRET time-courses for 28 dLNs from eight *uas-Epac1-camps(42A)/y;Cry(39)-Gal4* brains treated with vehicle (0.1% DMSO in HL3 saline).

Supplemental Figure S7

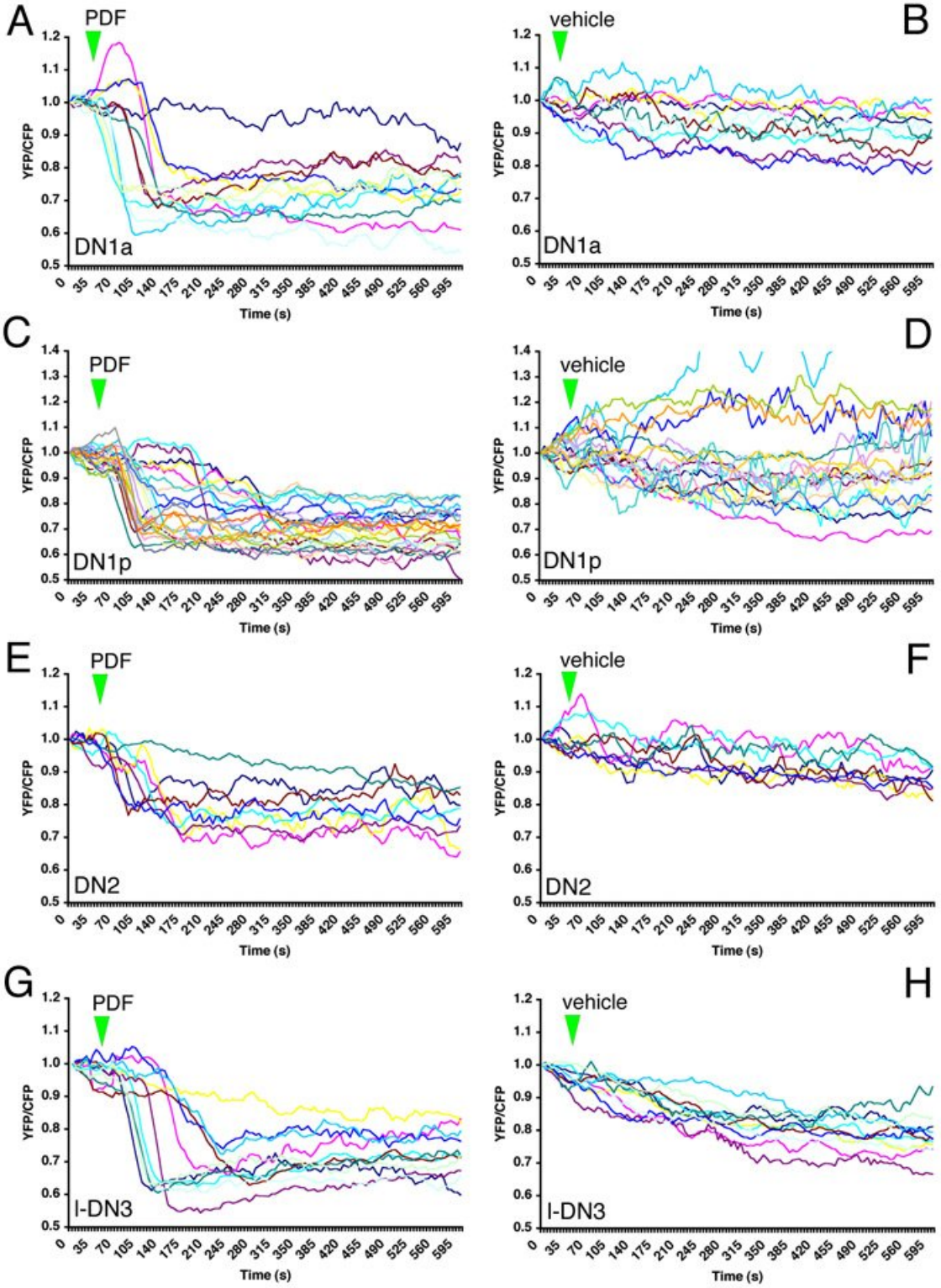

Figure S7. All classes of DN respond to bath-applied 10^{-5}M PDF with increases in cAMP. A. Epac1-camps FRET time-courses for 12 $DN1_a$s from seven *uas-Epac1-camps(42A)/y;Cry(39)-Gal4* brains treated with 10^{-5}M PDF. Only one neuron failed to respond to PDF. Green triangles indicate the time of bath application. B. Epac1-camps FRET time-courses for ten $DN1_a$s from 5 *uas-Epac1-camps(42A)/y;Cry(39)-Gal4* brains treated with vehicle (0.1% DMSO in HL3 saline). C. A. Epac1-camps FRET time-courses for 24 $DN1_p$s from seven *uas-Epac1-camps(42A)/y;Cry(39)-Gal4* brains treated with 10^{-5}M PDF. D. Epac1-camps FRET time-courses for 21 $DN1_p$s from 7 *uas-Epac1-camps(42A)/y;Cry(39)-Gal4* brains treated with vehicle (0.1% DMSO in HL3 saline). Note the large fluctuations and variance in cAMP dynamics. E. Epac1-camps FRET time-courses for eight DN2s from 6 *uas-Epac1-camps(42A)/y;Cry(39)-Gal4* brains treated with 10^{-5}M PDF. F. Epac1-camps FRET time-courses for eight DN2s from six *uas-Epac1-camps(42A)/y;Cry(39)-Gal4* brains treated with vehicle (0.1% DMSO in HL3 saline). G. Epac1-camps FRET time-courses for eleven l-DN3s from nine *uas-Epac1-camps(42A)/y;Cry(39)-Gal4* brains treated with 10^{-5}M PDF. Only one l-DN3 failed to respond to PDF. H. Epac1-camps FRET time-courses for eleven 1-DN3s from seven *uas-Epac1-camps(42A)/y;Cry(39)-Gal4* brains treated with vehicle (0.1% DMSO in HL3 saline).