Effect of Prenatal Cocaine Exposure on D2 Receptors and DAT in Rhesus Macaques

Jennifer Pryweller  School of Engineering of Vanderbilt University

Five adult rhesus monkeys with a history of prenatal cocaine exposure (PNCE) and two saline-exposed control animals were used to optimize positron emission tomography (PET) imaging methods and to gather preliminary data on the effects of PNCE on dopamine systems in the adult non-human primate central nervous system. Dynamic PET scans were performed on each monkey to measure the binding kinetics of PET ligands specific for dopamine D2/D3 receptors ([18F]fallypride) and dopamine transporter ([18F]FECNT). PET scans were coregistered to structural T1-weighted MR images to aid in identification of regions with significant dopamine innervation for tracer kinetic analysis. Brain regions of interest (ROI) included caudate, putamen, substantia nigra and anterior cingulate cortex. Kinetic modeling using a cerebellum reference region was implemented in the PMOD software package to determine ligand binding potentials for D2/D3 receptors and dopamine transporters (DAT) in each of the ROIs to test whether fallypride and/or FECNT binding potentials, measures of D2/D3 and DAT levels respectively, were influenced by prenatal cocaine exposure. Preliminary results suggest a trend of reduced D2/D3 binding potential with increased PNCE, but future research is necessary.

Despite some variability amongst individual studies, a consensus is emerging that prenatal cocaine exposure (PNCE) can have dose dependent effects on the development of dopaminergic circuits (Levitt et al. 1997). A range of impairments, including deficits in recognition memory, processing speed, task persistence, arousal, distractibility, and stress-responsiveness, have been reported in infants and children exposed to cocaine in utero (Azuma et al. 1993; Delaney-Black et al. 1996; Gingras et al. 1998; Karmel et al. 1996; Koren 1998; Mayes et al. 1995; Mayes et al. 1993; Mayes et al. 1998). These deficits, which are highly reminiscent of early attention difficulties of children who develop ADHD (Richardson et al. 1996; Verbaten et al. 1994), suggest that similar or overlapping neural circuits are disrupted in both conditions. Animal studies suggest a molecular basis for at least some of these impairments. Chronic PNCE in rabbits has pronounced effects on CNS development (Jones et al. 1996; Jones et al. 2000; Levitt et al. 1997; Murphy et al. 1997; Stanwood et al. 2001; Wang et al. 1995) and produces specific changes in the structure and function of neurons in the DA-rich ACC and medial prefrontal cortex (mPFC) (Stanwood et al. 2001). The vulnerability of these frontal DA-rich targets is consistent with the long-term impairments observed in discriminative tasks requiring attentional processing (Romano et al. 1998; Romano et al. 1996; Romano et al. 1996; Taylor et al. 1999;
Gabriel et al. 1998). These animals have modifications in neuronal responsiveness in the ACC (Gabriel et al. 1998; Taylor et al. 1999) and hippocampus (Little et al. 1998; Little et al. 1996) and altered regulation of DA release (Wang et al. 1995; Du et al. 1999). Furthermore, D1-mediated locomotor responses to amphetamine-induced DA release are greatly reduced in adult PNCE rabbits (Simansky et al. 1996; Simansky et al. 1998; Romano et al. 1998). While the ADHD-like phenotype observed in PNCE rabbits arises from a postsynaptic defect in dopamine neurotransmission, the long-term molecular effects of PNCE on dopamine neurotransmission remain unclear in humans.

Positron emission tomography (PET) can map the distribution of specific ligands in the brain with high sensitivity and spatial resolution. Effective \(^{18}\text{F}\) ligands for cortical and subcortical dopamine D2/D3 receptors (fallypride) and DAT (FECNT), but not D1 receptors, are available. The purpose of the present study was first to implement PET imaging of D2/D3 and DAT distributions in the rhesus monkey, then to apply it to gather preliminary data on the effects of PNCE on D2/D3 and DAT levels in adult PNCE rhesus. To this end, data obtained from five prenatally exposed rhesus were compared to those from two saline-treated control animals. It was hypothesized that prenatal cocaine exposure in rhesus macaques leads to altered dopamine neurotransmission in part by changes in dopamine D2 receptors and/or transporters, which persists into adulthood.

