Mesotocin influences pinyon jay prosociality.
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Abstract

Many species exhibit prosocial behavior, in which one individual's actions benefit another individual, often without an immediate benefit to itself. The neuropeptide oxytocin is an important hormonal mechanism influencing prosociality in mammals, but it is unclear whether the avian homologue mesotocin plays a similar functional role in birds. Here, we experimentally tested prosociality in pinyon jays (*Gymnorhinus cyanocephalus*), a highly social corvid species that spontaneously shares food with others. First, we measured prosocial preferences in a prosocial choice task with two different payoff distributions: Prosocial trials delivered food to both the subject and either an empty cage or a partner bird, whereas Altruism trials delivered food only to an empty cage or a partner bird (none to subject). In a second experiment, we examined whether administering mesotocin influenced prosocial preferences. Compared to choices in a control condition, we show that subjects voluntarily delivered food rewards to partners, but only when also receiving food for themselves (Prosocial trials), and administration of high levels of mesotocin increased these behaviors. Thus, in birds, mesotocin seems to play a similar functional role in facilitating prosocial behaviors as oxytocin does in mammals, suggesting an evolutionarily conserved hormonal mechanism for prosociality.

Keywords: altruism, corvid, prosocial behavior, prosocial choice task, mesotocin, oxytocin

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From helping injured nestmates in ants to donating to charities in humans, many species exhibit prosocial behavior, in which they behave in a way that benefits another individual [1]. In mammals, the neuropeptide oxytocin is a critical hormone regulating social behaviors, including prosociality. For example, administering oxytocin increases charitable donations in humans [2], social contact in marmosets [3], and levels of affiliation, social orientation, and approach behaviors in dogs [4], though see [5] for summary of contrasting results. Among birds, administering an oxytocin antagonist impairs pair bond formation in zebra finches [6], while administering mesotocin—the avian homologue of oxytocin—increases the preference to associate with a larger social group [7]. Therefore, mesotocin also plays a key role in the social behaviors of birds. However, it remains unknown whether mesotocin's role in avian social behavior carries over to prosociality.

Prosocial behavior is often measured experimentally using the prosocial choice task [8]: Subjects make a choice between two options that vary in their reward consequences to another individual. If subjects have prosocial preferences, then they will choose the option that delivers food to the other individual, sometimes even at a cost (altruism). Many corvids exhibit high rates of naturally occurring prosocial behaviors, such as voluntary food sharing [9–13]; however, only a handful of corvid species have been examined in experimental prosocial tasks [14–17]. Despite high rates of naturalistic food sharing, among these corvid species, only azure-winged magpies, *Cyanopica cyana,* have provided convincing evidence of prosociality in an experimental setting [18].

The current study aimed to test mesotocin as a hormonal mechanism of prosociality in pinyon jays, *Gymnorhinus cyanocephalus*, a highly social corvid species that voluntarily shares food [9]. Like magpies, pinyon jays exhibit facultative cooperative breeding [19], which may facilitate the expression of prosocial behavior [20]. Given their highly social nature and voluntary food sharing, our first experiment examined whether pinyon jays choose to provide benefits to same-sex partners in a prosocial choice task. Our second experiment then investigated whether administering mesotocin influenced the proportion of subjects' prosocial choices. We hypothesized that (1) pinyon jays would preferentially choose to provide benefits to another individual and (2) mesotocin administration would increase these prosocial choices.

METHODS
Subjects
In Experiment 1, we tested three female and six male captive adult pinyon jays. In Experiment 2, we tested the same individuals, except for two males. In Experiment 1, subjects rotated through three same-sex partners, whereas, in Experiment 2, they had a single same-sex partner (Table S1).

