

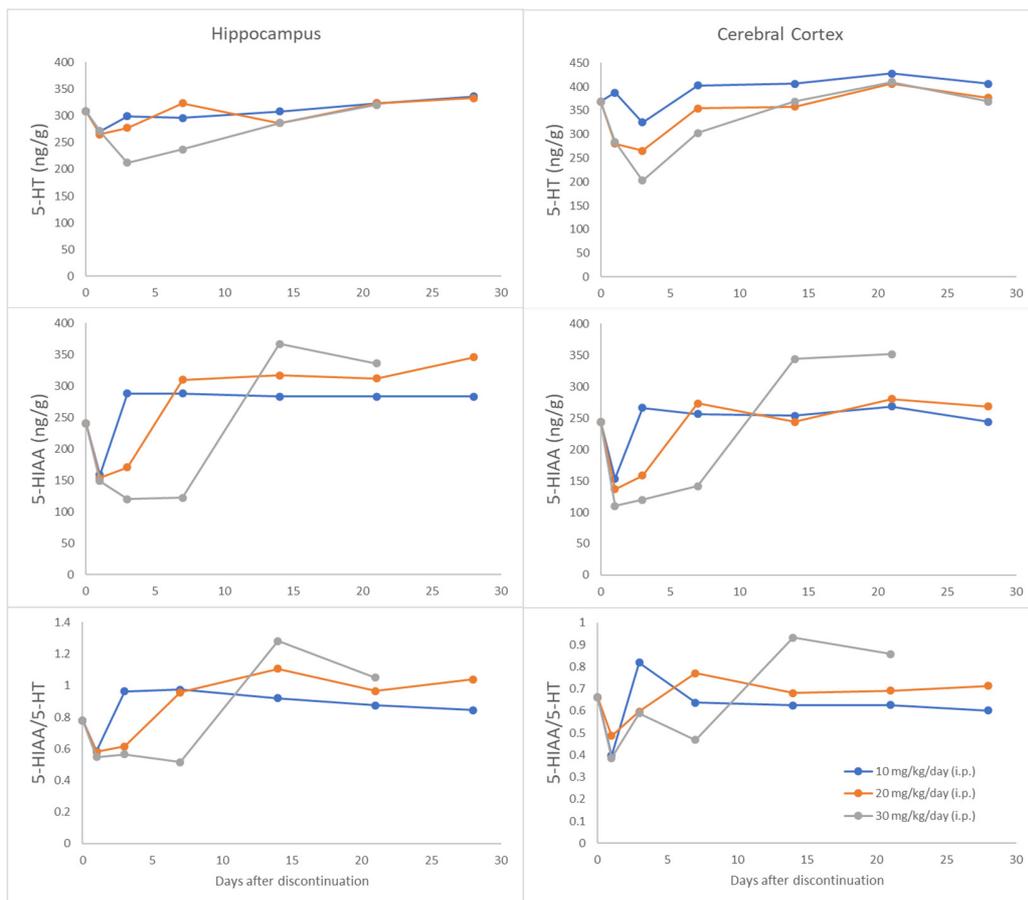
Supplementary Material

**Table S1.** Empirically based statements from researchers suggesting that serotonin is elevated in depression or that adaptations to ADMs are responsible for the antidepressant effect.

<b>Serotonin is elevated in depression</b>	
<p>“These data implicate a <i>5HT excess</i> in extracellular space” in response to inescapable shock. And, “some evidence points to a possible 5HT excess in depression.”</p>	<p>Petty et al. (1994, pp. 289-290, emphasis in original)<sup>1</sup></p>
<p>“[I]ncreased <i>5-HT</i> levels in specific brain regions might reflect depressive disorder.”</p>	<p>Zangen et al. (1997, pp. 2479-2480, emphasis in original)<sup>2</sup></p>
<p>“The marked reduction in serotonin turnover following SSRI treatment and the accompanying improvement in symptoms suggest that high brain serotonin turnover may be a biological substrate of MDD.”</p>	<p>Barton et al. (2008, p. 38)<sup>3</sup></p>

