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Committed for the long haul: Do nonapeptides regulate long-term pair maintenance in zebra finches?

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A B S T R A C T

The nonapeptides (oxytocin, vasopressin, and their non-mammalian homologs) regulate a number of social behaviors across vertebrates including monogamous pair bonds in mammals. Recent work on zebra finches has shown an important role for these neurohormones in establishing avian pair bonds as well. However, studies on the role of nonapeptides in maintaining pair bonds after pair formation are lacking. The goal of the present study was to investigate the effects of an oxytocin receptor antagonist (OTA) on pair maintenance behaviors in the monogamous zebra finch. I injected established zebra finch pairs over three days with either 5 μg of an OTA or a vehicle control, and separated the partners for one hour, after which partners were reunited and their reunion recorded on video for 30 min. Videos were then coded to measure singing, affiliative (allopreening, clumping, following), and aggressive (pecking) behaviors. These behaviors were also measured both on the day before injections to establish a pre-treatment level and two days after the last injection. Control and antagonist treated birds did not differ in the amount of time spent clumping or the frequency of pecking across the experiment. However, both male and female zebra finches that received OTA significantly reduced the amount of time spent following their partner. Females given the OTA treatment reduced allopreening and males given the OTA treatment reduced the frequency of singing bouts directed at their partners relative to controls. These results suggest that the nonapeptides play a role in regulating some, but not all, pair maintenance behaviors in experienced zebra finches.

1. Introduction

The nonapeptides oxytocin, vasopressin, and their non-mammalian homologs have been shown to regulate a wide range of social behaviors across vertebrates (Donaldson and Young, 2008; Choleris et al., 2013). These results suggest that the neurohormonal mechanisms underlying social behaviors are conserved among distantly related taxa. However, the nonapeptide mechanisms regulating pairing behavior are only well studied in rodents, especially the monogamous prairie vole (*Microtus ochrogaster*) (Young and Wang, 2004; Lim and Young, 2006). As social monogamy evolved independently multiple times across vertebrates (Whiteman and Côté, 2004; Adkins-Regan and Tomaszycki, 2007; Lukas and Clutton-Brock, 2013), the mechanisms of pair bonding likely differ between prairie voles and other mammals and non-mammals (Goodson and Thompson, 2010).

Pair bonds are established in prairie voles after mating occurs and are dependent on oxytocin (OT) for females (Insel and Hulihan, 1995; Liu and Wang, 2003) and vasopressin (AVP) for males (Liu et al., 2001; Lim and Young, 2004). Administration of either an OT receptor antagonist (OTA) for females or an AVP 1a receptor antagonist for males is sufficient to disrupt pair bond formation in naïve voles (females-Liu and Wang, 2003; males-Liu et al., 2001). However, disrupting nonapeptide action is not sufficient to block pair formation in other species. For example, in the socially monogamous black-tufted marmoset (*Callithrix penicillata*), administration of an OTA did not prevent pair bond formation, though it did significantly reduce partner directed affiliative behavior (Smith et al., 2010). Similarly, monogamous convict cichlids (*Amatitlania nigrofasciata*) treated with a non-specific nonapeptide antagonist delayed pair bonding, but ultimately did form pairs (Oldfield and Hofmann, 2011). Together, these studies support a general role for nonapeptides in pair bonding across vertebrates, but highlight species-specific regulation and the need to study a wide range of taxa to understand the neurohormonal mechanisms underlying monogamy.

The mechanisms regulating pair formation may also differ from those involved in pair maintenance. Dopamine and its receptors (D1 and D2) can either promote or inhibit pair formation in prairie voles, depending on what type of dopamine receptor is active. Activation of D2-like receptors significantly promoted pair formation while activation of D1-like receptors inhibited pair formation in prairie voles (Aragona et al., 2006). By contrast, pair maintenance involved a significant increase in D1-like (but not D2-like) receptors in the nucleus accumbens, leading to selective aggression towards non-partner voles (Aragona et al., 2006). Therefore, the same receptors can play opposing roles during pair formation and maintenance, with D1 inhibiting pair formation but promoting pair maintenance.

