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## Hippocampal serotonin-1A receptor function in a mouse model of anxiety induced by long term voluntary wheel running

Johannes Fuss<sup>1</sup>, Miriam A. Vogt<sup>1</sup>, Klaus-Josef Weber<sup>2</sup>, Teresa F. Burke<sup>3</sup>, Peter Gass<sup>#1</sup>, and Julie G. Hensler<sup>#3</sup>

<sup>1</sup>RG Animal Models in Psychiatry, Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, J 5, 68159 Mannheim, Germany

<sup>2</sup>Department of Radiooncology, Medical Faculty Heidelberg, Heidelberg University, Im Neuenheimer Feld 400, 69120 Heidelberg, Germany

<sup>3</sup>Department of Pharmacology, University of Texas Health Science Center-San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, United States

# These authors contributed equally to this work.

### Abstract

We have recently demonstrated that in C57/B16 mice long term voluntary wheel running is anxiogenic, and focal hippocampal irradiation prevents the increase in anxiety-like behaviors as well as neurobiological changes in the hippocampus induced by wheel running. Evidence supports a role of hippocampal 5-HT<sub>1A</sub> receptors in anxiety. Therefore, we investigated hippocampal binding and function of 5-HT<sub>1A</sub> receptors in this mouse model of anxiety. Four weeks of voluntary wheel running resulted in hippocampal subregion-specific changes in 5-HT<sub>1A</sub> receptor binding sites and function, as measured by autoradiography of [<sup>3</sup>H]8-OH-DPAT binding and agonist-stimulated binding of [<sup>35</sup>S]GTP S to G proteins, respectively. In the dorsal CA1 region, 5-HT<sub>1A</sub> receptor binding and function were not altered by wheel running or irradiation. In the dorsal dentate gyrus and CA2/3 region, 5-HT<sub>1A</sub> receptor function was decreased by running, but also by irradiation. In the ventral pyramidal layer, wheel running resulted in a decrease of 5-HT<sub>1A</sub> receptor function, which was prevented by irradiation. Neither irradiation nor wheel running affected 5-HT<sub>1A</sub> receptors in medial prefrontal cortex, or in the dorsal or median raphe nuclei. Our data indicate that down-regulation of 5-HT<sub>1A</sub> receptor function in ventral pyramidal layer may play a role in anxiety-like behavior induced by wheel running.

### Keywords

exercise; serotonin; neurogenesis; raphe nucleus; hippocampus

### INTRODUCTION

We have previously demonstrated that long-term voluntary wheel running increases levels of anxiety-like behavior in approach-avoidance tests in mice (Fuss *et al.*, 2010b). In this mouse model of anxiety, individual levels of anxiety were highly correlated with genesis of

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**Corresponding author** Johannes Fuss Central Institute of Mental Health J 5 68159 Mannheim Germany johannes.fuss@zi-mannheim.de Tel.: +49 621 1703 2931 Fax: +49 621 1703 2005 .

**CONFLICT OF INTEREST** None

new neurons in the hippocampus (Fuss *et al.*, 2010b), a brain region of particular interest involved in emotion (Fanselow and Dong, 2010; Gray and McNaughton, 2003). Moreover, ablation of hippocampal neurogenesis by focalized irradiation was sufficient to prevent the development of increased anxiety-like behaviors in dark-light box, o-maze and open field test (Fuss *et al.*, 2010a), indicating that hippocampal neurogenesis may be a crucial factor in the increase in anxiety-like behavior after long-term running. This finding was recently corroborated by others using a molecular approach to block neurogenesis (Onksen *et al.*, 2012). In our model running mice also have altered cellular activity, as measured by c-Fos activation, in hippocampal subfields in anxiogenic situations (Fuss *et al.*, 2010a). These effects of wheel running on anxiety-induced cell activation in hippocampus are prevented by focalized irradiation (Fuss *et al.*, 2010a).

Given the elevated levels of hippocampal neurogenesis and cell activation in this established model of exercise-induced anxiety, we hypothesized that additional neurochemical changes occur. The hippocampus receives a dense plexus of serotonergic innervation arising from serotonergic cell bodies in the dorsal and median raphe nuclei (see Molliver 1987). In order to begin to unravel mechanisms for the involvement of the serotonergic system in this animal model of anxiety, we focused our attention on the serotonin-1A (5-HT<sub>1A</sub>) receptor.

