



Response: Commentary: Chronic SSRI Stimulation of Astrocytic 5-HT_{2B} Receptors Change Multiple Gene Expressions/Edittings and Metabolism of Glutamate, Glucose and Glycogen: A Potential Paradigm Shift

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A Response to

Commentary: Chronic SSRI stimulation of astrocytic 5-HT_{2B} receptors change multiple gene expressions/editings and metabolism of glutamate, glucose and glycogen: a potential paradigm shift

by Banas, S. M., Diaz, S. L., Doly, S., Belmer, A., and Maroteaux, L. (2015). *Front. Behav. Neurosci.* 9:207. doi: 10.3389/fnbeh.2015.00207

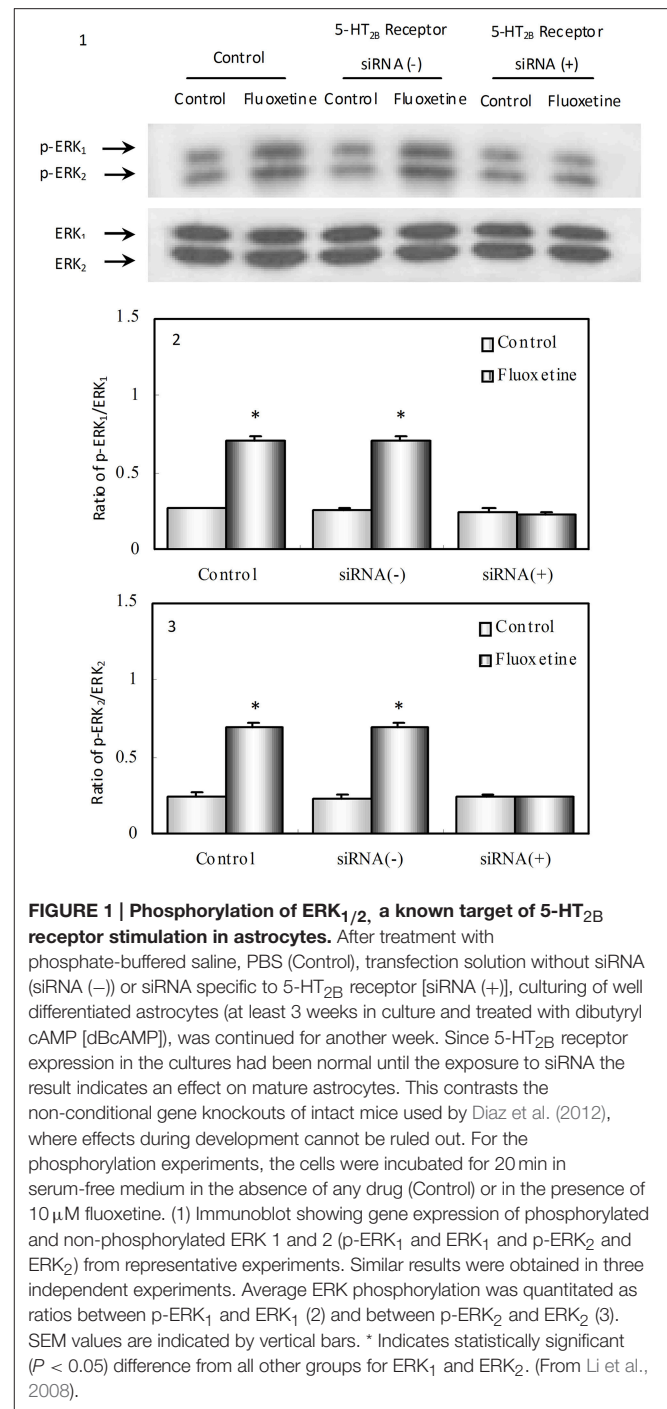
Hertz et al. (2015) provided data indicating that the indisputable SERT inhibition caused by SSRIs is an epiphenomenon. Instead we pointed toward acute and chronic effects of fluoxetine on 5-HT_{2B} receptor stimulation in astrocytes (in culture or freshly isolated from brains of mice treated with fluoxetine for 14 days). Stimulation by fluoxetine of the astrocytic 5-HT_{2B} receptor causes a multitude of effects that in astrocyte cultures could be prevented by drug- or siRNA-induced 5-HT_{2B} receptor inhibition (**Figure 1**). This stimulation activates complex signaling pathways, including EGF receptor transactivation, a signal pathway in which a G-protein-coupled receptor (GPCR) signal leads to release of a growth factor, which activates the EGF receptor-tyrosinekinase in the same or adjacent cells. Astrocytes express a number of GPCRs and play key roles in brain function (Hansson and Rönnbäck, 2004; Fields, 2009). The autocrine effects of growth factor release may regulate gene expression and alter cell functions in the astrocytes themselves and the paracrine effects provide opportunities for effects on their neuronal neighbors. Our studies in mice treated with fluoxetine for 2 weeks showed multiple gene upregulations and editings (Li et al., 2012; Hertz et al., 2015), which altered the function of the gene product in kainate receptors, 5-HT₂ receptors, phospholipase cPLA2, a Ca²⁺ L-channel gene and nucleoside transporter genes. These changes occurred mainly in astrocytes but some were neuronal. They occurred together with effects on metabolism of glucose and glycogen and turnover of glutamate and GABA, consistent with evidence of increased glutamatergic activity and decreased GABA-ergic activity in patients suffering from major depression, which are reverted by successful therapy; similarly, glucose metabolism is decreased in depressed patients and increases following treatment (Hertz et al., 2015).

