



Reaction to novelty as a behavioral assay of recognition memory in homing pigeons and Japanese quail

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Abstract

Spontaneous novelty preference is apparent in a wide array of animals, including mammals, birds, reptiles, and fish. This provides a powerful behavioral assay to assess whether an animal can recognize a diverse array of stimuli in a common paradigm. Surprisingly, no research has been conducted in birds using novelty approach under conditions comparable to the *spontaneous object recognition* (SOR) protocols that have become standard across other animals. To correct this, the current study adapts a number of SOR protocols commonly used in mammals to characterize novelty approach in Silver King pigeons and Japanese quail. We show that, in general, both quail and pigeons readily approach novel objects or locations when tested using SOR protocols, although pigeons show a neophilic response under some conditions in which quail do not. Neither quail nor pigeons readily approach objects in novel contexts or novel locations. These data show that SOR can be successfully adapted to birds, allowing for more direct comparison between mammals and birds in tasks of shared ecological relevance.

Keywords Spatial learning · Conjunctive encoding · Avian · Bird

Introduction

The ability to detect novelty has broad implications for survival. For example, the investigation of novel places and objects within an environment can create opportunities to gather information (Hughes, 1997). Consistent with this idea, many animals, including mammals, birds, reptiles, and fish, will spontaneously and preferentially spend more time dwelling near and investigating novel objects (Blaser & Heyser, 2015; Hughes, 1997). While investigation of novelty can be beneficial, avoidance of novelty may be favored by selection in predator-rich environments and is thought to drive species specific characteristics such as niche breadth, diet, and home range size (Greggor et al., 2016). Because there are large selective pressures driving the detection of novelty, capitalizing on this ability offers a robust behavioral assay to assess the extent to which different animals will spontaneously recognize an object as novel when either the object's physical characteristics or its relationship to its surroundings is altered.

Here we utilize a series of four tests commonly used to assess novelty detection: *spontaneous object recognition* (SOR; e.g., Bevins & Besheer, 2006; Ennaceur & Delacour, 1988), SOR with *systematic variation* (SOR-SV; e.g., Burke et al., 2011), *conjunctive object recognition* (COR; e.g., Eacott & Norman, 2004), and *Y-maze discrimination* (YMD, Lalonde, 2002). The SOR and SOR-SV protocols manipulate physical characteristics of an object (e.g., size, shape, configuration) while COR manipulates aspects of the object relative to its environment (e.g., location, context in which an object appeared). While SOR, SOR-SV, and COR rely on a subject's ability to remember characteristics of an object and detect novel changes within them, YMD relies on the ability to remember familiar spatial locations and demonstrate a reaction when a novel location is made available. For each of these tests, reaction to novelty is commonly described in one of two ways: a preference to explore the detected novelty (neophilia), or avoidance of novelty (neophobia). In both types of exploration, a deviation from random proximity to an object indicates that change is detected and the subject can differentiate between the novel and familiar stimuli (Bevins & Besheer, 2006).

Although these studies have been conducted over multiple taxa, comparison is difficult as methods and experimental design tend to vary within the literature. For this reason,

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the current study adapts protocols that have become the de facto standard for testing in mammals to characterize novelty approach in two species of birds – pigeons (*Columba livia*) and Japanese quail (*Coturnix japonica*). In many ways, birds are the ideal candidates for broadening standardized novelty detection paradigms to non-mammalian species. Birds (and pigeons in particular) have inspired an immense body of literature studying their perception of objects (reviewed in Soto & Wasserman, 2014). However, no research to date on object recognition in birds has explicitly tried to match testing conditions used within rodent studies, despite the potential for these data to provide a direct comparison across orders using a common task. Generating such data facilitates bridging the procedural gaps between the considerable literatures regarding rodent object memory and avian object perception.

Experiment 1: Spontaneous object recognition

To begin assessing avian novelty detection in a way comparable to existing mammalian literature, we began with the most basic of the tests selected: SOR. The most widely implemented variation of SOR utilizes a sample and choice phase. During the sample phase, the subject encounters two identical objects within an arena. The subject is then removed and placed in a holding cage before returning to the enclosure for the choice phase, in which the subject encounters a familiar object (i.e., an object that is identical to those used during the sample phase) and a visually distinct novel object. If the subject discriminates between the novel and familiar objects, then a behavioral response (typically in the form of novelty approach) is observed.

The simplicity of SOR's experimental design is one of the many reasons this paradigm has become one of the dominant means to assess a wide range of cognitive functions (Blaser & Heyser, 2015). Because SOR tests are predominantly one-trial memory tests that do not require learning, they can be rapidly assessed. Moreover, SOR is well suited for a number of manipulations to evaluate neural function, as this paradigm provides a single unambiguous window within which memory function can be facilitated or impaired (Ennaceur, 2010).

Testing SOR in a variety of mammals, including lab reared rats (e.g., Ennaceur & Delacour, 1988), mice (e.g., Dodart et al., 1997), and domesticated pigs (e.g., Moustgaard et al., 2002), reveals an ability for these subjects to discriminate between objects, commonly showing a tendency to explore novelty. Testing of avian species explicitly matching the rodent protocol outlined by Ennaceur and Delacour (1988), has yet to be conducted. Based on previous alternative tests in birds demonstrating avian novelty detection

(Mettke-Hofmann et al., 2013; Saint-Dizier et al., 2008; Sowards & Sowards, 2002), we predicted that quail and pigeons will react differently to novel and familiar objects during SOR testing. If these species respond to novelty in a way comparable to lab-reared mammals, then we expected to see a neophilic response.

Methods

Subjects

Twenty-two adult Japanese quail (Spring Creek Quail Farms, Saint Anns, ON, Canada) and 26 Silver King pigeons (Cober Farms, Wellesley, ON, Canada) were used in this experiment. All birds were group housed on a 12:12 light cycle with ad lib access to food and water. Prior to behavioral testing, all animals were handled 15 min per day for at least 7 days. All procedures were approved by the Animal Care Committee of Wilfrid Laurier University and conducted in accordance with Canadian Council on Animal Care regulations.

Materials

Testing occurred in a 50 × 76 × 20 cm (l × w × h) open field arena constructed from white corrugated plastic sheeting. The interior of one wall was covered in black Bristol board to serve as an orienting cue. The arena floor was covered in wood shavings that were redistributed between trials to control for scent trails.

