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Short communication

Chronic fluoxetine treatment attenuates post-septic affective changes in the mouse

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HIGHLIGHTS

- LPS-induced sepsis leads to long-lasting changes in affective behaviours.
- Chronic fluoxetine treatment following recovery from sepsis attenuates such changes in affective behaviours.
- Chronic fluoxetine treatment also attenuates post-septic decreases in cell proliferation in the dentate gyrus, decreased expression of EGR1 in the CA1 and increased microglial activation in the hippocampus.

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ABSTRACT

It has been previously demonstrated that the induction of sepsis in rodents results in persistent impairments in affective and cognitive domains. In this study we have examined the impact of chronic treatment with the antidepressant fluoxetine on affective behaviours and hippocampal neuroinflammation and stem cell proliferation in animals that have previously undergone sepsis induced by peripheral treatment with lipopolysaccharide. We find that fluoxetine significantly attenuates post-septic increases in behavioural despair and motivated exploration, whilst also reversing the effects of previous sepsis on activated microglia and stem cell proliferation. These results indicate that conventional antidepressants may be effective in the management of mood disorders in survivors of sepsis.

It is increasingly recognised that survivors of sepsis may experience a long-lasting syndrome of cognitive and behavioural changes of uncertain aetiology that can significantly impact on quality of life [1]. Similar changes in cognitive and behavioural domains can be described in animal models of sepsis-survival, indicating that such approaches may be useful in understanding mechanisms and factors that shape post-septic impairments [2,3]. We have recently described that in a mouse model in which sepsis is induced by a sub-lethal treatment with lipopolysaccharide (LPS) there are long-lasting changes in affective domains, including increased behavioural despair and increases in anxiety-like behaviours [4]. In post-septic animals there is also decreased cellular proliferation in the dentate gyrus, altered hippocampal expression of the neural plasticity-related immediate early genes EGR1 and ARC, and evidence of increased and long-lasting neuroinflammation [4].

There are emerging links between neuroinflammation and affective disorders such as major depression that suggest that neuroimmune factors may be therapeutic targets in the treatment of mood disorders [5,6]. The selective serotonin reuptake inhibitor fluoxetine, a commonly used anti-depressant, has been described to exert anti-neuroinflammatory properties [7–9], as well as exerting effects on neurogenesis and neural plasticity [10,11]. Given the need to identify potential therapeutic strategies for the alleviation of post-septic symptoms, in the current study we examined whether chronic fluoxetine treatment following recovery from LPS-induced sepsis could attenuate some of the previously described affective and neurochemical changes observed in the mouse.

For the purpose of all experiments male C57BL/6 mice (Charles River, Kent, UK; $N = 41$) were used. Animals were aged 8 weeks at the start of the experimental procedures. Animals were group housed in a 12:12 light:dark cycle for 2 weeks prior to LPS

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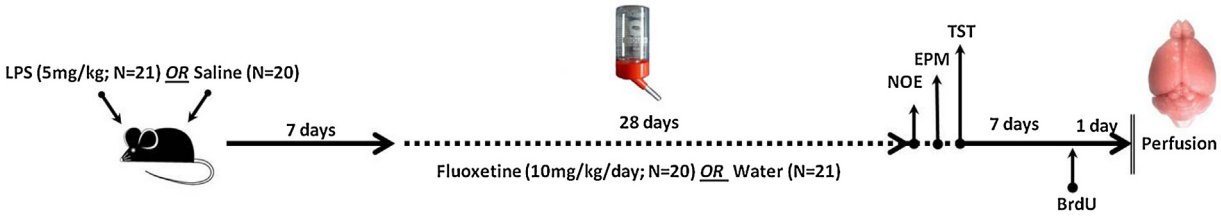

Fig. 1. Schematic illustrating the experimental design utilised in this study. Half of the study cohort received i.p. LPS (5 mg/kg), with the other half receiving vehicle. After 7 days the animals were commenced on fluoxetine treatment (10 mg/kg/day via drinking water; $N = 10$ or 11 for previously LPS-treated animals, $N = 10$ for previously vehicle treated animals) or control drinking water ($N = 10$ for previously LPS-treated animals, $N = 10$ for previously vehicle treated animals). After twenty eight days of treatment animals were tested on the novel object exploration task (NOE), the elevated plus maze (EPM) and the tail-suspension test (EPM). Treatment with fluoxetine or vehicle was then discontinued for 7 days. All animals then received an i.p. treatment of BrdU (50 mg/kg) and were perfused 24 h later.

administration. Food and water were available ad libitum and temperature was $21 \pm 1\,^{\circ}C$ and humidity was $50 \pm 10\%$. Animals remained housed in groups of 2–4 in polypropylene cages (33 cm long × 15 cm wide × 13 cm high) with wood chip bedding and environmental enrichment (shredded paper and cardboard tubes). All procedures were approved by the Research Ethics Committee, National University of Ireland Maynooth, and were licensed by the Department of Health and Children, Ireland under statutory instrument No. 543 of 2012 and the European directive 2010/63/EU.

