American crow (*Corvus brachyrhynchos*) thanatology

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Abstract

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Given the complexity with which some animals respond to dead conspecifics, how and why non-human animals respond to dead conspecifics is of increasing scientific interest. Among vertebrates, few experimental studies have been conducted to better understand the motivations and manifestations of behavioral responses towards dead conspecifics. Some members of the Corvidae family, including American crows (*Corvus brachyrhynchos*), are among the animals that respond strongly to their dead. Like some mammals, their responses can vary from alarm calling and group formation, to physical contact with corpses. In the following three chapters I use experimental approaches to explore aspects of American crow thanatology (the study of death) that improve our understanding of how crows respond to their dead, what motivates their responses, and how such behaviors are neurologically mediated. In the first chapter I investigate the prevalence and nature of tactile interactions between wild crows and taxidermy prepared crows positioned in "dead" or "life-like" postures, and taxidermy prepared "dead" heterospecifics. I find that tactile interactions with dead crows can take a variety of forms including exploratory, aggressive and sexual, but occur infrequently, do not appear to be food motivated, and are partly correlated with the onset of the breeding season. In addition, I find that life-like crows evoke responses more consistent with intruder eviction than do crows postured in death-like position. That tactile interactions with corpses are somewhat seasonally constrained suggests that breeding-season induced changes likely downregulate the ability of some birds to appropriately respond to complex stimuli. In the second chapter I explore if responses to dead crows are influenced by the season in which they are encountered or the age of the dead bird. I find that robust responses to dead crows wane during the breeding season, particularly in response to dead juveniles, which are a less salient cue of danger. How higher breeding season recruitment to adult corpses is facilitated remains unclear, but may be tied to differences in call rate or variability. In the last chapter, I find that observations of dead crows stimulate brain regions associated with executive function, and higher order decision-making (NCL; nidopallium caudolaterale), rather than emotional processing or a simple stimulus-fear mechanism. In addition, I find that crows rapidly learn from previous negative experiences, and show higher brain activity in the amygdala and to some extent the striatum during subsequent responses to nonthreatening stimuli. Together these finding suggest that, among crows, attention to dead conspecifics is driven by danger avoidance, and how they ultimately respond is the product of context-dependent decision-making and seasonal influences.

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General Introduction

As evidenced by the development of post-mortem burials practices, humans have been interested in our dead since at least the Paleolithic era (Renfrew et al., 2016). While death remains an intimately emotional and spiritual event, since that time it has also become the focus of scientific interest. By the early 1900's the transcendence of human death across so many disciplines including biology, medicine, psychology, social sciences and forensics, warranted a dedicated interdisciplinary field. Coined in 1903 by Metchnikoff, the field of thanatology, or the scientific study of death, its rites, and meanings, arose to meet such a demand (Fonsca and Testoni 2012). Since that time, the field has grown to include such specific areas of study as music thanatology, thanatology and social justice, and thanatology and tourism (Freeman et al. 2006; Fowler 2008; Stone and Sharpley 2008). Clearly, there is a vast body of literature dedicated to death among our own species. The study of death among non-human animals, or comparative thanatology, remains limited, however.

Perhaps the only exception to the dearth of scientific studies on comparative thanatology concerns insects. Given that even ancient people recognized the undertaking practices of colonial insects this may be unsurprising (Anderson, 2016). Furthermore, the highly stereotyped undertaking behaviors of bees, termites and ants lend themselves more easily to systematic studies. Through those efforts, it appears that their behaviors are motived by colony hygiene and are triggered by either the absence of certain chemical cues, or the presence of post-mortem chemical cues, depending on the species (Sun and Zhou 2013). Rats (*Rattus norvegicus)*, house mice (*Mus musculus*), and some birds like lesser black-backed gulls (*Larus fuscus*) also express patterned responses to dead conspecifics, though the number of individual studies is small (Kruuk 1976; Pinel et al. 1981; Prounis and Shields 2013). Among these animals, behaviors towards dead conspecifics appear motived by either hygiene or anti-predator measures. The behaviors of other animals, however, beg more complex questions.

Elephants, non-human primates, dolphins, and a variety of other species will spend minutes, even days, touching or carrying the bodies of their deceased (Douglas-Hamilton et al. 2006; Bearzi et al. 2018; Watson and Matsuzawa 2018). In other cases, however, they may ignore, bite, beat, or even engage in sexual activity (Piel and Stewert, 2016). Such a diversity of responses invites many subsequent questions, few of which have received scientific attention. Among them are whether these animals possess a concept of death, if they can feel and express grief, what motivates their interactions, and what influence issues of context may be, including manner of death, familiarity with the individual, environment, or age of the individual. Attempts to answer such questions may help address the wide variety of potential explanations for the observed interest in dead conspecifics. These include but are not limited to: danger learning and avoidance, attempts to identify the individual, a wait and see strategy, social signaling, mate signaling, learning to mother, feeding attempts, or the misuse of a response that would be appropriate if the individual were still living, such as territory defense, mating, or caretaking (Piel and Stewert, 2016; Watson and Matsuzawa 2018). For now, logistical and ethical barriers make addressing these questions and their potential explanations among large mammals challenging. Corvids, which likewise respond to dead conspecifics and share many similarities in relative brain size and social complexity to non-human primates (Emery and Clayton, 2004), may therefore make a more appropriate model to begin addressing such questions.

Previous work on common ravens (*Corvus corax*), California scrub-jays (*Aphelocoma californica*), and American crows (*Corvus brachyrhynchos*), indicate that they will reliably alarm call and gather in response to the discovery of a dead conspecific, and that these behaviors are extensions of anti-predator behaviors (Iglesias et al. 2012; Peterson and Colwell 2014; Swift and Marzluff, 2015). Whether, like some mammals, crows also touch their dead has so far been untested, and the observation of such behaviors has largely been preluded by the design of previous studies. Anecdotal accounts, however, suggest that crows display a wider variety of behaviors than are currently reported in the scientific literature including sexual behaviors, cannibalism, even burials (Bekoff 2009). The prevalence and nature of such interactions may have important implications on the motivations behind their interest in dead crows. Should danger avoidance continue to emerge as a primary driver, it stands that crows may attend to features of the carcass that convey risk related information such as the age of the individual at the time of death, but this is so far unknown. Whether their behaviors change seasonally may likewise reveal important insights into the causes or constraints on their response.

While observing the reactions to dead conspecifics is crucial to understanding the consequences of death on the behavior of the living, such observations provide a limited picture. By complimenting field studies with neurological ones, however, a more complete understanding can emerge. For example, such studies can reveal areas specialized to process particular stimuli, allow for more robust cross-species comparisons, and reveal insights in the way stimuli are perceived (i.e. as a feeding opportunity, a cue of danger, something that prompts affect, etc.). Although there are published accounts of the neurological effects that observing corpses has on humans (Liberzon et al. 2003; Wright et al. 2004; Taylor et al. 2000), no such study has been reported for a nonhuman animal.

The objective of the following three chapters is to contribute to the field of comparative thanatology by using wild American crows as models to explore questions aimed at quantifying and improving our understanding of how and why some animals respond strongly to their dead. In Chapter 1, I explore the prevalence and nature of tactile interactions between crows and dead conspecifics, and how these responses compare across dead crows presented in varying postures. In Chapter 2, I examine whether the season in which the body was encountered or the age of the individual at its time of death influences how robust the resulting defensive response is (as measured by the presence and intentisy of responses such as solcding, recruitment and mobbing). In addition, I explore how call structure aids in the recruitment of neighboring birds to cues associated with a higher threat. In Chapter 3, I use a functional imaging approach to identify what brain areas among five *a priori* selected regions are most strongly stimulated during the observation of a dead crow. Through these studies I seek to better understand if danger learning and avoidance continues to emerge as the primary driver of crows' response, and address other hypotheses. Together these studies provide a robust understanding of how American crows respond to their dead and what largely motivates such behaviors. These experiments not only serve to deepen our understanding of crows, but the field of comparative thanatology at large.

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Chapter 1: Occurrence and variability of tactile interactions between wild American crows and dead conspecifics[1]

Abstract

Observations of some mammals and birds touching their dead provoke questions about the motivation and adaptive value of this potentially risky behavior. Here we use controlled experiments to determine if tactile interactions are characteristic of wild American crow responses to dead crows, and what the prevalence and nature of tactile interactions suggests about their motivations. In Experiment 1 we test if food or information acquisition motivate contact by presenting crows with taxidermy prepared dead crows, and two species crows are known to scavenge: dead pigeons, and dead squirrels. In Experiment 2, we test if territoriality motivates tactile interactions by presenting crows with taxidermy prepared dead and life-like crows. In Experiment 1 we find that crows are significantly less likely to make contact but more likely to alarm call and recruit other birds in response to dead crows than dead pigeons and squirrels. In addition, we find that aggressive and sexual encounters with dead crows are seasonally biased. These findings are inconsistent with feeding or information acquisition. In Experiment 2 we find that crows rarely dive-bomb and more often alarm call and recruit other crows to dead vs. life-like crows, behaviors inconsistent with responses given to live intruders. Consistent with a danger response hypothesis, our results show that alarm calling and

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neighbor recruitment occur more frequently in response to dead crows than other stimuli, and that touching dead crows is atypical. Occasional contacts, which take a variety of aggressive and sexual forms, may result from an inability to mediate conflicting stimuli.

"Thereupon Allah sent forth a raven who began to scratch the earth to show him how he might cover the corpse of his brother. So seeing, he cried: 'Woe unto me! Was I unable even to be like this raven and find a way to cover the corpse of my brother'? Then he became full of remorse at his doing." - *Qur'an Sura 5.31*

Introduction

Within the field of comparative thanatology, observations of prolonged transport of dead infants, and affiliative, sexual or aggressive contact with dead adults call into question the understanding and motivations behind animal responses to conspecific (members of the same species) death (Pierce 2013; Anderson 2016). Tactile interactions with conspecific corpses have been widely observed among dolphins, elephants and non-human primates, though whether tactile interactions are a characteristic response of these species is unknown (Dudzinski et al. 2003; Anderson 2011). Prolonged contact may even continue over the course of days or weeks in cases involving dead infants (Warren and Williamson 2004; Biro et al. 2010). Among these mammals, tactile interactions may manifest as affiliative behaviors such as grooming and gentle touching, aggressive interactions, and sexual behaviors (Pinel and Stewert 2016). Touching or remaining close to dead conspecifics may expose animals to disease or increase the risk of attack from dangerous scavengers, such as stinging insects or larger predators (Perry and

Manson 2008; Alpers 2008; Heinze and Walter 2010; Strauss and Muller 2012). Therefore what purpose, if any, these interactions serve remains unclear.

Tactile interactions may aid the assessment of an individual's state or identity, or allow animals to acquire other important information such as cause of death (Harzen and dos Santos 1992; Pinel and Stewert 2016). Alternatively, they may be a byproduct of adaptive behaviors such as territory defense, mating, or caretaking (Dudzinski et al. 2003). To date, motivations behind mammalian contact with dead conspecifics remain largely untested. In contrast to large or marine mammals, systematic studies of death responses among birds are more feasible due to the fact that carcasses are smaller and easier to transport, and limited olfactory abilities enable realistic experiments using taxidermy specimens. Among birds, some corvids demonstrate consistent responses to dead conspecifics, though it remains unknown if they engage in tactile interactions.

Humans have long noticed the attendance of corvids near their dead, even incorporating them into their parables, as is evident in the epigraph we chose to start this paper. When confronting a dead conspecific, wild American crows (*Corvus brachyrhynchos*), common ravens (*Corvus corax*), and California scrub-jays (*Aphelocoma californica*) alarm call and recruit other birds to the area (Iglesias et al. 2012; Peterson and Colwell 2014; Swift and Marzluff 2015). Following these events, jays and crows avoid or show wariness in areas associated with conspecific death, and crows harass people they observed handling dead crows. These findings suggest that crows and some other corvids recognize dead conspecifics as cues of danger, and use such information to inform future actions and learn novel predators. During studies with California scrub-jays, dead jays were presented in the absence of predators that might otherwise keep observers away, but no episodes of contact akin to those described in some mammals were reported. Studies done on crows and ravens, however, have largely prevented such opportunities for contact due to the manner in which birds were presented (as hanging effigies or paired with predators; Peterson and Colwell 2014; Swift and Marzluff 2015).

Given the similarities between crows and the mammals for which contact has been repeatedly observed, crows make a viable model for exploring tactile interactions between wild animals and their dead. Like dolphins, elephants, and primates, crows share a large relative brain size and a complex social system (Jerison 1973; Emery and Clayton 2004; Clayton and Emery 2007). If interactions with dead conspecifics serve to provide key information to the investigating animal, it is possible that crows would seek the same kinds of information as these mammals. Interactions in this species may also simply be extensions of typical scavenging behaviors that include interest in dead animals. Alternatively, given that crows defend territory boundaries and female mates, particularly during the period of peak female sexual receptiveness between March-April, it is possible that contact with dead crows is the result of misguided attempts to evict intruders (Kilham 1989; Townsend 2009). Through two controlled experiments we seek to determine if tactile interactions between wild crows and dead conspecifics are a defining feature of crows' response repertoire, and if food, information acquisition, or territoriality are motivating factors.

To meet our objectives, we present wild crows four stimuli: a dead adult crow, a dead juvenile crow, a dead rock pigeon and a dead eastern grey squirrel (Experiment 1). Although in our area scavenging accounts for a minority of a crow's diet, crows are known to scavenge these heterospecific species (Marzluff et al. 2001; *KS Per. Obs.).* In contrast, crows are not known to regularly scavenge conspecifics (Anderson 2004). Given that taxidermy prepared animals are of no real food value, we expect contact with heterospecifics to lack alarm calling and neighbor recruitment, be exploratory and aggressive in nature, and only last for brief periods until crows determine they are of no value. If interactions with dead crows mirror those with squirrels and pigeons in these ways, it is likely that contact is food motivated. Alternately, efforts to glean information such as the individual's state, identity, or signs of depredation may be responsible for observed contact. If such information acquisition motivates tactile interactions, we expect that interactions will be coupled with alarm calling and recruitment, be primarily non-aggressive in nature, and occur frequently and uniformly across the study period. Finally, previous studies have suggested that danger learning and avoidance motivate crows' attraction to dead conspecifics (Swift and Marzluff 2015). If crows exhibit a danger response, we expect higher rates of alarm calling and recruitment coupled with lower rates of contact in contrast to heterospecifics.

We then determine what role stimulus posture has on the occurrence of interactions (Experiment 2). In contrast to food or information, crows may regularly contact dead crows because they fail to recognize their deceased state and mistake them for an intruder, provoking territoriality and mate guarding. To test this we present wild crows with a dead adult crow or a life-like upright mounted crow. We expect that the life-like crow will result in scolding and dive-bombing, but infrequent recruitment, and that contact will be primarily aggressive. If crows fail to correctly assess the state of the dead crow, then we expect to see similar behaviors between life-like and dead crows.

Lastly, because our initial crow stimulus resembles female copulation posture (wings outstretched with tail exposed) we wondered if any observed sexual responses in Experiment 1 were mediated by stimulus posture. To test this, in addition to evaluating if crows attempt to copulate with the life-like crow, we present crows with a dead adult crow with wings prepared close to the body ("tucked" crow in dead posture). If sexual behavior is released by stimulus posture, we expect that neither the tucked crow, nor the life-like crow will elicit copulation attempts.

Methods

General information

We conducted experiments at sites in Washington U.S.A. in the cities of Seattle, Bellevue, Issaquah, Renton, and Kent. For these experiments, we selected individual trial sites based on the presence of a territorial adult pair. We identified pairs based on seasonally relevant breeding activity including nest building, nestling provisioning, or the presence of a fledgling. In our area helping behavior occurs infrequently so we assumed that no more than two adults occupied a single territory (Marzluff and Withey 2004). Each pair only received one stimulus during the course of the study. All trials were spaced at least 300m apart in an attempt to prevent carry over effects and increase independence.

During experimental trials a single observer, KS, placed the appropriate stimulus 35-45m from the nest or fledgling in the absence of any adult birds. We attached all avian stimuli in dead posture to a lead weight via 1.5m of transparent fishing line to prevent removal by crows. The squirrel was prepared with an internal lead weight and packed with sand. Stimuli were maintained in roughly the same condition and replaced by an identically prepared skin if irreparably damaged. All specimens were collected outside of the study area and presumed to be unfamiliar individuals to the observing crows.

Once the specimen was in place the observer stood and recorded data from 15-25m away. The observer waited for up to 2 hours for the first adult bird to come within 25m of the stimulus and look in its direction (stimulus discovery). Once these criterion were met the observer recorded data for 30min, after which the stimulus was removed. The distance from nest, and 25m radius was determined using a TruePlus 200 Laser Rangefinder. We recorded all experiments with a tripod mounted JVC Everio camera.

Experiment 1: Tactile interaction with conspecifics and heterospecifics

During the breeding season of 2015 (March-August) and 2016 (March-April) we tested the response of wild American crows to the sight of either a taxidermy prepared adult crow in dead posture (N=78) a taxidermy prepared fledgling crow in dead posture (N=78) a taxidermy prepared adult rock pigeon (*Columba livia)* in dead posture (N=77) or a taxidermy prepared adult Eastern grey squirrel (*Sciurus carolinensis)* in dead posture (N=76; Fig 1.1). We prepared avian skins with their heads turned to the side with wings slightly outstretched, and placed ventral side down. The squirrel skin was prepared caudal side down and slightly curled. Once the stimulus was discovered by the first adult bird, we recorded the number of birds within 2 and 25m from the stimulus, the number of scolds emitted and whether dive-bombing or contact with the stimulus occurred. We defined scolding as harsh, unstructured calling. We defined mobbing as the presence of three or more adults engaging in scolding. We defined dive-bombing as u-shaped

Figure 1.1 Stimuli used in *Experiment 1*. Clockwise from top left: Adult American crow in standard dead posture, Juvenile American crow in standard dead posture, rock pigeon in standard dead posture, Eastern grey squirrel in dead posture.

swoops directed at a specific target that may or may not result in brief physical contact. If tactile interactions occurred, we used the video recordings to count the number of such instances, determine the amount of time spent with one or both feet on the stimulus (stand time), and categorize the nature of each contact into one of the following five categories:

1. Peck. We defined a peck as forceful contact by the living bird with its beak delivered to any part of the stimulus.
2. Touch. We identified this as non-forceful contact made by the living bird with any part of its body (bill or foot) to any part of the stimulus.
3. Drag. We considered dragging to be the intentional movement of the stimulus by the living bird via its beak. Dragging could occur over very short (several centimeters) or longer distances (a meter) and could include attempts to pick up and fly off with the stimulus.
4. Tissue pull/dismember: Tissue pulling included any instances where the living bird removed fur or feathers from the stimulus by grabbing the material in its bill and yanking forcefully. Dismemberment included removal of limbs or cotton stuffing from the stimulus.
5. Sexual behavior. We considered this to include: attempted copulation with the stimulus defined as the observation of the living bird mounting the stimulus and positioning the tail while attempting to, or successfully making, cloacal contact; copulation between mates occurring following the discovery of the stimulus and within 25m of it; and sexual solicitation posturing occurring on or within 25m of the stimulus, following stimulus discovery.

Experiment 2: Effect of stimulus posture on respondent behaviors.

During the breeding season of 2016 (July-August) and 2017 (April-June) we tested the response of wild American crows to the sight of either a 1) taxidermy prepared adult crow in dead posture with wings partially outstretched as in *Experiment 1* ("standard" crow in dead posture N=52) a 2) taxidermy prepared upright life-like crow mount (N=45), or a 3) taxidermy prepared adult crow in dead position but with the wings tucked close to the body ("tucked" crow in dead posture N=25; Fig. 1.2). We presented the third stimulus only during March-May of 2017. We prepared the upright crow mount with glass eyes and assuming a standing posture typical of a live American crow. To aid with stability, we attached the specimen to a 36 x 36cm piece of plywood via hidden screws in the feet. Site selection, stimulus presentation data collection, and contact analysis procedures matched that of Experiment 1.