Objects were selected using the criteria previously outlined in Winters and Reid (2010) and were an assortment of junk objects (e.g., candle sticks, dog toys) constructed from washable materials including plastic, glass, and aluminum. Careful consideration was taken when selecting objects to ensure all were devoid of biologically relevant features such as eyes and mouths, and likenesses to food or nesting materials. The objects ranged from 10 to 20 cm in height and varied in visual and tactile characteristics. Once an object was selected for use, three copies were obtained to be used across testing sessions so that the same object was never used twice for the same bird. All objects were affixed to the floor of the testing arena using strips of hook and loop tape, preventing object movement during testing. Objects were wiped with 70% ethanol before each phase of testing. All sessions were recorded using an overhead webcam.

Testing procedure

The testing protocol was adapted from rodent testing as described previously (Marrone et al., 2011). Briefly, birds were transported to the testing room in individual cages on a

rack containing all subjects. Subjects remained undisturbed on rack for 1 h prior to testing. Three habituation sessions occurred over three consecutive days during which birds were placed individually into the arena to explore freely for 10 min. The experimental protocol (Fig. 1a) consisted of a sample phase followed by a choice phase. During sample, birds were placed in the open field containing two identical objects. After 5 min, the bird was removed and placed into a transport cage for 1 min. During this time, stimuli in the open field were changed to contain an object identical to those used during sample and a novel object. The subject was then returned to the open field for 5 min and exploration was recorded according to the criteria provided below. The side of the arena in which the novel object was placed was

counterbalanced between subjects. The objects used were randomized.

Behavioral Scoring and Analysis

Exploration was defined as the bird spending time within 30 cm of an object while not preening or pecking at the surrounding walls. The time spent exploring the novel (N) and familiar (F) objects for all birds was converted into a discrimination ratio (DR) as follows: $DR = (N - F) / (N + F)$ (Bevins & Besheer, 2006). The DR scores range from -1 (which indicates that the bird explored the familiar object exclusively) to 1 (all exploration time was spent around the novel object). Finally, a DR of 0 would indicate an equal amount of time around both objects (consistent with random chance).

The DRs were analyzed using a one-way analysis of variance (ANOVA) across species, as well as a one-sample t-test within each species comparing performance to zero (chance investigation).

Results and discussion

When presented with objects to discriminate between (Fig. 1b) both quail ($t_{21} = 6.08$; $p < 0.001$) and pigeons ($t_{25} = 1.99$; $p = 0.03$) spent significantly more time interacting with the novel object, and no significant difference was seen in the DRs generated by the two species ($F_{1,46} = 3.20$; $p = 0.08$). These results suggest that quail and pigeons spontaneously discriminate between junk objects in an SOR paradigm, and react by spending a larger proportion of time in exploration actively investigating the novel object, demonstrating a neophilic response similar to that described in mammalian studies (Dodart et al., 1997; Ennaceur & Delacour, 1988; Moustgaard et al., 2002).

It is important here to stress what can and cannot be concluded from these results. When animals spend more time exploring an object, this can give an indication of what quail and pigeons spontaneously discriminate. However, when they do not differentially explore an object pair, this does not necessarily indicate that an *individual or species* cannot perceive or discriminate the objects. This is because the SOR task does not permit the dissociation of memory processes, perception, motivation, or other cognitive factors that go into performance on this task. Rewarded training would likely be required to attempt such dissociation. However, this task permits the application of novelty detection to a paradigm that is controlled, ecologically valid, and easily applied in a consistent manner across the animal kingdom. This last feature is particularly relevant considering several studies of novelty reactivity in wild-caught birds (e.g., Martin & Sherry, 2019; Mettke-Hofmann et al., 2002; Nilsson et al.,

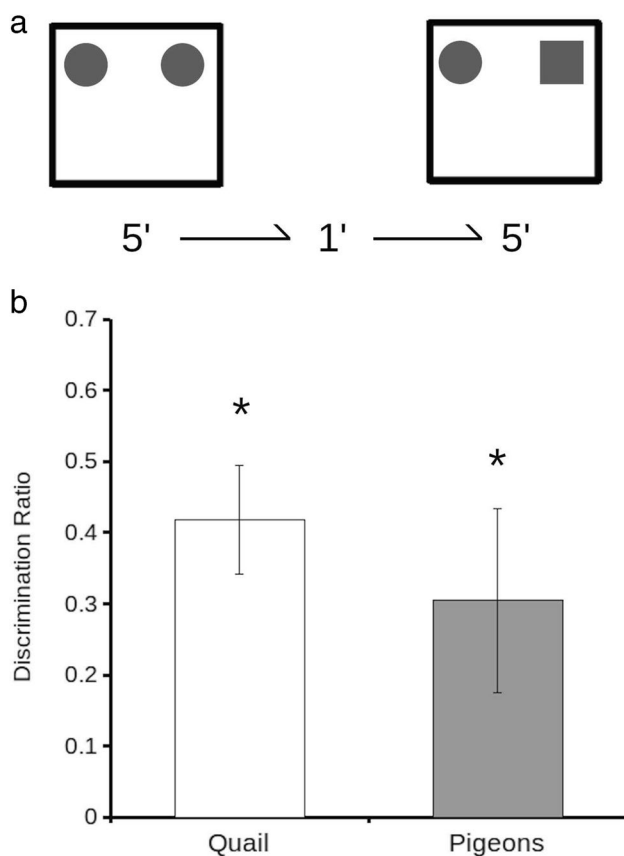


Fig. 1 Spontaneous object recognition (SOR) in pigeons and quail. A schematic (a) demonstrates the placement of objects and timing of trials in SOR. Following three days of habituation, birds received their first sample trial (left) in an open-field containing two identical novel objects (circles) for 5 min. After a delay of 1 min, birds received a second sample trial (right) in which an object that is identical to the two previously seen is presented alongside a distinct novel object (square). Calculation of a discrimination ratio (b) shows that both quail (white) and pigeons (grey) spend significantly more time investigating the novel object, since the *discrimination ratio* (DR) is greater than zero (bars show mean \pm SEM; * = $p < 0.05$ significant difference from random chance)

2010; Stöwe et al., 2006a, b) have generally reported strong neophobia, and many either state or imply that neophobia is endemic to Aves. However, avian studies of novelty reaction have typically involved placing a novel object into the bird's home cage or another location exceedingly familiar to the subject, in which no novel objects had a history of appearing. Under these same testing conditions, both wild (Cowan, 1976) and domesticated (Mislin & Ropartz, 1981) rodents are also neophobic, despite their robust neophilic response within a relatively novel testing environment (Ennaceur & Delacour, 1988). This suggests that the extent to which the response to novelty is neophobic or neophilic is the result of the testing protocol, rather than the species studied.

