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Imaging Sex Differences in Regional Brain Metabolism during Acute Opioid Withdrawal

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Abstract

The rate of opioid overdose continues to rise, necessitating improved treatment options. Current therapeutic approaches rely on administration of either a blocking agent, such as naloxone, or chronic treatment with replacement drugs, including methadone and/or buprenorphine. Recent findings suggest that males and females respond to these treatments uniquely. In an effort to better understand this sex-specific variation in treatment efficacy, we investigated the effects of acute opioid withdrawal in male and female rats using 18FDG and microPET. These data demonstrate that acute opioid withdrawal produces metabolic alterations in brain regions associated with reward and drug dependence, namely corpus striatum, thalamic nuclei, septum, and frontal cortex. Furthermore, certain changes are unique to males. Specifically, males demonstrated increased metabolism in the anterior cingulate cortex and the ventral hippocampus (CA3) following acute opioid withdrawal. If males and females exhibit sex-specific changes in regional brain metabolism following acute opioid withdrawal, then perhaps it is not surprising that they respond to treatment differently.

Keywords: Drug dependence; Opioid withdrawal; Brain metabolism

Introduction

The escalating use of prescription pain relievers has contributed to the current opioid abuse epidemic in the United States. This has resulted in a surge of acute intoxication related deaths [1-3]. Although naloxone (Narcan®) expansion and administration has proven effective as a short-term measure, saving countless lives, it fails to address the underlying issue [4,5]. Unfortunately, the development of effective treatment strategies targeting patients suffering from opioid abuse and withdrawal has lagged behind its clinical necessity. In fact, current options are limited to opioid replacement with methadone and buprenorphine. While these medications represent first-line treatments for opioid detoxification, their efficacy remains controversial. Furthermore, these replacement therapies retain the addictive liability and side effect profile associated with illicit opioids.

Interestingly, there is an understudied observation that males and females respond to these treatments uniquely, consistent with knowledge that both substance abuse and dependence rates vary between males and females [6-11]. Two studies, a 25-year follow-up of heroin-dependent patients treated with methadone and a seven-year follow-up of patients prescribed buprenorphine both found that women were significantly more likely than men to have stopped heroin use [12,13]. This could be attributed to differences in analgesic tolerance, which is known to vary between males and females [14-17].

In the current study, we investigated whether sex would influence the metabolic representation of opioid withdrawal. Specifically, we examined the effects of acute opioid withdrawal on drug-naïve adolescent animals via micro positron emission tomography (microPET). Using 18F-fluorodeoxyglucose (18FDG), we compared the regional metabolic effects of acute opioid withdrawal between sexes. We hypothesized that males and females would respond to opioid withdrawal uniquely as evidenced by regional differences in brain glucose metabolism.

Methods

Adolescent male (n=8) and female (n=8) Sprague-Dawley rats were acquired from Taconic Farms. Animals arrived on postnatal day (PND) 22. Animals were maintained on a 12-hour light-dark cycle and received food and water *ad libitum*. Following an acclimation period, all animals received baseline 18FDG microPET scans (PND 31, Scan 1). Injectable morphine sulfate (15 mg/mL, 20 mL/vial) was acquired from Sigma Aldrich. Animals received morphine treatment for 5 days at a dose of 10 mg/kg/day subcutaneously (PND 35-39). Following a two-day withdrawal period (PND 40-41), animals received a second 18FDG scan (PND 42, Scan 2).

Prior to scanning, animals were fasted for 12 hours to ensure blood glucose stability [18,19]. All images were acquired using a Siemens Inveon microPET. Each animal received a single intraperitoneal injection of 18FDG (1.8-2.0 mCi). After 18FDG administration, animals were left undisturbed in their home cage for 40 minutes to ensure radiotracer uptake. Animals were then transferred to a clear acrylic chamber, where isoflurane/oxygen was used to induce anesthesia. Five minutes post-induction, animals were transferred to the imaging platform and were secured. Continuous isoflurane/oxygen at 2.0-2.5% was administered via nasal cannula for the entire 10-minute static scan. These imaging protocols have been shown to effectively reflect brain glucose metabolism [20-22].

All microPET images were corrected for attenuation and then reconstructed using a Maximum a Posteriori (MAP) probability estimate with 20 iterations as described previously [23,24]. Raw data files were uploaded into Pixel-wise Modeling Tool software (PXMOD version 3.3, PMOD Technologies LLC), and were aligned to a reference template created using the Paxinos and Watson Sprague-Dawley rat brain atlas. After placement in anatomical space, images were skull-stripped to subtract extraneous metabolic activity, and then were corrected for injected dose to ensure comparability of regional uptake values [20,25]. Post-processing including realignment to an atlas, normalization to a mean template, and smoothing was accomplished using Statistical Parametric Mapping (SPM5, Welcome Trust Centre for Neuroimaging). Between and within group comparisons were carried out using paired and 2-sample T-tests, respectively. Post-processed images were aligned to the Paxinos and Watson rat brain atlas [26] and regions were identified using x, y, and z coordinates. Increases and decreases in relative brain glucose metabolism were visually represented using color mapping. Images were overlaid onto an anatomical cryostat template with increases set as hot (red-yellow), and metabolic decreases set as winter (blue-green). The color scale used represents all T distributions achieving statistical significance [27-29]. All corresponding brain areas are significant at a value of $p \leq 0.001$ (corrected) with a cluster-extent threshold of k=0 voxels.

