Supplementary Material

Supplementary Fig. 1: Gene structures of adult brain-expressed synapsins. The SYN1a and SYN1b variants are identical in all but the 3'end where the last exon of SYN1a is longer and the 3'UTR is shorter. The SYN2a and SYN2b variants are identical except in all but the 3'end where SYN2a has two extra coding exons and a completely different 3'UTR. The SYN3a and SYN3g variants are identical in the coding regions but SYN3g has an additional non-coding exon in the 5'end and consequently the two variants have distinct promoters.

Supplementary Table 1: Correlations between possible confounding factors and RQ expression values relative to GAPDH as an endogenous control. Linear regression was used for gender and Pearson's tests were used for age, pH, post-mortem delay, and RNA Integrity numbers. Only age is significantly correlated with some of the expression values, specifically for SYN1b, SYN3a, and SYN3g (see *). When age was included as a covariate in an ANCOVA analysis of differences between diagnostic groups, there was no change in significance levels from the reported ANOVA results in Table 2.

		Gender	Age	Brain pH	Post-Mortem Delay	RNA Integrity No
SYN1a RQ	r2	0,033	0,27	0,176	0,187	-0,006
	p-value	0,250	0,084	0,265	0,236	0,970
SYN1b RQ	r2	0,046	0,327	0,177	0,112	0,044
	p-value	0,168	*0.033	0,256	0,475	0,791
SYN2a RQ	r2	0,068	-0,129	-0,169	-0,107	-0,040
	p-value	0,103	0,421	0,291	0,505	0,810
SYN2b RQ	r2	0,000	0,033	0,072	-0,154	0,146
	p-value	0,996	0,83	0,64	0,319	0,375
SYN3a RQ	r2	0,023	0,324	0,09	0,059	-0,063
	p-value	0,339	*0.039	0,578	0,716	0,716
SYN3g RQ	r2	0,027	0,401	0,154	0,080	0,043
	p-value	0,307	*0.009	0,337	0,618	0,797

Supplementary Table 2: Correlations between possible confounding factors and ChIP/Input values for the different promoter regions. Linear regression was used for gender and Pearson's tests were used for age, pH, and post-mortem delay. Only gender is significantly correlated with the H3K4me3 enrichment at the SYN1a+b promoter (see *). When gender was included as a covariate in an ANCOVA analysis of differences between diagnostic groups, there was no change in significance levels from the reported ANOVA results in Table 3.

		Gender	Age	Brain pH	Post-Mortem Delay
SYN1a+b promoter	r2	0,161	-0,085	-0,064	-0,154
	p-value	*0.010	0,602	0,694	0,343
SYN2a+b promoter	r2	0,039	0,005	-0,122	-0,06
	p-value	0,313	0,979	0,536	0,761

Supplementary Method. Sample characterization:

Brains were collected in collaboration with the Quebec Coroner's Office after consent was obtained from next-of-kin and samples from brain tissue, peripheral blood and urine were collected for toxicological analysis. Two to 4 months later families were contacted and the person best acquainted with the deceased was recruited to undergo a series of structured interviews known as psychological autopsies (Dumais et al., 2005). The interviews were supplemented with information from archival material obtained from hospitals, the Coroner's office and other relevant sources. Following the interviews, clinical vignettes were produced and assessed by a panel of clinicians to generate DSM-IV diagnoses.

The controls were specifically selected to be psychiatrically healthy according to psychiatric autopsies and thus they had no history of psychiatric medication prescriptions. The effect of psychoactive drugs on synapsin gene expression and promoter H3K4me3 enrichment values was investigated both in terms of medical prescription history and toxicology at the time of death. Antidepressants were reported to be prescribed in 46% of the BD group and 40% of the MDD group in the last 3 months before death, and toxicology reports detected these drugs in 15 and 13% respectively. There was no significant correlation (Spearman's test) with expression or H3K4me3 enrichment values. Lithium was reported to be prescribed to 0% of the BD group and 6.7% of the MDD group in the last 3 months before death though in the toxicology report showed lithium in 2 of the 13 BD patients (15%) and none of the MDD or CTRL. Lifetime medication reports indicate some history of lithium in 38% of the BD group. This is unlikely affect gene expression levels at time of death, but it can explain why the patients had access to the drug. Toxicology levels of Li, but not current (last 3 months) or lifetime prescription history showed a significant correlation with expression of SYN1a (p-value = 0.025), SYN1b (p-value = 0.037), and SYN3a (p-value = 0.038), though with only 2 of 41 total subjects represented, no conclusion can be drawn as to its biological effect on synapsin gene expression.

Toxicology reports were also analyzed for tobacco, non-prescription drugs (cocaine, methamphetamine, opiates and cannabinoids detected), and alcohol use. There are no records of tobacco in toxicology reports. Non-prescription drugs were detected in 15% of the BD group, 33% of the MDD group and 15% of the CTRL group; however there was no significant correlation with synapsin gene expression or H3K4me3 promoter enrichment values. Alcohol was detected in 39% of the BD group, 40% of the MDD group and 0% of the controls. Spearman's tests revealed a significant correlation with expression of SYN1a (p-value = 0.025), SYN1b (p-value = 0.020), and SYN3a (p-value = 0.018). This is not surprising as presence of alcohol was restricted to the BD and MDD groups as a result of the ascertainment bias of selecting psychiatrically clean controls; they were also screened for alcohol abuse and dependence problems. If the correlation is computed without the control group, significance is lost: SYN1a (p-value = 0.345), SYN1b (p-value = 0.181), and SYN3a (p-value = 0.102).

References

Dumais, A, Lesage, AD, Alda, M, Rouleau, *et al.* (2005) Risk factors for suicide completion in major depression: a case-control study of impulsive and aggressive behaviors in men. *American Journal of Psychiatry*, 162(11): 2116-2124.