

The Ventral Tegmental Area has calbindin neurons with the capability to co-release glutamate and dopamine into the nucleus accumbens

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Table 1: Total number of different neuronal populations in the VTA

	CB only	VGluT2-CB	Total CB	Total VGluT2
Rat 1	2134.5	1318	3452.5	4466.5
Rat 2	942	464	1406	1487
Rat 3	2108.5	938.5	3047	3465
Rat 4	967.5	481	1448.5	1571.5
Total	6152.5	3201.5	9354	10990

Table 2: Number of total CB and VGluT2-CB neurons across different bregma levels

	Total CB neurons				
	4.92	5.2	5.4	5.64	6.48
Rat 1	101	585	902.5	624	398
Rat 2	70	209	374.5	438.5	214
Rat 3	292	746.5	1030.5	331	279
Rat 4	91	204.5	385	434.5	234

	VGluT2-CB neurons				
	4.92	5.2	5.4	5.64	6.48
Rat 1	18.5	277.5	380	223	102.5
Rat 2	62	159	79	131.5	31.5
Rat 3	73	288.5	293	141.5	55
Rat 4	55	112	89	150	71

Table 3: Number of VGluT2-CB neurons in rostral, medial and caudal VTA

	Total numbers of VGluT2-CB			
	Rostral	Medial	Caudal	Total
Subject 1	18.5	667.5	632	1318
Subject 2	62	179	223	464
Subject 3	101	605	232.5	938.5
Subject 4	55	131	295	481
Mean	59.125	395.625	345.625	3201.5
S.E.M.	16.9046	139.8531037	96.7848505	
Total	236.5	1582.5	1382.5	3201.5

Table 4: Number of VGluT2-CB neurons in different VTA nuclei

	RLi	IF	PN	PIF	PBP	CLi	Total
Subject 1	183.5	447.5	257.5	65.5	328	36	1318
Subject 2	88	182	61.5	39.5	77	16	464
Subject 3	257	328	124.5	54	175	0	938.5
Subject 4	111	110.5	57.5	58	128	16	481
Total	639.5	1068	501	217	708	68	3201.5

Table 5: Number of VGluT2-CB neurons at bregma level -5.28 mm in the RLi and IF nuclei

Bregma, 5.28		Total CB	Total VGluT2	VGluT2-CB	CB-only	VGluT2 only	double
Rat 1	RLi	62	152	55	7	97	55
Rat 2	RLi	44	115	41	3	74	41
Rat 4	RLi	34	112	29	5	83	29
	Total	140	379	125	15	254	125
Bregma, 5.28							
Rat 1	IF	59	80	40	19	40	40
Rat 2	IF	93	124	88	5	36	88
Rat 4	IF	49	79	41	8	38	41
	Total	201	283	169	32	114	169

Table 6: Total number of neurons in the VTA

	PV only	VGluT2-PV	Total PV	Total VGluT2
Rat 1	107	86	193	2985
Rat 2	89	74	163	3797
Rat 3	110	82	192	3556
Rat 4	37	112	149	3956
Total	343	354	697	14294

Table 7: Number of total PV neurons and VGluT2-PV neurons across different bregma levels in the VTA

	Total PV neurons				
	4.92	5.2	5.4	5.64	6.48
Rat 1	2	10	99	0	47
Rat 2	2	38	44	4	0
Rat 3	6	66	34	7	26
Rat 4	0	45	57	8	3

	VGluT2-PV neurons				
	4.92	5.2	5.4	5.64	6.48
Rat 1	0	6	67	0	4
Rat 2	2	19	34	3	0
Rat 3	6	28	23	7	0
Rat 4	0	35	52	6	0

Table 8: Number of VGluT2-PV neurons in rostral, medial and caudal VTA

	Total numbers VGluT2-PV			Total
	Rostral	Medial	Caudal	
Subject 1	0	52	34	86
Subject 2	4	50	20	74
Subject 3	9	45	28	82
Subject 4	4	68	40	112
Mean	4.25	53.75	30.5	
S.E.M.	1.84	4.97	4.27	
Total	17	215	122	354