Experimental Apparatus
We placed three adjacent cages in front of a choice apparatus with two trays resting on a shelf (Figure 1). Each tray contained two dishes in which food (a mealworm) could be placed. To begin a trial, both trays remained out of the birds' reach. Subjects chose by pecking one of two wires extending from the apparatus, which resulted in an experimenter pushing forward the corresponding tray, giving access to food dishes on that tray. Subjects chose from the center cage, with a partner in either the left or right cage (side counterbalanced across sessions).
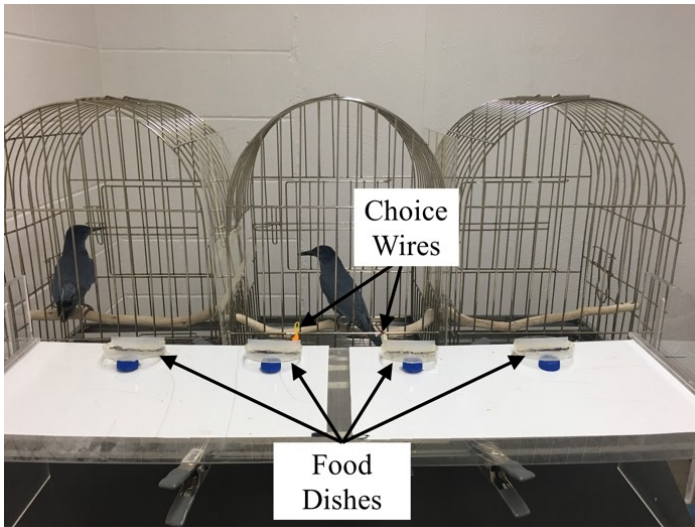
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Figure 1: Experimental apparatus. Subjects in center cage pecked one of two possible choice wires. An experimenter pushed forward the chosen side, thereby giving the subject access to one of the innermost food dishes and the partner access to an outermost food dish (if the tray on the partner's side was chosen). The trial type (Attention, Bias, Altruism, Prosocial) determined the distribution of food across food dishes.

Experimental Sessions

Subjects experienced training to ensure that they understood the consequences of their choices (see Supplementary Materials). All experimental sessions consisted of 16 trials: four Attention trials, followed by four Bias trials, and then four each, in pseudorandomized order, of Prosocial and Altruism trials (Table 1).

Table 1. Experimental trial types.

Trial Type	Reward Distribution (food dishes left to right: 1-food present; 0-absent)	Explanation	Purpose
Attention	0010 or 0100	One mealworm was placed on either the L- or R- center dishes.	These trials ensured that subjects started each session attending to where food rewards were distributed.
Bias	0110	One mealworm was placed on each of the center dishes, thus either an L- or R- choice resulted in a food reward.	Since the outcome to subjects is equivalent, these trials reflect (1) the overall preference for choosing left or right (side bias) and (2) the potential role of social facilitation, where the presence of a partner could influence which side the subject chooses.
Altruism	1001	One mealworm was placed on each of the outermost dishes. Though	Subjects do not get food regardless of side chosen, but if they prefer to be altruistic, they

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		neither an L- or R- choice would give the subject a reward, an L-choice would deliver one mealworm to the left cage and R-choice to right cage.	will choose the same side as the partner. That is, an altruistic choice would deliver no food to the subject, thus benefiting the partner at a low cost to subject.
Prosocial	1111	One mealworm was placed on all dishes. Any choice resulted in a food reward for subject; an L-choice would deliver one mealworm to the left cage and R-choice to right cage.	Subjects will get food regardless of side chosen, but if they prefer to be prosocial, they will choose the same side as the partner. That is, a prosocial choice would deliver food to both the subject and partner.

Measurement of Choice and Analyses

To account for potential biases in the subjects' prosocial and altruistic choices, such as social facilitation, we corrected the amount of matching (*i.e.*, choosing the tray on the same side as the partner) observed in Prosocial and Altruism trials by subtracting the amount of bias matching. For each comparison, we first calculated the absolute change in partner-side matching from Bias to Prosocial/Altruism trials (*absolute tendency*, see Pt in [21]). We also calculated a relative, *weighted tendency* (see Pt' in [21]); however, results from both measures agreed for all analyses, so we present only absolute tendency here (see Supplementary Materials). The greater the amount of prosocial/altruistic choices relative to their bias, the more positive a subject's tendencies will be (see Table S2 for definition of each term). To test whether the amount of matching differed from that observed in Bias trials, we compared the absolute and weighted tendencies against 0. We used Bayes factors (BF) to measure the strength of evidence for hypotheses of group differences over null hypotheses of no difference [22].