Consistent with the experiments on pair bonds in prairie voles,

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recent work in socially monogamous zebra finches (*Taeniopygia guttata*) found that peripheral administration of an OTA significantly reduced the likelihood of pairing in inexperienced birds (Pedersen and Tomaszycki, 2012). Chronic central administration of OTA also significantly disrupted pair formation in inexperienced zebra finches (Klatt and Goodson, 2013a). However, both studies found that after receiving OTA, some naïve zebra finches still did form pair bonds, suggesting that pair formation may be somewhat independent of nonapeptides. Based on these results and the work in prairie voles, the nonapeptides are likely involved in regulating pair maintenance behaviors in zebra finches (Prior and Soma, 2015).

Recent work in zebra finches also suggests that the nonapeptides may play a different role in initial pair formation than in pair maintenance (Lowrey and Tomaszycki, 2014). Zebra finches were allowed to pair naturally and then tested for the abundance of nonapeptide mRNA in the brain at either 48 h or two weeks after pair formation. In males, courtship behaviors (e.g., directed singing) during the first 48 h best explained increases in MT (mesotocin, avian homolog of oxytocin) and AVT (arginine vasotocin, avian homolog of vasopressin) in the paraventricular nucleus (PVN) and bed nucleus of the stria terminalis (BSTm) (Lowrey and Tomaszycki, 2014). While expression of MT and AVT in the PVN remained high at both 48 h and two weeks after pairing, expression of AVT in the BSTm was only high after 48 h and dropped by two weeks. There also was a sex effect of pairing as only clumping (partners perched at rest in direct physical contact) predicted variations in MT mRNA in females and no pair behaviors predicted AVT mRNA in either the PVN or BSTm (Lowrey and Tomaszycki, 2014). These results suggest that there may be differences in the mechanisms behind pair bonding and maintenance as well as sex differences in the effects of nonapeptides on pair behaviors. To the best of my knowledge, no study has yet directly examined the effect of the nonapeptides on pair maintenance behaviors in birds.

Although MT and AVT can potentially affect behavior via distinct mechanisms, due to the promiscuity of their receptors it is difficult to disentangle these distinct effects. Like most vertebrates, birds have multiple vasotocin/vasopressin receptors and one OT-like receptor (Baeyens and Cornett, 2006). In birds, V1a, VT1, and OT-like VT3 receptors are widespread throughout the brain, particularly in regions associated with social behaviors (Leung et al., 2011). In zebra finches the VT3 receptor has been shown to bind both MT and AVT (Baeyens and Cornett, 2006; Leung et al., 2009); therefore, blocking the VT3 receptor with an oxytocin receptor antagonist could inhibit the binding of both MT and AVT. For this reason, this experiment tests whether nonapeptides in general, and not MT or AVT specifically, regulate pair-maintenance behaviors by bonding to the OT-like VT3 receptors in the brain.

I tested whether the zebra finch behaviors involved in maintaining an established pair bond were regulated by nonapeptides MT and AVT by administering an OTA peripherally. I predicted that administration of an oxytocin receptor antagonist could disrupt pair maintenance behaviors in reproductively experienced pairs.

2. Methods

2.1. Subjects and housing

The control and experimental groups each included eight zebra finch pairs (16 males and 16 females across both treatments). All subjects had been bred in the lab. All birds had been freely allowed to choose their partners prior to the start of the study, and all pairs had successfully raised at least one clutch of offspring. Each pair had previously participated in a study on the effects of an oxytocin receptor antagonist (OTA) on parenting. After the parenting experiment, the subjects were kept with their partners in mixed-sex aviaries for at least two weeks to ensure no lasting effects of the previous treatment. The previous treatment groups of the subjects were randomized with respect to the current study. Individual age was also randomized with respect to treatment.

