

Using tandem behaviour-PET to examine dopaminergic signalling underlying exploration

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Abstract

Here, we examine the potential of positron emission tomography (PET), a non-invasive technique that detects the location of a small molecule within a subject in real-time with resolution in the micrometre range, in providing insight into the role of dopaminergic signalling in exploratory behaviours. Using a pilot of 5 adult mice, we recorded the behaviour of each subject during a 15 min free exploration period and then performed PET imaging with the F-labelled high-affinity dopamine D₂/D₃ receptor antagonist ¹⁸F-fallypride. A correlation matrix of behaviours and brain regions of interest revealed some interesting correlations. In particular, we find a decreased standardised uptake value (SUV) for ¹⁸F-fallypride in the hippocampal formation and amygdala in subjects that exhibited high levels of unassisted rearing. This finding suggests that either a higher concentration of dopamine in these areas, or lower D₂/D₃ receptor availability, is associated with increased exploratory behaviour. In contrast, we found that high SUVs for ¹⁸F-fallypride throughout the brain correlated most strongly with immobility and body grooming, suggesting these behaviours dominate during times of low global dopamine/dopamine receptor binding. This pilot study serves as an example of the potential for using tandem behaviour-PET to identify novel brain-behaviour interactions, but additional refinements to the methods are warranted before full-scale studies are engaged.

Introduction

As a non-invasive technique that permits high-resolution real-time visualisation of receptor density and distribution with the help of radioactively-labelled small molecules, small animal positron emission tomography (PET) is an ideal tool to examine metabolic demand, receptor expression and neurotransmitter signalling in the intact brain [1]. The general principle underlying PET is that the concentration of a trackable radiotracer depends on both the concentration of available binding sites (typically determined by receptor expression) and the amount of competing endogenous ligand. Although first developed for use in humans, PET is now also performed with non-human primates as well as smaller experimental subjects (usually rodents, termed “small animal PET”). As a result, PET is increasingly recruited to address fundamental questions in neuroscience. In particular, small animal PET has proved to be a powerful tool to determine phenotypic differences resulting from genetic modification [2] [3] and has been insightful in research examining ischaemia, tumorigenesis, glucose metabolism and drug-addiction. For example, exposure to an environment previously paired with cocaine leads to a reduction in striatal ¹¹C-raclopride binding, suggesting either a down-regulation of D₂-like receptors or an increase in dopamine release in the striatum when a cue for the drug is presented [4].

The use of small animal PET to directly study behaviour in healthy wildtype animals is far more rare, and no studies that we could find have examined correlations between the PET signal of specific receptor-binding radiotracers (as opposed to a general metabolic sensor) and the duration of time spent performing individual behaviours prior to PET imaging. Our study here was, therefore, an exploratory endeavour, aiming to determine the feasibility and potential of such an approach. We decided to focus on novelty driven exploration since it is a fundamental behaviour governed by dopaminergic signalling [5] [6], and the dopaminergic neurotransmitter system is ideal to examine with PET.

We therefore monitored the behaviour of mice in a novel, non-stressful environment and then performed small animal PET using ^{18}F -fallypride as our radioligand since it is a D2/D3 receptor-binding small molecule suitable for measurement of extrastriatal dopamine release [7]. The aim was to investigate the potential for this approach to identify novel brain regions in which dopamine signalling may underlie exploration or be involved in important mental processes that take place during the post-exploration period (e.g. memory consolidation). Briefly, we find there is some promise, but refinements in the experimental protocol warrant investigation before full-scale studies are initiated.

Objective

The general objective of this study was to examine if performing small animal PET immediately following a behavioural analysis provides any possibility to identify brain-behaviour interactions.