In many respects, the observations from Experiment 1 provide baseline data for further comparison. Objects presented in Experiment 1 differed across many characteristics, including size, shape, texture, and scent, offering multiple dimensions that could provide the basis for novelty discrimination. Now that it has been established that novelty can be detected under these conditions and elicits approach of the novel object in quail and pigeons, this positive control can be used to make further comparisons. In Experiment 2, we extend our observations by systematically varying stimuli across only the visual dimension.

Experiment 2: Spontaneous object recognition with systematic variation

Given that both species discriminate novel from familiar objects in simple SOR, next we assessed whether birds are sensitive to the degree of feature overlap in reliably detecting novelty. Although the discrimination in individual subjects is digital, the probability of a subject responding changes systematically with feature overlap, and as a result a graded DR is generated across levels of similarity. This graded response across the population can be used to assess manipulations that improve or degrade performance. In an effort to generate comparable graded responses in quail and pigeons, we created objects for the sample and choice phases out of LEGO® building blocks (Aggleton et al., 2010; Burke et al., 2011). This had the advantage of allowing us to assemble a number of identical objects, as well as affording the ability to have a series of objects all made of the same complement of building blocks, but with a set number of these blocks rearranged to create the novel object. To assess the degree of rearrangement needed for objects to be detected as novel, we implemented conditions in which 25%, 50%, and 100% of the blocks making up the structure were rearranged (Fig. 2a). If the subjects could discriminate between two objects based on the arrangement of building blocks, then we expected a larger proportion of time to be spent

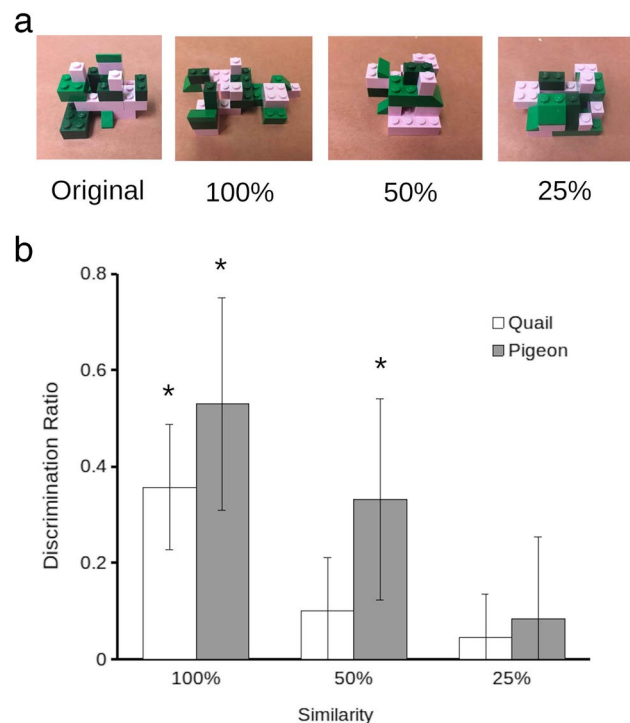


Fig. 2 Spontaneous object recognition (SOR) with systematic variation. Images (a) depict one of the arrangements of LEGO objects making up the sample object (left) and how the blocks were altered to create new objects to be differentiated by rearranging 100%, 50%, or 25% of the component blocks. Testing using the same paradigm described in Fig. 1 and calculating a *discrimination ratio* (DR) shows that (b) both quail (white) and pigeons (grey) are able to differentiate objects when 100% of the component blocks are rearranged, since the DR is greater than zero. Pigeons, but not quail, are able to differentiate an object with 50% of the component blocks rearranged from the original, while birds from neither species spend significantly more time investigating an object that has 25% of the component blocks rearranged relative to the original (bars show mean \pm SEM; * = $p < 0.05$ significant difference from random chance)

investigating the novel arrangement, comparable to findings in Experiment 1. We anticipated that the greater the percentage of re-arrangement of the building blocks, the more likely subjects would be to differentiate between them.

Methods

Subjects

Twenty-three adult Japanese quail and 15 Silver King pigeons were used in this experiment. Subjects were purchased from the same suppliers and were housed in the same conditions as those described in Experiment 1.

Materials

Testing took place in the same arena as described in Experiment 1. LEGO® objects were constructed so that each percent change condition (25%, 50%, 100%) had three identical sample objects and one novel object. The novel object changed only in the configuration of a given percentage of pieces, the rest of the pieces remained identical in placement to those in the sample object.

Testing procedure

Testing was conducted as described in Experiment 1 but with three conditions: a 25% change group in which 25% of the blocks in the LEGO® object were re-positioned, as well as a 50% change group, and a 100% change group (Fig. 2a). Testing consisted of a sample phase with two identical LEGO® objects followed by a choice phase in which one LEGO® object was identical to those encountered during sample and the other was manipulated based on the change condition. The order of these conditions was counterbalanced across subjects, and each test was separated by at least 24 h.

Behavioral scoring and analysis

Scoring was identical to that outlined in Experiment 1. A two-way mixed ANOVA was conducted comparing similarity (i.e., 25%, 50%, 100%) as a repeated factor, as well as species. In addition, a one sample t-test was conducted for each species at each similarity level relative to a DR of zero (chance exploration).

Results and discussion

Discrimination performance was affected by the degree of similarity between objects (main effect of similarity: $F_{2,72} = 7.77$; $p < 0.001$, Fig. 2b). The difference between species in this regard was not significant ($F_{1,36} = 1.28$; $p = 0.27$). Single sample t-tests showed that while both quail ($t_{22} = 5.02$; $p < 0.001$) and pigeons ($t_{14} = 9.11$; $p < 0.001$) were able to make this discrimination at the easiest level, in which 100% of the blocks are rearranged, quail did not show a significant preference for the novel object in the 50% condition ($t_{22} = 0.63$; $p = 0.53$), while pigeons did ($t_{14} = 1.99$; $p = 0.03$). In the 25% condition, both quail ($t_{22} = 0.81$; $p = 0.43$) and pigeons ($t_{14} = 0.509$; $p = 0.62$) failed to significantly prefer the novel stimulus.

These results suggest that the ability for both quail and pigeons to detect and react to a novel object declines as the objects become more physically similar. As fewer

pieces were rearranged, both species investigated the novel object less, suggesting increased difficulty in detecting change in these conditions. This trend is apparent in Fig. 2b; however, performance of quail in the 50% and 25% condition were not indicative of novelty detection.