Statistical analysis

We conducted all tests in SPSS v.19 (IBM, Armonk, NY, U.S.A). Apart from determining the likelihood of contact overall, all tests regarding contact omitted trials where no contact occurred, to control for zero-inflated data. In cases where there were only two stimuli such as heterospecifics and conspecific or life-like vs. dead we used Pearson's Chi-squared and Z-tests to determine likelihood differences among binomial measurements such as scolding, mobbing or contact. To evaluate differences in binomial responses among 3 or more stimuli we used Negative binomial regression. In *Experiment 1* we used logistic regression to determine if there were interaction effects

Figure 1.2 Stimuli used in *Experiment 2*. Clockwise from top: Adult crow in "standard" dead posture, upright, life-like adult crow, and adult crow in "tucked" dead posture.

between date and stimulus types. This test excluded data taken in 2016, since data collection in 2016 was limited to the months of March and April, and its inclusion may have biased the test's outcome. We used one-way ANOVAS to compare means. In n=1 case, a crow was able to remove the pigeon stimulus to a nearby rooftop. Because the bird was visible and audible for the duration of the trial, we included this point in binomial scolding, mobbing and contact tests, but not in tests of specific interaction types since the observer was unable to film them.

Results

Experiment 1: Tactile interaction with conspecifics and heterospecifics

The majority, 70%, of trials resulted in no contact between wild birds and the stimulus. With respect to conspecifics, the most typical reaction by wild birds was scolding (94% of 156 trials) often followed by mobbing (54% of trials). Among heterospecifics, scolding occurred less often than in response to crows (41% of 153 trials; Pearson's X^2_1=96.94, P<0.0001) and mobbing was only observed in a small number of cases (7% of trials).

Crows only contacted conspecifics in 24% of trials. Contact occurred more frequently with heterospecifics than with conspecifics (36% of heterospecific trials; Z=-2.22, P=0.026). Of the n=55 that contacted heterospecifics, 82% (n=45) engaged in pecking. During these encounters birds would often target eyes or other weak spots including ventral areas.

The contacting crow was more likely to scold during contact with a conspecific than a heterospecific (Pearson's X^2_1=38.97, P<0.0001). This finding was not driven only by a lack of a scolding response towards the squirrel, since crows were still more likely to scold during contact with conspecifics than with pigeons (partial X^2_1 conspecifics vs. pigeons=14.33, $P<0.0001$). Crows were no more likely to scold during contact episodes with adult vs. juvenile crows (partial $X^2_1=0.89$, $P=0.34$). In addition, among trials where contact occurred, mobs were significantly more likely to form in response to conspecifics (60% of n=38 contact trials) than to heterospecifics (7% of n=55 contact trials; *Z* test=5.56, $P<0.00001$). Wild crows contacted adult crows in dead posture in 25% of the 79 trials, juvenile crows in dead posture in 23% of the 77 trials, pigeons in dead posture in 34% of 77 trials, and squirrels in dead posture in 38% of 76 trials (Table 1.1). We observed crows engaging in all five categories of interaction with each stimulus, with the exception of sexual behaviors following presentations of the squirrel, which were never observed. Among the touch, drag, and tissue pull categories we observed no difference in responses between the four stimuli (touches: Wald $X^2_1=2.28$, $P=0.52$; drags: Wald $X^2_1=4.50$, $P=0.21$; tissue pulls: Wald $X^2_1=5.67$, $P=0.13$). We found no interaction effect of date and stimulus type on the occurrence of contact (Wald $X^2_3 = 3.20$, $P= 0.36$).

Crows were less likely to peck the adult crow in dead posture than the other three stimuli (Pearson's $X^2_1 = 8.34$, $P=0.004$), however when they did, they administered more pecks to adults (Table 1; mean±SD = 228.89±292.46). This difference was marginally significant (pecks: Wald $X^2_1=7.05$, $P= 0.070$). There was a significant interaction

			Exploratory behaviors			
	Total trials (N)	Occurrence of contact	Occurrence of touching	Average touches (±SD)	Occurrence of dragging	Average drags (±SD)
Adult crow	78	n=20, 25%	n=12, 60%	4.75±6.00	n=17, 85%	5.00±6.52
Juvenile crow	78	n=18, 23%	n=9, 50%	11.00±11.45	n=16, 89%	6.25±4.89
Pigeon	77	n=26, 34%	n=12, 46%	5.92±8.37	n=19, 73%	4.53±3.86
Squirrel	76	n=29, 38%	n=23, 79%	3.78±2.61	n=14, 48%	5.29±4.46
			Aggressive behaviors			
	Occurrence of pecking	Average Pecks (±SD)	Occurrence of tissue pulling	Average tissue pull (±SD)	Occurrence of sitting	Average stand time (sec±SD)
Adult crow	n=9, 45%	228.89±292.46	n=7, 35%	13.71±20.94	n=7, 35%	349.57±326.20
Juvenile crow	n=12, 67%	67.08±99.38	n=6, 33%	11.33±8.64	n=10, 55%	102.30±100.99
Pigeon	n=19, 73%	28.61±37.49	n=10, 38%	11.30±22.94	n=14, 54%	59.79±72.69
Squirrel	n=26, 90%	49.12±56.14	n=11, 38%	2.82±2.60	n=12, 41%	47.58±54.72
			Sexual behaviors			
	Total Sexual events (n)	Attempted copulation with stimulus (n)	Copulation between stimulus and >1 bird (n)	Copulation with mate* (n)	Solicitation posturing** (n)	
Adult crow	4	4	1	0	0	
Juvenile crow	2	2	1	0	0	
Pigeon	4	1	0	2	1	
Squirrel	0	0	0	0	0	

Table 1.1 Occurrence of tactile responses during *Experiment 1*. With the exception of ‘Occurrence of contact’, all other percentages are representative of the occurrence of the contact category out of the total trials in which contact occurred for each stimulus. Exploratory behaviors are highlighted in black and include touching and dragging. Aggressive behaviors are highlighted in medium grey and include pecking, tissue pulling and sitting. Sexual behaviors are highlighted in white. * = Defined as copulation occurring following stimulus discovery and within 25m of the stimulus. Identification of mated pair is based on nesting activity immediately before or after copulation event. ** = Defined as drooped vibrating wings and erect tail.

between date and stimulus on the likelihood of pecking (Date x stimulus: Wald X^2_3 = 8.13, P= 0.043). This finding appears to be driven by an early season (March-May) bias towards pecking conspecifics (Date x Con/Heterospecific: Wald X^2_1 = 4.63, P= 0.031; Fig. 1.3). Among cases where birds stood on the stimulus, there was a difference in stand time on each stimulus ($F_{3,39}$=7.56, P<0.0001). This was driven by responses to adult crows presented in dead posture, which were stood upon for the greatest length of time (Tukey's post hoc: Adult-Juvenile mean difference±SE = 247.27±71.97sec, Adult-Pigeon mean difference±SE = 298.79±67.60sec, Adult-Squirrel mean difference±SE = 301.99±69.46sec). We observed sexual behavior in n=10 trials: n=4 adult crows in dead posture, n=2 juvenile crows in dead posture, and n=4 pigeons in dead posture (Table 1.1). In two of these cases (n=1 adult crow in dead posture and n=1 juvenile crow in dead posture) a second live bird mounted and attempted to copulate during the initial bird's copulation event with the stimulus. It was not clear in these cases if the second bird was attempting to mate with the live bird or if it was also attempting to mate with the stimulus. Based on our knowledge of the territorial pair's whereabouts at the onset of the trial, in both these cases we believed that the second bird was mated to the first bird (however, the study birds were not marked). In cases where copulation occurred, in n=3 for adult crows in dead posture and n=2 for juvenile crows in dead posture, the copulating bird(s) scolded immediately before or after the event and pecked the stimulus aggressively during or after the event. Among sexual events during the presentation of the dead pigeon, n=1 was an attempted copulation with the stimulus and did not include scolding before, during, or after the event, n=2 were copulations between the presumed mated pair following stimulus discovery, and n=1was a bird making solicitation postures

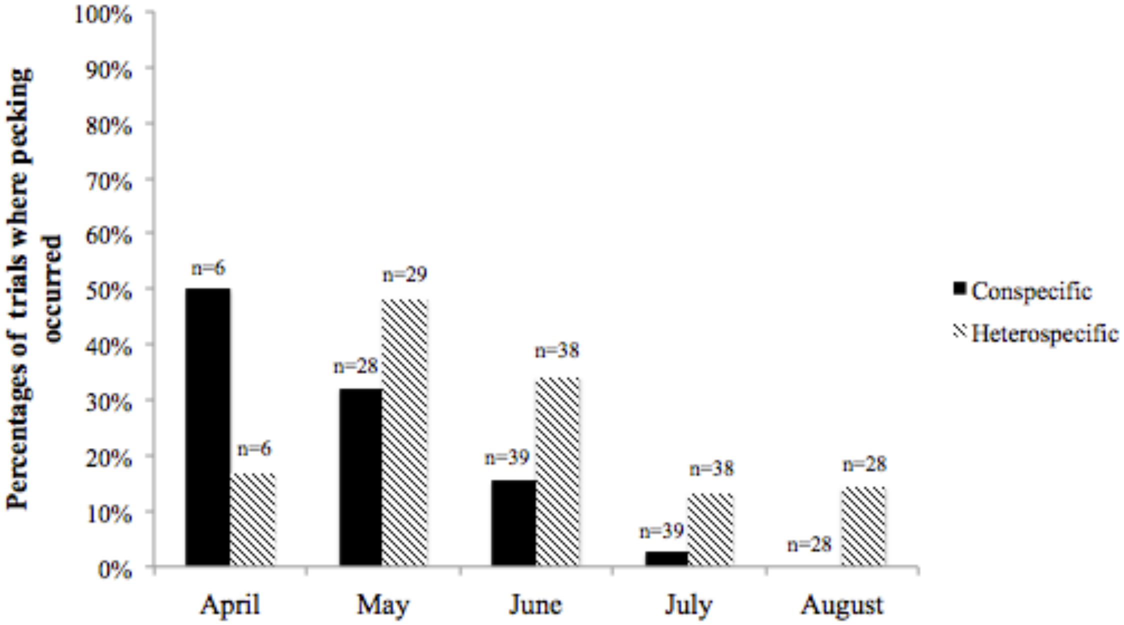

Figure 1.3 Pecking as a proportion of carcass-directed contact in Experiment 1. Graph shows what percentages* of trials resulted in pecking over each month of the 2015 field season. Black bars indicate trials using adult and juvenile crows presented in dead posture. Lined bars indicate trials using pigeons and squirrels in dead posture. The sample size per month for each stimulus is provided above the corresponding bar. *Percentage is calculated as the number of trials that resulted in pecking out of the total number of trials where contact occurred for each stimulus type.

while on top of the pigeon (Table 1.1). The observation of copulation behavior was biased toward the first half of the breeding season (March-May). Only one event was observed after the end of May, the observation of the bird soliciting from on top of the pigeon occurred June 18th, 2015.

Experiment 2: Effect of stimulus posture on respondent behaviors.

Crows did not appear to distinguish the adult crows in tucked dead posture (N=26) from those in standard dead posture (N=52). There were no differences in their response with respect to scolding (all trials resulted in scolding), mobbing (Pearson's $X^2_1=0.44$, $P=0.51$), overall contact (Pearson's $X^2_1=0.029$, $P=0.87$), or copulation attempts with the stimulus (n=1 tucked dead crow; n=4 with standard dead crow). As a result, we collapsed these stimuli into a single 'dead posture' category (N=78) for all subsequent analyses.

Crows distinguished between the adult stimulus in dead posture (N=78) and the life-like crow (N=45). Crows scolded in response to the dead crow in all cases but only scolded the life-like crow in 71% of cases (Pearson's $X^2_1=17.35$, $P<0.0001$). If scolding occurred, birds scolded more quickly after discovering the crow in dead posture (Mean±SD=39.94s±118.03sec) than they did when they encountered the life-like stimulus (Mean±SD=241.72±477.77sec; $F_{1,108}=12.24$, $P=0.001$). Mobbing also occurred more frequently in response to a conspecific presented in dead posture (63% of trials) than to a life-like crow (31% of trials; Pearson's $X^2_1=11.49$, $P=0.001$). In contrast, dive-bombing occurred most often in response to presentations of the life-like crow (Pearson's

X^2_1=15.08, P<0.0001). Crows dive-bombed the dead crow in 9% of trials and the life-like crow in 38% of trials.

Although crows more often contacted the life-like crow (49% of trials) than with crows presented in dead posture (33% of trials), this difference was marginally significant (Pearson's X^2_1=2.90, P=0.088). If contact occurred there was no observed difference with respect to pecking (Wald X^2_1=0.83, P=0.36) or touching (Wald X^2_1=0.97, P=0.33). Crows did, however, engage in more feather pulling/dismemberment with the upright stimulus than the dead stimulus (Wald X^2_1=3.89, P=0.049).

We observed n=8 sexual events in response to crows presented in dead posture and n=4 sexual events in response to life-like crows (Table 1.2). Of the n=3 attempted copulations with the upright mount, none of them were preceded or followed by scolding or pecking by the mating bird, whereas all n=5 attempted copulations with the crow in dead posture were. All copulation events were observed before the end of May, with the latest event occurring on May 25th 2017.

Discussion

Among some mammals including dolphins, elephants and non-human primates, there are dozens of accounts detailing tactile interactions between living individuals and a dead conspecific, but to date no systematic studies have been done to determine how typical this response is. Our experimental study demonstrates that among one group of animals, wild American crows, contact with dead conspecifics is not characteristic of their response, but when it occurs it is extremely varied. During these encounters we found that crows are much more likely to scold and mob, something we observed

	Total sexual events (n)	Attempted copulation with the stimulus (n)	Copulations with >1 bird (n)	Copulations with mate (n) *
Dead adult crow	8	5	0	3
Live adult crow	4	3	0	1

Table 1.2 Occurrence of sexual behaviors in *Experiment 2*. *Defined as copulation occurring following stimulus discovery and within 25m of the stimulus. Identification of mated pair is based on nesting activity immediately before or after copulation event.

significantly less often in response to dead heterospecifics or life-like crows. In addition, tactile interactions with crows were more often conflicting in nature (ex: aggressive and sexual) in contrast to other stimuli. Although infrequent, we found that inappropriate contact like prolonged pecking or copulation generally occurred in the first half of the breeding season. Given these findings, we suggest that tactile interactions with dead conspecifics do not represent attempts to assess key information, typical scavenging or territorial behaviors. Our results suggest that costs associated with contact precludes most individuals from touching dead crows but that in some birds, breeding-season induced changes may be responsible for overcoming more typical necrophobic responses.

Touching conspecific corpses may expose animals to disease or increase the risk of attack from dangerous scavengers, such as stinging insects or larger predators (Anderson 2011). To avoid these risks, social insects such as ants, termites and bees, have special undertaking procedures to efficiently dispose of conspecific corpses (Rosengaus and Traniello 2001; Choe et al. 2009; Sun and Zhou 2013. Rats (*Rattus norvegicus)* likewise bury cagemates that have been dead for more than 40 hours (Pinel et al. 1981). For these colony living animals, any contact that occurs is therefore associated with body removal and the ultimate driver is maintaining colony health. Why some animals are motivated to engage with dead conspecifics in contexts outside of body disposal remains less clear, but some have suggested they do it as a means to glean information from the body including attempts to identify the individual or assess its state (Harzen and dos Santos 1992; Anderson 2011). Our observation that crows primarily responded to dead crows by scolding and mobbing in the absence of contact, supports previous studies that crows and other corvids recognize dead conspecifics as indications of danger (Iglesias et al 2012; Swift and Marzluff 2015). The finding that contact was uncommon may suggest that crows do not collect information via tactile interactions, tactile interactions are not necessary to collect such information, or that the benefits of collecting such information are not outweighed by potential costs.

Although uncommon, we repeatedly observed wild crows make contact with dead conspecific stimuli including juveniles and adults in varying positions. Contact could be exploratory, aggressive or sexual in nature. To our knowledge this behavior has not been previously reported in wild American crows. In contrast, California scrub-jays are not known to touch dead jays (Iglesias et al. 2012). Further study is needed to determine if this behavior is unique to crows among other corvids.

Our finding that interactions by wild crows with heterospecifics differed from interactions with conspecifics with respect to scolding, mobbing, and the prevalence of aggressive and sexual behaviors, suggests that interactions with dead crows are not food motivated. Specifically, that crows were significantly more likely to scold and mob in response to dead conspecifics both overall and during trials where contact occurred, demonstrates a consistent danger response that was not observed in response to dead heterospecifics. This finding is supported by other observations that cannibalism is rare among passerines (Balda and Bateman 1976). To our knowledge only one documented case of adult-adult cannibalism has ever been reported in crows (Anderson 2004). Although most birds did not approach heterospecifics, this is not necessarily surprising given that in our area, predation and scavenging account for less than 25% of a crow's diet (Marzluff et al. 2001). Furthermore, crows prefer open carcasses, therefore carcass integrity may have kept some individuals away (Skagen et al. 1999). Those that did make contact appeared to be assessing and quickly determining that the specimens were of little food value.

Instead of foraging attempts, interactions with dead adults included behaviors more akin to territory defense such as intense physical aggression and scolding. In Experiment 2, however, we found that crows differed in their response to crows in a life-like vs. dead posture in several key ways. In contrast to presentations of life-like crows, when crows encountered dead crows they scolded more quickly and more often, were more likely to mob, and were less likely to dive-bomb. These behaviors are consistent with the danger response hypothesis and suggest that the crows correctly identified the state of the dead bird. These findings are supported by a study on California scrub-jays that showed jays responded with alarm to a dead jay, but aggressively towards an upright jay (Iglesias et al. 2012). Given that crows will attack intruders, and males appear to force extra pair copulations on non-receptive females, the finding that crows physically attacked or attempted to mate with the live mount is not unexpected (Townsend 2009). With respect to interactions with the dead crows, it remains unclear what might account for the inappropriate and often conflicting nature of tactile responses including carcass destruction or attempts to mate, but we can begin to speculate as to their cause.

Observations of post-mortem contact that can be described as aggressive or sexual have been observed in a variety of animals. Following the death of a Rhesus macaque (*Macaca mulatta*) Buhl et al. 2012 observed dominant group members interacting aggressively with the body including beating and biting for 50min. Stewart et al., 2012 described rough-handling and beating of a deceased female by young male chimpanzees after her death. Dolphins also sometimes direct aggressive and agitated behaviors towards dead conspecifics (Dudzinski et al. 2003). Such behaviors have been proposed as manifestations of frustration or confusion at being unable to elicit a response (Stewart et al. 2012). This would not account for the early breeding season bias when this behavior occurred, however. Rather, social theater or displacement behaviors that arise out of the heightened state of arousal experienced during the breeding season may better explain such observations (Pinel and Stewert 2016).

Across both experiments, we observed n=11 attempts to mate with a dead crow (4.7% of N=234 trials), 90% of which were coupled with scolding and all of which took place before the end of May. Sexual behaviors around dead conspecifics are rare, but not unique to crows. Hetero and homosexual necrophilia has been observed across a wide variety of taxa. Sexual arousal in response to dead conspecifics has been documented in bottle nosed dolphins (Dudzinski et al. 2003) and humpback whales (*Magaptera novaeangliae*; Pack et al. 1998). Mating attempts with dead conspecifics have been observed in Richardson ground squirrels (*Citellus richardsoni*), Mallards (*Anas platyrhynchos*), sand martins (*Riparia riparia*) cururu toads (*Rhinella steuvax*), and great ameivas (*Ameiva ameiva*; Dickerman 1960; Lehner 1988; Costa et al. 2010; Brito et al. 2012; Tomita and Iwami 2015) The copulation posture typical of dead birds has been proposed as the releasing factor for such inappropriate attempts to mate, particularly among monomorphic birds (Tomita and Iwami 2015). In Experiment 2, however, we show that crows attempted to mate both with a life-like crow in neutral standing posture and a dead crow with the wings tucked close to the body. These observations call into question the validity of posture as the primary releasing factor for copulation events between crows and dead crows, and warrant further investigation.

