Mesotocin influences pinyon jay prosociality.
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SUPPLEMENTARY MATERIAL
Methods
Subjects & Housing
We tested three female and six male adult pinyon jays in Experiment 1 from Mar-Jun 2016 and seven of those same jays in Experiment 2 from Apr-Jun 2017 at the University of Nebraska-Lincoln (two males, 768 and 761, died in between experiments).   In Experiment 1, subjects rotated through three same-sex partners, whereas in Experiment 2, they had a single same-sex partner. We did not use opposite-sex partners because pinyon jays form long term pair bonds and can show prosocial behavior during courtship and related periods. Since our birds are individually housed, pairing opposite sex individuals may induce courtship and other reproduction-related behaviors. Thus, prosocial behavior in a mating context is confounded by courtship and related processes and is easily explained by kin selection. Moreover, subjects were familiar with same-sex partner birds, and pinyon jays do not generally exhibit high levels of aggression. Therefore, we chose to pair subjects with partners of the same sex.

We maintained all subjects at 90% of their free-feeding weight and tested all subjects prior to their daily afternoon feeding to elicit a high motivation for food. After completing the daily sessions, subjects received their maintenance diet of Lafeber's Cockatiel and Parrot Pellets.

Researchers captured all birds in either Arizona or California (United States Fish and Wildlife permit MB694205) between 1996 and 2009 (Table S1). Experimenters individually housed all birds since capture to control for social experience. Home rooms were kept at 22° C with a 14:10 h light:dark cycle.

Table S1. Subject and partner information

Bird ID	**Sex**	**Capture Date**	**Capture Location**
402	Male	August 2009	Eagle Lake, California
404	Female	August 2009	Eagle Lake, California
405	Male	August 2009	Eagle Lake, California
412	Male	August 2009	Eagle Lake, California
518	Female	August 2009	Eagle Lake, California
761	Male	October 1996	Flagstaff, Arizona
768	Male	October 2003	Patterson, Arizona
780	Male	April 2006	Flagstaff, Arizona
782	Female	April 2006	Flagstaff, Arizona
408-partner	*Female*	*August 2009*	*Eagle Lake, California*
410-partner	*Male*	*August 2009*	*Eagle Lake, California*
779-partner	*Male*	*April 2006*	*Flagstaff, Arizona*
785-partner	*Female*	*October 2007*	*Arizona*
791-partner	*Female*	*October 2007*	*Arizona*
795-partner	*Male*	*October 2007*	*Arizona*

Cages and Prosocial Choice Apparatus

Subject and Partner Cages

We placed three side-by-side cages, each measuring 47 cm x 49 cm x 65 cm, 15 cm in front of the prosocial choice apparatus (Figure 1). This distance prevented birds from reaching the food dishes unless the tray containing the food dishes was pushed forward. A single perch was placed 10cm from the cage front in all three cages.

At the borders between adjacent cages, each cage had a small opening, 15.5 cm x 22 cm, allowing all three cages to be interconnected. We allowed subject birds to move between cages in some Training Phase trials (see below). However, in most trials, a transparent barrier between the cages prevented access to adjacent cages. In partner-present trials, the subject and partner birds could watch and vocalize to one another through the wire cages, but the barriers prevented any further interaction. Thus, unless otherwise noted, experimenters always placed subjects in the center cage and, in partner-present trials, a partner into one of the side cages, with neither bird having access to any other cage.

Prosocial Choice Apparatus

The prosocial choice apparatus consisted of two transparent shelves. The top shelf supported two trays each with two food dishes attached. The bottom shelf supported a plastic square with two wires attached (the left and right choice wires) that slid independently of the trays on the top shelf. By pulling back or pushing forward the plastic square, an experimenter could retract or present the choice wires, respectively. For each trial, an experimenter first baited the food dishes with a mealworm, then pushed forward the wires, thereby allowing subjects to make a choice by pecking one of the two wires. After subjects made a choice, the experimenter retracted the wires and slid the tray on the chosen side closer to the cages, making the dishes on that tray (and, if present, food) accessible. The center cage could access either of the two innermost dishes, while the side cages could only access the outermost dish on its side.

