The use of [^{18}F]4-MPPF as a novel PET ligand for the study of 5-HT receptors: Ex vivo preclinical studies

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Introduction

- Serotonin (5-HT) has multiple important roles in brain function and its dysfunction is linked to depression and anxiety.
- [^{18}F]4-MPPF is a novel positron emission tomography (PET) ligand with affinity for 5-HT_{1A} receptors(1)
- The aim of this study is to investigate the binding distribution and displaceability of [^{18}F]4-MPPF in the rat brain

Methods

- Rats were pre-treated with [cold]4-MPPF, fenfluramine (a 5-HT releasing agent) or vehicle 30 min or 1 hr. before treatment with [^{18}F]4-MPPF.
- 5 minutes after [^{18}F]4-MPPF administration, the rats were sacrificed and brains was removed snap frozen and cut into 50 µM sections
- Brain sections from frontal cortex, hippocampus, dorsal raphe and cerebellum were exposed to autoradiographic film overnight.
- The autoradiography films were developed manually (Fig 2) and the brain sections were stained used cresyl violet to reveal histological architecture(Fig 1).
- An ImageJ macro was used to measure grey density of the autoradiographic images after the different brain areas had been defined on the cresyl violet stained sections.
- The % change in grey density (from [^{18}F]4-MPPF) was calculate in the [cold]4-MPPF, or fenfluramine pretreated animal. Data were analysed single sample t-test.

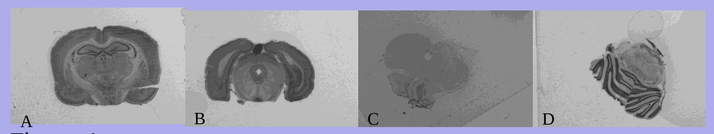


Figure 1
Cresyl violet stained brain sections containing- A the hippocampus, B the raphe nucleus, C the pre frontal cortex, D the cerebellum.

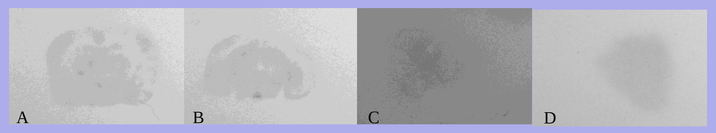
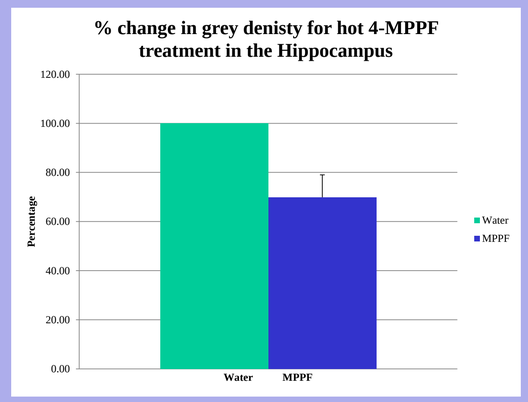
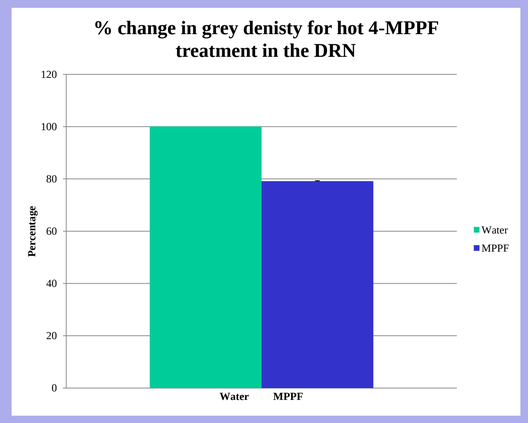


Figure 2
Autoradiography images showing [^{18}F]4-MPPF binding brain sections containing- A the hippocampus, B the raphe nucleus, C the pre frontal cortex, D the cerebellum.

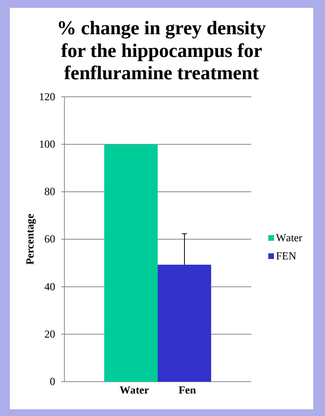
Results

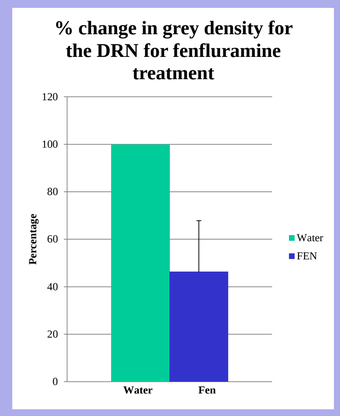
- The dorsal raphe, prefrontal cortex and hippocampus all showed high grey densities. These were higher than the low level signal in the cerebellum.
- Rats pretreated with [cold]4-MPPF had lower grey density in some area's than those that had been pretreated with vehicle (n=4 pairs). Single sample t-tests (compared to 100%) revealed significant effects of cold MPPF in the hippocampus (p= 0.023) and the raphe (p= 0.004)





- The above graphs show the change in grey density (specific binding) in the hippocampus and dorsal raphe nucleus after 30 minutes pre treatment with 4-MPPF. The non-specific binding in the cerebellum is used as a control and is removed from the other results to find the specific binding. The areas of high density are known to contain high levels of 5-HT_{1A} receptors





The % change in grey density can be seen in the hippocampus and the dorsal raphe nucleus due to fenfluramine treatment. Single sample t- tests (compared to 100%) revealed significant effects of the fenfluramine in the hippocampus (p= 0.03) but not in the dorsal raphe nucleus (p = 0.065)

Summary And Discussion

- The areas of high density are known to contain high levels of 5-HT_{1A} receptors
- The finding that excess 'cold' 4-MPPF displaced the hot [^{18}F]4-MPPF is consistent with the [^{18}F]4-MPPF PET ligand binding to the same receptor as the cold ligand
- The finding that pre-treatment with the 5-HT releasing agent fenfluramine displaced the hot [^{18}F]4-MPPF suggests that the binding of the [^{18}F]4-MPPF PET ligand can be displaced by an increased in endogenous 5-HT
- Current parallel in vivo PET studies have produced similar results to these and suggest that [^{18}F]4-MPPF may be a useful PET ligand for the study of 5-HT dysfunction

References

1 - Nicolas Aznavour and Luc Zimmer. (2007). [18F]MPPF as a tool for the in vivo imaging of 5-HT1A receptors in animal and human brain. *Neuropharmacology*. 52 (3), 695 – 707.

Acknowledgements

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