The unique distribution of 5-HT<sub>1A</sub> receptors in brain is consistent with its role in cognitive or integrative functions, as well as in emotional states. 5-HT<sub>1A</sub> receptors are present in high density in cortical and limbic areas, including the hippocampus (Hensler *et al.*, 1991, Kia *et al.*, 1996, Verge *et al.*, 1986). In these terminal field areas of serotonergic innervation, the 5-HT<sub>1A</sub> receptor is located postsynaptically to serotonergic neurons (Hensler *et al.*, 1991, Riad *et al.*, 2000, Verge *et al.*, 1986). Hippocampal 5-HT<sub>1A</sub> receptors play an important role in the maintenance of normal neuronal function (Sarnyai *et al.*, 2000), neurogenesis, and neuronal survival (Gould 1999, Fricker *et al.*, 2005, Santarelli *et al.*, 2003). While the activation of 5-HT<sub>1A</sub> receptors promotes neurogenesis in the adult hippocampus (Gould 1999, Banasr *et al.*, 2004), antagonists of the receptor inhibit cell proliferation in the dentate gyrus (Radley & Jacobs 2002).

Human and animal studies indicate that forebrain 5-HT<sub>1A</sub> receptor function is closely linked to anxiety symptoms (Andrews *et al.*, 1994, Spindelegger *et al.*, 2009). A number of studies in humans show an inverse correlation between 5-HT<sub>1A</sub> receptor binding and anxiety traits in healthy humans (Tauscher *et al.*, 2001) as well as reduced 5-HT<sub>1A</sub> binding in patients suffering from panic disorder (Nash *et al.*, 2008, Neumeister *et al.*, 2004) and social anxiety disorder (Lanzenberger *et al.*, 2007). Local injections of 5-HT<sub>1A</sub> receptor agonists into the hippocampus of rats reduces anxiety-like behavior in open field and elevated plus-maze tests (Kostowski *et al.*, 1989). In 5-HT<sub>1A</sub> knockout mice the absence of 5-HT<sub>1A</sub> receptors in all brain regions induces an animal model of increased anxiety (Ramboz *et al.*, 1998). However, a region-specific rescue of forebrain 5-HT<sub>1A</sub> receptors is sufficient to restore normal behaviour in these mice, further highlighting the important role of 5-HT<sub>1A</sub> receptor function in anxiety (Gross *et al.*, 2002).

Here, we measured both 5-HT<sub>1A</sub> receptor binding sites and function in forebrain regions, specifically medial prefrontal cortex and hippocampus, after voluntary wheel running. We also examined 5-HT<sub>1A</sub> receptor binding and function in serotonergic cell body areas, particularly the dorsal and median raphe nuclei, where these receptors function as somatodendritic autoreceptors (Aghajanian *et al.*, 1990, de Montigny *et al.*, 1984) and therefore control serotonergic neurotransmission. In our earlier experiments hippocampal irradiation prevented the increase in anxiety after wheel running. Hence, we also studied the effects of focalized irradiation on wheel running-induced changes in these 5-HT<sub>1A</sub> receptor populations. 5-HT<sub>1A</sub> receptor function was measured at the level of receptor-G protein

interaction using quantitative autoradiography of [<sup>35</sup>S]GTP S binding stimulated by the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT (Hensler, 2002; Hensler and Durgam, 2001). The coupled, high-affinity agonist state of the 5-HT<sub>1A</sub> receptor was measured by the binding of the agonist radioligand [<sup>3</sup>H]8-OH-DPAT (Chamberlain, et al., 1993; Verge et al., 1986).

## MATERIALS AND METHODS

### Animals

C57Bl/6J male mice (n = 40) were obtained at the age of 4 weeks from Charles River (Sulzfeld, Germany). Half of the mice (n=20) received hippocampus-focalized irradiation at the age of 4 weeks with a dose of 10 Gy (at a rate of 1 Gy/min). Irradiation was performed through a small stereotactically-defined opening (3.7 × 11 mm) sparing other brain regions from irradiation and allowing an even distribution of X-rays in the irradiated field (Fuss *et al.*, 2010a). Cells expressing neural differentiation marker doublecortin and proliferation marker Ki67 are evenly reduced in ventral and dorsal hippocampus after hippocampal irradiation (Fuss *et al.*, 2010a). Controls (n = 20) received a sham-treatment with the same anesthesia consisting of ketamine and xylazine (Fuss *et al.*, 2010a). Mice were housed in a light-dark cycle with lights on at 7 p.m. and provided with standard chow and water *ad libitum*. Following a recovery period of 4 weeks, irradiated and non-irradiated mice were single housed and 1 week later divided into runners and sedentary controls, the latter equipped with blocked wheels. After 4 weeks of daily running mice were sacrificed between 10 a.m. and 2 p.m. by decapitation and brain tissue prepared for autoradiographic studies. All procedures complied with the regulations covering animal experimentation within the EU (European Communities Council Directive 86/609/EEC) and Germany (Deutsches Tierschutzgesetz). Experiments were approved by the German animal welfare authorities (Regierungspräsidium Karlsruhe) and comply with the “Animal Research: Reporting In Vivo Experiments” (ARRIVE) guidelines. All efforts were made to minimize the number of animals used and the severity of procedures applied in this study.