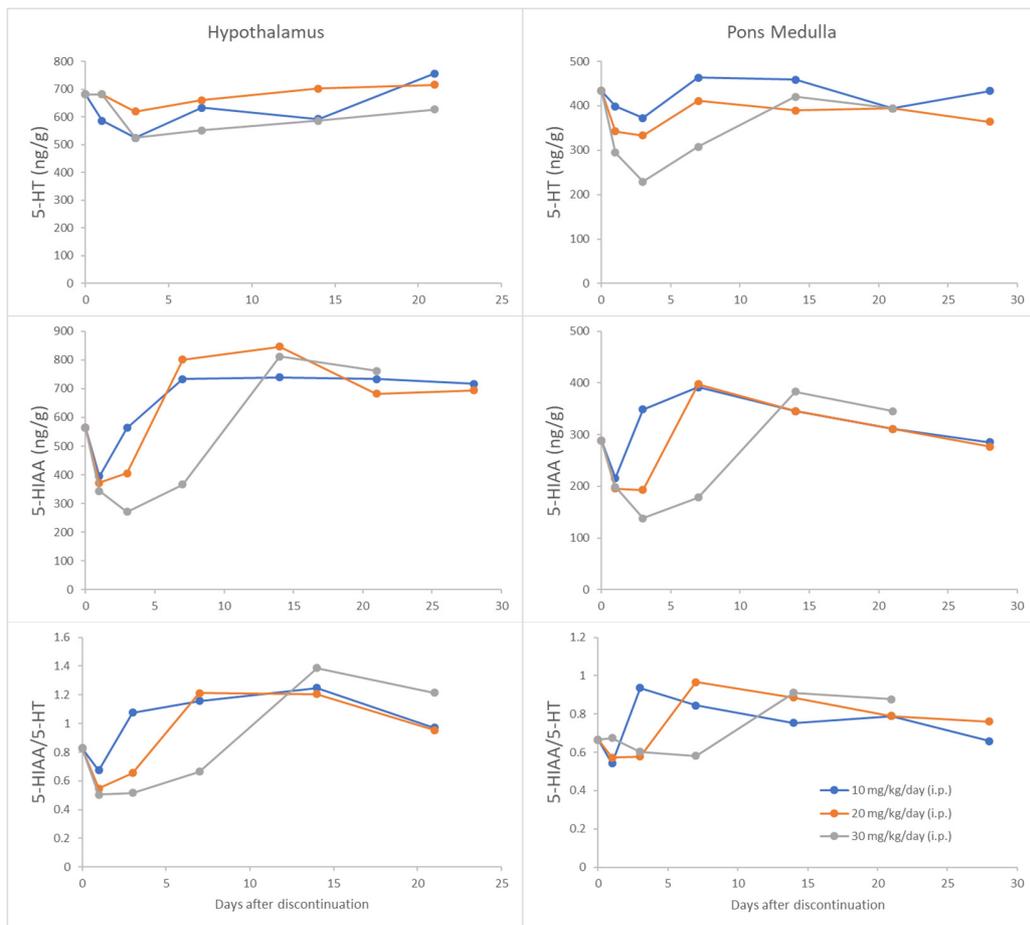
Detailed description of study by Trouvin et al. (1993)<sup>4</sup>

In a study by Trouvin and colleagues, rats were treated with fluoxetine for three weeks.<sup>4</sup> After the drug was discontinued, the rats were followed for varying periods of time and then sacrificed to measure serotonin and 5-HIAA concentrations in four brain regions—hippocampus, cortex, hypothalamus, and pons medulla (Figures S1 and S2).



**Figure S1.** Data are from Trouvin et al. (1993).<sup>4</sup> Rats were exposed to fluoxetine at 10, 20 or 30 mg/kg/day (i.p.) for 21 days, after which the drug was discontinued. The rats were followed for up to varying lengths of times after discontinuation (1, 3, 7, 14, 21, and 28 days), sacrificed, and then several brain regions (hippocampus and cerebral cortex shown here) were assayed for

serotonin (5-HT) and 5-hydroxy-indoleacetic acid (5-HIAA) content. The ratio of 5-HIAA/5-HT was calculated from this data. Day “0” represents the levels of the variables over 21 days of i.p. injection of saline. Since it was the control where no fluoxetine was administered, it represents the baseline.



