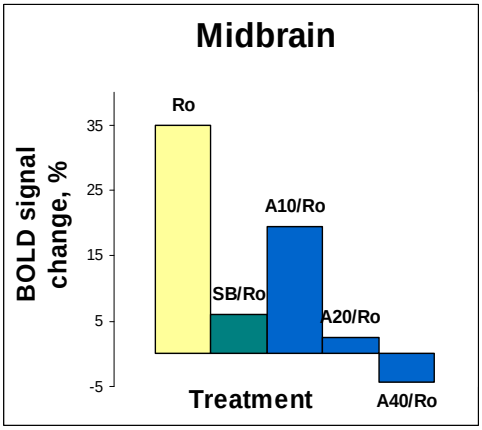
Characterization of a 5-HT2C antagonist profile of Agomelatine using challenge phMRI in the rat

I. Linnik[1], S. McKie[2], J. A. Stark[3], S. Luckman[3], B. Deakin[2], and S. R. Steve R. Williams[1]

[1]Imaging Science and Biomedical Engineering, Manchester University, Manchester, United Kingdom, [2]Neuroscience and Psychiatry Unit, Manchester University, Manchester, United Kingdom, [3]Faculty of Life Science, Manchester University, Manchester, United Kingdom

PURPOSE Agomelatine (S 20098) is a novel clinically active antidepressant drug.[1, 2] Agomelatine is a potent melatoninergic agonist at MT_1 and MT_2 sites [3] and it also acts in vitro as a serotoninergic antagonist of the 5-HT_{2C} receptors.[4] The antidepressant activity of agomelatine needs the combination of melatonin agonist activity and 5-HT_{2C} serotoninergic antagonist properties.[5]
In the present study selective 5-HT_{2C} agonist Ro60-0175 [6] was used to test the robustness of brain activation by 5-HT_{2C} antagonists (Agomelatine and SB 242084*) in vivo* using challenge phMRI. The aim of the study was to identify areas in which Ro60-0175-induced activation is modulated by pre-treatment with either agomelatine, or a specific $5HT_{2c}$ antagonist (SB 242084). This allowed us to detect the brain regions in which agomelatine modulates serotoninergic activity and to test the hypothesis that this is due to direct interaction with 5-HT_{2C} receptor subtypes.
METHODS ANIMAL PROTOCOL: Sprague-Dawley male rats (250 ± 40 g, Charles River Laboratories, Inc., Sandwich, UK) were. anaesthetized with α-chloralose and introduced into the bore of the 7T magnet. Temperature and respiration rate were monitored.
DRUGS: 5-HT_{2C} antagonists (1.Agomelatine: 10, 20 and 40 mg/kg (I.R.I.S.); 2. SB 242084: 10 mg/kg) and vehicle (HEC 1%).
5-HT_{2C} agonist (Ro60-0175: 3 mg/kg) and vehicle (saline + Tween 80).
MR PROTOCOL: Data were obtained on a 7T horizontal-bore magnet (Magnex Scientific Ltd., Abingdon, UK) interfaced to a SMIS console (Surrey Medical Imaging Systems Limited, Surrey, UK) with a transmit-receive surface coil. For functional images, a T_2*-weighted 2D gradient echo sequence was used: TR/TE = 172 ms/15ms, a matrix 128 x 64 x 4, 11 coronal slices of 1mm thickness. A total of 72 volumes were acquired. Antagonist or vehicle was injected (i.p.) during acquisition of volume 12, and agonist or vehicle was injected (s.c.) during acquisition of volume 24.
Data were analyzed using the p-block method.[7] Drug and time interactions were analyzed using a three-way ANOVA (antagonist x agonist x time). Any significant differences in the antagonist x agonist interaction indicating an antagonist effect on the Ro60-0175-induced BOLD signal. F-statistical parametric maps were overlaid onto an anatomical template image, with a threshold level of $p<0.01$ uncorrected. Paxinos and Watson (1998) atlas figures were used to identify the activated brain areas.

a



b

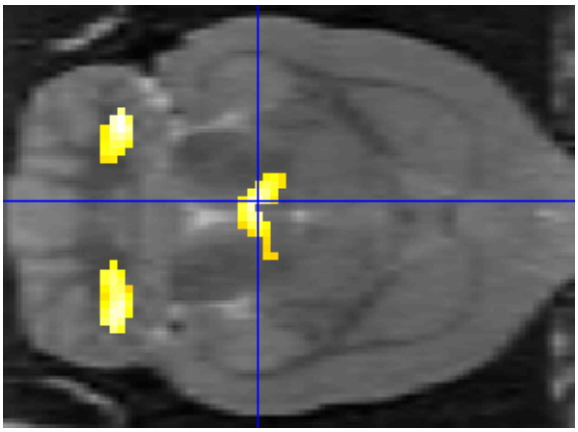


Fig. 1. a: *Significant changes in BOLD signal (%) in the rat brain using phMRI, showing the effects of Ro60-0175 (Ro) relative to appropriate controls and to pre-treatment with each of 2 antagonists: SB 242084 and agomelatine(A) at 10, 20, 40mg/kg in midbrain. The plots are total AUC from the single voxel at the position of the crosshairs (b).*

RESULTS SB 242084 and agomelatine significantly attenuated the BOLD response to Ro60-0175 in the following brain areas: cortex, midbrain (Fig.1), hippocampus, thalamic nuclei, pons and cerebellum. Figure 1 shows an example of the BOLD signal changes in the midbrain, the % signal change from pre-infusion baseline is shown as total area under the curve in a single voxel under the different conditions. It can be seen that there is a large increase in signal after Ro60-0175 alone, which is attenuated by increasing doses of agomelatine and by SB242084.
In a number of brain regions, the two antagonists have similar effects, with differences in magnitude presumably indicating different potency. In these brain areas we can conclude that the actions of agomelatine are due to $5HT_{2c}$ antagonism.
CONCLUSIONS This study has provided evidence for $5HT_{2c}$ antagonist activity of agomelatine *in vivo*, and has shown a convincing dose response in most of the brain regions in which it is active.
ACKNOWLEDGMENTS Funded by I.R.I.S. (Institut de Recherches Internationales Servier) - 6, place des Pléiades 92415 Courbevoie Cedex, France.
REFERENCES: 1. Lôo *et al*, *Encephale*; **29**:165-171(2003). 2. Kennedy *et al*, *Eur Neuropsychopharm;* **16**:93-100 (2006).
3. Masson-Pévet *et al*, *J. Pineal Res*; **25**:172-176 (1998). 4. Millan *et al*, *J Pharmacol Exp Ther*; **306**:954-964 (2003).
5. Papp *et al, Neuropsychopharmacology;* **28**: 694-703 (2003). 6. Martin *et al*, *J Pharmacol Exp Ther*;**286**: 913-924 (1998)
7. McKie *et al*, *Psychopharmacology;* **180**(4): 680-686(2005). ..