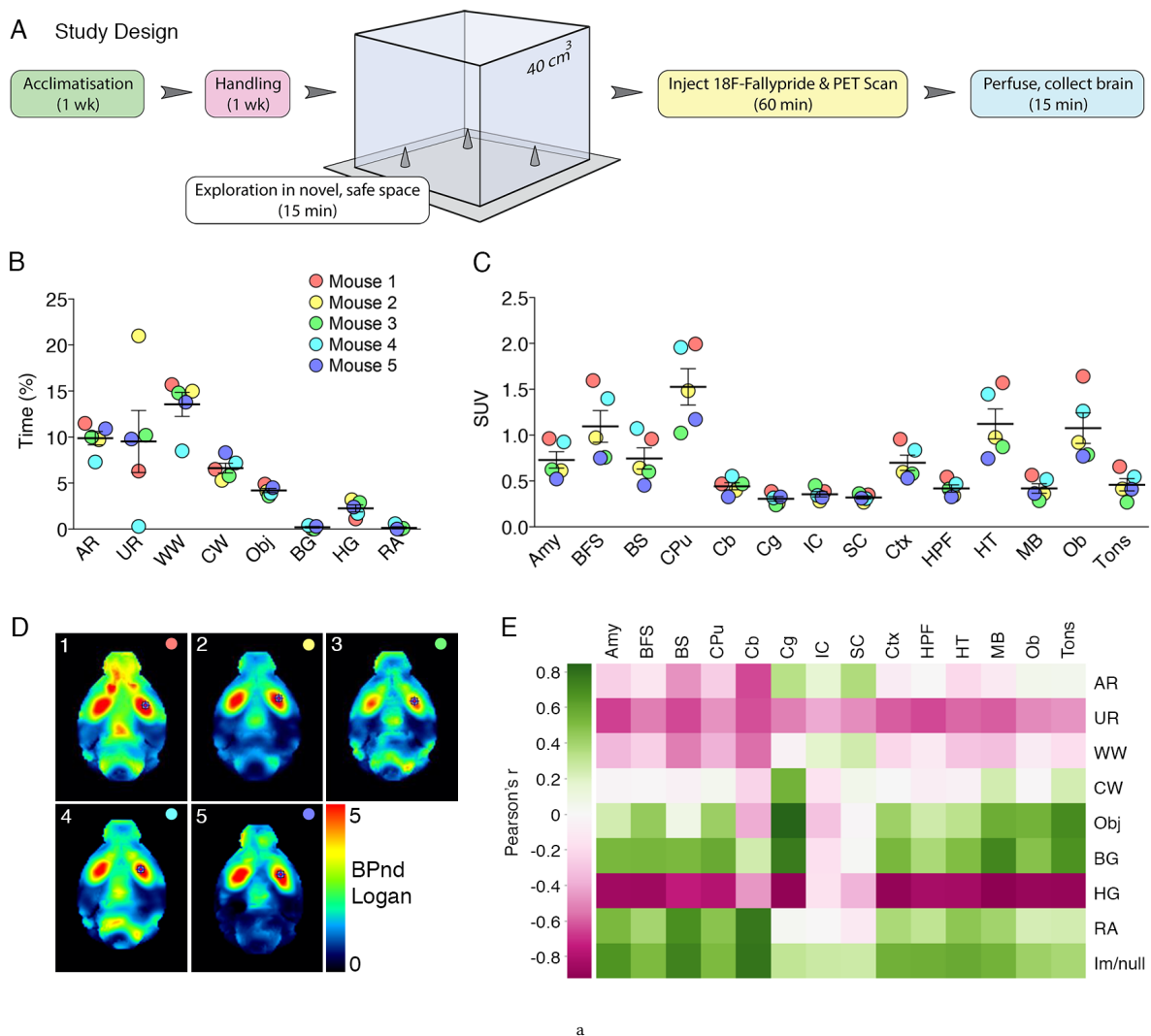


Figure Legend

Figure 1. Correlations of mouse behaviours in a novel, non-stressful environment with the regional distribution of ^{18}F -fallypride in the brain.

(A) Experimental study design.

(B) Individual behaviours performed by the 5 mice in the novel environment.

