Brain imaging reveals neuronal circuitry underlying the crow’s perception of human faces

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Crows pay close attention to people and can remember specific faces for several years after a single encounter. In mammals, including humans, faces are evaluated by an integrated neural system involving the sensory cortex, limbic system, and striatum. Here we test the hypothesis that birds use a similar system by providing an imaging analysis of an awake, wild animal’s brain as it performs an adaptive, complex cognitive task. We show that in vivo imaging of crow brain activity during exposure to familiar human faces previously associated with either capture (threatening) or caretaking (caring) activated several brain regions that allow birds to discriminate, associate, and remember visual stimuli, including the rostral hyperpallium, nidopallium, mesopallium, and lateral striatum. Perception of threatening faces activated circuitry including amygdalar, thalamic, and brainstem regions, known in humans and other vertebrates to be related to emotion, motivation, and conditioned fear learning. In contrast, perception of caring faces activated motivation and striatal regions. In our experiments and in nature, when perceiving a threatening face, crows froze and fixed their gaze (decreased blink rate), which was associated with activation of brain regions known in birds to regulate perception, attention, fear, and escape behavior. These findings indicate that, similar to humans, crows use sophisticated visual sensory systems to recognize faces and modulate behavioral responses by integrating visual information with expectation and emotion. Our approach has wide applicability and potential to improve our understanding of the neural basis for animal behavior.

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pathway, crows used their thalamofugal pathway (directly linking thalamus and hyperpallium) to perceive humans. Activation of the rostral nidopallium, which has strong connections with the entopallium and mesopallium (14) and access to sensory information from both the tectofugal and thalamofugal visual pathways (15), appears important to the recognition of human faces.

Crows quickly associated negative (capture) and positive (provision of food) experiences with a human face. In our stimulation protocol, familiar humans were seated and did not behave as expected, neither threatening nor providing care to crows during stimulation. This surprise, or prediction error, may account for the activation of the crows’ lateral striatum (Fig. 3), as it does in a variety of animals, including humans (16). The activated rostral mesopallium, an associative forebrain area that participates in rapid, multimodal learning and is enlarged in crows (17), may be especially important to the association of human faces with reward and punishment. Caudal regions of the nidopallium, mesopallium, and hippocampus—which are important to the recognition of biologically significant conspecific (18) and executive function (19)—were not consistently activated by the sight of a person.

Crows activated different regions when viewing a familiar threatening vs. a familiar caring person. Upon seeing the face of their captor, crows activated their nidopallium/mesopallium, arcopallium including the region containing the nucleus taeniae of the amygdala (TnA), and nuclei in the dorsal thalamus and brainstem (Fig. 4A-C). This response is commonly elicited by the emotion of fear (20, 21). Definitive identification of nuclei in the thalamus and brainstem was not possible given our resolution and the many small, densely packed nuclei, but likely candidates include those involved in visual pathways (nucleus dorsolateralis posterior thalami and nucleus isthmo-opticus) (22), learning pathways (substantia grisea centralis and locus ceruleus) (23), and emotional motor pathways that control vocal and postural responses to predators (area surrounding tractus occipitomesencephalicus, nucleus reticularis, and trigeminal nucleus) (21, 22). The neural response of crows viewing a human who cared for them daily, albeit in captivity, was distinct from the response of birds to a threatening person. The hyperpallium, mesopallium, preoptic area, and medial striatum were strongly activated (Fig. 4A and C). These areas are known to be important to associative learning, motivation, and autonomic functions including hunger in vertebrates (17, 24), which suggests that crows perceived the association established between their caretakers and food. In crows, as in other animals including humans, it appears that both the striatum and the amygdala are critical to associative learning, with the former apparently broadly attuned to prediction errors and the latter often attuned to the reliability of threatening cues (16, 20).