Using PET imaging the local concentration of specific radioactive tracer molecules can be evaluated in a target tissue. A specific tracer molecule can selectively elucidate physiological interactions on a molecular level. The acquisition of dynamic (time-sequence) PET imaging data in conjunction with the application of kinetic models can quantify in vivo tracer kinetics. \(^{18}\text{F}\)fallypride (Figure 1a) is a radioactive tracer molecule that binds to both striatal and cortical dopamine D2 and D3 receptors. Due to its high affinity for D2 and D3 receptors, fallypride provides for a higher signal-to-noise ratio than other D2-like ligands (Slifstein et al. 2004). \(^{18}\text{F}\)FECNT (Figure 1b) is a radioactive dopamine transporter (DAT) probe. The positron emitter fluorine-18, a cyclotron produced radioisotope, has a half life of approximately 110 minutes, allowing for adequate injection and imaging time. Using fallypride and FECNT, a measure of D2/D3 and DAT levels can be evaluated in targeted tissues. Quantification of this parameter, known as the binding potential (BP) carries the implicit assumption that there is a constant affinity of the ligand for the receptor across subjects.
In kinetic modeling, compartmental models describe molecular interactions and kinetics between two portions of a body system where each compartment is a functionally homogeneous domain, and exchanges between compartments are either clearances or deliveries of material. A plasma sample defining arterial input is used in most compartmental modeling to drive the system. Reference region models provide BP estimates with the same level of accuracy as do arterial input functions, but use a non-invasive method of reference region selection for system input. Similar levels of $[^{18}\text{F}]$fallypride are found in the cerebellum and in arterial plasma, indicating low levels of specific binding (Mukherjee et al. 2002). Similar results are obtained for the DAT ligand FECNT. Two reference region models in particular provide for excellent measures of binding potential. The original Logan Plot model uses graphical analysis to calculate the distribution volume (DV) in a reversible system. The Logan Non-Invasive Model amended the Logan Plot to allow for direct calculation of the distribution volume ratio (DVR) by using a reference region $[C'(t)]$ with a constant tissue-to-plasma efflux rate $[k'_{2}]$. The DVR, a linear function of receptor availability, corresponds to the ratio of the DV of a receptor-rich region to a reference region (receptor-less) and is used to estimate BP. The result is Equation 1, where DVR is the regression slope and the intercept $[\text{int'}]$ becomes constant after the time at which equilibrium is reached (Logan et al. 2001). This Logan Non-Invasive model is a gold standard in kinetic modeling analysis:

$$\int_{0}^{T} \frac{C(t)dt}{C(T)} = DVR \left[ \frac{\int_{0}^{T} C'(t)dt + C'(T)/k'_{2}}{C(T)} \right] + \text{int'}$$

(1)

The Ichise Multilinear Reference Tissue Model 2 (MRTM2, Equations 2 and 3) (Ichise et al. 2003) eliminates BP bias and variability arising in situations of low signal to noise ratios.

$$C(T) = -\frac{V}{V'b} \left( \int_{0}^{T} C'(t)dt + \frac{1}{k'_{2}} C'(T) \right) - \frac{1}{b} \int_{0}^{T} C(t)dt$$

(2)

$$BP = -\left( \frac{\gamma_{1}}{\gamma_{2}} + 1 \right) = -\left( -\frac{Vb}{V'b} + 1 \right) = \frac{V}{V'} - 1.0$$

(3)

The MRTM2 was developed as a second subsequent modification to the original Multilinear Reference Tissue Model (MRTM0) which was originally developed as a linearized reference tissue model for PET images of binding potential. The first modification, MRTM, allowed for the estimation of a reference tissue clearance rate $[k'_{2}]$. Holding this parameter constant resulted in the second
modification, MRTM2 (Equation 2), which reduced the number of parameters from three to two. The two regression coefficients are \( \frac{V}{V'b} \) and \( \frac{1}{b} \). The BP is then calculated using the MRTM2 by taking the ratio of the two regression coefficients (Equation 3). The MRTM2, a recent addition to the available reference tissue models, distinctly reduces binding potential bias from 12-70 percent as seen in the MRTM0, to 1-4 percent. At the same time it claims to reduce BP variability from that of several other reference region models by a factor of two to three (Ichise et al. 2003).

