Thought for food; brain activity and task performance of American crows (*Corvus brachyrhynchos*) in pursuit of food

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Abstract

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The last common ancestor shared by modern birds and mammals died out over 300 million years ago, yet both groups have convergently evolved more advanced intelligence than any other taxonomic class. With their enlarged forebrains, behavioral flexibility, and ability to navigate novel challenges in their environment, members of the Corvidae family are often compared to primates, yet we know considerably less about corvid intelligence than we do primate intelligence. In this dissertation, I explore the intelligence and brain activity of American crows (*Corvus brachyrhynchos*) as they pursue a food reward. First, I examine the individual and social factors which influence crows' ability to solve and master a string-pulling task. Although not statistically significant, I find that crows with large brain volumes and without a conspecific model consistently mastered the task in the minimum number of days, whereas those with conspecific models and smaller brain volumes required varying and sometimes a substantial number of days to master the task. Second, I work with the UW Department of Radiology to examine crow neural activity as they view a piece of food, listen to conspecifics vocalizing at a food source, or both stimuli presented simultaneously. I find two regions, the nucleus taenia of the amygdala (TnA) and a medial portion of the caudal nidopallium, that show increased activity in response to the multimodal combination of stimuli but not in response to either stimulus when presented unimodally, and significantly increased activity in the lateral septum and medially within the nidopallium in response to both the audio-only and the combined audio/visual stimuli. Finally, I merge the previous studies' methodologies and examine the individual factors and neurological activity as crows learn and master a task requiring the use of tools. I find that naïve crows predominantly use brain circuits associated with higher order thinking (the mesopallium, nidopallium, and nidopallium caudale) when first confronted with the task, yet their brain activity shifts to circuits associated with memory and motor control (hippocampus, tegmentum, nucleus basorostralis, and cerebellum) as they become proficient tool users. I additionally find that all crows that learned to use tools to solve the task were exclusively adults and predominantly females. This work showcases the capabilities, and limits, of the charismatic birds that live all around us.

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General Introduction

By the beginning of the 1900’s, scientists thought that birds were incapable of complex cognition. The term “bird brain” was synonymous with simpleton. This poor reputation was primarily due to the early work of Ludwig Edinger, a comparative anatomist. Although he would later go on to become a pioneer in the field of neuroanatomy (Stahnisch 2008), Edinger (1899) mistakenly concluded that most of the bird brain evolved from the striatum, a structure responsible for instinctual or species-typical behaviors. Edinger reasoned that if the avian brain contained little to no areas that resembled the mammalian cortex, then they must not be capable of higher thought processes and their behavior was driven by instinct alone. This idea remained the most dominant scientific model well into the 1900’s.

However, beginning in the mid-1900’s studies began to challenge the view that birds were simpletons. Skinner (1948) realized that pigeons (*Columba livia*) can develop ‘superstitious’ behavior by causally linking unassociated events together. Thorpe (1958) determined that birds must learn the songs sung by their species and began the study of bird song learning, and Marler & Tamura (1964) found regional song dialects spreading via cultural transmission throughout a population of white-crowned sparrows (*Zonotrichia leucophrys*). Hunt (1984) documented wild New Caledonian crows (*Corvus moneduloides*) making and using two different types of tools to capture prey, and Weir, Chappell, & Kacelnik (2002) observed a New Caledonian crow named Betty bend a straight piece of wire into a hook to solve a task. Pepperberg’s (1987) work with Alex the African grey parrot (*Psittacus erithacus*) revealed that he could categorize objects based on characteristics such as shape or color. Clayton & Dickinson (1998) discovered that Florida scrub-jays (*Aphelocoma coerulescens*) utilize episodic memory to recover cached food. Bird & Emery (2009) demonstrated that rooks likely understand cause & effect relationships. These studies and many more like them conclusively show that birds are capable of much the same cognitive feats found in mammals.

These behavioral observations have been supplemented by research examining the inner workings of the avian brain. The song control system has been explored and mapped (Brainard & Doupe 2013, Brenowitz & Beecher 2005, Konishi 1985), as well as the imprinting process (Bateson & Horn 1994, Bredenkötter & Braun 1997). The Avian Brain Nomenclature Forum devised new terminology to describe regions within the avian brain so that they were attributed to the pallium, not striatum (Reiner et al. 2004). Other studies found that bird brains are capable of things that mammalian brains are not, such as sleeping one hemisphere while the other remains half-awake to look for predators (Rattenborg et al. 1999), or seasonal neurogenesis of specific regions (Brenowitz & Larson 2015). Unfortunately, our understanding of the inner workings of the avian brain remains limited relative to mammals, and there is still much we do not know about how birds use their impressive brains to approach and resolve novel challenges in their environment.

American crows (*Corvus brachyrhynchos*) are an ideal species to study avian cognition. They and other members of family Corvidae are among the most intelligent birds (Lambert et al. 2019); they’re capable of long-term human facial recognition (Marzluff et al. 2010), understand

and respond to reward inequity (Wascher & Bugnyar 2013), assess risk (Dufour et al. 2019), track and remember the actions of others (Bobrowicz & Osvath 2020), and potentially possess a theory of mind (Bugnyar et al. 2016, though see Van der Vaart 2012). They've developed complex social dynamics from living in their fission-fusion societies; they guard territories and regularly fight amongst themselves, yet they also roost in large numbers and cooperate to mob predators (Marzluff & Angell 2005). They can use social learning to identify new dangers from both living (Cornell et al. 2011) and dead crows (Swift & Marzluff 2015), or alternatively learn that a perceived threat poses little actual danger (Marzluff et al. 2015). Though true tool-use is rare in wild American crow populations (Caffrey 2000), they practice many proto-tool behaviors (such as dropping hard shelled food onto a hard surface to crack it) and can learn to use tools in captivity (Cole 2004). Yet there's much we still don't know about crows, such as the purpose many of their vocalizations serve, the extent to which they use insight, or what other information they can learn via social learning

The overarching objective of the studies covered by this dissertation is to contribute to our understanding of how the avian brain processes the cognitively difficult challenges crows face in their environment, and what physical qualities are shared by successful crows. In the first chapter I examine whether crows can use social learning to solve a string-pulling task, and what individual characteristics (such as age, sex, or nervousness) influence task performance. In the second chapter, I use PET imaging to examine the crows' neural activity as they view a piece of food, listen to conspecifics vocalize at a food source, or both stimuli presented simultaneously. In the final chapter, I use PET imaging to examine crow neural activity as they view a task requiring them to use tools to solve (an Aesop tube task) under different knowledge and proficiency levels, and I additionally compare the physical characteristics of the birds that were most proficient at solving the task. Through this research I seek to learn more about the neural mechanisms of these intelligent birds and to probe the limitations of their impressive cognitive capabilities.

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CHAPTER 1: Individual and social factors affecting the ability of American crows to solve and master a string-pulling task.

ABSTRACT

Crows and other birds in the family Corvidae regularly share information to learn the identity and whereabouts of dangerous predators, but can they use social learning to solve a novel task for a food reward? I examined the factors affecting the ability of 27 wild-caught American crows to solve a common string-pulling task in a laboratory setting. I split crows into two groups; one group was given the task after repeatedly observing a conspecific model a solution, the other solved in the absence of conspecific models. I recorded the crows' estimated age, sex, size, body condition, level of nervousness, and brain volume using DICOM images from a CT scan. Although none of these variables were statistically significant, crows without a conspecific model and large brain volumes consistently mastered the task in the minimum number of days, whereas those with conspecific models and smaller brain volumes required a varying and sometimes substantial number of days to master the task. I found indirect evidence that body condition might also be important for motivating crows to solve the task. Crows with conspecific models were no more likely to initially solve the task than those working the puzzle without social information, but those that mastered the task usually copied the method most frequently demonstrated by their knowledgeable neighbors. These findings suggest that brain volume and possibly body condition may be factors in learning new tasks, and that crows can use social learning to refine their ability to obtain a novel food source, although they must initially learn to access it themselves.

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INTRODUCTION

Most vertebrates regularly encounter novel situations throughout their lives, and their ability to successfully navigate them directly benefits their survival and fitness (Cole & Quinn 2011, Cauchard et al. 2013, Wetzel 2017). Assessing how to acquire and process novel foods is one such challenge faced by many species, particularly generalists. There are many opportunities for individuals from social species to observe conspecific behavior, and those capable of using information obtained via social learning to improve their foraging efficiency and solve novel problems would have an advantage over those that cannot (Galef & Giraldeau 2001, Rieucau & Giraldeau 2011). One way that researchers can tease apart aspects of a species' capacity to decipher novel situations is to measure their ability to solve novel tasks of varying complexities; by modifying this approach to include other conspecifics, researchers can also test whether individuals learn the task by watching others interact with it.

Aside from social learning and information sharing, there are a variety of individual factors which are known to contribute to problem solving ability, but motivation is perhaps the most overarching factor. An animal's motivation can be affected by hunger and associated body condition; hungry animals will be more motivated and devote more time and attention to a task with a food reward compared to well-fed individuals (Birch 1945). An animal's temperament can affect its motivation: neophobia, for example, inhibits problem solving (Benson-Amram & Holekamp 2012) such that animals with greater degrees of neophobia are more reluctant to approach an unfamiliar object and require additional time before they attempt a novel task compared to bolder individuals (Heinrich et al. 1995, Heinrich 2000). Age might also affect motivation; juveniles tend to interact with the task more persistently due to playfulness (Vince 1958), although older animals are more cognitively developed and have amassed experience which may be applicable to the novel task (Mason & Harlow 1961).

Large brains are demonstrably advantageous in many species (Deaner et al. 2007, Benson-Amram et al. 2016); therefore, brain volume might affect an individual's ability to solve novel problems. Domestic dog breeds (*Canis lupus familiaris*) with larger brains perform better in cognitive tasks (Horschler et al. 2019), although there may be confounding factors stemming from other differences between breeds, such as body size or temperament. Additionally, large-brained guppies (*Poecilia reticulata*) had a higher survivorship then small-brained conspecifics, though this effect was limited to females (Kotrschal et al. 2015), suggesting that an individual's sex may also be a confounding factor when examining the effect of brain size. Alternatively, brain volume may not be as important as other factors; the brain is a complex organ, and aspects such as neuronal density, neuronal counts, or the relative size or complexity of specific circuits within the brain may be better indicators of the advantages that developed brains provide (Healy & Rowe 2006, Olkowicz et al. 2016, Jardim-Messeder et al. 2017).

Crows and other members of family Corvidae (corvids) are widely regarded as among the most intelligent bird species (Emery & Clayton 2004, Lambert et al. 2019); many are capable of long-term human facial recognition (Marzluff et al. 2010), understanding and responding to reward inequity (Wascher & Bugnyar 2013), assessing risk (Dufour et al. 2019), tracking and remembering the actions of others (Borborwicz & Osvath 2019), manufacturing rudimentary tools (Hunt 1996, Caffrey 2000, Shumaker et al. 2011, Uomini & Hunt 2017), and potentially possessing a theory of mind (Clayton et al. 2007, Ostojić 2013, Bugnyar et al. 2016, though see Van der Vaart 2012). Corvids are also capable of social learning; American crows (*Corvus* *brachyrhynchos*) learn new dangers and keep track of predators by observing conspecific behavior (Cornell et al. 2011, Marzluff et al. 2015, Swift & Marzluff 2015), while New Caledonian crows (*Corvus moneduloides*) and common ravens (*Corvus corax*) obtain information on how to acquire food socially (Heinrich et al. 1993, Holzhaider et al. 2010, Sierro et al. 2019). Crows possess many of the characteristics required for high intelligence: they have high annual survivorship (Kilham 1990, Marzluff & Neatherlin 2006), live in fission-fusion social groups (Cornell et al. 2011), and possess a large brain relative to their body size (Portmann 1946, Marzluff & Angell 2007). These qualities allow crows to amass and draw upon a great deal of experience (via individual and social learning) over the course of their long lives.

A common behavioral test given to birds to gauge their problem-solving ability is the string-pulling task: out-of-reach food is suspended from a perch by a string, and the subject must devise a method to retrieve it (Thorpe 1943, Jacobs & Osvath 2015). There is considerable debate concerning whether birds use insight (here defined as the use of mental visualization to suddenly solve a specific novel task without the incremental progression of trial-and-error learning nor pre-programmed instinctual responses) or other processes, such as operant conditioning or positive reinforcement, to solve the string-pulling task (Heinrich 1995, Heinrich & Bugnyar 2005, Taylor et al. 2010, Shettleworth 2012). However, because the task does not resemble anything most birds would encounter in the wild, a naïve bird must learn a new strategy to solve it rather than rely on experience (Jacobs & Osvath 2015). The most common solution is for the bird to "reach down from the perch, pull up on the string, place the pulled-up loop of string onto their perch, step on the loop with one foot, release the string from the bill, then reach down and pull up more string, etc., so that the food is drawn into reach" (Heinrich 1995), hereafter referred to as the string pull method. While common ravens are usually able to solve the task using a string pull method on their first try (Heinrich 1995), American crows typically require training before they can retrieve the food this way, although naïve crows have been observed solving the task by flying up and grabbing the food from the string while airborne (Heinrich 1999).

Here I expand on Heinrich's work with American crows by asking which factors (if any) affect the birds' abilities to solve the string-pulling task. I wondered if crows: 1) used social learning to aid their ability to solve this novel task and 2) whether individual factors (such as sex, age, motivation, or brain volume) affect the crows' ability to solve the task. Other bird species can learn how to acquire food from novel sources by watching knowledgeable conspecifics (Aplin et al. 2013), and American crows have demonstrated that they can acquire novel information by observing the behavior of other crows (Cornell et al. 2012). If crows can use social learning to solve novel tasks, then I predict that crows with a conspecific model will be more successful at solving and learning the task compared to crows without a model. Additionally, I predict that crows with the model will be more likely to adopt the method demonstrated to them by the model. If individual factors influence task performance, I make the following predictions: older, calmer individuals with lower body condition (smaller body mass than expected for a given body size, see Methods below) and larger brain volumes (either absolute or relative to body size) will be more likely to initially solve the task and will master it in less time compared to younger, nervous individuals with higher body condition and smaller brain volumes.

METHODS

Capturing and Housing Crows

I captured wild American crows as they departed a large communal roost at various locations in Seattle, Bothell, and Woodinville WA. I lured birds from flocks with bread and trapped them using a net launcher. I captured 27 subadult and adult crows (16 males, 11 females), which I categorized as being in their second year of life (n = 15 subadults) or older (n=12 adults) by a combination of plumage color and feather wear (Emlen 1936), as well as coloration of their mouth lining (fully black in birds older than two years). Due to potential differences in behavior and cognitive development, I released birds in their first year of life immediately upon capture. I caught birds outside of the breeding season and held them for several months each of three years (10 crows from February-March 2016, 9 from October-December 2017, and 8 from September-November 2018).

I kept captured crows in a protected outdoor aviary at the University of Washington, Seattle. The crows were housed individually and assigned to each cage randomly. The aviary contained ten adjacent cages separated by wire mesh; crows could see and hear their immediate neighbors (and potentially birds beyond their immediate neighbors' cages) but could not leave their own cage. Because the cages were arranged in a line, the birds at each end of the aviary (n=2 for each year, N=6 total) only had a single neighbor, but the remaining crows all had two neighbors (N=21). Each cage measured 1.8m wide x 2.1m tall x 2.4m deep and contained three perches, each approximately 1.2-1.5 m above the ground. The perch configuration was identical for each cage, and each bird was given the task within their own cage.

Crows were fed once per day and had full access to their food bowl for at least 6 hours. The 2016 group of crows were given a large amount of food immediately after participating in tasks within experiments described below, and their uneaten food was not removed until the following morning. Because half the birds in the 2016 group never solved the task (see Results below), I modified the food availability during subsequent years; the 2017 and 2018 groups received less food, were not fed until 2-3 hours after their task was completed, and had any uneaten food taken away later that evening. Theoretically, the 2017 and 2018 birds were more highly motivated by hunger to solve the tasks I describe compared to the 2016 group.

String-pulling Task

I suspended a preferred food item (a small piece of fried chicken) from the center of the perch nearest to the cage door using sisal rope twine. Even at full string length, the food remained approximately 1 meter above the ground and 50 cm away from the cage walls. I gave crows the task within their cage and they had the remainder of the day to solve it. I removed the food from unsolved tasks the following morning. When the task was first introduced to a crow, the food was suspended 45cm below the perch; I called this the "Naïve Full" stage. The purpose of the Naïve Full stage was to determine if and how crows can solve the task at full length without any prior training. The amount of time Model crows (defined below) spent in the Naïve Full stage varied from 3 days (2016 and 2017 cohorts) to 7 days (2018 cohort). Observer crows in 2016 spent up to 16 days in this stage, but this was reduced to 7 days in 2017 and 2018. After the Naïve Full stage was completed, the string length was shortened so that the food was tied directly to the perch (string length 0cm) within easy reach of the crow; this was the start of the "Learning" stage. During this stage, I lengthened the string by 7.5 cm each day if the crow previously succeeded in retrieving the food and reduced the string length by 7.5 cm each day if the crow failed. Once the string length reached 15 cm, the crows could not directly reach the food. I considered a crow to have mastered the task when it succeeded consistently enough to increase the string length back to 45cm (minimum 6 successes); all subsequent trials with the string back at full length were part of the "Mastery" stage.

I assigned crows to a role as Model (n = 13) or Observer (n = 14) at random based on their cage number (odd vs even). I immediately gave the Model group crows access to the task. The Observer crows did not initially have access to the task but could freely observe both of their Model neighbors as they interacted with the task (Observer crows housed in an end cage were only directly adjacent to a single Model, n = 4). Crows in the Observer group were only given access to the task after at least one of their Model neighbors had solved it seven times after reaching the Mastery stage.

The crows utilized a variety of strategies to solve the task, which I combined into three broad categories: attacking-string, food intercept, and string-pulling (Fig. 1.1). Each of the three broad categories include several specific methods, which I list from most to least common. Attacking-string occurs when the crows retrieve the food by knocking it to the ground, and includes the specific methods "string break", where the crow breaks the string at the base of the perch; and "shake free", where the crow's efforts to break/manipulate the string cause the food to slip free of the knot and fall to the ground. Food intercept occurs when the crow intercepts the hanging food from midair without interacting with the string, and includes the specific methods "fly up and grab**"**, where the crow starts on the ground and flies up to the food; "drop down and grab", where the crow starts at the perch and intercepts the food as it drops to the ground; and "cling and grab", where the crow clings to the side of the cage and jumps outward towards the food. String-pulling occurs when the crow pulls the hanging food towards itself, and includes the specific methods of "pull, step, pull", where the crow string pulls while remaining stationary; "side pull", where the crow string pulls while moving laterally along the perch; and "swing and catch", where the crow manages to swing the food within reach. During the beginning of the Learning stage, the string length was short enough (<15 cm) that the crows could directly grab the food from the perch- I called this the "within reach" method. Because this method was only possible for a limited amount of time and required relatively little cognitive ability to solve, I omitted it from all analyses which examine the methods used. Because they were wild caught, the crows were never tame enough to attempt the task while I was present, so I remotely monitored the crows' progress using a surveillance system.

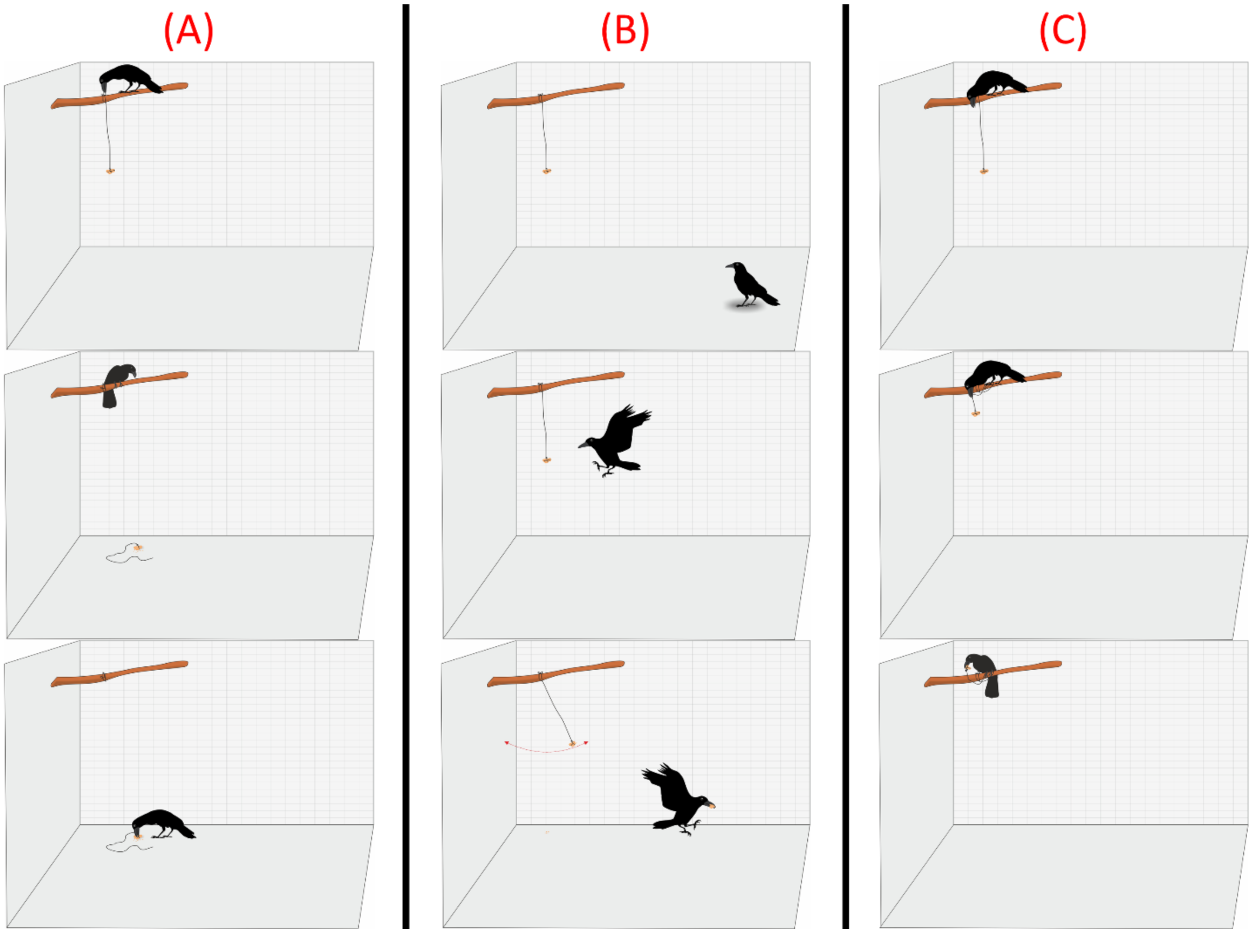

Figure 1.1. *Illustration of the three general methods used by the crows to solve the string-pulling task: (A) attacking string, (B) food intercept, and (C) string-pulling.*

Individual Measures

In addition to a crow's role as Model or Observer, I measured the following attributes of each bird: age (1-2 years-old = 'subadult', older = 'adult'), body condition, culmen length, level of nervousness, sex, and brain volume (both absolute and relative to body size). I calculated the crows' body condition (n=26) by extracting the residuals from a regression of their body weight upon capture (although see Discussion below for shortcomings to this method) against their culmen length (mm from the distal tip of the bill to the base of the feathers, n=26). I determined each bird's level of nervousness by standing 2 m away from each bird's cage while staring at a fixed point within the cage (not at the crow) and counting the bird's movements for 60 s. I assigned a numerical value based on the bird's perceived urgency to each move; walking along the perch = 0.5, while flying/hopping to another perch = 1. I obtained two such measurements for each bird (the first within two weeks of capture, the second within two weeks of release) and averaged them.

To determine the sex of the crows, I extracted approximately 20μL samples of blood from each bird via the brachial artery and stored them at -80°C. I used a QIAGEN® DNeasy® Blood & Tissue Kit to isolate genomic DNA from each blood sample, then amplified the target genes (CHD1-W and CHD1-Z) using polymerase chain reaction (PCR). I conducted agarose gel electrophoresis on the PCR product; males contain two Z chromosomes and produce a single band in the gel between 400-450 bp, whereas females contain a Z and a W chromosome and therefore produce an additional band in the gel between 300-325 bp (Griffiths et al. 1996).

As part of a related experiment, I imaged all but two crows (n=25) in a Siemens Inveon PET/CT system, and used the resulting CT images to calculate crow total brain volume (Fig. 1.2). I anesthetized (isoflurane) crows and secured them to a multimodality bed before conducting a 15 min microPET scan, followed by a CT scan in the docked and coregistered microCT scanner. The CT field of view was 7.9 cm x 13.3 cm and included the entire brain with a slice thickness of approximately 0.1 mm. I used open source DICOM viewer Horos version 3.2.1 (Horos 2019) to analyze the CT images and calculate brain volume. Brain segmentation was done using Horos's threshold-based 2D region of interest (ROI) utility on approximately 40% of the relevant slices. I edited these ROIs by hand before the software added interpolated ROIs on the missing slices. The generated ROIs were then inspected before I calculated the final volume (cm^3) with the built-in utility. As some of the brain segmentation is done manually and is thus subject to user bias, I and another researcher (A.L.L) independently analyzed several of the CTs; our findings differed by a very small amount (0.82% ± 0.62%, n = 11), thus I discounted user bias as minimal. To account for the allometric association of brain volume to body size (Fig. 1.3), I extracted the residuals from a regression of brain volume and culmen length (n=24) and used these residuals as a measure of relative brain volume during analysis in addition to raw absolute brain volume.

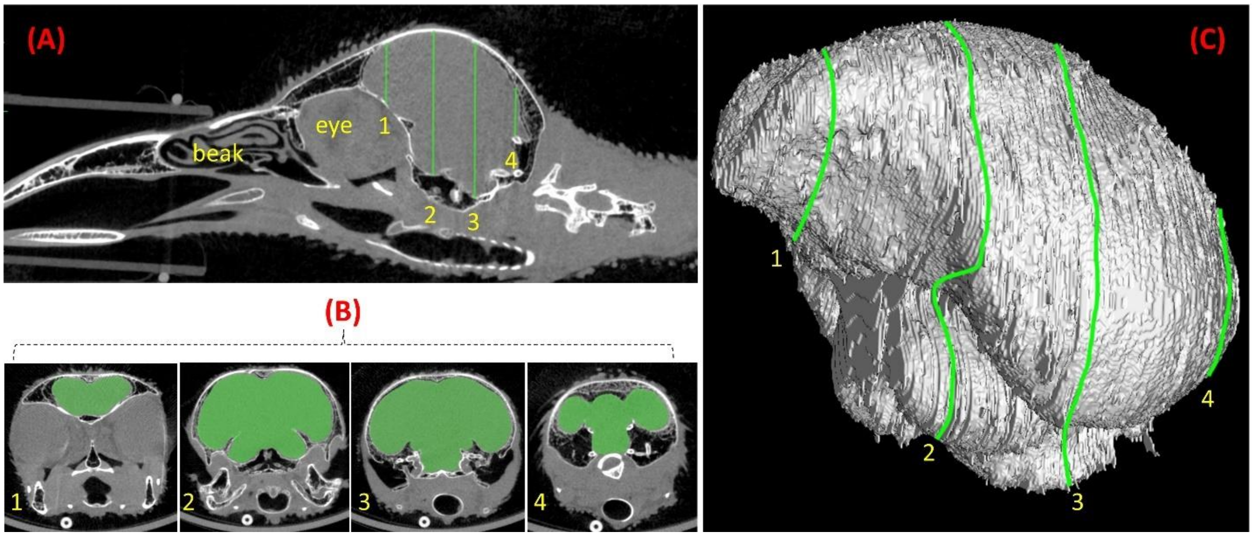

Figure 1.2. *DICOM image output from CT scan of a crow's head. For all viewpoints, the same regions of interest (indicated by numbers) are highlighted in green. (A) Medial slice of head from sagittal viewpoint. (B) Sample slices from coronal viewpoint progressing from rostral to caudal position within head. (C) 3D model of brain volume generated by the regions of interest from approximately 260 coronal slices.*

Analyses

I conducted all statistical analyses using RStudio version 1.0.136 (RStudio Team 2016). Because I examined eight different variables using a limited sample size, I constructed models using each single variable (along with an accompanying null model) and used AICc to determine which were most consistent with the data. I considered models to be competitive if they were within 2 AICc of the model with lowest AICc (Burnham & Anderson 1998). As I obtained many variables which could be correlated with each other (such as sex and culmen length; Clark et al. 1991), I tested correlation via Pearson's r. I used generalized linear models (GLM) to compare the individual factors affecting the crows' likelihood of solving the task during the Naïve Full stage (limiting this analysis to the first three days to account for the variable amount of time the different groups spent in this stage) and the time it took the successful crows to reach Mastery (progress fully through the Learning stage). I examined whether the methods used by the crows and those used by their neighbors affected their likelihood of success with Fisher's Exact tests.

I examined the congruency in methods used by the Models and Observers' by treating their similarity as a binomial and as a continuous variable. Because most Observer crows had two Model neighbors directly adjacent to them, which may have demonstrated different or the same methods, I considered the Observer to have copied a Model if it adopted either demonstrated method as its own. I conducted a binomial test (single tail because I predicted the observers would copy their neighbors with assumed probability 0.333 because there were three potential methods) to determine if the Observers and the Models used the same primary method more often than expected by chance. I assumed the most frequently used method was the primary method for this test. To account for all methods used by Observers and Models, I combined each of the three general methods' proportion of use by both the Observer and its Model to create a continuous "difference index" using the following equation: $\frac{|\alpha Obs\ AS-\ \alpha Mod\ AS|+|\alpha Obs\ FI-\alpha Mod\ FI|+|\alpha Obs\ SP-\ \alpha Mod\ SP|}{2}$ where AS, FI, and SP stand for the three general methods (attacking-string, food intercept, and string-pulling, respectively) and αObs and αMod are the proportion of successful trials where the Observer or Model used that method to solve the task. The index returns a value between 0 and 1, where 0 means the two birds solved the task using the exact same proportion of methods and 1 means the two birds had absolutely no overlap in methods used. For example, if the Observer and Model both exclusively used the food intercept method, the difference index would be $\frac{|0-0|+|1-1|+|0-0|}{2} = 0$, which reflects that both birds are perfectly aligned in method choice. If the Observer evenly preferred the food intercept and string-pulling methods while the Model evenly preferred the attacking-string and food intercept methods, the difference index would be $\frac{|0-0.5|+|0.5-0.5|+|0.5-0|}{2} = 0.5$, showing that there was 50% overlap in the methods used by the two birds. If the Observer exclusively used attacking-string and the Model evenly used food intercept and string-pulling methods, the difference index would be $\frac{|1-0|+|0-0.5|+|0-0.5|}{2} = 1$, demonstrating that the two birds used completely different methods. Although the index does not have a critical threshold for statistical significance (such as <0.05 for P-value) and tends to bias towards 1 (minor differences in the sample size of Observers and Models can only increase the score rather than decrease it), I present the difference index as a relative measure of method choice similarity instead of statistical tests of individual Model and Observer congruence (e.g., χ^2) because the repeated choice of methods by a particular bird are not independent of one another.

Because it is possible that the Observers copied the exact method most used by their Model neighbors to solve the task, I examined the use of specific, rather than general, methods using the six different methods the crows could use to solve the task during the Mastery stage (none of the crows used the cling and grab or swing and catch specific methods during this stage). As above, I calculated difference indices for the use of exact methods by Observers and

Models and used an exact binomial test to determine whether the crows are copying their neighbors. To reflect the six possible methods, I set the probability that crows copied their models due to chance to be 0.167.

Ethical Note

I captured, housed, and tested all crows (including PET/CT scans) in accordance with the Institutional Animal Care and Use Committee of the University of Washington (IACUC; protocol number 3077-01), Federal Fish and Wildlife Permit MB761139-0, and State of Washington Scientific Collection Permit 14-010. All were released back into the wild at the conclusion of the study.

RESULTS

General

My captive crows varied in size (culmen length: mean ± SE = 49.5 + 3.08 mm, n = 26), body condition (0.00 ± 38.15 g, n = 26), level of nervousness (29.11 ± 11.99 movements/min, n = 27), and absolute brain volume (Figure 1.3; 7.68 ± 0.64 cm^3, n = 25), although compensating for body size reduced the variation in brain volume somewhat (relative brain volume: 0.00 ± 0.48 cm^3, n = 24). The level of nervousness displayed by the crows did not significantly change over time (first measure: 29.74 ± 13.14 movements/min, second measure: 28.48 ± 13.62 movements/min, t_{26}= 0.55, P= 0.59). The crows were well fed during their time in captivity and gained a significant amount of weight by the time they were released (capture: 400 ± 46.16 g, release: 442.4 ± 59.77 g, t_{26}= -5.67, P < 0.001). While the Model birds were given the task within eight days of capture (1.45 ± 2.93 days), the Observers had to wait several weeks (19.07 ± 5.21 days) before they were given the task.

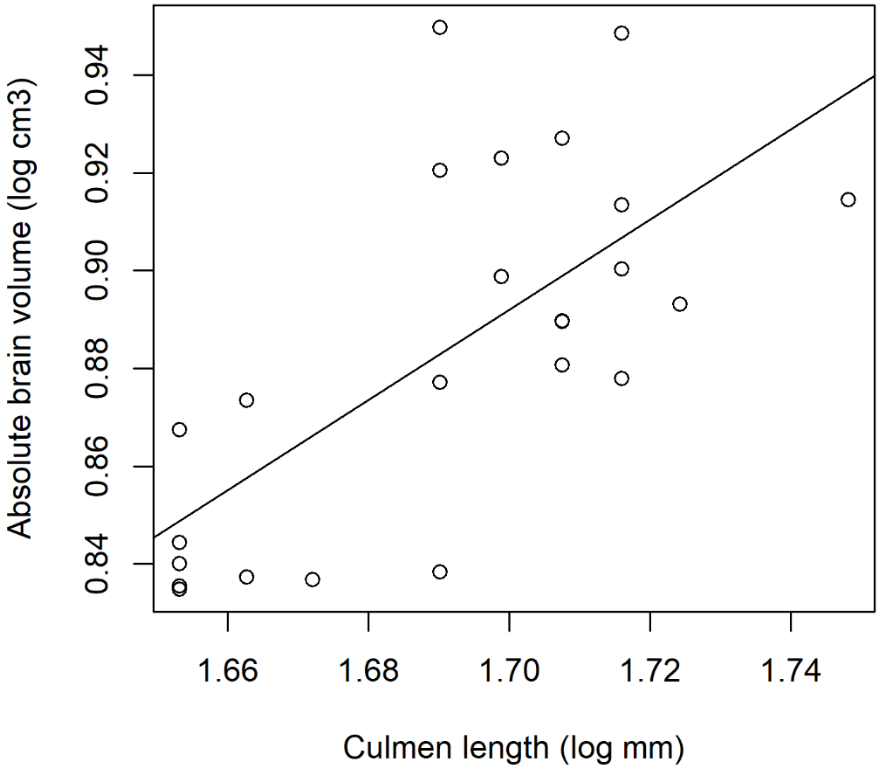

Figure 1.3. *Relationship between log-transformed absolute brain volume (cm^3) and body size (represented by culmen length, mm) of captive crows.*

My calculated crow brain volumes were larger than the endocranial volumes reported by Iwaniuk & Nelson (2002) (endocranial volume: 7.17 ± 0.55 cm^3), yet smaller than the brain volumes reported by Mlikovsky (2003) (endocranial volume: 8.7 cm^3, SE not provided). These discrepancies are likely due to differences in measurement technique and demonstrate that my virtual measurements are comparable to physical measurements of the brain.

Among the eight variables I obtained from the crows, I observed five cases where variables were strongly correlated, and two additional cases where there was notable correlation (Table 1.1). Absolute brain volume and relative brain volume are, unsurprisingly, highly correlated (t_{22} = 5.23, P < 0.001). Three of the variables (absolute brain volume, culmen length, and sex) are closely tied to the size of the bird and are all highly correlated with one another as a result; males have larger absolute brain volumes (t_{23} = 4.86, P < 0.001) and culmen lengths (t_{22} = 8.13, P < 0.001) than females, and birds with long culmen lengths had correspondingly large brains (t_{22} = 4.21, P < 0.001). Additionally, birds with poorer body condition were more nervous than those in good condition (t_{24} = -2.92, P= 0.007). The Model group contained more males (t_{25} = -1.85, P = 0.08) and had somewhat larger absolute brain volumes (t_{23} = -1.90, P = 0.07)) than the Observer group, although this was due to chance as I randomly assigned birds to their cages (and therefore, social role) prior to determining their sex and brain volume.