Hormonal Manipulation

For Experiment 2, an experimenter intranasally administered one of three possible solutions (high-mesotocin: 30-microgram (15 IU) dose; low-mesotocin: 15-microgram (7.5 IU); and a saline control) 30 minutes prior to each session. For each administration, an experimenter dripped the corresponding solution into the subject's nares using a needleless 1-mL syringe. We based administration time frames and dosages on mammalian oxytocin studies [3].

RESULTS

Experiment 1: Do pinyon jays preferentially deliver food to others?

Compared to Bias trials, pinyon jays increased their delivery of food to a partner by 7.1% in Prosocial trials (Figure 2a) and by 3.3% in Altruism trials. Therefore, there is evidence for pinyon jays choosing prosocially (prosocial absolute tendency; one sample t-test: $t(8) = 3.6$, BF = 8.4) but not altruistically (altruistic absolute tendency; $t(8) = 0.9$, BF = 0.5).
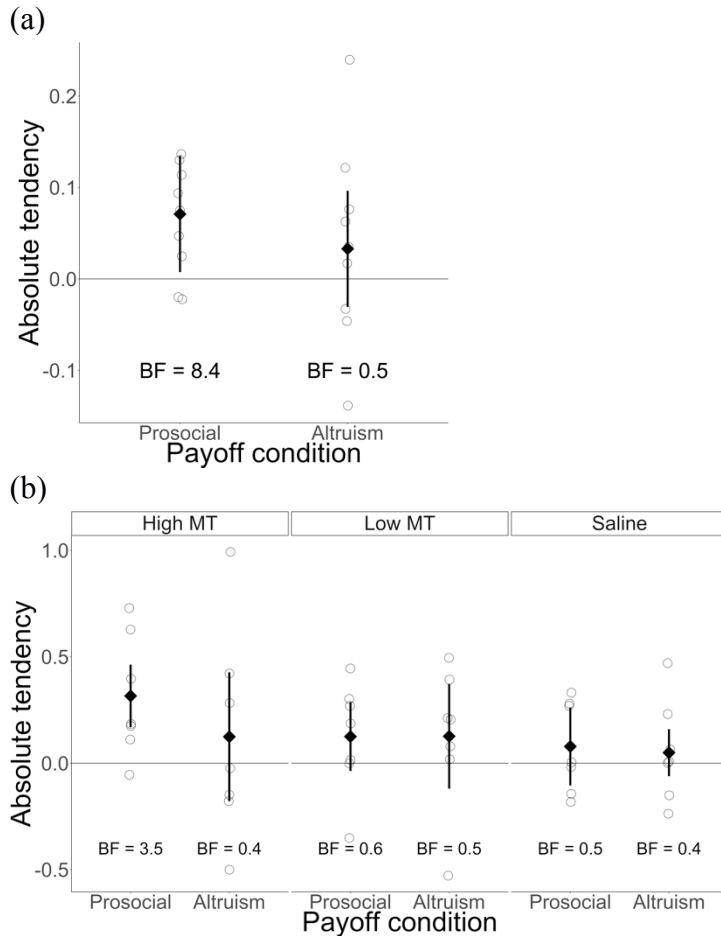
(a)

(b)

Figure 2: Absolute tendency for both experiments. (a) In Experiment 1, compared to Bias trials, subjects preferentially delivered food to partners in Prosocial but not Altruism trials. (b) In Experiment 2, subjects who were administered high levels of mesotocin preferentially delivered food to partners in Prosocial trials but not in any other condition. BF=Bayes factor, MT=mesotocin. Circles represent individual subjects' mean absolute tendency, diamonds represent the overall means, and bars represent within-subjects 95% confidence intervals.

