

The endocannabinoid system regulates some aspects of the brain's inflammatory response, including the release of pro-inflammatory cytokines and modulation of microglial activation.<sup>1–4</sup> The endocannabinoid system is composed of two G-protein-coupled receptors designated as CB1 and CB2 expressed throughout the body and notably by neural stem cells.<sup>5</sup> We have previously shown that stimulation of the CB1/2 receptors using a low dose of WIN-55,212-2 significantly reversed the LPS-induced microglia activation in young rats.<sup>2</sup> This anti-inflammatory effect was also found in aged rats, attenuating the age-induced performance impairment observed in the water pool task.<sup>3</sup> As normal aging is associated with increased levels of microglial activation and a decrease in neurogenesis, both probably contributing to the hippocampus-related memory deficit, we investigated the effects of an agonist of CB1/2 receptors on neurogenesis in the brain of normal aged rats.

A total of 12 old (23-month-old) and 6 young (3-month-old) male F-344 rats were chronically infused for 28 days subcutaneously using an osmotic minipump with WIN-55,212-2 (2 mg kg<sup>-1</sup> per day  $n=6$ ) or the vehicle ( $n=12$ ) into the dorsal abdominal area. Two injections of 50 mg kg<sup>-1</sup> i.p. of 5-bromo-2-deoxyuridine were made on day 1 and day 2 post surgery to track new cells' production. The rats were assigned to one of the following three groups: young-vehicle ( $n=6$ ), old + vehicle ( $n=6$ ) and old + WIN-55,212-2 2 mg kg<sup>-1</sup> per day ( $n=6$ ).

Doublecortin immunoreactivity was found only in the subgranular zone of the dentate gyrus (DG) of the hippocampus (Figure 1A). A significant difference (Figure 1Aa–d) in doublecortin immunoreactivity was found between young (3-month-old,  $73.125 \pm 22.8$  cells per DG) and old rats (23-month-old,  $3 \pm 1.8$   $P < 0.05$ ). The 4 weeks of WIN 55,212-2 infusion resulted in a significant increase in doublecortin immunoreactivity cells (+116%,  $F_{1,30} = 6.774$ ,  $P = 0.0142$ , ANOVA with Fisher's PLSD *post-hoc* test) as compared with that in old vehicle-treated controls (Figure 1B).

5-bromo-2-deoxyuridine immunoreactivity was found sparsely in the cortex and hippocampus of the old vehicle-treated controls. The number of 5-bromo-2-deoxyuridine immunoreactivity cells was counted in the DG; the 4 weeks of WIN-55,212-2 infusion produced a significant increase in 5-bromo-2-deoxyuridine immunoreactivity cells (+63%,  $F_{1,221} = 12.795$ ,  $P = 0.0004$  ANOVA with Fisher's PLSD *post-hoc* test) as compared with that in old vehicle-treated controls (Figure 1C). The proportion of newly produced neurons over glial cells was determined and showed no significant difference between control or treated aged rats (Figure 1D,  $P = 0.42$ ), confirming the increase in number of neurons engrafted in the DG after the 4-week treatment.

Our results confirm that neurogenesis is still present during normal aging in rats, although at a drastically reduced level as compared with that in

young rats, for review see Verret *et al.*<sup>6</sup> We now report that neurogenesis in aged rats can be significantly increased by a low, continuous, non-psychoactive dose of a cannabinoid receptor agonist, WIN-55,212-2. This report shows for the first time the potential therapeutic efficacy of endocannabinoid receptor stimulation in stimulating neurogenesis from proliferation to engraftment during normal aging *in vivo*. The current results, coupled with our previous observations regarding the role of endocannabinoid receptors,<sup>3,4</sup> underscores the potential clinical benefits of cannabinoid pharmacotherapies during normal and pathological brain aging.

### Conflict of interest

The authors declare no conflict of interest.

### Author's contributions

YM and GLW conceived and designed the study. YM and HMB performed the experiments. YM, HMB and GLW wrote the paper. All authors have read and approved the final paper.

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## Activation of brain interleukin-1 $\beta$ in schizophrenia <sup>MP</sup>Open

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The underlying pathophysiological cause of schizophrenia is essentially unknown. Increasing evidence suggests that immunological processes may contribute to the etiology of the disease. Owing to the lack of direct evidence of an infectious cause of schizophrenia much attention has been directed towards cytokines; molecules that initiate immunological

responses. However, previous studies measuring the level of cytokines in the serum of patients with schizophrenia have provided an inconsistent picture in this regard.<sup>1</sup> The relatively few studies focusing on the level of cytokines in the cerebrospinal fluid (CSF) have been limited to a restricted number of cytokines analysed, to the lack of a control group of healthy volunteers or to the sensitivity of the cytokine assays used. Here, a sensitive assay was used to analyse the CSF concentration of well-characterized cytokines in first-episode patients with schizophrenia and age-matched healthy volunteers.

