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PET IMAGING IS A PROMISING TECHNIQUE TO MEASURE BRAIN ACTIVITY DURING CYSTOMETRY IN RATS.

Hypothesis / aims of study

The bladder allows us to store urine until a socially convenient time to expel it. This simple function requires complex regulatory systems. Minor lesions result in overactive bladder syndrome (OAB) but the mechanisms are poorly understood. For a long time, research has focused on counteracting the efferent signal to the bladder. However, it now appears that not the efferent, but rather the afferent signal is key to the development of OAB. We know that the pontine micturition center (PMC) and the periaqueductal grey (PAG) are the most important brainstem-regions for bladder control. In turn, they are controlled by higher brain centers, such as the prefrontal cortex, thalamus, hypothalamus and basal ganglia. Brain imaging studies in patients have greatly contributed to our current knowledge, but voluntary control of the bladder can never be fully eliminated, which makes it more difficult to draw firm conclusions. In addition, genetic modifications, experimental surgical interventions and some pharmacological interventions cannot be performed in patients. Therefore, it is important that animal models for brain imaging exist. We investigated brain activity in normal rats, in relation to bladder function using small animal Positron Emission Tomography (PET) and 2-[^{18}F]-fluoro-2-deoxy-D-glucose ([^{18}F]-FDG).

Study design, materials and methods

We implanted a catheter (PE50, Clay Adams) into the bladder dome of 12 female Sprague-Dawley rats, all 13-14 weeks of age. 48 hours after surgery, all rats were anaesthetized (urethane I.P. 1.3g/kg) and installed in prone position on a thermal pad in the µPET scan. Glycemia was measured from the tail artery. We infused saline into the bladder at 0.1ml/min for 45minutes before start of the scan. Then, infusion was increased to 0.25ml/min in the test group and was stopped in the sham group. Subsequently, [^{18}F]-FDG (23.8±1.1 MBq) was dissolved in 500µl of saline and injected into the tail vein of the rat. Meanwhile, metabolic brain imaging was started, using a FOCUS 220 Positron Emission Tomograph (Siemens/Concorde Microsystems). Infusion rates were kept constant during 90 minutes of dynamic scanning. To analyse data, PET images were spatially normalized to a stereotactic space based on the rat brain Paxinos atlas, using PMOD® (version 3.2, PMOD Inc.). Two methods were used for further analysis. First, we used a template-based volume of interest (VOI) approach using PMOD®. Second, we analysed on voxel basis using SPM8 (Statistical Parametric Mapping version 8). For VOI analysis, a predefined map representing the major cortical and subcortical structures was loaded on all images. [^{18}F]-FDG uptake was measured in counts per voxel and averaged for each VOI. Relative glucose metabolism was determined by normalizing [^{18}F]-FDG data to the whole-brain uptake and statistical comparison was performed using a non-parametric t-test (Mann-Whitney u test). A p-value of 0.05 or lower was considered significant. For SPM analysis, we used a single factor design using the factor group. T-maps were interrogated at a peak voxel level of p=0.002 (uncorrected) with extent threshold kE>100 voxels. In both methods, differences in [^{18}F]-FDG uptake were compared between sham and test group.

Results

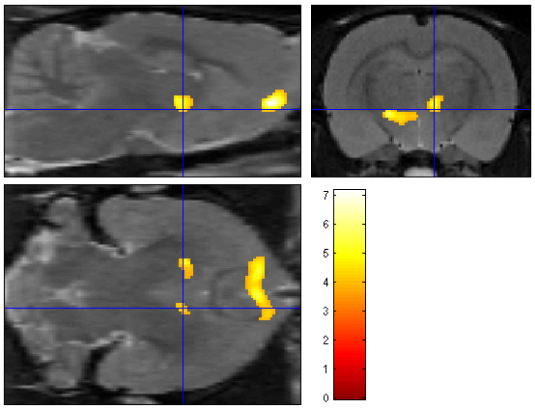
Significant differences in relative [^{18}F]-FDG uptake were observed in thalamus, frontal cortex and nucleus accumbens. In all these regions, uptake was higher in the test group. With VOI analysis, the difference amounted to 7.3% in the thalamus, 5.8% in the nucleus accumbens and 5.5% in the frontal cortex. Voxel-based analysis confirmed these findings: voxel-clusters that significantly differed from the sham group were shown in the frontal cortex and nucleus accumbens, using a threshold of p=0.001. When this was lowered to p=0.002 a voxel-cluster was also found in the thalamus. High saline infusion of 0.25 ml/min resulted in an average of 34 voidings during the scan. In the sham group, maximally 1 voiding was recorded.