### Tissue Preparation

Brains were rapidly removed, frozen on powdered dry ice and then stored at –80°C until sectioning. Coronal sections of 20 µm thickness were cut at –17°C in a cryostat microtome at the level of the medial prefrontal cortex (plates 13-14), dorsal hippocampus (plates 46-47), ventral hippocampus (plate 55) and dorsal raphe nucleus (plates 68-70) according to the atlas of the mouse brain by (Paxinos & Franklin 2001). Sections were thaw-mounted onto gelatin-coated glass slides, desiccated at 4°C for 18 hours under vacuum and then stored at –80°C until they were used in the autoradiographic experiments.

### [<sup>3</sup>H]8-OH-DPAT autoradiography

Autoradiography of the binding of [<sup>3</sup>H]8-OH-DPAT to 5-HT<sub>1A</sub> receptors in brain sections was performed as described with minor modification (Hensler *et al.*, 1991). Briefly, slide-mounted sections were thawed and desiccated at 4°C for 1 hour. Sections were pre-incubated for 30 minutes at 30°C in assay buffer (170 mM Tris-HCl; pH 7.6), and then incubated in assay buffer containing 2 nM [<sup>3</sup>H]8-OHDPAT for 60 minutes at room temperature. Non-specific binding was defined by incubating adjacent sections in the presence of 10 µM WAY 100635. Incubation was terminated by two washes for 5 minutes each in ice-cold 170 mM Tris-HCl buffer (pH 7.6), followed by a dip in ice-cold de-ionized water. Sections were dried on a slide warmer and exposed to Kodak BioMax MR Film for a period of 9 week to generate autoradiograms.

### [<sup>35</sup>S]GTP<sub>γ</sub>S autoradiography

Autoradiography of 8-OH-DPAT-stimulated [<sup>35</sup>S]GTP<sub>γ</sub>S binding in brain sections was performed as previously described (Hensler 2002; Hensler & Durgam 2001; Rossi *et al.*, 2006) with slight modifications. Briefly, slide-mounted sections were incubated in HEPES buffer containing GDP (2 mM), adenosine A1 receptor antagonist 1,3-Dipropyl-8-cyclopentylxanthine (DPCPX, 1 μM) and 40 pM [<sup>35</sup>S]GTP<sub>γ</sub>S, either in the absence or presence of racemic 8-OH-DPAT (1 μM), for 60 minutes at 25°C. This concentration of 8-OH-DPAT produces maximal stimulation of [<sup>35</sup>S]GTP<sub>γ</sub>S binding (Hensler & Durgam 2001). 8-OH-DPAT, which has high affinity for 5-HT<sub>1A</sub> receptors (K<sub>i</sub>=1 nM) (e.g. Sprouse *et al.*, 2004), also has moderate affinity for and agonist activity at 5-HT<sub>7</sub> receptors (K<sub>i</sub>=250 nM) (Sprouse *et al.*, 2004, Duncan & Franklin 2007, Hagan *et al.*, 2000, Hedlund *et al.*, 2004). However, the stimulation of [<sup>35</sup>S]GTP<sub>γ</sub>S binding by (1 μM) 8-OH-DPAT is completely blocked by the 5-HT<sub>1A</sub> receptor antagonist WAY 100635 (100 nM) in all areas of brain examined (Hensler & Durgam 2001), and is not altered by the selective 5-HT<sub>7</sub> receptor antagonist SB 269970 (100 nM) (Rossi *et al.*, 2006). Basal [<sup>35</sup>S]GTP<sub>γ</sub>S binding was defined by incubating adjacent sections in the absence of 8-OH-DPAT. Nonspecific [<sup>35</sup>S]GTP<sub>γ</sub>S binding was defined in the absence of 8-OH-DPAT and in the presence of 10 μM GTP<sub>γ</sub>S. The incubation was stopped by two washes for 5 minutes each in ice-cold 50 mM HEPES buffer (pH 7.4), followed by a brief immersion in ice-cold de-ionized water. Sections were dried on a slide-warmer and exposed to Kodak Biomax MR film for 48 hours.

### Image Analysis

Analysis of the digitized autoradiograms was performed using the image analysis program ImageJ, version 1.42q (National Institutes of Health, Bethesda, MD). Tissue sections were stained with thionin and the brain areas identified using the atlas of the mouse brain by (Paxinos & Franklin 2001).

Autoradiograms of [<sup>3</sup>H]8-OH-DPAT binding were quantified by the use of simultaneously exposed [<sup>3</sup>H] standards (ART-123, American Radiochemicals, St. Louis, MO, USA), which had been calibrated using brain-mash sections according to the method of Geary and colleagues (Geary *et al.*, 1985, Geary & Wooten 1983). This allowed the conversion of optical density measurements to femtomoles per milligram of protein. Specific binding was calculated by subtracting non-specific binding from total binding on adjacent sections.

