

Aspirin-Fluoxetine Interaction: Combined In Vivo Electrochemical and Electrophysiological Study of Raphe Dorsalis Nucleus activity and Cortical Serotonin in Rodents

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Abstract

Animal studies suggest that aspirin (ASA) may enhance the antidepressant effects of Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine. Recent work report that non-steroidal anti-inflammatory drugs (NSAIDs) have been studied as possible adjunctive therapy in the treatment of depression. However, administering non-steroidal anti-inflammatory drugs to increase the effectiveness of antidepressant has yielded inconsistent results.

In the present work experiments using electrochemical and concomitant electrophysiological methodologies have been performed in vivo to analyse the influence and putative interrelation of aspirin and fluoxetine upon cell firing in raphe dorsalis nucleus (RDN) and serotonin (5-HT) levels in the frontal cortex of rodents.

Key Words: in vivo voltammetry & electrophysiology; aspirin; fluoxetine; raphe nucleus; frontal cortex; rodents

Introduction

The link between serotonin, the dorsal raphe nucleus (DRN), and the frontal cortex constitutes a fundamental neuro-anatomical circuit in the pathophysiology of depression and the response to antidepressant treatments. In short, depression is often associated with dysfunction of this system, with reduced serotonergic activity originating in the brainstem and projecting to the cerebral cortex [1, 2].

Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine are first-line medications for the treatment of major depressive disorder and anxiety, working by increasing the availability of serotonin in brain synapses. They improve mood, reduce anxiety, and are preferred for their better tolerability compared to older antidepressants [3, 4].

Animal studies suggest that aspirin (ASA) may potentiate the antidepressant effects of fluoxetine [5]. However, recent work report that non-steroidal anti-inflammatory drugs (NSAIDs) have been studied as possible adjunctive therapy in the treatment of depression. However, administering NSAIDs to increase the effectiveness of antidepressant has yielded inconsistent results [6].

In the present work experiments using electrochemical and concomitant electrophysiological methodologies [7-10] have been performed in vivo to analyze the influence and putative interrelation of ASA and fluoxetine upon

cell firing in raphe dorsalis nucleus (RDN) and serotonin (5-HT) levels in the frontal cortex of rodents.

Methods

Male adult rats (Wistars, 250-280 g) were supplied by Charles-River (Italy) and kept in temperature- and humidity-controlled rooms (22 °C, 50%). All animal procedures were carried out in accordance with the Italian law (Legislative Decree no. 116, 1992) which acknowledges the European Directive 86/609/EEC. Furthermore, all efforts were made to minimize the number of animals and their suffering therefore the number of animals has been decided based upon the 3Rs, i.e. Reduction in the number of animals required; Refinement of the methodologies of analysis; Respect of the animals i.e. reducing their suffering as described [11].

For experiments each rat was anaesthetized using urethane (1.4 g/kg i.p.), placed on a stereotaxic apparatus (D. Kopf, USA). For stereotaxic preparation, micro-biosensors preparation and their insertion in relevant brain regions under stereomicroscopy see ref [7, 8].

In particular, one micro-carbon fibre electrode (μ CFE) was stereo-tactically inserted in the RDN i.e. cell body region, then, in the same animal, a second μ CFE was implanted within the frontal cortex following coordinates from Paxinos and Watson [12].

Both micro-biosensors were previously chemically treated with Nafion so that selective, voltammetric measurements of 5-HT could be performed [13, 14].

Successively, continuous real time in vivo amperometric measures of the neurotransmitter as well as electrophysiologic detection of cell firing have been performed as described earlier [7-10].

Treatments

Following a 30 min period of control/control measurements, control rats were treated with vehicle (1.4ml saline s.c.). Other rodents received selected doses of aspirin (45mg/Kg s.c.), fluoxetine (5mg/Kg s.c.) or aspirin + fluoxetine (45mg + 5mg/Kg s.c.)