Table 9: Number of VGluT2-PV neurons in different VTA nuclei

	RLi	IF	PN	PIF	PBP	CLi	Total
Subject 1	7	14	57	4	4	0	86
Subject 2	3	7	45	3	16	0	74
Subject 3	3	13	45	1	20	0	82
Subject 4	4	19	62	6	21	0	112
Total	17	53	209	14	61	0	354

Table 10: Number of VGluT2-PV neurons at bregma level -5.28 mm in the RLi and IF nuclei

RLi, Bregma 5.28				out of total VGluT2			
	Total PV	VGluT2-PV	PV only	% PV only neurons	% VGluT2-PV neurons	% PV+VGluT2 neurons/VGluT2	Total VGluT2
Rat 1	2	1	1	50	50	1.49	67
Rat 2	4	3	1	25	75	2.10	143
Rat 3	3	1	2	66.67	33.33	0.57	175
Total	9	5					385
Mean				47.22	52.78	1.39	
SD					20.97		
SEM					12.11	0.44	

IF, Bregma 5.28							
	Total PV	VGluT2-PV	PV only	% PV only neurons	% VGluT2/PV neurons	% PV+VGluT2 neurons/VGluT2	Total VGluT2
Rat 1	2	2	0	0	100	4.17	48
Rat 2	3	0	3	100	0	0	120
Rat 3	3	2	1	33.33	66.67	1.85	108
Total	8	4	4				276
Mean				44.44	55.56	2.01	
SD					50.92		
SEM					29.40	1.21	

Table 11: Total number of neurons in the VTA

	CR only	VGluT2-CR	Total CR	Total VGluT2
Rat 1	2017	11	2028	985.5
Rat 2	4174	20.5	4194.5	2559.5
Rat 3	4656	9	4664.5	2126
Rat 4	4075	17	4092	1896
Total	14922	57.5	14979	7567

Table 12: Number of VGluT2-CR neurons in rostral, medial and caudal VTA

	Total numbers VGluT2-CR			Total
	Rostral	Medial	Caudal	
Subject 1	7	4	0	11
Subject 2	2	15.5	3	20.5
Subject 3	1	6	2	9
Subject 4	1	14	2	17
Mean	2.75	9.875	1.75	
S.E.M.	1.44	2.86	0.63	
Total	11	39.5	7	57.5

Table 13: Number of VGluT2-CR neurons in different VTA nuclei

	RLi	IF	PN	PIF	PBP	CLi	Total
Subject 1	5	3	1	0	2	0	11
Subject 2	7.5	4	5	0	4	0	20.5
Subject 3	4	1	1	0	3	0	9
Subject 4	4	5	0	1	7	0	17
Total	20.5	13	7	1	16	0	57.5

Table 14: Number of sections from each subject covering entire VTA

	Number of sections
Rat 1	20
Rat 2	22
Rat 3	23
Rat 4	27
Mean	23
S.E.M.	1.472

Table 15: Number of VGluT2-CB neurons in the VTA coexpressing either TH or VGaT

	VGluT2+ CB	VGluT2+CB+TH (no VGaT)	VGluT2+CB+VGaT (no TH)	VGluT2+CB+TH+VGaT
Rat 1	101	23	16	2
Rat 2	137	18	17	10
Rat 3	163	19	16	16
Reg Rat 2	105	28	13	6
Total	506	88	62	34
Average %		18.56	12.61	6.20
S.E.M.		3.66	1.24	1.64

Table 16: Phenotype of retrogradely-labeled neurons in the VTA following Fluoro-Gold (FG) injections in the nucleus accumbens shell