Previous instrumental conditioning data in both species are consistent with the observed relationship between similarity and the ability to discriminate objects. In quail, trials to reach criterion was lowest for a color discrimination (red vs green), moderately higher for a pattern discrimination (horizontal vs vertical lines), and highest for a form discrimination (triangle vs. circle). Moreover, as the complexity of the objects increased, the ability of the quail to make pattern or form discriminations became worse, requiring more than 1,300 trials to reach a criterion of 15 consecutive correct responses in a form discrimination task (Fidura, 1969; Fidura & Grey, 1966). Although pigeons learn these discriminations somewhat faster, they show a comparable trend (Towe, 1954; Williams, 1972), requiring approximately 1,000 trials to reach a similar criterion in a form discrimination task. Thus, it is perhaps not surprising that a rearrangement of LEGO® objects, which keeps color consistent while altering form and pattern, creates a stimulus pair that neither bird spontaneously discriminates with limited experience under the most difficult condition. In fact, the observation that a pigeon can discriminate a 50% change in block configuration speaks to the speed with which data can be generated using the SOR paradigm. A single trial under conditions that more closely resemble foraging behaviors in the wild allows birds to demonstrate a discrimination that would require hundreds of instrumental conditioning trials to establish.

It is also notable that the current data mimic the small differences observed between quail and pigeons in instrumental tasks, with pigeons showing a significant preference for the novel configuration in the 50% when quail did not. Differences in performance between quail and pigeons were small and statistical evidence was mixed. Thus, until a wider array of species can be tested, results must be interpreted cautiously.

Collectively, Experiment 1 and Experiment 2 suggest that both quail and pigeons (a) discriminate between novel and familiar objects, even when novelty is based on the arrangement of components of the same shape and color, (b) generally exhibit neophilia when novelty is detected, and (c) are sensitive to the amount of feature overlap in their ability to make discriminations. To further extend our understanding of novelty detection in quail and pigeons, in Experiment 3, we were interested in determining if information about the object can be bound to information about location and context.

Experiment 3: Conjunctive object recognition

In a naturalistic setting, the context in which an object is encountered, including its physical location, and its relationships to other objects, is important for object recognition (reviewed by Ennaceur, 2010). To test the ability for subjects to bind these characteristics to form a representation of an object and detect change, we implemented a COR task similar to that described in Eacott and Norman (2004). If the subjects can recognize important contextual cues and bind them with object identity, then we expected them to spend a greater portion of time investigating a familiar object if that object is encountered in a new location or context. For all conditions of COR, Fig. 3 denotes the novel object with an N, this is the object expected to elicit a neophilic response.

Methods

Subjects

Twenty-two Japanese quail and 26 Silver King pigeons were used in this experiment. Subjects used were the same sample as those used in Experiment 1. Prior experience on Experiment 1 was not considered to affect performance on Experiment 3 as they were separated by several weeks, a different set of objects was used, and spontaneous novelty detection does not require rule learning (Blaser & Heyser, 2015).

Materials

This experiment consisted of two arenas, both identical in dimensions to those described in Experiment 1, in two

different rooms. These are referred to as Context A and Context B. While one arena was identical to Experiment 1, the other had three dark grey walls and one was covered in green Bristol board. Testing rooms were located across the hallway from one another, and each contained distinct visual cues on the walls. Objects used were selected based on the same parameters outlined in Experiment 1.

Testing procedure

Subjects were habituated to both contexts for 10 min a day for three consecutive days. To assess the extent to which approach could be stimulated by *conjunctive object recognition* (COR), subjects were exposed to novel conjunctions of objects, locations, and contexts, through a series of three conditions (Fig. 3) adapted from Eacott and Norman (2004).

In the Object/Location condition, subjects were placed in the open field containing two distinct objects for sample training. After 5 min, the bird was removed and placed in a transport cage for 1 min. During this time, one of the stimuli in the open field was exchanged for an object identical to the other sample object, so that now there were two identical objects in the open field. There was now, therefore, an object in the arena that was not novel in itself, and occupied a location in which the bird had previously seen an object, but the conjunction of object and location was novel. The bird was then returned to the open field for 5 min and their exploration was recorded.

In the Object/Context condition, birds were placed in the open field containing two identical objects for sample training, this will be referred to as Context A. After 5 min, the bird was removed and placed in their transport cage for 1 min. During this time, the animal was transported to a second room, Context B, with distinct visual cues on the walls and a second open

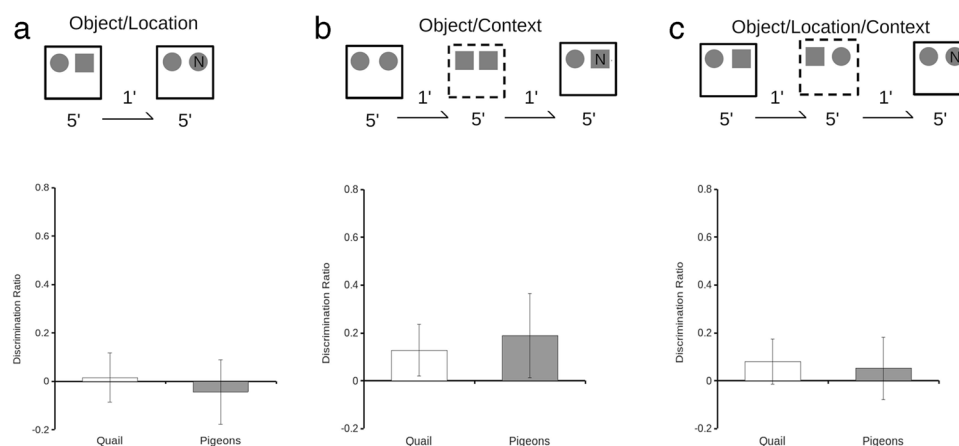


Fig. 3 *Conjunctive object recognition* (COR) in pigeons and quail. Schematics (top) demonstrate the placement of objects and timing of trials in the Object/Location test (a), the Object/Context test (b), and

the Object/Context/Location test (c). Neither quail (white) or pigeons (grey) spent significantly more time than expected by chance investigating the novel object (N) in any condition

field of the same dimensions as Context A, containing two identical objects that are distinct from those seen in Context A. After 5 min in Context B, the bird was removed and placed in a transport cage for 1 min, before being returned to Context A, which now contained one object identical to the objects encountered in Context A and one object identical to those encountered in Context B. Thus, neither object nor context are novel on their own, but one object is novel in this context. Note here that if the bird responds only to relative novelty, they will spend a lesser proportion of time around the most recently seen item, rather than the item that was not encountered in this context, indicating that the subject was not binding object and context information.

