Article type : Brief Communication (includes Case Reports)



Chronic fluoxetine treatment accelerates kindling epileptogenesis in mice independently of 5-HT_{2A} receptors

Crystal Li¹, Juliana Silva¹, Ezgi Ozturk^{1, 3}, Gabriella Dezsi^{1, 3}, Terence J. O'Brien^{1, 3}, Thibault Renoir², Nigel C. Jones^{1, 3}

¹Department of Medicine (Royal Melbourne Hospital), The University of Melbourne, Australia.

²The Florey Institute of Neuroscience and Mental Health, Melbourne Brain Centre, The University of Melbourne, Australia.

³Department of Neuroscience, Central Clinical School, Monash University and Department of Neurology, The Alfred Hospital, Melbourne, Victoria, 3004, Australia

Author for correspondence:

Associate Professor Nigel C. Jones.

³Department of Neuroscience, Central Clinical School, Monash University, Prahran, Victoria, 3004, Australia. Email: nigel.jones@monash.edu

Running Title: Fluoxetine accelerates kindling: no role for 5-HT_{2A}rs.

Key Words: epileptogenesis, antidepressant, fluoxetine, serotonin, 5- HT_{2A} receptor Number of text pages: 12

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the <u>Version of Record</u>. Please cite this article as <u>doi: 10.1111/epi.14435</u>

Number of words: 1935 Number of references: 15 Number of figures: 2

Summary

Patients with epilepsy often suffer from mood disorders, and these are commonly treated with antidepressant drugs. While these drugs are often successful in mitigating depressive symptoms, how they affect the epileptogenic processes has been little studied. Recent evidence has demonstrated that treatment with selective serotonin reuptake inhibitor (SSRI) antidepressant drugs adversely promotes epileptogenesis, which may be of great concern considering the number of patients exposed to these drugs. This study investigated 5-HT_{2A} receptor signalling as a potential mechanism driving the pro-epileptogenic effects of the prototypical SSRI fluoxetine. Male homozygous 5-HT_{2A} receptor knockout mice or wildtype littermates (n = 9-14/group) were treated with continuous fluoxetine (10 mg/kg/day s.c.) or vehicle and subjected to electrical kindling of the amygdala. Compared to vehicle, fluoxetine treatment accelerated kindling epileptogenesis (p < 0.001), but there was no effect of genotype (p = 0.75), or any treatment x genotype interaction observed (p = 0.90). Interestingly, fluoxetine treatment increased **afterdischarge** thresholds in both genotypes (p = 0.007). We conclude that treatment with fluoxetine promotes epileptogenesis in mice, but this effect is not mediated by 5-HT_{2A} receptors. This suggests that antidepressants may accelerate the onset of acquired epilepsy in patients who have experienced epileptogenic cerebral insults.

Introduction

It is now well-recognised that patients with epilepsy are at greater risk of experiencing psychiatric comorbidities than the general population, including depression, anxiety and suicidality¹. These comorbidities contribute substantially to reduced quality of life², and can predict poorer seizure outcomes³. It is also now apparent that bidirectional relationships exist This article is protected by copyright. All rights reserved

between these disorders, such that the psychiatric conditions often pre-date the onset of epilepsy by several years⁴. As such, appropriate diagnoses and adequate management of these comorbidities is now high priority. Newer generation antidepressant drugs, such as the selective serotonin reuptake inhibitors (SSRIs), constitute the primary pharmacological therapy for depressive disorders.

A wealth of experimental and clinical evidence reports that SSRIs do not aggravate seizures in established, chronic epilepsy at therapeutic doses^{5; 6}. However, an important aspect that remains largely unexplored is how SSRIs influence epileptogenesis – the transformation of a regular brain state into one susceptible to generating epileptic activity. This may be important since many patients at risk of developing epilepsy are treated with antidepressants prior to epilepsy onset, at a time when epileptogenesis is occurring. For example, psychiatric conditions often predate epilepsy onset⁴, inferring that patients may also be exposed to antidepressant drugs prior to epilepsy onset. Also, brain insults recognised to promote epilepsy, such as traumatic brain injury or stroke, are commonly associated with mood disorders⁷, and would likely require therapeutic intervention. In addition, while great progress is being made understanding the molecular mechanisms underpinning epileptogenesis, these largely remain mysterious, but are several reasons why exposure to antidepressants may interact with epileptogenic processes⁶. For example, neuroplasticity of limbic circuits – strongly implicated in antidepressant actions – is a central component of most theories explaining epileptogenesis.