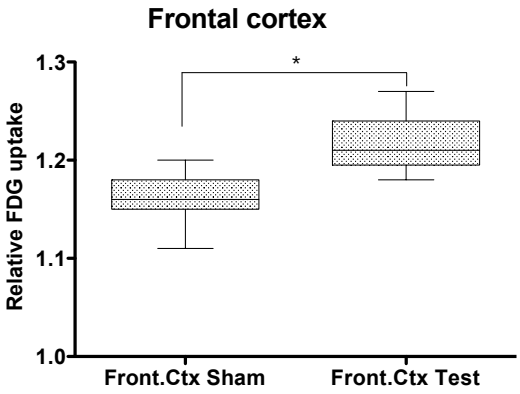
Interpretation of results

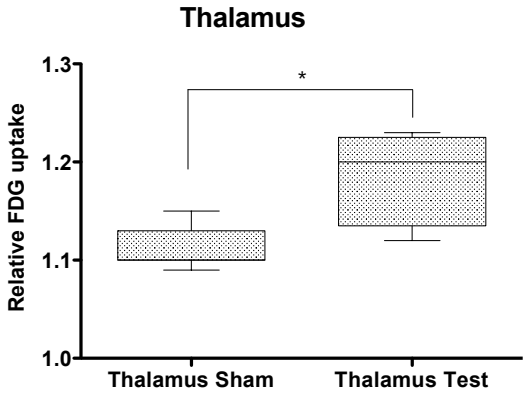
Our results are consistent with current literature. This strengthens our hypothesis that µPET scan can be used as a tool for measuring brain activity during cystometry in rats. However, not all previously reported regions could be detected. While in previous articles, obstruction of the urethra was often used to generate a high signal; we used a filling protocol that mimics physiological filling and emptying of the bladder. All brain regions we detected will therefore be more specific to bladder function, while confounding factors such as pain or attention have been eliminated. The main disadvantage is that it yielded a low magnitude of response and that no subdivision between storage and micturition was made. Therefore, some brain regions could not be detected. In the future, optimization of the protocol can improve on these drawbacks.

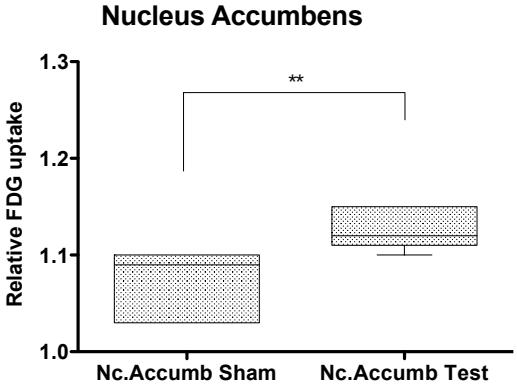
Concluding message

µPET scan with [^{18}F]-FDG is a promising technique to measure brain activity during cystometry in rats. The animal model carries advantages over brain imaging in patients. First, animals can be anesthetized to minimize environmental influences. Second, genetically modified animals can be used and last, pharmacotherapy can easily be tested. In addition, µPET scan is an accessible, relatively cheap technique, which is much easier to conduct than fMRI. On the other hand, signal to noise ratio is lower, but this can be improved by optimizing the protocol in future experiments.









Figures:
Brain regions selectively active in the sham group are shown in the top left picture. In all these regions, relative [^{18}F]-FDG uptake was higher in the test group. In the graphs, differences in uptake between sham and test group are shown. They amounted to 7.3% in the thalamus, 5.8% in the nucleus accumbens and 5.5% in the frontal cortex.

Disclosures
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