Fig. 1. Experimental protocol. Different rubber masks, molded from actual people, were used to create threatening and caring faces that single crows responded to during stimulation. During i.p. injection and induction, crow’s faces were covered to prevent them from glimpsing humans.
The neural responses of crows to threatening and caring faces differed in hemispheric bias, or lateralization, as hypothesized for humans. In support of the valence theory of emotional processing (5), crow responses to the caring (positive) face were predominantly left-hemisphere biased, whereas responses to the threatening face were predominantly right-hemisphere biased. These biases were strongest in the limbic and subpallial structures (threatening, amygdalar regions; caring, preoptic area and striatum; Fig. 4 A–C). Lateralization was less consistently right-hemisphere biased in the upper pallium (mesopallium, nidopallium, and hyperpallium) to all familiar faces (Figs. 3 A and 4A), perhaps reflecting specialization of the right hemisphere for recognition of familiar social companions (25)—or, alternatively, the tendency of subjects to perch on the right side of the chamber and view the stimulus with their left eye.

Under both experimental conditions and in the field, crows responded to perception of a threatening face with a fixed gaze (quantified as decreased blink rate). In nature, crows blinked less when they looked at threatening people than when they fed in conspecific flocks (Fig. 5B). During our experiments, crows froze, stared at the person, and flashed their conspicuous white nictitating membrane on average 29 times per min (SE = 4.4) upon seeing the threatening face. In contrast, when viewing the caring person, crows stared, blinked 41 times per min (SE = 3.4), and often swallowed (caring, two of four birds; threatening, one of five birds) and even defecated (caring, three of four birds; threatening, two of five birds). Reduced blinking at the sight of the threatening person relative to the caring person was as expected from field observations [Mann–Whitney U = 2.0; P = 0.03 (one-tailed)], but there was substantial variation.

Individual variation in blink rate was associated with distinct brain activity in the crows that viewed human faces. Increased blinking was correlated with increased activity in the hyperpallium and the lateral striatum near the nidopallium (Fig. 5 A and C). This finding is consistent with processing of visual information by both tectofugal and thalamofugal pathways and integration with expectation from learned associations (15). Reduction in blinking was correlated with increased activity in the brainstem (Fig. 5 A and D). Blinking and associated neural activity varied continuously among individual crows, suggesting that the birds varied in their perception of, or reaction to, risk and rewards associated with human faces.

The greater overall area of activation, including subthreshold pixels in the nidopallium and mesopallium in the group viewing the threatening vs. that viewing the caring face (Fig. 4), may represent heightened arousal and greater involvement of the crow forebrain to resolve negative vs. positive stimuli. Greater consistency in neural responses to the threatening face suggests differential arousal or attention, but the equal number of significant activation peaks between treatments and the similar demeanor of all crows during testing suggest that crows actually used a larger forebrain area to resolve threatening faces compared with caring faces. During stimulation, crows oriented toward the stimulus and did not fly, vocalize, or flick their wings or tails as is typical in agitated birds. This subdued response across treatments was likely due to the confining nature of the small cage we used. All but one bird moved occasionally during the stimulation protocol, either jumping between the cage floor and perch (n = 5) or shifting its head from side to side (n = 7). Most of these birds moved only once. However, a single bird in each treatment frequently moved between perch and floor (threatening, 19 movements; caring, 6 movements), and five birds shifted their heads frequently (threatening: mean = 55.5 shifts during stimulation, SD = 43.1, n = 2; caring: mean = 71.3, SD = 31.7, n = 3).
It is unlikely that differences in brain activity were due to extraneous factors. We eliminated variation in environmental factors known to affect brain activity by holding lighting, noise, time of day, room composition, position of observers, handling, and housing before and after treatment constant across trials. The blinking, swallowing, and defecating behavior of crows discussed above suggested that they perceived the difference in stimuli and that they were attentive to both threatening and caring faces. Documenting the neural responses of birds to a variety of threats and rewards could resolve the relative influence of the stimulus on the extent of forebrain activity.

Our results demonstrate how crows use a diversity of regions from the brainstem to the forebrain to distinguish and adjust their response to individual human faces. This finding is consistent with established and emerging views of how the subpallial limbic network interacts with the integrative forebrain to shape memory-based social behavior in vertebrates (17, 18, 26), including humans (27, 28). The use of cortical sensory processing, the striatum, and the limbic system suggests strong analogy and possible homology between avian and mammalian facial recognition and associative learning systems. Further studies are needed to determine whether the forebrain regions used by crows in our study are functionally analogous to facial recognition regions in humans and other mammals.