Finally, in the Object/Context/Location condition, birds were exposed to two distinct objects in the open field of Context A. After 5 min, the bird was removed and placed in a transport cage for 1 min. During this time, the animal was transported to a second room (Context B) with distinct visual cues on the walls and an open field of the same dimensions to Context A. Context B contained two objects identical to those observed in Context A, but here they were presented in the opposite orientation (the object on the left in Context A was now on the right and vice versa). After 5 min, the bird was removed and placed in a transport cage for 1 min, before being returned to Context A. The open field now contained two identical objects that are the same as one of the objects previously presented. Although both objects had been seen in both rooms and in both locations, one object had not been seen in this location in this room. The time spent exploring this object was recorded relative to the other object, over the course of 5 min. Objects used and the location of the novel object were counterbalanced for all conditions.

At least 24 h elapsed between each COR testing condition.

Behavioral scoring and analysis

Scoring was identical to that outlined in Experiment 1. Discrimination ratios were analyzed using a 3 (condition: Object/Context, Object/Location, Object/Context/Location) \times 2 (species) mixed ANOVA. Each individual species and condition were also evaluated using a one-sample t-test against a DR of zero (chance exploration).

Results and discussion

Analysis of COR (Fig. 3) revealed no significant main effect of condition ($F_{2,90} = 1.85$; $p = 0.16$) or species ($F_{1,45} = 0.01$; $p = 0.97$). One-sample t-tests verified that this is because neither quail nor pigeons approached any novel conjunction of an object with a location and/or context more than expected by chance ($p > 0.05$ in all conditions). In the Object/Location (Fig. 3a), Object/Context (Fig. 3b),

and Object/Location/Context (Fig. 3c) conditions, performance of quail and pigeons suggests that they were unable to detect novel changes. The observation that quail and pigeons do not approach novel conjunctions of object with their environment is not consistent with rodent literature. For instance, findings of Eacott and Norman (2004), which provided the basis for our experimental protocol, showed that rats could identify the novel change in all conditions, and consistently exhibited a neophilic response. In another comparable study by Dix and Aggleton (1999), rats reliably approached the novel element across a wide range of novelty discrimination tests incorporating elements of object location within an arena, object position relative to an array of objects, and the context in which an object was presented.

The current observations lead to two distinct possibilities: (a) that the conjunction of a familiar item with a novel location and/or context does not elicit the motivation to respond with exploration, or (b) that the novelty of these conjunctions of information cannot be detected. Although no comparable data exist testing feature binding in quail, several behavioral experiments in pigeons corroborate the latter interpretation. Although pigeons can be trained to make discriminations of object location (Leising et al., 2013), their performance decays to chance levels at presentation delays of less than 10 s – far less than the delays encountered in SOR. Our findings are also consistent with data on pigeons' performance in a what-where-when memory task (Skov-Rackette et al., 2006). Skov-Rackette and colleagues (2006) showed that, while pigeons could correctly indicate the location, identity, and time of appearance of a single cue, when they were required to respond to more than one of these features of a single item, a successful response on one feature did not predict success in the other. This suggests that although pigeons could retain information about the what, when, and where of objects, they did not bind this information together in memory. Similarly, Lazareva and Wasserman (2016) found no evidence of feature binding in pigeons across multiple versions of a change detection task, even with a delay of only 900 ms.

A potential explanation for an inability to detect novelty in this task could point to an inability to detect novel spatial locations. Perhaps detection of novelty in quail and pigeons does not extend beyond physical characteristics of the object itself. To test this further, we implemented a test in which novelty detection relied on differentiating between novel and familiar spatial locations in the absence of object information.

Experiment 4: Y-maze discrimination

After testing novelty seeking in relation to objects, we investigated if quail and pigeons had similar discrimination reactions to novel spatial locations. Additionally,

since neither species responded to novelty via location change in the COR test, we wanted to assess if the lack of response was due to an inability to detect novel spatial locations. Toward this goal, birds were tested using two versions of YMD: one utilizing a single Y-maze (Fig. 4a) and the other incorporating two Y-mazes in two distinct contexts (Fig. 4b). During commonly used YMD protocols, an arm of the maze is blocked during the sample trial and is opened during the choice trial (Lalonde, 2002). Consistent with mammalian experiments (Kraeuter et al., 2019; Lalonde, 2002), we were interested in the amount of time that subjects spend in the novel, previously blocked arm, relative to the proportion of time in the familiar, previously open arm. If subjects can detect spatial novelty in addition to the observed object novelty from Experiments 1 and 2, then we expected them to spend a larger proportion of time investigating the previously blocked arm. In the two Y-maze condition, subjects were challenged with remembering which arm was blocked in each of two contexts and were expected to explore the previously blocked arms in both contexts during the choice trial.

Methods

Subjects

Fifteen adult Japanese quail and 15 Silver King pigeons were used in this experiment and were the same sample as those

used in Experiment 2. Participation in Experiment 2 was not thought to affect performance on Experiment 4 as they were separated by several weeks, a variety of visual cues within the room and the testing apparatus were changed, and neither task required rule learning.

Materials

Two Y-mazes with arms measuring $60 \times 20 \times 30$ cm ($l \times w \times h$) were constructed from clear acrylic so that subjects could readily see the distinct visual cues present on all four walls of the rooms. Square rod styrene tracts with removable opaque acrylic guillotine doors were installed in the two exploration arms. The floor was constructed from black haircell acrylonitrile-butadiene-styrene (ABS) and covered with wood shavings.