All subjects were housed in a temperature and humidity controlled room on a 14:10 light:dark schedule for the duration of the experiment. Each individual had four unique leg bands; three colored and one silver ID band with an individualized ID code.

Prior to the start of the study, pairs were randomly selected and assigned to one of four aviaries such that each aviary consisted of four pairs. To control for any cage effects, two pairs from each cage were in the experimental group and the remaining two pairs were in the control group. All aviaries (0.94 m by 0.76 m by 0.94 m) were equipped with seed, grit, cuttlebone and water *ad libitum*. All aviaries were located within the same room. The Cornell University IACUC approved all methods and procedures of the study.

2.2. OTA injections

Pairs were randomly assigned to either the experimental group or a vehicle control group. Both males and females within a pair were given the same treatment. Each bird in the experimental group was given a 0.05 ml intramuscular injection into the pectoral muscle of 5 µg OTA ([d$(CH_2)^1_5$, Tyr(Me)^2, Thr^4, Orn^8, des-Gly-NH^9_2]-Vasotocin trifluoroacetate salt, Bachem) dissolved in 0.9% saline. In rats, this antagonist is 18 times more potent as an OT receptor antagonist than a V1a receptor antagonist (Manning et al., 2008) and has been highly effective in altering rat social behaviors (Neumann et al., 2003, 2006). This antagonist has also effectively disrupted social behaviors in zebra finches (Goodson et al., 2009; Pedersen and Tomaszycki, 2012; Klatt and Goodson, 2013a). Administration of 5 ug of this OTA through peripheral injection has been previously shown to be effective in disrupting zebra finch pair formation (Pedersen and Tomaszycki, 2012). Control subjects were injected with the same volume of 0.9% saline. Animals were only injected once per day for three days and the effects of each injection likely did not last the entire 24-hour period of each day (Pedersen and Tomaszycki, 2012).

2.3. Separation and reunion testing

To induce pair maintenance behaviors, I briefly separated each subject from their partner before reuniting them and video recording their behaviors. Birds were separated and then reunited and recorded on each of five days: Pre-treatment (Day 0, no injection), three consecutive days of injections (Days 1–3), and Post-treatment (Day 5, no injection, 48 h after the third injection). For each recording day, the partners were removed from their home cage and then either immediately placed into two separate small aviaries (Pre-treatment and Post-treatment) or injected (Days 1–3) and placed into two separate small aviaries for 60 min (Fig. 1). The same two small aviaries were used on each day. The small aviaries for both birds were placed across from their home aviary, and males and females could hear but not see their pair partners. Separation from the partner for as little as one hour induces a glucocorticoid stress response and also leads to an increase in affiliative behaviors upon reunion (Remage-Healey et al., 2003). Though previous studies using this OTA had intervals between injection and behavioral testing ranging from 10 to 30 min (Samuelsen and Meredith, 2011; Pedersen and Tomaszycki, 2012), I chose 60 min to increase the likelihood of affiliative pair maintenance behaviors (Baran et al., 2016; Prior et al., 2018).

After being separated for 60 min, both members of a pair were placed in a new aviary (0.94 m by 0.76 m by 0.94 m) within their homeroom and video recorded for 30 min. After recording, the subjects were returned to their home aviary. Videos were scored separately for each male and female by a single trained coder who was blind to treatment. Behaviors scored for pair maintenance (see Table 1) were based on previous studies of pairing behavior in zebra finches (Tomaszycki and Adkins-Regan, 2005; Pedersen and Tomaszycki, 2012; Baran et al., 2016). Specifically, I scored time spent perched in contact