To minimize distractions, we placed a white curtain separating the cages and apparatus from the experimenter. The experimenter could put his/her arms through open flaps in the curtain to bait food dishes and slide the wires or trays. An overhead camera recorded all sessions, and a live-feed to a monitor allowed experimenters to observe subjects' choices and behaviors despite the visual barrier of the curtain.

Trial Type Notation

Since there are four possible locations for food rewards, we denoted the distribution of food across the dishes by 1/0: the presence (1) or absence (0) of food. For example, 1111 denotes food in each of the food dishes, 1000 / 0001 denotes food in the leftmost or rightmost dish, respectively, and 1001 denotes food in both of the outermost dishes.

Procedure

Habituation and Training

Phase 1: Habituation to cages and apparatus

In Phase 1, we habituated subjects to the prosocial choice apparatus and reinforced for pecking choice wires. Initial trials consisted of baiting both center food dishes (0110). If subjects pecked a wire, an experimenter pushed forward the corresponding tray, and gave the subject 5 minutes

to eat the presented food. If the subject made no feeding attempt, the experimenter reset the tray and started the next trial. Sessions lasted up to 20 minutes, with an experimenter re-baiting food dishes and, when necessary, directing the subject's attention toward those food dishes by first waving the mealworm back and forth in the subject's line of sight.

Criterion: Subjects progressed to the next phase once they touched the wire in three successive sessions or touched three times within a single session.

Phase 2: Training for left or right wire
In Phase 2 trials, an experimenter baited either the center-left (0100) or center-right (0010) food dish (side pseudo-randomized each trial), pushed forward the choice wires, then gave subjects up to 45 seconds to make a choice. If subjects did not choose within 45 seconds, then the experimenter reset the food dishes and started the next trial. Once subjects made a choice and the corresponding tray was pushed forward, subjects could access the food dish for 30 seconds. Thus, during Phase 2 trials, subjects learned (1) that a choice needed to be made in <45 seconds and (2) the outcomes of pecking either wire: left wire results in left tray pushed forward, right wire right tray pushed forward.

Each daily session ended once subjects completed 10 trials in which they made a choice, up to a maximum of 20 total trials (10 trials without a choice). If subjects understood how choices influenced access to food dishes, then they should choose the wire on the same side as the tray with food more frequently than the wire/tray without food.

Criterion: Subjects progressed to the next phase once they chose the correct tray in at least 8 of 10 completed trials for two consecutive sessions.

Phase 3: Training to understand outcomes to side cages
In Phase 3 sessions, the experimenter removed either the left or right barrier between cages, allowing subjects to move into one of the side cages and access the food dishes on that side (in addition to the center food dish from the center cage). Subjects had to hop between the center cage, where they made a choice, and the accessible side cage to receive all the presented food. Thus, subjects experienced the full outcomes of choosing the left or right wire, because they had access to the food items delivered to both the donor and partner cages.

Each session consisted of 10 trials of pseudo-randomized 1000/0001 or 0100/0010. Over successive sessions, experimenters monitored whether subjects exhibited a bias toward choosing one side over another (*i.e.,* side bias). If a bias was observed over several prior sessions, the experimenter baited more food dishes on the opposite side to the bias in the following session. For example, if an individual began to always choose left, the following session consisted of 0001 or 0010 (both trial types which require choosing the right wire to obtain food). As subjects learned how to make a correct choice when only one food item was present, experimenters began to add Bias (0110), Altruism (1001), and Prosocial (1111) trials with one barrier removed (Table 1). If subjects understood which cages allowed access to which food dishes, they should have chosen the side that provided access to the side cage.