Cases (FG in nAcc Shell)	Total FG neurons	Total FG- VGluT2 neurons	FG-VGluT2 neurons coexpressing CB-IR	FG-VGluT2 neurons lacking CB- IR	FG-VGluT2- CB neurons coexpressing TH-IR	FG- VGluT2- CB neurons lacking TH- IR
Rat 8	551	255	57	198	32	25
Rat 6	406	49	17	32	17	0
Rat 2	311	72	16	56	15	1
Average	422.67	125.33	30	95.33	21.33	8.67
S.E.M.	69.78	65.15	13.50	51.80	5.36	8.17
Total	1268	376	90	286	64	26

GraphPad Prism Statistical analysis

Subregional comparison VGluT2-CB (4 rats)

Friedman test				
Table Analyzed	subregional comparison VGluT2-CB (4 rats)			
Repeated Measures ANOVA				
P value	0.0010			
P value summary	**			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	6			
F	7.535			
R square	0.7152			
Was the pairing significantly effective?				
R square	0.2706			
F	6.515			
P value	0.0049			
P value summary	**			
Is there significant matching? (P < 0.05)	Yes			
ANOVA Table				
	SS	df	MS	
Treatment (between columns)	161324	5	32265	
Individual (between rows)	83694	3	27898	
Residual (random)	64232	15	4282	
Total	309250	23		
Newman-Keuls Multiple Comparison Test				
	Mean Diff.	q	Significant? P < 0.05?	Summary
Cli vs IF	-250.0	7.641	Yes	***
Cli vs PBP	-160.0	4.890	Yes	*
Cli vs Rli	-142.9	4.367	Yes	*
Cli vs PN	-108.3	3.308	No	ns
Cli vs PIF	-37.25	---	No	ns
PIF vs IF	-212.8	6.502	Yes	**
PIF vs PBP	-122.8	3.752	No	ns
PIF vs Rli	-105.6	---	No	ns
PIF vs PN	-71.00	---	No	ns
PN vs IF	-141.8	4.332	Yes	*
PN vs PBP	-51.75	---	No	ns
PN vs Rli	-34.63	---	No	ns
Rli vs IF	-107.1	3.274	No	ns
Rli vs PBP	-17.13	---	No	ns
PBP vs IF	-90.00	---	No	ns

Rostromedial comparison VGluT2-CB (4 rats)

Table Analyzed	Rostral-medial-caudal comparison VGluT2-CB (4 rats)		
Repeated Measures ANOVA			
P value	0.0688		
P value summary	ns		
Are means signif. different? (P < 0.05)	No		
Number of groups	3		
F	4.320		
R square	0.5902		
Was the pairing significantly effective?			
R square	0.2725		
F	1.828		
P value	0.2425		
P value summary	ns		
Is there significant matching? (P < 0.05)	No		
ANOVA Table			
	SS	df	MS
Treatment (between columns)	263753	2	131876
Individual (between rows)	167388	3	55796
Residual (random)	183156	6	30526
Total	614296	11	

Subregional comparison VGluT2-CR (4 rats)

Table Analyzed	subregional comparison VGluT2-CR (4 rats)			
Repeated Measures ANOVA				
P value	0.0008			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	6			
F	7.987			
R square	0.7269			
Was the pairing significantly effective?				
R square	0.1065			
F	2.183			
P value	0.1324			
P value summary	ns			
Is there significant matching? (P < 0.05)	No			
ANOVA Table				
	SS	df	MS	
Treatment (between columns)	86.05	5	17.21	
Individual (between rows)	14.11	3	4.705	
Residual (random)	32.32	15	2.155	
Total	132.5	23		
Newman-Keuls Multiple Comparison Test				
	Mean Diff.	q	Significant? P < 0.05?	Summary
Cli vs Rli	-5.125	6.983	Yes	**
Cli vs PBP	-4.000	5.450	Yes	*
Cli vs IF	-3.250	4.428	Yes	*
Cli vs PN	-1.750	2.384	No	ns
Cli vs PIF	-0.2500	---	No	ns
PIF vs Rli	-4.875	6.642	Yes	**
PIF vs PBP	-3.750	5.109	Yes	*
PIF vs IF	-3.000	4.087	Yes	*
PIF vs PN	-1.500	---	No	ns
PN vs Rli	-3.375	4.598	Yes	*
PN vs PBP	-2.250	3.066	No	ns
PN vs IF	-1.500	---	No	ns
IF vs Rli	-1.875	2.555	No	ns
IF vs PBP	-0.7500	---	No	ns
PBP vs Rli	-1.125	---	No	ns