Table 1.1. *Correlation matrix of the variables I obtained from the crows. I calculated Pearson's correlation coefficient when comparing two continuous variables and reported t-score and P-values for all comparisons for all variables. Significant correlations are bolded.*

	Relative brain volume (n=24)	Absolute brain volume (n=25)	Body condition (n=26)	Culmen length (n=26)	Nervous-ness (n=27)	Sex (n=27)	Age (n=27)
Absolute brain volume (n=25)	**r = 0.74** **t_{22} = 5.23** **P < 0.001**						
Body condition (n=26)	r = -0.043 t_{22} = -0.20 P = 0.84	r = 0.005 t_{22} = 0.025 P = 0.98					
Culmen length (n=26)	r = 0.00017 t_{22} = 8.2 e-04 P = 0.99	**r = 0.67** **t_{22} = 4.21** **P < 0.001**	r < 0.001 t_{24} < 0.001 P = 1				
Nervous-ness (n=27)	r = 0.29 t_{22} = 1.44 P = 0.16	r = 0.14 t_{23} = 0.67 P = 0.51	**r = -0.51** **t_{24} = -2.92** **P = 0.007**	r = -0.038 t_{24} = -0.19 P = 0.85			
Sex (n=27)	t_{22} = 1.05 P = 0.30	**t_{23} = 4.86** **P < 0.001**	t_{24} = 0.25 P = 0.80	**t_{24} = 8.13** **P < 0.001**	t_{25} = 0.10 P = 0.92		
Age (n=27)	t_{22} = 0.36 P = 0.72	t_{23} = 0.91 P = 0.37	t_{24} = -0.007 P = 0.99	t_{24} = 0.75 P = 0.46	t_{25} = -0.16 P = 0.87	t_{25} = 0.08 P = 0.93	
Social role (n=27)	t_{22} = -1.55 P = 0.14	**t_{23} = -1.90** **P = 0.07**	t_{24} = -0.09 P = 0.93	t_{24} = -1.29 P = 0.21	t_{25} = -0.51 P = 0.62	**t_{25} = -1.85** **P = 0.08**	t_{25} = 0.93 P = 0.36

Initial Access to Task

Many of the crows initially struggled to obtain the food hung at full length from the perch; only nine Models and four Observers solved the task within the first three days. However, eight additional crows learned to solve the task after the first three-day period; three Observers given additional time in the Naïve Full stage solved it, and one Observer and four Models solved it during the Learning stage. In total, 21 of 27 birds were successful. The crows became increasingly successful at solving the task as they gained experience with it; all successful birds were nearly twice as likely to solve the task on any given day during the Mastery stage than during the Naïve Full stage (Naïve Full probability of solving: mean ± SE= 0.47 ± 0.9, Mastery: 0.91 ± 0.04, t_{20}= 4.82, P<0.001). Although almost all crows in the 2017 and 2018 cohorts learned to solve the task, only five of the crows I captured in 2016 solved it (all from the Model group). These birds had better overall body condition upon capture than both the 2017 (t_{23}=3.32, P=0.003) and 2018 (t_{23}=3.35, P=0.003) cohorts.

Almost all the crows that succeeded during the entire Naïve Full stage (n=16) used an attacking-string (n=5) or food intercept (n=9) method when they solved the task for the first time. The crows' social role significantly affected their method choice during this stage (Fisher's exact test, P= 0.037), with crows in the Model group preferring food intercept while Observer crows preferred the other two methods (Table 1.2). Contrary to my expectations, three crows from 2018 used a string pull method during the Naïve full stage; one male Model switched to this method after first solving the task using food intercept, and two female Observers used this method to solve the task for the first time. Afterwards, all three birds immediately adopted string-pulling as their primary method. Although one of the females had a notably small culmen (46 mm) and poor body condition (38 grams lower than expected, given culmen length), the remaining attributes of the three string pullers were within one standard deviation of the mean for the rest of the crows.

Table 1.2. *The most common method used by crows in each social group that solved the task during the Naïve Full stage.*

Primary method choice during Naïve Full stage	Model	Observer
Attacking-String	1	4
Food Intercept	7	1
String-Pulling	1	2

The most used method by the birds in the Model group did not significantly affect their Observer neighbors' likelihood of solving the task during the entire Naïve Full stage (Fisher's exact test; P = 0.48; Table 1.3), nor did it influence the general method used by the successful Observers (Fisher's exact test; P = 1.0; Table 1.3). Even after lumping the "copy/did not copy" data from all four possibilities for methods demonstrated by their neighbors (Table 1.3) into a single category, I found that successful Observers were not significantly more likely than expected by chance to adopt a method used by their Model neighbors (Exact binomial test, P = 0.54).

Table 1.3. *A comparison of the most common methods used by the Observer crows' neighbors during the entire Naïve Full stage, and whether the Observer crows' most used method was the same or different as their neighbors' during this stage. Note: during the Naïve Full stage, the Observers' primary method was identical to their first method. If the neighbors used different methods, we checked whether the Observer used either of the two modeled methods.*

Naïve Full Stage Only	Solved task		Failed to solve task
	Same method as neighbor	Different method from neighbor	
Neighbors primarily used different methods	1	1	3
Neighbor(s) primarily used String-Pulling	1	2	2
Neighbor(s) primarily used Food Intercept	0	2	1
Neighbor(s) primarily used Attacking-String	0	0	1
Total	2	5	7

Of the eight individual variables I modeled, social role best accounted for the likelihood of a crow succeeding during its first three days with the task, although it only garnered 42% of the weight of evidence and was not substantially better than the null model (Table 1.4). Specifically, the crows in the Observer group were *less* likely than their neighbors to solve the task during this stage (Models: mean = 0.75 likelihood success, Observers: 0.33 likelihood success, z_{23} = -1.98, P = 0.048). This relationship persisted, albeit not as intensely, even after I removed the 2016 group to control for the abysmal performance of that year's Observers (Models: 0.88, Observers: 0.44, z_{16} = -1.72, P = 0.086).

Table 1.4. *Individual variable model selection for a crow's likelihood of succeeding during the first three days after gaining access to the task (n=24). Intercept, coefficient, and SE estimates are given in logit scale. Best-fit model and competing models (< 2 Δ AICc) are highlighted in grey.*

Model	Δ AICc	W_i	Intercept ± SE	Coefficient ± SE	P
Social role (Model/Observer) †	0.00 ‡	0.40	1.10 ± 0.67	-1.79 ± 0.91	0.048
Null model	1.95	0.15	0.17 ± 0.41		
Body condition	2.44	0.12	0.14 ± 0.43	-0.016 ± 0.01	0.204
Relative brain volume	3.48	0.07	0.18 ± 0.42	+0.83 ± 0.93	0.371
Sex (Female/Male) †	3.79	0.06	-0.22 ± 0.67	+0.63 ± 0.85	0.461
Absolute brain volume	3.83	0.06	-3.37 ± 5.07	+ 0.46 ± 0.66	0.480
Age (Adult/Subadult) †	4.17	0.05	0.34 ± 0.59	-0.34 ± 0.82	0.682
Nervousness	4.29	0.05	-0.08 ± 1.19	+0.008 ± 0.04	0.822
Culmen	4.33	0.04	-0.10 ± 6.67	+0.005 ± 0.14	0.969

† Binomial variable coefficients are for Observer (social role), Male (sex), and Subadult (Age)

‡ AICc = 33.34

Learning and Mastery

After the Naïve Full stage ended, I trained the crows to consistently solve the task using a string-pulling method (time required: mean ± SE = 7.9 ± 0.73 days). Of the eight individual variables I modeled, social role, absolute brain volume, relative brain volume, and body condition best explained the number of days it took for a successful crow to progress through the

Learning stage and reach the Mastery stage (Table 1.5), although none of them were significant at the P = 0.05 level, nor substantially better than the null model. Specifically, crows in the Model group, birds with larger brain volumes (both absolute and relative to their body size), and those with good body condition tended to reach Mastery in fewer days than Observers, small-brained birds, and those with poor body condition (Fig. 1.4). The effect was strongest for social role; Observers on average required an additional 2.5 days more than the Models (z_{18}= 1.879, P= 0.06). Absolute brain volume was also notable; gaining 1 cm^3 of brain volume decreased the mean time required to master the task by 1.88 days (z_{18}= -1.746, P= 0.08). The models for relative brain volume (gaining 1 cm^3 relative to body size decreased time required by 1.77 days; z_{18}= -1.379, P= 0.168) and body condition (gaining 1 g decreased time required by 0.02 days; z_{18}= -1.286, P= 0.199) were weaker, and appear to be primarily driven by a few individuals (three small-brained birds and three poor body condition birds, respectively, see supplementary material 1). The only competitive multivariate models combined social role/body condition and absolute brain volume/body condition, but neither performed as well as the single variate models (Table 1.5). Altogether, the six competitive models cumulatively garnered 52% of the weight of evidence, but none were substantially better than the null model (Table 1.5).

Table 1.5. *Model selection for time required for a successful crow to progress through the Learning stage and reach the Mastery stage (n=19). Intercept, coefficient, and SE estimates are given in log scale. Best-fit model and competing models (< 2 Δ AICc) are highlighted in grey. Multivariate models are constructed using only the variables from competing models.*

Model	Δ AICc	W_i	Intercept ± SE	Coefficient 1 ± SE	P	Coefficient 2 ± SE	P	Coefficient 3 ± SE	P	Coefficient 4 ± SE	P
Social role †	0.0 ‡	0.15	1.93 ± 0.11	0.31 ± 0.16	0.060						
Abs. brain volume	0.35	0.12	3.78 ± 0.98	-0.23 ± 0.13	0.081						
Null model	0.96	0.09	2.06 ± 0.08								
Rel. brain volume	1.47	0.07	2.06 ± 0.08	-0.26 ± 0.19	0.168						
Body condition	1.71	0.06	2.04 ± 0.08	-0.003 ± 0.002	0.199						
Social role † + body condition	1.79	0.06	1.93 ± 0.11	0.28 ± 0.17	0.09	-0.002 ± 0.002	0.31				
Abs. brain volume + body condition	1.85	0.06	3.64 ± 0.98	-0.21 ± 0.13	0.10	-0.003 ± 0.002	0.26				
Sex †	2.03	0.05	2.17 ± 0.12	-0.20 ± 0.16	0.229						
Culmen	2.04	0.05	3.71 ± 1.37	-0.034 ± 0.028	0.230						
Social role † + abs. brain volume	2.05	0.05	3.01 ± 1.21	0.21 ± 0.21	0.28	-0.14 ± 0.15	0.37				
Social role † + rel. brain volume	2.24	0.05	1.95 ± 0.11	0.26 ± 0.18	0.15	-0.16 ± 0.20	0.44				
Rel. brain volume + body condition	2.43	0.04	2.04 ± 0.08	-0.26 ± 0.19	0.16	-0.003 ± 0.002	0.18				
Age †	2.98	0.03	2.01 ± 0.11	0.12 ± 0.16	0.480						
Abs. brain volume + rel. brain volume	3.19	0.03	3.67 ± 1.52	-0.21 ± 0.20	0.29	-0.03 ± 0.29	0.93				
Nervousness	3.43	0.03	2.02 ± 0.23	0.001 ± 0.007	0.845						
Social role † + rel. brain volume + body condition	4.25	0.02	1.95 ± 0.11	0.22 ± 0.18	0.23	-0.18 ± 0.20	0.38	-0.003 ± 0.002	0.28		
Social role † + abs. brain volume + body condition	4.27	0.02	2.98 ± 1.21	0.18 ± 0.20	0.36	-0.13 ± 0.15	0.38	-0.002 ± 0.002	0.32		

Abs. brain volume + rel. brain volume + body condition	5.03	0.01	3.29 ± 1.55	-0.16 ± 0.20	0.42	-0.08 ± 0.29	0.78	-0.003 ± 0.002	0.25		
Social role † + abs. brain volume + rel. brain volume	5.27	0.01	2.77 ± 1.73	0.22 ± 0.20	0.28	-0.11 ± 0.22	0.64	-0.06 ± 0.29	0.85		
Social role † + abs. brain volume + rel. brain volume + body condition	7.90	0.00	2.53 ± 1.76	0.19 ± 0.20	0.35	-0.07 ± 0.23	0.74	-0.11 ± 0.30	0.72	-0.003 ± 0.002	0.30

† Binomial variable coefficients are for Observer (social role), Male (sex), and Subadult (Age).

‡ AICc = 96.56

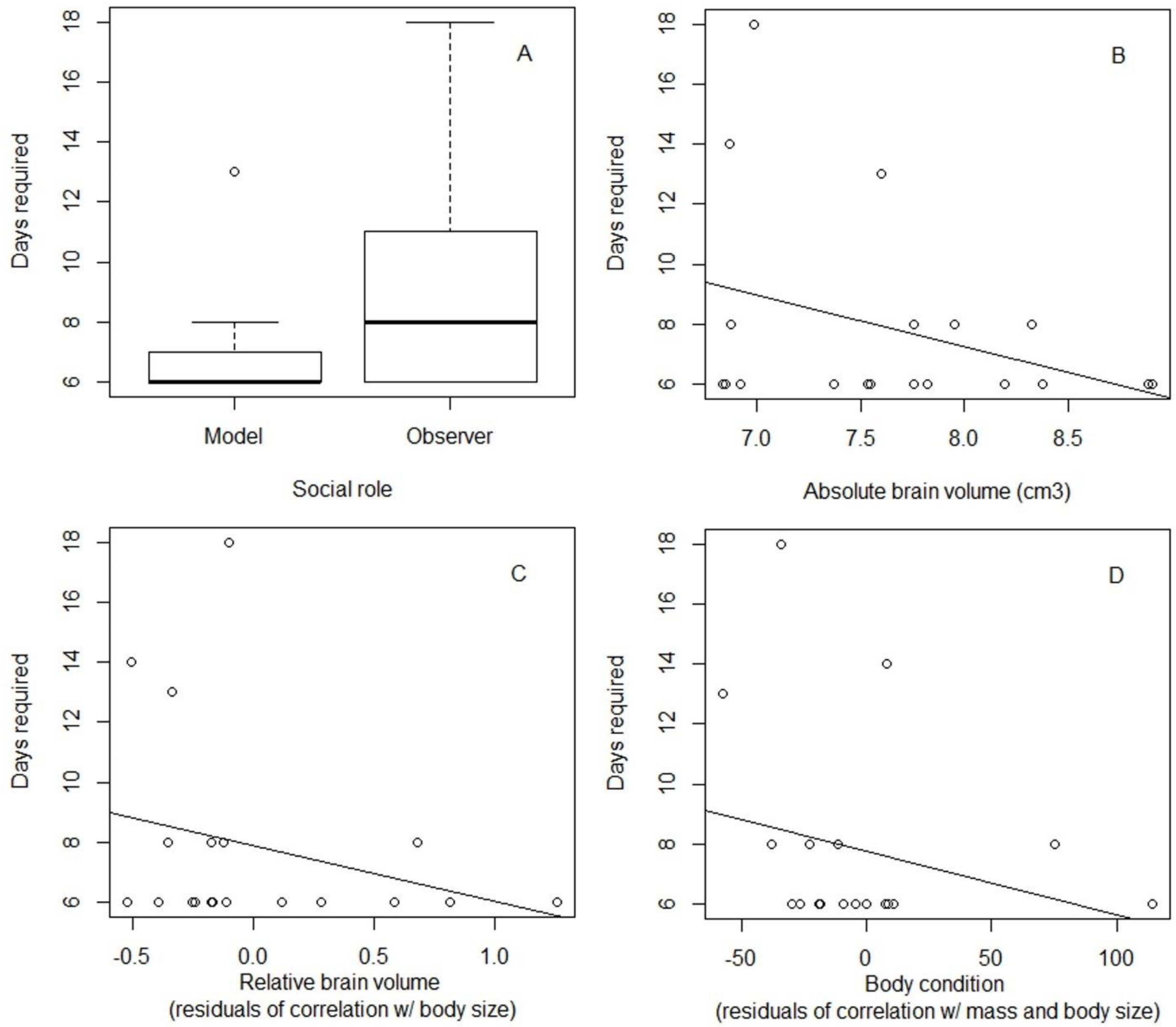

Figure 1.4. *The four leading variables for explaining the days required for a crow to progress through the learning stage and reach mastery: A) social role, B) absolute brain volume, C) relative brain volume, D) body condition.*

Many of the crows showed flexibility in method preference; a significant number of birds changed their most frequently used method after they progressed out of the Naïve Full stage (Fisher's exact test; P = 0.013), with more than half of the crows adopting the string-pulling method as their primary method overall (Fig. 1.5). Although the methods employed by the Model birds did not affect the likelihood that neighboring Observers would succeed overall (Fisher's exact test; P = 0.5; Table 1.6), those that did succeed were significantly more likely to adopt their Model neighbor's primary method as their own (Exact binomial test, P = 0.003; Table 1.6) after they entered the Mastery stage. This effect persisted even after removing the two Observers that were exposed to two differing favorite methods by their neighbors (Exact binomial test, P = 0.018) to account for them having a greater probability of copying one of the demonstrated methods by chance alone.

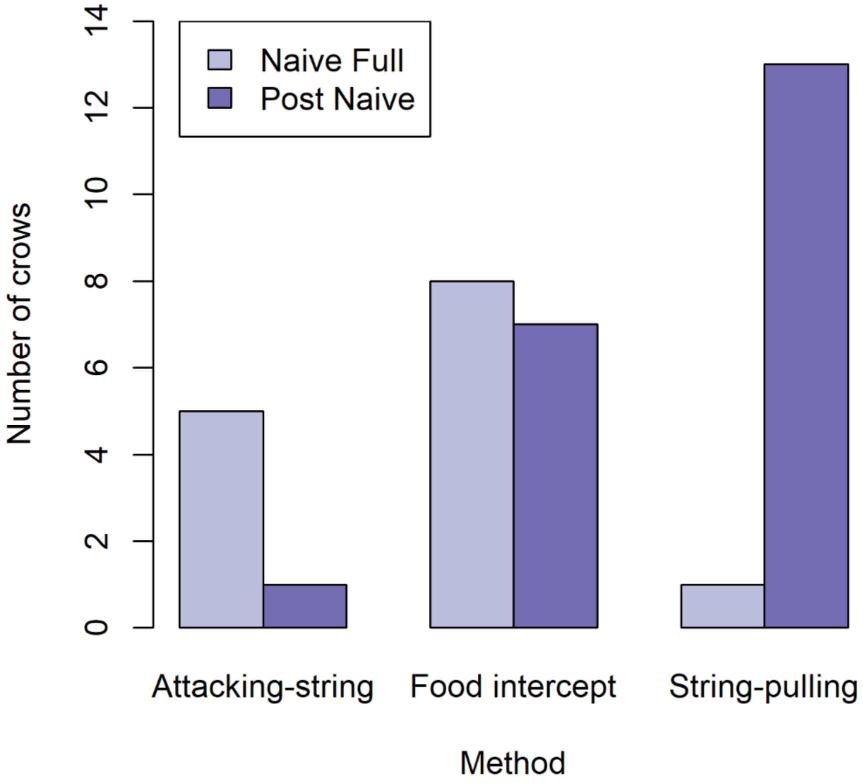

Figure 1.5. *A comparison of the number of crows that most frequently used each method during the Naïve Full stage and afterward.*

Table 1.6. *A comparison of the most common general methods used by the Observer crows' neighbors during the Mastery stage, and whether the Observer crows utilized the same or different primary method as their neighbors (or failed to solve the task). If the neighbors used different methods, we checked whether the Observer used either of the two modeled methods.*

Overall	Solved task		Failed to solve task
	Same method as neighbor	Different method from neighbor	
Neighbors primarily used different methods	2	0	3
Neighbor(s) primarily used String-Pulling	4	0	1
Neighbor(s) primarily used Food Intercept	1	1	1
Neighbor(s) primarily used Attacking-String	0	0	1
Total	7	1	6

During the Mastery stage, six of the eight successful Observers used nearly identical general methods as one of their Model neighbors to solve the task (difference index < 0.25, Table 1.7). All but two successful Observers most frequently used a string pull method, but the two that did not (Observer crows 4 and 5) closely matched their neighbors' use of a food intercept method (Table 1.7), even though my training emphasized string-pulling and Observer crow 4's other neighbor (Model crow 3) overwhelmingly solved via string-pulling. Another Observer (crow 3) used a wider variety of methods and had a difference index of nearly 0.5 with both of its neighbors, even though all three crows in that group most frequently solved with a string pull method. Only one Observer avoided using the same method as its neighbor (Table 1.6); that same bird (crow 8) was one of the three crows that began using a string pull method to solve the task prior to receiving any training, and it continued to exclusively use that method during the Mastery stage, whereas its Model only used attacking-string or food intercept methods (difference index = 1, Table 1.7). In addition to matching the general methods demonstrated by their neighbors, Observers were also more likely to copy one of the six specific methods modeled by their neighbors (Table 1.8 in supplementary material). Five of the eight successful Observers copied the most frequently demonstrated specific method (Table 1.8; Binomial P= 0.005). Five Observers were at least 50% similar to their neighbors in their choice of method and three mirrored their neighbors nearly perfectly (difference index < 0.15; Table 1.9). One Observer (Observer crow 2) had two Models that used different specific methods (Model crow 1 used pull, step, pull, and Model crow 2 preferred side pull; Table 1.9) and that Observer utilized both demonstrated methods to solve the task.

Table 1.7. *A comparison of how often each general method was used during the Mastery stage by the eight successful Observers and the Model birds they were adjacent to. Many of the Models were included more than once in this comparison, as they were adjacent to two successful Observers. Difference Index scores near to 0 indicate close alignment of method choice between the Observer and that Model, whereas scores close to 1 indicate the two birds were highly dissimilar in method choice.*

Group	Birds	# Trials Total	# Attacking-String	# Food Intercept	# String-Pulling	Difference Index
1	Obs 1	15	0	0	15	
	Mod 1	42	6	0	35	0.15
2	Obs 2	11	0	0	11	
	Mod 1	42	6	0	35	0.15
	Mod 2	39	1	0	34	0.03
3	Obs 3	22	3	8	11	
	Mod 2	39	1	0	34	0.47
	Mod 3	42	2	0	39	0.45
4	Obs 4	21	0	20	1	
	Mod 3	42	2	0	39	0.95
	Mod 4	31	0	29	1	0.01
5	Obs 5	9	0	7	1	
	Mod 4	31	0	29	1	0.09
6	Obs 6	18	0	0	16	
	Mod 5	38	0	38	0	1.00
	Mod 6	38	0	9	29	0.24
7	Obs 7	16	1	0	15	
	Mod 6	38	0	9	29	0.24
	Mod 7	38	3	0	35	0.02
8	Obs 8	18	0	0	18	
	Mod 8	37	13	23	0	1.00

DISCUSSION

While multiple studies have examined the role that cultural transmission, experience, and other individual factors play in an individual's ability to solve novel tasks (Beck 1973, Tomasello et al. 1987, Von Bayern et al. 2009, Holzhaider et al. 2010, Roth et al. 2010, Jacobs & Osvath 2015), fewer studies have simultaneously examined which of multiple factors best determine an individual animal's ability to solve such a task (though see Cole et al. 2011 and Papp et al. 2015). Here I expand on Heinrich's 1999 research on American crows by examining which individual and social factors in a long-lived, social animal have the greatest impact on their ability to solve a string-pulling task.

Observers Less Motivated than Models

Contrary to my predictions, crows that observed their neighbors master a task were not more likely to initially solve the task and took longer to master it than did crows without access to such information. The Observers performed measurably *worse* than the birds in the Model group.

I believe this apparent lack of social learning was due to the Observers being less motivated to retrieve the food- they spent roughly three weeks in captivity before being given the task, during which their body condition improved from regular food access. The 2016 group supports this hypothesis: they performed objectively worse (none of the Observers from that cohort ever solved the task), yet their initial body condition was greater (possibly due to their being captured later in the non-breeding season) than the groups from subsequent years. Furthermore, the statistical model containing body condition was one of the competitive models for predicting the amount of time required for a crow to reach the Mastery stage and was the most highly ranked of the non-competitive models for predicting the likelihood of initial success. Unfortunately, because I did not extract daily weights from the birds (I used weight on day of capture to calculate body condition) I cannot directly compare the body condition of Models versus Observers when each group first gained access to the task- future studies should consider regularly measuring the body condition of their study species as they attempt the task.

In addition to better body condition, the Observer crows may have been less motivated due to learning the routine of captivity. While the Model birds were newly captured from the wild (and still uncertain of when their next meal would come) when they were first given the task, the Observers had likely learned that food would be delivered at some point each day. While I attempted to motivate them by removing their food bowls prior to giving them the task, after several days I noticed that the crows had started caching food throughout their cage. Whether because of the natural corvid instinct to cache (Jacobs et al. 2014), or in anticipation of their food bowl being removed (Emery 2004), this caching behavior ensured that most crows had continuous access to food and were therefore less motivated to retrieve the hanging chicken (Birch 1945).

Finally, the Model group contained more males and a higher mean absolute brain size compared to the Observer group. This is due to chance, as I assigned crows to cages randomly at the beginning of the study and did not determine sex or brain volume until the study had concluded. Although the crows' sex did not affect any of the conditions I measured, absolute brain volume might play a role in the amount of time a successful crow needs to master the task.

Therefore, it is possible that the superior performance of the Model group compared to the Observer group may be due, in part, to the higher mean absolute brain volume of that group.

Social Learning

Although they did not master the task more rapidly, nor have a higher likelihood of initially solving it, the crows in the Observer group were more likely to copy the general and specific method most frequently used by their neighbors. This suggests that crows can use social learning to hone their technique *after* they've independently learned to solve the task. The ability to use social learning to refine a foraging technique would allow an observer to capitalize on a conspecific's innovation to improve its own foraging efficiency. Crows are certainly capable of this; carrion crow (*Corvus* corone) use of vehicles as nutcrackers is transmitted via social learning throughout Japan (Nihei & Higuchi 2001). The crows were already capable of cracking the nuts by repeatedly dropping them on a hard surface, but those that copy the 'car nutcracker' method demonstrated by conspecifics can save both time and energy.

My social learning results may have been limited by the crows' individual housing, a precaution taken to reduce social inhibition (Marzluff & Heinrich 1991) and to accurately monitor individual food consumption. Most research into corvid social learning allow the model and observer to freely interact with each other in a single chamber (Fritz & Kotrschal 1999, Bugnyar et al. 2007, Clayton et al. 2007). It is possible that the American crows might have had more success learning the string pull task socially if they had been allowed to interact with one another in a similar fashion. This could be considered in future studies. Additionally, the crows may be more likely to copy individuals of a particular social class (such as their mate or kin, see Clayton & Emery 2007), and I do not know the prior relationships (if any) our study animals shared prior to capture.

Brain Volume

My findings that American crows with smaller absolute brain volumes tended to have greater variability in the time required to master the task supports the hypothesis that larger brains are advantageous for complex cognitive tasks (Deaner et al. 2007). Brain tissue is metabolically expensive to grow and maintain (Aschoff et al. 1971, Hofman 1983), thus we should expect large brains to provide a proportionally greater benefit than their costs to overcome selection pressure. The variation in brain volume in my results and those reported by Iwaniuk & Nelson (2002) are comparable, indicating that the brain volume of American crows can vary in size by up to 8% between individuals. This variation would offer an avenue for natural selection to favor large or small-brained individuals under differing constraints; appropriate given that American crows are human commensal species that live in dynamic environments with ample opportunities to discover novel food sources (Kilham 1990).

However, there are significant caveats to claiming that within-species whole brain volume can be used to predict task performance. Primary among these caveats is that none of the models I used to compare each measured factor against the crows' likelihood solving or learning the task were substantially better than the null model, which severely limits my ability to apply these findings generally. Additionally, most of the brain regions responsible for higher-order cognitive tasks (such as the nidopallium and mesopallium) are specifically located in the avian pallium (Emery & Clayton 2004), whereas I measured the entire brain's volume. Furthermore, my method for calculating brain volume does not account for neuron density, which varies between and within species (Lange 1975, Collins et al. 2010, Olkowicz et al. 2016), and even within brain regions of a single individual (Collins et al. 2010).

Despite these caveats, there are numerous studies supporting brain volume as a predictor of cognitive ability. Recent research has emerged demonstrating the importance of neuronal counts over other brain-related metrics (such as relative brain volume or cortex/pallium mass) in predicting a species' cognitive capability (Herculano-Houzel 2017, Jardim-Messeder et al. 2017, Jacobs et al. 2019), with brain size being an appropriate predictor of neuron number ((Marhounova et al. 2019). Additionally, a close association has been found between brain size and neuron count for the regions in the brain involved with cognition, such as the telencephalon (Marhounova et al. 2019). I have demonstrated that the absolute brain size of American crows varies among individuals and provide some evidence that individuals with larger brain volumes tend to consistently require less time to master the task. While this might suggest that large-brained individuals contain greater neuronal counts compared to smaller-brained individuals of a similar size (supporting the theory that neuronal counts are a predictor of cognitive ability), factors such as neuronal size and density must also be considered (Herculano-Houzel et al. 2015, Herculano-Houzel 2017) both of which are currently unknown for American crows.

Method Choice

The methods used by the crows throughout the study matched earlier (Heinrich 1999) observations: while naive crows rarely pulled the string to initially solve the task (preferring instead to intercept the food from the air or attack the string), most crows readily switched to string-pulling with experience and training. Compared to other methods, string-pulling requires less aerial agility (especially challenging for larger birds) than food interception and less physical strength (especially challenging for smaller birds) than attacking the string. I regularly observed three crows from 2017 and two from 2018 that would first use a string pull method to retrieve the food, but afterwards would begin attacking the empty string until it broke. This may be a form of play behavior for bored captive crows. Contrary to expectations (Heinrich 1999), I found that some American crows are capable of using a string pull method without training, although I only observed three birds accomplish this and were unable to identify any characteristic which might be responsible.

Crows in the Model group overwhelmingly preferred the food intercept method when they were first given the task, whereas the Observer crows strongly preferred the other methods (Table 1.2). I don't believe that this discrepancy was caused by social learning (most successful Observers did not *initially* copy their neighbors' favorite method), but it could have been caused by the additional time the Observers spent in captivity prior to being given the task. While the aviary cages were spacious enough to allow limited flight, the crows did not receive nearly as much exercise in captivity as they would in the wild (for perspective, I re-sighted one of our study crows on a territory 24 km away from the roost where I initially captured her). If their flight muscles had atrophied to some degree, then intercepting the food from the air would become relatively more difficult than the other two methods.

The training I gave the crows during the Learning stage preferentially emphasized the string-pulling method; by shortening the string, I made that method both easier to learn and perceive, and more efficient to perform than the other two methods. I did not change the difficulty of the attacking-string method (the string material remained constant), and I increased the difficulty of the food intercept method by bringing the string closer to the perch relative to the ground. This is the most probable explanation for why most crows switched to using the string-pull method after beginning the Learning stage and presents a competing reason for why most Observers copied the method frequently demonstrated by their Model neighbors. However, three lines of evidence suggest that social learning was involved. First, while six of the eight successful Observers did preferentially use the string-pull method to solve the task after mastering it, only five of them had a neighbor that used that method. Second, of the seven Observers that favored the same method as their neighbors, two preferred the Food Intercept method, even though one of them had a second neighbor that opted to use the string-pull method. (Table 1.7). Third, in addition to adopting the most modeled method of problem solving, after mastering the task the variety of general and specific methods used by most Observers closely matched the frequency of method use by their Model neighbors (difference index, Table 1.7, S1.3).

There is much disagreement over whether birds solve the string-pulling task using spontaneous insight-like processes, or more methodical processes such as operant conditioning (Heinrich 1995, Heinrich & Bugnyar 2005, Taylor et al. 2010, Shettleworth 2012). My results favor the hypothesis that most crows learn to string pull using operant conditioning. Specifically, during the Learning stage, the crows learned to use a string pull method by receiving immediate positive reinforcement; the shortened string length meant that a single reach-down-and-pull sequence brought the food within or almost within reach of the hungry crow, motivating it to continue its behavior. However, some crows may use other methods, as evidenced by the three crows that learned to use a string pull method without going through any training. These three crows may have discovered the method via social learning or an insight-like process, although I have no way of knowing if they had any prior life experiences that would have prepared them to use this method.

Brain Volume Calculation

My method of using DICOM from CT scans to calculate the brain volume of crows generated a mean estimate that was within the endocranial volumes calculated by Iwaniuk & Nelson (2002) and Mlikovsky (2003), demonstrating that this virtual approach is comparable to direct estimation. The advantage of my approach is that no animals were sacrificed, and brain volume was unaffected by post-mortem fluid/blood pressure loss.

Caveats

I wanted to maximize the opportunities for an Observer crow to see their Model neighbors work the task, so I did not partition the cages into pairs or otherwise block any bird’s view of their conspecifics. While this meant that crows could observe both of their immediate neighbors, it also meant that they could potentially see the birds beyond their neighboring cages. Because the additional distance and layers of wire mesh would make it more difficult to see the details of distant bird’s activities, coupled with the poorer performance of Observers compared to Models, I do not believe this to be a major confounding factor.

Despite my efforts to ensure every year’s group of crows experienced the same conditions, I was inconsistent with the amount of time the crows were given to attempt to solve the task during the Naïve Full stage (caused by temporal constraints from a different experiment), forcing me to limit my analysis to the first three days of task access. Additionally, I captured the 2016 crows later in the non-breeding season than subsequent years’ cohorts, which may have played a role in their poor performance.

My findings that brain volume and body condition are weakly correlated with learning time are primarily driven by three individuals (Fig. 1.4B, C, D); if I remove them from the analysis, the effect vanishes completely. While I could disregard a single outlier, multiple individuals that span a range of sizes, ages, and genders (Table 1.10 in Supplementary material) behaving similarly indicate a possible effect of brain volume and body condition that could be investigated in future studies.

Finally, my inability to detect social learning may be due to the experimental setup. I used wild-caught rather than captive-reared crows for our study; while this ensured that the factors I measured were representative of the wild crow population, it also meant that my setup was less optimal than a typical cognitive study, and may have added unnecessary variation to the data and the crows' performance. For example, many studies examining social learning record and/or standardize additional variables, such as the relationship of the tutor/observer dyad, the amount of time the observers watch the demonstrators, how much time the demonstrator spent working on the task, and whether the observer had a preferred demonstrator (Hoppitt & Laland 2008, Nielsen et al. 2012). Social status also influences Corvid behavior (Miyazawa et al. 2019), but I did not know what (if any) social relationship my crows had with each other prior to capture, and had no opportunity to learn while they were captive because the birds were housed individually and had limited ability to interact. The crows remained wary of humans, so I had to observe them remotely via surveillance cameras; the video resolution was good enough to ascertain which method the crows used to solve the task, but not enough to determine how long the crows were actively watching others interact with the task. This lack of standardization may be responsible for my crows' lackluster performance compared to other studies which examined corvid problem solving (Heinrich & Bugnyar 2005, Jacobs & Osvath 2015). However, my setup was sufficient in demonstrating some aspects of social learning, such as the propensity of Observers to copy their neighbors most frequently modeled solution.

Conclusion

In conclusion, my results suggest that motivation (caused by hunger and associated body condition) is the most important factor in determining the ability of American crows to solve a string-pull task, although absolute brain volume and social learning may also play a role. Crows tested a few days after capture were more likely to initially solve the task compared to crows that were given the task after several weeks of captivity. This same group of crows also required consistently fewer days to master the task compared to the group tested after several weeks, but bigger-brained birds also mastered in consistently less time compared to the crows with smaller brains. Contrary to my expectations, the only effect of social learning I found was the crows given the opportunity to watch conspecifics solve the task tended to change their method to copy the solution demonstrated by their knowledgeable neighbors, although my inability to detect additional examples of social learning might be due to our experimental setup inadvertently stymying the crows' ability to learn socially. Finally, I also found that absolute brain volume was a better predictor of an individual's capacity to solve novel tasks than was relative brain volume. I encourage future researchers to take a holistic approach when examining cognition, as there are a variety of factors which may play a role in an individual's ability to solve problems.

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Chapter 1 Supplementary Materials

Supplementary Table 1.8. *A comparison of the most common specific methods used by the Observer crows' neighbors during the Mastery stage, and whether the Observer crows utilized the same or different primary method as their neighbors (or failed to solve the task). If the neighbors used different methods, we checked whether the Observer used either of the two modeled methods.*

Overall	Solved task		Failed to solve task
	Same method as neighbor	Different method from neighbor	
Neighbors primarily used different methods	2	1	4
Neighbor(s) primarily drop down and grab			
Neighbor(s) primarily used fly up and grab	1	1	1
Neighbor(s) primarily used string break			1
Neighbor(s) primarily used shake free			
Neighbor(s) primarily used pulls, step, pull	2		
Neighbor(s) primarily used side pull		1	
Total	5	3	6

Supplementary Table 1.9. *A comparison of how often each specific method was used during the Mastery stage by the eight successful Observers and the Model birds they were adjacent to. Crows on the edge of the aviary only had a single neighbor, while the rest had two. Many of the Models were included more than once in this comparison, as they were adjacent to two successful Observers. Difference Index scores near to 0 indicate close alignment of method choice between the Observer and that Model, whereas scores close to 1 indicate the two birds were highly dissimilar in method choice.*

Group	Birds	# Trials Total	# drop down and grab	# fly up and grab	# string break	# shake free	# pull, step, pull	# side pull	Difference index
1	Obs 1	15			8		7		
	Mod 1	42			33		2	6	0.42
2	Obs 2	11			4		7		
	Mod 1	42			33		2	6	0.59
	Mod 2	39			1	1	33		0.34
3	Obs 3	22		8	9	1	1	2	
	Mod 2	39			1	1	33		0.90
	Mod 3	42					39	2	0.90
4	Obs 4	21		20	1				
	Mod 3	42					39	2	1.00
	Mod 4	31		29			1		0.05
5	Obs 5	9		7					
	Mod 4	31		29			1		0.03
6	Obs 6	18			5		11		
	Mod 5	38		38					1.00
	Mod 6	38	2	7	28		1		0.66
7	Obs 7	16			15	1			
	Mod 6	38	2	7	28		1		0.26
	Mod 7	38			33		2	3	0.13
8	Obs 8	18			18				
	Mod 8	37		23				13	1.00

Supplementary Table 1.10. *Individual characteristics of the three birds that took >12 days to master the task.*

Crow ID	Social role	Sex	Age	Body condition	Culmen length (mm)	Nervous-ness	Absolute brain volume (cm^3)	Relative brain volume
L-Or 2017	Observer	Female	Subadult	7.88	47	14	6.867	-0.502
R-Gr 2017	Observer	Female	Adult	-34.34	45	36	6.988	-0.1
L-Ye 2017	Model	Male	Subadult	-57.67	51	42	7.596	-0.334

CHAPTER 2: American crow brain activity in response to conspecific vocalizations changes when food is present.