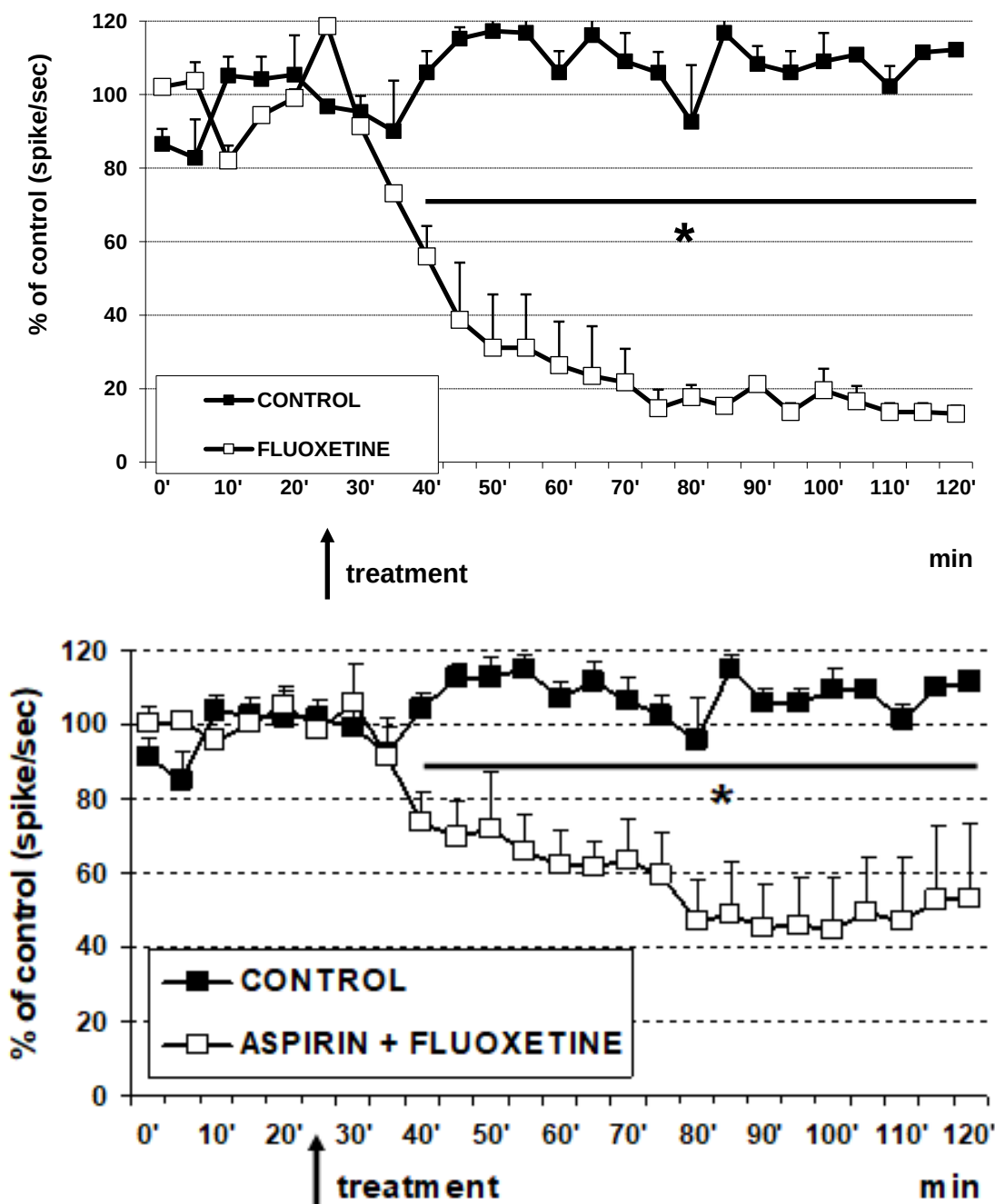
Statistics

Row data were subjected to ANOVA, with comparison between “control” (vehicle) and “treatment” values performed using the Tukey test. Then, the results were presented as % of control values, mean $\pm$ S.D., * $p < 0.05$.

Results

Electrophysiology

the present data indicate that fluoxetine alone decreases RDN firing to approximately 18% of control values within 60-70min, while the concomitant presence of aspirin blocks such decrease to approx. 45% of control after 60min and to approx 54% of control after 90min. These values are similar to those monitored 90 min after aspirin treatment alone (FIGURE 1 TOP, MIDDLE, BOTTOM, respectively).