Our approach that partners neuroscientists with ecologists could be used to better understand the neural bases of cognition in widely diverse animals (29). Current knowledge comes primarily from a few, well-studied, often domesticated species. Neuroimaging of wild animals to assess whole brain activity during complex behaviors, although presently limited to activities that can be elicited in a temporary captive setting, add substantially to traditional lesion, stimulation, and tracing studies (9). In vivo imaging and voxel-wise analyses of brain responses can be repeated longitudinally in the same animals, and when experiments...
are completed, the animals can be returned to the wild. Understanding how wild animals integrate perception, memory, and emotion to behave adaptively may allow researchers to generalize important findings across species and sensory modalities, develop strategies to lower stress in captive animals, shape animal actions to reduce human–wildlife conflicts, and engage the public to appreciate the cognitive capacity of other species.

Methods

We captured crows lured from large roosting and foraging groups on three separate occasions using a netlauncher and selected only large (likely male), black-mouthed (adult) birds to bring into captivity (30). None of the birds had previously been captured, and there was no evidence (presence of previously banded birds) that any of the birds resided in study sites that we have previously used for research. Because multiple groups of crows were captured over the course of the study, we counterbalanced the masks used: The threatening face was the mask used during the initial capture and when they were caught and moved to the PET laboratory. All masks were faces of actual people with neutral expressions; valence was conferred by our behavior, not by facial features.

The evening before imaging, the test subject was moved to a covered 0.5 × 0.5 × 1-m cage in the imaging facility to acclimate, undisturbed, overnight (Fig. 1 and SI Methods). In the morning, crows were blindfolded, removed from the covered cage, administered 1 mCi of FDG via i.p. injection, returned to the cage for a 2-min rest, and then shown the masked investigators in a new caring face, the mask worn at all times by the person feeding them and cleaning their cages. The threatening face was the mask used during the initial capture and when they were caught and moved to the PET laboratory. All masks were faces of actual people with neutral expressions; valence was conferred by our behavior, not by facial features.

High-resolution FDG-PET images were acquired using a Siemens Inveon PET system for 10 min from 27- to 37-min post-FDG administration (Fig. S1) followed by an ∼13-min attenuation scan and then reconstructed by using 3D ordered subsets expectation maximization/maximum a posteriori to a spatial resolution of 2.5 mm.
We stereotaxically aligned images to a jungle crow (Corvus macrorhynchos) atlas (32), facilitated by structural MRI of one American crow. Nine affine parameters were estimated and applied to images, for consistent stereotactic transformation of scans from the same subject (33, 34). Alignment precision was estimated to be 2–3 mm. After normalizing to global values, significant regional differences in cerebral metabolic rate were determined by using automated voxel-wise subtraction and Z-statistic mapping (NEUROSTAT) (8). Correlation with blink rate was obtained by a voxel-wise linear regression across all subjects exposed to face stimulation (35). We considered Z values that were >3.8 statistically significant, controlling the type I error rate approximately at \( p = 0.05 \) for multiple comparisons in a modified Bonferroni correction commonly used in imaging research (36). Volumes-of-interest (VOIs) for structures with a Z score of >3.8 were applied to individual images, and values were compared across groups by using a t test.

We directly observed blinking at close range during experiments and with the aid of 10× binoculars in the laboratory. In the laboratory, we counted each flash of the white nictitating membrane during each minute of stimulation and calculated the average of these as \( n = 7 \) counts per subject as the blink rate. We video-recorded laboratory trials, but resolution was insufficient to count blinking. From August 15 to 22, 2011, in the Seattle area or on nearby Vashon Island, we obtained up to five 1-min counts of individual wild crows blinking under three social settings: (i) as we held them during capture, (ii) eating food within a group of conspecifics and heterospecifics, and (iii) scolding a person who was close to the focal crow’s offspring. As in the laboratory, we averaged all blink counts obtained on a single bird to determine the subject’s blink rate. All blink rates were counted by J.M.M. to eliminate possible variation among observers.

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Supporting Information

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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]fluorodeoxyglucose (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 knelted 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 × 0.2 × 0.6 mm3 interpolated to 0.1 × 0.1 × 0.3 mm3).

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 $\geq 3.8$ were measured and statistically compared by either $t$ test or linear regression using a software program (SPSS; Version 11; SPSS Inc.).

**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**