Given the prevalence of scolding before, during or immediately following copulation events with dead but not life-like crows, alarm induced arousal rather than reproductive attempts, might better explain copulation with dead crows. Increased sexual behavior following alarm or excitement has been observed in the Zebra finch (*Taeniopygia guttata*), vermillion flycatcher (*Pyrocephalus rubinus*;) and pied avocet (*Recuruirostra avosetta*; Rand 1943; Morris 1954; Makkink 1936). Following the death of a group member, sexual behavior occurring outside the breeding season was observed in rhesus macaques (Buhl et al. 2012). Likewise we observed mating attempts between presumed pairs following discovery of a dead crow. It is possible in this context that distress induces arousal resulting in copulation attempts between mates if possible, but in the immediate absence of the mate results in displacement-mounting. In rooks (*Corvus frugilegus*), sexual displays by males sometimes stimulate reverse mounting by females (Coombs 1978). In our study, females witnessing male precopulatory behavior prior to mounting the stimulus may be responsible for the two possible instances of reverse mounting.

In addition to the multiple mating attempts with the dead and life-like crows, we also observed one attempted copulation with the dead pigeon. Attempts to mate with live heterospecifics have been observed in a variety of species including seals and non-human primates (Nico de Bruyn et al. 2008; Pelé et al. 2016). Although these events are rare enough that determining causal factors remains difficult, in many cases these were males that had restricted access to conspecific females. Such information about the crow involved in this case is not known.

Given that sexual and aggressive behaviors were often expressed simultaneously, it may be that breeding related endocrine changes down regulate the ability of some birds to process conflicting information. Among primates, ambiguous or conflicting stimuli are processed in the Anterior Cingulate Cortex (ACC). In humans it has been shown that individuals with high anxiety show reduced ACC recruitment during tasks involving threat related stimuli (Bishop et al. 2004). It is not yet known what area in birds may act as the functional homolog to the ACC, but the corticoidea dorsolateralis has been proposed as a possibility, given its similar pathways as the mammalian cingulate cortex (Csillag and Montagnese 2005). Further study is needed to determine if the CDL is responsible for processing such information in birds, and if some individuals show reduced activation and lower suppression of inappropriate responses during periods of high excitement such as the breeding season or when exposed to threats. In addition, exploring endocrine differences with respect to adrenocorticosteroid hormone, testosterone and corticosterone between birds that do and do not engage in contact will further elucidate breeding season induced changes on the release of inappropriate behaviors. Studies examining the change in rates and nature of contact within individuals across the breeding season will help verify that such behaviors wane in correlation to decreased reproductive activity.

This study is the first to demonstrate that American crows occasionally make contact with dead conspecifics. The nature of contact in crows can be exploratory, aggressive or sexual. We show that such behaviors are both atypical and, with respect to sexual and aggressive behaviors, seasonally biased. We suggest that rather than information acquisition, food, or territoriality, contact with crows is attributable to an inability among some birds to process conflicting stimuli resulting in inappropriate or conflicting displacement activities. Similar aggressive and sexual behaviors have been anecdotally observed among cetaceans, non-human primates, and elephants. It remains unknown, however, whether our findings apply to these animals. A crucial distinction between our study and the vast majority of observations among mammals is that most interactions involving mammals were between familiar individuals. The potential myriad ways this may affect the response of either mammals or birds are unknown. Given that crows maintain permanent pair bonds that can span over a decade, it is possible that responses to familiar individuals contrast with our findings, particularly with respect to affiliative behaviors. Understanding whether these differences exist and what form they take (which may be investigated in experiments employing sedation), will help us better elucidate the significance of death on group members and partners, and help guide best practices when we are confronted with animal death in captive settings.

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Chapter 2: Age at time of death and season of encounter influence the response intensity of wild American crows towards dead conspecifics[2]

Abstract

While humans are still considered the only animal to engage in spiritual rituals around the dead, there is increasing scientific attention on how non-human animals respond to their dead. Some animals display stereotyped undertaking behaviors in response to dead conspecifics whereas others respond in variable ways. What accounts for such variability is largely unknown. Here we test if either age at time of death or the season in which the body is encountered affect the responses of American crows (*Corvus brachyrhynchos*) to dead conspecifics and how such responses are mediated by alarm calls. We present wild crows with either a dead adult crow or a dead juvenile crow during both the breeding and non-breeding seasons and gauge their responses to playback calls uttered during these experiments. We find that whereas scolding occurs robustly and consistently across stimuli and seasons, the mobbing response is more constrained. Though large, long lasting mobs form in most cases regardless of stimulus during the non-breeding season, mobs are less likely to form during the breeding season both overall and especially in response to dead juveniles. When breeding season mobs form, they are also smaller and shorter lasting than their wintertime counterparts. What facilitates higher breeding-season mob formation in response to adults remains unclear. While we find that call rate influences the mobbing response overall, we do not find clear evidence there are differences in call rate between calls given in response to adult and juvenile carcasses. These results suggest that during the breeding season when engagement in mobbing is

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[2] This chapter will be published with Marzluff, J.M. as a coauthor.

riskier, crows attend to the relative risk indicated by a dead body and are less likely to mob if the body is weak indication of an environmental threat. Whether they advertise the relative danger indicated by carcass age with higher intensity calls warrants further investigation.

Introduction

A growing number of studies have documented the responses of wild animals towards their dead. Through these efforts it is evident that attention towards conspecific carcasses occurs in a variety of taxa including insects, mammals, birds and fish (Buhl et al. 20012; Sun and Zhou 2013; Oliviera et al. 2014; Swift and Marzluff 2015). Although systematic studies are limited, it appears that some organisms display highly stereotyped responses whereas others are highly variable. Among eusocial insects such as bees, termites and ants, for example, the presence of dead conspecific bodies provokes stereotyped undertaking behaviors such as consumption, burial, or physical removal (Sun and Zhou 2013). The adaptive value of such behavior appears tied to colony health (Sun and Zhou 2013). By contrast, observations of non-human primates, cetaceans and some birds responding to their dead are highly variable. These taxa may flee from, attend to, or touch bodies. When tactile interactions occur they can be aggressive, affiliative or sexual and occur very briefly or extend over the course of weeks (Harzen and dos Santos 1992; Anderson 2011; Swift and Marzluff 2018). What accounts for the differences in responses between and within certain species remains unknown, but issues of context such as age at time of death, environment, cause of death, time of year, and relationship to deceased have been proposed as influencing factors (Anderson 2011; Campbell et al. 2016).

Birds including crows, ravens and jays are among the animals that show strong responses to dead conspecifics. When presented with a conspecific carcass, American crows (*Corvus brachyrhynchos*), common ravens (*Corvus corax*) and California scrub-jays (*Aphelocoma californica*) will often alarm call and recruit other birds to the area (Iglesias et al. 2012; Peterson and Colwell 2014; Swift and Marzluff 2015). By contrast, when presented with a dead heterospecific such as a feral pigeon or squirrel, American crows do not show a robust alarm response (Swift and Marzluff 2015; Swift and Marzluff 2018). Previous studies have suggested that danger learning and avoidance drive such responses towards dead conspecifics (Iglesias et al. 2012; Swift and Marzluff 2015). Crows should therefore be attentive to carcass features that convey important risk related information. In our study area of the Pacific Northwest, adult crow survivorship is considerably higher than hatch year survivorship, so dead adults could be interpreted as stronger indications of risk (Withey and Marzluff 2005). If this is the case, then crows may advertise the discovery of a dead adult with alarm calls associated with a higher degree of danger. Although crows do not have threat-specific calls like some animals, they can vary the duration and rate of alarm calling as a means of communicating urgency and threat (Yorzinski and Vehrencamp 2009).

In an effort to address presently unanswered questions regarding the ability of wild animals to attend to and communicate context-dependent details regarding dead conspecifics, we aim to test the following hypotheses in free ranging American crows. 1) If crows do not have a more robust defensive response (i.e., higher likelihood of alarm calling and neighbor recruitment) towards dead adults, then we expect that they will not attend to the age of the dead bird as a salient cue of risk. 2) If crows do not have differences in their defensive response between seasons, then we predict that reproductive status will not influence their sensitivity to the presence of dead conspecifics. 3) If attendance to mobs is faciliaited by calls, then calls that resulted in mobbing will have a higher call rate and higher frequency, and larger bandwidth notes. 4) If call rate is what facilitates attendence to mobs, then removing or adding pauses to calls will render them more or less attractive. We conduct two experiments to test these hypotheses.

Methods

General Information

We conducted experimental tests across the cities of Seattle, Kirkland, Bellevue, Kent, Burien, Renton, Issaquah and Sammamish, WA, USA. We identified test locations by the presence of a bonded pair of crows, and spaced field sites 350 m apart to increase independence (Swift and Marzluff 2015). During the breeding season, we determined test locations by the presence of breeding behaviors such as nest construction, attendance, or the presence of fledglings (Kilham 1984). During the non-breeding season, we determined test locations by observing pairs for behaviors that suggested they were bonded territory holders including allopreening, territory defense and non-communal foraging (Kilham 1984). In our study area, helping behavior occurs infrequently so we assumed that no more than two adult birds occupied a test location (Withey and Marzluff 2005).

Stimuli included a sexually mature adult crow prepared in dead posture and an immature crow prepared in dead posture (Fig. 2.1). We identified adult carcasses by mouth lining color, and juvenile crows by gape and time of year when collected (Emlen 1936). All juvenile crows were less than four months old at time of death. We attached

Figure 2.1 Left is an adult crow prepared in “dead” posture. Right is a juvenile crow prepared in “dead” posture.

each stimulus to a lead weight via 1.5m of transparent fishing line to prevent removal by crows. We maintained stimuli in roughly the same condition and replaced them once they became irreparably damaged with an identically prepared specimen. All specimens were collected outside of the study area and presumed to be unfamiliar individuals to the observing crows.

Experiment 1: Responses to crow carcasses

During the breeding season (March-August 2015; March-April 2016) we presented N= 78 taxidermy prepared "dead" adult crows and N=78 taxidermy prepared "dead" juvenile crows at locations used by bonded pairs. Across all experiments, each pair was only visited once, and only received one stimulus. We placed the stimulus in an open area (typically a sidewalk) between 35-45m from the nest or fledgling and within 10m of a perch, such as a light post, where birds could observe the stimulus and alarm call. If the fledgling moved prior to stimulus discovery by the adult, the observer (KS) did not reposition the stimulus or terminate the trial. During the non-breeding season (December-February 2016) we presented N=15 "dead" adult crows and N=15 "dead" juvenile crows at locations with bonded pairs that were open and within 10m of a perch. During both seasons, we presented the stimulus in the absence of any adult crows, after which the observer retreated to a distance of 15-25m to record data. The observer was not hidden from attending crows. Data collection commenced once the first adult bird arrived within 25m of the stimulus and gazed towards it (stimulus discovery). Once stimulus discovery occurred, the observer recorded the presence of scolding, the number of birds within 25m of the stimulus, the amount of time birds spent there, and whether mobbing occurred. We defined scolding as harsh, unstructured calling (Chamberlain and Cornwell 1971). We defined mobbing as the presence of three or more adults engaging in

scolding. Thirty minutes following stimulus discovery we terminated data collection and removed the stimulus. This timing was determined by the observation that, in most cases, birds ceased their alarm response and left the stimulus area 20min following the stimulus presentation. After two hours if the stimulus had not been discovered the observer collected it and did not pursue a retest in that territory. We recorded all experiments with a JVC Everio camera.

To determine if call intensity varied between adults and juveniles, we analyzed calls collected during the 2015 field season. We selected calls from N=10 dead adult stimulus trials that resulted in mobbing, N=10 dead adult trials that did not result in mobbing, N=11 dead juvenile trials that did not result in mobbing, and N=7 dead juvenile trials that resulted in mobbing and We selected mobbing examples with the following criteria: mobbing occurred between 13-102s of the initial alarm call and maximum mob size exceeded 5 individuals. For the comparisons, we selected the first 60s of calls from the initial responding bird among trials that did not result in mobbing. Across both mobbing and non-mobbing examples, if two birds scolded simultaneously only the initial responding bird's calls were used. Using Raven Pro 1.5 we measured the following aspects for each call sample: number and duration of notes and between-note pauses (internote), number and duration of bouts and between bout pauses, 90% duration, peak frequency and center frequency (Fig. 2.2).

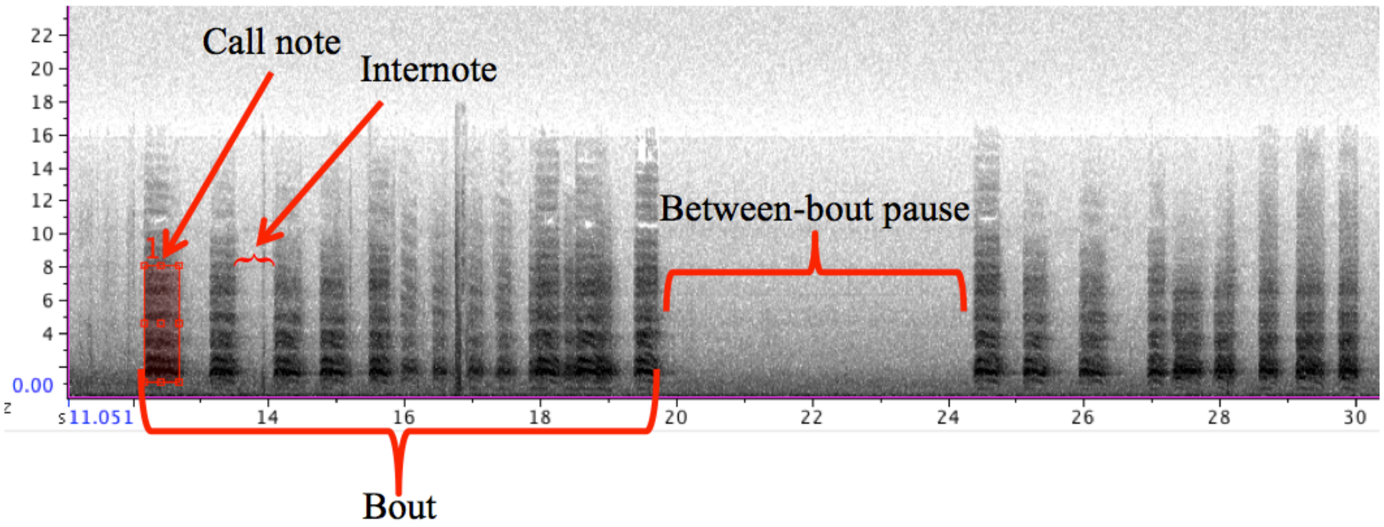

Figure 2.2 Spectrogram of crow scolding calls with indicators of gross call measurements. The x-axis shows time in seconds, and y-axis indicates frequency in kilohertz.

We defined a note as a single “caw” syllable and a bout as a series of call notes with internote durations lasting no longer than 1.9sec. We measured all calls notes manually in Raven Pro 1.5 (call analysis settings: data length=512 points, overlap=256, frequency range=1,000-8,000hz).

Experiment 2: Playback study

In order to obtain additional examples of adult scolding we presented N=62 adult crows in dead posture and N=23 juveniles in dead posture at locations with bonded pairs during the months of March and June, 2017. Site selection and manner of presentation for these tests were identical to those of Experiment 1. Once the stimulus was discovered we recorded the first 10 minutes of vocalizations with a Maranz PMD-671 solid-state recorder and a Sennheiser MKH 20-P48 microphone housed in a Universal Parabolic Dish-MK2 that the observer placed on the ground next to the recording camera, 15-25m from the stimulus. Using audio recorded in the 2015 and 2017 seasons, we selected n=7 examples of the initial attending crow’s first bout of scolding in response to a dead adult that successfully resulted in mobbing. We defined a scolding bout as a series of notes with intervening pauses lasting no longer than 1.9sec. We used only the first scolding bout because subsequent bouts were confounded by the vocalizations of recruited birds. Selected calls had the following characteristics: they lasted for 6.0-12.7s, included 10-22 individual notes, had an average between-note pause duration of 0.28s, and elicited mobbing within 188s (Adult Mob Calls). To make juvenile playback recordings, we selected N=8 examples of the first three scolding bouts given bny the initial responding crow after discovery of a dead juvenile that did not result in mobbing (Juvenile No-mob Calls). Juvenile bouts comprised 1-8 notes and lasted 0.51-7.5s. The average internote pause was 0.31s. Pauses between bouts lasted 2-17.3s. To test if varying the call intensity changed its effectiveness, we created manipulated playback tracks for both adult and juvenile response calls. To make manipulated adult calls (n=7 Adult Manipulated Calls), we inserted pauses between scolding notes to match bout patterns of Juvenile No-mob Calls. Each Adult Mob Call was manipulated to mimic the between-bout pause pattern of 1 of the 8 juvenile tracks. To make manipulated juvenile calls, we removed between-bout pauses (n=8 Juvenile Manipulated Calls). Each track repeated the vocalizations once a minute for 5min. We used Audacity 2.1.3 to manipulate and normalize tracks, and RavenPro 1.5 to manually measure call structure aspects including, number of notes, and between-note/between-bout pause lengths.

During August of 2017, we played Adult Mob Calls at N=21 sites, Adult Manipulated Calls at N=22 sites, Juvenile No-Mob Calls at N=21 sites, and Juvenile Manipulated Calls at N=23 sites using a Pignose 7-100 Legendary portable amplifier. Site selection was based on the presence of 1-2 individuals (as in non-breeding site selection in Experiment 1). Crows could be stationary or engaged in activity such as foraging. Once the test bird(s) was identified, the observer placed the amplifier 45-55m from the individual(s). If the bird(s) moved outside the 55m radius before the trial start, the observer terminated the trial. We measured distances to crows with a TruePulse 360 rangefinder. The observer concealed the amplifier in available vegetation, placed it within 10min of a perch such as a telephone pole or tree, and connected it via 25m of audio cable to a handheld mp3 player.

Over the course of conducting N=87 playback trials, each individual track was played 3-4 times. Calls were played at a minimum of 500m from their original collection area and assumed to be unfamiliar to the attending birds. The observer documented whether and how quickly a crow came within 25m of the speaker once playback commenced, how many total birds arrived, and whether they engaged in scolding.

Statistical analysis

In Experiment 1 we sought to identify if crows show a more robust defensive response (i.e., scolding and mobbing) toward dead adults or juvenile crows, and if these responses are seasonally influenced. To test this, we modeled binary presence/absence of scolding and mobbing with a logistic regression that included both ‘Season’ (breeding or nonbreeding) and ‘Stimulus’ (adult or juvenile) as covariates. To further understand within-season effects of stimulus (for example mobbing likelihood of adults vs. juveniles during the breeding season), we also conducted logistic regressions with a single ‘Stimulus’ covariate separately for both breeding and nonbreeding seasons. For all logistic regression tests, only main effects are reported in cases where interaction terms did not have a meaningful influence on the main effect ($P > 0.20$).

Beyond exploring simply if the proportion of trials that resulted in mobbing was different between stimuli and seasons, we also determined if mobs differed with respect to number of attending birds (mobbers). To do so, we used a negative binomial model with season and stimulus as covariates. This model was selected based on its ability to appropriately model over-dispersed count data. To assess the effects of season and stimulus on mob duration, we used linear regression with both of these variables as covariates, or for within-season tests, with just Stimulus as a predictor. Because interaction effects for this test were non-significant, only main effects are reported.