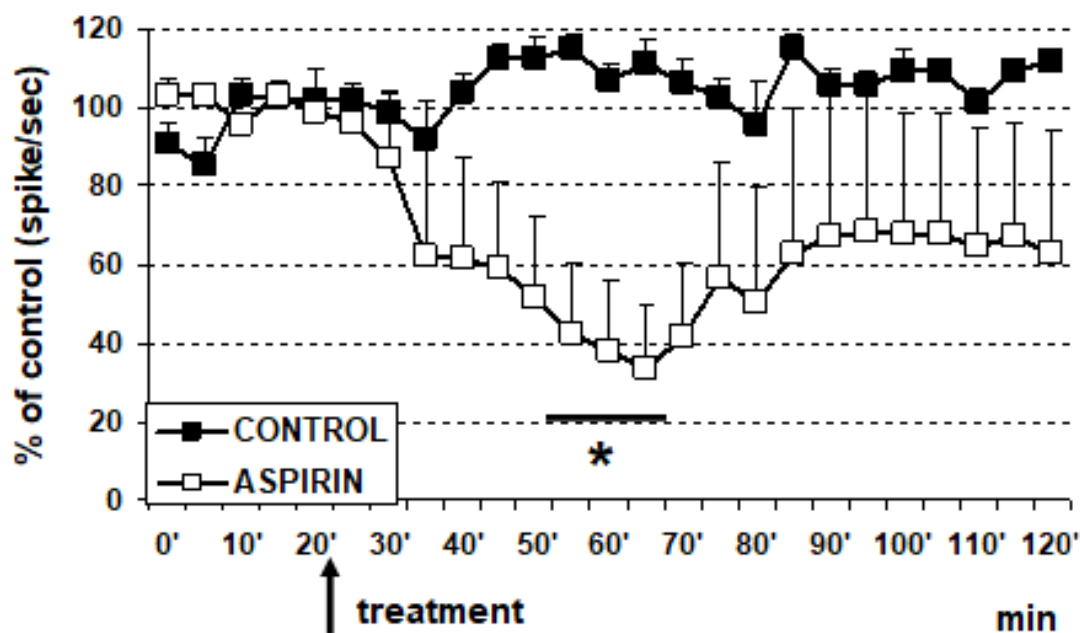


Figure 1: electrophysiological data monitored in the RDN of anaesthetised rodents treated with vehicle (n=3) or fluoxetine (n=3) TOP, aspirin + fluoxetine (n=3) MIDDLE, or aspirin (n=3) BOTTOM.

All treatments produced significant changes when compared to control, i.e

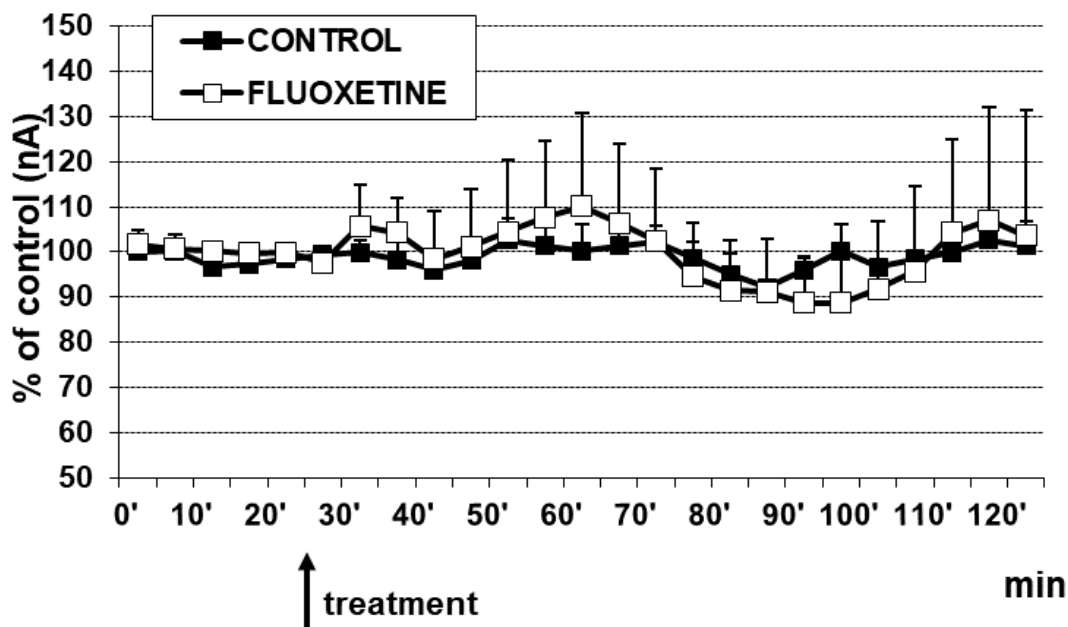
TOP mean±SD 2W ANOVA $F(24,96) = 2,0924$; $p=0,0000$ * $p<0.05$ Dunnett