Lumbar punctures were carried out on twenty-six infection-free, first-episode male Caucasians who later were diagnosed with schizophrenia (DSM-IV). Patients were treated with various antipsychotics from the day of hospitalization to lumbar puncture (about three weeks). Thirty healthy male Caucasian volunteers served as controls. Cytokines were analysed using a sandwich-immunoassay-based protein-array system, see Supplementary Materials.

Interleukin (IL)-1 $\beta$ , IL-6 and IL-8 were reliably detectable in CSF of both patients and controls. IL-2, IL-4, IL-5, IL-10, granulocyte-macrophage-colony stimulating factor, interferon- $\gamma$  and tumour necrosis factor- $\alpha$  were found in low concentrations (median <0.5 pg/ml) or were undetectable in both patients and controls. In patients, IL-1 $\beta$  concentrations were markedly elevated (median 4.37 pg/ml) compared with controls (median 0.78 pg/ml; Figure 1). Analysis of IL-6 and IL-8 showed no significant difference between patients (median 2.53 and 64.6 pg/ml, respectively) and controls (median 2.52 pg/ml and 89.0 pg/ml, respectively). No correlations were observed between cytokine concentrations and age, smoking or length/dosage of antipsychotic medication.

These results provide evidence for the activation of the brain immune system in first-episode schizophrenia. This is in contrast to previous studies examining CSF of chronic patients with regard to IL-1 $\beta$ .<sup>2</sup>



**Figure 1** Interleukin (IL)-1 $\beta$  in cerebrospinal fluid from healthy volunteers (mean age  $25.4 \pm 7.2$  years) and patients with schizophrenia (mean age  $27.5 \pm 6.6$  years). Each point represents the concentration of IL-1 $\beta$  (pg/ml) in a single cerebrospinal fluid (CSF) sample. Horizontal lines show median values for each group. The 75 percentiles are 0.97 (healthy volunteers) and 12.2 (patients with schizophrenia).  $P < 0.0001$  (Mann-Whitney  $U$ -test).

Possibly, the elevation of IL-1 $\beta$  may be normalized or downregulated along the disease progress or during prolonged antipsychotic treatment. Whether the elevation of IL-1 $\beta$  is congenital, acquired during prodromal phase or absent until the time of first psychotic episode remains to be elucidated.

Like other cytokines, IL-1 $\beta$  is released from microglia and astrocytes. However, as serum cytokines may access brain for example, through saturable carriers or a damaged blood brain barrier,<sup>3</sup> a peripheral contribution to CSF IL-1 $\beta$  levels should not be excluded. It should be noted that the selective elevation of IL-1 $\beta$  observed differs from the general activation of pro-inflammatory cytokines seen during a CNS infection and from the typical cytokine profile observed during an autoimmune response.

During infection, pro-inflammatory cytokines, including IL-1 $\beta$  are suggested to induce sickness behaviour for example, lethargy, anhedonia, cognitive impairment and social withdrawal.<sup>4</sup> Similar symptoms are also referred to as negative symptoms and cognitive deficits in schizophrenia. Schizophrenia is generally attributed to a dysfunction of brain dopaminergic and glutamatergic circuits. Interestingly, administration of IL-1 $\beta$  to rats is associated with an increased and decreased dopamine concentration in the nucleus accumbens<sup>5</sup> and prefrontal cortex, respectively,<sup>6</sup> conditions proposed to occur simultaneously in schizophrenia. With respect to glutamate neurotransmission the action of IL-1 $\beta$  seems complex and diversified, including both excitatory and inhibitory components. These involve direct actions on the intercellular brain signalling<sup>7</sup> as well as effects with delayed onset, like altered expression of genes encoding the enzymes regulating glutamate neurotransmission.<sup>8</sup> Clearly, IL-1 $\beta$  possesses properties possibly causing a dopaminergic and glutamatergic dysregulation as proposed to occur in schizophrenia.

This is the first study showing a marked elevation of IL-1 $\beta$  in CSF of male patients with first-episode schizophrenia. Regardless of whether such activation of the brain immune system is of infectious, genetic or other origin it is clearly associated with the onset of schizophrenia. Present data are interesting in view of genetic studies showing an association of polymorphisms in the IL-1 gene cluster in schizophrenia.<sup>9,10</sup> Our results may open up new therapeutic strategies in the treatment of the disease. However, it remains to be established if the activation of IL-1 $\beta$  is causally related to the development of schizophrenia or is tentatively a consequence of the dopaminergic/glutamatergic dysfunctions that characterize the disease.

### Conflict of interest

The authors declare no conflict of interest.

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