In light of this, we previously examined the influence of SSRI antidepressants on epileptogenesis. Using two different compounds – fluoxetine and citalopram – we found that continuous exposure to SSRIs accelerated kindling epileptogenesis⁸. This was the first study to examine this, and has triggered a range of subsequent clinical and preclinical investigations. A critical, yet unanswered question is the biological mechanisms underpinning this pro-epileptogenic effect. SSRI antidepressants act to increase synaptic serotonin levels by selectively blocking the serotonin reuptake sites on presynaptic nerve terminals. This leads to increased activation of serotonin receptors, and, since therapeutic improvement for depression symptoms typically takes a few weeks, is suggestive of some form of neuroplasticity of appropriate neural circuits. On first principles, understanding which serotonin receptor subtypes mediate the previously identified pro-epileptogenic effect of SSRIs would be a great advance to understanding the molecular mechanisms and downstream signalling pathways adopted. One receptor subtype which has received recent

attention is the 5- HT_{2A} receptor subtype. Dysfunction in this receptor has been linked to epilepsy and depression⁹, and stimulation of this receptor accelerates kindling epileptogenesis¹⁰. 5- HT_{2A} receptors are G-protein-coupled receptors linked to Gq/G11 to mediate excitatory neurotransmission, and are expressed in highly epileptogenic brain regions such as the amygdala and hippocampus¹¹. Further, a large body of research links this receptor subtype to seizure activity⁹.

With this in mind, we designed the current study with two aims: first, to attempt to replicate the observation that fluoxetine accelerates epileptogenesis, this time using mice; and second, to investigate the influence of 5-HT_{2A} receptors in the pro-epileptogenic effect of SSRI antidepressants. To achieve this, we used 5-HT_{2A} receptor knockout mice and wild-type littermates, and continuous treatment with fluoxetine or vehicle. We chose to explore genetic rather than pharmacological methods of examining 5-HT_{2A} receptors because the currently available ligands, such as DOI and ketanserin used in previous studies¹⁰, can also interact with other 5-HT receptor subtypes, making this a more direct approach. **Our hypothesis was that fluoxetine would accelerate kindling epileptogenesis in wild-type mice, but not in 5-HT2A receptor knockout mice, inferring that the pro-epileptogenic mechanism of action of fluoxetine is through this receptor subtype.**

Methods

Animals

Homozygous 5-HT_{2A} receptor knockout mice (5-HT_{2A}r^{-/-}) and **C57Bl/6** wildtype littermates, originally generated at Columbia University¹², were bred in the Bioresource Facility at The Florey Institute of Neuroscience and Mental Health (Australia), and transferred to the Biological Resource Facility at The Department of Medicine (Royal Melbourne Hospital), The University of Melbourne for all experiments. All procedures were approved by the Howard Florey Institute Animal Ethics Committee (#15-095).

Surgical implantation of electrodes and minipumps

Male mice aged 11-14 weeks underwent surgical implantation of electrodes as previously described¹³. This included a stimulating bipolar electrode into the left basolateral amygdala. During the same surgery session, an osmotic minipump (Alzet, Cupertino, CA, USA) was implanted subcutaneously beneath the scapula. Pumps were filled with either vehicle (50% This article is protected by copyright. All rights reserved

DMSO in sterile saline) or fluoxetine hydrochloride (Biotrend Chemicals, Switzerland). Mice were randomly assigned to treatment, leading to four experimental groups: wildtype + vehicle (n=10), wildtype + fluoxetine (n=9), 5-HT_{2A}r^{-/-} + vehicle (n=14), or 5-HT_{2A}r^{-/-} + fluoxetine (n=13). The concentration of fluoxetine was tailored to deliver ~10mg/kg/day to the mice for 30 days. This dose was chosen based on our previous study⁸, which showed the plasma levels achieved by this regime match human plasma levels of patients taking fluoxetine. Mice recovered from surgery for one week, at which time kindling commenced. Researchers who performed the kindling were blinded from genotype and treatment.