Once experimenters addressed strong biases and subjects paid attention to the distribution of food, we began training with one barrier removed. These sessions consisted of four Attention trials (0100/0010), followed by 12 other trials: four Bias (0110), four Altruism (1001), and four Prosocial (1111). We randomized the order of the eight Altruism and Prosocial trials, and subjects had 45 seconds to make a choice before the experimenter withdrew the choice wires and rebaited for the next trial (Table 1). Each session began with two left (0100) and two right (0010) Attention trials, order randomized. Subjects had to correctly choose in at least three of four Attention trials to proceed with Bias, Altruism, and Prosocial trials. Attention trials ensured that subjects began each session paying attention to the distribution of food. If subjects failed to meet the criterion, experimenters gave an additional four Attention trials and the new criterion to proceed became six or more of eight correct. Failure to meet six of eight correct resulted in the remaining 12 trials becoming Attention trials.

Criterion: Subjects progressed to the next phase once they chose the correct tray in at least 8 of 10 completed trials for two consecutive sessions.

Partner Training
Experimenters selected three male and three female partner birds for their ability to reliably eat food presented to them. Experimenters placed partner birds in one of the side cages, baited the same-side food dish with 2-5 mealworms, and pushed forward the tray on the partner side. Initially, experimenters gave birds up to 5 minutes to eat, but as training continued, experimenters reduced the number of worms to one and the amount of time the food dish was accessible to 30 seconds (*i.e.,* the same time window as subjects following a choice).

Phase 4: Experiment Trials
Experimental sessions, with both barriers present, began once subjects progressed through habituation and training (Phases 1-3). Sessions alternated between partner-present and partner-absent sessions. In partner-present sessions, experimenters placed one same-sex partner bird in a side cage. We randomized the side and the identity of the same-sex partner birds, but each subject cycled through all three partners before being paired with the same partner again.

Experimental sessions were the same as subjects experienced at the end of training: four Attention trials followed by 12 others; four each of Bias, Altruism, and Prosocial, order randomized, and subjects needed to make a choice within 45 seconds. Likewise, subjects had to correctly choose in at least three of four Attention trials to proceed with Bias, Altruism, and Prosocial trials. If the subject failed to meet the criterion, experimenters gave an additional four Attention trials and the new criterion to proceed became six or more of eight correct. Failure to meet six of eight correct resulted in the remaining 12 trials becoming Attention trials. If subjects failed to meet criterion, experimenters gave the same session the following day, and if failed again (*i.e.*, two consecutive failures), added the session to the end of the running schedule.

Experiment 2
After Experiment 1 completed, we attempted to reduce variance in prosocial / altruistic choices introduced by the use of multiple partners by selecting just one male and one male female partner based on which male and female most reliably ate accessible food in Experiment 1.

Experiment 2 procedures were the same as Experiment 1, except for hormonal administration. Thirty minutes prior to each session, an experimenter administered 100-125 microliters of either a high or low dose of mesotocin or a saline control by dripping the solution into the subject's nares using a needleless 1 mL syringe. We administered half of the solution into each of the nares. Mesotocin solutions (Bachem Inc., Torrance, CA: product number H-2505) were diluted in 100 microliters of saline solution. The high mesotocin dose was 30 micrograms (15 IU), and the low dose was 15 micrograms (7.5 IU). The additional 25 microliters were included to reduce any loss from spillage during the administration. We based administration time frames and dosages on mammalian oxytocin studies (*e.g.,* Smith et al 2010). Experimenters were blind to which hormone treatment subjects experienced.

To reduce the likelihood of carry-over hormone effects (*i.e.*, from one hormone condition to another), partner-absent and partner-present sessions alternated and subjects always received saline for partner-absent sessions. Thus, subjects always received saline in-between sessions with mesotocin (unless a subject failed to pass Attention trials for a mesotocin session and thus received the same mesotocin dose the following day). All analyses were conducted within partner-present sessions, therefore subjects' choices in partner-absent sessions are not discussed further.