ABSTRACT

Social interaction among animals can occur under many contexts, such as during foraging. Our knowledge of the regions within an avian brain associated with social interaction is limited to the regions activated by a single context or sensory modality. I used 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) to examine American crow (*Corvus brachyrhynchos*) brain activity in response to conditions associated with communal feeding. Using a paired approach, I exposed crows to either a visual stimulus (the sight of food), an audio stimulus (the sound of conspecifics vocalizing while foraging) or both audio/visual stimuli presented simultaneously and compared to their brain activity in response to a control stimulus (an empty stage). I found two regions, the nucleus taenia of the amygdala (TnA) and a medial portion of the caudal nidopallium, that showed increased activity in response to the multimodal combination of stimuli but not in response to either stimulus when presented unimodally. I also found significantly increased activity in the lateral septum and medially within the nidopallium in response to both the audio-only and the combined audio/visual stimuli. I did not find any differences in activation in response to the visual stimulus by itself. I discuss how these regions may be involved in the processing of multimodal stimuli in the context of social interaction.

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INTRODUCTION

Social animals must filter, process, and act upon a variety of information when they assemble and interact with one another; they send and receive signals across multiple sensory modalities, observe interactions between conspecifics, and evaluate the intentions of others towards themselves, all the while remaining vigilant for danger and attempting to maximize their access to any resources in the area. This cognitive demand requires a brain with a high degree of processing power (Dunbar 1998, Dunbar 2009). Most species known to possess such a brain are mammals (such as primates or cetaceans) or birds (such as corvids or parrots). Despite convergently evolving advanced cognitive capabilities, these two classes diverged approximately 300 million years ago (Burt et al. 1999), resulting in numerous structural differences between mammalian and avian brains (Shimizu 2001).

Aside from regions and systems homologous to both clades, our understanding of the inner workings of the avian brain is limited relative to mammals, although a surge of research conducted over recent decades has made considerable progress in filling this gap (Reiner et al. 2004, Jarvis et al. 2005, Wada et al. 2017, Ksepka et al. 2020). Much of this work has focused on determining the functions of, and connectivity between, individual brain regions (see Cowan et al. 1961, Karten et al. 1973, Nottebohm et al. 1976, Shanahan et al. 2013, Wild et al. 1993 as examples). Fewer studies have examined how systems within the avian brain function holistically, though our extensive understanding of the avian song control system (Brainard & Doupe 2013, Brenowitz & Beecher 2005, Konishi 1985) remains a notable exception. While scientists have uncovered numerous brain regions associated with the avian social network, such as the lateral septum, nucleus taenia of the amygdala (TnA), anterior hypothalamus, ventromedial hypothalamus, preoptic area, and potentially the dorsal arcopallium (Atoji et al. 2006, Cooper & Erickson 1976, Goodson 2005, Nishizawa et al. 2011, Ondrasek et al. 2018), how these regions interact with other areas of the brain under different social situations remains unclear. For example, counter-singing between neighboring rivals, fights over access to resources, courting a potential mate, and recruiting to food are all examples of social behavior, yet likely involve different regions/systems within the brain due to varying social contexts and sensory modalities. Scientists must also consider how the brain integrates multimodal sensory information, as animals regularly communicate social information using more than one modality (Horn 1983, Gopher et al. 1996) and usually pay more attention to multimodal signals, regardless of whether each modality is transmitting redundant or non-redundant information (Partan & Marler 1999).

American crows (*Corvus brachyrhynchos*) are songbirds noted for their intelligence and complex social dynamics; they guard territories and regularly fight among themselves (occasionally escalating to the death of one of the belligerents), yet they also cooperate to mob predators and roost communally in large numbers (Marzluff & Angell 2005). Much of this complex social interaction can be observed when crows congregate around an ephemeral food source; while they certainly spend time obtaining food, they also use these occasions as opportunities to gauge their position within the local dominance hierarchy, search for prospective mates, and learn about potential rivals (Kilham 1990, Marzluff & Angell 2005, Marzluff & Angell 2013). Crows exchange much information via vocalization; as a result, such gatherings can become quite noisy as crows communicate with one another (Pendergraft & Marzluff 2019).

To supplement insights from behavioral observation, Positron Emission Tomography combined with the radiotracer 18F-fluorodeoxyglucose (FDG-PET) can be used to better understand the brain activity of animals from various stimulus conditions. In this brain imaging modality, the FDG, a glucose analog, is injected into the body and distributes systemically. The uptake of FDG within the brain is preferential to regions of increased activity; therefore, the levels of radioactivity in a brain region (as measured by PET) act as a surrogate marker of brain activity (Jonides et al. 1993). An advantage of FDG-PET over other in vivo imaging modalities is that the subject can be awake (unanesthetized) and free from restraints or attached apparatus (which can cause stress to an unhabituated animal, confounding the results) during the stimulation period, as the circulating FDG is trapped in the tissue of the active brain region but cannot be further metabolized by glucose-6-phosphotase within the glycolytic pathway (Newberg et al. 2002). The subsequent PET imaging can be performed under anesthesia to assess the brain activity during the prior stimulation period (Marzluff et al. 2012).

This methodology has been used for various studies to identify distinct regions within the crow's brain that respond to specific stimuli (Marzluff et al. 2012, Cross et al. 2013, Swift et al. 2020). For example, wild crows respond to a variety of dangerous stimuli by giving alarm calls and mobbing the threat, yet an innate fear (a red-tailed hawk, *Buteo jamaicensis*) caused increased activity in the caudal nidopallium, whereas a learned fear (human who was previously antagonistic) activated the amygdala and a novel fear (unfamiliar human holding a dead crow) stimulated the hippocampus (Cross et al. 2013). While other methods can reveal activity at the regional/neuronal level or the connectivity between regions, such as implanting microelectrodes (Kita & Wightman 2008) or antegrade/retrograde degeneration (McGeer & McGeer 1980) respectively, PET imaging allows one to examine brain activity holistically (albeit indirectly via FDG uptake) and make inferences about regional connectivity based on the active regions.

Here, I conducted a 18F-fluorodeoxyglucose PET imaging study with the objective of determining how the American crow brain holistically functions in response to different sensory modalities associated with communal feeding events, with the secondary objective of understanding how the avian brain processes multimodal sensory information. I compared the baseline brain activity (as measured by the relative FDG activity) in wild crows during a control condition (viewing an empty stage) to their brain activity when hearing conspecifics foraging, seeing a preferred food item, or both hearing foraging and seeing food. I selected four regions *apriori* that I hypothesized would be activated in response to specific conditions. Because vocalizations encode social information, I expected the audio stimulus to cause an increase in activity in regions of the brain associated with social interaction, such as the 1) amygdala (specifically TnA) or the 2) lateral septum; multiple studies point to these regions as being involved in the vertebrate social network (Cooper & Erickson 1976, Goodson 2005, Nishizawa et al. 2011). If the calls encode information about food (such as presence, quantity, or quality), I hypothesized that the 3) hypothalamus, which is involved with motivation and food regulation (Wright 1968, Kuenzel 1994, Kuenzel et al. 1999, Primeaux et al. 2013), would also increase in activity in addition to the regions associated with social interaction; for the same reasons, I predicted this region would also increase in activity in response to the sight of a preferred food item. Finally, because the 4) thalamus filters, organizes, and relays information gathered by the senses to other brain regions (Bentivoglio et al. 1993), I expected to see increased activity here in response to the combined multimodal stimuli (sound of conspecific vocalizations and sight of food item), as this region will be processing additional information.

MATERIALS AND METHODS

Capturing and Housing Crows

I captured wild American crows near Woodinville, WA, USA, as they departed a large communal roost. I lured birds from flocks with bread, trapped them using a net launcher, and preferentially selected individuals that were likely adult males (determined by plumage color and wear, mouth color, and overall size, Emlen 1936). I caught two groups of crows outside of the breeding season and held them for several months each (9 crows from October-December 2015 and 8 crows from January-March 2016) in a protected outdoor aviary at the University of Washington, Seattle. The crows were individually housed in adjacent cages (measuring 1.8 x 2.1 x 2.4 m) separated by wire mesh. I provided crows with a rotating diet of assorted meats, eggs, grain, fruit, and dried dog food *ad libitum*. After identifying the crows' most preferred food item (half of a fried chicken patty) by observing which food item was preferentially consumed first, I began wrapping it in plastic food film prior to giving it to them to match the food presentation during the imaging process. Crows easily removed the plastic film prior to consuming the chicken, and they habituated to receiving their favorite food item in this presentation.

Imaging the crows

I imaged up to three crows per day, using a Siemens Inveon PET/CT system. The scanning process consisted of a 20 min microPET scan, followed by a CT scan in the docked and coregistered microCT scanner. The scanners share a multimodality bed and have a bore diameter of ~12 cm. The PET field of view was approximately 8 x 13 cm^2 while the CT field of view was 7.9 cm x 13.3 cm; both included the entire brain with a slice thickness of approximately 0.1 mm. The scanner bed contained a pressure pad, which I used to monitor the crow's breathing (and thus depth of anesthesia) during the scan process.

The evening before a bird was scanned (typically 1600-1700), I removed it from its aviary cage, placed it in a sock to keep it docile, and carried it across campus to the imaging laboratory, where I placed it in a small wire cage (1 x 0.5 x 0.5 m, hereafter imaging cage) within a fume hood to acclimate overnight. The imaging cage contained water but not food, ensuring crows fasted for at least 14 hours prior to imaging to control for variable blood glucose levels influencing FDG uptake. I covered the cage with a blanket to keep the bird calm; it could hear the ambient noise of the equipment but could not see into the imaging lab.

On the morning of the experiment, I removed the acclimated crow from its cage, covered the bird's head with a cloth to calm it, and gave it an interperitoneal injection of approximately 1 mCi of [^{18}F] Flourodeoxyglucose (FDG) (exact volume adjusted to account for radioactive decay and the bird's weight, ranging from 0.05 to 0.10 mL). After injection, I returned the crow to the covered imaging cage. During the next three minutes, I positioned the stimulus stage (see *Experimental stimuli* below) in front of the covered cage and removed the blanket covering the wire cage- the crow remained in relative darkness because the fume hood blocked the view to the lab while the stimulus stage's closed sliding panels prevented the crow from seeing the illuminated stage interior (Fig. 2.1). Three minutes post-injection, I opened the sliding panels to reveal the stimulus (see *Experimental stimuli* below). For the following 10 min (hereafter referred to as the "stimulus phase", see Table 2.1 in supplementary materials), I used the sliding doors to alternatively reveal the stimulus to the crow for 60 s, then hide it for 30 s (seven exposures and six associated breaks total). After the stimulus phase ended at 13 min post-injection, I again removed the crow from the cage, covered its head with a cloth, and anesthetized it via a custom nose cone with 5% isoflurane in oxygen with a flow rate of 300-800 mL/min before placing it in the scanner (I reduced isoflurane concentration to 2.5-3% after the crow was fully induced). I used Velcro straps to secure the anesthetized crow to the scanner bed before starting the imaging process 26 mins post-injection. After the scan was complete, I secured the crow in hand until it fully recovered from anesthesia (indicated when it regained the ability to grip with both feet), before returning it to the cage. I kept the crows in the imaging lab for 20 hours (the time required for ^{18}F radioactivity to decay to acceptable levels), after which I returned them to the aviary.

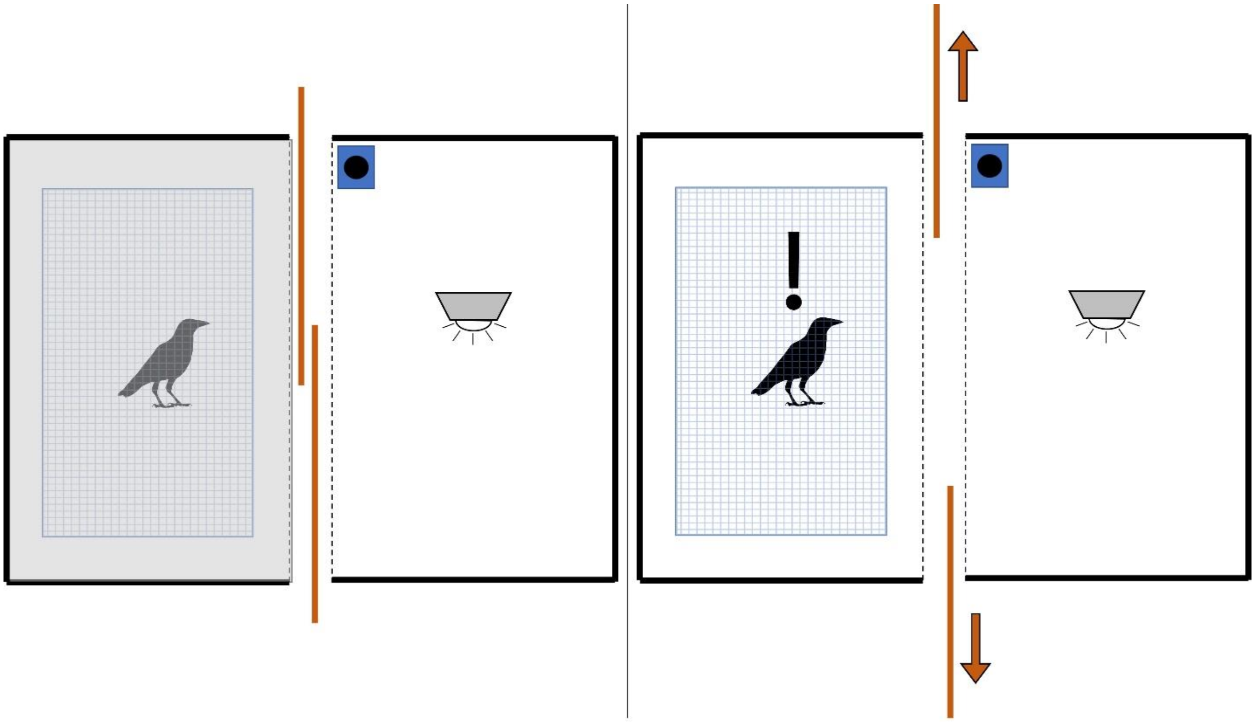

Figure 2.1. *Graphic of experimental layout during stimulus phase (top-down view). The fume hood surrounding the crow's cage blocked all view of the surrounding laboratory. When I closed the sliding panels between the crow's cage and the stimulus stage (left), the crow was plunged into relative darkness. I revealed the interior of the well-lit stage (and any stimulus within it) by opening the sliding panels (right).*

I imaged all crows twice- the first scan for all birds was the control while the second scan introduced one of three different stimuli (see *2.3 Experimental stimuli* below). This ordering was to prevent carryover of the crow's previous experience biasing the control scan (Swift et al. 2020). I waited at least one week between scans for most crows, although one crow received its second scan four days after the first due to logistical constraints.

Experimental stimuli

As part of the imaging process (see *Imaging the crows* above), I presented all experimental stimuli within a (1.2 x 0.6 x 0.45 m) wooden stage designed to block the crow's view of the imaging laboratory/personnel (thereby removing potential confounding sources of distraction) and to standardize the background color, light intensity, and light angle between trials (Fig. 2.1). The stimulus stage always contained an LED light on the ceiling and a Bem wireless HL2022A speaker placed far enough to the side to be considered out-of-view for the experimental crow. The front of the stimulus stage had two overlapping sliding panels, which I used to reveal or hide the stage interior- in addition to blocking the crow's view of the stimulus, the panels also blocked nearly all the light from the internal LED, increasing the visual contrast between showing and hiding the stimulus. The panels opened from the center of the crow's view of the stage so that the crow's eyes received equal stimulation. I used this configuration as the control stimulus (n=13).

During the crows' second scan, I introduced either a visual stimulus (n=4), auditory stimulus (n=5), or combination of audio/visual stimulus (n=4) to the stimulus stage interior. For the visual food-associated stimulus, I placed a fried chicken patty directly underneath the LED light in the center of the stage. I wrapped the food in plastic food film to reduce its scent profile, which matched how the crows received this item in the aviary. For the auditory food-associated stimulus, I used the internal wireless speaker to play a 60 s recording (see Fig. 2.6 in supplemental materials for a sample audio stimulus spectrogram) of roughly 30-40 crows vocalizing as they foraged around a food source (a pile of bread), which was synchronized to begin when the stimulus stage interior was revealed (see *Imaging the crows* above and Table 2.1 in supplementary materials) and end when it was hidden. I recorded 22 minutes of crows vocalizing at the capture site (one day prior to capture) in WAV format using a Marantz PMD-671 solid-state recorder and a Sennheiser MKH 20-P48 microphone contained within a Telinga Universal Parabolic Dish MK2 housing. From the 22-minute master track, I selected ten 60 s duration intervals which were relatively free of other noises, such as passing cars. I controlled stimulus amplitude by normalizing the peak amplitude using Audacity (Audacity Team 2015) and keeping the source and speaker volume consistent between trials (mean= 73 dB, SD= 3 dB). I reduced other potential acoustic confounding factors by excluding recordings with crow alarm vocalizations and randomly assigning a unique exemplar to each crow that received either an auditory or combined stimulus. For the combined A/V stimuli, I simultaneously showed the wrapped fried chicken while playing the assigned audio file of conspecifics vocalizing during each reveal of the stimulus stage interior.

Behavior during Imaging

To better gauge their level of attention towards the stimulus stage and to control for factors which may influence FDG uptake within the brain (Marzluff et al. 2012, Cross et al. 2013), I used a GoPro Hero 4 camera to record (30 fps) the gaze time, blink rate, and amount of movement of each crow during the stimulus phase of the imaging process.

Avian brains are highly lateralized (Rogers & Ansem 1979), so I measured the gaze time from each eye to verify that any observed differences in hemispherical activity were not due to the bird preferentially using one eye to view the stimulus over the other (Mench & Andrew 1986). I tracked each eye's gaze time independently from the other eye e.g., I added gaze time to each eye if the bird binocularly gazed directly into the stage. I also used gaze time to measure a crow's level of interest in the stimulus being presented, and thus only recorded gaze when the stimulus stage's interior was revealed and visible to the crow (see *2.2 Imaging the crows* and Table 2.1 in supplemental material).

I measured blink rate to verify that the crows were not threatened by any of the presented stimuli or prior experience in the scanning apparatus, as previous studies have established a relationship between blink rate and the crow's perceived sense of danger; specifically, blink rate is negatively correlated with activity in fear-associated brain regions, and crows decrease blink rate when faced with a threatening stimulus compared to while foraging (Marzluff et al. 2012, Cross et al. 2013). Although the image resolution was sufficient to see the white flash of the crow's nictating membrane, the birds sometimes turned their heads such that their eyes were no longer visible, so I calculated an observed blink rate by dividing the number of observed blinks by the amount of time the eye was visible. The cage interior became too dark to observe blinks when the panels to the stimulus stage were closed, so I only calculated blink rate when the stimulus stage was visible during the seven reveals of the stimulus phase.

I measured the crows' movement because physiological activity can confound the amount and location of FDG uptake within the brain (Bhargava et al. 2011). I quantified the following actions as 1 unit of movement: crow moved 5-50 cm laterally along the perch (did not count if it moved <5cm, counted as 2 units if moved >50cm), crow rotated its body 180^o to face the opposite direction, and crow hopping from the perch to the cage floor (or vice-versa). Because I measured movement to account for possible confounds to FDG uptake activity, I counted movement throughout the entire 10 minutes of the stimulus phase, including when the stimulus stage interior was hidden from the crow's view.

Image Processing

After each crow was imaged, I conducted a 13 min attenuation scan, then reconstructed the image using the vendor-supplied 3D OSEM/MAP algorithm to an isotropic spatial resolution of 2.5 mm full width at half maximum, with attenuation and scatter corrections applied to the data. The image matrix was 128 x 128 x 159. I exported reconstructed images using DICOM for the statistical parametric analysis software.

I imported the raw DICOM data to ImageJ (Schneider et al. 2012), manually aligned their orientation to match the jungle crow (*Corvus macrorhynchos*) brain atlas established by Izawa & Watanabe (2007) and adapted for PET by Marzluff et al. (2012), and trimmed the images to include only the brain. I stereotactically aligned the scans by estimating and applying nine affline parameters to the images using algorithms originally designed for automated human brain analysis (NEUROSTAT, University of Utah; Minoshima et al. 1992), which have been adapted for crow brains analysis. I estimated alignment precision to be one-two pixels. Finally, I normalized all uptake values to a global brain FDG uptake.

Although I used Izawa and Watanabe's (2007) atlas as a guide to identify the regions significantly activated by each stimulus, I did not use it as the sole determinant. This was because jungle crows are larger than American crows (Jungle crow mean male weight: 680 g, American crow: 450 g, Kitagawa 1990, Kilham 1990). More importantly, the atlas was based on a sectioned brain, whereas our activation foci were based on in vivo imaging. Sectioned brains tend to "flatten" (reduced Y-axis length, increased X- and Z- axis lengths) after being extracted from the skull and are vulnerable to other artifacts which can further alter the original shape (Rolls et al. 2008). Therefore, I scaled Izawa and Watanabe's (2007) atlas for use with American crow brains and used it in conjunction with the shape and extent of the total activation (not just the focal coordinates) to determine the activated regions.

Statistical Analyses

Due to the small sample size, I calculated differences between the first and second scan's stimulus phase behaviors (blink rate, gaze, and movement) using a paired samples t-test, correlation between FDG uptake and blink rate/movement using a Pearson correlation test, and differences between the different stimuli of the 2nd scan using a linear model, all in RStudio version 1.0.136 (RStudio Team 2016). I determined significant differences in regional activity within the brain using an automated voxel-wise subtraction and Z-statistic mapping algorithm originally designed for automated human paired-brain analysis (NEUROSTAT, University of Utah; Minoshima et al. 1992). This algorithm conducts a paired Z-test comparing the study population's total difference in FDG signal strength between each individual subject's first (control) and second (stimulus) scans against the study population's pooled variance; it does this for each voxel coordinate throughout the entire brain. Due to the large number of comparisons made, the algorithm calculated a Z-threshold for statistical significance using a modified Bonferroni correction commonly utilized in imaging research (Friston et al. 1991). Because this threshold is conservative (Cross et al. 2013), I also report any regions with a Z-score more than 3.0, as these regions may be worth examining in greater detail in future studies. I verified the voxel-wise results by obtaining spherical volumes of interest (VOIs; 2-voxel radius) centered around the significant coordinates to determine if said results are driven by outlying individual scans.

Ethical Note

I captured, housed, and tested all crows (including PET/CT scans) in accordance with the Institutional Animal Care and Use Committee of the University of Washington (IACUC; protocol number 3077-01), Federal Collecting Permit MB761139-0, and State of Washington Scientific Collection Permit 14-010. I released all crows back into the wild at the location where they were captured at the conclusion of the study.

RESULTS

I was only able to obtain usable imaging data from 13 of the 17 captured crows; the other four individuals had one of their scans invalidated by mechanical/software issues with the imaging system. Additionally, I was unable to obtain blink rate data from one bird's 2nd scan (audio stimulus) due to it positioning itself with its eye remaining out of the camera's field of view throughout the stimulus phase.

Differential Brain Activity

Crows that were exposed to the unimodal visual stimulus showed no notable increases in brain activity relative to their initial baseline scan, even at the coordinates of peak differential activity for the combined stimulus (Fig. 2.7 in supplementary material). See Fig. 2.8 (supplementary material) for differential activity patterns throughout the entire brain in response to the visual stimulus.

By contrast, crows that were exposed to the unimodal audio stimulus showed significantly increased activity in the medial portion of the nidopallium in their left hemisphere compared to their baseline control scan (10.6% increase, $Z = 4.57$, $P < 0.001$, Fig. 2.2 and Fig. 2.3); this region includes or is adjacent to Field L and possibly the lateral septum. This activity extends along the anterior-posterior axis with the most visible at A11.8. While no other regions' increase in activity in response to the audio stimulus exceeded the critical Z-threshold ($Z = 4.08$), there was a notable increase in FDG uptake within a part of the medial striatum ventral to the highly activated medial nidopallium, particularly in the right hemisphere (10.2% increase, $Z = 3.58$, $P < 0.001$, at A11.0). I observed another low threshold (but notable) activity increase in the caudal nidopallium ventral to the HVC region in the left hemisphere (7.5% increase, $Z = 3.33$, $P < 0.001$, at A9.4). See Fig. 2.9 (supplementary material) for differential activity patterns throughout the entire brain in response to the audio stimulus.

Simultaneous presentation of preferred food item and food-associated vocalizations induced higher FDG-uptake activity in those areas responding to audio stimuli in the left hemisphere (Fig. 2.4 and 2.5), including the medial nidopallium (11.6% increase, $Z = 4.23$, $P < 0.001$, at A11.4), lateral septum (11.8% increase, $Z = 4.00$, $P < 0.001$ at A12.6), and the caudal nidopallium (13.1% increase, $Z = 3.66$, $P < 0.001$, at A5.8) though the latter two were low threshold increases (Z-threshold = 4.14). I also observed significant increased activity in the TnA of the right hemisphere (14.7% increase, $Z = 4.27$, $P < 0.001$, at A6.6). See Fig. 2.10 (supplementary material) for differential activity patterns throughout the entire brain in response to the combined A/V stimulus.

Stimulus phase behavior

The crows visually attended to the stimulus stage whenever it was revealed, regardless of what was inside; they stared into the stage for a majority of the time (right eye: mean ± SD; 373.0 ± 48.0 s; left eye: 357.3 ± 87.3 s) that it was visible (420 s), with no significant difference in gaze time between their two scans (left eye: $t_{26} = 1.51$, $P = 0.14$; right eye: $t_{26} = 0.56$, $P = 0.58$) nor between any of the stimuli during their second scan (left eye: $F_{2,11} = 1.35$, $P = 0.30$; right eye: $F_{2,11} = 0.31$, $P = 0.83$). Their mean blink rate remained steady (30.2 ± 8.3 blinks/min), with no significant change between the 1st and 2nd scans ($t_{11} = 0.83$, $P = 0.43$) nor any of the different stimuli presented during the 2nd scan ($F_{2,10} = 0.07$, $P = 0.93$). Most crows moved little during the stimulus phase, though there were several outlier individuals with a high degree of movement

(3.4 ± 7.6 total movement, max= 39); there was no change in movement between the two scans (t_{12}= 1.23, P= 0.24) nor any of the different stimuli during the 2nd scan ($F_{2,11}$= 2.12, P= 0.17). See Fig. 2.11 in supplementary materials for details on behavioral changes between scans and stimuli. I did not observe any significant correlation between blink rate or movement with the rate of FDG uptake (see Fig. 2.12 and 2.13 in supplementary material for details).

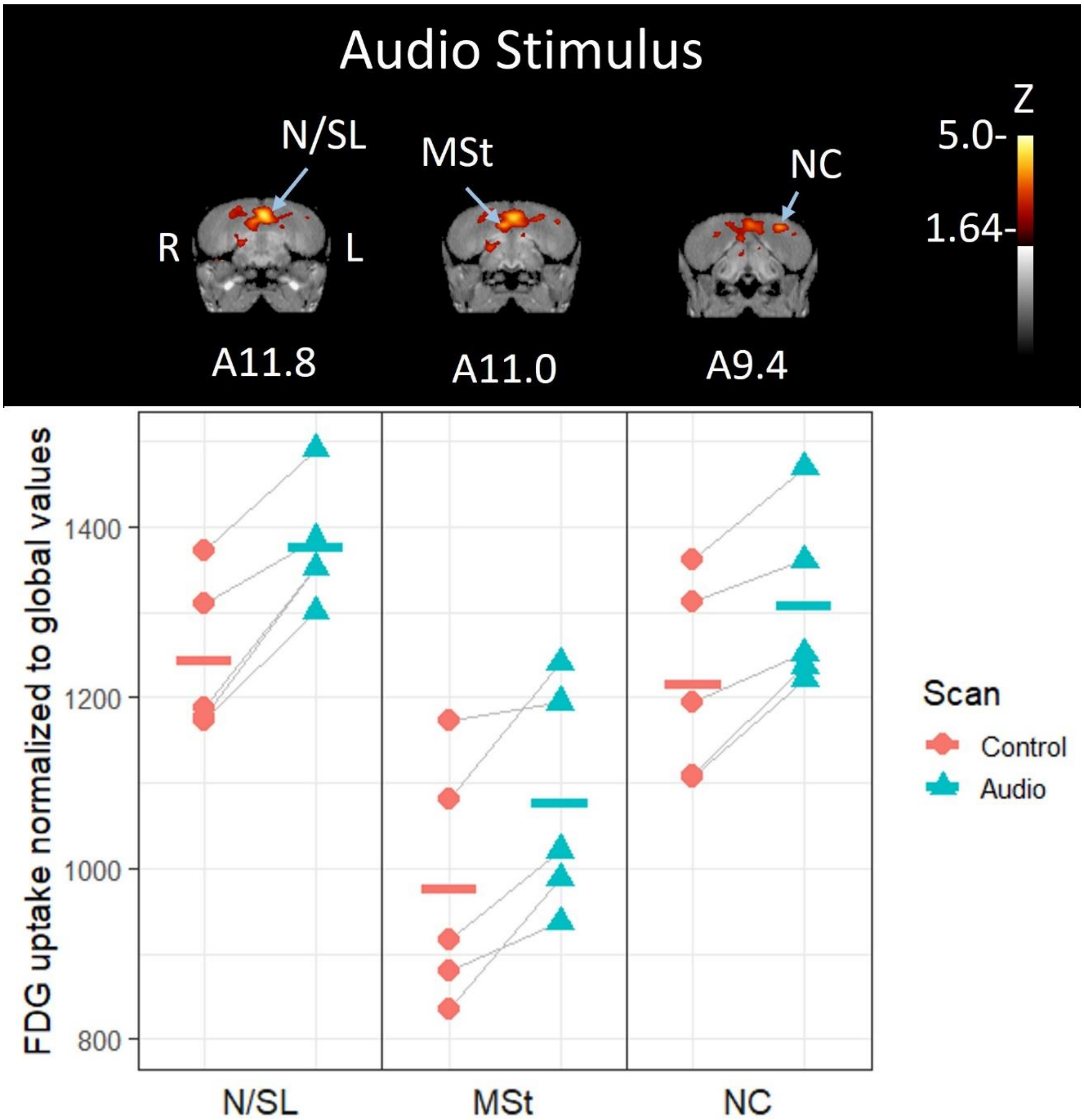

Figure 2.2. ***Top****: Coronal view of voxel-wise subtractions (converted to Z-scores) showing differential brain activity at the indicated region for all crows exposed to the vocalizations of feeding crows during their stimulus scan (n=5). Brain activity is superimposed atop a composite (n=4) structural MRI of the American crow brain. Slice coordinates (A11.8, A11.0, and A9.4) refer to the Izawa & Watanabe (2007) jungle crow atlas.* **Bottom:** *individual normalized (global) uptake values obtained from VOI's centered on peak activation coordinates. Horizontal lines indicate group means. Note that only the nidopallium/lateral septum border (N/SL) showed significant increases in brain activity; the medial striatum (MSt) and caudal nidopallium (NC) did not meet the critical Z-threshold.*

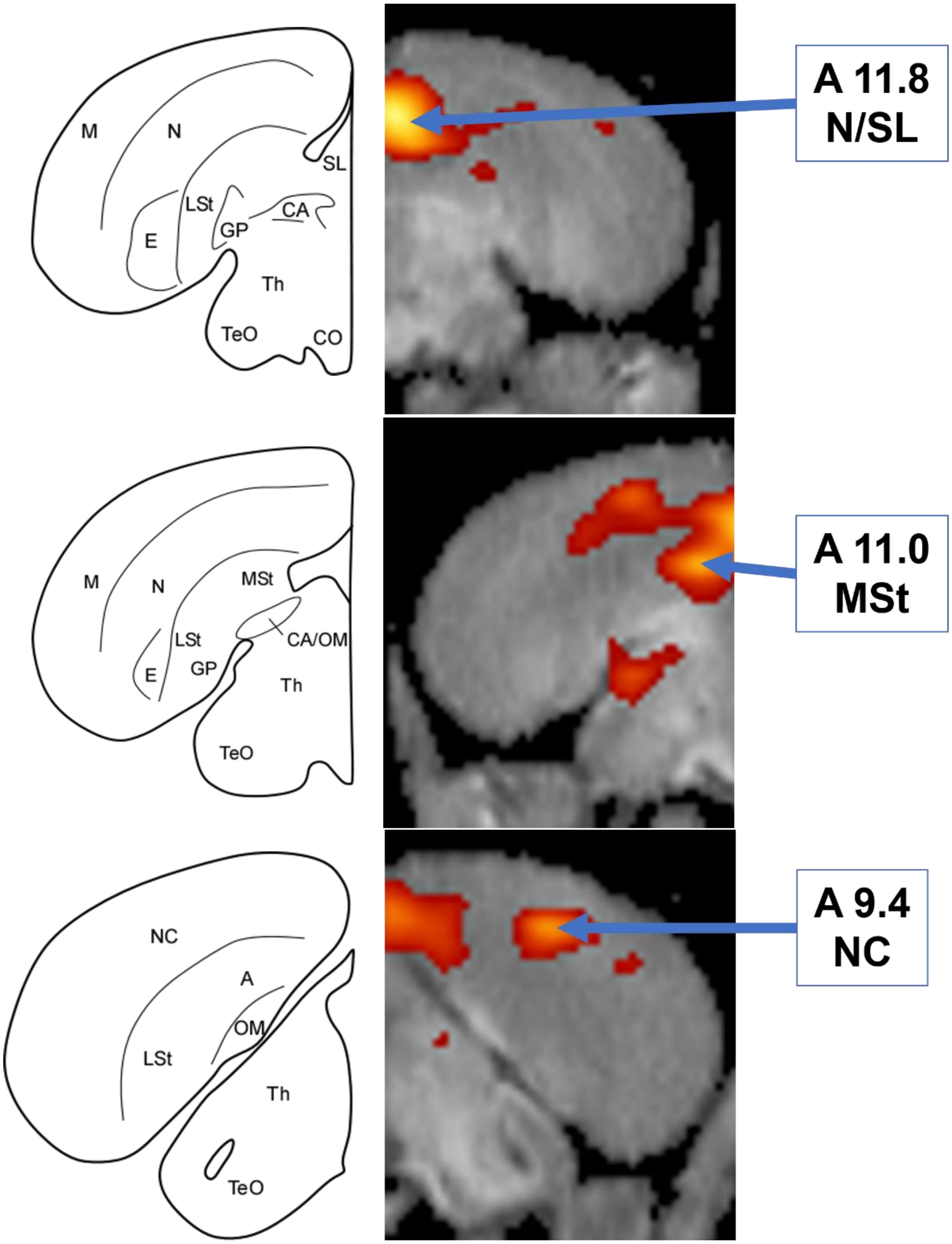

Figure 2.3. ***Left:*** *Schematic coronal hemisections of the American crow brain, drawn based on structural MRI of the American crow brain and Izawa & Watanabe jungle crow atlas. A: Arcopallium, CA: Anterior Commissure, CO: Optic Chiasm, E: Entopallium, GP: Globus Pallidus, LSt: Lateral Striatum, M: Mesopallium, MSt: Medial Striatum, N: Nidopallium, NC: Caudal Nidopallium, OM: Occipito-mesencephalic Tract, SL: Lateral Septum, TeO: Optic Tectum; Th: Thalamus.* ***Right:*** *Coronal view of voxel-wise subtractions (converted to Z-scores) showing differential brain activity at the indicated region for all crows exposed to the audio stimulus (n=5). Brain activity is superimposed atop a composite (n=4) structural MRI of the American crow brain.*

