Resting-state fMRI and pharmacological MRI of changing dopaminergic activity in the developing rat brain

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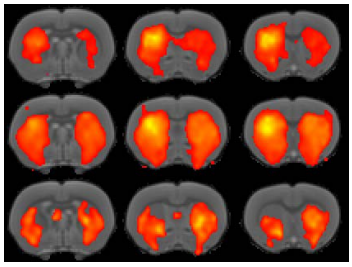
Introduction Dopamine is a considered a key neuromodulator involved in the control of corticostriatal synchronization[1,2]. As sensitive periods of brain development are characterized by profound dopaminergic changes, early dopamine dysfunction is associated with altered corticostriatal processing implicated in neuropsychiatric diseases such as attention deficit hyperactivity disorder (ADHD) and schizophrenia. Animal studies have revealed that maturation of corticostriatal function continues after infancy[3]. Correspondingly, neuroimaging techniques employing BOLD- or CBV-contrast have demonstrated age-dependent increases in response-amplitudes after application of various psychostimulant drugs[4]. However, as transient stimulation may mask persistent developmental differences in baseline dopaminergic modulation, it remains unclear whether corticostriatal maturation is also reflected in stimulus-free measures of functional organization.

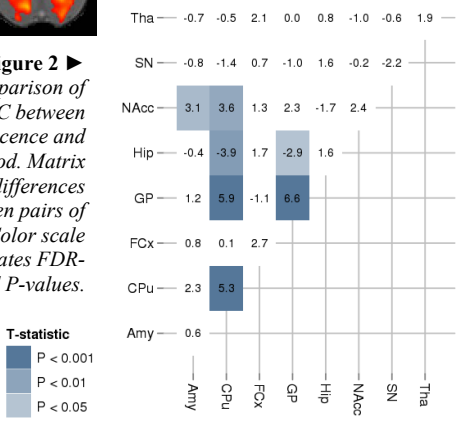
The aim of the present study was therefore to characterize the age-dependent differences in measures of functional organization of the dopamine-regulated corticostriatal circuitry in the rat brain that occur as a result of normal maturation during, and after adolescence. To that end we applied resting-state fMRI (rs-fMRI)-based functional connectivity (RSFC) analysis, and pharmacological MRI (phMRI) with a D-amphetamine challenge, to study the developmental effects on baseline neural processing, in relation to dopamine-stimulated neural functioning. We hypothesized that a stronger D-amphetamine-induced neural activation in adult rats would be paralleled by a strengthened coupling between key corticostriatal regions after adolescence.



◄ **Figure 1** *Group-level statistical RSFC maps overlaid on three adjacent slices from a T2-weighted rat brain template. Color-coded voxels exhibit significant RSFC with seed in left caudate-putamen (P<0.01, cluster correction P<0.001) in adolescents (top) and adults (middle). RSFC is significantly higher in adults (bottom).*

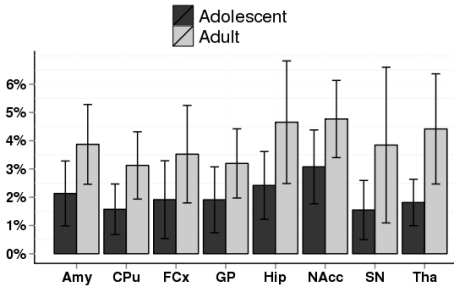
Methods MRI was conducted in adolescent (P48-52, n=12) and adult (P83-87, n=15) male Wistar rats. Animals were anesthetized with 5% isoflurane (2-2.5% maintenance). Two tail veins were cannulated for administration of propofol (20 mg/ml) and D-amphetamine sulfate dissolved in saline (1-1.6 mg/ml). Measurements were conducted on a 4.7T horizontal bore Varian MR system, with use of a 90-mm diameter Helmholtz volume coil and an inductively coupled surface coil (25 mm diameter) for signal excitation and detection, respectively. Rats were immobilized in a MR-compatible stereotactic holder and mechanically ventilated with an air/O_2 (2:1) mixture when anesthesia was switched to propofol (15 mg/kg induction, 60 mg/kg/h continuous infusion). Blood oxygen saturation and heart rate, and expired CO_2, were monitored during MRI, and body temperature was maintained at 37.0 ± 0.5 °C. First, 10 minutes of rs-fMRI was performed using a T2*-weighted single-shot gradient echo EPI sequence (TR/TE=600/25 ms; 50° flip angle; 64×64 matrix; 0.5×0.5 mm^2 voxels; 13×1.5 mm coronal slices; 1000 BOLD images). Subsequently, T2*-weighted phMRI with a 4-shot gradient-echo EPI sequence was performed (TR/TE=1000/25 ms; 60° flip angle; 64×64 matrix; 0.5×0.5 mm^2 voxels; 19×1.0 mm coronal slices) with 10 minutes (150 images) of baseline measurements followed by 35 minutes (525 images) acquisition after intravenous injection of 1 mg/kg D-amphetamine. Anatomical images for registration purposes were obtained with a 3D gradient-echo sequence (TR/TE=6/2.576 ms; 40° flip angle; 256×128×128 matrix; FOV=60×40×40 mm^3).