MIDDLE mean±SD 2W ANOVA $F(24,96) = 3,4435$; $p=0,00001$ * $p<0.05$ Dunnett

BOTTOM mean±SD 2W ANOVA $F(24,120) = 5,8355$; $p=0,00000$ * $p<0.05$ Dunnett

Voltammetry

Amperometric measurements show that fluoxetine alone does not modify 5-HT levels compared to the control treated group, while aspirin alone is transiently reducing significantly the levels of the neurotransmitter to approximately 72% of control values within 40min, returning to controls within approximately 1 hour post treatment. Finally the combination of the two treatments were showing no effect upon 5-HT release when compared to saline treated rats (FIGURE 2 TOP, MIDDLE, BOTTOM, respectively).



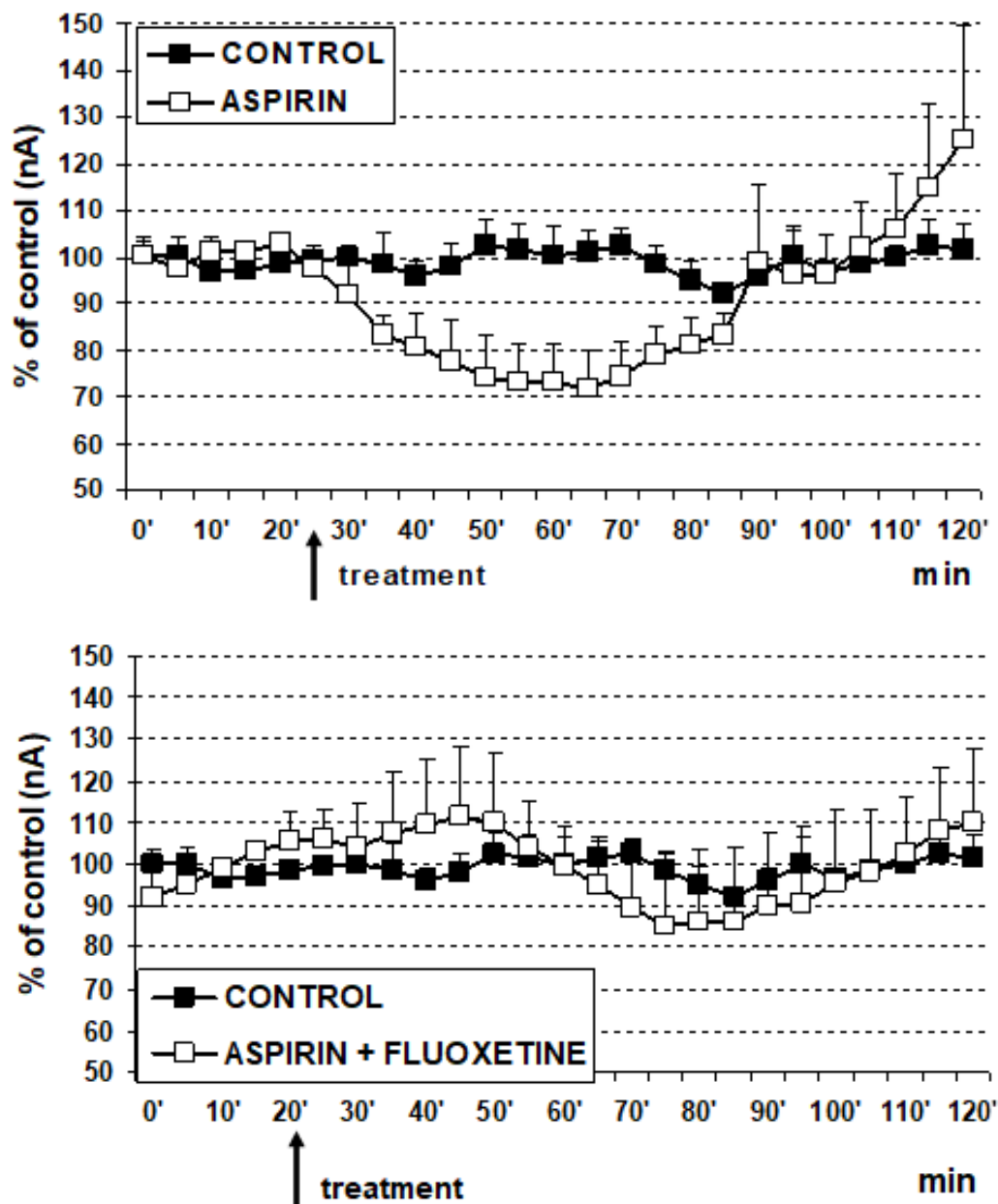


Figure 2: amperometric levels of 5-HT monitored in the frontal cortex of the rodents treated as described above. No significant changes were detected, only aspirin was determining a transient decrease of the voltammetric signal mean $\pm$ SD 2W ANOVA $F(24,96) = 3,3294$; $p=0,00002$

Discussion

Neuropharmacological studies have shown that aspirin administration leads to a suppression of the firing activity of 5-HT neurons in the dorsal raphe (15, 16).

Systemic intravenous administration of Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine dorsal raphe nucleus cell firing in a dose-dependent manner (17, 18).

Specific research (such as that conducted by Warner-Schmidt et al. 19) has shown that nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin can reduce the biochemical and behavioral efficacy of SSRIs. At the RDN level, the addition of aspirin can counteract the fluoxetine-induced increase in serotonin, attenuating it through modulation of the neuronal activation necessary for long-term therapeutic effect.

Accordingly, the electrophysiological data indicate a significant modulation of aspirin upon fluoxetine influence on RDN cell firing.