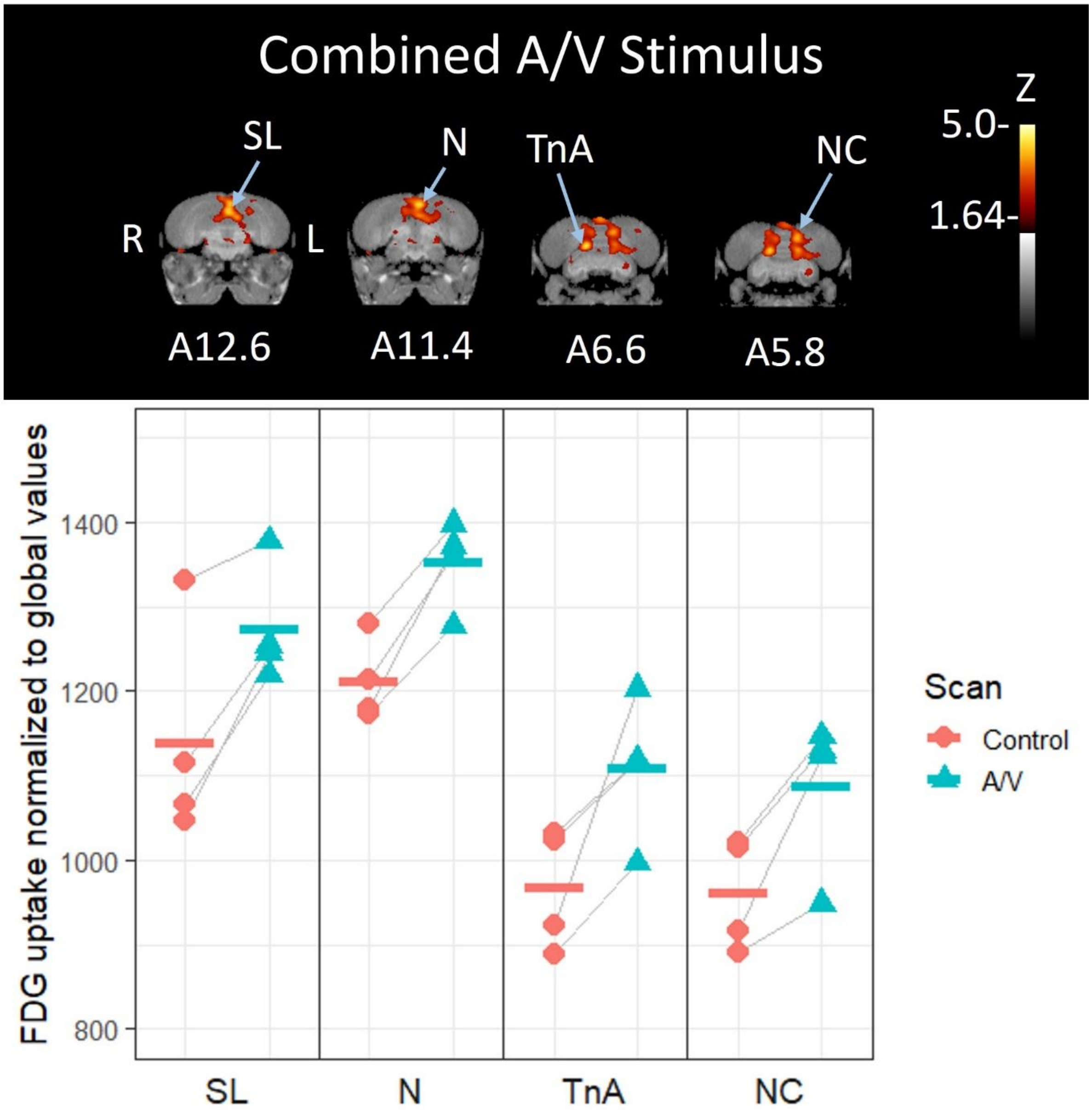

Figure 2.4. ***Top:*** *Coronal view of voxel-wise subtractions (converted to Z-scores) showing differential brain activity at the indicated region for all crows simultaneously exposed to the vocalizations of feeding crows and the sight of their preferred food item during their stimulus scan (n=4). Brain activity is superimposed atop a composite (n=4) structural MRI of the American crow brain. Slice coordinates (A12.6, A11.4, A6.6, and A5.8) refer to the Izawa & Watanabe (2007) jungle crow atlas.* ***Bottom:*** *individual values for normalized (global) uptake obtained from VOI's centered on peak activation coordinates. Horizontal lines indicate group means. Note that while the nucleus taeniae of the amygdala (TnA), and nidopallium (N) showed significant increases in FDG uptake, the lateral septum (SL) and caudal nidopallium (NC) did not meet the critical Z-threshold.*

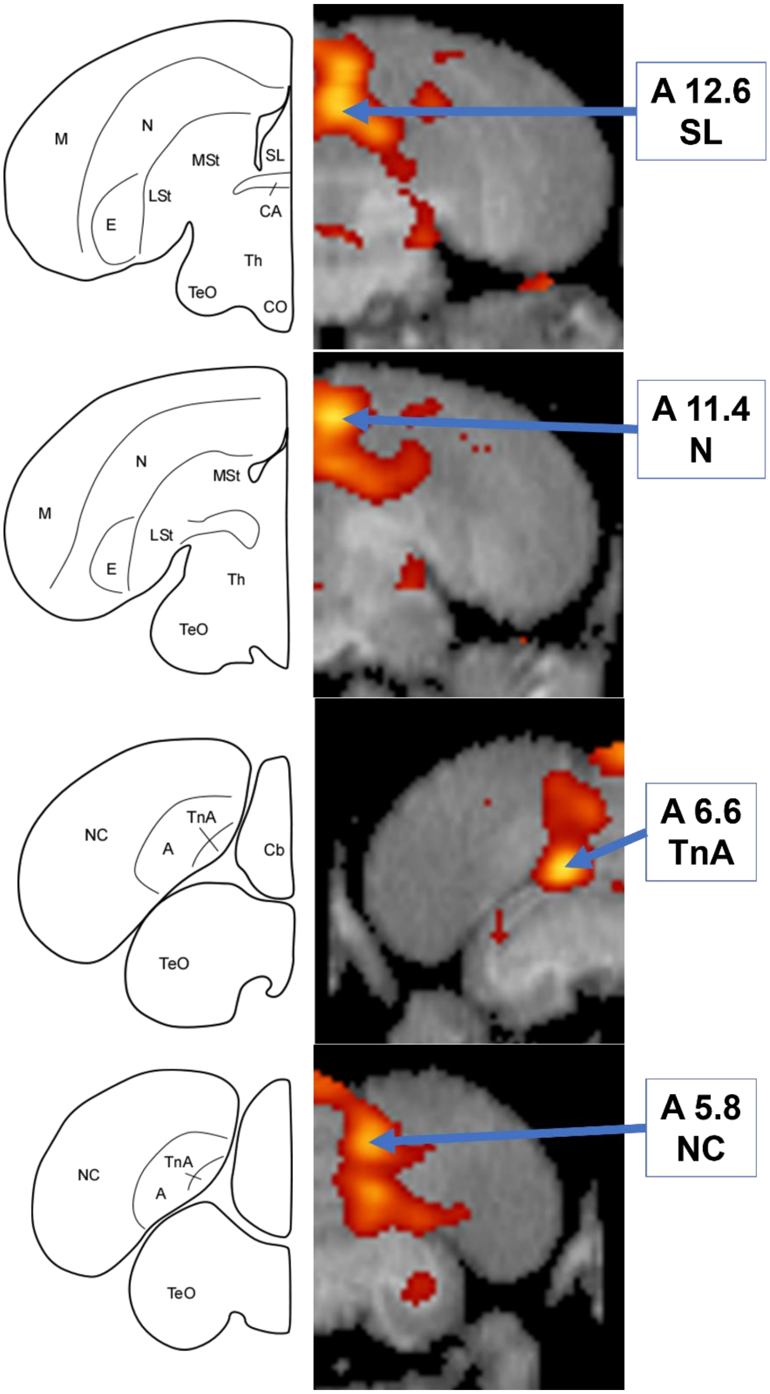

Figure 2.5. ***Left:*** *Schematic coronal hemisections of the American crow brain, drawn based on structural MRI and Izawa & Watanabe jungle crow atlas. Cb: Cerebellum, TnA: nucleus taeniae of the amygdala. For other abbreviations, see Fig.2.3.* ***Right:*** *Coronal view of voxel-wise subtractions (converted to Z-scores) showing differential brain activity at the indicated region for all crows simultaneously exposed to the audio and visual stimuli (n=4). Brain activity is superimposed atop a composite (n=4) structural MRI of the American crow brain.*

DISCUSSION

When social animals gather around a food source, they must divide their attention between the activity of their fellow conspecifics and the food itself; the mental demand required to successfully navigate these situations likely necessitates increased neural activity in multiple regions and systems throughout the brain. Adult American crows have ample experience navigating the social milieu around a communal food source thus their relevant neural circuitry should be well-developed compared to other songbirds.

Combined A/V & emergent activity

The combined A/V stimulus mimicked the conditions of a social feeding event; focal crows heard conspecifics vocalizing while simultaneously visually observing a preferred food item. These conditions triggered increased activity in two regions (the TnA and caudomedial nidopallium) that were not active in response to either unimodal stimulus, and further defined the activity of two regions (the lateral septum and medial nidopallium) that were active for the unimodal audio stimulus.

The largest increase in activity occurred in the TnA, a region associated with processing social information (Cheng et al. 1999, Mayer et al. 2019) and one of the regions I hypothesized would be active in response to hearing conspecific vocalizations. This suggests that the TnA is involved in integrating sensory information with social stimuli, which supports existing evidence linking the TnA with controlling social foraging behavior (Cheng et al. 1999, although see Xin et al. 2017). As the amygdala is also associated with processing a learned fear response (Cross et al. 2013), this result could alternatively be explained as the crows being especially frightened by some confounding factor associated with the combined A/V stimulus that was absent for the other stimuli. However, frightening stimuli usually decreases the blink rate of observing crows (Marzluff et al. 2012, Cross et al. 2013), which was not strong in our experiments (I observed only a slight reduction FDG uptake in the TnA; see Fig. 2.12 in supplementary material). Finally, neither of the unimodal conditions prompted increased TnA activity. I therefore conclude that the TnA activity during the multimodal stimuli was not caused by a strong fear response.

The other region notably stimulated by the combined A/V stimulus, but not by either unimodal stimulus, was located medially within the caudal nidopallium. The caudomedial nidopallium is involved in avian auditory processing (Atoji & Wild 2009, Moorman et al. 2012), and recent evidence suggests it may be a passerine-specific enlargement of the avian prefrontal area and involved with multimodal processing (von Eugen et al. 2020), which our results support.

The addition of the visual stimulus slightly enhanced the activity of the medial nidopallium and lateral septum; I observed their activity as a merged entity for the audio-only stimulus and separately as two distinct entities for the combined A/V stimulus. As in mammals, the avian lateral septum is a part of descending connections from the hippocampus to the brainstem limbic centers and is involved in regulating aggression (Cooper & Erickson 1976, Goodson et al. 1998, Goodson 2005). This is consistent with the fact that fights and status displays were common among crows jockeying for access to food when I recorded the stimulus audio, so there is a high likelihood that the audio stimuli contained aggressive vocalizations. The other region stimulated by both audio-associated conditions, the medial nidopallium, has been implicated with A/V stimulus processing in imprinting learning (Bredenkötter & Braun 1997, Wallhäusser & Scheich 1987), though those studies focused on precocial chicks of a species belonging to a different taxonomic order. Further studies are needed to determine whether the medial nidopallium in different avian species are equivalent in function.

While I observed activity in two *apriori* hypothesized regions associated with social behavior (the TnA and lateral septum), I did not observe any activity in the thalamus, the region I predicted would be active during the multimodal stimulus. The thalamus is involved with filtering and relaying sensory information to other brain regions (Bentivoglio et al. 1993); it's possible that there was not enough contrast in sensory information presented between the control and stimulus scans. Although the audio stimulus increased the amplitude of the crow's sensory environment, I kept the amount of light, non-stimulus ambient sound, and temperature constant for all scans, potentially masking any effect the added stimulus sounds might have had on thalamus activity.

Response to unimodal audio stimulus

In contrast to the previous regions, the medial striatum was only notably stimulated by the unimodal audio stimulus; this activity was not present when food visually accompanied the vocalizations. The avian medial striatum, extending in the antero-posterior axis, corresponds to the mammalian ventral striatum. In birds, the anterior portion is involved in sensory processing (e.g., area X in songbirds), though the activated area observed in the present study is in the limbic posterior region including the avian nucleus accumbens (Husband & Shimizu, 2011).

The other region notably activated by the audio-only condition is located centrally between the nidopallium and caudal nidopallium, ventral to the HVC; this area is most likely a lateral area of Field L. Like the caudomedial nidopallium, Field L is heavily involved in songbird auditory processing and filtering conspecific vocal signals from other sounds (Grace et al. 2003, Nagel et al. 2011, Wild et al. 1993, Zaretsky 1978). Despite its central role in the avian auditory network, Field L was not stimulated when the listening crows could also see food, which further emphasizes the importance of context in songbird auditory processing of a vocal signal.

Lateralization of activity

Avian brains are highly lateralized (Mench & Andrew 1986), and I observed some bias in hemispherical activity in all observed regions. Most of the strongest observed activity occurred in the left hemisphere, though the TnA and medial striatum were most active in the right hemisphere. As there was no significant difference in gaze direction (see Fig. 2.11 in supplementary material), this asymmetry cannot be explained because of directional sensory bias. Previous studies have found that the caudomedial nidopallium tends to be more active in the left hemisphere for passerine songbirds when processing audio information (Moorman et al. 2012, Ocklenburg et al. 2013), which is consistent with our findings.

Lack of response to unimodal visual stimulus – why more activity for vocalizations?

In contrast to the multiple active regions that I observed in response to conspecific vocalizations, I did not observe any areas of increased activity (*apriori* hypothesized hypothalamus or otherwise) in response to the unimodal visual stimulus of a preferred food item. This lack of activity was not the result of a single outlier individual's contrary activity masking a majority trend (Fig 2.7 in supplementary material). While it's possible that some unknown factor was occurring to reduce food-associated brain activity (such as imaging-associated stress inhibiting appetite) or the metabolic uptake of the radiotracer (such as increasing blood flow to the GI tract in anticipation of a meal), I believe this is unlikely for the simple reason that the food caused observable changes in brain activity when added to the auditory-associated stimuli.

Why did the food, something necessary for the crow's survival, evoke less activity throughout the brain than the vocalizations? I posit that the food was less interesting to the crows and required less cognitive power to neuronally process than the calls. The food did not add light to the crows' sensory environment (only a negligible increase in visual signals sent to the brain), remained static (less cognitive processing required), and, because they were fed after their previous control scan, was something they had already experienced in the context of the imaging lab (less stimulating). By contrast, the calls added sound to the crows' sensory environment (large increase in auditory signals sent to the brain), were dynamic (more cognitive processing required), and were likely much more surprising to hear within the setting of the imaging lab (more stimulating). When a more interesting stimulus (the vocalizations) was present to maintain the crows' attention, this may have had the secondary effect of encouraging the crows to pay more attention by proxy to the food than they would otherwise.

The social aspect of the vocalizations is another factor to consider. Although I recorded the stimulus vocalizations from crows as they gathered around a food source, crows in such contexts usually do not limit their communication to food-associated information; for example, they also vocalize to announce their presence, assert dominance, recruit allies, etc. (Pendergraft & Marzluff 2019). In addition to the overt signals, their vocalizations also contained characteristics that can be used to identify the caller's sex and identity (Mates et al. 2015). The amount of social information contained within the vocalizations, combined with the information being conveyed indirectly (they contained information *about* things, whereas the food *was* food) is a likely reason why the calls elicited significant increases in neural activity throughout the brain, yet the food did not.

The amount of neuronal processing required to extract all the social information from a vocal signal is supported by behavioral observations, as most birds closely attend to conspecific vocalizations (Dooling & Prior 2017). Pinyon jays (*Gymnorhinus cyanocephalus*), another Corvidae species, pay most attention to conspecific vocalizations when they recognize the caller as belonging to their own flock (Marzluff & Balda 2010), which may be applicable here: there's evidence that American crows can use acoustic properties of the vocalization to identify the caller (Mates et al. 2015) and I observed increased activity in the caudomedial nidopallium- a region associated with vocal recognition of known individuals (Bolhuis & Gahr 2006, Chew et al. 1996). As I had pre-baited the capture site each day of the week preceding their capture and many of the crows had fallen into the habit of visiting the location each morning, it's possible that the experimental crows may have recognized some of the callers from the stimulus tracks, which would further motivate them to attend the audio stimulus.

No confounding effects of repeated testing

In a prior study where the presentation of four stimuli to crows was balanced, researchers observed that after crows experienced a potentially dangerous stimulus in one trial, their subsequent responses were biased toward fearful reactions (Swift et al. 2020). I did not observe such carryover effects in the present study; neither blink rate nor movement varied from the first to the second scan. This is likely due to the benign, non-threatening stimulus presented during the control scan as well as the limited number of presentations (two) each bird received.

Conclusion

Taken as a whole, the six distinct regions activated by the sound of conspecifics vocalizing at a food source are associated with either processing auditory sensory data or social information. This would suggest that all the identified regions are either involved in a larger socio-auditory processing system or, more likely, are components of two or more brain systems that are triggered when the bird needs to process additional modalities or contextual information. Although multiple studies have examined avian brain activity in response to social interactions, this study is the first to use functional imaging to holistically measure activity in response to social signals under the context of communal foraging, one of many social contexts that birds regularly encounter in nature. The visual presence of food, despite not causing any notable changes in activity on its own, significantly alters the neural activity triggered by the sound of conspecifics vocalizing and even stimulates activity in regions not activated by either modality alone. This demonstrates that the context associated with a stimulus matters to the neuronal processing of that information, especially for something as varied as social interaction.

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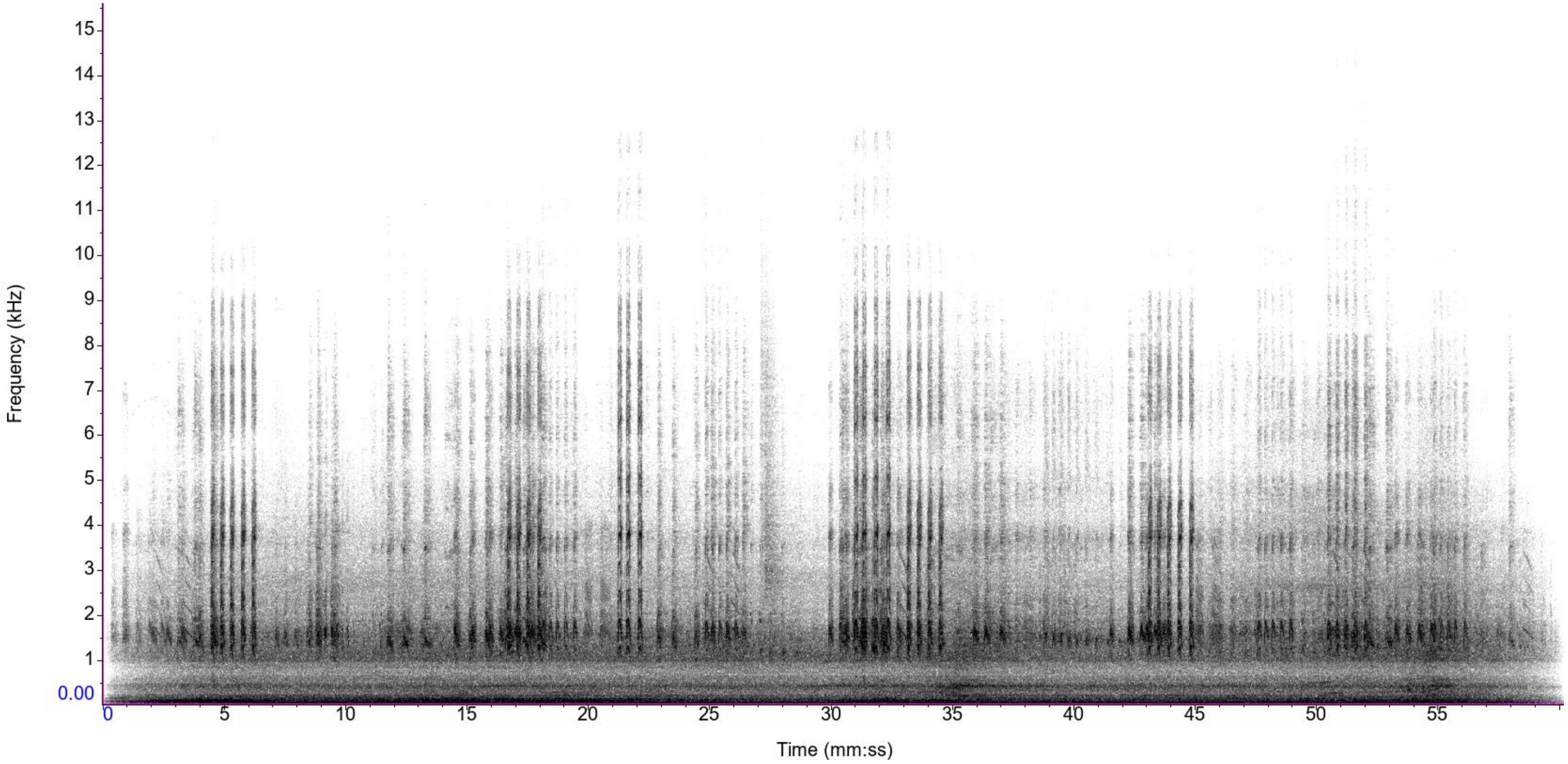
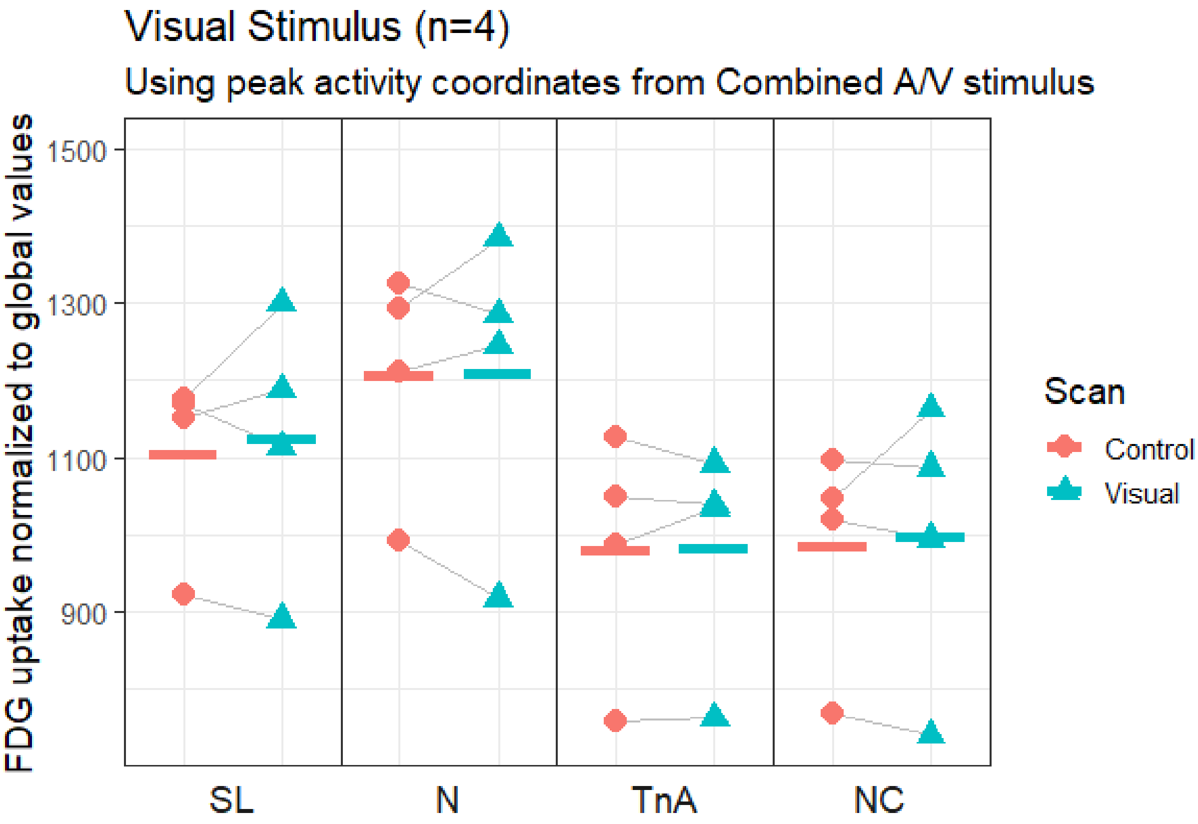
Chapter 2 Supplementary Materials

Supplementary Table 2.1. *Timeline of the experimental methodology between FDG injection and start of imaging process.*

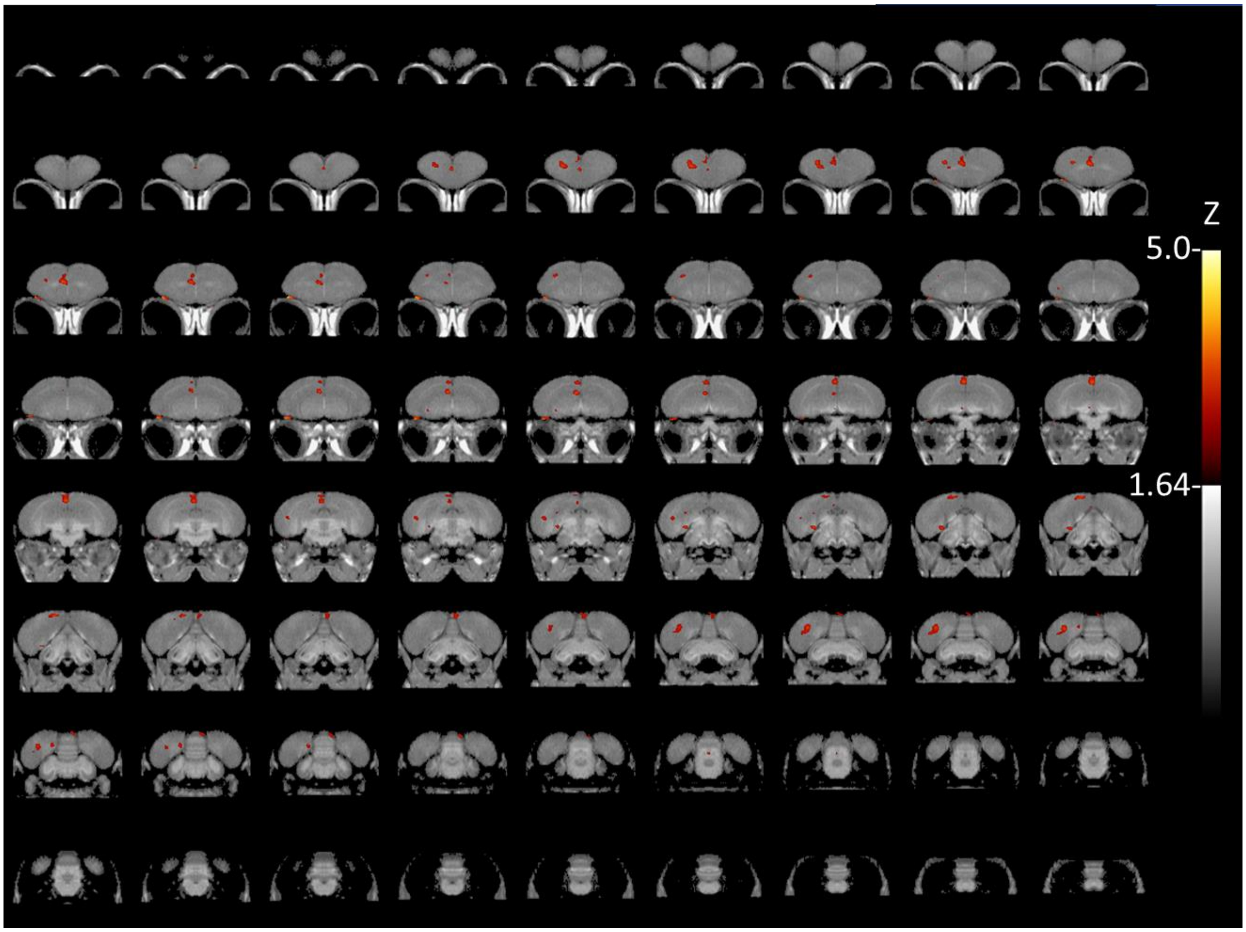
Name	Action	Time (mm:ss)
Injection	Interperitoneal FDG injection	00:00
Pre-stimulus phase	Crow returned to covered cage, stimulus stage is moved into position	00:00 – 03:00
Stimulus phase	Open panels, revealing stimulus stage interior	03:00
	Close panels, hiding stimulus stage interior	04:00
	Open panels, revealing stimulus stage interior	04:30
	Close panels, hiding stimulus stage interior	05:30
	Open panels, revealing stimulus stage interior	06:00
	Close panels, hiding stimulus stage interior	07:00
	Open panels, revealing stimulus stage interior	07:30
	Close panels, hiding stimulus stage interior	08:30
	Open panels, revealing stimulus stage interior	09:00
	Close panels, hiding stimulus stage interior	10:00
	Open panels, revealing stimulus stage interior	10:30
	Close panels, hiding stimulus stage interior	11:30
	Open panels, revealing stimulus stage interior	12:00
	Close panels, hiding stimulus stage interior	13:00
Pre-imaging phase	Crow removed from cage and induced with isoflurane. Anesthetized crow secured to scanner bed and positioned within imager field of view	13:00 – 26:00
Imaging phase	Begin micro-PET scan	26:00



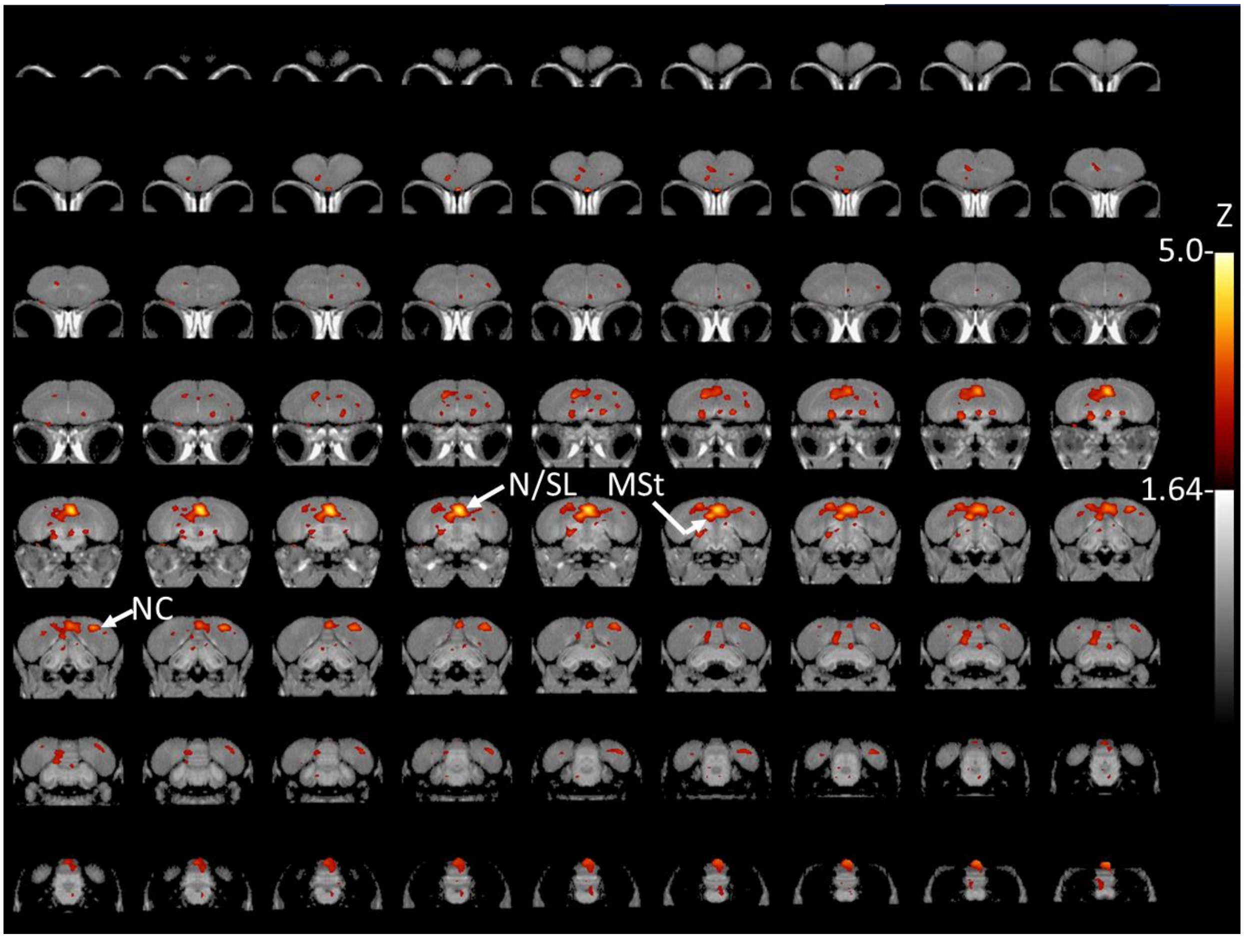
Supplementary Figure 2.6. *Spectrogram of audio stimulus exemplar.*



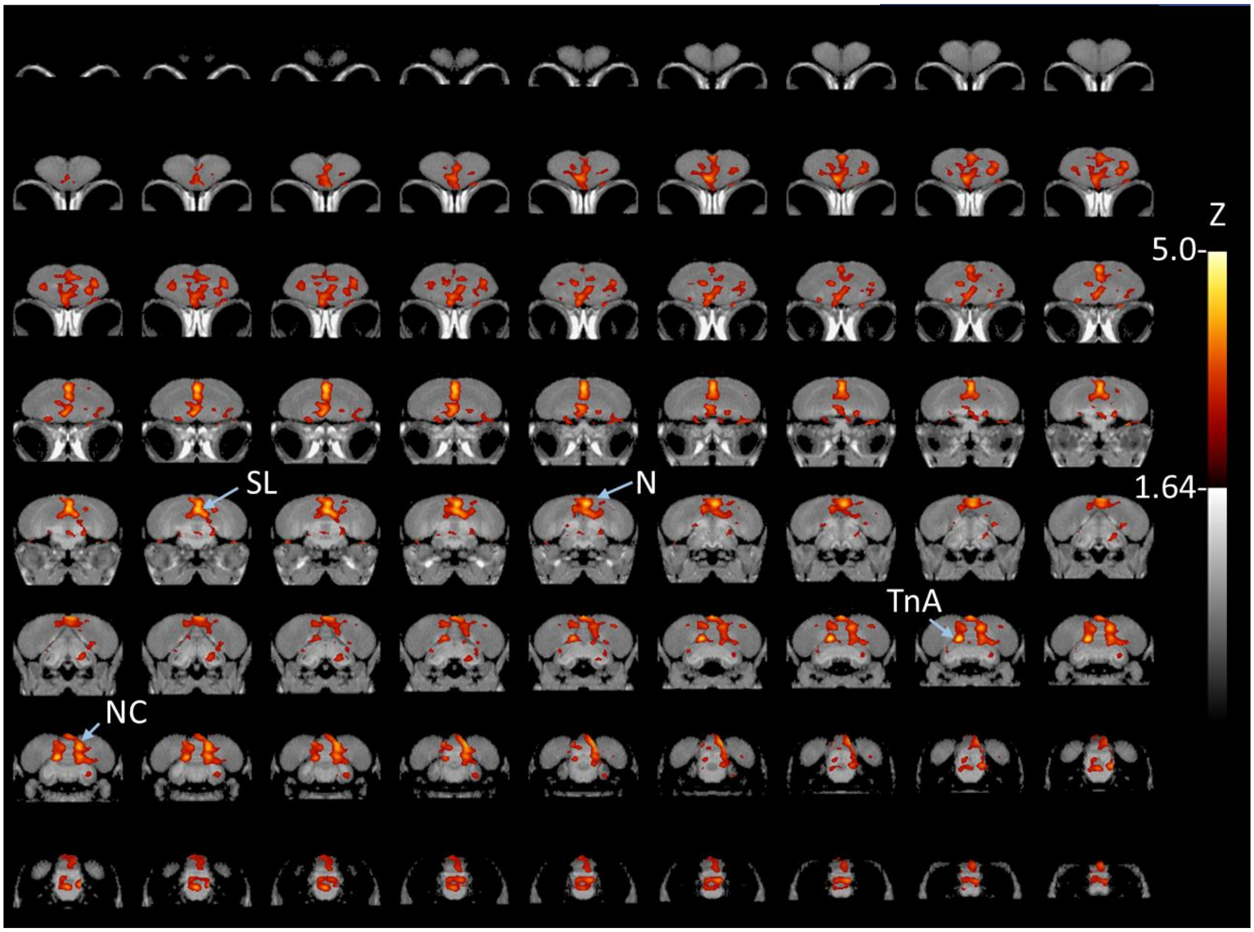
Supplementary Figure 2.7. *Individual values for normalized (global) uptake of the birds exposed to the visual-only stimulus obtained from VOI's centered on peak activation coordinates from the Combined A/V stimulus. Horizontal lines indicate group means.*