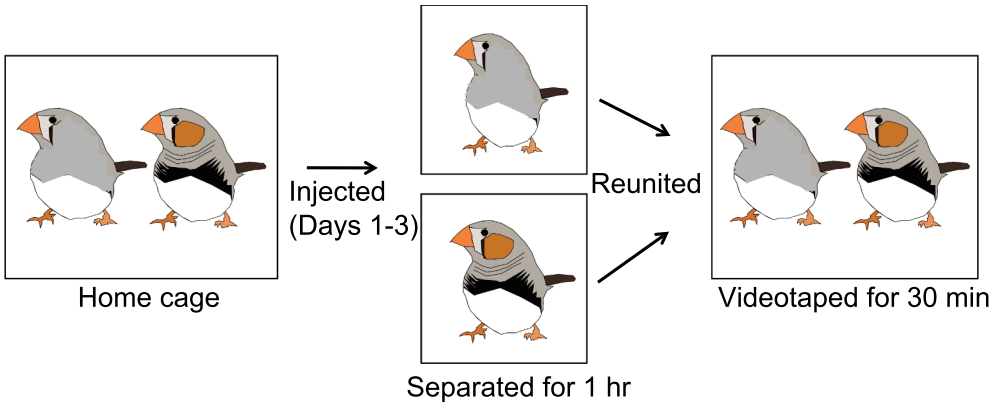

Fig. 1. Time sequence of injections and separations for each pair across the experiment. Birds were caught in their home cages and then either immediately placed in separate small aviaries (Pre-Treatment and Post-Treatment) or injected (Days 1–3) and then placed in separate small aviaries for 1 h. After being separated for 1 h, the partners were reunited and filmed in the reunion aviary.

(clumping), allopreening bouts by both birds (allopreen), frequency of song bouts (song bout) and total time spent singing (total song) by males, and following the partner to a new perch within three seconds (follow). Three seconds was chosen based on previous observations of zebra finches during pair formation where individuals either immediately followed the bird they were interested in (< 3 s) and perched near them or flew to the perch later (> 3 s) and did not perch near the other bird. A song bout was considered over when a male stopped singing for at least 1 s (Okubo et al., 2015; Norton and Scharff, 2016; Bruno and Tchernichovski, 2017). I also scored number of pecks (pecking), an aggressive behavior (Table 1).

2.4. Data analysis

Data was analyzed using linear mixed-effect models to determine how treatment affected pair maintenance behaviors. All models were run in R (version R 3.4.0) using the "lme" package. I used nested random-effects terms to address both the repeated measurement of individual birds and the potential non-independence of an individual's behavior due to how their partner behaved or what cage they were in.

For linear mixed effects models of allopreening, follow, and pecking, Bird ID (unique code for each bird) was nested within Pair ID (unique identifier for each pair) and Pair ID was nested within Cage ID (unique code for each cage) as random intercepts and random slopes for the change in behavioral duration or frequency with day. For linear mixed effects models of singing, which is performed only by males (so that only one bird in each Pair ID was repeatedly measured), Bird ID was nested within Cage ID as random intercepts and random slopes for the change in behavioral duration and frequency with day. For linear mixed effects models of clumping (a single measurement of a behavior exhibit by both birds in a pair) Pair ID was nested within Cage ID as random intercepts and random slopes for the change in behavioral duration or frequency with day.

All models initially included fixed main effects of sex and treatment and 2- and 3-way interactions between sex by treatment and sex by treatment by day. I then removed insignificant fixed effects in a backwards, stepwise manner until only significant terms remained. Treatment and day were treated as fixed effects for all models. Control and OTA groups were considered significantly different from each other if there was a significant treatment by day interaction, as determined by analysis of variance (ANOVA) (Table 2).

For allopreening, the behavior with a significant 3-way sex by treatment by day interaction, a post-hoc Tukey's Test was used to determine if significant differences exist in the slopes of the change in the behavioral duration vs. day between sexes in the same treatment (2 contrasts) and between treatments of the same sex (2 contrasts, for a total of 4). In the absence of significant sex main- or interaction-effects in the ANOVA of the linear mixed effect models, the reported F-tables are for behaviors measured in both males and females (except for singing, which is performed only by males) (Table 2).