Though fallypride is a newer tracer there is substantial literature reporting regional BP values of D2 receptors. Mukherjee et al. (2002) performed human studies using \( [18F] \)fallypride to evaluate D2/D3 receptor distribution in normal subjects 21-63 years of age. Reported BP values for the caudate and putamen were in the range of 16-32 and 19-37 respectively, and values for the substantia nigra were in the range of 1-1.9 or approximately five percent of that found in the putamen. Kinetic analysis of data was performed using the original Logan Plot model and showed decreasing receptor concentration with increasing age. Slifstein et al. (2004) investigated D2/D3 BP values in baboons using \( [18F] \)fallypride using a two-compartment kinetic model. The range of reported BP values of the striatum, which encompasses both the caudate and putamen, was 10-54. Siessmeier et al. (2005) compared three different D2 ligands to quantify binding potential in the human brain using six different techniques to analyze each data set. Using \( [18F] \)fallypride as the ligand the overall range of BPs found in the striatum inclusive of all six analysis techniques was 17.6-40.3. For kinetic analysis, the use of the Logan Non-Invasive model and the Simplified Reference Tissue Model (SRTM), thought to be comparable to the MRTM0, both provided BP ranges of 18-28.

**Methods**

Five adult rhesus monkeys (three females, two males) with a history of prenatal cocaine exposure were studied: one high dose (1.0 mg/kg t.i.d.); one intermediate dose (0.5 mg/kg t.i.d.); three low dose (0.3 mg/kg t.i.d.). Exposure to cocaine was present throughout the entire gestational period. These animals were compared with two saline-exposed controls (both males). All monkeys were 12-15 years of age. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC).

The first phase of the experiment was to obtain and register MRI and PET scans from each of the seven monkeys. High-resolution T1-weighted structural images were obtained from each monkey using a Philips 3T Achieva MRI scanner. Dynamic PET scans were performed on each monkey to measure the binding kinetics of specific PET ligands for the dopamine D2 receptor (\( [18F] \)fallypride) and DAT (\( [18F] \)FECNT) using a Concorde microPET Focus 220 scanner. Tracers were injected using a single bolus which had a specific activity of between 2.3 and 4.3 mCi. Fallypride scans were limited to three hours and FECNT scans to two hours—the times after which equilibrium was reached. All MRI and PET studies were performed under propofol anesthesia, as isoflurane has been shown to modulate DAT in non-human primates (Votaw et al. 2003). Structural T1-weighted MR images were
NOTE: Static (0-50min) filtered back projection PET scan images show differences in tracer distribution between $^{18}$F-fallypride (a) and $^{18}$F-FECNT (b). High levels of D2 receptors and DAT seen in subcortical grey matter: caudate (a), putamen (b) and substantia nigra (c).

registered to images from each PET scan. Rigid image registration was performed using the AMIR software package.

After image registration, regions of the brain with significant dopamine innervation were identified for tracer kinetic analysis. Brain regions of interest (ROI) included the caudate, putamen, substantia nigra and anterior cingulate cortex (ACC). The cerebellum was identified as a region of interest for use as the reference region in kinetic modeling. ROIs were manually drawn on the coronal plane of each static PET scan image using the PMOD software package. ROI selections were three-dimensional, encompassing the entire volume of the identified brain structure. The right and left portions of the caudate, putamen, substantia nigra and cerebellum were outlined as two separate volumes to exclude extraneous tissue. The anterior cingulate cortex was sub-divided into two distinct volumetric regions for ROI analysis. The anterior region included the pregenual and subgenual portions and the posterior region consisted of the supragenual portion of the anterior cingulate cortex. Each ROI was overlaid on the corresponding T1-weighted, registered image to validate structural accuracy of the ROI identification.

Dynamic PET scans for each monkey were analyzed using the Ichise Multilinear Reference Tissue Model 2 and the Logan Non-Invasive Model, implemented in the PMOD software package, to determine binding potentials for the D2/D3 receptors and DAT in each of the ROIs.¹

Results and Discussion

Static PET scan images, which summed the scans from the first 50 minutes of the dynamic PET scan, were evaluated to identify brain regions with the highest levels of dopamine innervation. When compared, the spatial distribution of dopamine D2/D3 receptors and DAT was found to be very similar. Figure 2 shows a fallypride static image (left) and an FECNT static image (right) from the same monkey. The image size and resolution are [128, 128, 95] and [1.898mm, 1.898mm, 0.815mm], respectively.
Arrows a, b and c point to the caudate, putamen and substantia nigra, which are three of the most highly innervated dopaminergic structures. The high intensity values seen in these subcortical grey matter structures validate their selection as regions of interest. The anterior cingulate cortex was also selected as a region of interest, as it has been implicated in the dopaminergic circuitry related to ADHD, and shows altered dopaminergic properties in PNCE rabbits (Levitt et al. 1997). Upon examination of PET scan images, a distinct difference in spatial distribution between the anterior and posterior regions of the anterior cingulate cortex was seen in both fallypride and FECNT images. Neuro-anatomical examination showed that the anterior region, with higher intensity values, corresponded to the pregenual and subgenual structures of the anterior cingulate cortex. The posterior region corresponded to the supragenual portion of the anterior cingulate cortex.

