

Supplemental Information: **Characterization of dopamine releasable and reserve pools in *Drosophila* larvae using ATP/P2X₂ mediated stimulation**

Ning Xiao and B. Jill Venton

Table S1 Statistics for dopamine release during stimulations repeated at 0.5, 1, 2, 5 or 10 min intervals.

Two way ANOVA shows a significant interaction of stimulation interval and stimulation number ($F[28,220] = 5.291$, $p < 0.0001$) and significant main effects of stimulation interval ($F[4,220] = 236.7$, $p < 0.0001$) and stimulation number ($F[7,220] = 14.98$, $p < 0.0001$). The table below shows Bonferroni post test comparisons. Significant values are highlighted.

30 s vs 1 min			1 min vs 2 min			2 min vs 10 min		
Stim Number	t	P value	Stim Number	t	P value	Stim Number	t	P value
Stim 1	0	P > 0.05	Stim 1	0.000	P > 0.05	Stim 1	0.000	P > 0.05
Stim 2	3.674	P < 0.01	Stim 2	1.369	P > 0.05	Stim 2	4.411	P < 0.001
Stim 3	2.85	P < 0.05	Stim 3	1.400	P > 0.05	Stim 3	5.986	P < 0.001
Stim 4	2.092	P > 0.05	Stim 4	2.254	P > 0.05	Stim 4	6.228	P < 0.001
Stim 5	2.664	P > 0.05	Stim 5	2.059	P > 0.05	Stim 5	6.152	P < 0.001
Stim 6	2.619	P > 0.05	Stim 6	1.703	P > 0.05	Stim 6	6.524	P < 0.001
Stim 7	2.492	P > 0.05	Stim 7	1.910	P > 0.05	Stim 7	6.431	P < 0.001
Stim 8	2.48	P > 0.05	Stim 8	1.827	P > 0.05	Stim 8	6.634	P < 0.001
30 s vs 2 min			1 min vs 5 min			5 min vs 10 min		
Stim Number	t	P value	Stim Number	t	P value	Stim Number	t	P value
Stim 1	0.000	P > 0.05	Stim 1	0.000	P > 0.05	Stim 1	0.000	P > 0.05
Stim 2	5.042	P < 0.001	Stim 2	4.464	P < 0.001	Stim 2	1.216	P > 0.05
Stim 3	4.250	P < 0.001	Stim 3	5.449	P < 0.001	Stim 3	1.814	P > 0.05
Stim 4	4.346	P < 0.001	Stim 4	6.447	P < 0.001	Stim 4	1.875	P > 0.05
Stim 5	4.619	P < 0.001	Stim 5	7.300	P < 0.001	Stim 5	1.101	P > 0.05
Stim 6	4.219	P < 0.001	Stim 6	7.231	P < 0.001	Stim 6	1.198	P > 0.05
Stim 7	4.304	P < 0.001	Stim 7	6.803	P < 0.001	Stim 7	1.716	P > 0.05
Stim 8	4.211	P < 0.001	Stim 8	7.278	P < 0.001	Stim 8	1.382	P > 0.05
30 s vs 5 min			1 min vs 10 min					
Stim Number	t	P value	Stim Number	t	P value			
Stim 1	0.000	P > 0.05	Stim 1	0.000	P > 0.05			
Stim 2	7.993	P < 0.001	Stim 2	5.726	P < 0.001			
Stim 3	8.188	P < 0.001	Stim 3	7.332	P < 0.001			
Stim 4	8.457	P < 0.001	Stim 4	8.393	P < 0.001			
Stim 5	9.860	P < 0.001	Stim 5	8.443	P < 0.001			
Stim 6	9.747	P < 0.001	Stim 6	8.474	P < 0.001			
Stim 7	9.197	P < 0.001	Stim 7	8.584	P < 0.001			
Stim 8	9.661	P < 0.001	Stim 8	8.712	P < 0.001			

30 s vs 10 min			2 min vs 5 min		
Stim Number	t	P value	Stim Number	t	P value
Stim 1	0.000	P > 0.05	Stim 1	0.000	P > 0.05
Stim 2	9.256	P<0.001	Stim 2	3.149	P < 0.05
Stim 3	10.07	P<0.001	Stim 3	4.104	P<0.001
Stim 4	10.40	P<0.001	Stim 4	4.281	P<0.001
Stim 5	11.00	P<0.001	Stim 5	5.051	P<0.001
Stim 6	10.99	P<0.001	Stim 6	5.326	P<0.001
Stim 7	10.98	P<0.001	Stim 7	4.715	P<0.001
Stim 8	11.10	P<0.001	Stim 8	5.252	P<0.001

Table S2 Statistics for cocaine and 3-iodotyrosine on repeated stimulations.