Results

There were no regional differences in brain metabolism between males and females at baseline (Figure 1A). However, acute opioid withdrawal produced significant changes in both cortical and subcortical brain metabolism (Figure 1B). When all animals were grouped together and compared to baseline, subjects experiencing acute morphine withdrawal demonstrated bilateral metabolic increases in the corpus striatum and thalamic nuclei, as well as in prelimbic and frontal cortices. Additionally, marked decreases were observed in the septum, ventral striatum, and ventral hippocampus compared to baseline (Figure 1B). When separated by sex, male and female groups exhibited significant differences. Specifically, males demonstrated increased metabolism in the anterior cingulate cortex and the dorsal hippocampus (CA3) compared to females (Figure 1C). No significant decreases in glucose metabolism were noted between males and females.

All reported increases and decreases were significant at a strict p-value threshold of $p \leq 0.001$ (corrected) with a cluster-extent threshold of k=0 voxels. These constraints were chosen based on previous studies where liberal primary cluster extent thresholds were kept at a minimum. These parameters ensure the statistical validity of reported regions of interest by eliminating large activations in overlapping anatomical areas [30].

Discussion

In the present study, no regional differences in brain metabolism were observed between adolescent males and females at baseline. However, following acute opioid withdrawal, brain metabolism was altered both cortically and subcortically. Specifically, metabolic increases were measured in the corpus striatum and the deep thalamic nuclei, in addition to increases in both the prelimbic and frontal cortices. Furthermore, metabolic decreases were noted in the septum, ventral striatum and ventral hippocampus. These findings reflect metabolic averages of male and female animals grouped together. When images were disaggregated according to sex, males
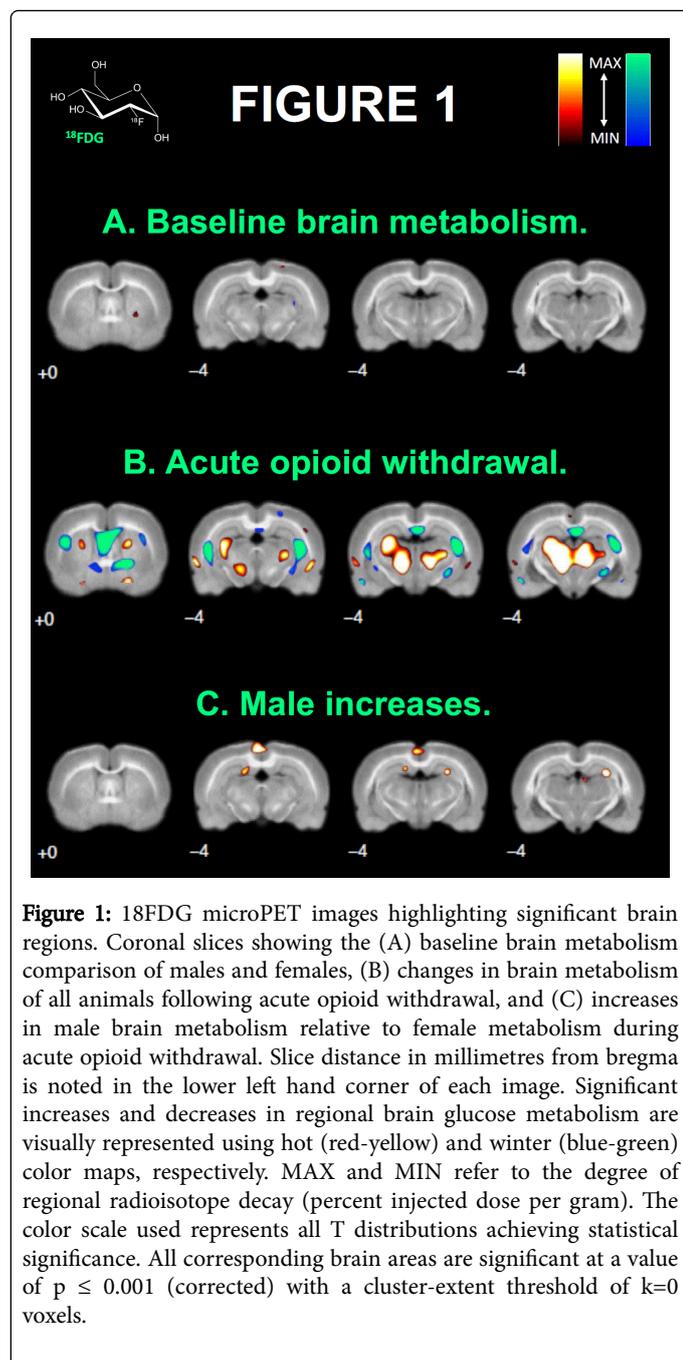
demonstrated increased metabolism in the anterior cingulate and the dorsal hippocampus (CA3) compared to females. There were no metabolic decreases observed between sexes.