Autoradiograms of 8-OH-DPAT-stimulated [<sup>35</sup>S]GTP<sub>γ</sub>S binding were quantified by the use of simultaneously exposed [<sup>14</sup>C] standards (ARC-146, American Radiochemicals). Standard curves were fitted to pixel data obtained from [<sup>14</sup>C] standards and tissue equivalent values (nCi/g) provided by American Radiochemicals, and were used to transform the actual regional densitometric values into relative radioactivity measures. Non-specific binding of [<sup>35</sup>S]GTP<sub>γ</sub>S was subtracted from basal binding and from binding in the presence of 8-OH-DPAT. Specific 8-OH-DPAT-stimulated binding was expressed as % above basal.

Basal [<sup>35</sup>S]GTP<sub>γ</sub>S binding was determined in tissue sections adjacent to those used to determine agonist-stimulated [<sup>35</sup>S]GTP<sub>γ</sub>S binding. Determination of basal binding in these sections serves as a control for any potential differences in tissue handling (e.g. dissection, freezing, sectioning). We and others commonly express agonist stimulated [<sup>35</sup>S]GTP<sub>γ</sub>S binding as % above basal, which serves not only to control for potential differences in tissue handling, but also controls for brain region-specific differences in basal [<sup>35</sup>S]GTP<sub>γ</sub>S binding. It is important to note that neither irradiation nor exercise resulted in changes in basal binding (Table 1).

## Statistical Analyses

Statistical analysis was carried out using SPSS 16.0 (SPSS Inc., Chicago, IL). All data are reported as means  $\pm$  S.E.M.. Differences between groups were detected using two-factorial analysis of variance followed by Fischer's LSD post hoc analysis. Significance was evaluated at a probability of 5% or less ( $<0.05$ ).

## Materials

[<sup>35</sup>S]GTP S (1250 Ci/mmol) was purchased from PerkinElmer (Waltham, MA). [<sup>3</sup>H]8-OH-DPAT (210 Ci/mmol) was purchased from Amersham Biosciences (Piscataway, NJ). Racemic 8-OH-DPAT hydrobromide, and 1,3-Dipropyl-8-cyclopentylxanthine (DPCPX) were purchased from Tocris (Ellisville, MO). WAY 100635 maleate, GDP (disodium salt) were purchased from Sigma/RBI (St. Louis, MO). GTP S (tetralithium salt) was purchased from Roche/Boehringer-Mannheim (Indianapolis, IN).

## RESULTS

First, our objective was to discover the impact of voluntary wheel running on 5-HT<sub>1A</sub> receptors in the dorsal and median raphé nuclei, where 5-HT<sub>1A</sub> receptors function as somatodendritic autoreceptors. Alterations in 5-HT<sub>1A</sub> receptor function or binding sites in these serotonergic cell body areas would be expected to directly alter the serotonergic tone in brain regions that receive projections from these raphé nuclei. Neither voluntary wheel running, nor focal hippocampal irradiation affected the 5-HT<sub>1A</sub> receptor binding in the dorsal and median raphé nuclei as measured by the binding of [<sup>3</sup>H]8-OH-DPAT (Table 2a). Moreover, we did not find significant changes in 5-HT<sub>1A</sub> receptor function as indicated by 8-OH-DPAT (1  $\mu$ M)-stimulated [<sup>35</sup>S]GTP S binding in either of the raphé nuclei (Table 2b).

The main focus of our the present work, however, was on the hippocampal formation (Fig. 1), since our previous studies had demonstrated a specific role of the hippocampus in voluntary exercise-induced anxiety, with a plethora of cellular and neurochemical alterations occurring specifically in the hippocampus following wheel running (Biedermann *et al.*, 2012, Fuss *et al.*, 2010b). In dentate gyrus of dorsal hippocampus (Fig. 1a), [<sup>35</sup>S]GTP S binding stimulated by 8-OH-DPAT (1  $\mu$ M) was decreased by long term wheel running, but also by irradiation (Fig 2a). Two-way ANOVA revealed an interaction effect of the factors running and irradiation ( $F(1,35) = 6.41$ ;  $p = 0.01$ ). Post-hoc comparisons showed a significant reduction in 8-OH-DPAT -stimulated [<sup>35</sup>S]GTP S binding in sham-treated runners compared to sham-treated sedentary mice ( $p = 0.04$ ). 8-OH-DPAT-stimulated [<sup>35</sup>S]GTP S binding was also decreased by focal irradiation in sedentary mice ( $p = 0.006$ ). [<sup>3</sup>H]8-OH-DPAT binding to 5-HT<sub>1A</sub> receptors was not significantly altered by voluntary wheel running or by focal irradiation (Fig. 2a). Our data indicate that 5-HT<sub>1A</sub> receptor function, at the level of receptor-G protein interaction is reduced in dentate gyrus following long term wheel running, but also by irradiation.