(C) Mean ^{18}F -fallypride standardised uptake values (SUVs) in various regions of interest (ROIs) for the 40–60 min post-injection period.

(D) Maximum intensity projections of ^{18}F -fallypride binding potential immediately after a 15 min exposure to the novel, non-stressful environment.

(E) Correlation matrix of duration spent on specific behaviours and ^{18}F -fallypride SUV.

Abbreviations: AR: Assisted rearing; UR: Unassisted rearing; WW: Wall walk; CW: Centre walk; Obj: Object interaction; BG: Body grooming; HG: Head grooming; RA: Risk assessment; Im/null: Immobility/null; Amy: Amygdala; BFS: Basal forebrain/sep-tum; BS: Brain stem; CPu: Caudate putamen (striatum); Cg: Central gyrus; Cb: Cerebellum; IC: Colliculus, inferior; SC: Colliculus, superior; Ctx: cortex; HPF: Hippocampal formation; HT: Hypothalamus; MB: Midbrain; Ob: Olfactory bulb; Tons: Thalamus.

Individual data points and mean \pm SEM are shown.

Results & Discussion

During the 15 min free exploration period (Fig. 1A), all 5 mice spent at least 25% of the time actively engaged in a specific behaviour other than sitting (“Im” or “immobility”) or transitioning between behaviours (“null”). Unassisted rearing, ranging from 0.3% to 21.0% (CV = 0.79), exhibited the most variation of the behaviours performed by all 5 animals (Fig. 1B). The behaviour with the least variation was object interaction (CV = 0.12). All subjects spent more time walking near the walls of the arena than in the centre, as expected, and overall behavioural performance was consistent with other recent investigations [6] [8]. In the subsequent PET scan, regional distribution of ^{18}F -fallypride-binding potential among a set of regions of interest (ROIs) was consistent with previous studies, exhibiting strongest uptake in regions such as the striatum where D2/D3 receptors are highly expressed, and whole-brain standardised uptake value (SUV) variation (Fig. 1C, D) was within the typical range [9].

We next used a correlation matrix to perform unbiased comparisons between the two datasets and observed both expected and unexpected outcomes (Fig. 1E).

First, unassisted rearing (canonical exploratory behaviour performed by mice [6] [10]) demonstrated an inverse correlation with ^{18}F -fallypride SUV throughout the brain, suggesting either a brain-wide internalisation of D2/D3 receptors or an increase in dopamine release throughout most of the brain. We favour the latter interpretation since novel environments like the one employed in this study are known to increase the firing rate of ventral tegmental area (VTA) dopamine neurons [11]. What our study adds to this knowledge is the potential that the degree of VTA activation corresponds to the degree of exploration within a novel environment, even when the degree of absolute novelty remains constant. Of the many ROIs demonstrating a negative correlation between ^{18}F -fallypride SUV and unassisted rearing, the hippocampus and amygdala showed the strongest Pearson’s r . Dopamine projections to these specific regions may, therefore, be the most strongly activated by sensorium-enriching exploratory behaviours.

The association between dopaminergic signalling and the hippocampus helps confirm an already established interaction [5] [6], but the strong correlation with the amygdala was more surprising. While VTA-amygdalar dopamine transmission is proposed to signal danger [12] and increased dopamine release occurs in response to stress [13], more unassisted rearing in the safe novel environment employed here would not be expected to indicate more stress. Unassisted rearing is unlikely a proxy for danger in our experiment. More likely, the lack of ^{18}F -fallypride in the amygdala reflects memory support mechanisms consistent with reduced amygdalar SUVs observed in humans during word pair association tasks [14].

Second, we observed that SUVs within the superior and inferior colliculi did not correlate strongly with any of the scored behaviours, suggesting D2/D3 receptors in the colliculi do not play a major role in these behaviours under the conditions used in this study. The special lack of correlations for ^{18}F -fallypride uptake in the colliculi also points towards a functional separation of dopamine neurons that project to the colliculi compared to other target regions. Both of these ideas are consistent with the seemingly specific role for dopamine signalling in the colliculi in behavioural responses to aversive or fearful stimuli [15] [16]. Our experiment examined well-handled subjects in a non-fearful environment. In this context, D2/D3 receptor expression level and/or dopamine release in the colliculi would not be expected to play any major role.