**Figure S2.** Data for the hypothalamus and the pons medulla are from Trouvin et al. (1993).<sup>4</sup> See the Figure S2 legend for other details.

In Figures S1 and S2, day “0” represents the average levels of the variables over 21 days of intraperitoneal injection of saline. It can be considered the premedication baseline. Day “1”

represents the system one day after the drug had been discontinued. Based on the half-life data for fluoxetine and its active metabolite (norfluoxetine) in rats,<sup>5</sup> the rats barely had enough time to eliminate most of the drug from their body by Day “1”. (At least this would be true of the 10 mg/kg/day dose. The higher dosages may have taken longer to metabolize.) In other words, the system did not have much time to re-equilibrate, so the serotonin content at that time is probably close to the value that had equilibrated over the course of chronic fluoxetine treatment. Thus, it appears that for most fluoxetine dosages in all four brain regions, chronic fluoxetine treatment resulted in a decline in total serotonin brain content, consistent with the other research described in the paper.

By day 3, nearly all of the fluoxetine and norfluoxetine should have been metabolized. As the SERT sites in each brain region became unblocked, extracellular serotonin should have declined as it re-entered the pre-synaptic neurons. This could explain why there was often a further drop in serotonin content at day 3 in most brain regions. However, by day 7, serotonin content started to increase and eventually returned to the pre-drug (day 0) baseline value. In other words, the loss of serotonin content that occurred during chronic fluoxetine administration was eventually reversed after the drug was discontinued, and this presumably involved an upregulation in the synthesis of serotonin.

The researchers did not report the 5-HIAA/5-HT ratio, but they did report 5-HIAA and 5-HT concentrations separately, which allowed for the calculation of the ratio (Figures S1 and S2). The day 1 baseline value represents the system shortly after fluoxetine had been discontinued and it shows that serotonin neurotransmission has been suppressed (relative to the unmedicated

baseline) in all four brain regions. This is consistent with research discussed in the main text (see Figure 3). However, as fluoxetine clears from the system by day 3, serotonin transmission increases. The increase in transmission, coupled with the increase in synthesis, must have helped return forebrain serotonin concentrations to normal.

Other results in this study are worth noting. First, after discontinuation, serotonin neurotransmission exhibited an overshoot, which is consistent with our hormetic predictions. Second, in three of the four brain regions, the largest serotonin overshoot occurred at the highest fluoxetine dose, which is also consistent with our predictions. Third, in all four regions, the greater the dose, the more delayed the overshoot. Although unexpected, it is consistent with our predictions. Finally, after the overshoot, there appears to be a gradual decline in serotonin transmission, and it eventually returned to pre-fluoxetine, baseline conditions.

### References

- 1 Petty F, Kramer G, Wilson L, Jordan S. In vivo Serotonin release and learned helplessness. *Psychiatry Res* 1994; **52**: 285–93.
- 2 Zangen A, Overstreet DH, Yadid G. High Serotonin and 5-Hydroxyindoleacetic Acid Levels in Limbic Brain Regions in a Rat Model of Depression; Normalization by Chronic Antidepressant Treatment. *J Neurochem* 1997; **69**: 2477–83.
- 3 Barton DA, Esler MD, Dawood T, *et al.* Elevated brain serotonin turnover in patients with depression: Effect of genotype and therapy. *Arch Gen Psychiatry* 2008; **65**: 38–46.
- 4 Trouvin JH, Gardier AM, Chanut E, Pages N, Jacquot C. Time course of brain serotonin metabolism after cessation of long-term fluoxetine treatment in the rat. *Life Sci* 1993; **52**: PL187–92.
- 5 Qu Y, Aluisio L, Lord B, *et al.* Pharmacokinetics and pharmacodynamics of norfluoxetine in rats: Increasing extracellular serotonin level in the frontal cortex. *Pharmacol Biochem Behav* 2009; **92**: 469–73.