Figure 2 ► *Comparison of RSFC between adolescence and adulthood. Matrix depicts differences between pairs of ROI's. Color scale indicates FDR-corrected P-values.*



Preprocessing for rs-fMRI entailed motion correction (FSL FLIRT, www.fmrib.ox.ac.uk/fsl), spatial smoothing, data correction with linear regression against the mean signal, and band-pass filtering ($0.01 < f < 0.1$ Hz). With use of elastix[5], anatomical images were registered non-rigidly to a reference image, which was matched to a 3D model of a rat brain atlas[6]. RSFC was measured as the Fisher *z'*-transformed correlation coefficient. Preprocessing for phMRI entailed linear drift correction, spatial smoothing and calculation of BOLD signal changes relative to the mean baseline value. Regions-of-interest (ROIs) within the (fronto-)corticostriatal circuit, i.e. prefrontal cortex, caudate-putamen, nucleus accumbens, globus pallidus, substantia nigra, amygdala, thalamus and hippocampus were projected from the atlas onto the functional time-series for seed-based RSFC and phMRI analysis. Each ROI's average D-amphetamine response was fitted with a gamma distribution, from which the peak amplitude was calculated for statistical testing.

Results Rs-fMRI analysis showed consistent interhemispheric RSFC between homologous cortical and subcortical regions involved in fronto-corticostriatal signaling pathways (e.g., Figure 1). Adult animals displayed a significantly stronger RSFC between bilateral caudate-putamen structures and increased connectivity between amygdala and nucleus accumbens, while RSFC between hippocampus and caudate-putamen was slightly higher in adolescent animals. No changes were observed in RSFC between frontocortical regions and basal ganglia (Figure 2). Gamma fits of phMRI measurements revealed a strong response with largest signal change in nucleus accumbens acutely following intravenous D-amphetamine administration. Adult animals showed a significantly higher response amplitude than adolescent rats in all areas except for the frontal cortex and substantia nigra (Figure 3).



▲ **Figure 3** *PhMRI response to D-amphetamine as the maximum change in signal relative to baseline.*

Discussion Our phMRI-results are in agreement with other studies reporting an age-dependent increase in response to D-amphetamine. Large increases in rCBV in adult rats evoked by D-amphetamine have been attributed to activation of dopamine D1 receptors. A relative shift from post-synaptic D2- to D1-like receptor expression, with hypofunctionality of D1 receptors, best explains the lower phMRI response in adolescent rats[4]. The rat frontal cortex exhibits a different pattern of dopamine receptor development, characterized by protracted pruning of D1 receptors between P40 and P120 days, which may explain the absence of frontocortical age effects[7]. Normal maturation of the caudate-putamen has been shown to involve increased spatial segregation of cortical inputs[3]. Our rs-fMRI data suggest that this is accompanied by increased neural synchrony between bilateral caudate-putamen, during normal development from adolescence to adulthood[8]. Although a strong synchronous response could be attributed to a more patterned cortical input that is observed during anesthesia[9], we could not determine a difference between adults and adolescents in RSFC strength among caudate-putamen and cortical areas, probably because of aforementioned differences in patterns of dopamine receptor development. In conclusion, our study demonstrates that combined pharmacological MRI and rs-fMRI provide complementary insights into the ontogeny of dopamine modulated functional brain networks.

References 1. Greengard *Science* 2001; 2. Raz et al. *J Neurophysiol* 1996; 3. Galiñanes et al. *J Neurosci* 2009; 4. Chen et al. *Dev Neurosci* 2010; 5. Klein et al. *IEEE TMI* 2010; 6. Paxinos & Watson *The rat brain* 2005; 7. Andersen et al. *Synapse* 2000; 8. Uhlhaas et al. *Trends Cogn Sci* 2010; 9. Mahon et al. *J Neurosci* 2006.