Measurement of Choice and Analyses
Measurement of prosocial or altruistic choices must account for potential biases that predispose an individual to choose one side over another (*e.g.*, an increased likelihood of choosing the partner side due to social facilitation). Therefore, we used Bias trials to correct each individual's measure of prosocial and altruistic behavior (see Massen, et al. 2011). Unlike Massen et al. (2011), however, every experimental session included Bias trials that immediately preceded Prosocial/Altruism trials. Thus, we quantified biases *within* sessions, which allowed us to correct our measures of altruistic and prosocial behavior in the same set of partner-present sessions.

Specifically, for each individual and trial type (Bias, Prosocial, Altruism) combination, we calculated the proportion of choices made for the same side as the partner (*i.e.*, partner-side matching). We then calculated prosocial/altruistic *absolute tendency* (see Pt in Massen, et al. 2011) by subtracting the amount of matching in Bias trials from the amount of matching in Prosocial/ Altruism trials. Therefore, a subject's absolute tendency reflects the absolute amount of change from their bias. The more positive the value, the more subjects chose to reward the partner beyond their baseline biases.

However, since individuals can differ in their biases, the total amount by which those individuals can increase or decrease their matching in Prosocial or Altruism trials can differ as well. We therefore calculated a relative, *weighted tendency* (see Pt' in Massen, et al. 2011) that reflects the subject's magnitude of change from bias matching weighted by the degree of bias and direction of change (*i.e.*, with or against the bias). This was done by dividing the absolute tendency by the amount of remaining "space" available in the direction of the change. Thus, to calculate the weighted tendency we divided a positive absolute tendency by the proportion of non-matching in Bias (1-Bias matching), and, if negative, divided by the proportion of matching in Bias.

For example, if a subject partner-side matched in 55% of Bias trials and increased to 65% in Prosocial trials (*i.e.*, 0.1 prosocial absolute tendency), then there is 45% "space" available in the direction of the change (increased matching in this example). Thus, the 0.1 prosocial absolute tendency would be divided by 0.45, to obtain a 0.222 prosocial weighted tendency. If the subject had decreased to 45% instead, they would have a -0.1 prosocial absolute tendency, but 55% space available and therefore a -0.182 prosocial weighted tendency. See Table S2 for definition of each term.

Lastly, it was possible for subjects to not make a choice in a trial. In some cases, no choices were made within a session for a particular trial type (Prosocial/Altruism). Therefore, bias matching was calculated separately for the two trial types and only from the subset of sessions in which subjects made at least one choice for that trial type.

Table S2. Definitions for all measures.

Measure	Definition
Matching	Proportion of choices made for the same side as the partner (B = bias, P = Prosocial, A = Altruism)
Absolute Tendency	Absolute change from bias matching to prosocial or altruistic matching; bias matching is calculated from the same subset of sessions for prosocial/altruistic matching (*i.e.*, sessions in which subject made at least one choice) Prosocial absolute tendency = $P - B$ Altruistic absolute tendency = $A - B$
Weighted Tendency	Absolute change in matching from Bias trials to Prosocial or Altruism trials (absolute tendency) divided by the available amount of proportion remaining in the direction of the change. • If positive, then divide by (1-bias matching) • If negative, then divide by (bias matching) Prosocial weighted tendency = $(P - B) / (1 - B)$, if $P - B > 0$ Prosocial weighted tendency = $(P - B) / B$, if $P - B < 0$ Altruistic weighted tendency = $(A - B) / (1 - B)$, if $A - B > 0$ Altruistic weighted tendency = $(A - B) / B$, if $A - B < 0$

Data Analysis

We assessed whether there was evidence that the average absolute and weighted tendencies differed from 0. If pinyon jays are prosocial or altruistic, then there will be evidence for an increased tendency (*i.e.*, evidence that the average value is greater than 0). We used Bayes factors (BF) to measure the strength of evidence for hypotheses of group differences over null hypotheses of no difference (Wagenmakers, 2007). For example, BF = 12 means there is 12 times more evidence for the alternative hypothesis than the null hypothesis. Bayes factors above 3 are considered moderate evidence and, above 10, strong evidence (Andraszewicz et al., 2015).