In particular, electrophysiological analyses indicate that aspirin antagonizes the effect of fluoxetine in the dorsal raphe nucleus, reducing its ability to lower neuronal firing rates. The combination of treatments shows that aspirin dominates the electrophysiological profile, attenuating the neuronal response to fluoxetine, with aspirin also causing a transient reduction in serotonin. Although the combination of the two drugs negates this effect, leading to a 5-HT release comparable to that of a saline solution (no net change).

The present data indicate that aspirin "blocks" the reduction in neuronal firing induced by fluoxetine. This phenomenon has been observed in studies that suggest that NSAIDs may interfere with the biochemical mechanisms of SSRIs, potentially making them less effective in treating depression in some patients [19].

However, in some experimental and clinical models, low-dose aspirin appears to accelerate or enhance the effect of fluoxetine due to its anti-inflammatory and antioxidant properties, which appear to reduce the inflammatory state associated with depression (5, 20).

In both tests (electrophysiology and voltammetry), when the drugs are administered together, the final result resembles the profile of aspirin alone much more closely than that of fluoxetine.

No effect of fluoxetine on 5-HT release in this model: The voltammetric data show that fluoxetine, in this specific experimental setting, does not alter serotonin levels compared to the control.

This is possibly related to the present acute administration and should be verified in future experiments with repeated injections as it is known that the efficacy of such compounds upon the 5-HT system is related to chronic treatment [21, 22].

Nonetheless, the data reflect a real and complex scientific debate about a possible negative interaction between SSRI antidepressants (such as fluoxetine) and nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin.