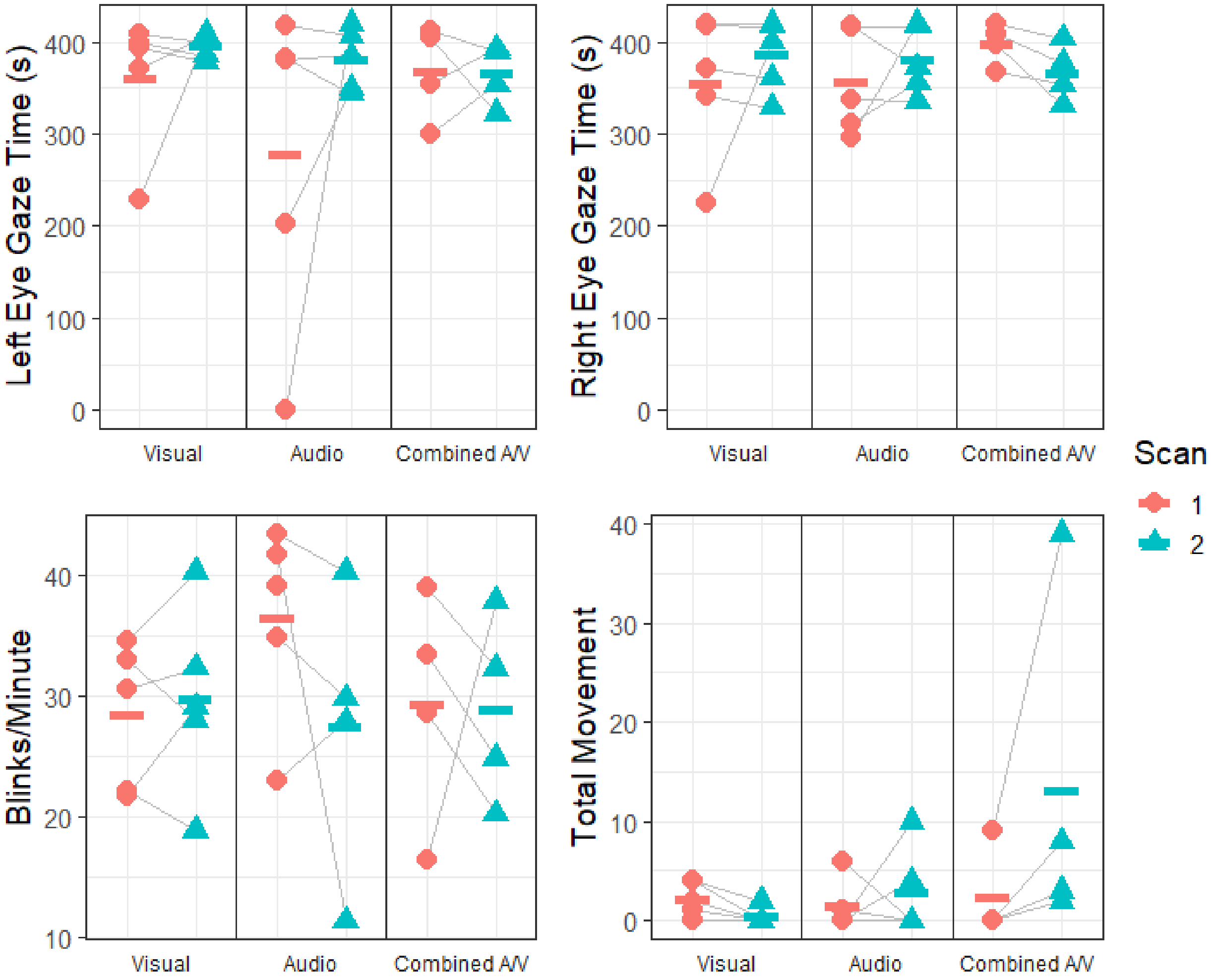
Supplementary Figure 2.8. *Voxel-wise subtractions (converted to Z-scores) showing differential activity patterns throughout the brain for all crows (n=4) exposed to the sight of their preferred food item during their stimulus scan. Brain activity is superimposed atop a composite (n=4) structural MRI of the American crow brain.*



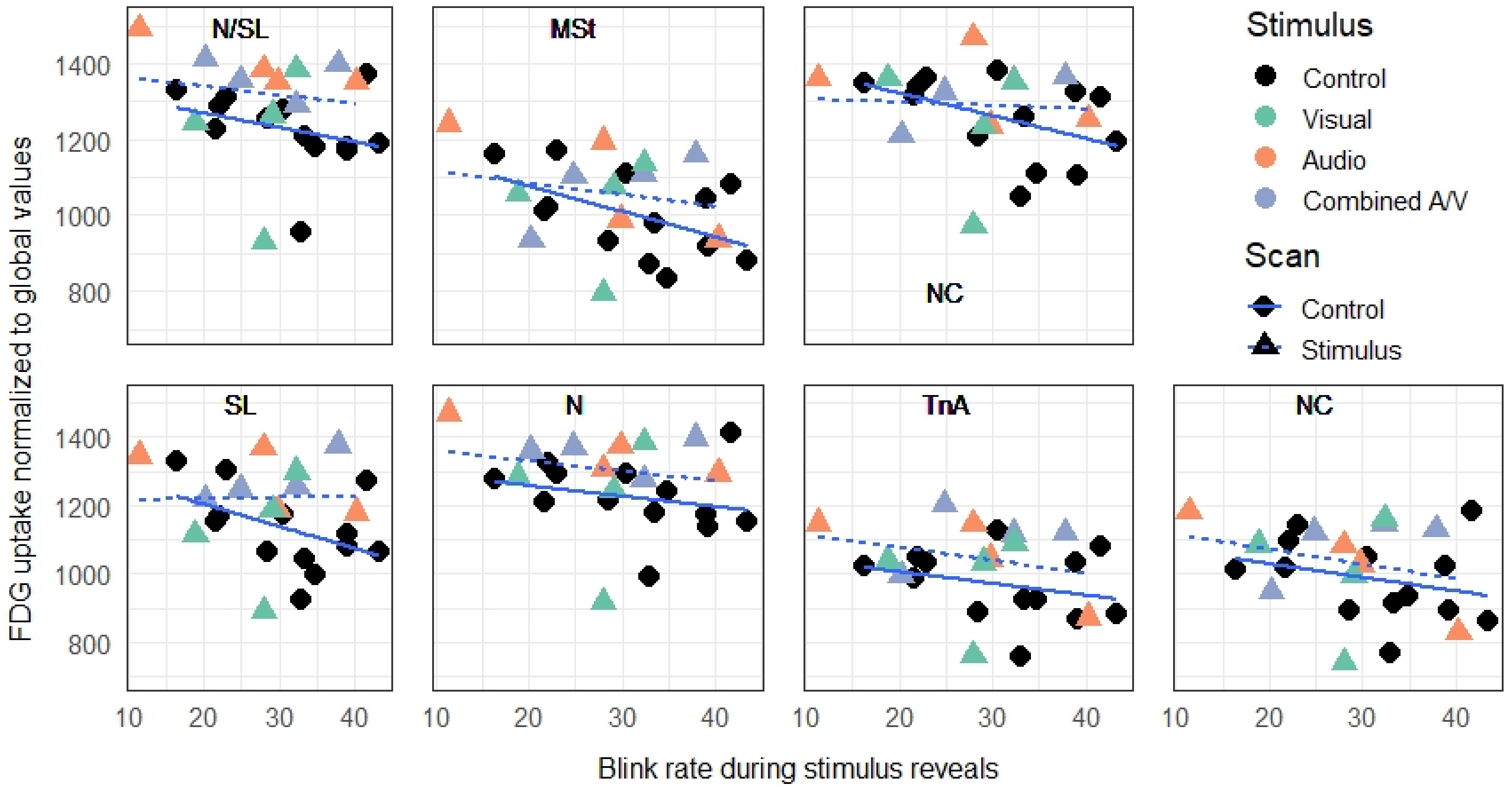
Supplementary Figure 2.9. *Voxel-wise subtractions (converted to Z-scores) showing differential activity patterns throughout the brain for all crows (n=5) exposed to the vocalizations of feeding crows during their stimulus scan. Brain activity is superimposed atop a composite (n=4) structural MRI of the American crow brain. Note that only the nidopallium/lateral septum border (N/SL) showed significant increases in brain activity; the medial striatum (MSt) and caudal nidopallium (NC) did not meet the critical Z-threshold.*



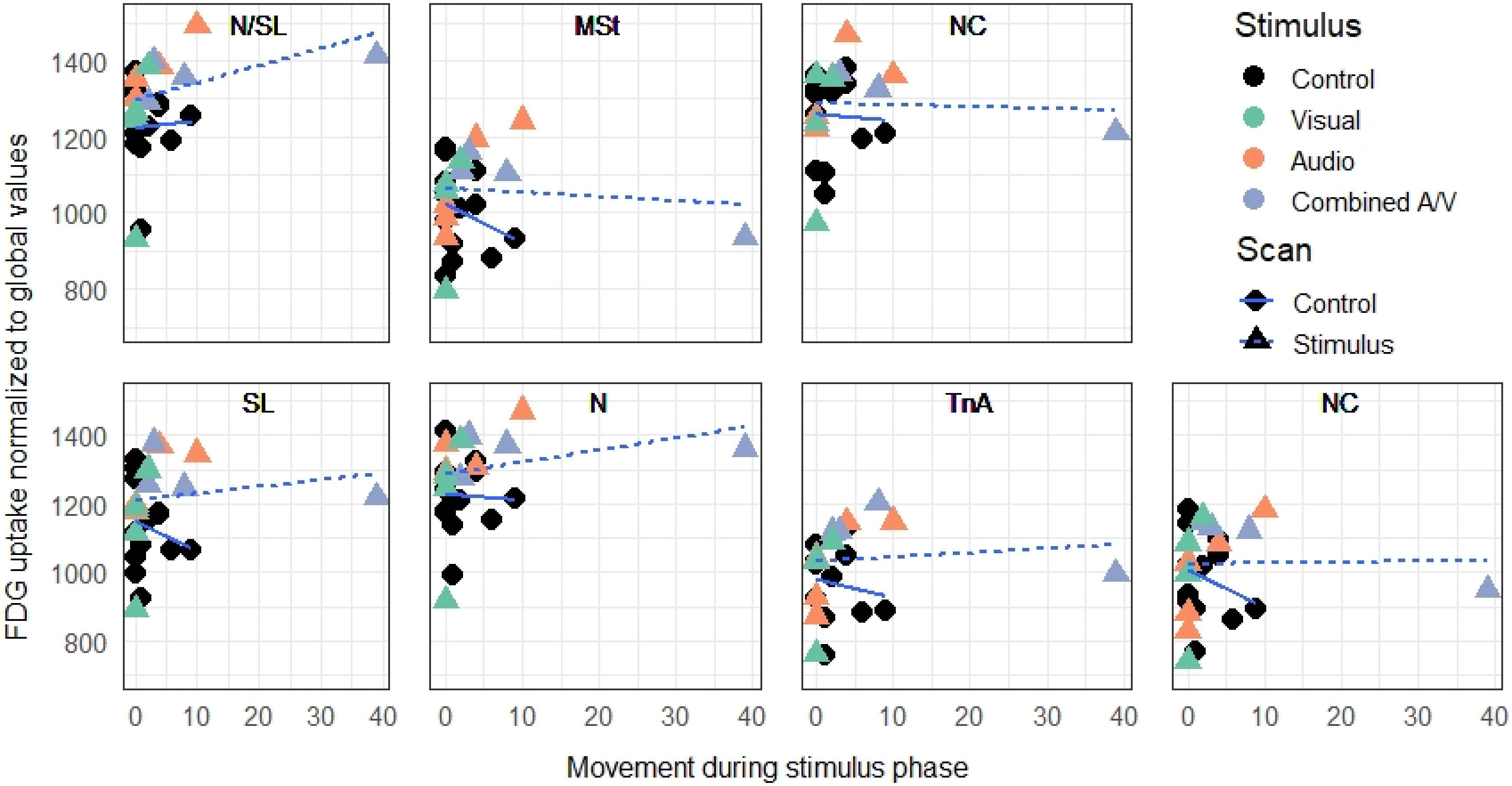
Supplementary Figure 2.10. *Voxel-wise subtractions (converted to Z-scores) showing differential activity patterns throughout the brain for all crows (n=4) simultaneously exposed to the vocalizations of feeding crows and the sight of their preferred food item during their stimulus scan. Brain activity is superimposed atop a composite (n=4) structural MRI of the American crow brain. Note that while the nucleus taeniae of the amygdala (TnA), and nidopallium (N) showed significant increases in FDG uptake, the lateral septum (SL) and caudal nidopallium (NC) did not meet the critical Z-threshold.*



Supplementary Figure 2.11. *Changes in behavior between the first and second scan of each individual crow for blink rate (top left), total movement (top right), and gaze time (left eye: bottom left; right eye: bottom right). Horizontal lines indicate group mean.*



Supplementary Figure 2.12. *Results of correlational analyses between blink rate and FDG uptake. Blink rate was not significantly correlated with FDG uptake in any region, for either the first control scan or second stimulus scan. Top row: regions significantly activated by the audio stimulus; N/SL (control scan: r= -0.31, t_{11}= -1.09, P= 0.31; stimulus scan: r= -0.13, t_{10}= -0.42, P= 0.68), MSt (control scan: r= -0.51, t_{11}= -0.98, P= 0.07; stimulus scan: r= -0.20, t_{10}= -0.65, P= 0.53), and NC (control scan: r= -0.46, t_{11}= -1.73, P= 0.11; stimulus scan: r= -0.06, t_{10}= -0.18, P= 0.86). Bottom row: regions significantly activated by the combined A/V stimulus; SL (control scan: r= -0.46, t_{11}= -1.71, P= 0.11; stimulus scan: r= 0.02, t_{10}= 0.06, P= 0.95), N (control scan: r= -0.25, t_{11}= -0.86, P= 0.41; stimulus scan: r= -0.17, t_{10}= -0.56, P= 0.59) TnA (control scan: r= -0.28, t_{11}= -0.98, P= 0.35; stimulus scan: r= -0.24, t_{10}= -0.79, P= 0.45), and NC (control scan: r= -0.28, t_{11}= -0.98, P= 0.35; stimulus scan: r= -0.25, t_{10}= -0.83, P= 0.42).*



Supplementary Figure 2.13. *Results of correlational analyses between movement and FDG uptake. Movement was not significantly correlated with FDG uptake in any region, for either the first control scan or second stimulus scan. Top row: regions significantly activated by the audio stimulus; N/SL (control scan: r= 0.05, t_{11}= 0.17, P= 0.87; stimulus scan: r= 0.36, t_{11}= 1.27, P= 0.23), MSt (control scan: r= -0.26, t_{11}= -0.91, P= 0.38; stimulus scan: r= -0.09, t_{11}= -0.32, P= 0.76), and NC (control scan: r= -0.05, t_{11}= -0.15, P= 0.88; stimulus scan: r= -0.04, t_{11}= -0.14, P= 0.89). Bottom row: regions significantly activated by the combined A/V stimulus; SL (control scan: r= -0.21, t_{11}= -0.71, P= 0.49; stimulus scan: r= 0.16, t_{11}= 0.55, P= 0.59), N (control scan: r= -0.05, t_{11}= -0.16, P= 0.87; stimulus scan: r= 0.28, t_{11}= 0.97, P= 0.35), TnA (control scan: r= -0.15, t_{11}= -0.50, P= 0.63; stimulus scan: r= 0.11, t_{11}= 0.36, P= 0.73), and NC (control scan: r= -0.27, t_{11}= -0.91, P= 0.38; stimulus scan: r= 0.02, t_{11}= 0.07, P= 0.94).*

CHAPTER 3: Physical and neurological factors associated with proficiency by American crows proficiency at a tool-use task

ABSTRACT

Tool-use behavior affords many benefits to the species that practice it and has been convergently adopted by multiple taxonomically distant groups. However, we know comparably little about how non-primate species transition from proto-tool behaviors to true tool-use behaviors. I taught American crows (*Corvus brachyrhynchos*) to solve a task requiring the use of stone tools with varying degrees of proficiency and used 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) to compare the brain activity of naïve vs knowledgeable birds. I found that naïve and low proficiency crows primarily use neural circuits associated with executive planning and higher-order thinking (the mesopallium, nidopallium, and nidopallium caudale) when confronted with the task, whereas the highly proficient individuals show increased activity in circuits associated with motor learning and tactile control (hippocampus, tegmentum, nucleus basorostralis, and cerebellum). I also found the crows that mastered the tool-use task were exclusively adults and primarily female. I discuss how the crows' brain activity at varying tool-use proficiencies is comparable to the activity of human novices vs experts, and how female crows face additional pressure to use more cognitively complex strategies to obtain food in their environment.

INTRODUCTION

Tool use was once thought to be an ability unique to humans, yet it is now known from multiple vertebrates, and even among some invertebrates (Beck 1980). Multiple authors have defined tool use, but Beck's (1980) definition is perhaps the most widely used:

> "… tool use is the external employment of an unattached environmental object to alter more efficiently the form, position, or condition of another object, another organism, or the user itself when the user holds or carries the tool during or just prior to use and is responsible for the proper and effective orientation of the tool." (p. 10)

A behavior that meets all of Beck's criteria is considered true tool use, whereas behavior that meets most of the criteria (such as birds cracking open shells by dropping them onto a hard surface) can be considered "proto-tool" behavior (Lefebvre et al. 2002). There have been more reported cases of species engaging in proto-tool behaviors than true tool use (Beck 1980). Many species that exhibit proto-tool behaviors possess the necessary morphology (hands, bills, prehensile snout, etc.) to also utilize true tools, as evidenced by the many species that do not normally use tools in wild populations which yet can be trained to use tools in captivity (see Amodio et al. 2019, Okanoya et al. 2016, and Tebbich et al. 2010 for examples). What characteristics might be advantageous in pushing an individual from proto-tool behavior into true tool-use behavior? An individual's hunger and associated body condition (starved, overweight, etc.) could provide the necessary motivation to try novel techniques to access food that might otherwise remain inaccessible; for example, hungry animals are more successful in solving novel tasks in captivity (Birch 1945). The individual's age might be important, as the accumulated experiences of older individuals could be applicable in learning a new behavior. However, younger individuals tend to interact with objects more persistently due to playfulness (Vince 1958), which could also lead to the development of new behaviors. Temperament is another factor to consider, as animals which are especially nervous or neophobic are less capable of solving problems and less likely to approach novel objects (Benson-Amram & Holekamp 2012, Heinrich 2000). Tool use in some species is biased in favor of females (Gruber et al. 2010, Smolker et al. 1997), thus an individual's sex may be a factor. An animal's position within the dominance hierarchy could promote the innovation necessary to refine proto-tool use into true tool use, as subordinate individuals often have limited access to resources compared to more dominant individuals. Because learned tool use is associated with animals of increased intelligence (Emery & Clayton 2004), an individual with comparatively higher cognitive processing power (alternatively quantified using brain volume relative to body size: Deaner et al. 2007, Benson-Amram et al. 2016; or neuronal counts/density: Jardim-Messeder et al. 2017, Olkowicz et al. 2016; although see Healy & Rowe 2006) should be more capable of learning to use tools compared to its peers.

Tool-use behavior may be rare because of cognitive constraints, i.e., few animals have the necessary brainpower to develop tool-use behaviors (Hunt 2005, though see Hansell & Ruxton 2008 for dissenting argument). Although advanced cognition and behavioral flexibility are not requisite for tool use, as demonstrated by genetically heritable and stereotyped tool-use behaviors found among arthropods and fish, they do appear to be common traits in species that must learn to use tools, such as among primates and birds. Therefore, researchers may be able to explore the question of why more animals don't use tools by examining the regions within the brain that are active when the subject uses a tool. Specifically, comparing the neural activity of proficient tool users against less proficient tool users of the same species can reveal the regions or circuits which are necessary for true tool use to develop.

Studies on humans have found two distinct neural circuits involved with tool use; one circuit is responsible for the semantic knowledge of the tool and its uses, while the other controls the learned motor skills required to effectively use the tool (Johnson-Frey 2004, Orban & Caruana 2014). A similar system appears to operate in non-human primates (Iriki 2006). Unfortunately, our knowledge of the neurological underpinnings of avian tool use remains limited. Birds with regular and widespread tool use throughout their wild populations show increased encephalization in regions involved with associating/memorizing diverse stimuli and executing complex motor output (Mehlhorn et al. 2010a), which suggests that the brains of avian tool users operate similarly to humans and primates. However, we do not currently know if those regions are specifically active during tool use.

Because multiple circuits are involved in tool use, researchers should attempt to examine brain activity holistically, making *in vivo* methods ideal. Positron Emission Tomography, combined with the radiotracer 18F-fluorodeoxyglucose (FDG-PET), is a brain imaging modality that works by injecting the subject with FDG, a radioactive glucose analog. The injected FDG distributes systemically and is preferentially absorbed within the brain in regions of increased synaptic activity. Therefore, the relative level of radioactivity measured by PET is a surrogate measure of brain activity (Jonides et al. 1993). An advantage of FDG-PET over other *in vivo* imaging modalities is that the subject can be awake and free from restraints or attached apparatus (which can confound the results by causing stress to an unhabituated animal) during the stimulation period, as the circulating FDG is trapped in the tissue of the active brain region but cannot be further metabolized by glucose-6-phosphatase within the glycolytic pathway (Newberg et al. 2002). The subsequent PET imaging can be performed under anesthesia to assess the brain activity during the prior stimulation period (Marzluff et al. 2012).

The intelligence of crows and other members of the avian family *Corvidae* (corvids) is advanced enough that they are often compared to the great apes; both groups possess comparable neuronal counts (Olkowicz et al. 2016), can learn and solve similar novel challenges with comparable speed and flexibility (Emery & Clayton 2004), and possess the capacity to use tools (McGrew 2013). In addition to proto-tool behavior, such as battering or dropping food items on the ground, corvids have been observed using many true tools, such as stones to hammer open hard shells, spines/edged leaves to probe for/extract insects, and pulling fishing lines to extract fish and bait, among others (Lefebvre et al. 2002). Like other groups, tool use among corvids is not universal; some species show widespread tool use throughout their wild populations (such as the New Caledonian crow, *Corvus moneduloides;* Bluff et al. 2010) while it is far less common among others (such as the American crow, *Corvus brachyrynchos;* Caffrey 2000). Although there have been reports of American crows using true tools in nature (Beck 1980, Boswall 1978, Caffrey 2000, Cristol et al. 1997), these accounts are noteworthy partially because they are rare among the greater American crow population. However, despite rarely using tools in the wild, American crows can learn to use a variety of tools in captivity (Cole 2004). Additionally, American crows have been subject to multiple prior studies involving PET imaging (Marzluff et al. 2012, Cross et al. 2013, Swift et al. 2020, Pendergraft et al. 2021), creating a foundational knowledge upon which new research can build, and making American crows an ideal study species to examine avian brain activity in response to a tool-use task under varying knowledge conditions.

Here, I used behavioral observation, computerized tomography (CT) imagery, and 18F-fluorodeoxyglucose PET imaging to explore the physical and neurological factors associated with American crow proficiency at learning a task requiring the use of a novel tool; the Aesop fable paradigm task (hereafter Aesop task). This task requires the subject to drop stones into water to raise the water level and obtain an out-of-reach food item (satisfying the requirements for tool use) and has been used to test the cognitive capabilities of multiple corvid species (Jelbert et al. 2015). My objective is to explore how tool use might develop in a species by examining the correlation between the crows' proficiency with the Aesop task and their individual factors (such as sex, body size, and brain volume) as well as their brain activity when confronted with the task.

If individual factors influence their task proficiency, I predict that older, calmer females with lower body condition (smaller body mass than expected for given body size, see Methods) and larger brain volumes (both absolute and relative to body size) will be more likely to achieve high proficiency compared to younger, nervous males with higher body condition and smaller brains. For brain activity, I predict that crows that are highly proficient at the task will show increased activity (compared to naïve or less proficient crows) in regions previously identified as enlarged in corvids with more proficient tool use: the mesopallium, striatal complex, septum, and tegmentum (Mehlhorn et al. 2010a). I additionally expect to see activity in the nidopallium caudolaterale (NCL) due to its association with tool use (Lefebvre et al. 2002), and the hippocampus due to its association with memory (Clayton 1998).

METHODS

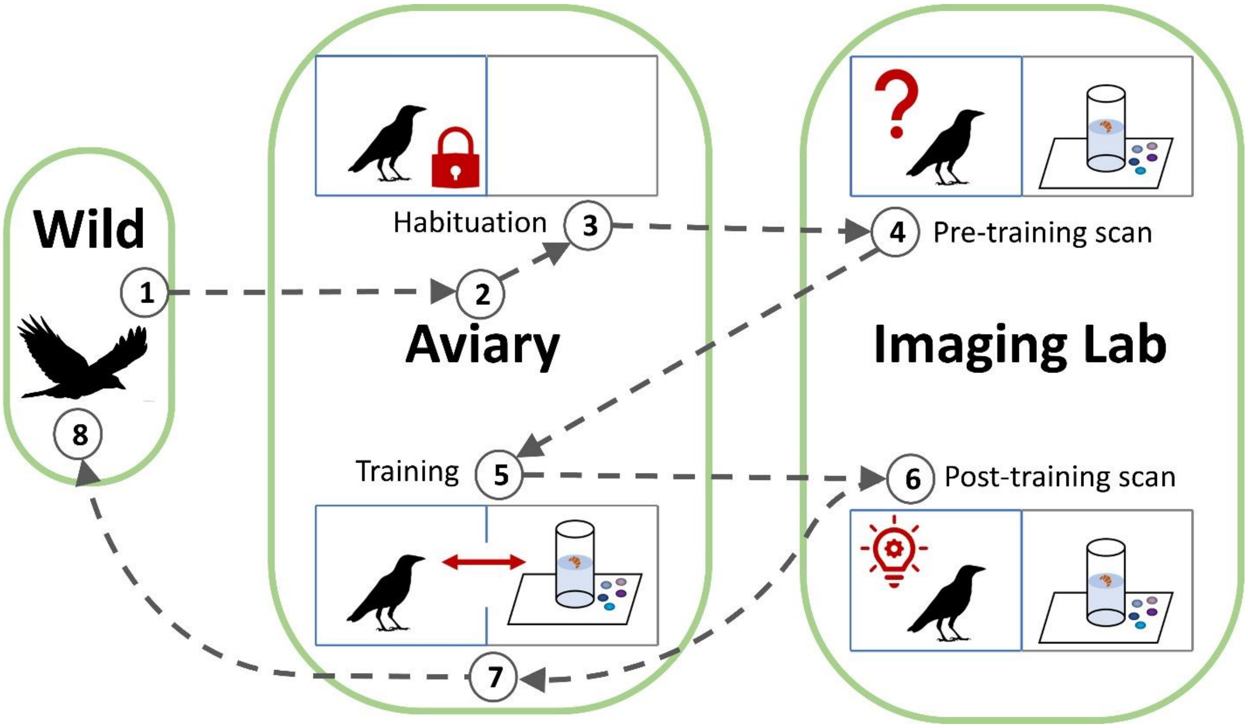


Figure 3.1. *Overview of my methodology. 1) I captured crows from the wild and 2) housed them in an outdoor aviary. 3) I regularly habituated the crows to the conditions of the imaging process, but they could not leave training cage and I did not give them a stimulus. 4) I transported crows from the aviary to the imaging lab for their baseline scan; this was the first time the naïve crows were exposed to an Aesop tube task. 5) I returned the crows to the aviary and modified the habituation process so that crows had full access to an Aesop task; the crows learned to solve the task to varying degrees. 6) I returned the crows to the imaging laboratory and scanned them again; this time they were familiar with the stimulus Aesop tube task. 7) I returned crows to the aviary and 8) released them back to the wild several days after the final scan.*

Capturing and Housing Crows

I captured 16 wild American crows as they departed a large communal roost at various locations in Seattle, Bothell, and Woodinville WA, by luring them from flocks with bread and then trapping them using a net launcher. Due to potential differences in behavior and cognitive development, I released birds in their first year of life immediately upon capture. I caught the birds outside of the breeding season and held them for several months each of two years (n = 8 from October 2017-February 2018, and n = 8 from September 2018-February 2019).

I kept captured crows in a protected outdoor aviary at the University of Washington, Seattle (Fig 3.1). The crows were individually housed in adjacent cages (measuring 1.8 x 2.1 x 2.4 m; hereafter aviary cage) separated by wire mesh; crows could see and hear their neighbors but could not leave their cage. I provided crows with a rotating diet of assorted meats, eggs, grain, fruit, and dried dog food *ad libitum* for several hours each day.

Aesop Task Apparatus

I constructed an Aesop task apparatus using clear plastic acrylic tubes with an internal diameter of 5.1 cm mounted to the center of a (15.2 x 20.3 x 0.6 cm) polycarbonate sheet (Fig. 3.2). Because larger birds were capable of reaching deeper into the tube's interior, I standardized the "difficulty" of the water depth by using shorter tubes (17.8 cm length) for smaller crows and longer tubes (20.3 cm length) for larger individuals. I measured water depth from the top of the tube (e.g. filling the tube to the rim had 0 mm of depth) using a ruler and adjusted the interior water level using a syringe. I opted to use cheese puffs as the food reward because they float on water for an extended time period, are a food item the crows preferentially selected over other options, and possess a bright orange color which can be easily seen. I used plastic aquarium stones with this task; they were relatively light (thus easy for a crow to lift), colored green or blue (increasing their contrast with the white arena), and, when dropped, displaced water within the tube by roughly 5-9 mm.

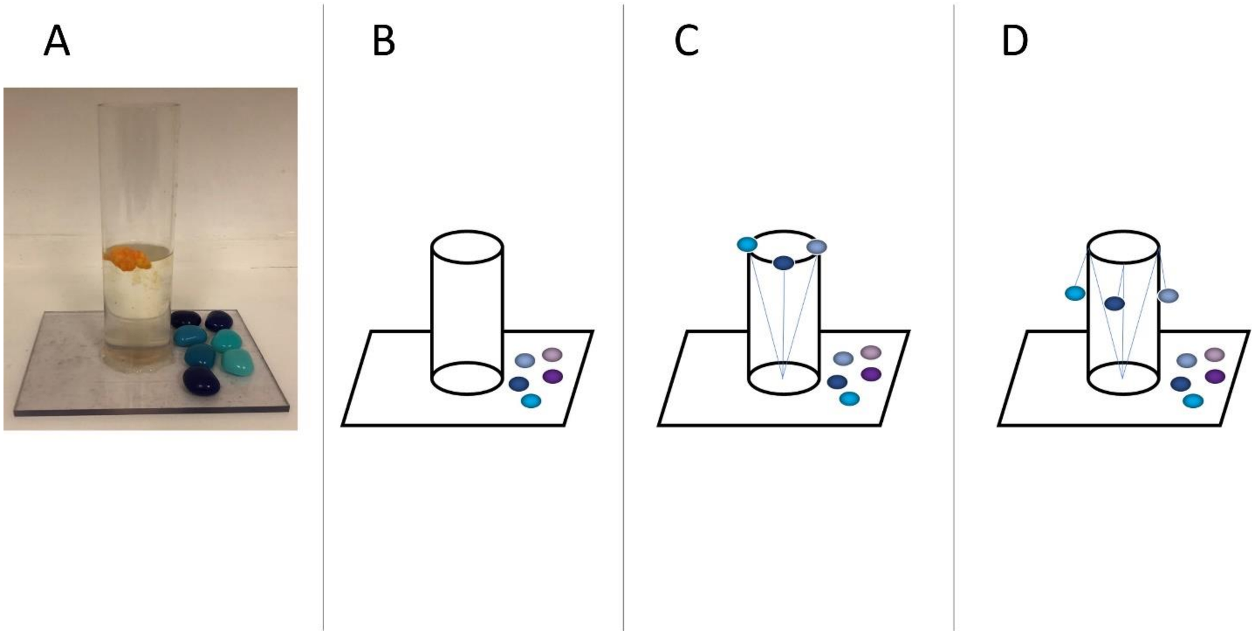


Figure 3.2. ***A***: *photo of an Aesop tube task baited with a cheese puff and aquarium stones alongside. I used this arrangement as the stimulus for all imaging sessions.* ***B-D***: *graphic of different Aesop task stone configurations.* ***B***: *the standard apparatus, without any training stones.* ***C***: *training stones are balanced along the edge of the tube.* ***D***: *the training stones are suspended along the outside of the tube. Training stones are connected to the tube's interior via fishing lines, which prevented them from falling outside the tube.*

Habituation / Learning the Aesop Task

Several days post-capture, I began regularly (every 1-2 days) habituating crows to the unfamiliar conditions they would experience during the imaging process (see Fig. 3.1 and *Imaging the crows* below). I simulated the radiotracer injection by removing crows from their aviary cage, covering their heads with a cloth, laying them on their back, spraying their belly with water (to simulate the alcohol used as an antiseptic), and lightly pinching the future injection site for several seconds (to simulate the injection). After the simulated radiotracer injection, I replicated the stimulus phase of the imaging process by moving the crow to a secluded area away from the main aviary and placing it inside a smaller cage (1 x 0.5 x 0.5 m;

hereafter training cage), before moving an identically-sized hollow wooden arena (hereafter the training arena) in front of the training cage (Fig. 3.3A). I deliberately mimicked the conditions crows would experience during the stimulus phase of the scans; I placed the training arena directly adjacent to (and flush with) the front of the training cage and covered the cage's walls so that the crow's only external view was the interior of the arena, painted the arena white, and kept the arena's interior well-lit by a ceiling-mounted LED light source (whereas the cage's interior was relatively dark due to the wall coverings).

During the pre-scan habituation process, I kept the training arena's interior empty and prevented crows from leaving the training cage by keeping the cage door closed. All crows experienced >7 such sessions (mean ± SD: 10.2 ± 4.5 days) before their first scan. I habituated crows before they received their daily food to replicate the time after the last eating that they would experience before the scan. After 2-3 hours in the training cage, I returned crows to their main cages and fed them. I habituated up to six crows per day: three in the morning and three more in the afternoon. Because birds that were habituated in the afternoon did not receive their daily food until approximately 1500-1600h, I placed all crows on a rotating schedule (day 1: morning, day 2: afternoon, day 3: no habituation, day 4: morning, etc.) to reduce bias and hunger stress.

After they were exposed to an Aesop tube task during their first imaging session, I modified the habituation process to train each crow to solve the task. I continued to simulate the radiotracer injection before placing crows inside the training cage. However, unlike their pre-scan habituation, I now added an Aesop task to the interior of the training arena and opened the door to their training cage; crows could now freely enter the arena and interact with the task (Fig. 3.3B.). As in the habituation process, I could train up to six birds per day: three in the morning and three more in the afternoon. I did not feed crows before training, ensuring they were motivated to retrieve the food reward. I returned crows to their cage and fed them after 2-3 hours or after they solved the task, whichever occurred first. I regularly checked on their progress remotely without disturbing them using wide-angle peephole door viewers mounted to the training arena and recorded their various attempts using a small camera placed inside the training arena.

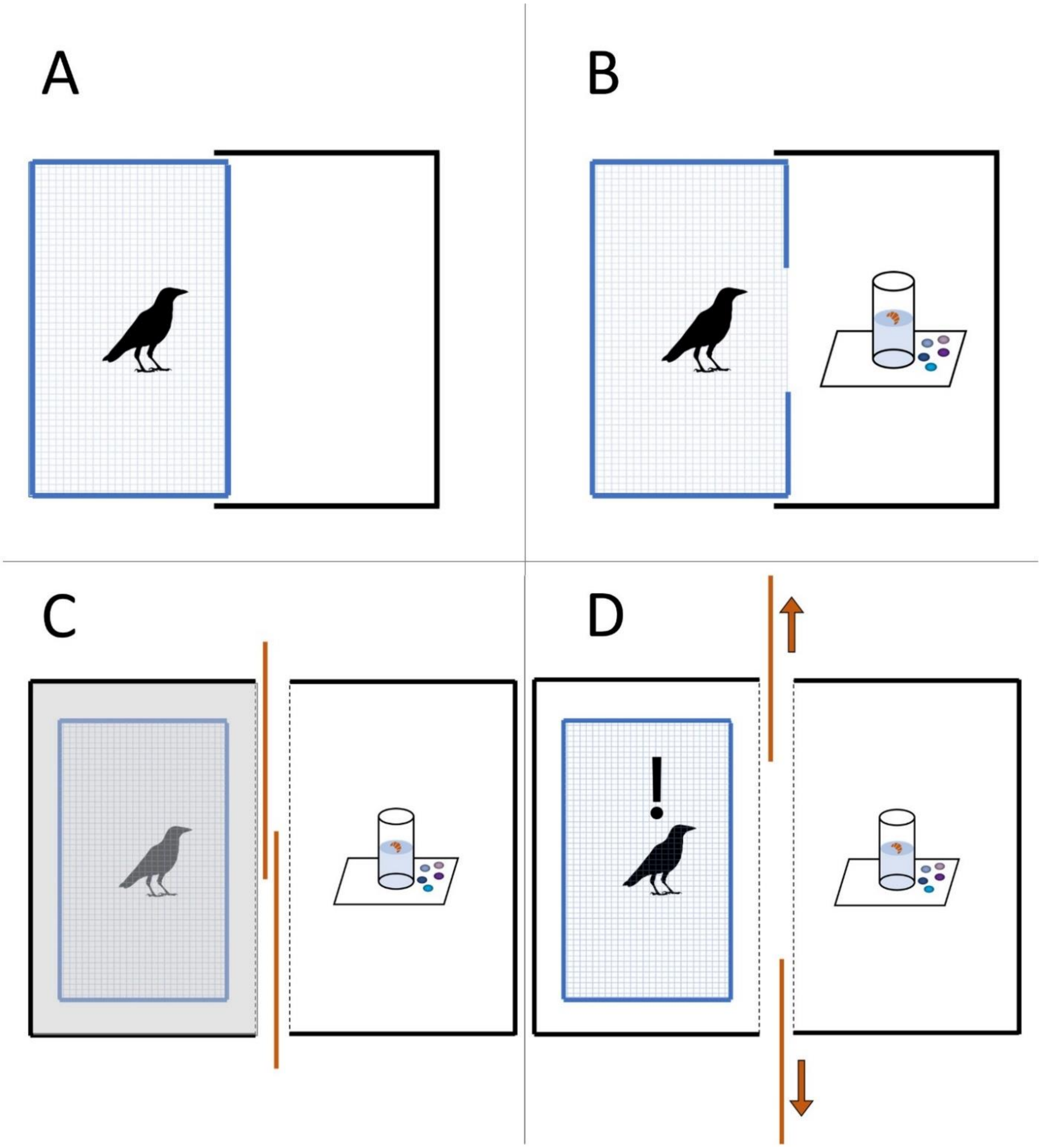


Figure 3.3. ***A-B***: *graphic of training cage and training arena arrangement.* ***A***: *before the first imaging session, the training arena was empty, and crows could not leave the training cage.* ***B***: *after their first imaging session, crows could freely enter the training arena and interact with the Aesop task within.* ***C-D***: *graphic of imaging cage and imaging stage arrangement during the stimulus phase (top-down view) of the imaging process. The panels and blanket surrounding the crow's cage blocked all view of the surrounding laboratory.* ***C***: *when I closed the sliding panels between the crow's cage and the stimulus stage, the crow was plunged into relative darkness.* ***D***: *I revealed the interior of the well-lit stage (and the Aesop task stimulus within) by opening the sliding panels.*

I trained crows to solve the Aesop task using incremental steps; every time a crow succeeded in retrieving food, I increased the difficulty. If crows failed to solve for 3 consecutive days, I reduced the difficulty. I started by leaving 2-3 cheese puffs on the ground of the training arena to entice crows to enter the arena. After a crow began retrieving food from the arena floor, I added a baited Aesop tube with water filled to the brim (depth 0 mm), along with multiple stones along the base of the tube. Each time a crow successfully retrieved food from the tube, I increased the water depth until I had established the maximum reachable depth for that crow. I then attached fishing line to the interior of the tube and secured the other end of the line to stones balanced along the top of the tube; the line prevented the stones from falling outside the tube, but they could be freely dropped into the tube, raising the water level (hereafter “training stones”; Fig. 3.2C). At this stage, I lowered the water depth to 5mm below the crow's maximum reachable distance; as crows attempted to reach the food, they usually dropped one or more of the balanced training stones into the tube, bringing the water level up to a reachable distance. I next began increasing the length of the training stone line, lowering the training stones along the tube exterior, forcing the crows to either lift the stones into the tube or pull them inside using the line before the water level was high enough for them to reach (Fig. 3.2D). Finally, I removed the training stones so that the crow was forced to pick stones off the ground and drop them into the tube before retrieving the food (Fig. 3.2B); I considered any crow that reached this stage to have mastered the task.

