Spontaneous changes in brain striatal dopamine synthesis and storage dynamics *ex vivo* reveal end-product feedback-inhibition of tyrosine hydroxylase

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SUPPLEMENTARY MATERIAL

TABLE S1. Inclusion list for mass spectrometry method. In order to detect specifically the sites of interest the protein was virtually digested and a list with the theoretical tryptic peptides containing the modification sites was generated

modification	sequence	z=1	z=2	z=3
	(K)GFRRAVSEQDAK(Q)	1363.7077	682.35385	455.2359
1Phospho	(K)GFRRAVSEQDAK(Q)	1443.674	722.337	481.891333
	(R)RAVSEQDAK(Q)	1003.5167	502.25835	335.172233
1Phospho	(R)RAVSEQDAK(Q)	1083.483	542.2415	361.827667
	(R)RAVSEQDAKQAEAVTSPR(F)	1942.9941	971.99705	648.331367
1Phospho	(R)RAVSEQDAKQAEAVTSPR(F)	2022.9604	1011.9802	674.9868
2Phospho	(R)RAVSEQDAKQAEAVTSPR(F)	2102.9268	1051.9634	701.642267
3Phospho	(R)RAVSEQDAKQAEAVTSPR(F)	2182.8931	1091.94655	728.2977
	(R)AVSEQDAK(Q)	847.4156	424.2078	283.138533
1Phospho	(R)AVSEQDAK(Q)	927.3819	464.19095	309.793967
	(R)AVSEQDAKQAEAVTSPR(F)	1786.893	893.9465	596.297667
1Phospho	(R)AVSEQDAKQAEAVTSPR(F)	1866.8593	933.92965	622.9531
2Phospho	(R)AVSEQDAKQAEAVTSPR(F)	1946.8256	973.9128	649.608533
3Phospho	(R)AVSEQDAKQAEAVTSPR(F)	2026.792	1013.896	676.264
	(R)AVSEQDAKQAEAVTSPRFIGR(R)	2260.168	1130.584	754.056
1Phospho	(R)AVSEQDAKQAEAVTSPRFIGR(R)	2340.1344	1170.5672	780.711467
2Phospho	(R)AVSEQDAKQAEAVTSPRFIGR(R)	2420.1007	1210.55035	807.3669
3Phospho	(R)AVSEQDAKQAEAVTSPRFIGR(R)	2500.067	1250.5335	834.022333
	(K)QAEAVTSPR(F)	958.4952	479.7476	320.165067
1Phospho	(K)QAEAVTSPR(F)	1038.4616	519.7308	346.820533
2Phospho	(K)QAEAVTSPR(F)	1118.4279	559.71395	373.475967
	(K)QAEAVTSPRFIGR(R)	1431.7703	716.38515	477.923433
1Phospho	(K)QAEAVTSPRFIGR(R)	1511.7366	756.3683	504.578867
2Phospho	(K)QAEAVTSPRFIGR(R)	1591.7029	796.35145	531.2343
	(K)QAEAVTSPRFIGRR(Q)	1587.8714	794.4357	529.957133
1Phospho	(K)QAEAVTSPRFIGRR(Q)	1667.8377	834.41885	556.612567
2Phospho	(K)QAEAVTSPRFIGRR(Q)	1747.8041	874.40205	583.268033
	(R)FIGRRQSLIEDAR(K)	1560.8605	780.93025	520.9535
1Phospho	(R)FIGRRQSLIEDAR(K)	1640.8268	820.9134	547.608933
	(R)RQSLIEDAR(K)	1087.5854	544.2927	363.195133
1Phospho	(R)RQSLIEDAR(K)	1167.5518	584.2759	389.8506
	(R)RQSLIEDARK(E)	1215.6804	608.3402	405.893467
1Phospho	(R)RQSLIEDARK(E)	1295.6467	648.32335	432.5489
	(R)QSLIEDAR(K)	931.4843	466.24215	311.161433
1Phospho	(R)QSLIEDAR(K)	1011.4507	506.22535	337.8169
	(R)QSLIEDARK(E)	1059.5793	530.28965	353.859767
1Phospho	(R)QSLIEDARK(E)	1139.5456	570.2728	380.5152
	(R)FEVPSGDLAALLSSVR(R)	1660.8905	830.94525	554.296833
	(R)TGFQLRPVAGLLSAR(D)	1585.9173	793.45865	529.305767