We analysed the data using R Statistical Software version 3.4.3 (R Core Team, 2017) and packages *BayesFactor* (Morey & Rouder, 2015), *car* (Fox & Weisberg, 2011), *papaja* (Aust & Barth, 2017), and *tidyr* (Wickham & Henry, 2017). Data and R code are available in the Supplementary Materials, on the Dryad Data Repository (https://doi.org/10.5061/dryad.g38qb00), and the Open Science Framework (https://osf.io/358hs/).

Experiment 1
To test whether pinyon jays preferentially delivered food rewards to partners, we conducted four one-sample Bayesian t-tests. We compared prosocial and altruistic absolute tendencies against 0 to test the amount of evidence for a difference in partner-side matching from Bias to Prosocial/Altruism trials. If pinyon jays preferentially chose to deliver food rewards to a partner bird, then there will be evidence for an increased absolute tendency (*i.e.*, evidence for a difference greater than 0). The same analyses were conducted for weighted tendency (and are only reported here in Supplementary Results).

Experiment 2
To test whether our hormonal manipulations influenced prosocial decisions, we conducted the same analyses as in Experiment 1 for each hormone condition (six Bayesian t-tests for each measure, absolute and weighted, and trial type, Altruism and Prosocial).

Results
Experiment 1: Do pinyon jays preferentially deliver food to others?
Out of 107 total sessions in Experiment 1, subjects made at least one choice during Prosocial trials in all sessions but did not make any choices during Altruism trials in 16 sessions. Subjects' prosocial and altruistic matching varied across the three partners (Table S3). Consistent with all analyses on absolute tendency (Figure 2a), after weighting each subject's magnitude of change by their degree of initial bias and direction of change (more or less matching from bias), pinyon jays showed a prosocial weighted tendency of 12.9% and altruistic weighted tendency of 5.4% (Figure S1b). Thus, there is evidence for pinyon jays choosing prosocially (prosocial weighted tendency; one sample t-test: $t(8) = 3.6$, $BF = 8$) but not altruistically (altruistic weighted tendency; $t(8) = 1.0$, $BF = 0.4$).

