Supporting Information

Marzluff et al. 10.1073/pnas.1206109109

SI Methods

Housing and Care of Subjects. Crows were maintained in individual cages on the University of Washington campus and fed an ad libitum mixture of dry dog food, peanuts, meat, cheese, French fries, bread, fruits, and vegetables. Exposure of birds to people was minimal during captivity (the cages are atop a building only accessed by research staff). Daily, one person, wearing a mask (the caring face) fed the birds and washed their cages. The cages had no bottoms but sat directly on concrete that we cleaned using water pressure sufficient to rinse feces and food scraps out of the cage. During this cleaning, the crows sat calmly on a high perch partially concealed from their keeper by a tarp.

Experimental Exposure to Faces. On the evening before an experiment, a single crow was captured from its holding cage by a person wearing the threatening face, placed in a sock to calm it, and carried across campus to a small wire cage in a fume hood of the imaging laboratory. Water, but no food, was available. We draped a blanket across the front of the cage to prevent the bird from seeing out into the laboratory and to keep it calm as it acclimated overnight without food.

On the day of an experiment, we reached under the blanket draping the experimental cage, removed the crow while covering its eyes with a hood, and injected [F-18]fluordeoxyglucose (FDG) into the peritoneum. All crows remained passive and relaxed during this procedure with no visible signs of stress or struggle. We returned the crow back into the blanket-draped cage and played recorded crow calls (contact kaws, no alarm calls) for 2 min, after which we started experimental treatments. Each exposure was interspersed with a corresponding 1-min-long break during which time the blanket was replaced on the cage, and the crow was allowed to relax out of view of the stimulus. To expose the crow to the human face, two researchers were in the laboratory. One researcher sat 0.5 m from the cage facing the crow, while the second researcher removed the blanket and kneeled next to the sitting person. Both people wore the same mask and were fully visible to the crow. After the 14 min of the experiment (seven exposures and seven breaks), we again took the crow out of the cage by reaching under the blanket, covered its eyes with a hood, induced sedation with 3–3.5% isoflurane, and placed it in the scanner.

PET Scanning Procedure. The crows were anesthetized with 2.5–3.5% isoflurane during the imaging procedure. A special nose cone fabricated from a 50-mL syringe tube was used to accommodate the crow's beak. The Inveon PET scanner collects data in a list mode format that allows custom time binning of the data after it has been acquired. In addition to the emission data, a transmission scan for attenuation correction was collected for each crow. Each crow's breathing was monitored by using a Biopac respiratory monitor.

For the first crow imaged, a 120-min imaging study was conducted to determine the time that the FDG activity peaks in the brain. Under isoflurane anesthesia, the crow was placed in the scanner and injected with FDG simultaneously with scan start. The data were binned into 24 time frames of 5 min each, and images were reconstructed by using Fourier rebinning of the data and 2D filtered backprojection. The time activity curve including decay correction of the FDG uptake in the crow's brain is illustrated in Fig. S1. The result from the curve indicates that the FDG activity in the crow's brain peaks at ∼25 min after injection. The observed FDG washout from the crow's brain is very rapid compared with mammals, even mice (1). This fast washout is a physiologic event and warrants further comparative studies between the brain glucose metabolism in birds vs. mammals.

After exposure to an experimental stimulus (see above), the crow's head was imaged for 16–20 min. After the emission data acquisition, a transmission scan was taken of the crow's head. Finally, an emission image of the crow's torso was taken to verify that there was a clean i.p. injection.

Images were reconstructed for the 10-min time frame starting 27 min after the time of injection. The images were reconstructed by using the vendor-supplied 3D ordered subsets expectation maximization/maximum a posteriori (MAP) algorithm with attenuation and scatter correction applied to the data. The image matrix was $128 \times 128 \times 159$. A zoom factor of 1.302 and a beta of 0.25 were used for the MAP smoothing parameter. After the images were reconstructed, they were exported by using DICOM for the statistical parametric analysis software.

Image Analysis. We obtained a structural MRI of one subject's brain using a 3-T MR scanner (Philips Achieva; Philips Healthcare) and a commercial coil (Philips Healthcare); T1-weighted MPRAGE (TR/TE = 10.8/5.1 ms; Ti = 1,000 ms; FA = 9 deg acquired matrix 512×512 mm over 110 slices, voxel $0.2 \times 0.2 \times 0.6$ mm3 interpolated to $0.1 \times 0.1 \times 0.3$ mm^3).

PET image sets were normalized to the global activity to semiquantitatively and sensitively analyze regional metabolic alterations. A global activity was defined by an atlas-based delineation of the brain.

A Z statistic map is widely used in brain mapping as a method by which one can objectively and statistically evaluate consistent group-wise changes from one condition to another on a pixel-by-pixel basis over the entire brain (2). Group-wise subtraction analysis allows the statistical comparison of different activation paradigms. Two-sample t statistic values were calculated across groups for each subtracted pixel value. The calculated t statistic values were converted to Z statistic maps by using a probability integral transformation (3). The resultant Z statistic maps represent the extent and significance of regional brain activity averaged across groups under different stimulation paradigms. Coordinates for which Z values was >3.8 were considered statistically significant, controlling the type I error rate approximately at $P = 0.05$ for multiple comparisons (4). The resultant z-score maps were superimposed on to the atlas-aligned MRI template for anatomical localization of activated structures.