I created an ordinal proficiency scale to quantify crow progression towards mastering the task; each stage within this scale is representative of a significant obstacle that crows had to overcome (Table 3.4 in supplementary material). Because all (but one) of the crows were ultimately distributed among three proficiency levels (Table 3.5 in the supplemental material), I merged the seven proficiency levels into three categories: crows that never dropped any training stones were low proficiency, crows which occasionally dropped training stones while they were balanced on the tube lip but never progressed further were medium proficiency, and crows that fully mastered the task were high proficiency.

Individual Measures

To examine which (if any) individual characteristics were correlated with task proficiency, I measured and compared the following attributes from each bird: age, body condition, culmen length, level of nervousness, sex, and brain volume (both absolute and relative to body size).

For age, I categorized birds as being in their second year of life (subadult) or older (adult) using a combination of plumage color (dark brown/dull black for subadults, glossy black for adults), mouth coloration (traces of pink for subadults, fully black for adults), and feather wear (uniformly worn for subadults, mostly new for adults) (Emlen 1936). I calculated the crows’ body condition by extracting the residuals from a regression of their body weight upon capture against their culmen length (mm from the distal tip of the bill to the base of the feathers). I determined each bird’s level of nervousness by standing 2 m away from each bird’s cage while staring at a fixed point within the cage (not at the crow) and counting the bird’s movements for 60 s. I assigned a numerical value based on the bird’s perceived urgency to each move; walking along the perch = 0.5 or flying/hopping to another perch = 1. I obtained two such measurements for each bird (the first within two weeks of capture, the second within two weeks of release) and averaged them.

To determine the sex of the crows, I extracted approximately 20µL samples of blood from each bird via the brachial artery and stored them at -80^{o}C. I used a QIAGEN® DNeasy® Blood & Tissue Kit to isolate genomic DNA from each blood sample, then amplified the target genes (CHD1-W and CHD1-Z) using polymerase chain reaction (PCR). I conducted agarose gel electrophoresis on the PCR product; males contain two Z chromosomes and produce a single band in the gel between 400-450 bp, whereas females contain a Z and a W chromosome and therefore produce an additional band in the gel between 300-325 bp (Griffiths et al. 1996).

I calculated brain volume as in Pendergraft et al. (2020). I used an open-source DICOM viewer (Horos version 3.2.1; Horos 2019) to analyze the CT images obtained during the birds' first imaging session. I conducted brain segmentation on approximately 50% of the relevant slices using Horos's threshold-based 2D region of interest (ROI) utility, though I avoided the regions (cerebellum and brainstem) caudal to the nidopallium caudale; I edited these ROIs by hand before the software added interpolated ROIs on the missing slices. I inspected all generated ROI's before calculating the final brain volume (cm^3) with the built-in utility. Because some of the brain segmentation was performed manually (and thus subject to user bias), I and another researcher independently analyzed several of the same CTs; because our findings differed by a very small amount (0.82% ± 0.62%, n = 11), I discounted user bias as minimal. To account for the allometric association of brain volume to body size, I extracted the residuals from a regression of brain volume and culmen length and used these residuals as a measure of relative brain volume during analysis in addition to raw absolute brain volume. Finally, I discovered that the crow's brain volumes were smaller during their post-training scan than they were during their pre-training scan (see Results), so I included the % reduction in volume between the two scans as a final individual measure against task proficiency.

Imaging the crows

I imaged up to three crows per day, using a Siemens Inveon PET/CT system. The scanning process consisted of a 20 min microPET scan, followed by a CT scan in the docked and coregistered microCT scanner. The scanners share a multimodality bed and have a bore diameter of ~12 cm. The PET field of view was approximately 8 x 13 cm^2 while the CT field of view was 7.9 cm x 13.3 cm; both included the entire brain with a slice thickness of approximately 0.1 mm. The scanner bed contained a pressure pad, which we used to monitor the crow's breathing (and thus the depth of anesthesia) during the scan process.

The evening before a bird was scanned (typically 1600-1700), I removed it from its aviary cage, placed it in a small animal carrier, and carried it across campus to the imaging laboratory, where I transferred it to a small wire cage with identical dimensions as the training cage (1 x 0.5 x 0.5 m; hereafter referred to as imaging cage) in a separate holding room to acclimate overnight (Fig. 3.1). The imaging cage contained water but not food, ensuring crows fasted for at least 14 hours before imaging to control for variable blood glucose levels influencing FDG uptake. The holding room's lights were set to follow a 12-hour day/night timer so the ambient light did not disrupt the crows' sleep cycle, and two sets of doors separated the holding room from the scanning room, ensuring the birds received minimal disturbance from the scanner or personnel.

The following morning, twenty minutes before the experiment, I covered the crow's imaging cage with a blanket (preventing it from looking out) and moved it into the scanning room to acclimate to the ambient noise of the room. To administer the radiotracer, I reached under the covering into the cage, grabbed the crow, covered its head with a cloth to calm it, placed it on its back, sprayed its belly with a disinfectant, and administered an intraperitoneal injection of approximately 1 mCi of [^{18}F] Fluorodeoxyglucose (FDG) (exact volume adjusted to account for radioactive decay and the bird's weight, ranging from 0.05 to 0.10 mL). After injection, I returned the crow to its covered cage and removed the cloth covering its head.

I presented the experimental stimuli within a wooden stage (1.2 x 0.6 x 0.45 m; hereafter the stimulus stage), the purpose of which was to block the crow's view of the imaging laboratory/personnel (thereby removing potential confounding sources of distraction) and to standardize the background color, light intensity, and light angle between trials. The stimulus stage was painted white to meet facility regulations for disinfecting porous surfaces and contained a ceiling-mounted LED light source directly above the stimulus. The front of the stimulus stage had two overlapping sliding panels, which I used to reveal or hide the stage interior; in addition to blocking the crow's view of the stimulus, the panels also blocked nearly all the light from the internal LED, increasing the "contrast" between showing and hiding the stimulus (Fig. 3.3C, D). The panels opened from the center of the crow's view of the stage so that the crow's eyes received equal stimulation, thus ensuring my methodology was not responsible for any bilateral differences in activation between the two brain hemispheres.

During the 3 min immediately after injecting the radiotracer, I positioned the stimulus stage in front of (and adjacent to) the covered imaging cage and removed the blanket from the cage side facing the stage; the crow remained in relative darkness because the remaining blanket blocked all exterior view of the lab while the stimulus stage's closed sliding panels prevented the crow from seeing the illuminated stage interior. At 3 min post-injection, I opened the sliding panels to reveal the experimental stimulus: a baited Aesop tube task with water level 12 cm from the top accompanied by seven stones. For the following 10 min (hereafter the "stimulus phase"), I used the sliding doors to alternatively reveal the stimulus to the crow for 60 s, then hide it for 30 s (seven exposures and six associated breaks total). After the final exposure ended at 13 min post-injection, I again removed the crow from the cage, covered its head with a cloth, and anesthetized it via a custom nose cone with 5% isoflurane in oxygen with a flow rate of 300-800 mL/min before placing it in the scanner (I reduced isoflurane concentration to 2.5-3% after the crow was fully induced).

I used Velcro straps to secure the anesthetized crow to the multimodality bed before starting the scanning process 26 mins post-injection. The multimodality bed contained a pressure pad, which I used to monitor the crow's breathing (and thus the depth of anesthesia) during the scan process. After the scan was complete, I secured the crow in hand until it fully emerged from anesthesia (indicated when it regained the ability to grip with both feet), before returning it and the imaging cage to the holding room. I kept the scanned crows in the holding room for 20 hours (the time required for ^{18}F radioactivity to decay to acceptable levels), after which I returned them to the aviary.

I scanned all crows twice; first before they had ever been exposed to the Aesop task (hereafter "Pre-Training scan"), and second after the birds were trained to retrieve food from the Aesop tube (hereafter "Post-Training scan"). Thus, although the scanning procedure and stimulus remained the same, the crows' experience with the task changed; crows were naïve to the task during the first scan but had achieved varying levels of proficiency with the task for the second scan.

Image Processing

After I imaged each crow, I conducted a 13 min attenuation scan, then reconstructed the image using the vendor-supplied 3D OSEM/MAP algorithm to an isotropic spatial resolution of 2.5 mm full width at half maximum, with attenuation and scatter corrections applied to the data. The image matrix was 128 x 128 x 159. I exported reconstructed images using DICOM for the statistical parametric analysis software.

I imported the raw DICOM data to ImageJ (Schneider et al. 2012), manually aligned their orientation to match the jungle crow (*Corvus macrorhynchos*) brain atlas established by Izawa & Watanabe (2007) and adapted for PET by Marzluff et al. (2012), and trimmed the images to include only the brain. I stereotactically aligned the scans by estimating and applying nine affline parameters to the images using algorithms originally designed for automated human brain analysis (NEUROSTAT, University of Utah; Minoshima et al. 1992), which have been adapted for crow brains analysis. The stereotactic alignment also corrected for differences in brain volume. I estimated alignment precision to be one-two pixels. Finally, I normalized all uptake values to a global brain FDG uptake.

Although I used Izawa and Watanabe’s (2007) atlas as a guide to identify the regions significantly activated by each stimulus, I did not use it as the sole determinant. This was because jungle crows are larger than American crows (Jungle crow mean male weight: 680 g, American crow: 450 g, Kitagawa 1990, Kilham 1990). More importantly, the atlas was based on a sectioned brain, whereas my activation foci were based on in vivo imaging. Sectioned brains tend to “flatten” (reduced Y-axis length, increased X- and Z- axis lengths) after being extracted from the skull and are vulnerable to other artifacts which can further alter the original shape (Rolls et al. 2008). Therefore, I scaled Izawa and Watanabe’s (2007) atlas for use with American crow brains and used it in conjunction with the shape and extent of the total activation (not just the focal coordinates) to determine the activated regions.

Behavior immediately before Imaging

To better gauge their level of attention towards the stimulus stage and to control for factors that may influence FDG uptake within the brain (Marzluff et al. 2012, Cross et al. 2013), I used a GoPro Hero 4 camera to record (30 fps) the gaze time, blink rate, and amount of movement of each crow during the stimulus phase of the imaging process.

Avian brains are highly lateralized (Rogers & Ansem 1979), so I measured the gaze time from each eye to verify that any observed differences in hemispherical activity were not due to the bird preferentially using one eye to view the stimulus over the other (Mench & Andrew 1986). I tracked each eye’s gaze time independently from the other eye e.g., I added gaze time to each eye if the bird binocularly gazed directly into the stage. I also used gaze time to measure a crow’s level of interest in the stimulus being presented, and thus only recorded gaze when the stimulus stage’s interior was revealed and visible to the crow (see *Imaging the crows*).

I measured blink rate to verify that the crows were not threatened by any of the presented stimuli or prior experience in the scanning apparatus, as previous studies have established a relationship between blink rate and the crow’s perceived sense of danger; specifically, blink rate is negatively correlated with activity in fear-associated brain regions, and crows decrease blink rate when faced with a threatening stimulus compared to while foraging (Marzluff et al. 2012, Cross et al. 2013). Although the image resolution was sufficient to see the white flash of the crow’s nictating membrane, the birds sometimes turned their heads such that their eyes were no longer visible, so I calculated an observed blink rate by dividing the number of observed blinks by the amount of time the eye was visible. The cage interior became too dark to observe blinks when the panels to the stimulus stage were closed, so I only calculated blink rate when the stimulus stage was visible during the seven reveals of the stimulus phase.

I measured the crows' movement because physiological activity can confound the amount and location of FDG uptake within the brain (Bhargava et al. 2011). I quantified the following actions as 1 unit of movement: crow moved 5-50 cm laterally along the perch (did not count if it moved <5cm, counted as 2 units if moved >50cm), crow rotated its body 180^o to face the opposite direction, and crow hopping from the perch to the cage floor (or vice-versa). Because I measured movement to account for possible confounds to FDG uptake activity, I counted movement throughout the entire 10 minutes of the stimulus phase, including when the stimulus stage interior was hidden from the crow's view.

Statistical analysis

Due to the small sample size, I tested changes in stimulus phase behaviors (blink rate, gaze, and movement) between scans using a Student's t-test, between general proficiency groups using a linear model, and correlated FDG uptake and blink rate/movement using a Pearson correlation test, all in R version 3.6.3 (R Core Team 2020). To determine which (if any) of the seven individual factors were associated with a crow's proficiency at solving the task, I constructed multinomial models using R package plyr (Wickham 2011) for every individual variable (along with an accompanying null model) and used AICc to determine which model was most supported by the data. I considered models to be competitive if they were within 2 AICc of the model with the lowest AICc (Burnham & Anderson 1998). Because the high proficiency birds were exclusively adults (see Results), I conducted a second model selection using only adult birds and the binomial "did/did not reach high proficiency" variable using generalized linear models (family = "binomial") to identify common characteristics of successful adult crows. Both models used a logit link. As I obtained many variables which could be correlated with each other (such as sex and culmen length; Clark et al. 1991), I report the Pearson's r between variables.

Because the crows' brains decreased in volume in the time interval between their pre- and post-training scans, I treated the two scans as independent for the voxelwise statistical comparison, despite having come from the same bird. I determined significant differences in regional activity within the brain using an automated voxel-wise subtraction and Z-statistic mapping algorithm originally designed for automated human brain analysis (NEUROSTAT, University of Utah; Minoshima et al. 1992). This algorithm conducts a Z-test comparing the study population's globally normalized difference in FDG uptake between the first (control) and second (stimulus) scans against the study population's pooled variance; it does this for each voxel coordinate throughout the entire brain using a modified Bonferroni correction with a smoothing factor. I only report the activity with a Z-score greater than 4.0 to reduce type I errors and to be consistent with previous imaging studies (Marzluff et al. 2012, Cross et al. 2013, Swift et al. 2020, Pendergraft et al. 2021). I also report changes in activity with a Z-score between 3.7 – 4.0 if the extent of activity is consistent with the size/location of a known brain region, as this may be worth examining in greater detail in future studies. As an additional verification of the voxelwise results, I ran an independent analysis, which sampled all the image sets from all subjects using spherical volumes of interest (VOIs; 2-voxel radius) centered around the significant peak coordinates. I plotted these VOI uptake values as a distribution to determine if said results are driven by outlying individual scans.

Ethical Note

I captured, housed, and tested all crows (including PET/CT scans) in accordance with the Institutional Animal Care and Use Committee of the University of Washington (IACUC; protocol number 3077-01), Federal Collecting Permit MB761139-0, and State of Washington Scientific Collection Permit 14-010. All were released back into the wild after the study.

RESULTS

General Crow Information

My sample group of crows consisted of 10 males and 6 females, with the sexes equally divided by age (n=3 adult and 3 subadult females, n=5 adult and 5 subadult males). They varied in size (culmen length: mean ± SD= 50.0 ± 2.9 mm), body condition (0.00 ± 18.08 g), level of nervousness (31.94 ± 10.95 movements/min), and brain volume during first scan (absolute brain volume: 7.56 ± 0.68 cm^3; relative brain volume: 00 ± 0.50 cm^3). There was no significant change in the crows' level of nervousness during their time in captivity (first measure: 38.00 ± 11.69 movements/min; second measure: 33.50 ± 13.17 movements/min; $t_{15} = 1.94$, $P = 0.07$). Despite only having access to food for several hours each day, the crows gained a significant amount of weight during their time in captivity (capture: 387.5 ± 31.4 g; release: 415.0 ± 37.87 g; $t_{15} = 6.12$, $P < 0.001$). The crows' brains shrank in the time between their pre-training and post-training scans (5.2% ± 2.7%, min= 0.18%, max= 9.15%). See Table 3.5 in the supplemental materials for the individual measures for each crow.

I observed multiple instances of significant correlation among the seven individual measures obtained from the crows (Table 3.1). Absolute brain volume, culmen length, and sex are all correlated due to their close association with general bird size; crows with smaller culmen length also have smaller absolute brain volumes ($r = 0.68$, $t_{14} = -3.51$, $P = 0.003$), and females have both smaller culmen lengths ($t_{14} = -10.85$, $P < 0.001$) and smaller absolute brain volumes ($t_{14} = -4.45$, $P < 0.001$) compared to males. Birds with larger absolute brain volumes tend to have correspondingly larger relative brain volumes ($r = 0.73$, $t_{14} = 3.98$, $P = 0.001$), though this was unsurprising as I calculated relative brain volume from absolute brain volume. Crows with large reductions in brain volume between scans were more nervous than those with less brain volume shrinkage ($r = 0.55$, $t_{14} = 2.45$, $P = 0.03$), whereas crows with high body condition scores were less nervous than those with low scores ($r = -0.58$, $t_{14} = -2.69$, $P = 0.017$).

Table 3.1. *Correlation matrix of the variables I obtained from the crows. I calculated Pearson's correlation coefficient when comparing two continuous variables and reported t-score and P-values for all comparisons for all variables. Sex and age are both categorical variables, so I did not include that comparison here (sex was exactly balanced across both age categories). Significant correlations are* ***bolded.***

	Relative brain volume	Absolute brain volume	Brain volume change	Body condition	Culmen length	Nervous
Absolute brain volume	**r = 0.73** **t_{14} = 3.98** **P = 0.001**					
Brain volume change	r = 0.32 t_{14} = 1.26 P = 0.23	r = 0.23 t_{14} = 0.87 P = 0.40				
Body condition	r = 0.03 t_{14} = 0.12 P = 0.91	r = 0.02 t_{14} = 0.09 P = 0.93	r = -0.11 t_{14} = -0.42 P = 0.68			
Culmen length	r = -3.77 e-10 t_{14} = -1.41 e-09 P = 1	**r = 0.68** **t_{14} = 3.51** **P = 0.003**	r = -0.01 t_{14} = -0.03 P = 0.97	r = 9.35 e-12 t_{14} = 3.50 e-11 P = 1		
Nervous	r = 0.49 t_{14} = 2.08 P = 0.06	r = 0.43 t_{14} = 1.78 P = 0.10	**r = 0.55** **t_{14} = 2.45** **P = 0.03**	**r = -0.58** **t_{14} = -2.69** **P = 0.017**	r = 0.11 t_{14} = 0.41 P = 0.69	
Sex	t_{14} = 0.62 P = 0.55	**t_{14} = 4.45** **P < 0.001**	t_{14} = 0.48 P = 0.64	t_{14} = -0.37 P = 0.72	**t_{14} = 10.85** **P < 0.001**	t_{14} = 1.39 P = 0.19
Age	t_{14} = 0.17 P = 0.87	t_{14} = 0.18 P = 0.86	t_{14} = -0.28 P = 0.78	t_{14} = -0.39 P = 0.70	t_{14} = 0.08 P = 0.94	t_{14} = 0.79 P = 0.44

Learning the Task

Only four of the 16 crows fully mastered the Aesop task (high proficiency). Of the 12 birds that did not master, four were of low proficiency and eight were of medium proficiency. Although the 2017 cohort of birds received a balanced number of training days (mean ± SD= 51.4 ± 0.5 days), the 2018 cohort's training time was more variable due to logistical constraints (53.8 ± 13.6). The crows quickly progressed through the initial proficiency levels, with the low and medium proficiency birds reaching their maximum proficiency in 7.0 ± 3.7 days and 17 ± 10.5 days, respectively. However, it took much longer (48.0 ± 9.8 days) for the high proficiency crows to deliberately drop hanging training stones into the apparatus, though they rapidly progressed from there to dropping stones from the ground into the tube (3.0 ± 1.0 additional days). See Figure 3.12 in the supplementary materials for the crows' learning curve.

Individual Factors and Task Proficiency

A crow's estimated age was the most important factor in predicting its general proficiency; it was the only competitive model and garnered 90% of the weight of evidence, primarily because the high proficiency group consisted exclusively of adults (Table 3.3, Fig. 3.4). Although half of the adults achieved high proficiency, three of the four remaining adults never progressed beyond low proficiency, whereas seven of the eight subadults achieved medium proficiency with the task. None of the individual characteristics I measured significantly differed between the adults and subadults (Table 3.1).

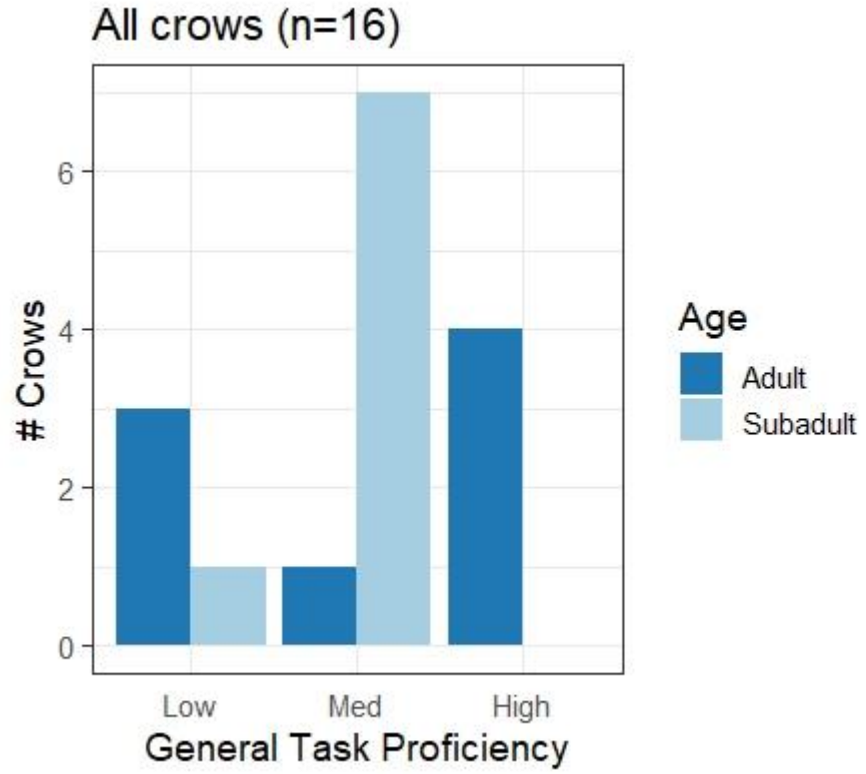


Figure 3.4. *The number of adults and subadults in each task proficiency group. This model garnered 90% of the weight of evidence for a crow's general proficiency level.*

Because no subadults achieved high proficiency, I further examined the factors that best explained which adults mastered the task. Within the adult crows, the models for absolute brain volume, sex, and body size were all competitive, collectively garnering 81% of the weight of evidence, although the model for sex failed to converge properly because all the adult females solved the task (Table 3.3). The successful adult crows were primarily female (did solve: 3 females + 1 male; did not solve: 4 males), and tended to have smaller culmen length (did solve: mean ± SD= 46.5 ± 3.0 mm; did not solve: 51.3 ± 0.96 mm) and absolute brain volume (did solve: 7.05 ± 0.45 cm^3; did not solve: 8.00 ± 0.64 cm^3) (Fig. 3.5). Although not competitive, the adults that mastered the task also had a slight tendency to have better body condition (did solve: 8.39 ± 17.68 g; did not solve: -4.79 ± 3.67 g), less nervousness (did solve: 25.5 ± 7.56 move/min; did not solve: 34.0 ± 11.75 move/min), and less variable relative brain volume (did solve: -0.12 ± 0.33 cm^3; did not solve: 0.08 ± 0.65 cm^3) compared to the other adults (Fig 3.13 in supplementary materials).

Table 3.2. *Individual variable model selection (multinomial) for a crow's likelihood of progressing beyond low task proficiency. Most competitive model in bold. The model for age failed to converge properly because there were 0 instances of a subadult mastering the task. Intercept, coefficient, and SE estimates are given in logit scale.*

Model	AICc	Δ AICc	Wi	Intercept ± SE	Coefficient ± SE
Age †	**29.62**	**0.00**	**0.90**	**Med: -1.10 ± 1.15** **High: 0.29 ± 0.76**	**Med: 3.04 ± 1.57** **High: -8.87 ± 73.18**
Size	37.13	7.52	0.02	Med: 0.21 ± 12.17 High: 21.62 ± 14.60	Med: 9.61e-03 ± 0.24 High: -4.50e-01 ± 0.30
Absolute brain volume	37.27	7.65	0.02	Med: 3.04 ± 7.56 High: 18.11 ± 11.52	Med: -0.30 ± 0.97 High: -2.45 ± 1.57
Null	37.27	7.65	0.02	Med: 6.31e-01 ± 0.61 High: -3.38e-06 ± 0.71	
Sex †	38.10	8.48	0.01	Med: 0.96 ± 1.22 High: 1.10 ± 1.15	Med: -1.92e-05 ± 1.41 High: -2.20e00 ± 1.63
Nervousness	38.99	9.37	0.01	Med: 1.52 ± 2.21 High: 3.20 ± 2.53	Med: -0.02 ± 0.06 High: -0.11 ± 0.08
Body condition	39.46	9.84	0.01	Med: 0.80 ± 0.66 High: -0.03 ± 0.79	Med: 0.03 ± 0.04 High: 0.06 ± 0.05
Brain volume change	40.74	11.12	0.00	Med: 0.08 ± 1.37 High: 2.02 ± 1.45	Med: 11.55 ± 23.66 High: -4.28 ± 26.66
Relative brain volume	40.74	11.12	0.00	Med: 0.72 ± 0.62 High: -0.01 ± 0.73	Med: -0.51 ± 1.23 High: -1.10 ± 1.56

Table 3.3. *Individual variable model selection (binomial) for an adult crow's likelihood of fully mastering the task Most competitive models in bold. The model for sex failed to converge properly because there were 0 instances of a female failing to master the task. Intercept, coefficient, and SE estimates are given in logit scale.*

Model	AICc	Δ AICc	Wi	Intercept ± SE	Coefficient 1 ± SE	P
Absolute brain volume	**11.22**	**0**	**0.29**	**76.32 ± 95.71**	**-10.22 ± 12.79**	**0.42**
Sex †	**11.4**	**0.18**	**0.27**	**19.57 ± 6208.83**	**-20.95 ± 6208.83**	**0.99**
Size	**11.5**	**0.28**	**0.25**	**46.97 ± 39.73**	**-0.95 ± 0.78**	**0.23**
Null	13.76	2.54	0.08	7.85e-17 ± 7.07e-01		1
Body condition	15.13	3.91	0.04	-0.11 ± 0.83	0.10 ± 0.08	0.20
Nervousness	15.78	4.56	0.03	3.22 ± 2.83	-0.11 ± 0.09	0.25
Brain volume change	16.84	5.62	0.02	1.11 ± 1.61	-20.54 ± 26.19	0.43
Relative brain volume	17.11	5.89	0.02	-0.03 ± 0.73	-0.97 ± 1.62	0.55

† Binomial variable coefficients are for Subadult (Age) and Male (sex)

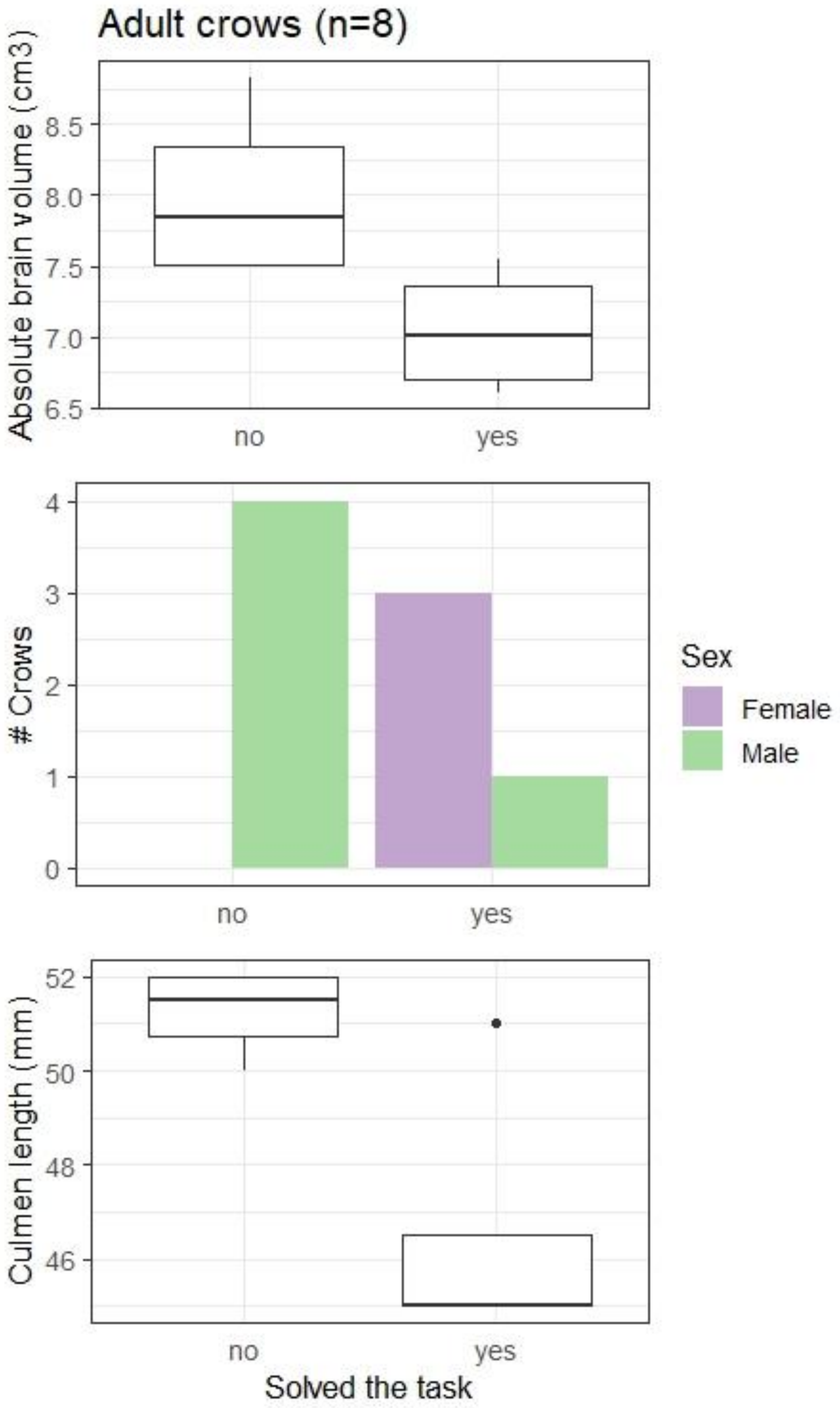


Figure 3.5. *The leading variables that best explain which adult crows will solve the task:* ***Top:*** *absolute brain volume,* ***Mid:*** *sex,* ***Bottom:*** *culmen length. All three models collectively garnered 81% of the weight of evidence.*

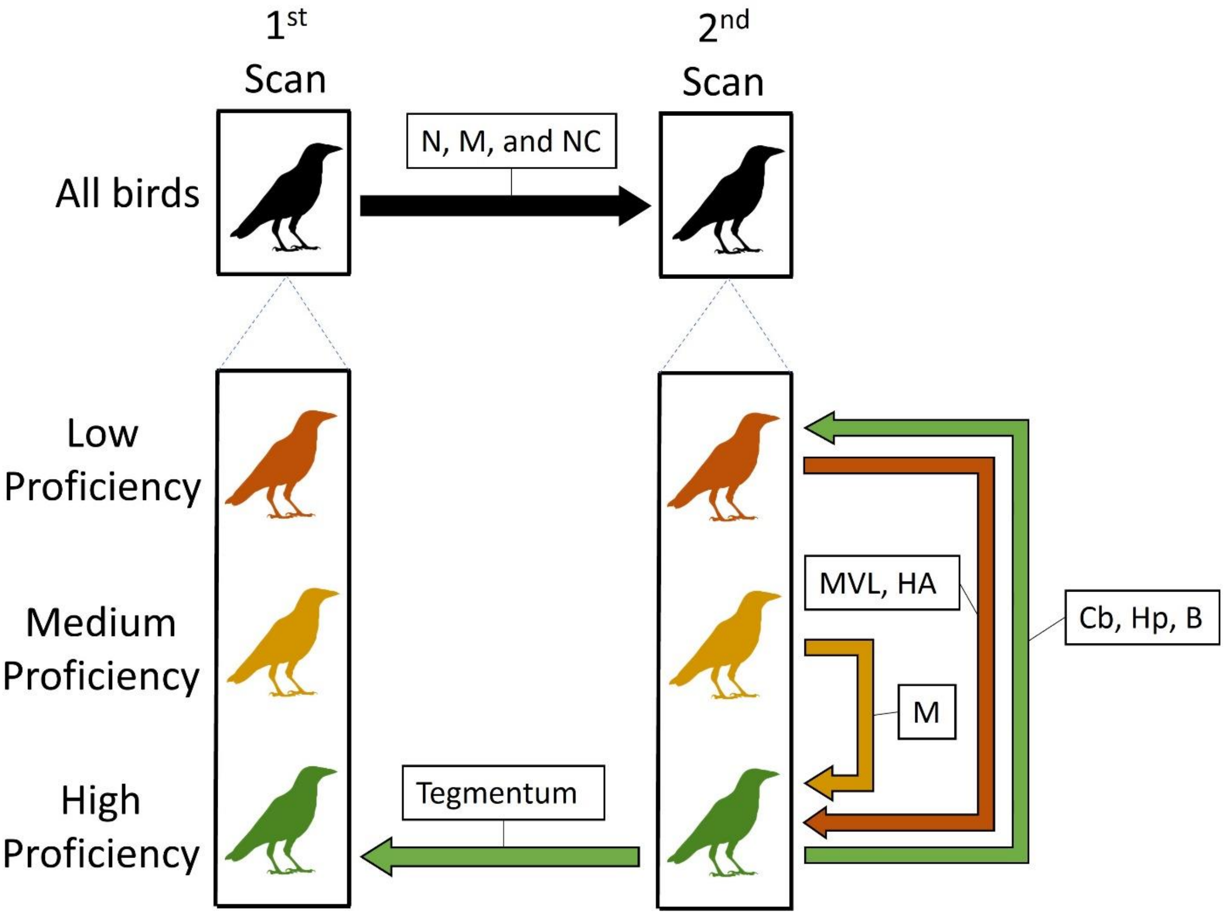


Figure 3.6. *Graphic representation of all observed differences in brain activity between the two scans and three general proficiency levels (Black: all proficiencies merged, Dark orange: low proficiency, Gold: medium proficiency, Green: high proficiency). Arrows indicate the direction of comparison; the group at the base of the arrow has greater activity within the indicated region in comparison to the group at the arrow point. B: nucleus basorostralis, Cb: cerebellum, HA: hyperpallium apicale, Hp: hippocampus, M: mesopallium, MVL: mesopallium ventro-lateralis, N: nidopallium, NC: nidopallium caudale.*

Pre- and Post-training Differential Brain Activity

I was able to acquire imaging data from only 14 of the 16 captured crows; the other two individuals (n=1 low proficiency and n=1 medium proficiency) had one of their scans invalidated by mechanical/software issues with the imaging system. I compared all proficiency groups between and within the pre-training and post-training scans; see Fig. 3.6 for a visual representation of all the differences I observed.