FIG. S1

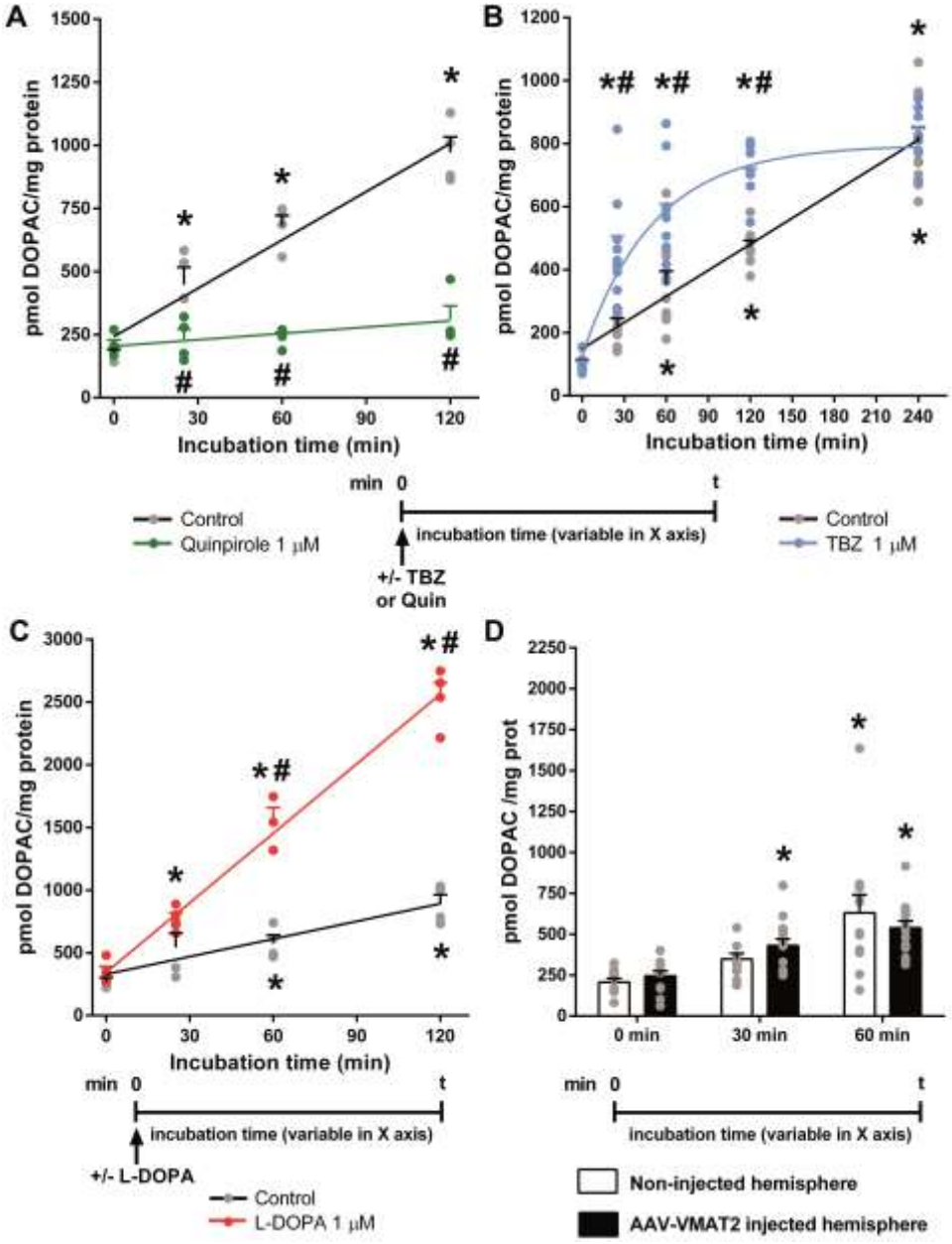
A
pmol DOPAC/mg protein
Incubation time (min)
Control
Quinpirole 1 µM
B
pmol DOPAC/mg protein
Incubation time (min)
Control
TBZ 1 µM
min 0
t
incubation time (variable in X axis)
+/- TBZ
or Quin
C
pmol DOPAC/mg protein
Incubation time (min)
min 0
t
incubation time (variable in X axis)
+/- L-DOPA
Control
L-DOPA 1 µM
D
pmol DOPAC /mg prot
0 min
30 min
60 min
min 0
t
incubation time (variable in X axis)
Non-injected hemisphere
AAV-VMAT2 injected hemisphere