Testing procedures

The testing protocol used here was adapted from rodent testing as described previously in Marrone et al. (2011). The YMD tasks consisted of two conditions, a single Y-maze condition, and another in which two Y-mazes were utilized in two separate rooms. In the first single Y-maze condition, subjects underwent three consecutive days of 10-min habituation sessions. During the sample trial, birds were given 5 min of exploration with one arm of the maze blocked off by a guillotine door. Birds were then removed for 1 min, during which time the door was removed and the bedding in the maze

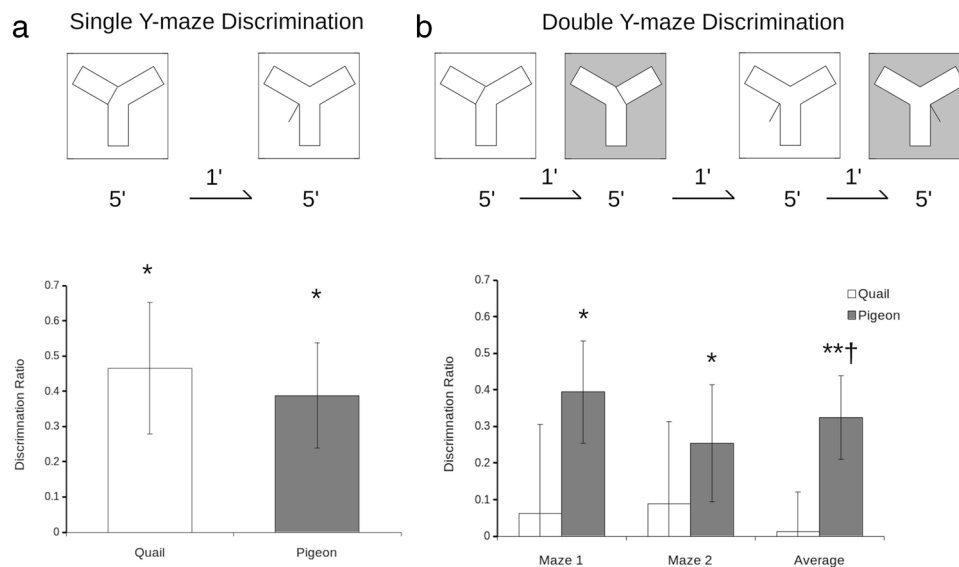


Fig. 4 Reaction to novel spatial locations in pigeons and quail. Schematics are presented above to demonstrate the timing of trials in each variation of the spatial recognition task, while data are presented below. In the single-Y-maze condition (a), both quail (white) and pigeons (grey) spend significantly more time in the arm of the maze

that had previously been blocked. In the two-Y-maze condition (b), pigeons spent significantly more time in the previously blocked arm in both Y-mazes, while quail did not (bars show mean \pm SEM; * = $p < 0.05$; ** $p < 0.01$, significant difference from random chance; † = $p < 0.05$ significant difference between species)

was replaced to remove scent cues. Birds were then returned to the maze for a 5-min choice trial. Which arm was blocked during the sample trial was counterbalanced across subjects.

In the two-Y-maze condition, birds were sequentially placed in two different rooms (Context A and Context B) containing distinct visual stimuli. The task consisted of four trials: two sample trials (one in each context), and two choice trials (one in each context), in the same order as during sample. In the first sample trial, the subject was placed in the start arm of the maze in Context A, facing away from the center, while one arm of the Y-maze (either left or right) was blocked with a guillotine door. Subjects were permitted to explore the Y-maze for 5 min. After being placed in a transport cage over a 1-min delay, the same procedure was followed in Context B. Again, after a 1-min delay, the subject underwent the first choice trial in which they were placed back into Context A but this time with all arms open. Following this, they were removed, placed in a transport cage for a 1-min delay and underwent the final choice trial in Context B. The sequence of exposure to the two contexts was counterbalanced between subjects. The floor of each maze was covered with bedding, which was replaced between trials in order to eliminate olfactory cues. Recordings were taken using an overhead camera.

Behavioral scoring and analysis

Manual scoring of videos recorded the time that the subject spent in each arm as a proportion of their total exploration time. The subject was considered to be exploring an arm if their entire torso was inside of the arm. The time spent exploring the novel and familiar arm (excluding the start arm) for all subjects was converted into a DR as described in Experiment 1. In the single Y-maze test, DRs were compared across species by one-way ANOVA. In the double-Y-maze, analysis consisted of a 2×2 mixed ANOVA comparing species in addition to maze as a repeated factor. Each individual species and condition were also evaluated using a one-sample t-test against a DR of zero (chance exploration).

Results and discussion

The pattern of behavior observed in the COR may suggest that novel spatial information does not elicit a spontaneous approach response in these bird species. Testing this hypothesis with spatial recognition refutes this possibility; both pigeons ($t_{14} = 2.51$; $p = 0.01$) and quail ($t_{14} = 2.30$; $p = 0.02$) spent more time in the previously blocked arm in the single Y-maze condition (Fig. 4a) than expected by chance, and the performance of the two species did not differ significantly ($F_{1,28} = 0.084$; $p = 0.77$). In the two Y-maze condition (Fig. 4b), pigeon performance differed from quail, as shown

by a significant species difference ($F_{1,26} = 4.51$; $p = 0.04$). One-sample t-tests verify that pigeons significantly preferred the novel arms (mean DR: $t_{14} = 3.38$; $p = 0.002$), while quail did not (mean DR: $t_{14} = 1.34$; $p = 0.10$).

These results demonstrate that while both quail and pigeons spent a larger proportion of time in the previously blocked arm, consistent with novelty detection and neophilia for novel spatial locations. This pattern is inconsistent with the suggestions that novel spatial locations cannot be detected or do not elicit approach from birds, corroborating that the failures to approach novelty in Experiment 3 were the result of an inability to detect novel conjunctions of item and context information.

Notably, only pigeons made this discrimination in the two Y-maze conditions. Comparing results to mammalian literature, rodents readily discriminate novel from familiar arms within a Y-maze in both a single maze (Kraeuter et al., 2019; Lalonde, 2002), and a two-maze condition (Marrone et al., 2011). Until additional species are tested, an explanation of why pigeons preferred the novel arms when presented two Y-mazes while quail did not is purely speculative. However, it is possible that species-related differences in this task may result from species-related differences in foraging strategies (Charnov, 1976; Reiss, 1987). For example, a species with a win-shift strategy might be more likely to investigate the novel arm, while a win-stay species may demonstrate hesitancy. Pigeons have been noted as having a win-shift strategy when tested within a T-maze (Olson & Maki, 1983; but see Hughes, 1989). Although information for Japanese quail is lacking, other Galliformes have a win-stay strategy (Hayes & Warren, 1963). It should be noted, however, that Hayes and Warren (1963) urged caution in this interpretation, positing that exploration of the maze may be a stressful experience and as a result removal from the maze may serve as a reward that reinforces entering the arm that the subject was last removed from on a previous trial.

Conclusions

The current findings show that reaction to novelty can be successfully used to assess novelty detection for both discrete objects (Experiments 1 and 2) and spatial locations (Experiment 4) in both pigeons and quail. Observations of novelty detection tests described here, support their use in at least some avian species with minimal changes in protocol relative to that used for rodents. Moreover, the fact that these effects are consistently observed in two families of birds (i.e., Galliformes and Columbiformes) suggests that approach to novelty may provide a robust behavioral assay across Aves.