Our second goal in *Experiment 1* was to determine if any measured aspects of call structure differed between trials that did or did not result in mobbing. Specifically, we were interested in comparing gross call features including note duration, internote duration, and call rate, as well as microstructure properties including peak frequency, center frequency and 90% bandwidth. We ran one logistic regression to test for the effect of gross call features on the presence of mobbing, and a second to test for the effect of microstructure properties on the presence of mobbing. To control for pseudoreplication between individual internote and note durations, we averaged values across individuals. Values for peak frequency, center frequency and 90% bandwidth were radmonly selected from one of the three terminal notes for each individual.

In Experiment 2, our goal was to evaluate whether more intense calls produced mobs more effectively, and if by removing or adding pauses we could manipulate calls to become more or less attractive. In the field, we tested the attractiveness of more or less intense calls by presenting Adult Mob Calls, Juvenile No-Mob Calls, Adult Manipulated Calls Juvenile Manipulated Call and observing how birds responded. We used independent logistic regressions to test the effect of call type on the probability of recruitment, scolding and mobbing. We used independant linear regressions to test the effect of call type on continuous response variables including time to scold, time to mob, and number of mobbers across the four call types. We used ANOVAs to determine if there were differences between any of the call types among each of the response variables.

We followed a statistical inference approach as proposed by Fisher (1925). Namely, we considered p values <0.05 as evidence that an effect should be confirmed by other studies, and p values between 0.05 and 0.20 as evidence of effects that should be tested in future studies with increased replications or other design improvements. We considered p values >0.20 to indicate that the effect is too small to be detected by the current experimental design. We performed all tests in RStudio 1.1.383 using the MASS package (Venables and Ripley 2002) and the Reshape package (Wickham 2007) was used to format data.

Results

Experiment 1: Responses to crow carcasses

Across seasons, crows scolded in response to 98% of dead adults (N=93) and 91% of dead juveniles (N=93). Between adults and juveniles, there was some evidence of a difference in the likelihood of scolding (logistic regression P=0.07; Table 2.1). Crows were more likely to mob in response to adults (70% of trials) than juveniles (47% of trials; logistic Regression P=0.002; Table 2.1). Mobs that formed in response to adults were significantly larger than those that formed in response to juveniles (Negative Binomial Regression P=0.02; Fig. 2.3). There was some evidence that mobs formed in response to seeing a dead adult crow lasted longer than those formed in response to a dead juvenile (linear regression P=0.06; Fig. 2.4)

During the breeding season, crows displayed some differences in their scolding response towards adults (97% of N=78) and juvenile carcasses (90% of N=78; logistic regression P=0.07; Table 2.1). Whether those scolds resulted in mobbing also differed

Independent logisitic regression tests	**Parameter estimates**	**SE**	**P**
Effect of stimulus and season on presence of scolding	Intercept: 3.64 StimulusJuvies: -1.47 SeasonNonbreeding: 16.84	0.72 0.81 1903.13*	<0.001 0.07 0.99
Effect of stimulus and season on presence of mobbing	Intercept: 0.69 StimulusJuvie: -0.99 SeasonNonbreeding: 1.26	0.23 0.31 0.50	0.003 0.002 0.01
Within breeding-season effect of stimulus on scolding	Intercept: 3.63 SitmulusJuvie: -1.47	0.72 0.81	<0.001 0.07
Within breeding-season effect of stimulus on recruitment of at least one bird	Intercept: 1.00 StimulusJuvie: -1.04	0.026 0.34	<0.001 0.002
Within breeding season effect of stimulus on mobbing	Intercept: 0.75 StimulusJuvie: -1.11	0.24 0.33	0.002 <0.001

Table 2.1 Results of all logistic regression models used to evaluate differences in scolding, recruitment, and mobbing response across seasons (breeding and non-breeding) and stimuli (dead adult crows and dead juvenile crows) in *Experiment 1*. The last three test are within breeding-season tests of the effect of stimulus. *Inflated SE is due to 100% scolding across both stimuli in nonbreeding season. Parameter estimates are reported on the logit scale.

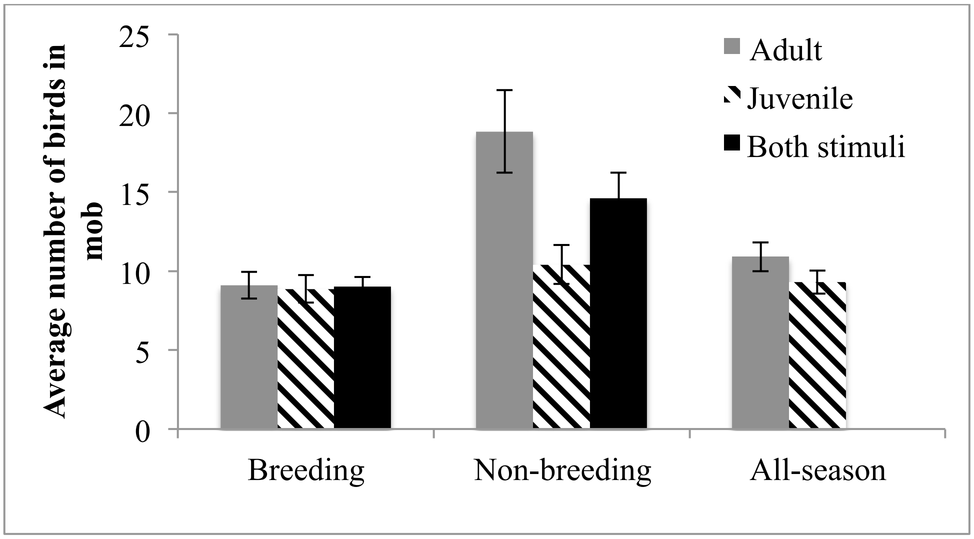

Figure 2.3 Seasonal and stimulus-induced differences in mob size. A mob comprises >3 adult birds all scolding, and can include the territorial pair. “Both stimuli” represents mob size across both stimuli combined. “All-season” represented each respective stimulus’ mob size across both seasons combined. Error bars indicate standard error of the mean.

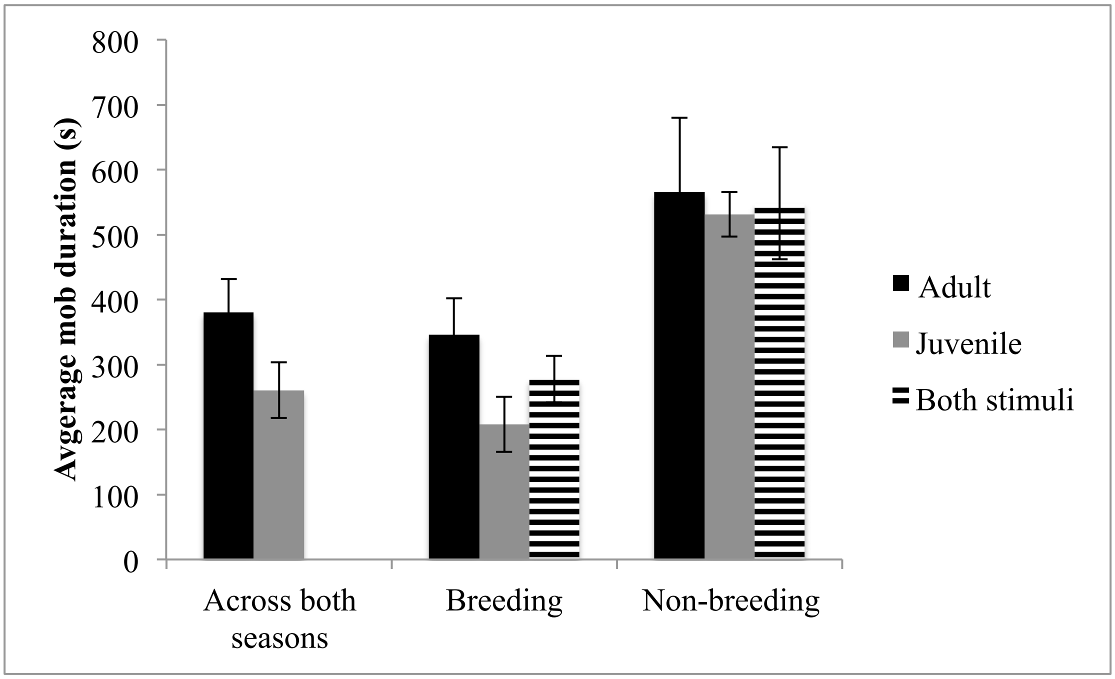

Figure 2.4 Seasonal and stimulus-induced differences in mob duration. A mob comprises more than three adult birds all scolding, and can include the territorial pair. "Both stimuli" represents mob size across both stimuli combined. "All-season" represented each respective stimulus' mob size across both seasons combined. Error bars indicate standard error of the mean.

between the two stimuli. Namely, dead adult crows were significantly more likely to trigger attendance by at least three adults birds to within 25m of the stimulus than dead juveniles (logistic regression P=0.01; Table 2.1) and to elicit mobbing (i.e., at least three birds attending *and scolding*; 68% of adult trials vs. 41% of juvenile trials; logistic regression P=0.0009; Table 2.1). If mobbing occurred, there was some difference with respect to the number of birds that participated in mobs that formed in response to adults or juveniles (negative binomial regression P=0.06; Fig. 2.3); and mob duration was higher for adults than juveniles (linear regression P=0.04; Fig. 2.4).

During the non-breeding season alarm responses toward adults and juveniles were equivalent. Crows scolded adults (N=15) and juveniles (N=15) in 100% of cases, and mobbed both stimuli in 80% of cases. There was also not a difference between the two stimuli in the mob duration (Linear Regression P=0.85; Fig. 2.4), but some evidence that the number of mobbers was different (Negative Binomial Regression P=0.12; Fig 2.3.).

Across stimuli, there was no difference in the likelihood of scolding between the seasons (logistic regression P=0.99; Table 2.1). Birds were more likely to mob during the non-breeding season (logistic regression P=0.01; Table 2.1). In addition, mobs that formed during the non-breeding season were both larger than those during the breeding season (negative binomial regression P=0.007; Fig. 2.3) and longer lasting (linear regression P=0.003; Fig. 2.4).

Experiment 1: Call analysis

We found that call rate among calls that resulted in mobbing (N=17) were signifigantly different than for calls that did not result in mobbing (N=21). Call rate was higher for calls that resulted in mobbing (Mob X̄=0.95notes/sec±0.06) than for those that did not (No-Mob X̄=0.67±0.11; p=0.041; Table 2.2). We did not find any other differences with respect to gross call features or microstructure features (Table 2.2). We were curious, however, if when comparing only Adult Mob Calls and Juvenile No-Mob calls if we would continue to find differences in call rate. We found that the rate among calls given in response to adults was somewhat higher than for juveniles and warrants further investigation (Adult Mob Calls: X̄=0.96±0.27, Juvenile No-Mob Calls: X̄=0.65±0.47).

Experiment 2: Playback study

At least one adult crow was recruited to the playback area in 57% of Adult Mob Call trials (N=21), 55% of Adult Manipulated Call trials (N=22), 57% of Juvenile No-Mob Call trials (N=23), and 48% of Juvenile Manipulated call trials (N=21), but these differences were not signifigant (ANOVA $F_{3,83}$=0.16, P=0.92; Fig 2.5). We found no differences between the four call types in whether scolding occurred (Scolding ANOVA $F_{3,83}$=11.55, P=0.38; Table 2.3; Fig. 2.5). If scolding occurred, there was no difference in how long (sec) until the bird first scolded (Adult Mob X̄=76.80s±26.04, Adult Manipulated X̄=80.17±33.62, Juvenile No-Mob X̄=96.20±36.82, Juvenile Manipulated X̄=109.75±29.11 ANOVA $F_{3,83}$= 0.28, P=0.84), nor was there a difference between call types in terms of whether the trial resulted in mobbing (ANOVA $F_{3,83}$=1.42, P=0.24;

logistic regression tests	Parameter estimates	SE	P
Effect of gross call features on mobbing			
Intercept	-6.04	2.38	0.01
Note duration	2.68	1.1	0.11
Internote duration	2.33	2.47	0.35
Call rate	7.18	4.5	0.01
Effect of microstructure on mobbing			
Intercept	8.84	4.49	0.05
Center Frequency	-0.003	0.003	0.18
Peak frequency	-0.002	0.002	0.42
90% Bandwidth	-0.0003	0.0003	0.38

Table 2.2 Results of two logistic regression tests looking at the effects of gross call features on the presence of mobbing, and the effect of call microstructure properties on mobbing between combined adult and juvenile selected calls that resulted and mobbing (N=17) that resulted in mobbing and combined calls that did not result in mobbing (N=21). Parameter estimates are reported on the logit scale.

Logistic regresstion tests	Parameter estimates	SE	P
Effect of call type on scolding	Adult Mob: -0.10 Adult Manipulated: -0.98 Juvenile No-Mob: -1.16 Juvneile Manipulated: -063	0.44 0.48 0.51 0.44	0.83 0.04 0.15 0.02
Effect of call type on mobbing	Adult Mob: -1.45 *Adult Manipulated: -19.56 Juvenile No-Mob: -1.79 Juvenile Manipulated: -1.90	0.56 2292.76 0.62 0.62	0.009 0.99 0.002 0.004
Effect of call type on recruitment of at least one adult crow	Adult Mob: 0.29 Adult Manipulated: 0.18 Juvenile No-Mob: -0.10 Juvenile Manipulated: 0.26	0.44 0.43 0.44 0.42	0.51 0.67 0.83 0.53

Table 2.3 Logistic regression tests on effects of call type on binomial responses including scolding, mobbing, and recruitment of at least one adult crow to within 25m of the speaker during *Experiment 2*. *Inflated standard error is to due no mobbing during Adult Manipulated trials. Parameter estimates for logistic regression tests are reported on the logit scale.

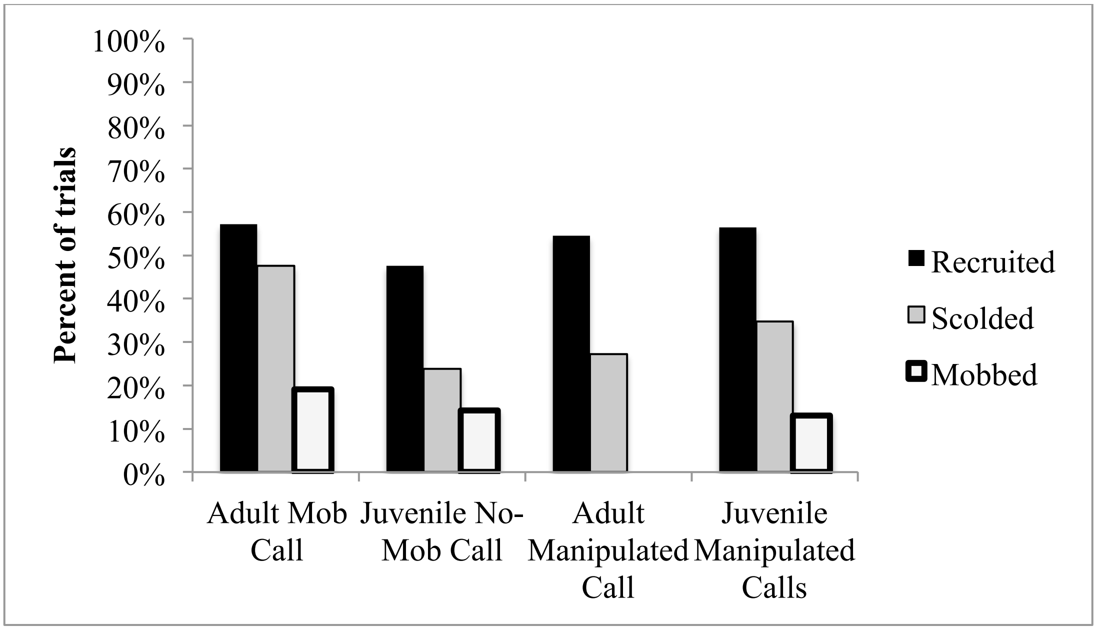

Figure 2.5 Effects of call type on recruitment, scolding and mobbing within 25m of the playback speaker in *Experiment 2*.

Table 2.3; Fig. 2.5). If mobbing occurred, we found no difference in mob size (Adult Mob $\bar{X}$=5.50±2.02, Juvenile No-Mob $\bar{X}$=8.67±2.33, Juvenile Manipulated $\bar{X}$=7.33±2.33 ANOVA $F_{2,7}$=0.54, P=0.61), but did find a difference in how quickly birds were recruited to the mob (Adult Mob $\bar{X}$=214.50±24.94, Juvenile No-Mob $\bar{X}$=101.67±28.79, Juvenile Manipulated $\bar{X}$=73.67±28.79, ANOVA $F_{3,7}$=30.77, P=0.0020; Fig 2.6).

Discussion

The ways in which context may affect how non-human animal respond to dead conspecifics have yet to be resolved. For example, Anderson (2011) suggested that the manner of death (violent or peaceful) may influence how aggressive the subsequent response by conspecifics is, but this explanation was based exclusively on the published observations of others. Given the unique responses to dead infants by attending adults in various cetaceans, primates and some other species, it has also been suggested that there may be age specific responses, but to date this idea remains largely speculative (Anderson 2016). Here we use systematic studies to test if either the age of the crow at time of death or season in which it is encountered affect how strongly wild American crows respond to their dead, and how such responses may be mediated by resulting calls. In Experiment 1 we show that while crows frequently and consistently alarm call, the occurrence of mobbing wanes during the breeding season, especially in response to juveniles. This finding suggests that mobbing may be most costly during the breeding season, and crows respond by limiting their participation to stimuli that convey higher environmental danger. How higher breeding season recruitment to adult carcass is actually facilitated, however, remains unresolved. While an analysis of call structure in

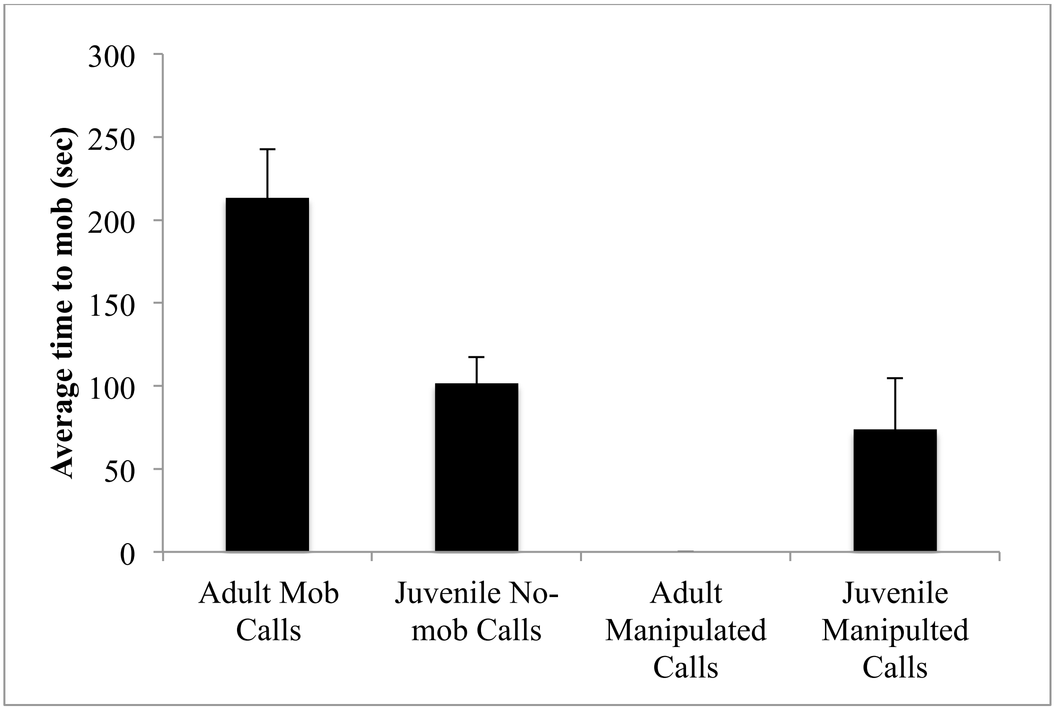

Figure 2.6 Average time to mob under the four different playback conditions in *Experiment 2*. Error bars indicate standard error of the mean. Adult Manipulated Calls never resulted in mobbing.