Figure 3a shows a sagittal midline section of a monkey brain from a fallypride PET scan. Arrows (1), (2) and (3) denote the supragenual, pregenual and subgenual structural regions of the anterior cingulate cortex. As a result, two volumetric ROIs were drawn on the PET images for each monkey, and subsequent analyses were performed for each ROI separately. When the two distinct ROIs for the anterior cingulate cortex, drawn on the PET image, were propagated onto the registered T1-weighted MRI image (Figure 3b), perfect structural correlation was confirmed.

Initial analyses of the caudate and putamen resulted in D2 receptor BP values significantly lower than those reported in literature. Further investigation showed higher than desirable levels of specific
binding in the cerebellum. An anatomical investigation of the cerebellum shed light on the problem. While the cerebellum has a low level of non-specific dopaminergic binding in the lobes, the vermis region, which lies in the center of the cerebellum, has high levels of specific dopaminergic binding. The decision to exclude the vermis region from subsequent cerebellar ROI selections was supported by previous research (Mukherjee et al. 2002). Subsequently, reference regions were outlined as two separate volumetric regions as outlined in Figure 4 where a coronal slice with the cerebellar lobes is outlined in blue and the excluded vermis region is outlined in red.

Post-processing, cerebellar time-activity curves for several of the fallypride and FECNT scans revealed unexpected oscillations in the first 50 minutes of the scan. As seen in Figure 5, the cerebellum time-activity curve should consist of an initial spike, when the tissue is saturated by the bolus injection, followed by decreasing counts across time, as the cerebellum only provides non-specific binding. Neither standard deviations nor a pattern in scan dates explained the oscillations. An FDG phantom (a tube containing $^{18}$F positron emitter with high specific activity) was dynamically scanned in the Focus 220 and validated the accuracy of the scanner's calibration. Using ASIpro, the software package inherent to the data acquisition system, cerebellar time-activity curve waveforms revealed similar oscillations, ruling out the PMOD software program as the source of error. It was concluded that the source of oscillations was the method of bolus injection. The scans were performed using a single bolus injection but the bolus was not tight enough, causing injection inconsistency. For future studies, the procedure for bolus injection should be altered to include a tighter bolus. Regions other than the cerebellum, including the anterior cingulate cortex, caudate, putamen and substantia nigra,
showed adequate and expected time-activity curves for the fallypride (Figure 5) as well as FECNT PET scans.

Analyzing data with two different reference region models, the MRTM2 and the Logan Non-Invasive model, provided a basis for comparison of the two models. Most ROIs yielded similar values for binding potential, but the BPs obtained in the anterior cingulate cortex depended on the method of kinetic analysis used. To resolve this conflict, the inherent properties of each model were evaluated. The Logan Non-Invasive model, based on the Logan Plot analysis, is a graphical method that transforms time-sequence data and input function into a linear plot. This method does not require a specific model structure, which may seem to be an advantage, but the lack of model specificity could create a bias in BP output. Noisy data, a problem inherent to PET scans, is also a cause for binding potential bias in the Logan plot. The MRTM2 model is based on the original linearized reference tissue model—MRTM. The biggest difference in the methodology of kinetic analysis between the two models lies in the estimation of the reference region efflux rate parameter. While the Logan model uses data with an average efflux rate, the MRTM2 holds this parameter constant basing the value on an estimate from MRTM calculations. The difference in this parameter estimation accounts for the ability of the MRTM2 model to significantly reduce binding potential bias and variability based on noise. Therefore, the MRTM2 estimated value of binding potential was accepted when both models presented similar but conflicting BP results. Although noise is accounted for to some degree in both models, measures were taken to reduce noise in the data.

While smoothing the data is one technique to overcome unwanted noise, it is a subjective task, which may result in the loss of signal, providing for an inaccurate evaluation of BP. A different data manipulation technique is the summing of time-sequence data, which introduces multiple data sets and eliminates noise through averaging. The data for this study was obtained by outlining ROIs on static PET images which were the sum of the first 50 minutes of time-sequence data. Though two hour (FECNT) and three hour (fallypride) dynamic scans were obtained; the first 50 minutes show peak subcortical uptake of the tracers.