Two way ANOVA shows a significant interaction of stimulation number and drug ($F[14,168] = 3.47, p < 0.0001$) and significant main effect of stimulation number ($F[7,168] = 86.58, p < 0.0001$) and drug ($F[2,168] = 67.61, p < 0.0001$) for the 1 min interval stimulation. There are also a significant interaction of stimulation number and drug ($F[14,121] = 11.77, p < 0.0001$) and significant main effect of stimulation number ($F[7,121] = 24.26, p < 0.0001$) and drug ($F[2,121] = 405.17, p < 0.0001$) for the 5 min interval stimulation. The table below shows Bonferroni post test comparisons. Significant values are highlighted.

Stimulations 1 min apart								
buffer vs cocaine			buffer vs 3-iodotyrosine			cocaine vs 3-iodotyrosine		
Stim Number	t	P value	Stim Number	t	P value	Stim Number	t	P value
Stim 1	0.000	P > 0.05	Stim 1	0.000	P > 0.05	Stim 1	0.000	P > 0.05
Stim 2	6.381	P < 0.001	Stim 2	1.137	P > 0.05	Stim 2	5.406	P < 0.001
Stim 3	5.880	P < 0.001	Stim 3	1.462	P > 0.05	Stim 3	4.541	P < 0.001
Stim 4	4.839	P < 0.001	Stim 4	1.889	P > 0.05	Stim 4	3.007	P < 0.05
Stim 5	4.496	P < 0.001	Stim 5	2.398	P > 0.05	Stim 5	2.109	P > 0.05
Stimulations 5 min apart								
buffer vs cocaine			buffer vs 3-iodotyrosine			cocaine vs 3-iodotyrosine		
Stim Number	t	P value	Stim Number	t	P value	Stim Number	t	P value
Stim 1	0.000	P > 0.05	Stim 1	0.000	P > 0.05	Stim 1	0.000	P > 0.05
Stim 2	4.230	P < 0.001	Stim 2	6.859	P < 0.001	Stim 2	2.888	P < 0.05
Stim 3	3.705	P < 0.01	Stim 3	9.004	P < 0.001	Stim 3	5.299	P < 0.001
Stim 4	4.586	P < 0.001	Stim 4	10.77	P < 0.001	Stim 4	6.186	P < 0.001
Stim 5	5.967	P < 0.001	Stim 5	12.86	P < 0.001	Stim 5	6.897	P < 0.001

Table S3 Statistics for cocaine and 3-iodotyrosine on repeated stimulations.

For cocaine, two way ANOVA shows a significant interaction of stimulation number and stimulation interval ($F[7,106] = 12.01, p < 0.0001$) and significant main effect of stimulation number ($F[7,106] = 58.25, p < 0.0001$) and stimulation interval ($F[1,106] = 480.94, p < 0.0001$).

For 3-iodotyrosine, there is no significant interaction of stimulation number and stimulation interval ($F[7,95] = 0.48, p = 0.8491$) and no significant main effect of stimulation interval ($F[1,95] = 7.68, p = 0.0067$) but a significant main effect of stimulation number ($F[7,95] = 405.17, p < 0.0001$). The table below shows Bonferroni post test comparisons. Significant values are highlighted.

Cocaine 1 min vs 5 min			3-iodotyrosine 1 min vs 5 min		
Stim Number	t	P value	Stim Number	t	P value
Stim 1	0.000	P > 0.05	Stim 1	0.000	P > 0.05
Stim 2	9.673	P < 0.001	Stim 2	0.078	P > 0.05
Stim 3	10.62	P < 0.001	Stim 3	0.731	P > 0.05
Stim 4	9.885	P < 0.001	Stim 4	0.821	P > 0.05
Stim 5	9.133	P < 0.001	Stim 5	1.399	P > 0.05