Experiment 1 suggested that faster call rates may be responsible, playbacks in *Experiment 2* showed mixed support. In both cases small sample sizes may have precluded clearer findings, and further investigation into the role of call structure is warranted.

The stronger mobbing response observed during the non-breeding season in *Experiment 1* may be attributable to seasonal differences in territory defense, mate guarding, and group foraging. Although crows maintain territories year round, they defend them less aggressively and spend more time off the territory in larger foraging groups during the non-breeding season (McGowan 2001). The abundance of local foraging flocks may bolster non-breeding mobbing responses. By contrast, during the breeding season crows more fiercely defend territories and will more often attack and will occasionally kill each other during altercations (Kilham 1984; *per. obs.*). Crossing territory boundaries to join mobbing efforts may therefore present a mortal risk. Furthermore, if the incubating female does not follow her partner, his absence may allow extra-pair copulation attempts by neighboring males or male helpers (Townsend 2009; Townsend et al. 2009). Our finding of lower breeding season mobbing responses is consistent with some other birds that defend territories only seasonally, such as black-throated blue warblers (*Dendroica caerulescens*), black-throated green warblers (*Dendroica virens*), and pinyon jays (*Gymnorhinus cyanocephalus*). Among these species recruitment to alarm calls outside the listener's territory occurs only rarely, or less often than during the non-breeding season (Cully and Ligon 1986; Bretts et al. 2005). Other studies of birds, however, have shown higher incidences of predator mobbing during the breeding season. The intensity of predator mobbing by barn swallows

(*Hirundo rustica*), for instance, is highest when the probable reproductive value of young is highest (Shields 1984). Such between-species differences in seasonal mobbing rates may be attributable to differences in vulnerability to nest predation or parasitism (Krama and Krams 2004; Welbergen and Davies 2008).

That we found crows were less likely to mob in response to juveniles during the breeding season is likely tied to differences in relative danger indicated by juvenile vs. adult carcasses. Previous work has demonstrated that danger avoidance is an underlying factor in mobbing responses by crows and California scrub-jays towards dead conspecifics (Iglesias et al. 2012; Swift and Marzluff 2015). In our study area, annual adult survivorship is 81.3%, whereas juvenile survivorship is only 45% (Marzluff et al. 2001; Withey and Marzluff, 2005). Because hatch year birds are killed more often, and thus more frequently encountered, they may not be as strong of an indicator of danger as a dead adult. This finding contrasts with observations in some mammalian animals that show prolonged interest in dead infants. For example, in Japanese macaques (*Macaca fuscata*), 10% of infants are carried for around 3 days following their death (Sugiyama et al. 2009). Among mammals, such behaviors may be manifestations of an emotional response or a "wait and see" strategy where adults, unsure of the state of the dead offspring, do not abandon it in case it recovers (Campbell et al. 2016).

Although we found differences in recruitment patterns to adult vs. juvenile bodies during the breeding season, if and what aspects of call structure facilitate this recruitment remain unclear. Among animals that live in social groups, the ability to impart the listener with functionally referential (threat-specific) information can help make the defense response most effective (Seyfarth 1980). To this end, some species such as Campbell's monkeys (*Cercopithecus cambelli*), vervet monkeys (*Cercopithecus aethiops*), and red squirrels (*Tamiasciurus hudsonicus*) can produce predator-, or predator class-, specific alarm calls (Seyfarth 1980; Greeneand and Meaghar 1998; Zuberbühler 2000). Among birds, only Japanese great tits (*Parus major minor*) have been shown to use predator specific calls, whereas other birds such as black-capped chickadees (*Poecile atricapilla*) can convey other relevant information about predator size (Templeton et al. 2005; Suzuki 2013). Despite the number of vocalizations American crows can make they do not appear to have threat-, or threat-class-, specific alarm calls (Chamerlain and Cornwell 1971). Instead they appear to vary the rate of alarm calls to convey urgency (Yorzinski and Vehrencamp 2009). Here we found that although call rates were higher for trials that resulted in mobbing overall, we did not find significant differences between calls given in response to adults vs. juveniles, nor did we find any differences in other aspects of call structure or microstructure.

Our finding that crows did not appear to have specific call structure or microstructure properties seems somewhat supported by the results of our playback experiment. In *Experiment 2*, we found that calls that had previously resulted in mobbing (Adult Mob Calls) did not more effectively attract birds than calls that had not (Juvenile No-Mob Calls). Nor did we find that removing pauses from previously unattractive Juvenile No-Mob Calls made them more attractive. However, we did find that inserting pauses into previously attractive Adult Mob Calls resulted in no mobbing. That we didn't find more consistent mobbing patterns among attractive and unattractive calls was surprising given that a previous study on crows found that removing or inserting pauses between calls altered the call's effectiveness at recruiting other individuals (Richards and

Thompson 1978). In that study, however, periods of calling lasted longer (30s) and were stitched together with only 5s intervening pauses. It may be that, in our study, bursts of 6-12sec of scolding followed by pauses lasting over 30sec were not attractive enough because they were too short and infrequent. A follow up study with a larger sample size and playback tracks that include longer periods of un-looped calling would clarify this issue. It is also possible that crows simply have different means of detecting or advertising the age identify of the dead crow than was explored here.

It is possible that crows do not rely on specific calls to trigger stereotyped defensive responses, but rather seek a variety of information before responding in a context dependent way. When exposed to alarm calls given in response to a dead crow, crows show activation in their nidopallium caudolaterale (NCL), an area of the avian brain associated with higher order decision-making (*See chapter 3;* Güntürkün 2005). In the wild, in addition to attending to conspecific alarm calls, crows may visually investigate the cause of alarm calls at a spatial scale greater than was captured in this study before deciding to attend to alarm calls or not. In seeking such visual information they may be looking for cues such as the identity of the threat and which or how many other individuals are participating. If this is the case, then the lack of observed differences in call structure would be better explained. Alternatively, crows may also communicate aspects of threat identity or level in ways that have not been captured with traditional methods. Explorations into functionally referential calling typically rely on detecting stimuli induced patterns in call structure or microstructure properties (Yorzinski and Vehrencamp 2009). It may be that rather than having specific call rates or patterns associated with dead adult vs. juvenile crows, changes in call variability may communicate urgency (Richard and Thompson 1978). Alternatively, it may be that while the initiatory calls have some threat-specific consistency, call structure changes either with time and/or as a result of attendance by other birds, such as the mate. Audience influenced alarm calling has been observed in chickens (*Gallus gallus*), though traditional ‘audience effect’ studies have focused on whether alarm calling occurs in the absence of an audience rather than changes to call structure as time or audience size increases since the initial vocalization (Karakshian et al. 1998).

This is the first systematic study to address if either age at time of death or season of encounter influence how a wild animal responds to an unfamiliar, dead conspecific. We found that alarm calling occurred frequently, year-round, and irrespective of carcass age at time of death. Recruitment to join in mobbing, however, was apparently constrained during the reproductive period by season-induced risks. We found that during the breeding season, crows responded most strongly to dead adults, which reflect higher environmental threats. The mechanism by which crows communicate the relative threat indicated by the age of the dead bird, or independently identify it, was not revealed here. Further playback studies utilizing a larger sample size and exploration into the variation, rather than consistency, of different alarm calls may help address this remaining knowledge gap. Together, these results are among the first to demonstrate that contextual features influence the response of how a non-human species responds to dead conspecifics and helps underscore the complexity and with which crows engage with their environment.

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Chapter 3: Brain activity underlying American crow processing of encounters with dead conspecifics[3]

Abstract

Among free-ranging crows, exposure to a dead conspecific can prompt alarm calling, mobbing, and occasionally physical contact. What brain regions may mediate this diversity of responses has so far been speculative. Using Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET) we contrast the metabolic response to visual and auditory cues associated with a dead conspecific among five *a priori* selected regions in the American crow (*Corvus brachyrhynchos*) brain: the hippocampus, nidopallium caudolaterale, striatum, amygdala, and the septum. Using a longitudinal, fully balanced approach, we exposed crows to four stimuli: a dead conspecific, a dead song sparrow (*Melospiza melodia*), conspecific alarm calls given in response to a dead crow, and conspecific food begging calls. We find that in response to observations of a dead crow, instead of activity suggesting conditional fear learning or an emotional response, crows show significant activity in areas associated with higher-order decision-making (NCL), and some evidence of prediction error (striatum). We do not find strong differences in activation between hearing alarm calls and food begging calls; both activate the NCL and striatum. Lastly, repeated exposures to negative stimuli had a marginal effect on the subjects' sensitivity in response to neutral stimuli, suggesting that crows might quickly learn from such experiences.

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[3] This chapter is to be submitted for publication with additional authors including: Shimizu, T., Cross, D., Templeton, C., and Marzluff, J. M.

Introduction

In humans, the observation of a dead body can prompt a variety of responses including disgust, grief, stress, fear, sadness and empathy, depending on the familiarity of the individual and the context in which it is being observed (Curtis et al. 2003; Proverbio et al. 2009; Bernhardt et al. 2012). To better understand how such a diversity of responses is mediated, functional neuroimaging studies have been used to examine what areas of the brain process images of the deceased (Taylor et al. 2000; Liberzon et al. 2003; Wright et al. 2004). Through these studies, several areas have been implicated including the sublenticular/extended amygdala region, the anterior insula, the right superior parietal cortex, and the orbitofrontal cortex. By contrast, the emotional states that are prompted by the observation of a dead conspecific in non-human animals remain unknown, but there is mounting evidence that some animals express intense interest in their dead (Anderson 2016).

In some animals such as eusocial insects the presence of a conspecific body prompts stereotyped responses such as burial, removal or ingestion (Sun and Zhou, 2013). Among other animals, particularly social mammals with large relative brain sizes such as cetaceans, primates, and elephants, observations of captive or free ranging individuals responding to conspecific corpses are both numerous and variable. These animals may explore, guard, transport, or attack the body, engage in caretaking behaviors such as grooming, or become sexually aroused (Douglas-Hamilton et al. 2006; Ritter 2007; Stewart et al. 2011; Campbell et al. 2016;).

Although observations of animals responding to their dead are key to elucidating the range of resulting behavioral expressions, such accounts cannot reveal more nuanced aspects of how animals perceive their dead or the neural mechanisms that underlie resulting behaviors. How the brain responds to audio and visual information, on the other hand, can reveal much more depth behind how humans and other animals process information. For example, dogs are well known for their attentiveness to humans, but functional magnetic resonance imaging (fMRI) studies were the first to reveal that dogs identify human faces with a specialized, face-selective region in the temporal cortex similar to one in primates, sheep and crows (Kendrick and Baldwin 1987; Perrett et al. 1988; Marzluff et al. 2012; Dilks et al. 2015). In addition to revealing specialized areas or allowing for cross-species comparisons, functional imaging studies can reveal important within-species insights into the differences in the brain's response even when the behavioral response appears similar.

When presented with a red-tailed hawk (*Buteo jamaicensis*) or a human holding a dead crow, wild American crows (*Corvus brachyrhynchos*) alarm call and recruit other birds to the area to mob (Marzluff et al. 2015; Swift and Marzluff 2015). Existing whole brain neural imaging studies on crows demonstrate that these threats are not equal and in fact trigger distinct neural circuits. Whereas novel humans holding dead crows show high activation in the avian hippocampus, an area associated with conditional and spatial learning, red-tailed hawks activate the caudal medial nidopallium, an area associated with higher-order decision-making (Güntürkün 2005; Cross et al. 2013). These studies demonstrate that behavioral observations provide an incomplete picture of the complexity in how crows perceive different dangers.

Like some mammals, some species in the Corvidae family (crows, jays, ravens, etc.) also respond strongly to dead conspecifics. American crows (*Corvus* *brachyrhynchos*), California scrub-jays (*Aphelocoma californica*), and common ravens (*Corvus corax*) will alarm call and recruit other birds to the area when a dead conspecific is discovered (Iglesias et al. 2012; Peterson and Colwell 2014; Swift and Marzluff 2015). In some cases, crows may even make exploratory, aggressive or sexual contact with dead crows (Swift and Marzluff 2018). In addition, crows demonstrate the ability to learn features associated with the dead crow after only one exposure, including the location where a dead crow was discovered, as well as the ability to learn humans seen holding them (Swift and Marzluff 2015). Given this complexity of responses, crows make a viable model for a functional neuroimaging study designed to explore what areas of the brain in a non-human animal facilitate diverse responses to conspecific bodies.

Utilizing a longitudinal Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET) imaging study, we aim to reveal neurological aspects of how American crows process the presentation of a dead crow. In addition, since attendance to the body of a dead crow appears to be mediated by individuals responding to alarm calls, we also explore patterns of activation associated with the auditory cues that follow the discovery of a dead crow. To meet these objectives, we present crows with cues associated with dead conspecifics and corresponding neutral (control) stimuli including: 1) an unfamiliar dead crow; 2) a dead song sparrow; 3) alarm calls recorded from free ranging birds during encounters with dead crows; and 4) crow begging sounds. We compare activation levels (as measured by the relative FDG uptake) between the paired threatening and neutral stimuli among five *a priori* determined brain regions (Fig. 3.1): the hippocampus, the nidopallium caudolaterale (NCL), the striatum, the amygdala, and the septum. We explore these regions based on their role in the following hypotheses:

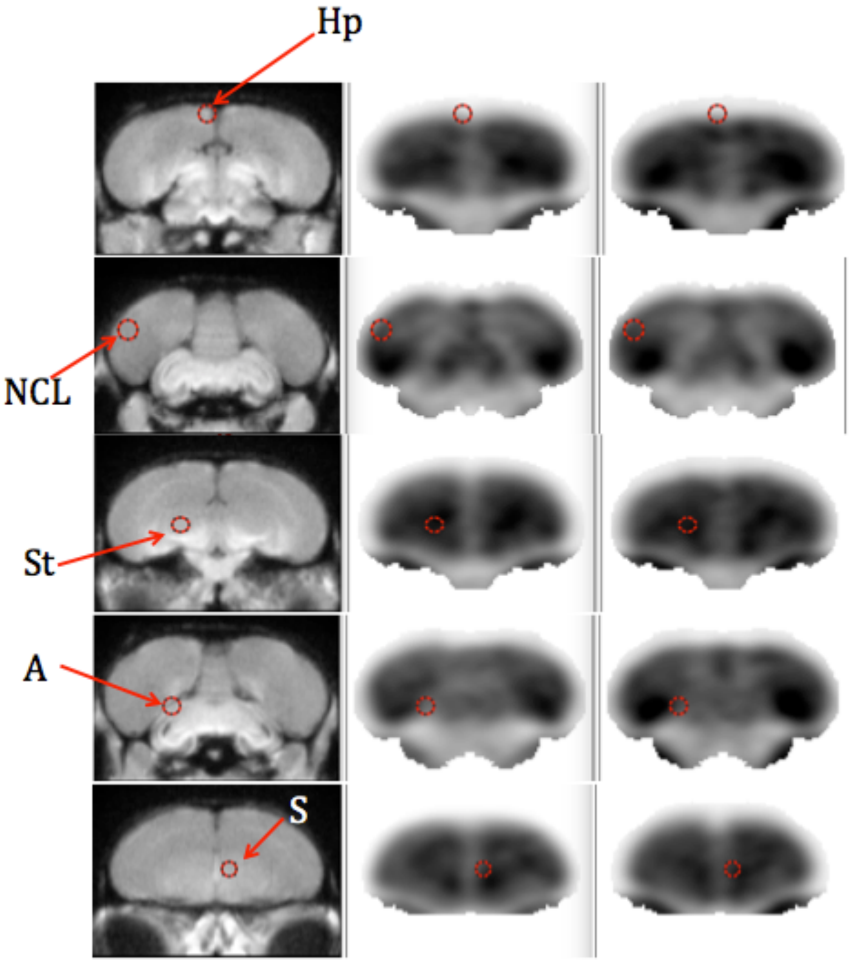

Figure 3.1 Left image is a structural Magnetic Resonance Image (MRI) of the crow brain. Center and right images are co-registered Positron Emission Tomography (PET) images from two subjects in the current study. Red circles indicate *a priori* regions of interest: Hp=Hippocampus, NCL= Nidopallium caudolaterale, St=Striatum, A=Amygdala, S=Septum. The size of the red circle has no relationship to the size of the structure. Given that the hippocampus is a smaller, more peripheral structure relative to the other four stuctures we investigate, this structure is more prone to type II errors resulting from misregistration errors.

If cues associated with dead crows primarily stimulate fearful recall and learning, we will see activation in areas associated with these processes. If cues associated with conspecifics trigger recognition circuits we will see activation in areas responsible for conspecific recognition. If cues associated with dead crows stimulate integration from multiple sensory inputs resulting in higher order decision making, we will see activation in areas associated with exectutive function. If cues associated with dead crows prompt affect, we will see activation in areas associated with emotional processing. Lastly, if rapid learning occurs between imaging session, we will see effects from the number of previous imaging session on activation levels. These hypotheses give rise to the following eight predictions: 1) When exposed to conspecific cues, crows will show activation in the Septum, which is responsible for conspecific recognition (Nishizawa et al. 2011). 2) Since dead crows are cues of danger, we expect that dead crows and alarm calls associated with them will provoke stronger activation relative to the neutral stimuli in the associative learning areas including the hippocampus, amygdala and striatum (Reilly and Good 1989; LaBar et al. 1998; Li et al. 2011). 3) Given the strong social bonds of crows, we expect that the sight of a dead crow will result in activation of the amygdala, which in humans is responsible for emotional processing (Phelps and LaDoux 2005). 4) Previous studies have shown that crows respond in variable ways to dead crows, and that dead crows appear to present as a complex stimulus (Swift and Marzluff 2018). Given these findings we expect to see activation in their nidopallium caudolaterale (NCL), which mediates executive function (Güntürkün 2005). 5) Since crows do not appear to respond in stereotyped ways to conspecific alarm calls, exposure to dead crow induced alarm calls will require integration into a multimodal pathway including the NCL. 6) We expect to see evidence of heightened or depressed FDG-uptake in proportion to the number of previous experiences, as a result of crows' ability to learn rapidly from previous experiences (Marzluff et al. 2010). 7) Given that observations of dead crows and the vocalizations associated with them indicate a mortal threat, we predict that the number of previous experiences with dangerous stimuli will have a greater effect in this respect than the number of previous neutral stimuli. 8) Furthermore, we expect that if learning from previous experiences is happening it will primarily manifest as increased sensitivity (i.e., an increase in responsiveness to novel or strong stimuli (Duerr and Quinn 1981) as indicated in this study by increased local FDG uptake) rather than habituation (i.e., a decrease in response as a result of repeated stimulation (Duerr and Quinn 1981) as indicated in this study by depressed local FDG uptake).

Methods

Animal collection and housing

On July 6th, 2014 we captured N=7 American crows (N=4 adults, N=3 sub-adults; N=5 males, N=2 females) using a net launcher from a bait site located near a large roosting center in Bothell, Washington, USA. We based age and sex determinations on body weight, tarsus width, and mouth lining color (Dos Anjos et al. 2009). None of the crows had dependent young and all had completed their annual molt. Following capture, we housed the crows for 2 weeks in adjacent outdoor aviary cages measuring 1 x 2 x 2 m. We provided a diet of meat, fruit, eggs, seeds and dried dog food *ad libitum*. We collected and cared for the crows in accordance with University of Washington Institutional Animal Care and Use Committee Protocol 3077-01, 1, Washington Scientific Collection Permit 11-359, and US Scientific Collection Permit MB761.