To demonstrate the progressive nature of metabolic alterations relative to average number of blinks per minute (index of attentiveness to perceived threat), a voxel-wise regression analysis was performed, and correlation coefficients were converted to Z values by using the variance map generated in the regression analysis as described (5). The resultant z-score maps were superimposed on to the atlas-aligned MRI template for anatomical localization of activated structures.

To confirm the findings of the voxel-wise analyses outlined above, stereotactically defined volume-of-interest analysis for structures with a Z score of ≥3.8 were measured and statistically compared by either t test or linear regression using a software program (SPSS; Version 11; SPSS Inc.).

1. Dos Anjos L, Debus SJS, Madge SC, Marzluff JM (2009) *Handbook of Birds of the World*, eds del Joyo J, Eliott A, Christie DA (Lynx Edicions, Barcelona), pp 494–641.
2. Jonides J, et al. (1993) Spatial working memory in humans as revealed by PET. *Nature* 363:623–625.
3. Friston KJ, et al. (1989) Localisation in PET images: Direct fitting of the intercommissural (AC-PC) line. *J Cereb Blood Flow Metab* 9:690–695.
4. Petrie EC, et al. (2009) Preclinical evidence of Alzheimer changes: Convergent cerebrospinal fluid biomarker and fluorodeoxyglucose positron emission tomography findings. *Arch Neurol* 66:632–637.
5. Cross DJ, et al. (2004) Statistical mapping of functional olfactory connections of the rat brain in vivo. *Neuroimage* 23:1326–1335.

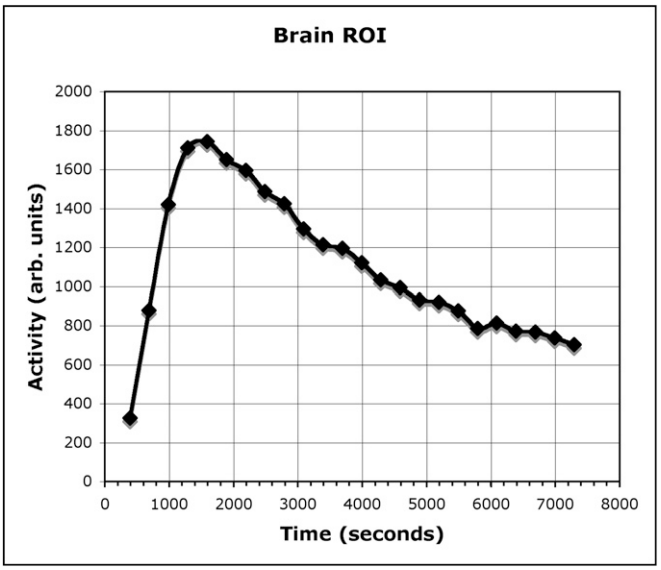
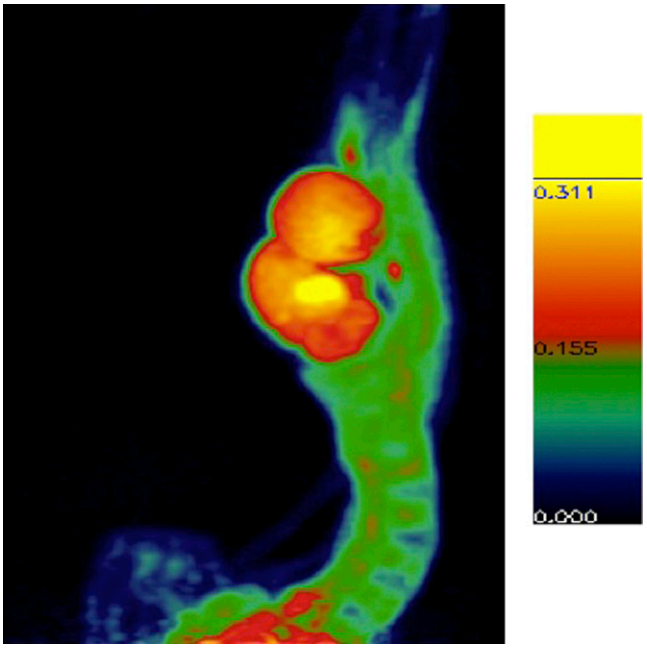


Fig. S1. [F-18]FDG activity during a 2-h-long dynamic scan of a single crow.



Movie S1. The 3D reconstruction of [F-18]FDG activity in the brain of an awake crow viewing a threatening person. The movie is the maximum intensity projection (MIP) of the 3D reconstructed image viewed at 30 different view angles. The color lookup table (LUT) for the movie is provided. The yellow and red areas correspond to regions of higher FDG uptake; green is areas of moderate FDG uptake; and blue is regions of low FDG uptake. The image was formed from the 27- to 37-min emission data.

Movie S1