Following Kline (2004), non-standardized effect sizes in their original units are reported, rather than approximations of effect size statistics such as Cohen's *d*, due to the difficultly of estimating the pooled variance of groups of semi-independent measurements in models with complicated random-effect structures (*see also* Nakagawa and Cuthill, 2007). Thus, the effect size is a measurement of the difference in slopes of the change in the behavioral duration or frequency vs. day. For example, for a contrast of behavioral duration between the control and OTA treatments, an effect size of 100 (s/day) means that, on average, the birds in the control treatment increased the behavior by an additional 100 s/day relative birds in the OTA treatment.

In order to assess the sign, magnitude, and uncertainty around the treatment effects, 95% confidence intervals were generated using the "lsmeans" packaged in R for the difference in slopes between the OTA and control treatment for the change in duration or frequency of a behavior vs. day (Table 3). The reported estimates indicate both the magnitude and sign of treatment effects (positive value, behavior increased in OTA vs. control; negative value, behavior decreasing in OTA vs. control). The associated p-values give the significance of each slope contrast; in general, when the 95% confidence interval does not overlap with zero, the contrast is significant at $p < 0.05$. For allopreening bouts, 95% confidence intervals were constructed around the difference in slopes of the change in the behavioral duration vs. day between sexes in the same treatment (2 contrasts) and between treatments of the same sex (2 contrasts), with a Tukey-correction for 4-contrasts to both the reported p-values and the width of the reported confidence intervals. These values were used to create the figures in the results, figures of the raw data for each behavior are found in the supplemental section.

Table 1
Pair maintenance and aggressive behavior coded for zebra finches. Pecking is aggressive.

Behavior	Sex	Duration or Frequency	Description
Clumping	Both	Duration	Perched at rest in direct physical contact
Allopreening	Both	Frequency	Grooming of partner
Song bouts	Male	Frequency	Number of song bouts directed at partner
Total Song	Male	Duration	Total time spent singing directed at partner
Follow	Both	Frequency	Follow partner within three seconds to new perch
Pecking	Both	Frequency	Sharp beak jab at partner

Table 2
ANOVA statistics for linear mixed models for all behaviors. Bird ID, Pair ID, and Cage ID were random effects for all models. Bolded P values are significant ($P < 0.05$).

Behavior	Fixed Effect	Sum SQ	Mean SQ	DF Num	DF Den	F value	P value
Clumping	Treatment	4646	4646	1	9.401	0.037	0.851
	Trial day	771,241	771,240	1	13.847	6.169	**0.026**
	Treatment × Trial day	50,101	50,101	1	13.922	0.401	0.536
Allopreen	Treatment	1.571	1.571	1	12.49	0.37	0.554
	Trial day	1.485	1.485	1	6.283	0.349	0.574
	Sex	0.935	0.935	1	38.668	0.22	0.641
	Treatment × Trial day	12.098	12.098	1	13.89	2.849	0.113
	Treatment × Sex	0.02	0.02	1	38.668	0.005	0.945
	Trial day × Sex	23.609	23.609	1	43.641	5.56	**0.023**
	Treatment × Trial day × Sex	19.264	19.264	1	43.641	4.537	**0.039**
Follow	Treatment	5.9977	5.9977	1	12.598	2.353	0.149
	Trial day	9.471	9.471	1	37.019	3.716	0.061
	Treatment × Trial day	13.9404	13.9404	1	47.605	5.47	**0.023**
Pecking	Treatment	1.863	1.863	1	14.596	4.547	**0.05**
	Trial day	0.132	0.132	1	14.084	0.322	0.578
	Treatment × Trial day	0.385	0.385	1	14.084	0.94	0.348
Song Bout	Treatment	6.963	6.963	1	14.229	0.679	0.423
	Trial day	56.549	56.549	1	19.218	5.521	**0.029**
	Treatment × Trial day	45.355	45.355	1	19.218	4.428	**0.048**
Total Song	Treatment	68.37	68.37	1	14.351	0.244	0.629
	Trial day	1593	1593	1	24.889	5.68	**0.025**
	Treatment × Trial day	837.48	837.48	1	24.889	2.986	0.096

3. Results

3.1. Pair behaviors

3.1.1. Clumping

Control pairs and OTA pairs did not significantly differ in the amount of time spent clumping over the course of the experiment ($F_{13.922} = 0.401$, $P = 0.536$) Fig. 2A).