Stimulus presentation protocol

We imaged three subjects per day. The night before imaging, we moved the test subject to a smaller, covered 0.5 x 0.5 x 1 m cage housed within the PET laboratory room to allow for acclimation. We did not provide food in the 12 hours prior to imaging, but did provide water *ad libitum*. The day of imaging, we removed the subject from the cage while covering its face with a hood and administered one mCi of 18F-fluorodeoxyglucose (FDG) via i.p. injection, and then returned the subject to the covered cage for a two min rest. Following the rest period the cover was removed and the bird was exposed to one of four stimulus options that included two “dangerous” stimuli and two corresponding neutral stimuli (Fig. 3.2). The visual dangerous/neutral stimuli included a prepared “dead” crow skin and a prepared “dead” song sparrow skin. The audio dangerous/neutral stimuli included an audio recording of crows giving alarm calls in response to a dead crow and an audio recording of a juvenile crow begging sound. We recorded stimulus calls at a minimum of 32 km from the site where we collected the subjects, within the greater Seattle area. We assumed that all calls and skins were from birds unfamiliar to the test subjects. We used Syrinx (John Burt,www.syrinxpc.com, Seattle, WA, U.S.A) to removed background noise from recordings and Audacity 2.1.3 to normalize peak amplitude to 0dB. All tracks lasted one minute and were spaced to mimic natural call patterns. We played all tracks at an equivalent volume using a Bem wireless HL2022A speaker. All stimuli (i.e., bird skin or audio speaker) were presented on top of a white sheet of cardboard placed on a stool 1 m in front of the cage. We used multiple

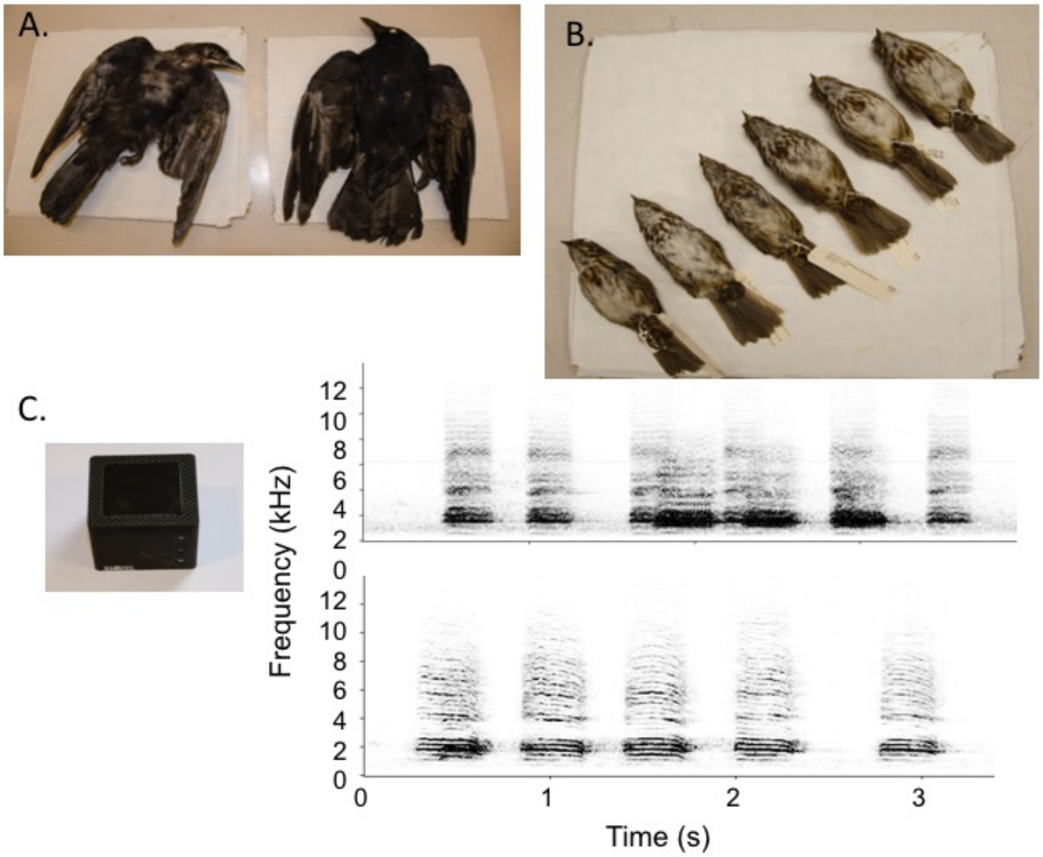

Figure 3.2 All subjects were exposed to four stimuli: A) an unfamiliar dead crow, B) an unfamiliar dead song sparrow, and C) a speaker playing one of two conspecific vocalizations including alarm calls given in response to a dead crow and food begging calls. The spectrograph shows differences in call structure between alarm calls (top) and food begging calls (bottom).

exemplars of each stimulus in a random order (N=3 dead crows, N=7 dead song sparrows, N=7 begging calls, N=6 mobbing calls). Stimulation lasted 10.5 min and included seven one minute presentations of the stimulus followed by 30 sec of rest in the cloaked cage. Over the course of four separate testing days, each crow was exposed to all four stimuli in a balanced design (N=4 exposure trials per subject; N=28 total trials). Three of the subjects received one of the two dangerous stimuli first and the other four the neutral stimulus. Across the seven subjects we presented stimuli in every possible sequence.

Imaging and Image Processing

Once the exposure protocol was complete, we hooded and removed the subject from its cage, anesthetized it with five percent isoflurane in oxygen with a flow rate of 300-800 mL/min, and positioned it in the scanner. We obtained high resolution FDG-PET images using a Siemens Inveon PET system for 10 min, starting 25 min after FDG injection (except for one subject, which was imaged after 27 min). Following imaging, we executed a 13 min attenuation scan and then reconstructed using vendor supplied 3D OSEM/MP algorithm with attenuation and scatter corrections applied to the data. The image matrix wa3s 128 x 28 x 159. We stereotaxically aligned the PET images to the jungle crow (*Corvus macrorhynchos*) brain atlas (Marzluff et al. 2012; Izawa and Watanabe 2007). For consistent stereotactic transformations of scans from the same subject, we estimated and applied nine affine parameters to the images using algorithms for automated human brain image analysis adapted for crow brains (NEUROSTAT, University of Utah; Minoshima et al. 1992). We estimated that aligned precision was one to two millimeters.

Statistical procedure

After normalizing to global brain FDG uptake (a process that controls for between-individual differences in injection dose and subject weight; Friston 1990), we conducted volume of interest (VOI) analysis to extract FDG uptake values from our five *a priori* selected regions across each subject's four stimulus tests. As the amount of metabolic FDG uptake is used to infer the relative level of activation, moving forward we will refer to these data as "brain activity", "activation level" etc., in the main text, rather than as "FDG uptake". To account for the longitudinal design we used repeated-measures ANOVAs to compare activations between the sight of a dead crow and a dead sparrow, or activations between hearing alarm and begging calls across each region. These results are reported as the within-subjects main effects.

To account for the effect of possible learning between trials, we included a covariate to test for the interaction between the main (stimulus) effect and the effect of the previous number of exposures. For example, a subject that received stimuli in the order dead crow, begging, scolding, dead sparrow, would have seen one dangerous stimulus prior to the neutral trial when evaluating responses to auditory stimuli, but two dangerous stimuli prior to the neutral test when evaluating responses to visual stimuli. Thus we included either the number (0-2 possible) of previous dangerous exposures or the number of (0-2 possible) of previous neutral exposures, to determine which experience had a greater effect on the overall response.

We used the between-subjects effect (which is funcationally a linear regression of the covariate on the activation level) along with the size and direction of the between-subjects parameter estimates (beta value) associated with each stimulus (i.e., dead crow and dead sparrow or scolding and begging) to evaluate the influence of presentation order on the subjects' sensitivity or habituation to repeated exposure. We defined "sensitivity" as increased activity with the number of previous (dangerous/neutral) stimuli exposures and "habituation" as decreased activity correlated with the number of previous (dangerous/neutral) exposures. We discuss the outcome of both these covariate approaches in detail in the "Learning from prior experience during experimentation" section in the Results. Because we found that the number of dangerous exposures was more often significant or marginally significant when looking at the between-subjects effect, we only report main effects with tests using this as a covariate, as this seemed more conservative (see "Differential response to dangerous and neutral stimuli" in the Results).

We used a one-way ANOVA to compare each dangerous and corresponding neutral stimulus against the values from three crows that saw only an empty room from an initial baseline study (Marzluff et al. 2012). To facilitate this comparison, the stimulus presentation and imaging protocol were designed to match the protocol from this initial study. Only higher activity resulting from the stimulus (i.e., dangerous stimulus compared to neutral, or any stimulus compared to empty room) is reported. Results where the control showed significantly higher activation than the stimulus are not discussed due to our inability to determine if such activity was because of higher activation in the control relative to the stimulus, or due to stimulus induced deactivation (Shulman et al. 1997).

We adopted an approach to statistical inference as proposed by Fisher (1925). We considered p values <0.05 as evidence that an effect should be confirmed by other studies, and p values between 0.05 and 0.20 as evidence of effects that should be tested in future studies with increased replications or other design improvements. We consider p values > 0.20 to indicate that the effect is too small to be detected by the experimental design. We used SPSS v.19 (IBM, Armonk, NY, U.S.A.) to conduct all statistical tests.

Results

Learning from prior experience during experimentation

Crows showed some degree of sensitization and habituation to repeated exposure to stimuli. In our longitudinal study these forms of learning were indicated by between-subjects effects of presentation order in the amygdala, the hippocampus and the striatum. These responses did not appear to be more common during auditory vs. visual tests. Only in the left-hemisphere of the amygdala did we find a significant overall effect of presentation order across both stimulus groups (Dead crow/dead sparrow between-subjects $F_{1,5}$=22.30, p=0.005, Fig. 3.3; scolding/begging, $F_{1,5}$=16.08, p=0.010, Fig. 3.4). In both of these cases, we found evidence of sensitization indicated by the positive relationship between the number of dangerous exposures (a dead crow or playback of scolding calls) and the activation level during exposure to a neutral stimulus (Dead sparrow beta=17.81, begging beta=34.96). Sensitization was also suggested by some evidence of effects in the right hemisphere of the striatum (Between-subjects $F_{1,5}$=F5.16,

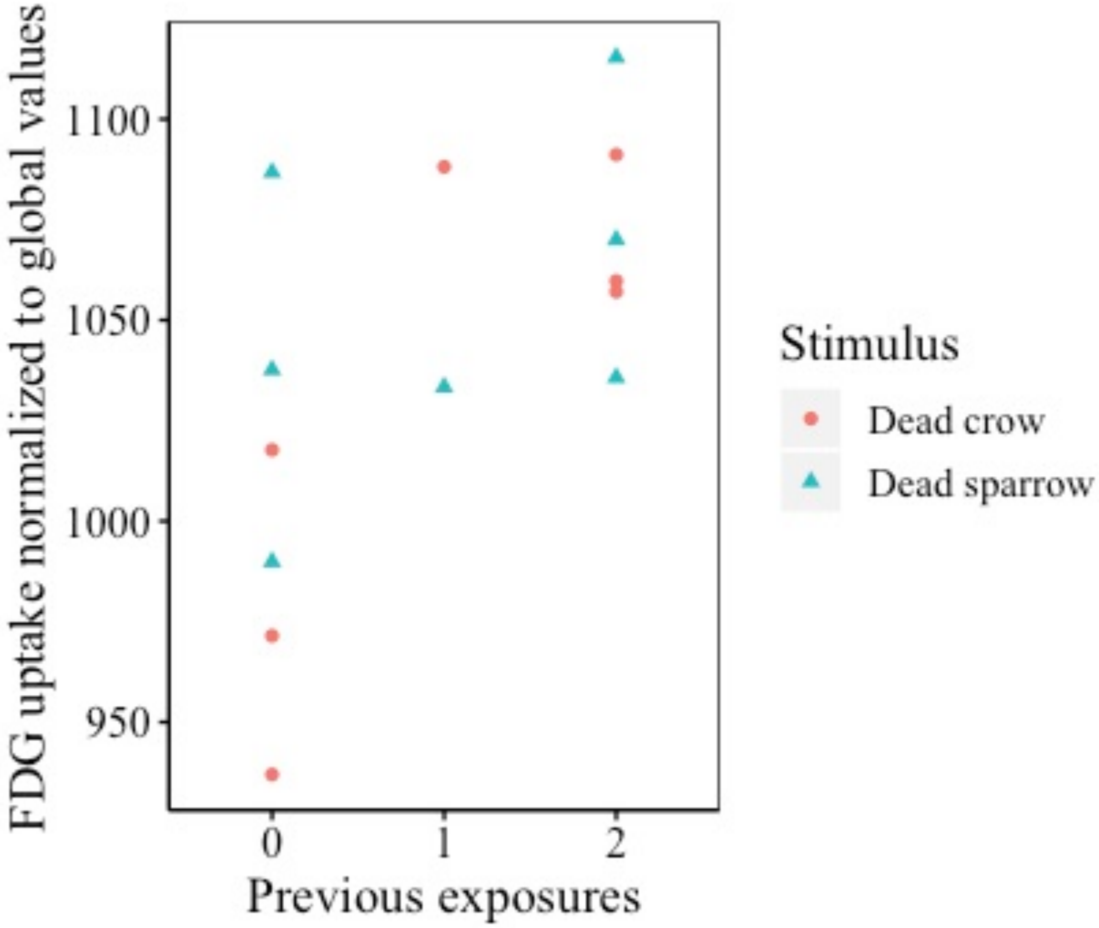

Figure 3.3 Effect of number of previous exposures to dangerous stimuli (either dead crow, alarm calls, or both) on FDG uptake in left hemisphere of the amygdala during experiences with visual stimuli.

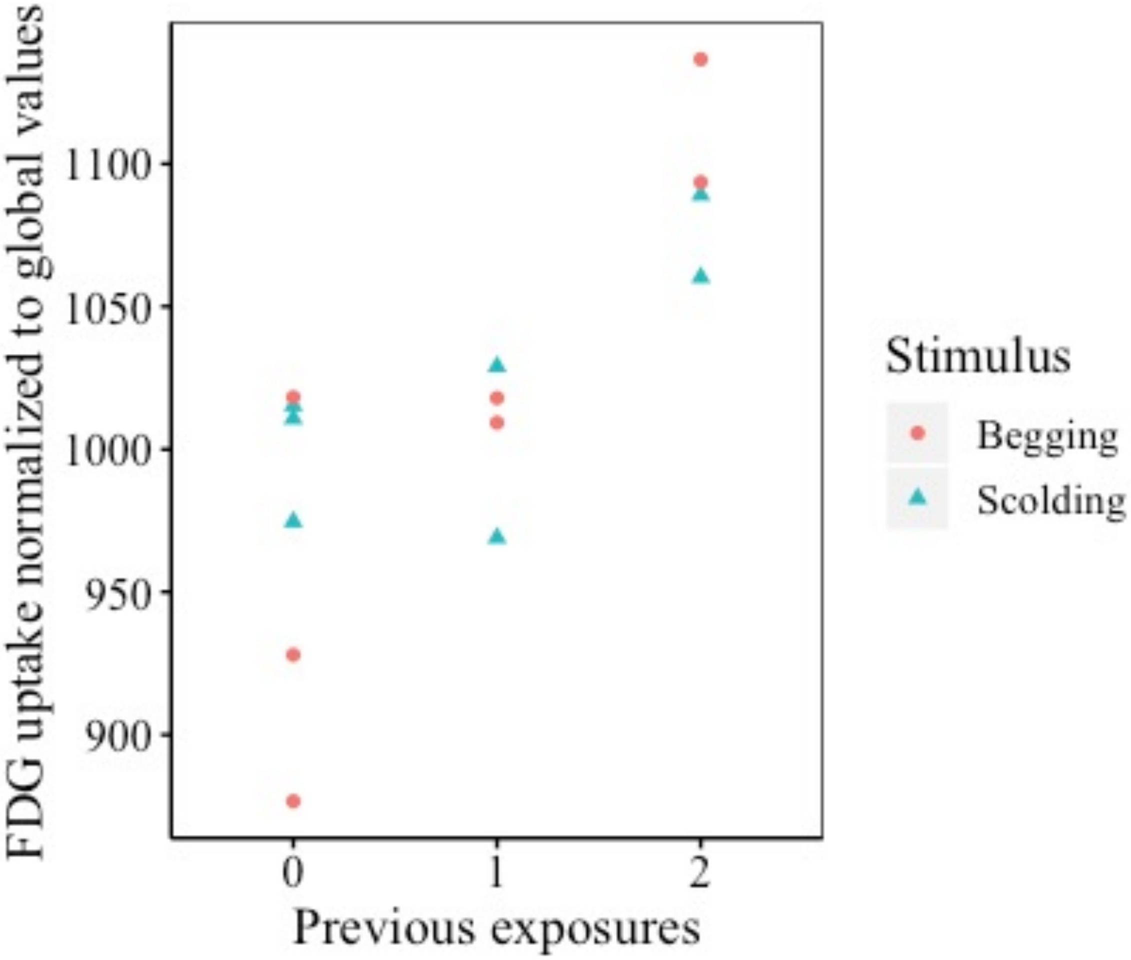

Figure 3.4 Effect of number of previous exposures to dangerous stimuli (either dead crow, alarm calls, or both) on FDG uptake in left hemisphere of the amygdala during experiences with auditory stimuli.

p=0.072; beta=12.05) during visual experiments. We also found some evidence of an effect of presentation order in the left and right hemispheres of the hippocampus during auditory experiments (Left: between-subjects $F_{1,5}$=4.42, p=0.089; Right: $F_{1,5}$=5.01, p=0.076). In these cases, exposure to a dangerous stimulus prior to hearing begging calls dampened activation during exposure to begging calls, suggesting habituation (Hp left begging: beta=-40.75; Hp right begging: beta=-63.84).

Exposure to neutral stimuli prior to dangerous stimuli was associated with less frequent learning than the reverse. We found some evidence of habituation in the left hemisphere of the amygdala during auditory tests (Between-subjects $F_{1,5}$=4.57, p=0.087, scolding beta=-16.56). We also found some evidence of sensitization in the right hemisphere of the striatum (Between-subjects $F_{1,5}$=5.16, p=0.072; dead crow beta=55.31).