Experiment 2: Does administration of mesotocin increase prosocial and altruistic choices?
Out of 126 total sessions in Experiment 2, subjects made at least one choice during Prosocial trials in all but one session but did not make any choices during Altruism trials in 30 sessions. Consistent with all analyses on absolute tendency (Figure 2b), after weighting each subject's magnitude of change by their degree of initial bias and direction of change, pinyon jays showed a prosocial weighted tendency of 39.8% in the high-mesotocin condition ($t(6)=3.6$, $BF=6.3$; Figure S1d), 13.7% in the low-mesotocin condition ($t(6)=1.2$, $BF=0.6$), and 2.1% in the saline condition ($t(6)=0.16$, $BF=0.4$). 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 weighted tendency; High-mesotocin: mean=4%, $t(6)=0.2$, $BF=0.4$; Low-mesotocin: 14.5%, $t(6)=1.0$, $BF=0.5$; Saline: 2.7%, $t(6)=0.2$, $BF=0.4$).
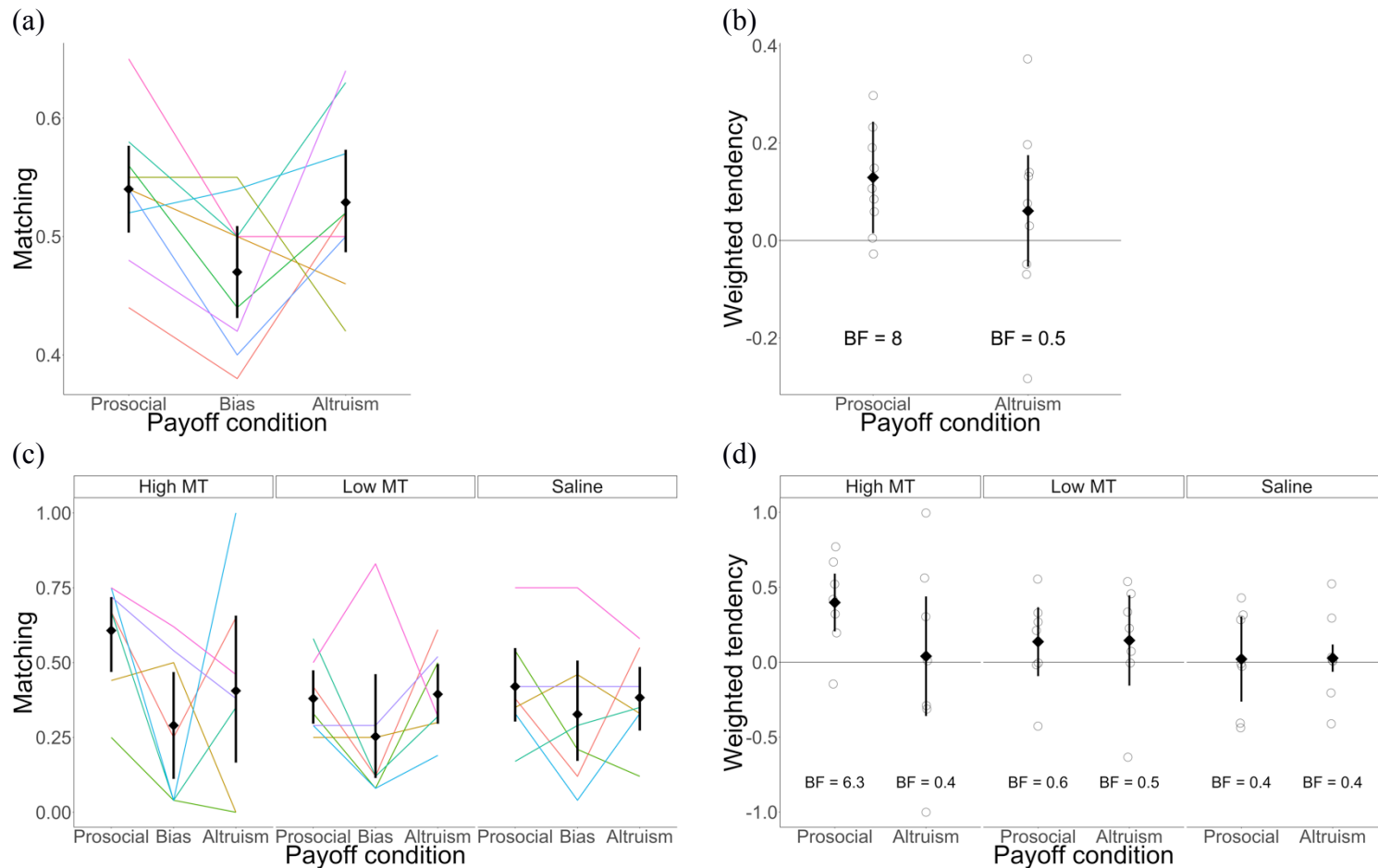
Figure S1: Matching and weighted tendency of each subject's choices. For the raw matching values for Experiment 1 (a) and Experiment 2 (c), each line is an individual subject with its partner-side matching in Bias trials presented in the middle to visualize whether each subject increased or decreased the proportion of partner-side choices in Altruism or Prosocial trials. (b) In Experiment 1, weighted tendencies show that subjects preferentially delivered food to partners in Prosocial but not Altruism trials. (d) In Experiment 2, administering high levels of mesotocin increased delivery of food to partners for Prosocial trials but not in any other condition. BF=Bayes factor, MT=mesotocin. Circles represent individual subjects' weighted tendency, diamonds represent the overall means, and error bars in (a) and (c) represent bootstrapped 95% confidence intervals and in (b) and (d) represent within-subjects 95% confidence intervals.