Electrical Amygdala Kindling

Kindling was conducted using a twice daily stimulation protocol, as previously described¹³. On the first day, the afterdischarge threshold was determined, defined as the minimum current required to produce a >6 sec electrographic seizure. Seizures were stimulated using an Accupulser Pulse Generator/Stimulator (A310, World Precision Instruments, USA) with the following stimulation parameters: 60 Hz, 1 second duration, 1 millisecond biphasic square wave pulse. Electrographic activity was visualised with LabChart software (ADInstruments, Bella Vista, NSW, Australia). Seizure severity was scored according to the Racine Scale. At the conclusion of kindling, electrode location was histologically verified. Animals with incorrect positioning of the bipolar electrode were excluded from all analyses.

Statistical analysis

Afterdischarge thresholds, kindling rates and seizure durations were analysed using mixedmodel ANOVA with Bonferroni post-hoc tests, where appropriate. The two independent variables in all analyses were genotype and treatment. Data is presented as group mean and error bars represent S.E.M., and statistical significance was set at p<0.05.

Results

Afterdischarge threshold is increased with fluoxetine treatment, but is not influenced by 5-HT_{2A} receptors

The afterdischarge threshold was significantly higher in fluoxetine-treated mice than in vehicle-treated mice ($F_{(1,42)}$ =8.10, *p*=0.007; Figure 1B), suggesting that fluoxetine acutely

reduces neuronal excitability. However, this measure was not influenced by genotype $(F_{(1,42)}=1.91, p=0.17)$, and no significant interactions between treatment and genotype were observed $(F_{(1,42)}=0.47, p=0.50)$.

Chronic fluoxetine treatment accelerates kindling epileptogenesis independently of 5-HT_{2A} receptors

Chronic fluoxetine treatment accelerated kindling epileptogenesis when compared to vehicletreated animals, as evidenced significantly fewer stimulations required to reach the successive stages of kindling ($F_{(1,42)}$ =10.03, p=0.003; Figure 2A). However, we did not observe any effect of genotype on this outcome ($F_{(1,42)}=0.01$, p=0.992; Figure 2A), and there was no interaction between treatment and genotype ($F_{(1,42)}=0.24$, p=0.62). In addition, when assessing the average seizure class elicited by successive stimulations, this was also accelerated by fluoxetine treatment ($F_{(1,42)}=5.05$, p=0.03; Figure 2B). This effect was particularly evident in the early stages of kindling before the ceiling was reached, verified by a significant interaction between treatment and stimulation ($F_{(14, 588)}$ =4.27, p< 0.001). Posthoc analyses revealed that fluoxetine treatment accelerated kindling epileptogenesis when compared to vehicle-treated groups in the initial stages of kindling (Figure 2B). The absence of 5-HT_{2A} receptors did not influence the rates of kindling epileptogenesis ($F_{(1,42)}=0.01$, p=0.99; Figure 2B), and no interaction between treatment and genotype (F_(1,42)=0.13, p=0.72) was observed. Neither fluoxetine treatment ($F_{(1,42)}=0.22$, p=0.64; Figure 2C), nor the absence of 5-HT_{2A} receptors ($F_{(1,42)}$ =0.03, p=0.87), influenced seizure duration, and no significant interaction between treatment and genotype was observed ($F_{(1,42)}=1.91$, p=0.17).

Discussion

Here we demonstrate the pro-epileptogenic effects of the SSRI antidepressant fluoxetine in mice, consistent with what we have previously identified in rats⁸. In addition, we show that this effect is not mediated by 5-HT_{2A} receptors. Indeed, the presence of these receptors does not influence kindling rates at all, evidenced by the overlapping kindling curves in 5-HT_{2A} receptor null and wildtype mice. This finding reinforces the notion that SSRI antidepressant medications may have negative repercussions on the onset and/or progression of acquired epilepsy in patients, but that the specific serotonin receptor subtype(s) responsible remain undisclosed.