In CA2/3 region of the dorsal hippocampus (Fig. 1a) [<sup>35</sup>S]GTP S binding stimulated by 8-OH-DPAT (1  $\mu$ M) was decreased by long term wheel running, but also by irradiation (Fig 2b). Two-way ANOVA revealed an interaction effect of the factors running and irradiation ( $F(1,35) = 10.88$ ;  $p = 0.002$ ). Post-hoc comparisons showed a significant reduction in 8-OH-DPAT-stimulated [<sup>35</sup>S]GTP S binding in sham-treated runners compared to sham-treated sedentary mice ( $p = 0.02$ ). 8-OH-DPAT-stimulated [<sup>35</sup>S]GTP S binding was also decreased by focal irradiation in sedentary mice ( $p = 0.04$ ). These decreases in 5-HT<sub>1A</sub> receptor function were accompanied by an increase in 5-HT<sub>1A</sub> receptor binding. [<sup>3</sup>H]8-OH-DPAT binding to 5-HT<sub>1A</sub> receptors was increased by voluntary wheel running in both sham-treated



and irradiated mice ( $F(1,36) = 10.06$ ;  $p = 0.003$ ), and by irradiation in running as well as in sedentary mice ( $F(1,36) = 6.02$ ;  $p = 0.01$ ) (Fig. 2b). 5-HT<sub>1A</sub> receptor function was restored in irradiated runners compared to irradiated sedentary mice ( $p = 0.02$ ). This increase in 5-HT<sub>1A</sub> receptor function in irradiated runners was accompanied by an increase in [<sup>3</sup>H]8-OH-DPAT binding.

In CA1 region of dorsal hippocampus (Fig. 1a), [<sup>35</sup>S]GTP S binding stimulated by 8-OH-DPAT (1 μM) was not significantly altered by voluntary wheel running or by focal irradiation (Fig 2c). [<sup>3</sup>H]8-OH-DPAT binding to 5-HT<sub>1A</sub> receptors was also not altered (Fig 2c). Two-way ANOVA revealed an interaction effect of the factors running and irradiation for [<sup>35</sup>S]GTP S binding ( $F(1,35) = 3.90$ ;  $p = 0.05$ ), Post-hoc comparisons indicated no significant reduction in dorsal CA1 ( $p = 0.23$ ). These data indicate that in the dorsal CA1 region, 5-HT<sub>1A</sub> receptor binding and function were not altered by wheel running or irradiation.

In the pyramidal layer of ventral hippocampus (Fig. 1b), [<sup>3</sup>H]8-OH-DPAT binding to 5-HT<sub>1A</sub> receptors was decreased by irradiation ( $F(1,30) = 10.52$ ;  $p = 0.003$ ), but not by wheel running (Fig. 2d). Although irradiation caused a reduction in 5-HT<sub>1A</sub> receptor binding in ventral pyramidal layer of (Fig 2d) there was no change in 8-OH-DPAT-stimulated [<sup>35</sup>S]GTP S binding, suggesting a compensatory increase in 5-HT<sub>1A</sub> receptor function. [<sup>35</sup>S]GTP S binding stimulated by 8-OH-DPAT (1 μM) however was decreased by wheel running (Fig 2d). Two-way ANOVA revealed an interaction effect of the factors running and irradiation ( $F(1,30) = 5.47$ ;  $p = 0.02$ ). Post-hoc comparisons showed a significant reduction in [<sup>35</sup>S]GTP S binding stimulated by 8-OH-DPAT (1 μM) in sham-treated runners compared to sham-treated sedentary mice ( $p = 0.005$ ). These data indicate that 5-HT<sub>1A</sub> receptor function, at the level of receptor-G protein interaction is reduced in ventral hippocampus following long term wheel running. Focal irradiation prevented the decrease in 5-HT<sub>1A</sub> receptor function observed in this brain region of runners.

To demonstrate the regional selectivity of our findings we measured 5-HT<sub>1A</sub> receptor binding sites and function in another serotonergic projection region, namely the medial prefrontal cortex (mPFCtx). Here, neither 5-HT<sub>1A</sub> receptor function (Table 2b) nor the number of 5-HT<sub>1A</sub> receptor binding sites as measured by the binding of [<sup>3</sup>H]8-OH-DPAT (Table 2a) were affected by wheel running or by focal hippocampal irradiation. In conclusion, our observations seem to be restricted to the hippocampus as a core structure influenced by long-term voluntary wheel running.