Third, the strongest correlations between behaviour and ^{18}F -fallypride SUV emerged

when examining head grooming (grooming anywhere in the face and head region while standing upright on hind legs). In behavioural literature, head grooming is rarely scored on its own, even when grooming microstructure is being examined as a proxy for internal states like anxiety [17] [18]. However, grooming initiation is known to be governed by dopaminergic signalling [19], and mice always start grooming at the face. The strong correlations between global dopamine signalling and head grooming would make sense in this context. In addition, head grooming could contribute to exploration as mice sample scents collected on their paws from the environment.

Finally, we assessed the feasibility of merging small animal PET and behavioural analyses using a Pearson's r power analysis [20]. Without considering multiple comparisons, sample sizes of 10 to 15 animals would be needed to obtain statistical significance for stronger correlations (e.g. hippocampus ^{18}F -fallypride and unassisted rearing). When considering multiple comparisons, the sample size required becomes highly dependent on correlative strength. For weaker correlations in the 0.6 range, multiple comparisons inflate the required sample size up to 25–30 subjects. Stronger correlations (Pearson's $r > 0.8$) are hardly affected by multiple comparisons, indicating sample sizes in the 10–15 range would still be adequate.

Conclusions

The current study describes the results of a correlation matrix derived from behavioural analysis in a novel safe environment and small animal PET imaging with the D₂/D₃ receptor radioligand ^{18}F -fallypride. The data are promising with respect to the possibility for tandem behaviour-PET to reveal novel brain-behaviour interactions. Even with its limited parametric power, the current study identified known interactions between dopaminergic signalling in the hippocampus and unassisted exploratory rearing. This is not to say that the other specific correlations revealed in this pilot study should be considered definitive. Prior to engaging in full studies, it would be prudent to first examine additional experimental refinements, such as administering the radioligand before behaviour.

Limitations

First, as is the case for all PET experiments, SUVs are determined by both the concentration of endogenous ligand and the surface expression of the target receptor. Histological assays, *in vivo* microdialysis and other complementary approaches are, therefore, needed to decipher between these sources of signal change.

Second, this study is parametrically limited. Only 5 male mice of a single strain were employed. Direct conclusions drawn from high Pearson's r values in the correlation matrix are strong candidates for false-positives. The primary value of the study is, therefore, not in drawing immediate conclusions on dopaminergic signalling underlying exploration but rather in assessing the possibility of doing so following a larger study.

Finally, the inherent delay in performing tandem behaviour-PET makes it difficult to determine if any observed correlations with specific behaviours are driven by dopamine signalling during free exploration or result from post-exploration processes such as memory consolidation. This issue could, at least in part, be resolved by simultaneously performing behaviour and PET using head-fixed awake subjects behaving on a rotating ball [21] or miniature PET equipment that can be carried directly on a rat's head [22]. However, these newer technologies are not without their own disadvantages, including complicated set-ups, large number of training hours needed before experimentation can begin and restricted subject manoeuvrability. We therefore felt that if insightful correlations could be made using standard small animal PET, this would represent an exciting opportunity in neuroscience.

Additional Information

Methods and Supplementary Material

Please see <https://sciencematters.io/articles/201702000008>.

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AMH, SMA and BJS designed experiments. AMH, SB and BJS performed experiments. AMH, H-II and BJS analysed data. All authors discussed the results, contributed to the manuscript and declare no conflicts of interests.

Ethics Statement

All experiments and manipulations conformed to the guidelines set by the Animal Care Commission of Switzerland and were covered under the authority of animal permit ZH263/2014. All possible measures were taken to ensure minimal pain and discomfort.

Citations

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