A commentary by Banas et al. (2015) claim that we forgot important information provided by Diaz et al. (2012), including absence of antidepressant effects of fluoxetine or the 5-HT_{2B} agonist BW723C86 in mice lacking the serotonin transporter (SERT) or differentiated serotonergic neurons. They allege that these data rule out that the antidepressant effects of fluoxetine or BW723C86 could be SERT-independent (as claimed by us) and show that serotonergic neurons expressing SERT are necessary for the 5-HT_{2B} receptor effects exerted by fluoxetine (and other 5-HT_{2B} receptor agonists). They further remind us that Launay et al. (2006) in neuronal cultures from raphe nuclei demonstrated 5-HT_{2B} receptor-mediated control of SERT activity via 5-HT_{2B} receptor-promoted SERT phosphorylations. This should explain the finding by Diaz et al. (2012) that there are no antidepressant effects of either fluoxetine or BW723C86 in mice knocked-out for SERT or lacking differentiated serotonin neurons. These data should also rule out that the antidepressant effects of 5-HT_{2B} agonists, including fluoxetine, could be independent of SERT and explain the conclusion by the Maroteaux group (Diaz et al., 2012) that fluoxetine acts by 5-HT_{2B} receptor-mediated regulation of SERT in a cell autonomous manner.

We were well aware of the lack of antidepressant effect in SERT knock-outs described by Diaz et al. (2012) and of their reference to the paper by Launay et al. (2006), but did not comment on these points, because they are irrelevant. The lack of effect in the knock-outs does not prove any dependence on SERT, because Qu et al. (2005) showed that the direct DOI-mediated stimulation of 5-HT₂ receptor activation of phospholipase A₂ (cPLA₂) and subsequent arachidonic acid release and metabolism seen in normal mice is abolished in mice lacking SERT. There is no information in the literature that DOI should interact with SERT. Although Qu et al. indicated DOI as a 5-HT_{2A/C} agonist it also activates the 5-HT_{2B} receptor (Pineda-Farias et al., 2015). Stimulation of this receptor is the most likely reason for the response in wild-type animals, because fluoxetine acutely stimulates astrocytic 5-HT_{2B} receptors in cultured astrocytes (Li et al., 2008; Qiao et al., 2015) and after chronic administration (14 days) to mice upregulates this receptor in astrocytes but not in neurons (Li et al., 2012; Hertz et al., 2015). Moreover, cPLA₂-mediated signal transduction is increased by acute fluoxetine administration in unanesthetized rats (Qu et al., 2003) and phospholipase A₂ activity is potently stimulated by the SSRI sertraline in yeast (Rainey et al., 2010). Since direct stimulation with an agonist has no effect in these animals, the lack of fluoxetine effect in the knockouts does *not* prove fluoxetine dependence on SERT in adult brain.