## DISCUSSION

Our objective in the present study was to investigate 5-HT<sub>1A</sub> receptor binding and function in an animal model of anxiety. In this mouse model, long term voluntary wheel running is anxiogenic. Focal hippocampal irradiation prevents the increase in anxiety-like behaviors in a variety of tests as well as neurobiological changes in the hippocampus induced by wheel running (Fuss *et al.*, 2010a, b). In hippocampus, we observed subregion-specific changes in 5-HT<sub>1A</sub> receptor binding sites and function, as measured by [<sup>3</sup>H]8-OH-DPAT binding and agonist-stimulated binding of [<sup>35</sup>S]GTP S to G proteins. In the ventral pyramidal layer, wheel running resulted in a decrease of 5-HT<sub>1A</sub> receptor function, which was prevented by irradiation. Our data suggest an association between decreased 5-HT<sub>1A</sub> receptor function in ventral hippocampus and anxiety-like behavior in this model of anxiety.

In the dentate gyrus and CA2/3 region of dorsal hippocampus, 5-HT<sub>1A</sub> receptor function was decreased by running, and also by irradiation. In the CA2/3 region, the decrease in 5-HT<sub>1A</sub> receptor function induced by running was accompanied by an increase of the binding

of [<sup>3</sup>H]8-OH-DPAT to the high affinity agonist state (i.e. the coupled state) of the receptor (Chamberlain, *et al.*, 1993; Verge *et al.*, 1986). As we are using [<sup>3</sup>H]8-OH-DPAT at a concentration near the K<sub>d</sub>, this could be interpreted as an increase in the affinity 5-HT<sub>1A</sub> receptors for agonist, or an increase in the number of 5-HT<sub>1A</sub> receptors in the coupled state. Taken together, these data suggest that the running-induced decrease in 5-HT<sub>1A</sub> receptor function in the dorsal CA2/3 region may not be at the level of the receptor (e.g. receptor affinity for agonist or receptors coupled to G protein), but may be due to changes in G protein function (e.g. the capacity of G protein to be activated by agonist binding to the receptor). Interestingly, running reversed the deficit in 5-HT<sub>1A</sub> receptor function in the CA2/3 region caused by irradiation and resulted in an up-regulation of [<sup>3</sup>H]8-OH-DPAT binding in irradiated mice. These data suggest that in irradiated mice, running induces an increase in the affinity of the receptor for agonist or increases the number of 5-HT<sub>1A</sub> receptors coupled to G proteins, which is accompanied by an increase in receptor function.

Voluntary exercise promotes neuronal activity in the hippocampus and increases the differentiation and proliferation of progenitor cells in the subgranular layer of the dentate gyrus (Matsumoto *et al.*, 2011, van Praag *et al.*, 1999). We and others have previously shown a strong correlation of running-induced hippocampal neurogenesis and anxiety-like behavior of mice (Fuss *et al.*, 2010 a,b; Onksen *et al.*, 2012). However, some investigators have found no relation of physical exercise and increased anxiety-like behavior (Dubreucq *et al.*, 2011), or have found a decrease in anxiety-like behavior after exercise (Salam *et al.*, 2009; Duman *et al.*, 2008; Brocardo *et al.*, 2012). A possible explanation for these discrepant findings might be differing degrees of running, and whether wheel running is voluntary or forced (Leasure and Jones, 2008). In our model C57Bl/6J mice performed excessive levels of running at an average of 10 km / active phase (Fuss *et al.*, 2010a, b). Differing levels of running might underlie motivational aspects mediated for example by the endocannabinoid system (Dubreucq *et al.*, 2010; Fuss *et al.*, 2010c). Furthermore strain, housing conditions (group versus single) and cage enrichment of control groups (blocked wheel versus no wheel) appear to impact anxiety-like behavior of mice (Chourbaji *et al.*, 2008; Dubreucq *et al.*, 2011).

When hippocampal neurogenesis is eliminated by focal irradiation or molecular approaches, running mice no longer exhibit an increase in anxiety-like behavior, indicating that hippocampal neurogenesis may be an important factor for the development of this anxiety-like phenotype (Fuss *et al.*, 2010a; Onksen *et al.*, 2012). Irradiation specifically targets cells with high proliferation rate like neural progenitors in the dentate gyrus (Jenrow *et al.*, 2010). Interestingly the production of other cell lines like astrocytes and oligodendrocytes seems to be less affected by irradiation (Mizumatsu *et al.*, 2003). We have previously reported a dramatic reduction in proliferating cells and newborn neurons 5 and 10 weeks after irradiation (Fuss *et al.*, 2010a). Running was not sufficient to compensate for this decrease. Therefore only sham-treated runners have increased numbers of newborn neurons following 4 weeks of exercise.