One factor that limits the generalization of these results, however, is the fact that both species of bird tested here are

highly domesticated. As pointed out by Blaser and Heyser (2015), domestication is a major predictor for rodents' reaction to novelty (Barnett, 1958; Minckler & Peaseh, 1938; Orgain & Schein, 1953). Similarly, studies of novelty reactivity in wild-caught birds generally reported strong neophobia (e.g., Martin & Sherry, 2019; Mettke-Hofmann et al., 2002; Nilsson et al., 2010; Stöwe et al., 2006a, b), and a bird's neophobia may be predicted by the nature of the habitat from which the bird was caught, migratory strategy, and diet breadth (Mettke-Hofmann et al., 2013; Sol et al., 2011). Although there is evidence to suggest that testing procedures may account for this difference (as described in Experiment 1), the neophilic reaction observed in Experiments 1, 2, and 4 should be replicated in wild-caught species. It will be important to assess behavioral differences in domesticated and wild birds during comparable novelty testing – the responses of wild birds under these standardized protocols remains to be addressed.

Despite this open question, it is clear that in Experiments 1, 2, and 4, both novel objects and novel locations readily elicit a neophilic response in both pigeons and quail. These observations lay the foundation for further apples-to-apples comparisons of the neurobiology of novelty detection across taxa using SOR. The neural circuits underlying these behaviors are very well characterized in the rodent, in part because of dissociation that can be observed by varying standardized testing protocols. Many of the variations in novelty detection tasks (including those used here) exist in part because interventions that perturb only one of these circuits alter performance on some variations of this task and not others. Searching for similar dissociation in birds can provide unique insight into the functional homologies that exist across taxa and allow the placement of object recognition memory within the framework of an evolutionary basis of multiple memory systems (Sherry & Schacter, 1987).

The fact that our findings show similar results across taxa also raises the question of whether novelty detection and neophilia in general are evolutionarily conserved or if these traits have independently evolved in two classes. The observation of neophilic responses to novel objects in fish, reptiles, amphibians, and a variety of invertebrates (reviewed in Blaser & Heyser, 2015) suggests that this may be a trait shared by much of the animal kingdom. More importantly, it suggests that the response to novelty when it is detected (i.e., approach or avoidance) is likely the product of the exact testing procedures and behavioral history of individual animals. The systematic manipulation of these conditions within the framework of standardized testing holds the most promise of understanding novelty detection and object recognition across taxa. It is the outliers that will provide the greatest insight into the basis for this cognitive ability and the circumstances under which adaptive specialization might sculpt it, in much the same way that insight into spatial cognition in

birds has been gained largely through the study of birds with exceptional spatial abilities, such as food caching (Sherry, 2014a, 2014b; Sherry & Hoshoooley, 2007).

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Code availability N/A

Authors' contributions CCD, NM, and DFM designed the experiment, CCD conducted the research, CCD, NM, and DFM analyzed the data, CCD, NM, and DFM wrote the manuscript.

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Declarations

Competing interests The authors declare that they have no competing interests.

Ethics approval All procedures were approved by the animal care committee of Wilfrid Laurier University and were carried out in accordance with the guidelines of the Canadian Council on Animal Care.

Consent to participate N/A

Consent for publication All authors consent to the contents of this publication

References

- Aggleton, J. P., Albasser, M. M., Aggleton, D. J., Poirier, G. L., & Pearce, J. M. (2010). Lesions of the rat perirhinal cortex spare the acquisition of a complex configural visual discrimination yet impair object recognition. *Behavioral Neuroscience*, 124(1), 55.
- Barnett, S. A. (1958). Experiments on 'neophobia' in wild and laboratory rats. *British Journal of Psychology* 49, 195–201. <https://doi.org/10.1111/j.2044-8295.1958.tb00657.x>
- Bevins, R. A., & Besheer, J. (2006). Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. *Nature Protocols*, 1(3), 1306–1311.
- Blaser, R., & Heyser, C. (2015). Spontaneous object recognition: a promising approach to the comparative study of memory. *Frontiers in Behavioral Neuroscience*, 9, 183.
- Burke, S. N., Wallace, J. L., Hartzell, A. L., Nematollahi, S., Plange, K., & Barnes, C. A. (2011). Age-associated deficits in pattern separation functions of the perirhinal cortex: a cross-species consensus. *Behavioral Neuroscience*, 125(6), 836.
- Charnov, E. L. (1976). Optimal foraging, the marginal value theorem. *Theoretical Population Biology*, 9(2), 129–136.
- Cowan, P. (1976). The new object reaction of *Rattus rattus* L.: the relative importance of various cues. *Behavioral Biology* 16, 31–44. [https://doi.org/10.1016/s0091-6773\(76\)91095-6](https://doi.org/10.1016/s0091-6773(76)91095-6)

- Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behavioural Brain Research*, 99(2), 191–200.
- Dodart, J. C., Mathis, C., & Ungerer, A. (1997). Scopolamine-induced deficits in a two-trial object recognition task in mice. *Neuroreport*, 8(5), 1173–1178.
- Eacott, M. J., & Norman, G. (2004). Integrated memory for object, place, and context in rats: a possible model of episodic-like memory?. *Journal of Neuroscience*, 24(8): 1948–1953. <https://doi.org/10.1523/JNEUROSCI.2975-03.2004>
- Ennaceur A. (2010). One-trial object recognition in rats and mice: methodological and theoretical issues. *Behavioural Brain Research*, 215(2), 244–254. <https://doi.org/10.1016/j.bbr.2009.12.036>
- Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research*, 31(1), 47–59. [https://doi.org/10.1016/0166-4328\(88\)90157-x](https://doi.org/10.1016/0166-4328(88)90157-x)
- Fidura, F. G. (1969). Selective attention and complex discrimination learning in the Japanese quail. *Psychonomic Science*, 15(3), 167–168.
- Fidura, F. G., & Gray, J. A. (1966). Visual discrimination of color, pattern, and form in the Japanese quail *Coturnix coturnix japonica*. *Psychonomic Science*, 5(11), 427–428.
- Greggor, A. L., Jolles, J. W., Thornton, A., & Clayton, N. S. (2016). Seasonal changes in neophobia and its consistency in rooks: the effect of novelty type and dominance position. *Animal Behaviour*, 121, 11–20.
- Hayes, W. N., & Warren, J. M. (1963). Failure to find spontaneous alternation in chicks. *Journal of Comparative and Physiological Psychology*, 56(3), 575.
- Hughes, R. N. (1989). Lack of spontaneous alternation in favor of perseveration in domestic fowls and pigeons. *Behavioural Processes*, 20(1-3), 85–92.
- Hughes, R. (1997). Intrinsic exploration in animals: motives and measurement. *Behavioural Processes* 4: 213–226
- Kraeuter, A. K., Guest, P. C., & Sarnyai, Z. (2019). The Y-maze for assessment of spatial working and reference memory in mice. In *Pre-Clinical Models* (pp. 105–111). Humana Press.
- Lalonde, R. (2002). The neurobiological basis of spontaneous alternation. *Neuroscience & Biobehavioral Reviews*, 26(1), 91–104.
- Lazareva, O. F., & Wasserman, E. A. (2016). No evidence for feature binding by pigeons in a change detection task. *Behavioural Processes*, 123, 90–106.
- Leising, K. J., Elmore, L. C., Rivera, J. J., Magnotti, J. F., Katz, J. S., & Wright, A. A. (2013). Testing visual short-term memory of pigeons (*Columba livia*) and a rhesus monkey (*Macaca mulatta*) with a location change detection task. *Animal Cognition*, 16(5), 839–844. <https://doi.org/10.1007/s10071-013-0644-9>
- Marrone, D. F., Adams, A. A., & Satvat, E. (2011). Increased pattern separation in the aged fascia dentata. *Neurobiology of Aging*, 32(12), 2317.e23–2317.e2.317E32. <https://doi.org/10.1016/j.neurobiolaging.2010.03.021>
- Martin, R.J. & Sherry, D.F. (2019). Overwinter temperature has no effect on problem solving abilities or responses to novelty in Black-capped chickadees (*Parus atricapillus*). *Behavioural Processes*, 162, 72–78.
- Mettke-Hofmann, C., Winkler, H., and Leisler, B. (2002). The significance of ecological factors for exploration and neophobia in Parrots. *Ethology* 108, 249–272. <https://doi.org/10.1046/j.1439-0310.2002.00773.x>
- Mettke-Hofmann, C., Winkler, H., Hamel, P. B., & Greenberg, R. (2013). Migratory New World blackbirds (icterids) are more neophobic than closely related resident icterids. *PLoS One*, 8(2), e57565.
- Minckler, J., and Peaseh, F. D. (1938). A colony of albino rats existing under feral conditions. *Science* 87, 460–461. <https://doi.org/10.1126/science.87.2264.460>
- Misslin, R., and Ropartz, P. (1981). Responses in mice to a novel object. *Behaviour* 78, 169–177. <https://doi.org/10.1163/156853981x00301>
- Moustgaard, A., Lind, N. M., Hemmingsen, R., & Hansen, A. K. (2002). Spontaneous object recognition in the Göttingen minipig. *Neural Plasticity*, 9(4), 255–259.
- Nilsson, A. L., Nilsson, J. A., Alerstam, T., and Bäckman, J. (2010). Migratory and resident blue tits *Cyanistes caeruleus* differ in their reaction to a novel object. *Naturwissenschaften* 97, 981–985. <https://doi.org/10.1007/s00114-010-0714-7>
- Olson, D. J., & Maki, W. S. (1983). Characteristics of spatial memory in pigeons. *Journal of Experimental Psychology: Animal Behavior Processes*, 9(3), 266.
- Orgain, H., and Schein, M. W. (1953). A preliminary analysis of the physical environment of the Norway rat. *Ecology* 34, 467–473. <https://doi.org/10.2307/1929719>
- Reiss, M. J. (1987). Optimization theory in behavioural ecology. *Journal of Biological Education*, 21(4), 241–247.
- Saint-Dizier, H., Leterrier, C., Lévy, F., & Richard, S. (2008). Selection for tonic immobility duration does not affect the response to novelty in quail. *Applied Animal Behaviour Science*, 112(3-4), 297–306.
- Sewards, T. V., & Sowards, M. A. (2002). Innate visual object recognition in vertebrates: some proposed pathways and mechanisms. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 132(4), 861–891.
- Sherry, D.F. (2014a). Decisions, memory, and the neuroecology of food-storing birds. In: Preston, S.D., Kringelbach, M.L. & Knutson, B. (Eds.) *The Interdisciplinary Science of Consumption* (pp. 111–125) MIT Press.
- Sherry, D.F. (2014b). How animal remember places and find their way around. Yasukawa, K. (Ed.) *Animal Behavior Volume 3* (pp 269–287). Praeger.
- Sherry, D.F. & Hoshooley, J.S. (2007). The neurobiology of spatial ability. In: Otter, K. (ed.) *Ecology and Behavior of Chickadees and Tits: An Integrated Approach* (pp.9–23) Oxford University Press.
- Sherry, D.F., & Schacter, D.L. (1987). The evolution of multiple memory systems. *Psychological Review*, 94, 439–454.
- Skov-Rackette, S. I., Miller, N. Y., & Shettleworth, S. J. (2006). What-where-when memory in pigeons. *Journal of Experimental Psychology: Animal Behavior Processes*, 32, 345–358. <https://doi.org/10.1037/0097-7403.32.4.345>
- Sol, D., Griffin, A. S., Bartomeus, I., & Boyce, H. (2011). Exploring or avoiding novel food resources? The novelty conflict in an invasive bird. *PLoS One*, 6, e19535.
- Soto, F. A., and Wasserman, E. A. (2014). Mechanisms of object recognition: what we have learned from pigeons. *Front. Neural Circuits* 8:122. <https://doi.org/10.3389/fncir.2014.00122>
- Stöwe, M., Bugnyar, T., Heinrich, B., & Kotrschal, K. (2006a). Effects of group size on approach to novel objects in ravens (*Corvus corax*). *Ethology*, 112(11), 1079–1088.
- Stöwe, M., Bugnyar, T., Loretto, M. C., Schloegl, C., Range, F., & Kotrschal, K. (2006b). Novel object exploration in ravens (*Corvus corax*): effects of social relationships. *Behavioural Processes*, 73, 68–75.
- Towe, A.L. (1954). A study of figural equivalence in the pigeon. *Journal of Comparative and Physiological Psychology* 47, 284–287.
- Williams, D. I. (1972). Discrimination learning in the pigeon with two relevant cues, one hard and one easy. *British Journal of Psychology*, 63(3), 407–409.
- Winters, B. D., & Reid, J. M. (2010). A distributed cortical representation underlies crossmodal object recognition in rats. *Journal of Neuroscience*, 30(18), 6253–6261.