Rostromedial comparison VGluT2-CR (4 rats)

Table Analyzed	Rostral-medial-caudal comparison VGluT2-CR (4 rats)			
Repeated Measures ANOVA				
P value	0.0500			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
Number of groups	3			
F	5.142			
R square	0.6315			
Was the pairing significantly effective?				
R square	0.1923			
F	1.292			
P value	0.3599			
P value summary	ns			
Is there significant matching? (P < 0.05)	No			
ANOVA Table				
	SS	df	MS	
Treatment (between columns)	135.0	2	67.52	
Individual (between rows)	50.90	3	16.97	
Residual (random)	78.79	6	13.13	
Total	264.7	11		
Newman-Keuls Multiple Comparison Test				
	Mean Diff.	q	Significant? P < 0.05?	Summary
Caudal vs Medial	-8.125	4.484	Yes	*
Caudal vs Rostral	-3.000	1.656	No	ns
Rostral vs Medial	-5.125	2.829	No	ns

Subregional comparison VGluT2-PV (4 rats)

Table Analyzed	subregional comparison VGluT2-PV (4 rats)			
Repeated Measures ANOVA				
P value	< 0.0001			
P value summary	****			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	6			
F	60.95			
R square	0.9531			
Was the pairing significantly effective?				
R square	0.01699			
F	1.842			
P value	0.1828			
P value summary	ns			
Is there significant matching? (P < 0.05)	No			
ANOVA Table				
	SS	df	MS	
Treatment (between columns)	7453	5	1491	
Individual (between rows)	135.2	3	45.06	
Residual (random)	366.8	15	24.46	
Total	7955	23		
Newman-Keuls Multiple Comparison Test				
	Mean Diff.	q	Significant? P < 0.05?	Summary
Cli vs PN	-52.25	21.13	Yes	***
Cli vs PBP	-15.25	6.168	Yes	**
Cli vs IF	-13.25	5.359	Yes	**
Cli vs Rli	-4.250	1.719	No	ns
Cli vs PIF	-3.500	---	No	ns
PIF vs PN	-48.75	19.72	Yes	***
PIF vs PBP	-11.75	4.752	Yes	*
PIF vs IF	-9.750	3.943	Yes	*
PIF vs Rli	-0.7500	---	No	ns
Rli vs PN	-48.00	19.41	Yes	***
Rli vs PBP	-11.00	4.449	Yes	*
Rli vs IF	-9.000	3.640	Yes	*
IF vs PN	-39.00	15.77	Yes	***
IF vs PBP	-2.000	0.8089	No	ns
PBP vs PN	-37.00	14.96	Yes	***

Rostromedial comparison VGluT2-PV (4 rats)

Table Analyzed	Rostral-medial-caudal comparison VGluT2-PV (4 rats)			
Repeated Measures ANOVA				
P value	0.0002			
P value summary	***			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	3			
F	51.44			
R square	0.9449			
Was the pairing significantly effective?				
R square	0.04948			
F	1.889			
P value	0.2324			
P value summary	ns			
Is there significant matching? (P < 0.05)	No			
ANOVA Table				
	SS	df	MS	
Treatment (between columns)	4907	2	2453	
Individual (between rows)	270.3	3	90.11	
Residual (random)	286.2	6	47.69	
Total	5463	11		
Newman-Keuls Multiple Comparison Test				
	Mean Diff.	q	Significant? P < 0.05?	Summary
rostral vs medial	-49.50	14.34	Yes	***
rostral vs caudal	-26.25	7.602	Yes	**
caudal vs medial	-23.25	6.733	Yes	**