In dentate gyrus and CA 2/3 region of dorsal hippocampus, irradiation as well as wheel running decreased 5-HT<sub>1A</sub> receptor function. The reduction in 5-HT<sub>1A</sub> receptor function in these subregions of dorsal hippocampus of sedentary mice as a result of irradiation was comparable to that seen in sham-treated, wheel running mice. We and others have previously demonstrated that wheel running increases hippocampal neurogenesis (Onksen *et al.*, 2012; Fuss *et al.*, 2010a,b). Data from multiple anxiety tests indicate that mice with increased neurogenesis exhibit heightened anxiety (Onksen *et al.*, 2012; Fuss *et al.*, 2010a,b). Blockade of neurogenesis by irradiation (Fuss *et al.*, 2010a,b) or molecular approaches (Onksen *et al.*, 2012) prevents this anxiety-like phenotype. Taken together these data suggest that in the dorsal hippocampus decreases in 5-HT<sub>1A</sub> receptor function occur

independently of neurogenesis, and may be unrelated to the anxiety-like phenotype in this model.

Hippocampus-focalized irradiation is widely used to generate control groups to dissect neurogenesis-dependent and -independent mechanisms in emotional behaviors (compare e.g. Surget *et al.*, 2011). And it is argued that irradiation specifically targets proliferating cells. Yet in the present experiment we demonstrate that irradiation also affects 5-HT<sub>1A</sub> receptor function in dorsal hippocampus. However, we cannot conclude from our data if this effect is due to the reduction of newborn neurons, or a neurogenesis-independent effect of irradiation that affects 5-HT<sub>1A</sub> receptors directly. Given that earlier studies also employing an irradiated control group have demonstrated a neurogenesis-dependent effect of serotonergic drugs (namely selective serotonin reuptake-inhibitors) on emotional behavior (Santarelli *et al.*, 2003), our findings of attenuated 5-HT<sub>1A</sub> receptor function should be taken into consideration in future experimental interventions. To understand how irradiation blocks emotional consequences of treatments stimulating neurogenesis, the role of 5-HT<sub>1A</sub> receptors needs to be evaluated in future experiments.

5-HT<sub>1A</sub> receptor function and binding in medial prefrontal cortex and serotonergic cell body areas (i.e. dorsal and median raphe nuclei) were not altered by wheel running or by focal hippocampal irradiation. This is in marked contrast to what we observed in hippocampus, and may be related to region-specific differences in the coupling of 5-HT<sub>1A</sub> receptors to subtypes of G protein belonging to the Gi/Go family (Mannoury la Cour *et al.*, 2006). In hippocampus, 5-HT<sub>1A</sub> receptors interact mainly with G<sub>o</sub> and weakly with G<sub>i3</sub>. This is in contrast with what is found in the cortex, where 5-HT<sub>1A</sub> receptors interact equally with G<sub>o</sub> and G<sub>i3</sub>. By contrast, in the anterior raphe, the 5-HT<sub>1A</sub> receptor couples exclusively with G<sub>i3</sub> (Mannoury la Cour *et al.*, 2006). The differences in G protein coupling between hippocampus, cortex and anterior raphe may be relevant to the region-specific regulation of 5-HT<sub>1A</sub> receptor function.

In conclusion, we found a region-specific modulation of hippocampal 5-HT<sub>1A</sub> receptor function in this mouse model of anxiety. In the dorsal dentate gyrus and CA2/3 region, changes in 5-HT<sub>1A</sub> receptor function appear to be unrelated to neurogenesis and the anxiety-like phenotype associated with this model of anxiety. In the ventral pyramidal layer however, wheel running resulted in a decrease of 5-HT<sub>1A</sub> receptor function, which was prevented by irradiation. These data suggest an association between decreased 5-HT<sub>1A</sub> receptor function in ventral hippocampus with anxiety-like behavior in this model. Gene expression studies and anatomical data support a functional distinction between the dorsal and ventral hippocampus. The dorsal hippocampus performs primarily cognitive functions, whereas the ventral hippocampus plays a critical role in emotion and the regulation of stress responses (Fanselow and Dong, 2010). Future pharmacological studies will help to further clarify the role of post-synaptic 5-HT<sub>1A</sub> receptors in the hippocampus in anxiety. For example, local injection of 5-HT agonist in ventral and dorsal hippocampus (Jolas *et al.*, 1995) may clarify the role of post-synaptic 5-HT<sub>1A</sub> receptors in hippocampal subregions in the development of running-induced anxiety-like behavior in this model.