Table S3. Matching values received by partners in Experiment 1.

Part- ner	Prosocial Matching	Altruistic Matching
408	0.19	0.18
410	**0.04**	**0.00**
779	0.11	0.17
785	0.08	-0.22
791	**-0.01**	**0.01**
795	0.04	0.02

Note: Bolded birds reflect the partners used in Experiment 2.

Table S4. Raw matching values and absolute and weighted tendency measures for each subject and condition in Experiment 1.

Subject	Overall Bias Matching	Prosocial Matching	Altruistic Matching	Prosocial Absolute	Altruistic Absolute	Prosocial Weighted	Altruistic Weighted
402	0.38	0.44	0.52	0.06	0.11	0.1	0.18
404	0.5	0.54	0.46	0.04	0.02	0.08	0.03
405	0.55	0.55	0.42	0	-0.15	0	-0.27
412	0.44	0.56	0.52	0.12	0.09	0.21	0.16
518	0.5	0.58	0.63	0.08	0.06	0.17	0.14
761	0.54	0.52	0.57	-0.02	0.03	-0.04	0.06
768	0.4	0.54	0.5	0.15	-0.03	0.24	-0.05
780	0.42	0.48	0.64	0.06	0.22	0.11	0.38
782	0.5	0.65	0.5	0.15	-0.05	0.29	-0.08

Table S5. Raw matching values (a) and absolute and weighted tendencies (b) for each subject and condition in Experiment 2.

(a)

Sub-ject	Overall Bias Matching			Prosocial Matching			Altruistic Matching		
	High MT	Low MT	Saline	High MT	Low MT	Saline	High MT	Low MT	Saline
402	0.25	0.12	0.12	0.67	0.42	0.38	0.65	0.61	0.55
404	0.5	0.25	0.46	0.44	0.25	0.35	0	0.3	0.33
405	0.04	0.08	0.21	0.25	0.33	0.54	0	0.5	0.12
412	0.04	0.12	0.29	0.67	0.58	0.17	0.35	0.32	0.35
518	0.04	0.08	0.04	0.75	0.29	0.33	1	0.19	0.33
780	0.54	0.29	0.42	0.72	0.29	0.42	0.38	0.52	0.42
782	0.62	0.83	0.75	0.75	0.5	0.75	0.46	0.32	0.58

(b)

Sub-ject	Prosocial Absolute			Altruistic Absolute			Prosocial Weighted			Altruistic Weighted		
	High MT	Low MT	Saline	High MT	Low MT	Saline	High MT	Low MT	Saline	High MT	Low MT	Saline
402	0.42	0.29	0.25	0.4	0.49	0.45	0.56	0.33	0.29	0.54	0.56	0.50
404	-0.06	0	-0.2	-0.5	0	-0.25	-0.11	0	-0.44	-1	0	-0.43
405	0.21	0.25	0.33	0	0.42	0.06	0.22	0.27	0.42	0	0.45	0.07
412	0.62	0.46	-0.12	0.3	0.22	0	0.65	0.52	-0.43	0.32	0.24	0
518	0.71	0.21	0.29	1	0.06	0.25	0.74	0.23	0.3	1	0.07	0.27
780	0.18	0	0	-0.17	0.22	0	0.4	0	0	-0.31	0.31	0
782	0.12	-0.33	0	-0.17	-0.51	-0.17	0.33	-0.4	0	-0.27	-0.62	-0.22

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