We were motivated to target the 5-HT_{2A} receptor in this study as a driver of SSRI effects for several reasons: this subtype has been suggested as a potential mediator of pathogenic processes in both depression and epilepsy⁹; the expression patterns of 5-HT_{2A} receptors include cortical and limbic regions, and these regions are also likely to be involved in acquired epileptogenesis; and, a previous study demonstrating that activation of this receptor can accelerate epileptogenesis¹⁰. Although scant evidence links this receptor subtype to the mechanism of action of SSRI antidepressants, this may not be relevant, since the pro-epileptogenic and anti-depressant actions of these compounds may be driven via independent mechanisms. Based on this, we anticipated that fluoxetine treatment would facilitate kindling epileptogenesis in wildtype mice, but not in mice lacking 5-HT_{2A} receptors. However, our study conclusively rules out this receptor as a possible mediator of this property of SSRIs. Another strong candidate receptor, or alternatively the serotonin transporter, since these proteins have also received much attention in epilepsy/depression research¹⁴.

We chose to deliver fluoxetine via continuous infusion in this study to model the typical exposure of human patients in which fluoxetine levels are in steady state throughout the day. This is in contrast to acute injections normally delivered in rodent studies, which are rapidly metabolised leading to high peaks in blood concentration, and also periods of low or no circulating drug. This strategy introduces a potential confound for interpretation of any observed effects on epileptogenesis, since the drug is circulating when the stimulations are given, and therefore may influence the acute seizure severity, and hence kindling rate. However, in addition to accelerated rates of kindling, we also show here an acute reduction in neuronal excitability of fluoxetine, as measured by the increase in after-discharge threshold. This paradoxical finding therefore removes any potential confounds of interpretation of the kindling data here due to constantly circulating fluoxetine levels, since we observe pro-epileptogenic effects of fluoxetine, opposite in nature to the heightened threshold to elicit afterdischarges. Of note, this was a different finding from our previous report on the pro-epileptogenic effects of SSRIs, which found no change in afterdischarge threshold in treated rats - it is unclear why this phenotype is not consistent, but may be related to species differences. In addition, it should be recognised that, while SSRIs appear to be safe to use in chronic epilepsy, and possibly have anticonvulsant properties (as identified here), this appears to be separate to the proepileptogenic effects. Acute effects of seizure sensitivity may be related to innate excitability of seizure circuitry, whereas epileptogenesis involves alterations involving changes to gene expression and the structure and function of neural circuits¹⁵. Antidepressants are well-placed to influence epileptogenesis⁶ since these are recognised to modulate a range of different aspects of neuroplasticity related to their antidepressant actions, although it should not be assumed that mechanisms which evoke anti-depressant activity overlap with the observed pro-epileptogenic actions of SSRIs.

To conclude, here we demonstrate that chronic treatment with the SSRI fluoxetine accelerates kindling epileptogenesis in mice, but that 5-HT_{2A} receptors are not necessary for these pro-epileptogenic effects. Future studies are required to identify the receptor, and downstream signalling pathways adopted by this drug, and also establish whether this is a unique feature of SSRIs, or represents a common property of all modern antidepressants. **Our findings also motivate investigation of this phenomenon in clinical populations.**

Acknowledgements

This work was supported by an NHMRC project grant (#1059860).

Disclosure of Conflicts of Interest

None of the authors have any conflicts of interest, financial or otherwise, to disclose.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.**References**

- 1. Tellez-Zenteno JF, Patten SB, Jette N, et al. Psychiatric comorbidity in epilepsy: a population-based analysis. *Epilepsia* 2007;48:2336-2344.
- Kanner AM. Depression in epilepsy: prevalence, clinical semiology, pathogenic mechanisms, and treatment. *Biol Psychiatry* 2003;54:388-398.
- 3. Petrovski S, Szoeke CE, Jones NC, et al. Neuropsychiatric symptomatology predicts seizure recurrence in newly treated patients. *Neurology* 2010;75:1015-1021.
- 4. Hesdorffer DC, Ishihara L, Mynepalli L, et al. Epilepsy, suicidality, and psychiatric disorders: A bidirectional association. *Ann Neurol* 2012;72:184-191.