Before treatment with morphine, both males and females exhibited similar patterns of brain glucose metabolism. However, following a five-day challenge with morphine and subsequent acute spontaneous withdrawal, males and females exhibited significantly different metabolic profiles, notably increased metabolism in anterior cingulate cortex among males. This is interesting given that disruption of the cingulate cortex can lead to an imbalance in dopaminergic signaling. This has been associated with impairment of executive function, reward-directed behavior, and conditioning, all of which have been implicated in impulsivity, compulsive drug use, and addiction [31-33]. More recently, Zakiniaeiz et al. demonstrated that the cingulate cortex may be a key region in the disruption of functional connectivity during cue-induced processing, while changes in its function may serve as a marker of subsequent alcohol relapse [34].

As noted earlier, previous studies suggest that sex differences likely affect the successful treatment of opioid abuse [12,13]. Here we demonstrate that it also impacts the primary metabolic representation of opioid withdrawal. The effects of opioids on the brain have been studied extensively [35]. Our findings support previous data indicating that opioids disrupt known reward pathways, notably in the corpus striatum [36]. Additionally, opioid withdrawal produces increases in thalamic cyclic AMP, which likely plays a role in the behavioral physiology of withdrawal [37]. The septum is also an integral part of the neurocircuitry underlying reward, pleasure, and drug seeking [38]. However, despite this knowledge, recent studies have shown that sex likely influences these pathways, and may affect treatment outcomes [17].

The morphine dose used in the present study was selected based on data indicating that a dose of 10 mg/kg was adequate to achieve conditioned place preference within this time period [39,40]. A single dose of morphine (10 mg/kg) was able to elicit conditioned place avoidance after a naloxone challenge [41]. Further, morphine, at this dose for this same period of time, also produced analgesic tolerance [42], and after only 4 days, produced withdrawal behaviors including increased defecation, urination, salivation, jumping, and wet dog shakes [43]. Finally, this dosing schedule activated glial cells and enhanced proinflammatory cytokine expression in the spinal cord, which has been implicated in morphine tolerance and withdrawal-induced hyperalgesia [39].

The 18FDG doses used are consistent with those reported previously using rats/mice and microPET [44-46]. This 18FDG dosing was designed to produce count rates that do not exceed the dead time correction capabilities of our scanner and images that could be reconstructed using an iterative method (i.e., maximum a posteriori). Relative to body weight, 18FDG is injected at significantly higher doses in rodents than in humans. These higher doses are necessary to achieve sufficient counting statistics and maximal spatial resolution in the substantially smaller brains of rodents [47]. Additionally, published reports have established that roughly the same amount of radiotracer used in humans should be used in rodents, since higher doses are necessary for equivalent image quality [48].

Figure 1: 18FDG microPET images highlighting significant brain regions. Coronal slices showing the (A) baseline brain metabolism comparison of males and females, (B) changes in brain metabolism of all animals following acute opioid withdrawal, and (C) increases in male brain metabolism relative to female metabolism during acute opioid withdrawal. Slice distance in millimetres from bregma is noted in the lower left hand corner of each image. Significant increases and decreases in regional brain glucose metabolism are visually represented using hot (red-yellow) and winter (blue-green) color maps, respectively. MAX and MIN refer to the degree of regional radioisotope decay (percent injected dose per gram). The color scale used represents all T distributions achieving statistical significance. All corresponding brain areas are significant at a value of $p \leq 0.001$ (corrected) with a cluster-extent threshold of k=0 voxels.

To our knowledge, this is the first study to assess and demonstrate sex-specific changes in regional brain metabolism following acute opioid withdrawal in drug-naïve adolescent animals. These findings are consistent with earlier reports suggesting that sex differences play an important role in the clinical presentation of opioid use disorders, specifically regarding drug craving and impairment of function [49]. Sex also appears to alter the expression of spontaneous withdrawal. Males often experience more severe and prolonged withdrawal compared to females [50]. However, while men often experience higher rates of substance abuse, women generally experience more adverse outcomes, significant impairment, and comorbid psychiatric disturbances [51], thus requiring more individualized approaches to treatment [52]. Future studies should examine both gender and sex

differences, and importantly similarities, to build a better profile of the neurobiological, psychiatric, and sociocultural factors characterizing male and female opioid dependent states [53,54]. Only then can we begin to devise more effective, and perhaps sex-specific, treatment strategies designed to address this urgent healthcare concern.

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