With respect to the paper by Launay et al. it 2006 deals with extremely young cells. It does therefore not show that fluoxetine stimulates the 5-HT_{2B} receptor in mature individuals, because Homberg et al. (2011) and Sarkar et al. (2014) have shown that the serotonergic system in the immature brain functions in a completely different manner than in the mature brain.

Banas et al. also claim we changed results. However, the figure in Hertz et al. (2012) and in Kong et al. (2002) is—of course—the same and any reader has the opportunity to calculate K_i values based on the graph and the concentrations of mesulergine. The Zhang et al. (2010) paper, referred to



in the Banas commentary clearly indicates the difference between SSRI affinity during acute and chronic treatment. There is no major difference between the acute affinity found by ourselves and others, and we are the only authors who have measured affinities for SSRIs in chronically treated cells. The suggestion that effects on muscarinic acetylcholine and histamine receptors or other monoamine transporters should explain our observations independently of “putative” direct

agonist effects at 5-HT_{2B} receptors is invalidated by the observation by Li et al. (2008) that “ERK phosphorylation was abolished by SB204741, a universal 5-HT₂ receptor antagonist, and in 5-HT_{2B} receptor-depleted cells (see **Figure 1**), but unaffected by 5-HT_{2A} or 5-HT_{2C} receptor antagonists.” Fluoxetine stimulation of astrocytic 5-HT_{2B} receptors was

confirmed by Qiao et al. (2015). The “putative” receptors were demonstrated on both well-differentiated astrocyte cultures and freshly isolated astrocytes (Li et al., 2008, 2012; Peng et al., 2010; Zhang et al., 2010).

The commentary by Banas et al. has accordingly not altered our original conclusions but rather strengthened it.