- 5. Kanner AM. Most antidepressant drugs are safe for patients with epilepsy at therapeutic doses: A review of the evidence. *Epilepsy Behav* 2016;61:282-286.
- Cardamone L, Salzberg M, O'Brien T, et al. Antidepressant therapy in epilepsy: can treating the comorbidities affect the underlying disorder? *Br J Pharmacol* 2013;168:1531-1554.
- 7. Moldover JE, Goldberg KB, Prout MF. Depression after traumatic brain injury: a review of evidence for clinical heterogeneity. *Neuropsychol Rev* 2004;14:143-154.
- 8. Cardamone L, Salzberg MR, Koe AS, et al. Chronic antidepressant treatment accelerates kindling epileptogenesis in rats. *Neurobiol Dis* 2014;63:194-200.
- 9. Guiard BP, Di Giovanni G. Central serotonin-2A (5-HT2A) receptor dysfunction in depression and epilepsy: the missing link? *Front Pharmacol* 2015;6:46.
- 10. Wada Y, Shiraishi J, Nakamura M, et al. Role of serotonin receptor subtypes in the development of amygdaloid kindling in rats. *Brain Res* 1997;747:338-342.
- 11. Bombardi C. Neuronal localization of 5-HT2A receptor immunoreactivity in the rat hippocampal region. *Brain Res Bull* 2012;87:259-273.
- 12. Gonzalez-Maeso J, Yuen T, Ebersole BJ, et al. Transcriptome fingerprints distinguish hallucinogenic and nonhallucinogenic 5-hydroxytryptamine 2A receptor agonist effects in mouse somatosensory cortex. *J Neurosci* 2003;23:8836-8843.
- 13. Liu S, Shen Y, Shultz SR, et al. Accelerated kindling epileptogenesis in Tg4510 tau transgenic mice, but not in tau knockout mice. *Epilepsia* 2017;58:e136-e141.
- Hasler G, Bonwetsch R, Giovacchini G, et al. 5-HT1A receptor binding in temporal lobe epilepsy patients with and without major depression. *Biol Psychiatry* 2007;62:1258-1264.
- 15. Goldberg EM, Coulter DA. Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. *Nat Rev Neurosci* 2013;14:337-349.



Figure legends

Figure 1: (A) An example of an electrographic seizure induced by electrical stimulation of the amygdala. The minimum current required to induce this seizure was defined as the

afterdischarge threshold (ADT). Scale bar in bottom right represents 1 second and 1 mV, and solid line above the start of trace represents the stimulus artifact. (B) The effect of fluoxetine treatment (FLX - 10mg/kg/day s.c.) on the ADT of 5-HT2A^{-/-} mice (open symbols) and WT littermates (solid symbols). Fluoxetine-treated mice displayed higher ADTs than vehicle-treated mice (VEH), consistent with an anti-convulsant drug action. No effects of genotype were observed. Data represent group mean +/- S.E.M. n=9-14 mice/group. **p<0.001.

Figure 2: The effect of fluoxetine and 5-HT2A receptor deletion on amygdala kindling. (**A**) Fluoxetine treatment (FLX) accelerates kindling epileptogenesis in both wildtype (WT) and 5-HT2Ar^{-/-} mice compared to vehicle treated mice (VEH), as evidenced by fewer stimulations required to reach each stage of kindling. No effect of genotype was observed. Sample sizes are given in parentheses. (**B**) The average seizure class induced by subsequent stimulations also indicates faster acquisition of kindling in fluoxetine-treated mice, particularly in the early stages of kindling. (**C**) No difference in seizure duration was observed. In panels B and C, the seizure class/duration resulting from 2 successive stimulations have been averaged for each animal for ease of viewing. Data represent group mean +/- S.E.M. *p<0.05, **p<0.05 represent significant main effect of treatment. #p<0.05 indicates significant difference between fluoxetine and vehicle treated groups following post-hoc evaluation in the early stages of kindling.

Author











epi_14435_f2.tif