Compared to their post-training scan, the crows showed three distinct brain areas of significantly higher FDG uptake during their pre-training scan, regardless of their eventual proficiency with the task. The most widespread activity extended dorsally throughout much of the right hemisphere mesopallium (7.4% more activity, Z= 4.82, Fig. 3.7); this activity extended caudally through the mesopallium into the dorsal nidopallium (7.2% more activity, Z= 5.24, Fig.

3.7), medially into the nidopallium caudale (6.9% more, Z= 4.31, Fig. 3.7) and almost reaching the border of the arcopallium. This increased activity was also present to a more limited degree within the left hemisphere but was only significantly greater within the mesopallium (5.1% more, Z= 4.27, Fig. 3.7). The second region of increased activity was also located within the mesopallium, but more rostral to the previous region. This activity was bilateral within both hemispheres and was located laterally within the mesopallium (left hemisphere: 8.2% more, Z= 4.53; right hemisphere: 8.6% more, Z= 4.22, Fig. 3.7) near the border with the nidopallium. The final region of significantly increased activity extended along the medial-lateral axis of the right hemisphere nidopallium caudale (7.8% more activity, Z= 4.57, Fig. 3.7); this activity was also present in a more limited degree within the left hemisphere nidopallium caudale (8.9% more activity, Z= 4.01, Fig. 3.7). There was an additional region of notably higher activity that did not exceed the Z-threshold of 4.0; it was also located within the nidopallium caudale, though more rostrally to the previous region and primarily limited to the left hemisphere (8.2% more, Z= 3.80, Fig. 3.7). See Figure 3.14 in the supplemental material for differential activity patterns throughout the entire brain.

In contrast, I found no areas of increased activity during the crows' post-training scan (compared to their pre-training scan) for the entire group of crows. However, when I subdivided the crows into their three task proficiency groups, I found that the high proficiency birds showed significantly increased activity medially within their left hemisphere tegmentum after their training (8.2% more, Z= 4.16). See Figure 3.15 in the supplemental material for differential activity patterns throughout the entire brain.

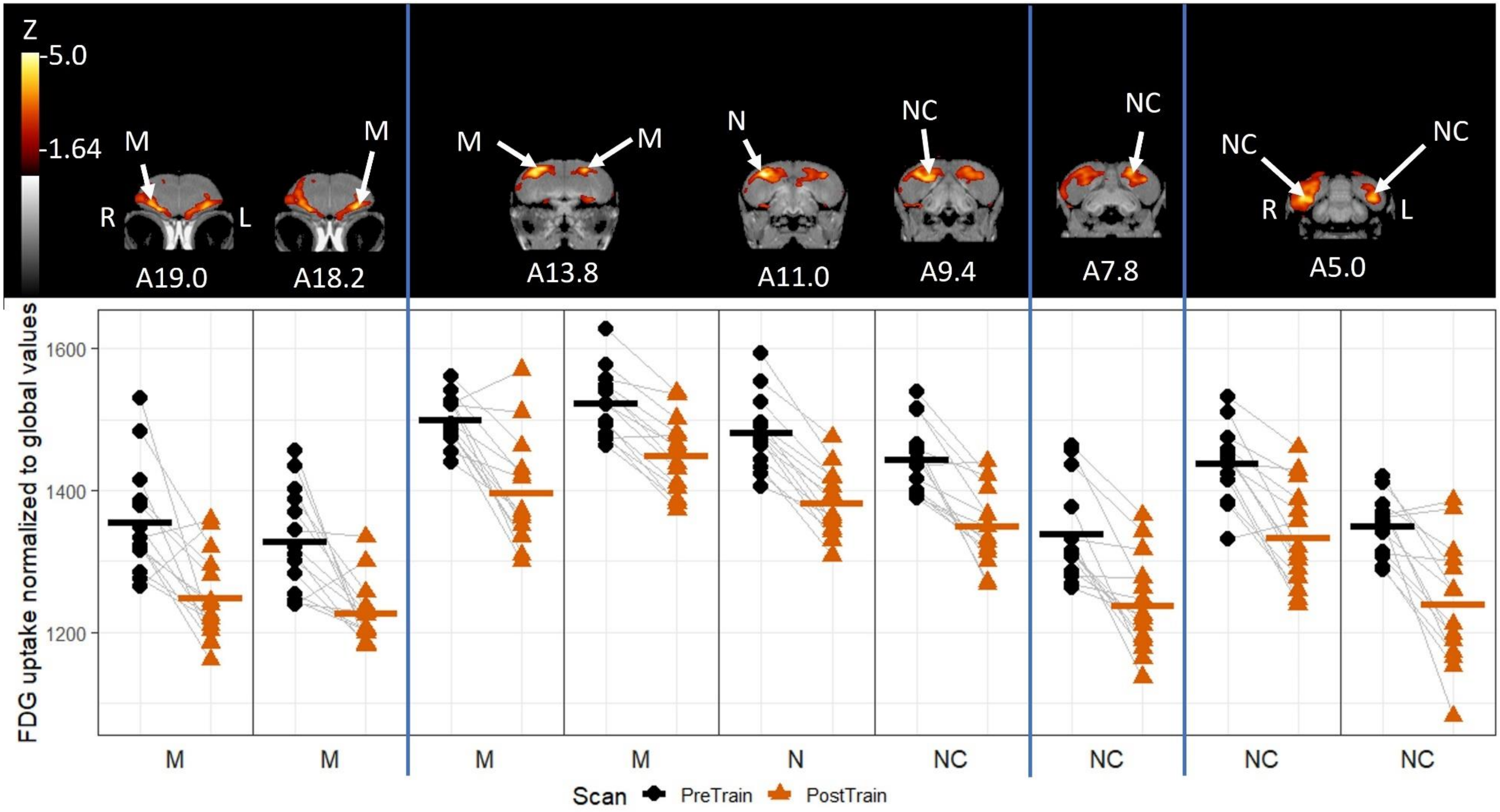


Figure 3.7. ***Top:*** *Coronal view of voxel-wise subtractions (converted to Z-score maps) showing differential FDG uptake at the indicated region for all the crows' pre-training scan compared to their post-training scan (n=14). M: mesopallium, N: nidopallium, NC: nidopallium caudale. Z-score map is superimposed atop a composite (n=4) structural MRI of the American crow brain. Slices are arranged from most rostral to most caudal, and slice coordinates refer to the Izawa & Watanabe (2007) jungle crow atlas.* ***Bottom:*** *individual normalized (global) uptake values obtained from VOI's centered on peak activation coordinates. Horizontal lines indicate group means. Vertical blue lines separate distinct regions of activity. Note that the activity at A7.8 within the nidopallium caudale did not exceed the Z-threshold for statistical significance.*

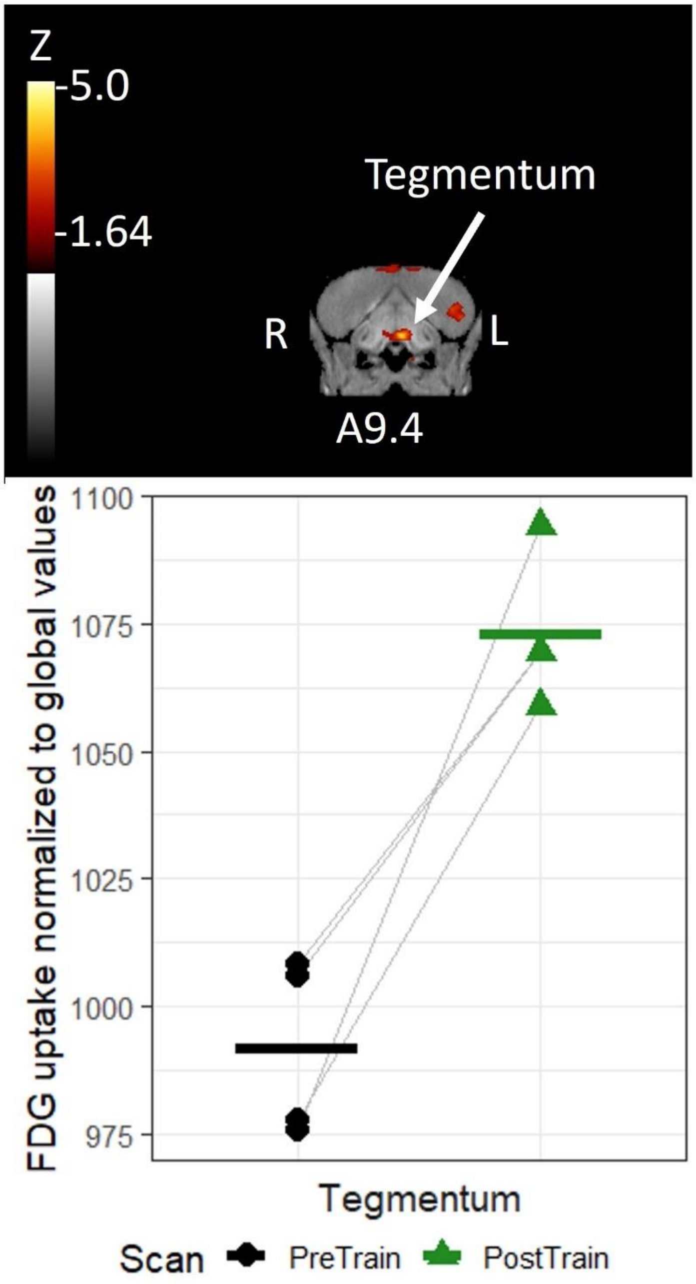


Figure 3.8. ***Top:*** *Coronal view of voxel-wise subtractions (converted to Z-score map) showing differential FDG uptake at the indicated region for the high task proficiency crows' post-training scan compared to their pre-training scan (n=4). Z-score map is superimposed atop a composite (n=4) structural MRI of the American crow brain. Slice coordinates refer to the Izawa & Watanabe (2007) jungle crow atlas.* **Bottom:** *individual normalized (global) uptake values obtained from VOI's centered on peak activation coordinates. Horizontal lines indicate group means.*

Proficiency-based Differential Brain Activity

After training, crow proficiency at solving the Aesop task was associated with differences in brain activity. Compared to the birds with low proficiency, crows that were highly proficient at solving the task showed significantly more activity within the right hemisphere hippocampus (9.1% higher activity, Z= 4.44, Fig 3.9) and dorsally within both hemispheres of the cerebellum (left: 9.8% more activity, Z= 4.89; right: 15% more activity, Z= 4.23, Fig 3.9), along with notably higher activity in the left hemisphere nucleus basorostralis (3.3% more activity, Z= 3.77, Fig 3.9) upon seeing the Aesop task during their post-training scan. By contrast, low proficiency crows showed notably more activity within the left hemisphere mesopallium, adjacent to (and possibly including) the mesopallium ventro-lateralis (MVL) (10.3% more activity, Z= 3.92, Fig 3.10), and the left hemisphere hyperpallium apicale (21.6% more, Z= 3.79, Fig 3.10) compared to the high proficiency birds, though neither region exceeded the Z-threshold for statistical significance (Z= 4.0). Finally, crows with medium proficiency had notably more activity laterally within the left hemisphere mesopallium (23.9% more activity, Z= 3.90, Fig 3.11) compared to the highly proficient birds. See Figs. 3.16, 3.17, and 3.18 in the supplementary material for differential brain activity throughout the entire brain for each comparison.

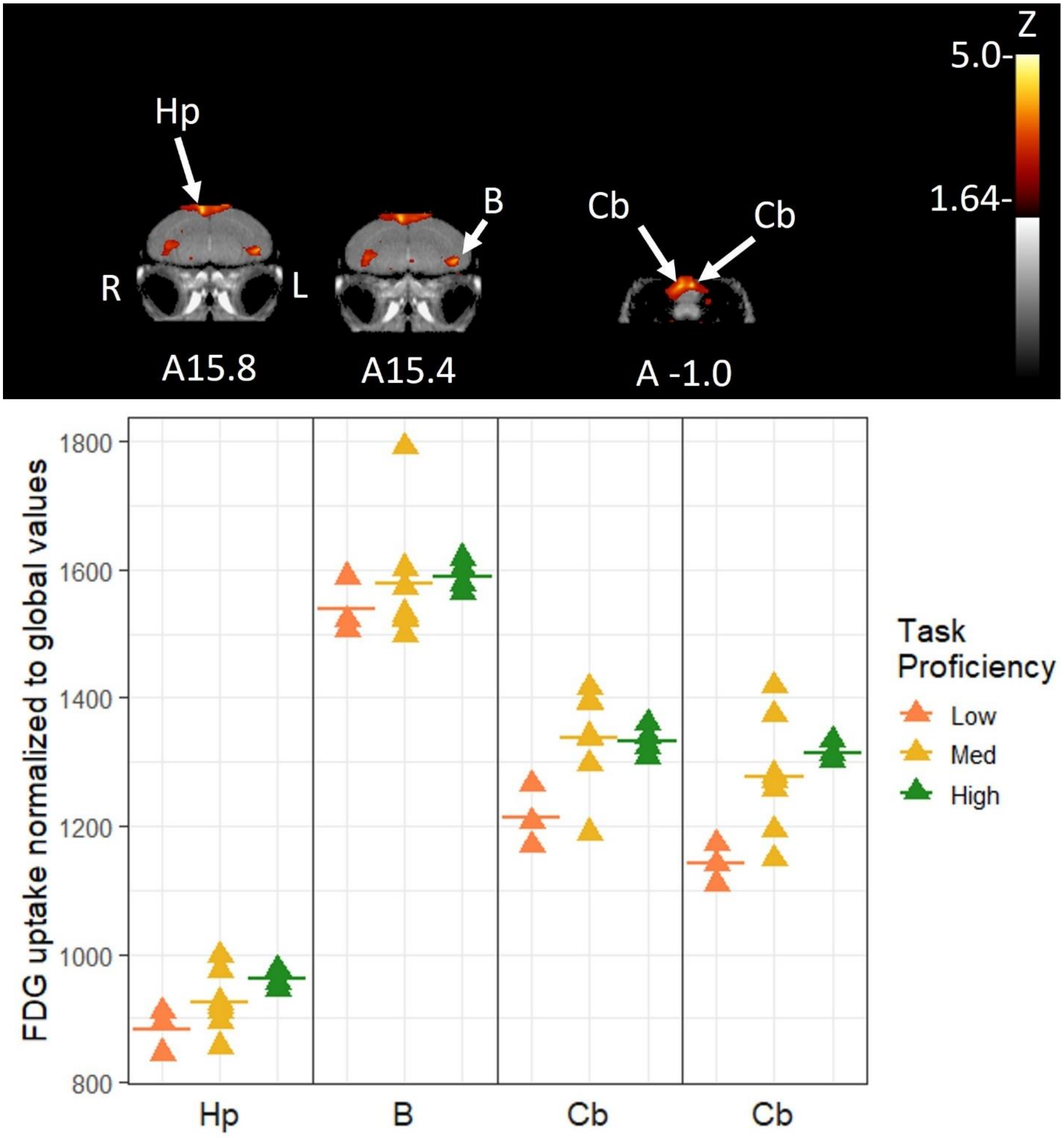


Figure 3.9. ***Top:*** *Coronal view of voxel-wise subtractions (converted to Z-score maps) showing differential FDG uptake at the indicated region for the high proficiency crows' post-training scan compared to the same for low proficiency birds (n=7). Hp: hippocampus, B: nucleus basorostralis, Cb: cerebellum. Z-score map is superimposed atop a composite (n=4) structural MRI of the American crow brain. Slices are arranged from most rostral to most caudal, and slice coordinates refer to the Izawa & Watanabe (2007) jungle crow atlas, although the peak activity within the cerebellum is more caudal than the atlas border (it is to scale)***. Bottom:*** *individual normalized (global) uptake values from all crows (n=14) obtained from VOI's centered on peak activation coordinates. Horizontal lines indicate group means. Note that the nucleus basorostralis did not exceed the Z-threshold for statistical significance.*

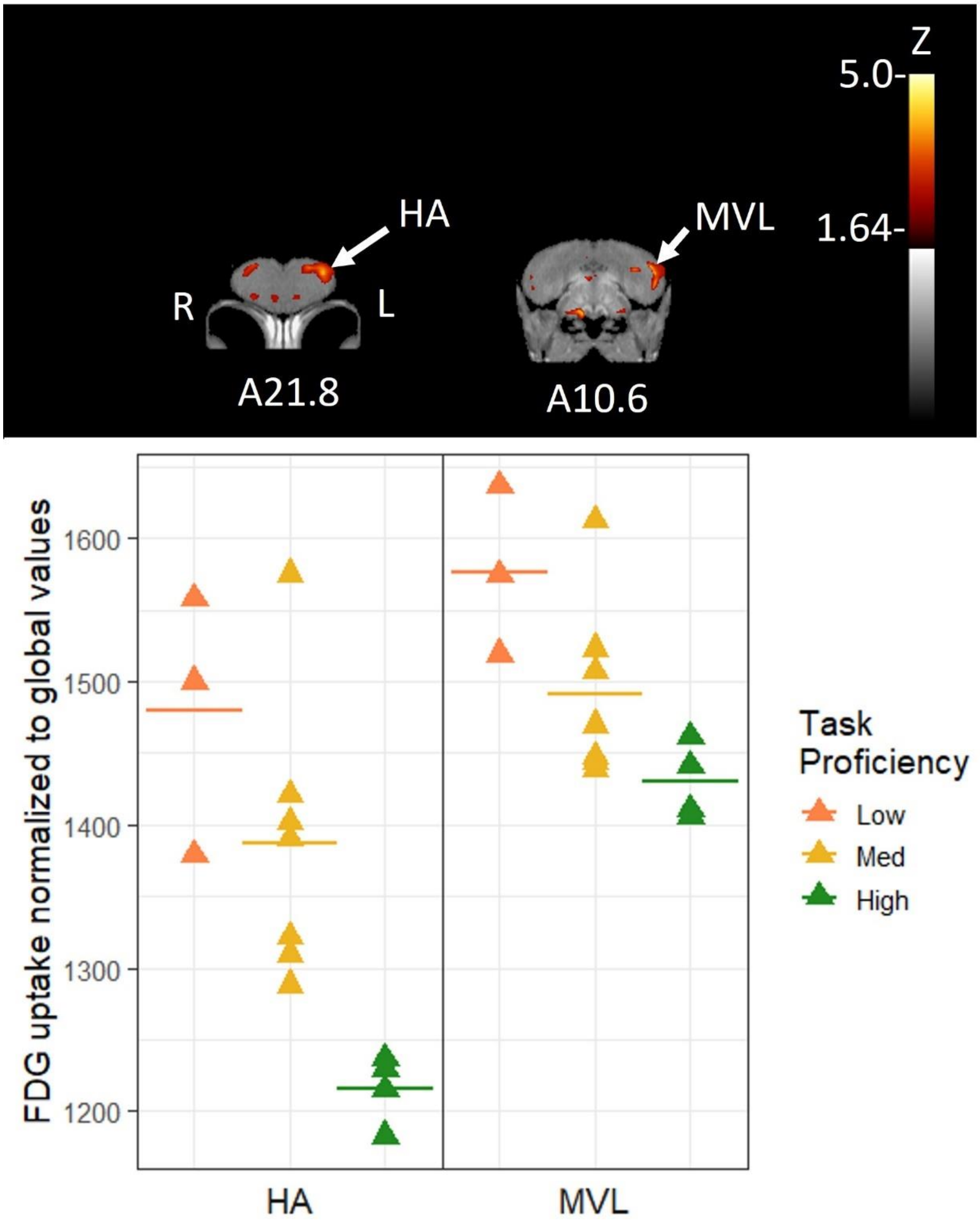


Figure 3.10. ***Top:*** *Coronal view of voxel-wise subtractions (converted to Z-score maps) showing differential FDG uptake at the indicated region for the low proficiency crows' post-training scan compared to the same for high proficiency birds (n=7). HA: hyperpallium apicale, MVL: mesopallium ventro-lateralis. Z-score map is superimposed atop a composite (n=4) structural MRI of the American crow brain. Slices are arranged from most rostral to most caudal, and slice coordinates refer to the Izawa & Watanabe (2007) jungle crow atlas.* ***Bottom:*** *individual normalized (global) uptake values from all crows (n=14) obtained from VOI's centered on peak activation coordinates. Horizontal lines indicate group means. Note that neither region exceeded the Z-threshold for statistical significance.*

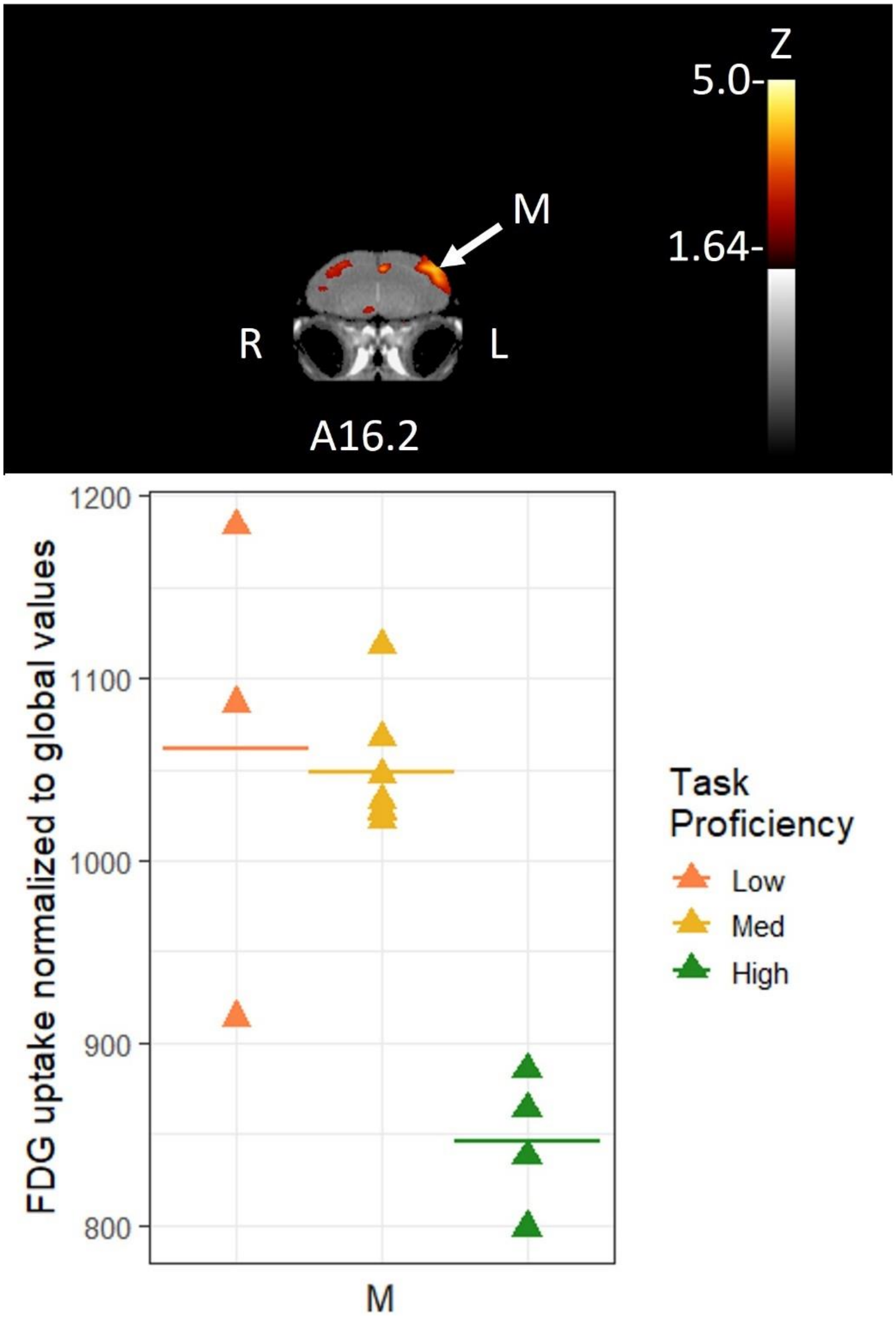


Figure 3.11. ***Top:*** *Coronal view of voxel-wise subtractions (converted to Z-score map) showing differential FDG uptake at the indicated region for the medium proficiency crows' post-training scan compared to the same for high proficiency birds (n=11). M: mesopallium. Z-score map is superimposed atop a composite (n=4) structural MRI of the American crow brain. Slice coordinate refers to the Izawa & Watanabe (2007) jungle crow atlas.* ***Bottom:*** *individual normalized (global) uptake values from all crows (n=14) obtained from VOI's centered on peak activation coordinates. Horizontal lines indicate group means. While notable, this region did not exceed the Z-threshold for statistical significance.*

Stimulus Phase Behavior

I was unable to obtain gaze and blink rate data from two birds' 1st scan (n=1 from low proficiency and n=1 from medium proficiency groups) and a third bird's 2nd scan (high proficiency) due to their eyes remaining out of the camera's field of view throughout the stimulus phase.

The crows visually attended to the Aesop tube whenever it was revealed, gazing into the stimulus stage for much of the time (left eye: mean ± SD; 383.2 ± 37.5 s; right eye: 368.7 ± 49.6 s) that it was visible to them (420 s) without favoring one eye over the other (t_{24}= 1.45, P= 0.16) and with no significant difference in gaze time between the two scans (left eye: t_{23}= 0.44, P= 0.66; right eye: t_{23}= 0.33, P= 0.74). Their mean blink rate remained steady (23.38 ± 8.12 blinks/min) and did not change between each scan (t_{23}= 0.30, P= 0.77). Although a single outlier individual from the low proficiency group paced constantly and moved more than five times the amount of any other crow (398 movements), the remaining birds moved substantially less often during the stimulus phase (without outlier: 21.73 ± 22.78 movements, max= 75). See Fig. 3.19 in the supplementary material for behavioral changes between scans and general proficiencies.

DISCUSSION

Tool use is evidently a difficult concept for captive American crows to learn; task proficiency was not universal, high proficiency birds required several weeks of training to reach their level of proficiency, and some crows never progressed beyond low proficiency. Yet, despite this difficulty, some crows successfully adopted tool use to solve the task. These high proficiency individuals shared important features in common; they were exclusively adults and primarily females. This sex bias in tool use is consistent with other animals. Additionally, high proficiency crows relied more heavily on neural regions associated with muscle memory when they encountered the task, as opposed to the higher order cognitive regions favored by their less proficient peers; this shift in active brain regions is comparable to the changes in human brain activity after mastering a skill.

Increased higher-order brain activity for naïve and less proficient crows

Crows were first exposed to the Aesop tube task during their pre-training scan, and they showed significantly increased FDG uptake, a surrogate of brain activaty, throughout their mesopallium, nidopallium, and nidopallium caudale (regardless of their later proficiency) compared to after spending weeks regularly interacting with the task. This naïve activity was nearly universal among all crows, regardless of their eventual task proficiency. The low and medium proficiency birds continued to show elevated activity in their mesopallium during their second scan (compared to the high proficiency birds), although the activity of the low proficiency birds varied greatly compared to the medium and high proficiency groups. All three regions are part of the avian pallium and are known to be involved with higher-order cognitive ability. The mesopallium is an associative forebrain region that does not receive any direct sensory information and is correlated with behavioral innovation and flexibility (Timmermans et al. 2000). The nidopallium and its various sub-regions, such as the nidopallium caudolaterale (NCL), have been compared to the mammalian prefrontal cortex in controlling executive function, working memory, planning, flexible thinking, and attending objects of interest, particularly if the object is associated with a reward (Güntürkün 2005, Herold et al. 2011, Kasties et al. 2016). Although some of this activity may be caused by the neophobic crows reacting to the novelty of the apparatus (particularly the nidopallium caudale, Cross et al. 2013), this trend of using higher-order cognitive circuits continued into the post-training scan for some of the less knowledgeable birds, even after they'd spent weeks interacting with the task. This suggests that these birds were still attempting to decipher how to retrieve food from the apparatus.

Increased activity in memory, motor, and tactile regions for high proficiency crows.

After they learned to fully solve the Aesop task, high proficiency crows showed increased activity in their cerebellum, hippocampus, and nucleus basorostralis during their post-training scan compared to the low proficiency crows. The hippocampus is strongly associated with memory (Clayton 1998), as is the cerebellum (Katajamaa et al. 2021). The cerebellum is additionally associated with motor learning, motor control, and perception (Ito & Ito 1984, Paulin 1993). The nucleus basorostralis is a somatosensory region associated with processing sensory information from the bill (Cunningham et al. 2013, Wild 2015). Additionally, high proficiency crows showed increased activity within their tegmentum after they mastered the task compared to their naïve brain activity. The tegmentum is generally involved with motor control, though it also contains sensory nuclei and autonomous control centers (Nieuwenhuys et al. 1998).

The activity in the memory, motor, and tactile regions suggest that much like the mental prep of a ski racer entering the starting gate- visualizing a path through the course, the location of each bump and obstacle, the areas to speed up or slow down – the high proficiency birds were mentally preparing to enter the stimulus stage and solve the task. This is further reflected in how the two groups behaviorally interacted with the task in the weeks preceding their second imaging session. While the low proficiency birds occasionally attempted to retrieve the food (without interacting with the stones), they usually gave up after a few attempts and retreated to the training cage for the remainder of the training session. By contrast, the high proficiency crows quickly, consistently, and repeatedly picked up and dropped stones into the tube to bring the food within reach. This deliberate and repetitive action is highly conducive to memory formation and motor learning (Magill & Anderson 2010). Unlike the consistent activity of the high and low proficiency groups, the medium proficiency crows showed a large degree of variability in activity in the cerebellum, hippocampus, and nucleus basorostralis. This is likely because these birds varied in their interactions with the task; some medium proficiency birds quickly gave up their attempts to retrieve the food after determining it was beyond their reach, while others persisted in repeatedly attempting to retrieve the out-of-reach food. This persistence was reinforced by the occasional success because I lowered the task difficulty if the crow failed to solve for three sessions in a row.

Executive planning becomes muscle memory

These findings partially support my *a priori* hypotheses of which regions would be more active for the high proficiency crows compared to the less proficient crows; highly proficient birds did utilize their hippocampus and tegmentum compared to less proficient (or naïve) birds, yet the mesopallium and NCL were primarily active in *naïve* individuals as opposed to proficient crows. Why did the brain activity of tool-using crows favor motor, tactile, and memory processing instead of higher-order cognitive processing? While naïve crows were still determining what the Aesop apparatus was and whether it posed a threat to them (a relatively demanding cognitive process), the knowledgeable and highly proficient individuals had been consistently retrieving food from it during each of their training sessions before the imaging session. These results suggest that the high proficiency crows no longer devote higher-order cognitive power to the task; they already knew how to solve it.

The changing neural activity of the crows as they progressed from naïve to varying degrees of proficiency with tools is similar to the differential neural activity observed between novice and elite human athletes (Kim et al. 2014, Callan & Naito 2014). Compared to more experienced athletes, novices have greater activity in the inferior frontal gyrus, superior frontal gyrus, and prefrontal cortex, regions which have been likened to the avian nidopallium (Güntürkün 2005). However, as the athletes become more skilled, their neural activity shifts away from executive function to supplementary motor areas and the cerebellum. Evidently, highly proficient human athletes and crow tool-users both rely more on muscle memory instead of executive planning once they have overcome challenges in their respective areas of expertise.

Increased activity in visual associated regions for the low-proficiency birds

Compared to their high proficiency conspecifics, the low proficiency crows showed greater activity in the hyperpallium apicale and MVL, both of which are associated with vision. The hyperpallium apicale is homologous to the mammalian visual cortex and appears to be involved with spatial orientation (Barkan et al. 2017, Medina & Reiner 2000). The MVL receives projections directly from the entopallium and is involved with categorizing an object and processing its form, color, and motion (Anderson et al. 2020, Stacho et al. 2016). While Anderson et al. (2020) noted that the MVL was the only visual region they examined without a strong left-hemisphere bias, I observed a strong left-hemisphere bias for *both* the MVL and hyperpallium apicale. The activity in these two regions was proportional to the proficiency level of each group; it was highest for the lowest proficiency group, intermediate for the medium proficiency group, and lowest for the high proficiency crows (Fig. 3.10). Unlike the other proficiency groups, the low proficiency birds were rarely attempting to solve the task during the training sessions preceding their second imaging session, thus these results may be due to the low proficiency crows visually attending to the Aesop task without any intention of interacting with it.

Lateralization of activity

Avian brains are highly lateralized (Mench & Andrew 1986), and I found differing levels of hemispherical bias in the active regions of this study, which are consistent with previous studies using American crows and PET imaging (Pendergraft et al. 2021). I found no difference in the crows’ gaze direction (Figure 3.19 in supplemental materials), thus this hemispherical bias in activity cannot be explained from asymmetrical sensory input. Low proficiency birds showed a left hemisphere bias in hyperpallium apicale and MVL activity, which is consistent with previous research demonstrating that left-hemisphere visual object processing is dominant in the left hemisphere (Rogers 1996). Research on domestic chicks shows that the right hemisphere hippocampus is more sensitive to geometric spatial information (Tommasi et al. 2003); this could indicate that the crows were mentally recalling their direction of approach and position of the apparatus when they interacted with the Aesop task during their training in the aviary. Finally, pigeons with experience in long distance navigation show increased volume in their right hemisphere mesopallium and nidopallium compared to pigeons without experience (Mehlhorn et al. 2010b); this bias in favor of the right hemisphere for cognitively demanding challenges supports my own findings.

High proficiency crows all adults and mostly female

The most important factor in predicting which birds would fully master the task was age; while half of the adult crows mastered the task, none of the subadults did. Why are adult crows better at solving the task compared to subadults? One explanation is that an older crow has had more opportunities to encounter challenges during its life (especially in a highly variable urban or suburban environment) which provides them with an advantage in learning novel tasks. For example, although it’s highly unlikely that any of the crows had prior experience with the Aesop task itself (none spontaneously solved, all required multiple weeks of training), it is more likely that some have already learned the concept of picking up/dropping objects for a food reward via the proto-tool foraging practice of dropping hard-shelled items (such as eggs, nuts, or bivalves) onto a surface to crack them open. By this logic, older adults should be more likely to solve the task than younger adults, which may explain why some of the adults failed to solve the task. Unfortunately, I could not determine an adult crow's exact age, only that it was in its third year of life or older (Emlen 1936), therefore I cannot examine this hypothesis further.

Female adult crows were more proficient at solving the task than were males. The three adult females that solved the task represented 100% of that demographic, whereas the single male in this proficiency group represented only 20% of the adult male demographic in my sample group. Body size and absolute brain volume are also correlated with adult task proficiency, yet this relationship likely stems from the strong correlation between sex, culmen length, and brain volume (Table 3.1); female American crows are documented to be smaller than males (Clark et al. 1991) and smaller animals tend to have correspondingly smaller brains (Tsuboi et al. 2018). Relative brain volume corrects for this allometry, and that variable shows no relationship with task proficiency (Table 3.3, Fig. 3.13 in supplementary materials).

Why are small adult females so proficient at solving the Aesop task? In wild populations, female crows are typically subordinate to male crows, primarily because of the size difference between the sexes. As a result, the larger males can often simply rely on their dominant status resulting from their size and strength to gain access to resources, whereas the smaller females must intelligently select an appropriate strategy from a large repertoire to compete. For example, when many crows gather around an ephemeral food source, dominant males can immediately access the food by simply displacing their smaller conspecifics, whereas the smaller crows are forced to utilize more cognitively complex strategies; they can patiently wait until all the larger birds become sated and depart, they can attempt to recruit allies to collectively displace the more dominant individuals (Heinrich & Marzluff 1991), they can dart in to grab food while the dominant birds are distracted, they can attempt to steal food from a satellite conspecific which has succeeded in acquiring food from the main source, they can mentally calculate whether it would be more efficient to leave in search of a different source of food, etc. Despite being the smallest birds in my sample group, three of the four high proficiency crows had relatively high body condition upon capture (Table 3.5 in supplementary materials), suggesting that they were better able to creatively identify (or create) successful strategies and capitalize upon them in the wild compared to their peers. This is further supported by other species with a female bias for tool use; female chimpanzees (*Pan troglodytes*), bonobos (*Pan paniscus*), and bottlenose dolphins (*Tursiops sp.*) are both smaller than males (Plavcan 2012, Samuels & Gifford 1997) and more likely to use tools (Gruber et al. 2010, Smolker et al. 1997).

Although I hypothesized that the level of nervousness, body condition, and relative brain volume may also play a role in influencing a crow's eventual task proficiency, I did not observe any meaningful effect of those factors on tool proficiency. My experimental design may have minimized any effect these factors may otherwise have had on tool proficiency. The habituation process and lengthy amount of time each crow was given to interact with the task during their training sessions may have allowed even the most nervous crows ample opportunities to interact with the task. I calculated the crows' body condition using their weight upon capture, yet they had regular access to food in the days between their capture and their first imaging session, which meant the "low body condition" crows had likely improved their body condition by the time they began learning the task. Finally, although brain volume has been found to correlate with the ability to solve cognitive tasks (Horschler et al. 2019), other factors such as neuronal count, neuronal density, relative size of specific regions, or relative complexity of specific circuits within the brain might have been better measures of cognitive ability (Healy & Rowe 2006, Jardim-Messeder et al. 2017, Olkowicz et al. 2016).

Shrinking brain volume?