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References

1. Ressler, K. J., & Nemeroff, C. B. (2000). Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. *Depression and anxiety*, 12(S1), 2-19.
2. Zhou, L., Zhang, C., Xie, Z., Yu, Q., Wang, J., Gong, Y., ... & Shi, Y. (2024). Neural Circuit Mechanisms of Sinisan formula for the Treatment of adolescent Depression: prefrontal cortex to dorsal raphe nucleus. *Journal of Ethnopharmacology*, 334, 118529.
3. Owens, M. J. (2004). Selectivity of antidepressants: from the monoamine hypothesis of depression to the SSRI revolution and beyond. *Journal of Clinical Psychiatry*, 5-10.
4. Harmer, C. J., & Cowen, P. J. (2013). 'It's the way that you look at it'—a cognitive neuropsychological account of SSRI action in depression. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1615).
5. Brunello, N., Alboni, S., Capone, G., Benatti, C., Blom, J. M., Tascedda, F., ... & Mendlewicz, J. (2006). Acetylsalicylic acid accelerates the antidepressant effect of fluoxetine in the chronic escape deficit model of depression. *International clinical psychopharmacology*, 21(4), 219-225.
6. Alboni, S., Benatti, C., Capone, G., Tascedda, F., & Brunello, N. (2018). Neither all anti-inflammatory drugs nor all doses are effective in accelerating the antidepressant-like effect of fluoxetine in an animal model of depression. *Journal of Affective Disorders*, 235, 124-128.
7. Crespi F. (2002). In vivo voltammetry and concomitant electrophysiology at a single biosensor to analyse ischaemia, depression and drug dependence. *J Neurosci Methods*, 119:173-184
8. Crespi F. (2010). SK channel blocker apamin attenuates the effect of SSRI fluoxetine upon cell firing in dorsal raphe nucleus: A concomitant electrophysiological and electrochemical in vivo study reveals implications for modulating extracellular 5-HT. *Brain Research*, 1334:1-11.14.
9. Crespi F. (2018). Concomitant Behavioral, Electrochemical and Electrophysiological Study in Real Time on the Role of CRF and CRF Antagonist(s) in Anxiety and Depression: Possible Association CRF + 5-HT Receptor Antagonists? *Int J Brain Disord Treat*, 4:022.15
10. Crespi F. (2019). Concomitant in Vivo Voltammetric and Electrophysiological Analysis Indicate that Nociceptin/Orphanin FQ Affects Dopamine and then Serotonin Activities in Brain Substantia Nigra. *International Physiology Journal*, 2,3:1-9.16.
11. Crespi F, Congestri F, Formenti F. (2019). Near infrared spectroscopy (NIRS) to attain reduction-refinement-respect, the three Rs towards ANIMAL WELFARE in preclinical research. *Neurol Neurol Sci Open Access*, 2(1): 1008.17.
12. Paxinos G, Watson C (1986) The rat in stereotaxic coordinates. (2nd ed), Plenum Press, New York.
13. Crespi F, Martin K, Marsden CA (1988) Measurement of extracellular basal levels of serotonin in vivo using nafion-coated carbon fibre electrodes combined with differential pulse voltammetry. *Neuroscience* 27: 885-896.
14. Crespi F (2013) Serotonin, how to find it. Xjenza, Malta Chamber of Scientists, 14-22
15. Baraban, J. M., & Aghajanian, G. K. (1980). Suppression of firing activity of 5-HT neurons in the dorsal raphe by alpha-adrenoceptor antagonists. *Neuropharmacology*, 19(4), 355-363.
16. McDevitt, R. A., & Neumaier, J. F. (2011). Regulation of dorsal raphe nucleus function by serotonin autoreceptors: a behavioral perspective. *Journal of chemical neuroanatomy*, 41(4), 234-246.
17. Smith, J. E., & Lakoski, J. M. (1997). Electrophysiological effects of fluoxetine and duloxetine in the dorsal raphe nucleus and hippocampus. *European journal of pharmacology*, 323(1), 69-73.
18. Czachura, J. F., & Rasmussen, K. (2000). Effects of acute and chronic administration of fluoxetine on the activity of serotonergic neurons in the dorsal raphe nucleus of the rat. *Naunyn-Schmiedeberg's archives of pharmacology*, 362(3), 266-275.
19. Warner-Schmidt, J. L., Vanover, K. E., Chen, E. Y., Marshall, J. J., & Greengard, P. (2011). Antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) are attenuated by anti-inflammatory drugs in mice and humans. *Proceedings of the National Academy of Sciences*, 108(22), 9262-9267.
20. Sun, Y., Narayan, V. A. & Wittenberg, G.M. Side effect profile similarities shared between antidepressants and immunomodulators reveal potential novel targets for treating major depressive disorders. *BMC Pharmacol Toxicol* 17, 47 (2016).
21. Jones, B. J., & Blackburn, T. P. (2002). The medical benefit of 5-HT research. *Pharmacology Biochemistry and Behavior*, 71(4), 555-568.
22. Ślifierki, G., Król, M., & Turło, J. (2021). 5-HT receptors and the development of new antidepressants. *International journal of molecular sciences*, 22(16), 9015.



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