Experiment 2: Does administration of mesotocin increase prosocial and altruistic choices?
Compared to Bias trials, pinyon jays increased prosocial matching by 31.6% in the high-mesotocin condition (prosocial absolute tendency; t(6)=3.0, BF=3.5; Figure 2b), by 12.5% in the low-mesotocin condition (t(6)=1.3, BF=0.6), and by 7.9% in the saline condition (t(6)=1.0, BF=0.5). Therefore, there is evidence for pinyon jays choosing prosocially only in the high mesotocin condition. There is no evidence for altruism in any condition (altruistic absolute tendency; High-mesotocin: mean=12.4%, t(6)=0.7, BF=0.4; Low-mesotocin: 12.6%, t(6)=1.0, BF=0.5; Saline: 5.0%, t(6)=0.5, BF=0.4).

DISCUSSION
In Experiment 1, pinyon jays preferentially chose to deliver food rewards to a partner but only in trials when also receiving benefits for themselves (*i.e.*, in Prosocial but not Altruism trials). In Experiment 2, when given a high dose of mesotocin, subjects preferentially chose to deliver food during Prosocial trials. However, there was no evidence of preferentially delivering food when given a low dose of mesotocin or a saline control. Lastly, pinyon jays did not preferentially deliver food in Altruism trials

regardless of hormone condition. Thus, pinyon jays are prosocial, but not altruistic, in a prosocial choice task, and mesotocin can enhance prosocial behavior.

These data are important in at least two ways. First, our measures of prosocial and altruistic tendency account for individual biases, such as local enhancement and social facilitation, and our results do not change whether we account for the initial degree of bias or not. Thus, pinyon jays join magpies [18] in corvids that show evidence of prosocial behavior not due to social facilitation in an experimental setting, which is consistent with the notion that cooperatively breeding species tend to exhibit unsolicited prosociality [20]. Second, this study is the first to show that mesotocin, the avian homologue of mammalian oxytocin, influences prosocial behavior in birds. Thus, whereas others have shown that mesotocin and oxytocin play a similar functional role in other social behaviors across birds and mammals [7], we provide the first evidence that the similarity extends to prosociality as well. This suggests that oxytocin and mesotocin may serve as an evolutionarily conserved hormonal mechanism for prosociality across mammals and birds.

Despite evidence for choosing prosocially in Experiment 1, the pinyon jays did not show this in the saline condition of Experiment 2, which most closely resembled Experiment 1. Characteristics of the subject, partner, and their interaction, such as degree of affiliation, could mediate decisions in the prosocial choice task, as well as the behavioral effects of mesotocin administration. Indeed, individuals showed considerable variation in their preferences in both experiments (Tables S4 and S5), and partner identity influenced their decisions (Table S3), replicating the variability in food sharing that donors exhibit across recipients [9]. In Experiment 2, we reduced the number of partners to decrease variation in the data. However, the partners chosen for Experiment 2 happened to receive fewer prosocial choices than other partners in Experiment 1 (Table S3). Thus, we may have biased subjects' decisions toward fewer prosocial choices, leading to this discrepancy.

Another possible cause of this discrepancy is that handling the subjects when administering the hormones may have elevated stress, which could have disrupted prosocial behavior. In mammals, oxytocin buffers stress responsiveness [23], which could explain why our high dose of mesotocin resulted in prosocial preferences. Thus, both handling stress and partner preferences may have contributed to a reduction in overall prosocial preferences in Experiment 2.

In mammals, contextual factors and individual differences (*e.g.*, familiarity of partners and genetic variation) moderate how oxytocin influences behavior [24]. Here, though mesotocin administration influenced prosociality, subjects differed in how they responded to this hormone (Table S5). Future studies exploring how contextual and individual characteristics influence prosocial preferences, as well as how different individuals respond to hormonal administration, may reveal the factors that underlie variation in avian prosociality.

Ethical Statement
The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved this project (protocol #1354).

Data Accessibility
Summary tables for subjects and partners, data, and R code are available in the Supplementary Materials, on the Dryad Data Repository [25], and the Open Science Framework [26].

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