I was surprised to discover that the crow's brain volumes had declined during their time in captivity. Some birds show seasonal plasticity in the volume of certain brain regions, such as the hippocampus (Guigueno et al. 2016, Smulders et al. 1996). However, the reduction in volume seems too large (5.2% ± 2.7%, max= 9.15%) to be the result of plasticity within a single region. It's possible that the relative lack of mental stimulation or physical exercise during their captivity, compared to what wild crows regularly experience, may be driving this reduction in brain volume (Balcarcel et al. 2021, Laberge et al. 2021, Tarr et al. 2009).

Caveats

Crows are social animals that are capable of social learning, such as discovering novel sources of danger by observing conspecifics mob the subject (Cornell et al. 2011). It's possible that teaching the crows to solve the task in isolation was not the most conducive way to train crows. However, previous research has shown that crows do not easily learn to solve tasks using social learning, although they will use it to refine their technique (Pendergraft et al. 2020).

Additionally, PET imaging results are susceptible to confounding influence from the subject's physiology and mental state. FDG is an analogue for glucose, so anything that affects the body's metabolism – blood sugar, body mass, tissue inflammation, healing injuries, etc. – can impact the amount of FDG absorbed by the brain (Bhargava et al. 2011, Sprinz et al. 2018). If the animal is especially frightened or stressed, their mental state will redirect FDG towards the corresponding brain regions instead of the circuits that would normally be activated by the stimulus. I attempted to address these factors in my experimental design; I controlled the time since last meal for all crows before they were imaged, used the bird's weight to calculate the dosage, and kept the imaging experience as consistent and stress free as possible.

Conclusion

Despite possessing the necessary mental ability and physical adaptations, American crows struggled to learn true tool use in my study. This may be because most American crows have little need or opportunity to use tools in their environment (Seed & Byrne 2010), unlike the more tool-adept New Caledonian crows (Hunt & Gray 2002). However, smaller adult females have comparably more need than the larger males to creatively examine their environment for novel ways to access otherwise inaccessible food sources, which would account for their success at solving the Aesop task. My findings support Mehlhorn et al.'s (2010a) conclusion that the enlarged regions of a New Caledonian crow's brain – the mesopallium, striatal complex, septum, and tegmentum – are heavily utilized as birds learn, practice, and master new tools in their environment, and further reveals that these regions are not all active for all stages of learning. Crows use their renowned intelligence to initially learn to use tools, but they switch to circuits associated with motor learning and memory as they grow more familiar with them. This suggests that even less cognitively advanced species can master tool use on their own if they are helped through the initial learning phase, such as when humans train other animals. Even though their common ancestors diverged more than 300 million years ago (Kapusta et al. 2017), birds and mammals show remarkably similar brain activity as they learn and master cognitively difficult tasks such as tool use.

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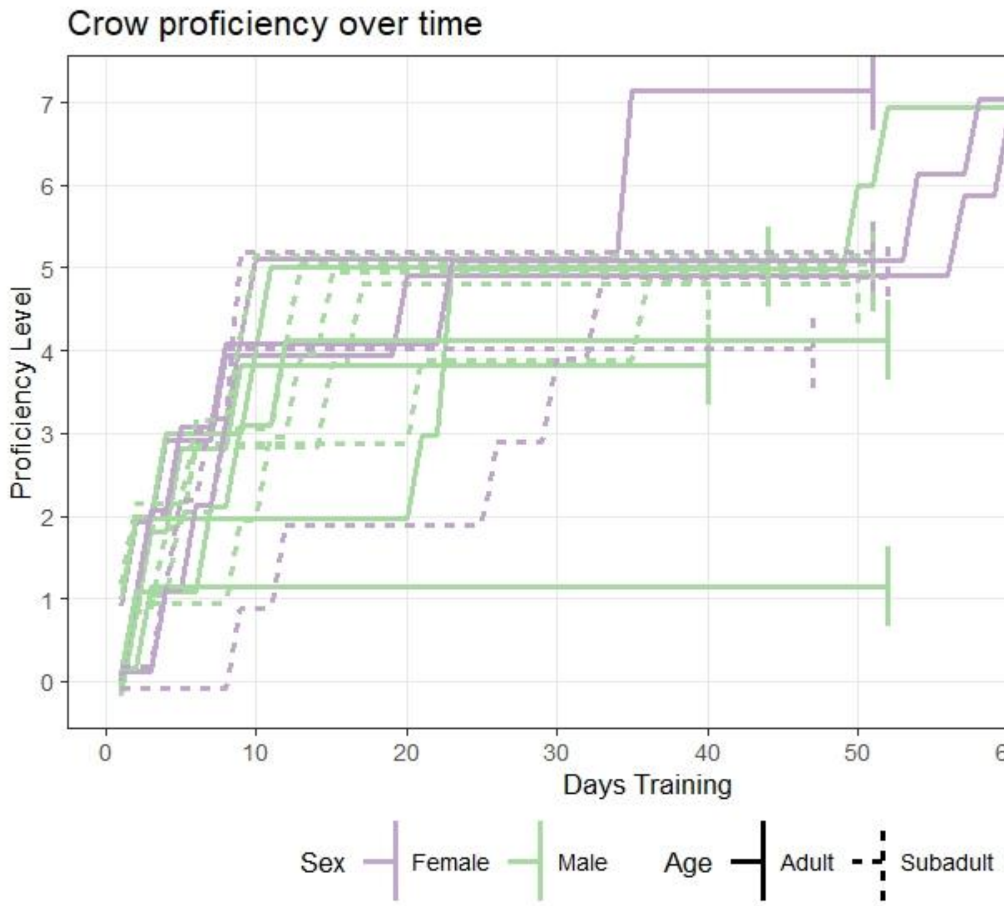
Chapter 3 Supplementary Materials

Supplementary Table 3.4. *Crow proficiency scale. Each stage within this scale is representative of a significant obstacle that crows had to overcome as they learned to solve the task. Because all (but one) of the crows were ultimately distributed among three proficiency levels (Table 3.5 in supplementary materials), I merged the seven proficiency levels into three general proficiencies: crows which never dropped any training stones (levels 1-4) were low proficiency, crows which occasionally dropped training stones while they were balanced on the tube lip but never progressed further (level 5) were medium proficiency, and crows that fully mastered the task (level 7) were high proficiency. I classified any crows which reached high proficiency as having fully solved the task.*

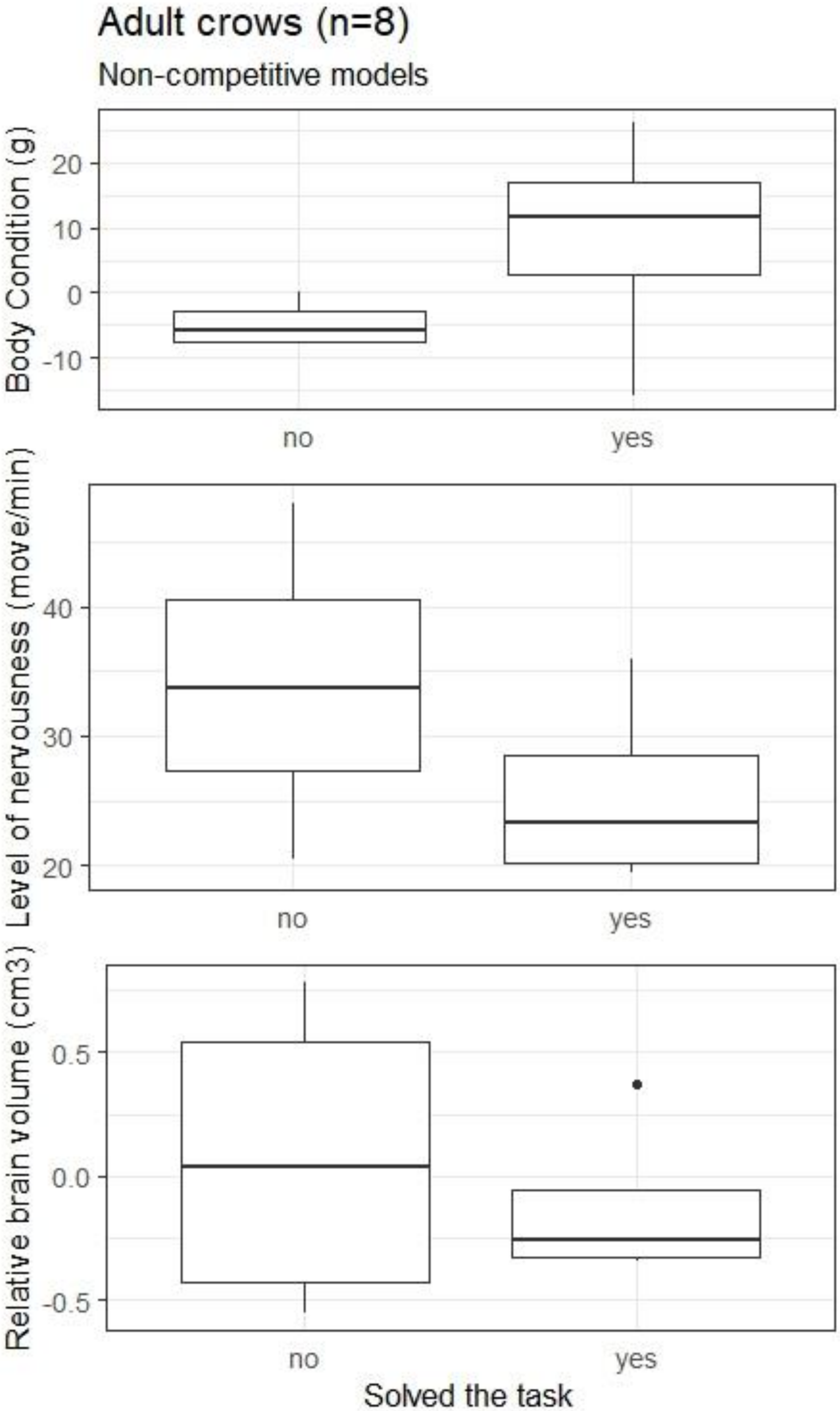
Proficiency Scale	General Proficiency	Criteria	Significance
0	None	Crows have not yet entered the training arena.	The starting point for naïve crows
1	Low	Exited training cage, entered the white arena, ate food from the ground	Crow overcame its neophobia of the training arena
2		Ate from Aesop tube w/ water level 0 mm from the top (tube filled)	Crow overcame its neophobia of the Aesop tube
3		Ate from Aesop tube w/ water level 50 mm from top	Crow overcame its reluctance to insert its head entirely inside the Aesop tube
4		Maximum reachable distance established	Maximum possible progression without dropping any stones into the tube
5	Med	Ate from the tube after dropping training stones balanced on the tube lip inside	Crow has dropped a stone into the tube, but likely did so by accident
6		Ate from the tube after lifting and dropping hanging training stones inside	Crow regularly observes that stones must be dropped inside before it can reach food
7	High	**Full mastery** - ate from the tube after lifting and dropping stones from the ground.	Crow associates dropping stones as a necessary step that must be accomplished before it can retrieve food

Supplementary Table 3.5. *Task proficiency and individual factors for all the crows used in my study.*

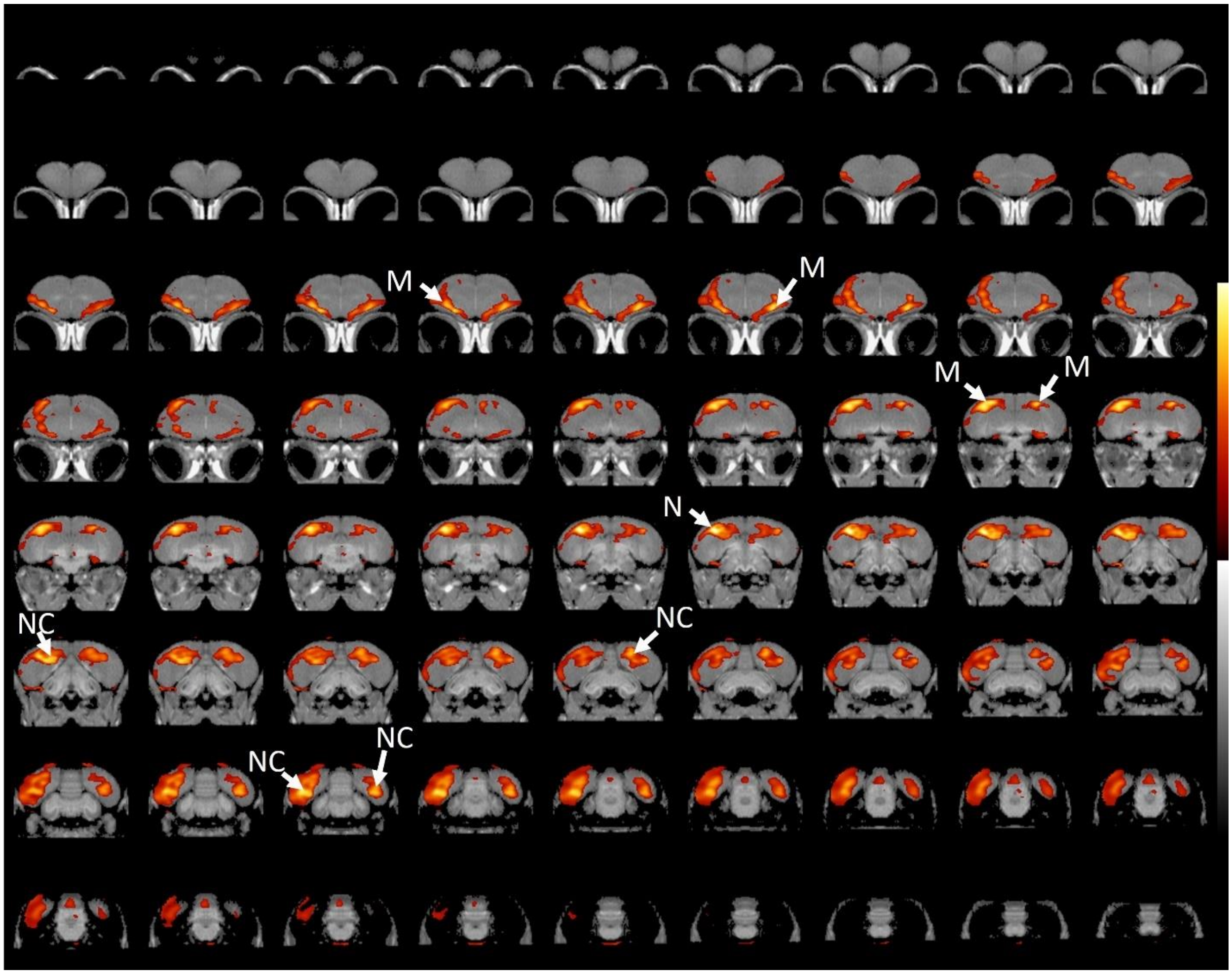
Bird ID	Prof. level	General Prof.	Age	Sex	Level of Nervous (move/min)	Culmen Length (mm)	Abs. brain volume (cm^3)	Rel. brain volume (cm^3)	Brain volume % reduction	Capture Weight (g)	Release Weight (g)	Body Condition (g)
L-Or 2017	5	Med	Subadult	Female	14	47	6.867	-0.473	5.50%	390	410	26.48
L-Re 2017	5	Med	Subadult	Male	23	52	8.191	0.009	5.15%	435	505	27.339
L-Ye 2017	5	Med	Subadult	Male	42	51	7.596	-0.418	5.58%	360	401	-38.833
R-Bl 2017	4	Low	Adult	Male	48	50	8.375	0.53	7.88%	390	425	-0.005
R-Gr 2017	7	High	Adult	Female	36	45	6.988	-0.015	7.81%	330	360	-15.864
R-Re 2017	1	Low	Adult	Male	38	51	7.754	-0.26	9.01%	395	385	-3.833
R-Sk 2017	5	Med	Subadult	Male	37.5	52	7.949	-0.233	4.51%	415	440	7.339
R-Wh 2017	5	Med	Subadult	Female	39	45	7.369	0.366	6.39%	345	360	-0.864
L-Bl 2018	4	Low	Adult	Male	29.5	52	8.882	0.7	2.46%	400	430	-7.661
L-Br 2018	5	Med	Subadult	Male	51.5	49	8.907	1.23	9.15%	370	455	-11.177
L-Gr 2018	5	Med	Subadult	Male	38.5	50	7.92	0.075	3.58%	385	435	-5.005
L-Or 2018	7	High	Adult	Female	20.5	45	6.846	-0.157	1.47%	355	385	9.136
L-Re 2018	7	High	Adult	Male	19.5	51	7.756	-0.258	1.81%	425	460	26.167
L-Sk 2018	5	Med	Adult	Male	20.5	52	7.549	-0.633	5.48%	400	420	-7.661
L-Wh 2018	4	Low	Subadult	Female	27.5	46	6.876	-0.296	0.18%	335	410	-19.692
L-Ye 2018	7	High	Adult	Female	26	45	6.835	-0.168	7.27%	360	395	14.136



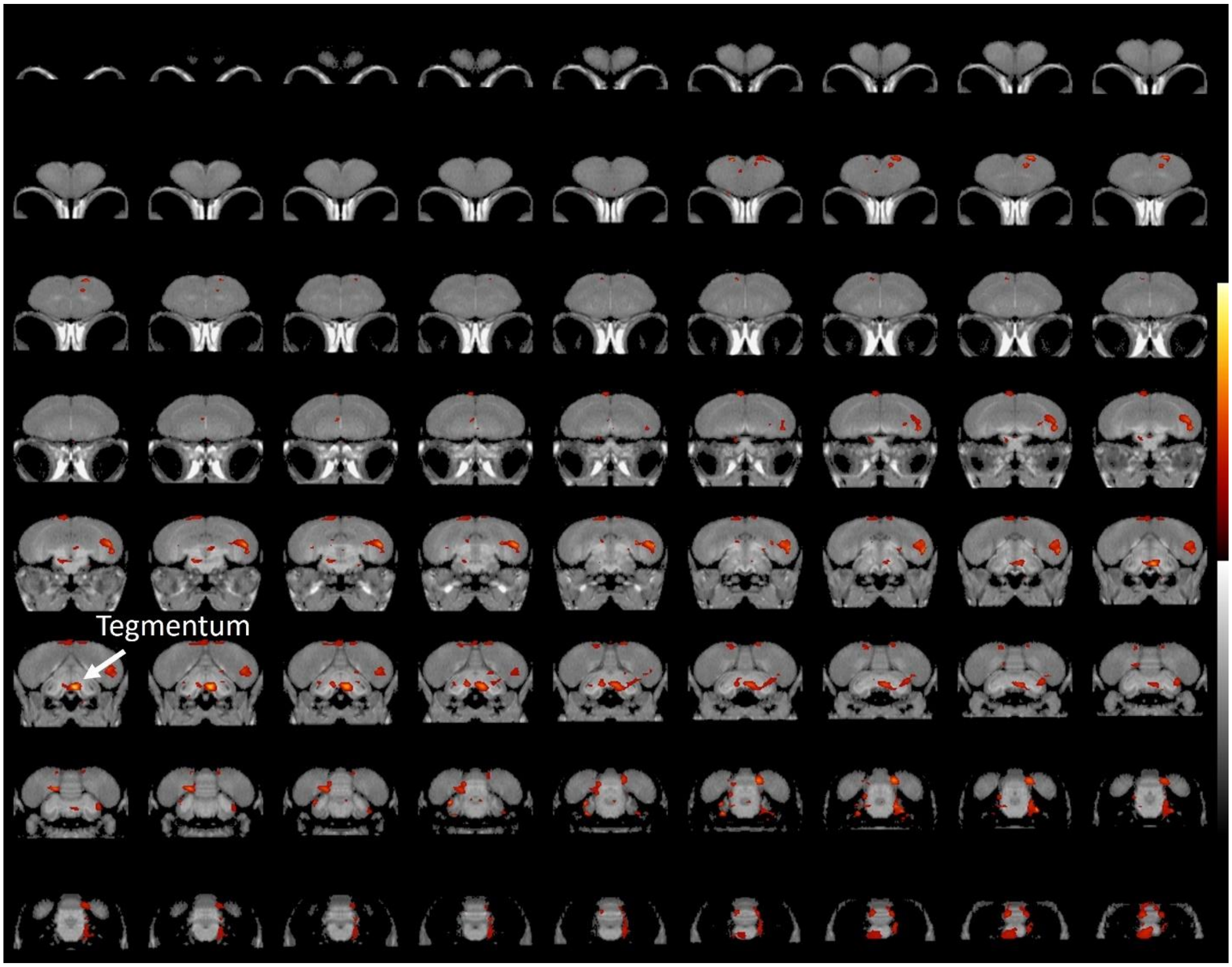
Supplementary Figure 3.12. *Crow task proficiency over time.*



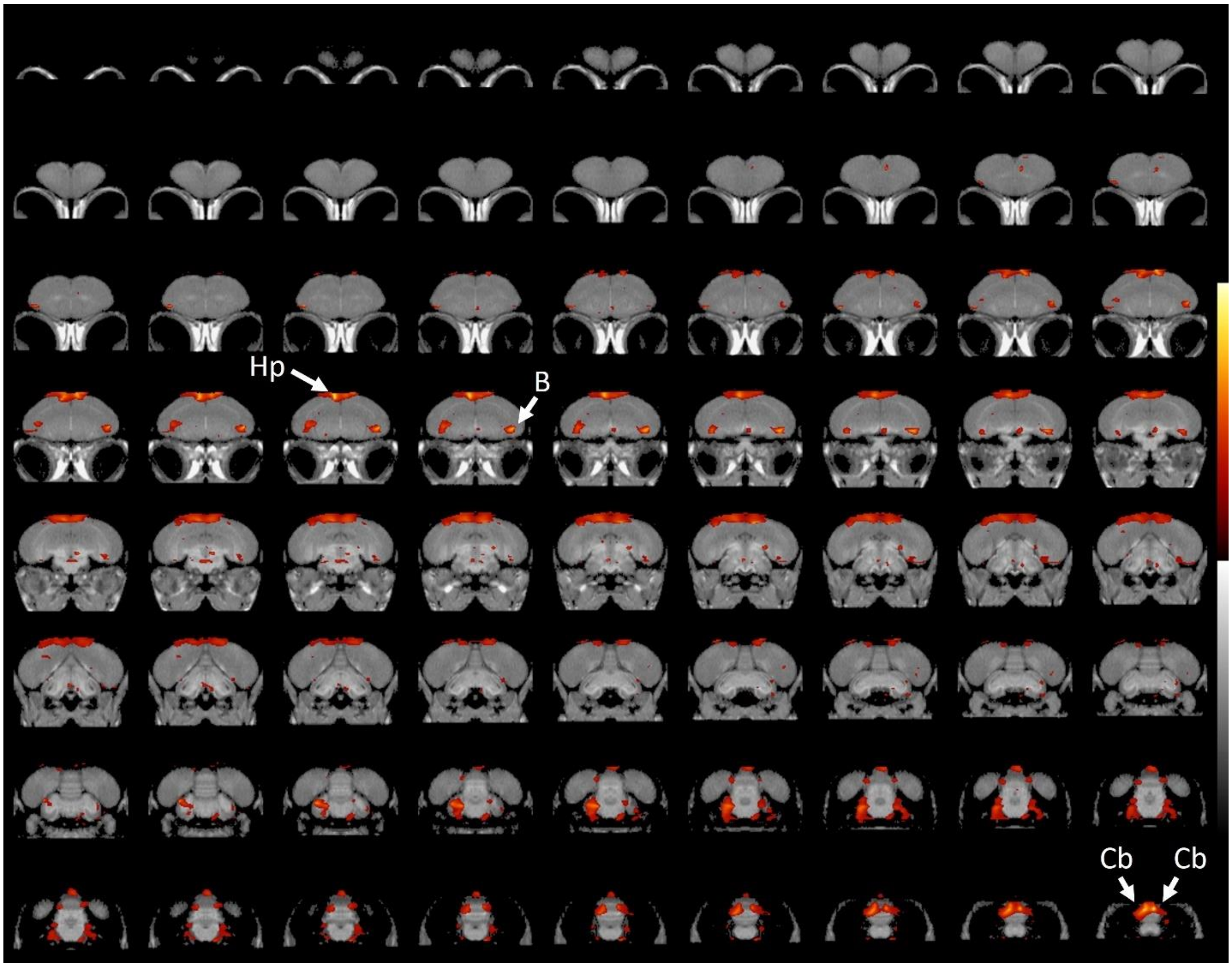
Supplementary Figure 3.13. *The remaining non-competitive variables attempting to predict which adult crows will solve the task: Top: body condition Mid: level of nervousness, Bottom: relative brain volume.*



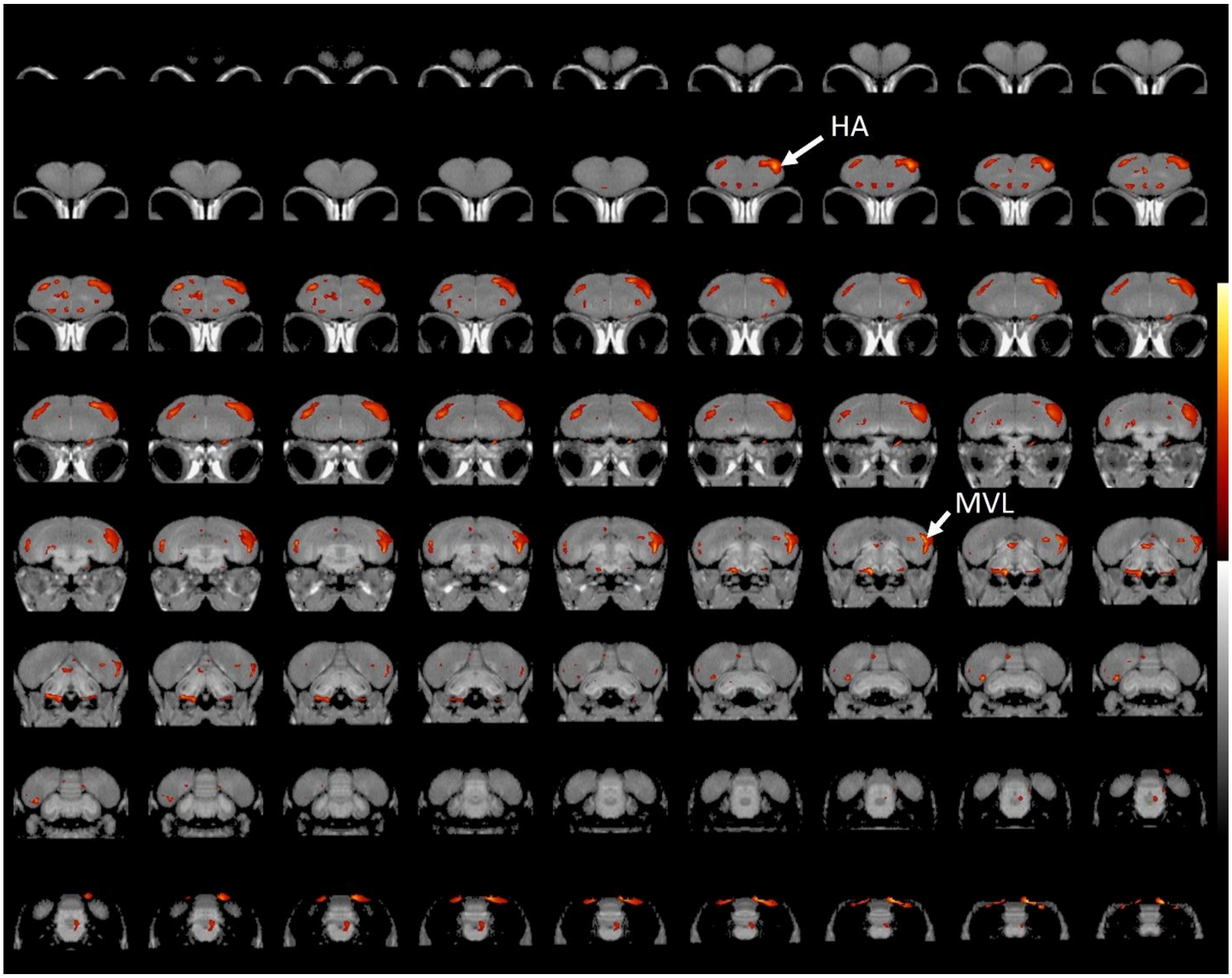
Supplementary Figure 3.14. *Coronal view of voxel-wise subtractions (converted to Z-score map) showing differential FDG uptake for all the crows' pre-training scan compared to their post-training scan (n=14). M: mesopallium, N: nidopallium, NC: nidopallium caudale. Z-score map is superimposed atop a composite (n=4) structural MRI of the American crow brain. Note that the left hemisphere NC activity at slice 49 (6th row, 5th column) did not exceed the Z-threshold for statistical significance.*



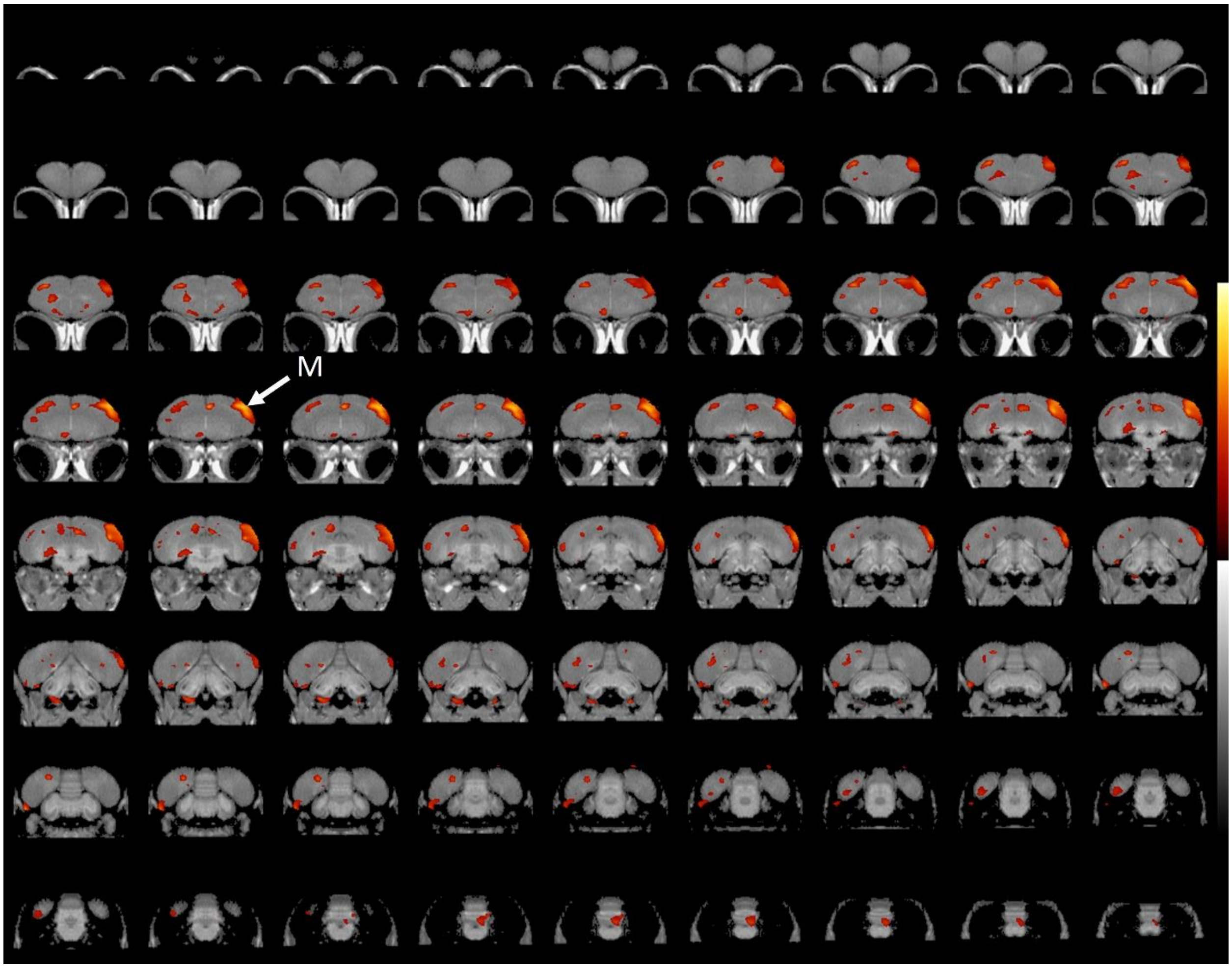
Supplementary Figure 3.15. *Coronal view of voxel-wise subtractions (converted to Z-score map) showing differential FDG uptake for the high task proficiency crows' post-training scan compared to their pre-training scan (n=4). Z-score map is superimposed atop a composite (n=4) structural MRI of the American crow brain.*



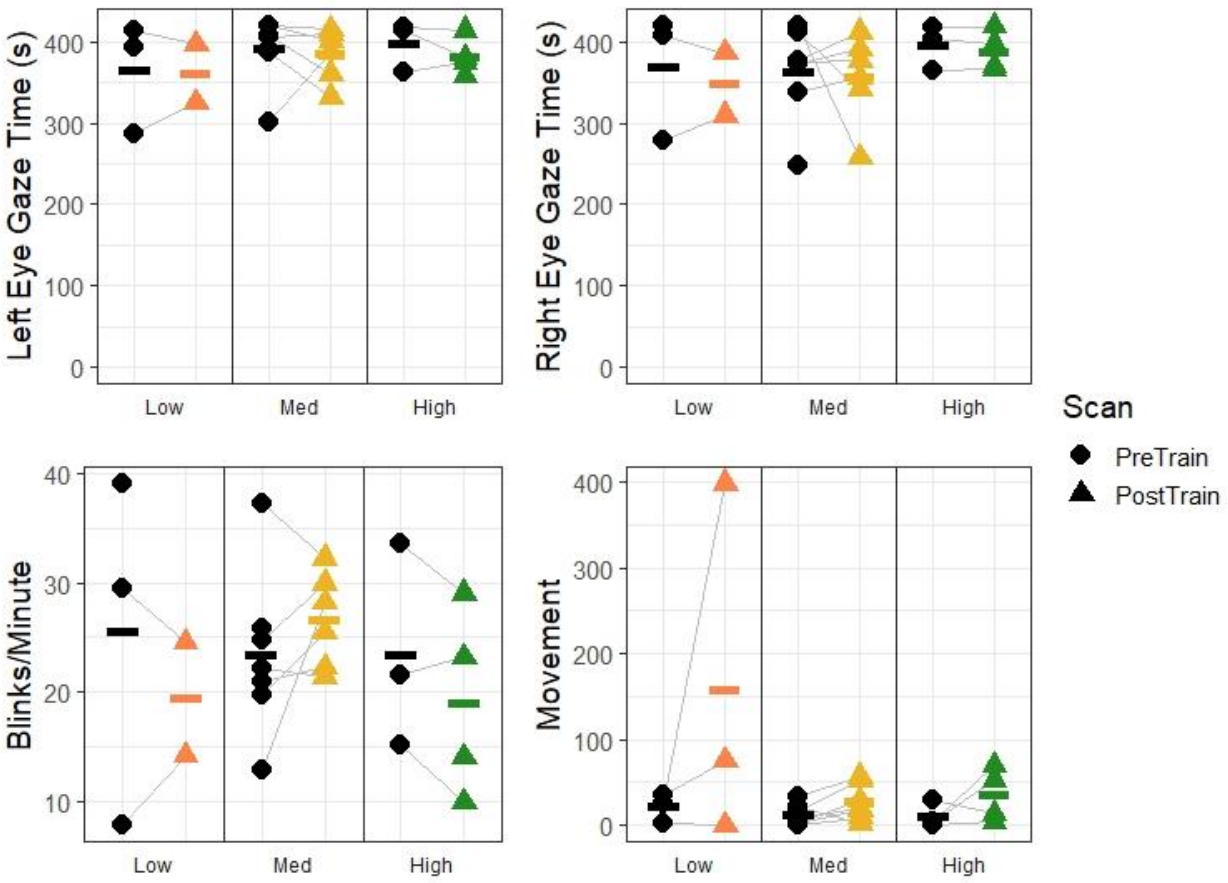
Supplementary Figure 3.16. *Coronal view of voxel-wise subtractions (converted to Z-score map) showing differential FDG uptake for the high proficiency crows' post-training scan compared to the same for low proficiency birds (n=7). Hp: hippocampus, B: nucleus basorostralis, Cb: cerebellum. Z-score map is superimposed atop a composite (n=4) structural MRI of the American crow brain. Note that the nucleus basorostralis did not exceed the Z-threshold for statistical significance.*



Supplementary Figure 3.17. *Coronal view of voxel-wise subtractions (converted to Z-score map) showing differential FDG uptake for the low proficiency crows' post-training scan compared to the same for high proficiency birds (n=7). HA: hyperpallium apicale, MVL: mesopallium ventro-lateralis. Z-score map is superimposed atop a composite (n=4) structural MRI of the American crow brain. Note that neither region exceeded the Z-threshold for statistical significance.*



Supplementary Figure 3.18. *Coronal view of voxel-wise subtractions (converted to Z-score map) showing differential FDG uptake for the medium proficiency crows' post-training scan compared to the same for high proficiency birds (n=11). M: mesopallium. Z-score map is superimposed atop a composite (n=4) structural MRI of the American crow brain. While notable, this region did not exceed the Z-threshold for statistical significance.*



Supplementary Figure 3.19. *Changes in behavior between the first and second scan of each crow for blink rate (top left), total movement (top right), and gaze time (left eye: bottom left; right eye: bottom right). Horizontal lines indicate group means.*