Fig. S1. DOPAC content in brain striatal minces incubated in the presence of quinpirole, TBZ or L-DOPA and from VMAT2-overexpressing animals. DOPAC accumulation was measured during variable incubation times of brain striatal minces with 1 µM quinpirole (A); 1 µM TBZ (B) or 1 µM L-DOPA (C) and during the incubation of left or right striatum one month after the injection of AAV-hVMAT2 viral vector unilaterally in the right *substantia nigra* (D). The experimental design is shown in the timeline. Data and the means ± SEM of N equal to A) 4 (control), 4 (Quin); B) 6-10 (control), 10 (TBZ); C) 4 (control), 3-4 (L-DOPA) and D) 12-14 (non-injected), 11-15 (injected) brain striatal tissue incubations are represented. In B) 1 and D) 2 incubations were excluded from the analysis after values were considered outliers by the ROUT test. Control curves of DOPAC accumulation (A, B, C) adjusted to a linear equation (r^2 0.97, 0.98 and 0.93, respectively); regression also followed by Quinp (A) and L-DOPA (C) (r^2 0.84 and 0.99, respectively). Finally, TBZ (B) effects fit a one-phase association regression (r^2 0.98). Two-way ANOVA showed in A) a significant effect of Treatment ($F(1,24) = 104.3$; $p < 0.0001$) and Time ($F(3,24) = 36.1$; $p < 0.0001$), and a significant interaction between these two factors ($F(3,24) = 21.9$ $p < 0.0001$); in B) a significant effect of Treatment ($F(1,85) = 31.9$; $p < 0.0001$) and Time ($F(4,85) = 95.3$; $p < 0.0001$), and a significant interaction between these two factors ($F(4,85) = 4.5$ $p < 0.0001$); in C) a significant effect of Treatment Time ($F(1,23) = 157.0$; $p < 0.0001$) and Time ($F(3,23) = 112.4$; $p < 0.0001$), and a significant interaction between time and treatment ($F(3,23) = 40.5$ $p < 0.0001$) and in D) a significant effect of Time ($F(2,67) = 19.8$; $p < 0.0001$); *$p < 0.05$, vs. 0 min, ANOVA plus Dunnett's multiple comparisons test; $^{\#}$ $p < 0.05$, vs. data in control curve, ANOVA plus Sidak's multiple comparisons test.

FIG. S2.