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## ABBREVIATIONS

5-HT<sub>1A</sub>                      serotonin-1A



**GTP S**                    guanosine 5'-O-[gamma-thio]triphosphate  
**8-OH-DPAT**            8-Hydroxy-2-(di-n-propylamino)tetralin

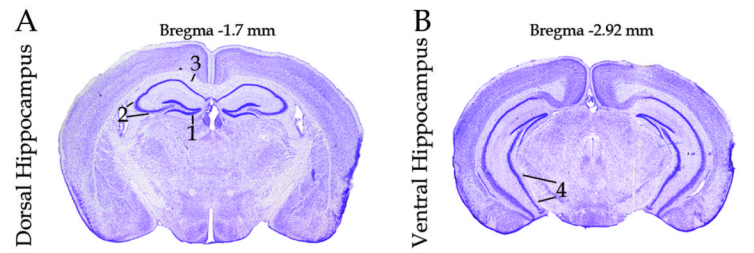
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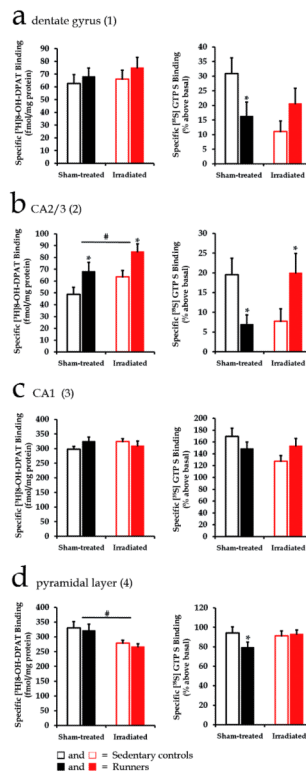
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**Figure 1.** Representative sections of hippocampus illustrating regions of interest. A. Dorsal hippocampus, (1) dentate gyrus, (2) CA2/3 regions, (3) CA1 region. B. Ventral hippocampus, (4) pyramidal layer. (adapted from Paxinos and Franklin, 2001)





**Figure 2.** 5-HT<sub>1A</sub> receptor function and binding in the dorsal (panels A, B and C) and ventral (panel D) hippocampal subregions. [<sup>35</sup>S]GTP S binding was stimulated by the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT (1 μM), and is expressed as % above basal. Specific binding of [<sup>3</sup>H]8-OH-DPAT (2 nM) is expressed as fmol/mg protein. \* indicates a significant post-hoc difference between runners and sedentary mice within groups of sham-treated or irradiated mice. # indicates significant differences between groups of sham-treated and irradiated mice in two-way ANOVA. Columns present means ± SEM.

**Table 1**

Basal binding of [<sup>35</sup>S]GTP S in specific brain regions of sham-treated (SS and SR) and irradiated, sedentary or running mice (IRS and IRR). Basal binding is expressed as tissue equivalent values (nCi/g). Values represent mean ± S.E.M. n = 9 per experimental group.

*Basal binding of [<sup>35</sup>S]GTP S*

	SS	SR	IRS	IRR
<b>Dorsal Hippocampus</b>				
CA1 region	169 ± 11.1	172 ± 8.4	176 ± 10.6	176 ± 7.3
CA2/3 region	173 ± 11.6	180 ± 7.9	177 ± 10.4	183 ± 6.8
Dentate gyrus	160 ± 11.9	156 ± 8.5	162 ± 11.3	150 ± 7.1
<b>Ventral Hippocampus</b>				
Pyramidal layer	265 ± 13.9	252 ± 11.1	251 ± 9.9	253 ± 12.1

**Table 2**

5-HT<sub>1A</sub> receptor binding sites and function are not altered in the dorsal raphe nucleus (DRN), median raphe nucleus (MRN) or medial prefrontal cortex (mPFCTx) by voluntary wheel running or hippocampus-focalized irradiation. Specific binding of [<sup>3</sup>H]8-OH-DPAT (2 nM) (a) is expressed as fmol/mg protein. [<sup>35</sup>S]GTP S binding (b) was stimulated by the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT (1 μM), and is expressed as % above basal.

**a 5-HT<sub>1A</sub> receptor binding sites (fmol/mg protein)**

	SS	SR	IRS	IRR	Running	Irradiation	Interaction
DRN	218 ± 16	215 ± 16	217 ± 16	218 ± 16	F < 1	F < 1	F < 1
MRN	45.5 ± 6.9	49.8 ± 7.3	38.3 ± 7.3	44.8 ± 6.9	F < 1	F < 1	F < 1
mPFCTx	120 ± 9	120 ± 9	122 ± 10	120 ± 9	F < 1	F < 1	F < 1

**b 5-HT<sub>1A</sub> receptor function (% above basal)**

	SS	SR	IRS	IRR	Running	Irradiation	Interaction
DRN	36.9 ± 4.6	29.3 ± 4.4	45.5 ± 4.4	37.1 ± 4.4	F = 3.2 p=.08	F = 3.5 p=.07	F < 1
MRN	29.1 ± 3.4	21.7 ± 3.4	20.1 ± 3.4	20.2 ± 3.4	F = 1.2 p=.29	F = 2.4 p=.13	F = 1.2 p=.28
mPFCTx	52.1 ± 6.2	56.5 ± 6.2	49.6 ± 6.2	58.7±6.2	F = 1.1 p=.29	F < 1	F < 1