Differential responses to dangerous and neutral stimuli

When confronted with a dead conspecific, crows showed higher relative activity in their nidopallium caudolaterale (NCL) than when presented with a dead song sparrow (Right-hemisphere: $F_{1,5}$=22.6 p=0.005; Left-hemisphere: $F_{1,5}$=19.22, p=0.007; Fig. 3.5). In neither case did learning from prior trials strongly influence the differential response of the NCL (Right-hemisphere: interaction of covariate with main effect $F_{1,5}$=0.84, p=0.40, Dead sparrow beta=7.78,; Left-hemisphere: $F_{1,5}$=4.36, p=0.09, Dead sparrow beta=-11.89,). Likewise, we found significant right-hemisphere NCL activity when comparing presentations of the dead crow to crows that had seen only an empty room ($F_{1,8}$=135.93, P<0.001). We found evidence of increased activity in the right-hemisphere

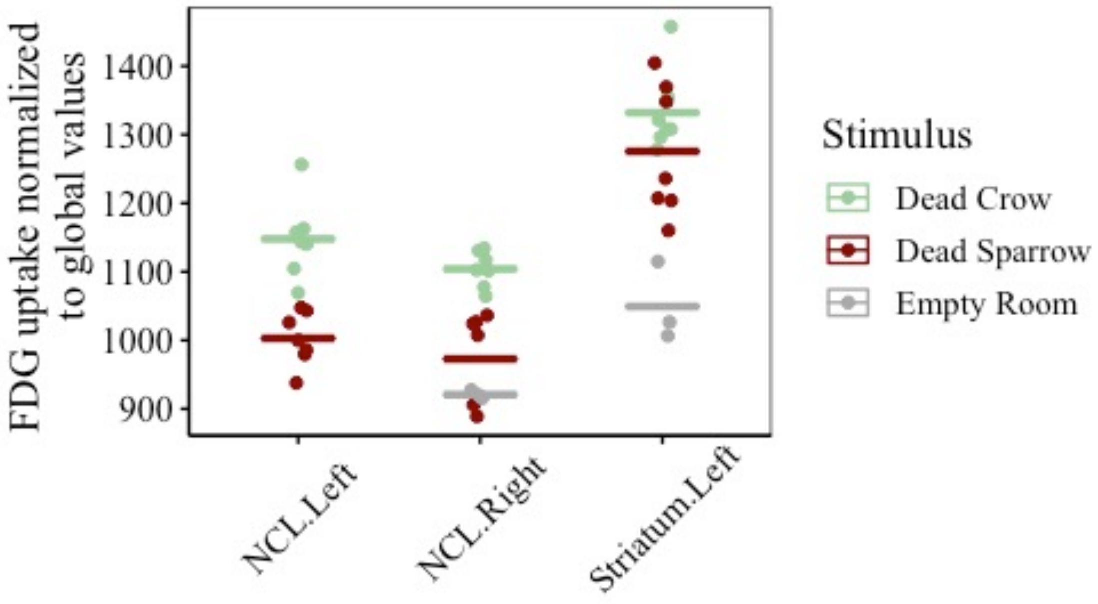

Figure 3.5 Individual values for FDG uptake normalized to global values in each structure and corresponding hemisphere where activation in response to the stimulus (i.e., dead crow in the case of dead crow vs. dead sparrow or dead sparrow in the case of dead sparrow vs. empty room) met the threshold for statistical significance (P<0.05). Horizontal lines indicate group mean. Individual data points represent uptake values for each subject (N=7 Dead crow, N=7 Dead sparrow, and N=3 Empty Room subjects). In all cases, Dead Crow was significantly higher than the control stimuli shown (Dead Sparrow and/or Empty Room). In one case (NCL, Right hemisphere) Dead Sparrow was not significantly different from Empty Room subjects. Empty Room data are omitted from structures where stimulus activation was never higher.

of the septum when crows viewed a dead conspecific relative to when they viewed a dead sparrow ($F_{1,5}$=3.77, P=0.11; interaction of covariate with main effect $F_{1,5}$=1.71, P=0.25, dead sparrow beta=25.46; Fig. 3.5). However, the response of the septum to the dead crow did not differ from the response to an empty room (Right-hemisphere $F_{1,8}$=0.096, P=0.764; left hemisphere: $F_{1,8}$=0.038, P=0.55; Fig. 3.5). We found some evidence of activity in the right hemisphere of the striatum when comparing dead crows and dead sparrows ($F_{1,5}$=3.87, p=0.11; no significant interaction with prior experience; Fig. 3.5). Interestingly, though, when comparing dead crows and dead sparrows to the empty room, both showed activity in the left, but not right, hemispheres (Dead crow vs. empty room: $F_{1,8}$=47.3, P<0.001; Dead sparrow vs. empty room $F_{1,8}$=13.9, P=0.006; no significant interaction with prior experience; Fig. 3.5). We did not find that dead crows stimulated significantly higher activity in the hippocampus amygdala relative to either dead sparrows or the empty room (See Tables 3.1 and 3.2 for full test results).

Comparing presentations of crow alarm and begging vocalizations, we found activity in both the right and left-hemispheres of the septum (Right hemisphere: $F_{1,5}$ =14.98, P=0.012; Left hemisphere: $F_{1,5}$=4.5, p=0.087). In both cases, this activity was influenced by prior experience (Right hemisphere: interaction of covariate with main effect, $F_{1,5}$=6.94, P=0.046; Left hemisphere: interaction of covariate with main effect: $F_{1,5}$=7.97, p=0.037). In this case, crows appeared to become more sensitive when exposed to the neutral begging call (Right: begging beta=33.82; Left: begging beta=48.36). We also found some evidence of activity in the left hemisphere of the striatum ($F_{1,5}$=3.95, p=0.10; no significant interaction with prior experience). We did see

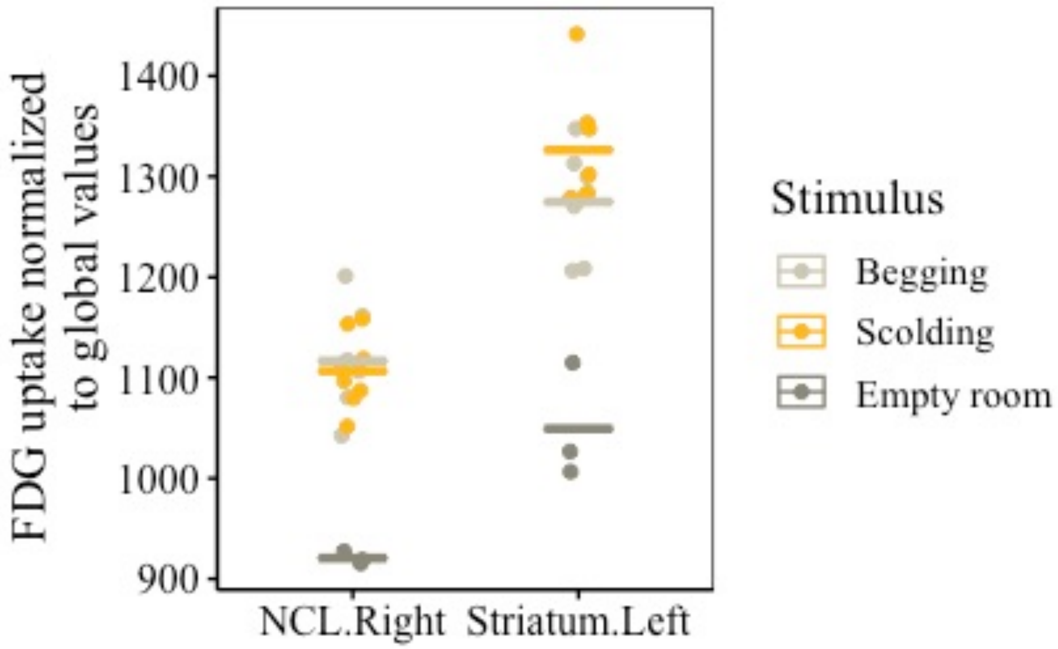

Figure 3.6 Individual values for FDG uptake normalized to global values in each structure and hemisphere where activation in response to the stimulus met the threshold for statistical significance ($P<0.05$). Horizontal lines indicate group mean. Individual data points represent uptake values for each subject (N=7 Begging, N=7 Scolding, and N=3 Empty room subjects). In all structures, Begging and Scolding showed higher activation relative to the empty room, but not each other.

Dead crow VS dead sparrow			
ROI	Test result	Right-hemisphere	Left-hemisphere
Hippocampus	Main Effect	F=6.89, P=0.047	F=7.22, P=0.043
	Covariate	F=0.28, P=0.62	F=0.03, P=0.87
NCL	Main Effect	F=22.36, P=0.005	F=19.22, P=0.007
	Covariate	F=0.84, P=0.40	F=4.36, P=0.091
Striatum	Main Effect	F=3.87, P=0.11	F=0.59, P=0.48
	Covariate	F=1.06, P=0.35	F=0.08, P=0.78
Amygdala	Main Effect	F=2.62, P=0.34	F=1.53, P=0.27
	Covariate	F=0.14, P=0.73	F=0.97, P=0.37
Septum	Main Effect	F=3.77, P=0.11	F=0.016, P=0.90
	Covariate F,P	F=1.71, P=0.25	F=0.50, P=0.51

Begging VS scolding			
ROI	Test	Right-hemisphere	Left-hemisphere
Hippocampus	Main Effect F,P	F=0.02, P=0.90	F=3.03, P=0.14
	Covariate F,P	F=0.15, P=0.72	F=0.16, P=0.71
NCL	Main Effect F,P	F=1.36, P=0.30	F=0.62, P=0.47
	Covariate F,P	F=1.36, P=0.30	F=9.32, P=0.028
Striatum	Main Effect F,P	F=2.16, P=0.30	F=3.95, P=0.10
	Covariate F,P	F=0.69. P=0.12	F=0.79, P=0.41
Amygdala	Main Effect F,P	F=0.015, P=0.91	F=4.26, P=0.09
	Covariate F,P	F=2.83, P=0.36	F=5.57, P=0.065
Septum	Main Effect F,P	F=14.98, P=0.012	F=4.5, P=0.087
	Covariate F,P	F=6.936, P=0.046	F=7.97, P=0.037

Table 3.1. Result of repeated measures tests from all paired dangerous/neutral stimulus tests. Green shading indicates tests with p values <0.05. Blue indicates significant (p<0.05), but unreported result due to activation in the wrong direction (i.e mean activation of neutral stimulus is higher than dangerous). All F statistics have df=1,5.

	Hippocampus	NCL	Striatum	Amygdala	Septum
	Empty Room vs. Scold				
Right	F=40.5, P<0.001	F=62.49, P<0.001	F=0.49, P=0.50	F=27.5, P=0.001	F=0.35, p=0.57
Left	F=33.1, P<0.001	F=0.88, p=0.38	F=45.8, P=0.00	F=0.60, P=0.46	F=1.19, P=0.31
	Empty Room vs. Beg				
Right	F=24.4, P=0.001	F=39.8, P<0.001	F=0.002, P=0.97	F=20.22, P=0.002	F=1.99, P=0.20
Left	F=17.227, P=0.003	F=0.06, p=0.82	F=36.9, P<0.001	F=0.46, P=0.52	F=0.49, P=0.51
	Empty Room vs. Dead Crow				
Right	F=19.1, p=0.002	F=135.9, P<0.001	F=0.21, p=0.66	F=22.74, P=0.001	F=0.096, P=0.76
Left	F=12.7, p=0.007	F=0.045, P=0.84	F=47.3, P<0.001	F=0.21, P=0.66	F=0.38, P=0.55
	Empty Room vs. Dead Sparrow				
Right	F=0.086, P=0.78	F=1.81, P=0.22	F=1.18, P=0.31	F=5.7, P=0.04	F=1.4, P=0.27
Left	F=4.86, P=0.06	F=40.46, P<0.001	F=13.9, P=0.006	F=0.002, P=0.97	F=1.45, P=0.26

Table 3.2. Results of ANOVA tests between stimuli and empty room subjects. Green indicates significant tests as reported in Results. Blue shading indicates significant, but unreported result due to activation in the wrong direction (i.e mean activation of empty room is higher than stimulus). All F statistics have df=1,8. Left or right-hemisphere indicated in left-most column.

some differences between the auditory stimuli and the empty room control, however. Crows showed significant right-hemisphere activation of their NCL during both types of playback (Scold: $F_{1,8}$=62.49, P<0.001; Beg: $F_{1,8}$=39.8, P<0.001; Fig. 3.6). In addition, during alarm call playback trials we found higher left-hemisphere activation in their striatum ($F_{1,8}$=45.82, P<0.001; Fig. 3.6). Likewise, begging calls showed higher left-hemisphere activation in the striatum ($F_{1,8}$=36.86, P<0.001; Fig. 3.6; see Supplementary figures S3.1-S3.5 for mean activation across all tested regions).

Discussion

Some nonhuman animals including cetaceans, elephants and primates have repeatedly been observed expressing a diversity of responses towards conspecific carcasses, but the drivers of these behaviors are unknown (Anderson 2016, Watson and Matsuzawa 2018). An understanding of brain activity during these encounters may help provide clarity, but no such studies have been undertaken in a nonhuman mammal. Likewise, wild American crows also express a diversity of responses to dead conspecifics including alarm, rapid, long-term memory formation, and as well as variety of physical interactions (Swift and Marzluff 2015; Swift and Marzluff 2018). In the present study we sought to explore how such a diversity of responses may be explained by what brain areas process visual and auditory information associated with dead crows. In response to visual stimuli, we found that crows showed no significant activity in areas associated with learning like the amygdala and hippocampus. Instead, observations of dead crows, but not dead sparrows, provoked activation in the NCL, an area associated with complex decision-making. In addition, we found some support for activation in the striatum and septum, which regulate prediction errors and conspecific recognition, respectively. In response to auditory stimuli, we found that hearing scolding calls corresponded with higher levels of activation in septum, relative to hearing begging calls, but found no differences in the other four regions. When comparing the two calls types against the empty room, we found consistent activation of the NCL and striatum. During both visual and auditory tests, we found evidence of a lateralized response across most regions, where either the left or right hemisphere was more strongly activated. Finally, we found that crows showed some evidence of learning with each trial, most commonly manifesting as increased sensitivity. Together, these results suggest that cues associated with dead crows stimulate higher order brain regions that allow for context dependent decisions about how to respond, along with other areas important for regulating predictions and conspecific recognition. This process may help explain why, in the wild, crows display such a diversity of responses towards visual and auditory cues associated with dead conspecifics (Swift and Marzluff 2018).

Wild American crows are wary in areas where a conspecific body was previously discovered and remember people they saw handling them (Swift and Marzluff 2015). That they learn threats associated with dead crows was further demonstrated in a previous FDG-PET study wherein crows showed significant activity in their hippocampus, an area associated with conditional fear learning, when viewing a novel person holding a dead crow (Reilly and Good 1989; Cross et al. 2013). Given these findings, we predicted that the observation of a dead crow, even in the absence of a predator, would activate the hippocampus. Contra to this predication, we did not find higher levels of hippocampal activation relative to either seeing a dead song sparrow or to birds that saw only the empty room. Since dead crows are innate cues of threat, and the hippocampus is associated with forming new memories, it is possible this region is only weakly recruited when birds are faced with familiar dangers (Strasser and Bingman 1999). Perhaps to detect a stronger associative learning response, presentations of the dead crow needed to include an element that was unique to only those trials, such as a novel person, or possibly even an object, since birds may exhibit attendance to the imaging room across all trials. It is also possible that low detection of hippocampal activation is due to the fact that the structure itself is quite small and located near the periphery of the brain, which makes it more vunerable type II errors resulting from misregistration errors.

In humans, the amygdala plays a central role in fear signaling and affect processing including grief (LaBar et al. 1998; Grundel 2003; Wang et al. 2005; Sergerie et al. 2008). Among birds and non-human mammals, the amygdala has likewise been implicated in acquiring and expressing conditioned fear (Blanchard and Blanchard 1972; Philips Yougran 1986; Miserendino et al. 1990). Although some scientists assert support for emotional intelligence, including grief, among non-human animals this idea remains controversial (Poole 1998; Bekoff 2000). Given the analogous function of the amygdala in birds and humans, evidence of an emotional response to the sight of a dead crow may therefore be present in the amygdala. A previous whole-brain imaging study found inconsistent support for amygdaloidal activation during observations of a dead crow, but in that study crows were paired with novel humans who may have acted to down regulate activation in the amygdala in favor of other associative learning pathways like the hippocampus and cerebellum (Cross et al. 2012). We addressed these effects by presenting dead crows in the absence of a potentially confounding predator. Yet, we did not find evidence of amygdala activation in comparison to either the observation of a dead song sparrow or when compared to birds that saw an empty room. This finding may be because crows do not express affiliative responses towards dead crows, or because they do not do so towards unfamiliar crows. Lack of amygdaloidal activation is also surprising given its role in the expression of fear responses in birds and the previously demonstrated activation during encounters with conditioned fears (Saint-Dizier et al. 2009; Marzluff et al. 2012). Given that birds do not express a typical fear response (alarm calling and movement away from the body) when confined to the imaging cage, they may not show strong amygdaloidal activation. In addition, although dead crows provoke defensive responses associated with fear (alarm calling, mobbing, etc.), they do not provoke a stereotyped response, suggesting that other brain areas may be more important than the amygdala in processing this complex stimulus.

In the wild crows demonstrate variable responses towards dead conspecifics (Swift and Marzluff 2018). They may alarm call, mob, touch, or in rare cases do nothing. Among birds that touch dead crows, they may do so in an exploratory, violent or more rarely, even a sexual manner (Swift and Marzluff 2018). From these observations it is clear that dead crows present as a complex stimulus, evoking fear, aggression, and sometimes sexual arousal. Furthermore, crows appear to be attentive to issues of context such as whether there is a predator with the dead crow, the age of the dead crow at time of death, and the season (breeding or nonbreeding) in which it is encountered (Swift and Marzluff 2015; *See Chapter 2*). By implication, responses to dead crows are not produced via a simple stimulus-response mechanism and require higher-order processing.

Crows observing a dead conspecific showed significant activation in the nidopallium caudolaterale (NCL) when contrasted against the empty room and the dead sparrow. This result suggests that NCL activity is not stimulated simply by the observation of corpses alone. The NCL has been proposed as an analologous region to the human prefrontal cortex (PFC) and is suggested to mediate executive functions in birds (Güntürkün 2005; Moll and Neider 2015). For example, the ability of corvids to demonstrate insight, episodic memory, and other features of cognitive flexibly has been largely attributed to their NCL (Nieder 2017). In humans, the prefrontal cortex plays an important role in the circuit responsible for the regulation of fear and negative affect, including the withdrawal system, which organizes appropriate responses to threat cues (Davidson 2001). When crows are exposed to another innate threat, a red-tailed hawk, they also show activation of the NCL. These findings suggest that, rather than simple stimulus-response behaviors, unconditioned threats prompt complex decision making allowing crows to respond in a variety of ways. This hypothesis may explain why a broad diversity of responses including no response, scolding, and different kinds of touching have all been observed among wild crows in response to a dead conspecific (Swift and Marzluff 2018).

It is worth considering that, in humans, the relationship between the PFC and the amygdala in terms of how they regulate fear and negative affect is complex and not fully understood. What is becoming clear is that, despite there being no direct connection between the lateral/dorsal PFC and the amygdala, these areas can respectively down regulate one another in certain kinds of tasks such as willful suppression or enhancement of negative emotions (Phan et al. 2005; Urry et al. 2006). Whether non-human animals are capable of such tasks is unknown, but the relationship between the human PFC and the amygdala raises the question as to whether the observed lack of amygdaloidal activation in our study is related to the activity in the NCL and warrants further study.

When compared to the empty room control, crows showed left-hemisphere activation of the striatum in response to both the dead crow and dead sparrow. The striatum plays a key role in associative learning, particularly with respect learning from prediction errors (i.e., incorrect guesses about future rewards and punishments; Li et al. 2011). Its activation in response to the dead crow may have resulted from the unexpected lack of alarm calling or the presence of a predator. Its activation in response to the sparrow may have resulted from failing to get the anticipated reward of eating it. Our finding that activation was left-hemisphere biased seems to supports our interpretation that the striatum was activated as apart of a learning mechanism. For example, in response to visual stimuli, before and especially after conditioned learning, pigeons show higher left-hemisphere neural recruitment (Verhaal et al. 2012).

We found some evidence of activity in the septum when comparing responses to dead crows against dead song sparrows, but not when comparing responses to dead crows against the empty room. Given the well-established role of the septum in conspecific recognition and social behavior, we expected a stronger than observed response (Nishizawa et al. 2011; Mayer et al. 2017). It may be that a larger sample size would reveal more activity in this brain region, and so further study in this area is warranted. Alternatively, to our knowledge, studies exploring the neural substrates of conspecific recognition have done so with individuals observing living, often moving, conspecifics (Mayer et al. 2017). Thus, it is possible that in cases where the conspecific is dead, and therefore presents as a threat rather than social opportunity, the pathway is different.

When exposed to a crow alarm call given in response to a dead crow, subjects showed some activity in the right-hemisphere of the septum. That we saw activity in the septum is consistent with previous studies demonstrating the role of the avian septum in modulating behavior, particularly aggression (Goodson et al. 1999). Because birds were collected during the breeding season, they may show a stronger aggressive reaction to conspecific calls, especially when the calls are associated with a threat, than if we had tested birds outside of the breeding season.