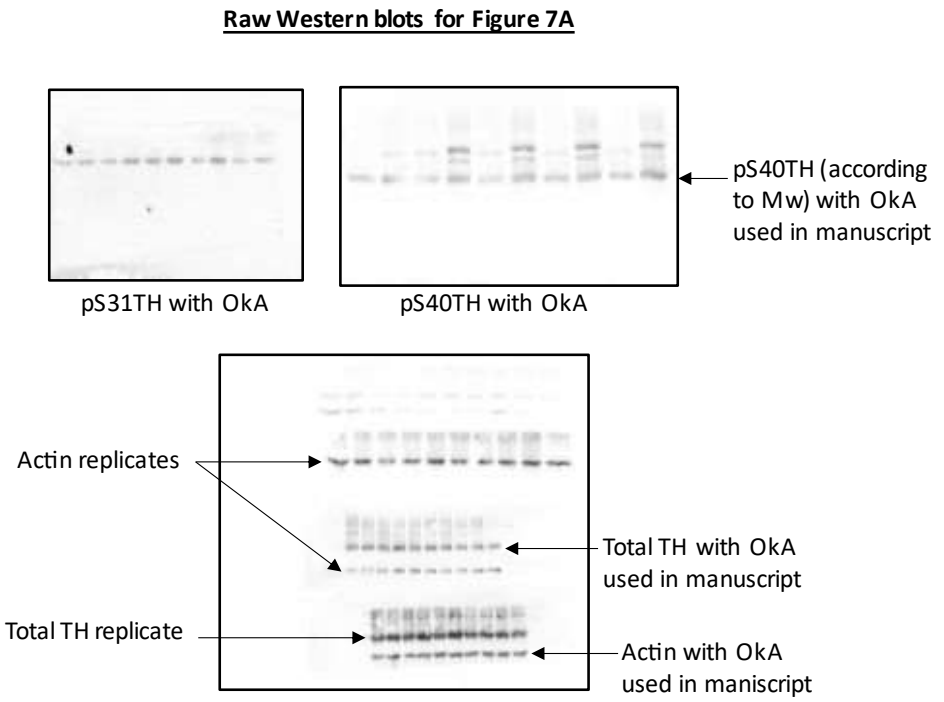

FIG. S2. Full raw western blot used in figure 7.

FIG. S3

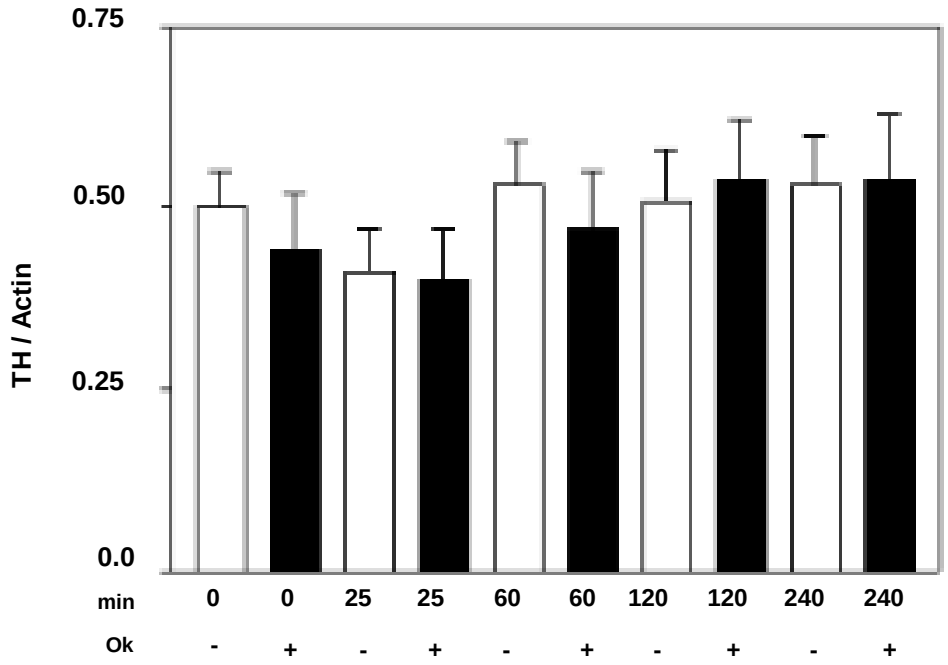

Fig. S3. TH / actin ratio in response to okadaic acid (Ok, 1 µM) at different times. Ratios shown were calculated using the same western blots used in figure 7 (A, B, and C). Mean optical densities were standardized to arbitrary units. Data represent the means ± SEM of N equal to 4-5 brain striatal tissue incubations in all graphs. 0 min samples were not incubated. ANOVA plus Sidak's multiple comparisons test was used to perform statistical analysis. No significances were found between treatments.