The experimental regime employed is represented in Fig. 1. For the induction of sepsis, animals were given a single i.p. treatment of LPS (5 mg/kg; serotype 0111.B4, Sigma Ireland) or control animals were treated with vehicle sterile saline. One week after LPS/vehicle treatment, animals were treated with fluoxetine (10 mg/kg, Tocris Bioscience, UK) in standard drinking water, or just water for control. Fresh fluoxetine was given every second day; animals and water were weighed to ensure the correct dosage was maintained. Animals were given fluoxetine or vehicle for 28 days before behavioural testing began and throughout the duration of behavioural testing. Animals were tested on the novel object exploration task followed by the elevated plus maze and then the tail suspension test. All behavioural tests were carried out as previously described, and the selection of behavioural tests was made on the basis of results from our previous study regarding post-septic affective and cognitive changes in the mouse [4]. Briefly, for the tail suspension test mice were attached to a support raised to a height of 121 cm using tape placed 1 cm from the tip of their tales for six minutes. Mice were suspended for six minutes each, with immobility (complete absence of movement) being recorded throughout the entire six minutes. For the novel object exploration task animals were first habituated to an arena of 50 cm and then underwent 5 trials lasting two minutes being habituated to two objects. On the fifth trial, one familiar object was replaced with a novel object. Animals were measured on their time touching, and number of nose touches for each object. As such we primarily use this task as a measure of exploratory motivation, but also calculate preference ratios for exploration of the novel object in the probe trial as a measure of working memory. For the elevated plus maze, the maze consisted of a centre area of diameter 13.5 cm at a height of 20 cm, from which four arms extended of length 34.5 cm, width 5 cm. Two arms were open without walls, while the other two were enclosed by high walls. Entrance to an arm was counted where all four of an animals' paws were within the arm. Animals underwent one five-minute testing session each.

To assess the rate of cellular proliferation in the hippocampus mice were injected i.p. with 50 mg/kg BrdU one week after the conclusion of behavioural testing. 24 h later mice were terminally anaesthetised with 0.1 ml sodium pentobarbital (Euthatal, Merial Animal Health, UK) and perfused transcardially with 0.9% saline, with brains fixed in 4% paraformaldehyde (Sigma Ireland) in 0.1 M phosphate buffer (PB), pH 7.4 at 4 °C. Brains were then cryoprotected in 0.1 M PB containing 30% sucrose, frozen and sectioned coronally at 30 μm. Immunohistochemistry for IBA1 (1:3000, rabbit polyclonal primary antibody, Wako, Denmark; 019-19741), EGR1 (1:3000, rabbit polyclonal primary antibody, Santa Cruz Biotechnology; sc-189) and BrdU (1:200, rat monoclonal primary antibody, AbD Serotec, Oxford, UK; MCA2060) was carried out following a standard Avidin–Biotin Complex/Nickel DAB protocol as previously described [4,12]. Photomicrographs of sections were taken using a digital camera connected to an Olympus BX-51 light microscope under constant light intensity. Morphology and number of active microglia (stages 3–5 of Kreutzberg [13]) stained with IBA1 were assessed under 100× magnification within a pre-defined area. EGR1 immunoreactivity was assessed by optical density with ImageJ 1.43 (NIH, USA). BrdU immunopositive cells in the dentate gyrus were counted by eye. All image assessment was carried out by an experimenter blind to the experimental manipulation.

Data from the behavioural experiments and neurochemical data were assessed via a factorial multivariate analysis of variance (MANOVA). The dependent variables for the behavioural data were time spent immobile on the tail suspension test, time spent in open arms on the elevated plus maze and touches to both objects on the probe trial of the novel object exploration task. The factors were prior LPS treatment or vehicle and fluoxetine treatment or vehicle. For immunohistochemical data the dependent variables were cells incorporating BrdU, optical density for EGR1 and the number of IBA1-positive microglia with activated-like morphology. For post-hoc analysis Bonferroni corrections were applied. $P < 0.05$ was considered statistically significant. All data are presented as means and standard error of the mean.

As previous data from our laboratory had demonstrated that LPS-induced sepsis was associated with a persistent increase in immobility in the tail suspension test, decreased object exploration in the probe trial of a novel object exploration task and decreased time spent in the open arms of the elevated plus maze, we examined whether these post-sepsis-associated changes would be ameliorated by chronic fluoxetine treatment (Fig. 2). MANOVA results from the behavioural experiments indicate that there is a statistically significant interaction between LPS treatment and subsequent fluoxetine treatment, and that the magnitude of such an effect is large ($F_{3,37} = 20.43$, $P < 0.001$, partial eta squared = 0.629). There were also significant main effects of both LPS treatment ($F_{3,37} = 22.17$, $P < 0.001$, partial eta squared = 0.65) and fluoxetine treatment ($F_{3,37} = 5.5$, $P < 0.01$, partial eta squared = 0.32). Considering the outcomes of the individual behavioural tests, there were statistically significant interactions between LPS treatment and fluoxetine treatment for the tail suspension test ($F_{1,37} = 36.1$, $P < 0.001$, partial eta squared = 0.49) and the novel object exploration test ($F_{1,37} = 5.27$, $P < 0.05$, partial eta squared = 0.12), but not the elevated plus maze ($F_{1,37} = 1.88$, $P = 0.163$). For the novel object exploration test, there were no significant effects on the preference for exploration of the novel object versus the familiar object, as has been