We found no other differences among our four other brain regions between trials where birds heard alarm calls produced in response to a dead crow and food begging calls. Previous field studies have shown that, among free ranging crows, playback of conspecific alarm calls and food-begging calls produce distinct behavioral responses (e.g., Frings et al. 1958). Our results suggest that whereas crows have a variety of calls used for communicating different aspects of their state or environment, and can respond in variety of ways, similar pathways ultimately mediate these auditory cues. This conclusion is supported by our findings that alarm and begging calls provoked identical patterns of activation when compared to the empty room. We found that both calls showed left-hemisphere activation in the striatum. Among other mammalian and avian vocal learners, the striatum shows some gene expression related to vocal learning and auditory processing, however the level of expression in the striatum is low relative to other areas of the telencephalon (Wang et al. 2014).

It is also possible that the absence of a visual stimulus contributed to the similarities in response to different kinds of vocal playback. Perhaps crows would show more dramatic differences if they could observe the vocalizing bird, especially because in the wild both begging and scolding calls are accompanied by visual cues that underscore their meaning (Kilham 1990). The surprise of hearing calls in such a strange context may have likewise contributed to a possibly atypical neurological response.

Significant activation of the NCL during exposure to different conspecific calls suggests that crows do not respond to conspecific calls in fixed ways but rather integrate information from a variety of sensory modalities and respond in a context-dependent way. For example, during the stimulus exposure protocol when crows are in small enclosures with movement is limited, crows do not alarm call in response to even the most salient threats suggesting that they are able to evaluate that such a response would be inappropriate or dangerous (Marzluff et al. 2012; Cross et al. 2013). In the wild, the ability to use executive functions when processing alarm calls may allow crows to ignore calls given inappropriately to benign threats, or when responding may be more costly (*See Chapter 2*; Marzluff et al. 2015).

Across both visual and auditory trials we found evidence of hemispheric lateralization among all tested brain regions. Lateralization is widespread among vertebrates and aids in a variety of tasks including object discrimination, predator aversion, and the ability to attend to multiple stimuli (Rogers 2002; Rogers et al. 2004). Although visual lateralization has been the most predominately studied form, there is also evidence of other sensory lateralization including audio and olfactory (Rogers, 2008; Mooreman et al. 2012; Vallortigara and Andrew, 1994). Here we found that when comparing dead crows and sparrows, the NCL showed unilateral activation, but when comparing dead crows to the empty room, we found a clear right-hemisphere bias. Likewise, we found marginal right-hemisphere activity in the septum when comparing dead crows and sparrows. We also found consistent activity in the right-hemisphere of the NCL when comparing scolding and begging calls against the empty room, as well as in the septum when comparing scolds and begs against one another. These findings are consistent with previous studies showing that the right-hemisphere controls fear and escape responses (Rogers 2008). For example, Dharmaretnam and Rogers (2005) found that chicken chicks (*Gallus gallus domesticus*) will reorient in order to be able to view predators with their left eye (right-hemisphere). Marzluff et al. (2012) also found general right-hemisphere biased activation in the crow brain among subjects viewing familiar faces of threatening people; however, as in our study lateralization was inconsistent in the nidopallium. Why we found right-hemisphere-biased activity during conspecific playbacks is somewhat puzzling, though, considering the robust literature on left-hemisphere processing of conspecific calls (Moorman et al. 2012).

In the striatum, we found left-hemisphere bias when comparing all four stimuli against the empty room. Among vocal playback trials, higher left-hemisphere activity is consistent with previous studies showing that harpy eagles (*Harpia harpyja*) and California sea lions (*Zalophus californianus*) orient to familiar conspecific sounds with their right ear (left-hemisphere; Palleroni and Hauser 2003; Böye et al. 2005). Furthermore, auditory processing as well as song and speech learning generally appear to be left-side biased in both birds and humans (Moorman et al. 2012). In response to the dead sparrow, the left sided activation of our subjects is consistent with previous studies demonstrating that reward-associated cues prompt increased left hemisphere activity (Verhaal 2012). Why a threat associated cue (the dead crow) provoked similar asymmetry warrants further study, however.

We found some evidence of learning occurring between stimulus exposures over the course of our longitudinal study. The effect of previous experiences appeared to be more influential if they were negative rather than neutral. This pattern was most evident in the left hemisphere of the amygdala and to some extent the right hemisphere of the striatum, and both hemispheres of the hippocampus. The amygdala and striatum showed increased sensitivity in response to the number of previous dangerous exposures, whereas the hippocampus showed marginal evidence of habituation. Given that repeated exposures to cues of danger may indicate that a particular space (the imaging room) or experience (stimulus presentation protocol) is of mortal threat, it is unsurprising that crows more often showed increases in sensitivity following exposures to dangerous stimuli, rather than habituation. That crows will rapidly learn from dangerous experiences has been previously demonstrated. For example, crows will learn the faces of people who capture them or who are seen holding dead crows after only a single exposure (Marzluff et al. 2010; Swift and Marzluff 2015). Such rapid learning is also of social value to crows and other corvids. After only a single experience, common ravens (*Corvus corax*) will learn and remember people who were unfair during an exchange task, and will choose not to work with them in the future (Müller et al. 2017). Our finding that crows learned from previous experiences underscores the care with which longitudinal studies using crows must be conducted. Therefore, as in our study, future experiments with crows must fully balance the order in which stimuli are presented and statistically account for previous experiences.

To our knowledge this is the first functional imaging study to examine the response of a non-human animal to the presentation of a dead conspecific, or to conspecific alarm calls. In response to viewing a dead crow, we found activation patterns consistent with higher-order decision-making but not learning or an emotional response. Further studies presenting sedated, familiar individuals may help reveal if in this study the unfamiliarity of the presented individual influenced the regional brain activity. In response to dead crow induced conspecific alarm calls, we found no activation when compared to a neutral conspecific call, however both auditory stimuli provoked significant activation in areas associated with executive function and auditory processing when compared to the empty room. Together these results demonstrate that, among crows, the range of behavioral response to cues associated with different threats may be somewhat limited but their response is far from fixed.

Supplemental material

The following figures show test results across all regions of interest and hemispheres relating to the experiment presented in the paper titled "Brain activity underlying American crow processing of dead conspecifics". In figures S3.1-S3.5 we present the mean and standard error for normalized FDG uptake in each brain region.

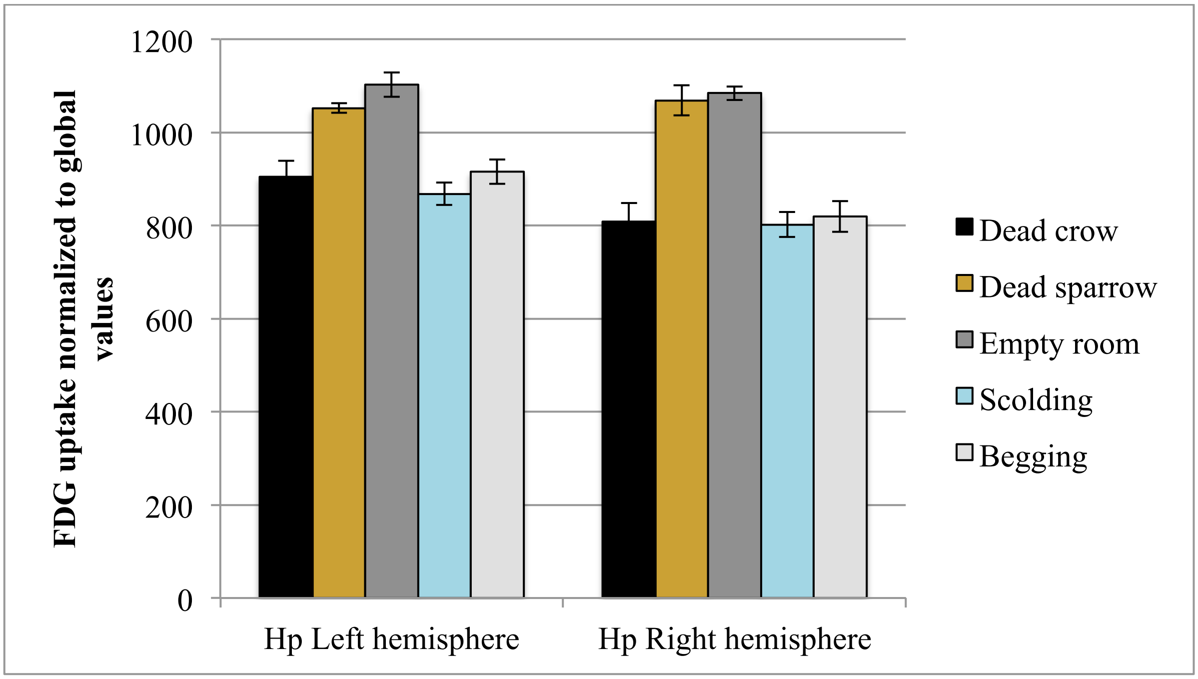

Figure S3.1 Mean FDG uptake across all stimuli and both hemisphere of the hippocampus. Error bars show standard error.

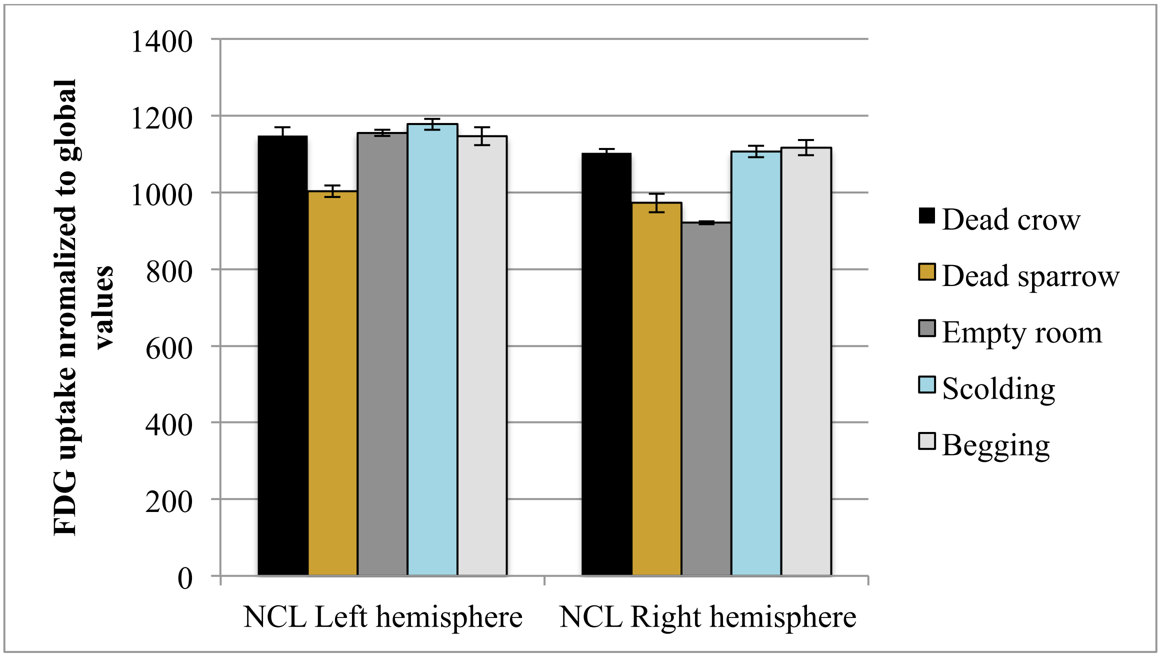

Figure S3.2 Mean FDG uptake across all stimuli and both hemisphere of the NCL. Error bars show standard error.

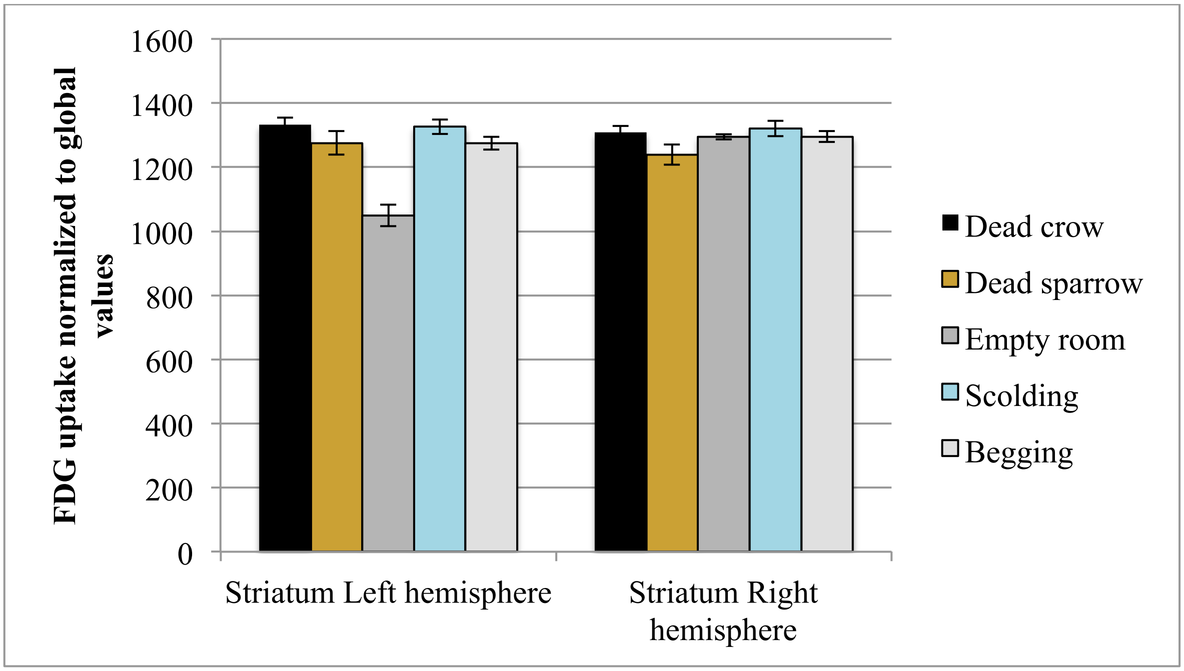

Figure S3.3 Mean FDG uptake across all stimuli and both hemisphere of the striatum. Error bars show standard error.

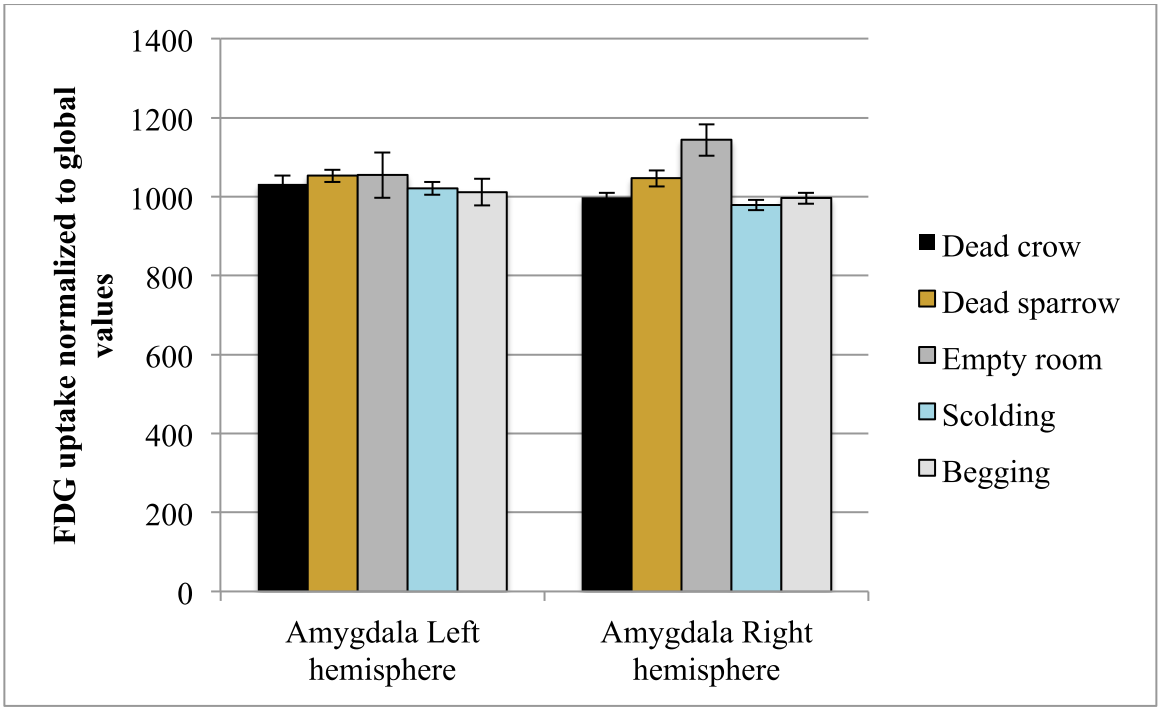

Figure S3.4. Mean FDG uptake across all stimuli and both hemisphere of the amygdala. Error bars show standard error.

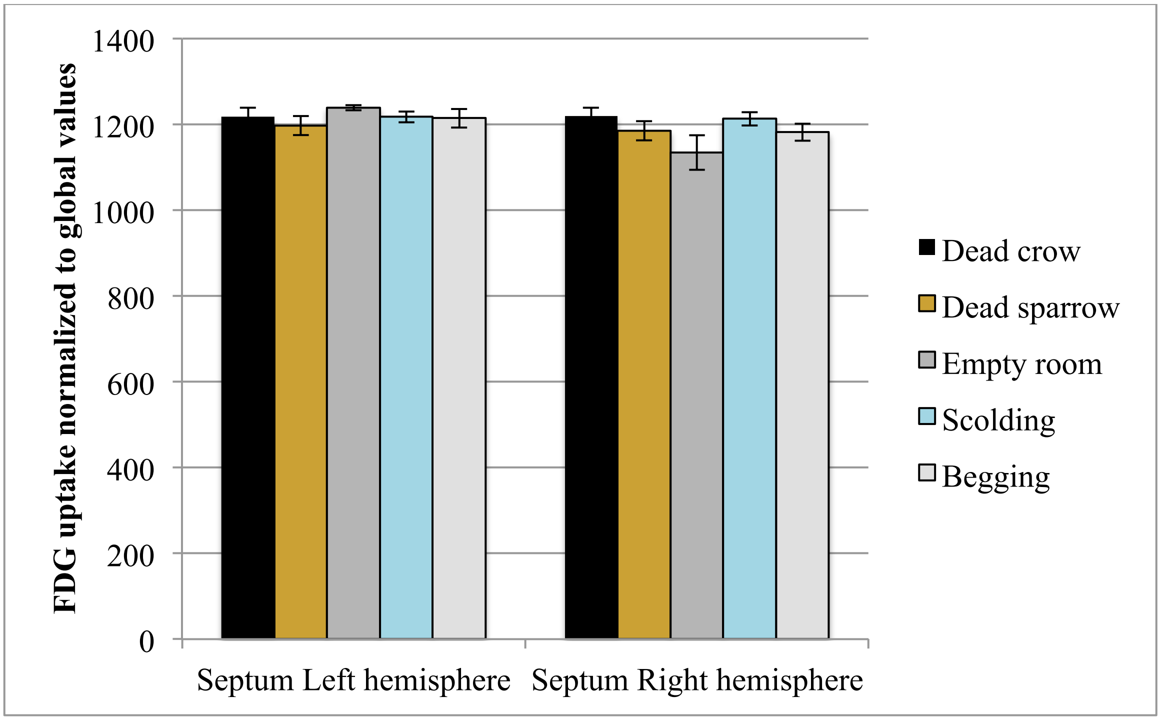

Figure S3.5. Mean FDG uptake across all stimuli and both hemisphere of the septum. Error bars show standard error.

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