REFERENCES

- Banas, S. M., Diaz, S. L., Doly, S., Belmer, A., and Maroteaux, L. (2015). Commentary: chronic SSRI stimulation of astrocytic 5-HT_{2B} receptors change multiple gene expressions/editings and metabolism of glutamate, glucose and glycogen: a potential paradigm shift. *Front. Behav. Neurosci.* 9:207. doi: 10.3389/fnbeh.2015.00207
- Diaz, S. L., Doly, S., Narboux-Nême, N., Fernández, S., Mazot, P., Banas, S. M., et al. (2012). 5-HT(2B) receptors are required for serotonin-selective antidepressant actions. *Mol. Psychiatry* 17, 154–163. doi: 10.1038/mp.2011.159
- Fields, R. D. (2009). *The Other Brain: From Dementia to Schizophrenia, How New Discoveries about the Brain Are Revolutionizing Medicine and Science*. New York, NY: Simon and Schuster.
- Hansson, E., and Rönnebeck, L. (2004). “Astrocytic receptors and second messenger systems,” in *Non-neuronal Cells of the Nervous System: Function and Dysfunction II*, ed L. Hertz (Amsterdam: Elsevier), 475–501.
- Hertz, L., Li, B., Song, D., Ren, J., Dong, L., Chen, Y., et al. (2012). Astrocytes as a 5-HT_{2B}-mediated SERT-independent SSRI target, slowly altering depression-associated genes and function. *Curr. Signal Transduct. Ther.* 7, 65–80. doi: 10.2174/1574362799278154
- Hertz, L., Rothman, D. L., Li, B., and Peng, L. (2015). Chronic SSRI stimulation of astrocytic 5-HT_{2B} receptors change multiple gene expressions/editings and metabolism of glutamate, glucose and glycogen: a potential paradigm shift. *Front. Behav. Neurosci.* 9:25. doi: 10.3389/fnbeh.2015.00025
- Homberg, J. R., Olivier, J. D., Blom, T., Arentsen, T., van Brunshot, C., Schipper, P., et al. (2011). Fluoxetine exerts age-dependent effects on behavior and amygdala neuroplasticity in the rat. *PLoS ONE* 6:e16646. doi: 10.1371/journal.pone.0016646
- Kong, E. K., Peng, L., Chen, Y., Yu, A. C., and Hertz, L. (2002). Up-regulation of 5-HT_{2B} receptor density and receptor-mediated glycogenolysis in mouse astrocytes by long-term fluoxetine administration. *Neurochem. Res.* 27, 113–120. doi: 10.1023/A:1014862808126
- Launay, J. M., Schneider, B., Loric, S., Da Prada, M., and Kellermann, O. (2006). Serotonin transport and serotonin transporter-mediated antidepressant recognition are controlled by 5-HT_{2B} receptor signaling in serotonergic neuronal cells. *FASEB J.* 20, 1843–1854. doi: 10.1096/fj.06-5724com
- Li, B., Dong, L., Wang, B., Cai, L., Jiang, N., and Peng, L. (2012). Cell type-specific gene expression and editing responses to chronic fluoxetine treatment in the *in vivo* mouse brain and their relevance for stress-induced anhedonia. *Neurochem. Res.* 37, 2480–2495. doi: 10.1007/s11064-012-0814-1
- Li, B., Zhang, S., Zhang, H., Nu, W., Cai, L., Hertz, L., et al. (2008). Fluoxetine-mediated 5-HT_{2B} receptor stimulation in astrocytes causes EGF receptor transactivation and ERK phosphorylation. *Psychopharmacol. (Berl.)* 201, 443–458. doi: 10.1007/s00213-008-1306-5
- Peng, L., Li, B., Du, T., Kong, E. K., Hu, X., Zhang, S., et al. (2010). Astrocytic transactivation by alpha2A-adrenergic and 5-HT_{2B} serotonergic signaling. *Neurochem. Int.* 57, 421–431. doi: 10.1016/j.neuint.2010.04.018
- Pineda-Farías, J. B., Velázquez-Lagunas, I., Barragán-Iglesias, P., Cervantes-Durán, C., and Granados-Soto, V. (2015). 5-HT_{2B} receptor antagonists reduce nerve injury-induced tactile allodynia and expression of 5-HT_{2B} receptors. *Drug Dev. Res.* 76, 31–39. doi: 10.1002/ddr.21238
- Qiao, J., Wang, J., Wang, H., Zhang, Y., Zhu, S., Adilijiang, A., et al. (2015). Regulation of astrocyte pathology by fluoxetine prevents the deterioration of Alzheimer phenotypes in an APP/PS1 mouse model. *Glia*. doi: 10.1002/glia.22926. [Epub ahead of print].
- Qu, Y., Chang, L., Klaff, J., Seemann, R., and Rapoport, S. I. (2003). Imaging brain phospholipase A2-mediated signal transduction in response to acute fluoxetine administration in unanesthetized rats. *Neuropsychopharmacology* 28, 1219–1226. doi: 10.1038/sj.npp.1300177
- Qu, Y., Villacreses, N., Murphy, D. L., and Rapoport, S. I. (2005). 5-HT_{2A/2C} receptor signaling via phospholipase A2 and arachidonic acid is attenuated in mice lacking the serotonin reuptake transporter. *Psychopharmacol. (Berl.)* 180, 12–20. doi: 10.1007/s00213-005-2231-5
- Rainey, M. M., Korostyshevsky, D., Lee, S., and Perlstein, E. O. (2010). The antidepressant sertraline targets intracellular vesiculogenic membranes in yeast. *Genetics* 185, 1221–1233. doi: 10.1534/genetics.110.117846
- Sarkar, A., Chachra, P., and Vaidya, V. A. (2014). Postnatal fluoxetine-evoked anxiety is prevented by concomitant 5-HT_{2A/C} receptor blockade and mimicked by postnatal 5-HT_{2A/C} receptor stimulation. *Biol. Psychiatry* 76, 858–868. doi: 10.1016/j.biopsych.2013.11.005
- Zhang, S., Li, B., Lovatt, D., Xu, J., Song, D., Goldman, S. A., et al. (2010). 5-HT_{2B} receptors are expressed on astrocytes from brain and in culture and are a chronic target for all five conventional ‘serotonin-specific reuptake inhibitors’. *Neuron Glia. Biol.* 6, 113–125. doi: 10.1017/S1740925X1000141

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