Though conservative precautions were taken to eliminate noise, residual noise inevitably affects the signal. When estimating BPs in the anterior cingulate cortex, where D2/D3 and DAT densities and consequently the signal to noise ratios are low, the Logan model failed to evaluate the data, yielding negative binding potentials (which are not theoretically possible) or failing to converge. MRTM2 analysis of the same anterior cingulate cortex ROIs provided plausible binding potential values which were in agreement with previous research (Suhara et al. 2002; Hagelberg et al. 2004; Talvik et al. 2003; Kaasinen et al. 2000). Based on the consistent response of the data to the MRTM2 analysis, but not to the Logan model analysis, it was conjectured that high levels of noise were present in the anterior cingulate cortex. Two approaches to data manipulation were taken to compensate for higher noise levels. The first approach was to outline the ROIs using the structural MRI image. This approach was
FIGURE 6

**NOTE:** D2 receptor binding potential as a function of prenatal cocaine dosage, by region: pre/subgenual (anterior) portion of ACC (a), supragenual portion of ACC (b), caudate (c), putamen (d), substantia nigra (e). Blue dots represent control monkeys, red represent low dose, open circle represents intermediate dose and orange represents high dose. Due to a technical failure one of the monkeys from the 0.3 mg/kg group was excluded from data analysis.

intended to more accurately define the ROI based on structure and eliminate noise problems potentially resulting from a less accurate ROI which was defined by regional signal intensity. This approach worked for only half of the data sets. A subsequent approach involved a 3mm Gaussian smoothing of the static PET scan image to improve signal to noise ratios in anterior cingulate.

In the saline control monkeys, BPs of both D2 receptors and DAT for the caudate, putamen and substantia nigra were within accepted ranges comparable to previous research (Slifstein et al. 2004; Mukherjee et al. 2002; Siessmeier et al. 2005; Carbon et al. 2004). The BP values of D2 receptors in the ACC of the control monkeys were on the higher end of reported BP ranges, but still comparable (Suhara et al. 2002; Hagelberg et al. 2004; Talvik et al. 2003; Kaasinen et al. 2000). Though there is only a small amount literature to form the basis of comparison, DAT BPs for the ACC are within a previously reported range for the caudate in humans. There is not substantial literature to support the findings of a lower D2 receptor and DAT BP in the posterior portion, as compared to the anterior portion, of the ACC.

For each region of interest, the binding potential of each monkey was plotted as a function of level of prenatal cocaine exposure. Across all regions of interest, there was a trend for the binding potential of
FIGURE 7

NOTE: DAT binding potential as a function of prenatal cocaine dosage, by region: pre/subgenual (anterior) portion of ACC (a), supragenual portion of ACC (b), caudate (c), putamen (d), substantia nigra (e). Blue dots represent control monkeys, red represent low dose, open circle represents intermediate dose and orange represents high dose. Due to a technical failure one of the monkeys from the 0.5 mg/kg group was excluded from data analysis.

D2 receptors to decline with increasing PNCE (Figure 6). $R^2$ values for D2 receptors vs PNCE exposure were as follows: anterior cingulate cortex (anterior portion) = 0.20, anterior cingulate cortex (posterior region) = 0.26, caudate = 0.36, putamen = 0.14, substantia nigra = 0.34. A power analysis must be performed to determine the number of monkeys that must be tested in future experiments to provide for statistically significant results. These correlation values must also be compared to other published findings for further validation. The existence of a trend across all regions of interest, including both regions of the anterior cingulate cortex, provides support for further investigation of anterior cingulate cortex involvement in ADHD dopaminergic circuitry. There was no significant effect of PNCE on levels of dopamine transporters (Figure 7).

Conclusion

Although repeating the study with a larger cohort is a primary goal, methodological changes must be evaluated before future studies continue. These data suggest the MRTM2 model, following Gaussian spatial smoothing of the PET data should be adopted for future studies. Preliminary dose response trends suggest a reduction of caudate and anterior cingulate cortex D2/D3 receptor levels with increasing levels of in utero cocaine exposures, but no effect of prenatal cocaine exposure on DAT.
Notes

1. Fallypride ((S)-5-(3-fluoropropyl)-2,3-dimethoxy-N-((2S)-1-(2-propenyl)-2-pyrrolidinyl)methyl]benzamide) is a dopamine D2/D3 receptor antagonist.

2. FECNT (N-Fluoroethyl-3-β-(4-chlorophenyl)nortropan-2-β-carboxylic acid methyl ester) is a dopamine transporter ligand.

3. “t.i.d.” is Latin for ter in die, meaning three times per day.

4. PMOD software version 2.65 © 1996-2005 by PMOD Technologies, Ltd.

References