3.2. Individual behaviors

3.2.1. Allopreening

For allopreening bouts, there was a significant three-way interaction of treatment by sex by day ($F_{43.641} = 4.537$, $P = 0.039$). Allopreening bouts for OTA females did not change across the experiment whereas control females significantly increased the number of allopreening bouts performed across the experiment [Difference in Slope-DSlope: -0.904 ± 0.343 bouts/day (difference ± SE for this and all other reported effect sizes), $T_{20.77} = -2.589$, $P = 0.05$] (Fig. 2B). Control females also significantly increased the number of allopreening bouts they performed compared to control males (DSlope: 0.931 ± 0.294 bouts/day, $T_{14.14} = 3.163$, $P = 0.011$) (Fig. 2B). Male OTA and control birds did not significantly differ from one another (DSlope: -0.02 ± 0.343 bouts/day, $T_{20.77} = -0.058$, $P = 1$) (Fig. 2B). Female and male OTA birds also did not significantly differ from one another (DSlope: 0.047 ± 0.293 bouts/day, $T_{13.85} = 0.162$, $P = 1$) (Fig. 2B).

3.2.2. Follow

There was a significant day by treatment interaction for follow bouts ($F_{47.605} = 5.47$, $P = 0.023$), with OTA birds reducing follow bouts relative to control birds (DSlope: -0.381 ± 0.163 bouts/day) (Fig. 3A).

3.2.3. Pecking

Pecking bouts for control birds and OTA birds did not significantly differ over the course of the experiment ($F_{14.084} = 0.679$, $P = 0.423$) (Fig. 3B).

3.3. Male singing

3.3.1. Song bouts

There was a significant day by treatment interaction for song bouts ($F_{19.218} = 4.428$, $P = 0.048$), with the number of song bouts performed by OTA males declining relative to control males (DSlope: -1.111 ± 0.528 bouts/day, $T_{19.22} = -2.104$, $P = 0.048$) (Fig. 4A).

3.3.2. Total song

Total time spent singing across the experiment for control and OTA

Table 3
95% Confidence intervals for the difference in slopes (ΔSlope) between the OTA and control treatments for the change in behavioral duration or frequency vs trial day. For Allopreening, differences are reported between the slopes of different treatment groups within a sex (e.g., OTA females vs Control females) and for different sexes within a treatment group (Control females vs. males), with a Tukey-adjustment to the p-values for multiple contrasts.

Behavior	Contrast	ΔSlope	SE	DF	Lower 95% Cl	Upper 95% Cl	T ratio	P value
Clumping	OTA vs Control	−31.233	49.336	13.92	−137.104	74.639	−0.633	0.537
Allopreen	OTA vs Control females	−0.904	0.343	29.36	−1.815	−0.007	−2.632	**0.05**
	OTA vs Control males	−0.02	0.343	29.36	−0.931	0.891	−0.059	1
	OTA females vs males	0.047	0.293	43.25	−0.729	0.823	0.162	1
	Control females vs males	0.931	0.294	44.03	0.151	1.712	3.165	**0.011**
Follow	OTA vs Control	−0.381	0.163	47.61	−0.708	−0.053	−2.339	**0.024**
Pecking	OTA vs Control	−0.084	0.08676318	14.08	−0.27	0.102	−0.97	0.348
Song Bout	OTA vs Control	−1.111	0.5278916	19.22	−2.215	−0.007	−2.104	**0.048**
Total Song	OTA vs Control	−4.311	2.495	19.62	−9.45	0.828	−1.728	0.096

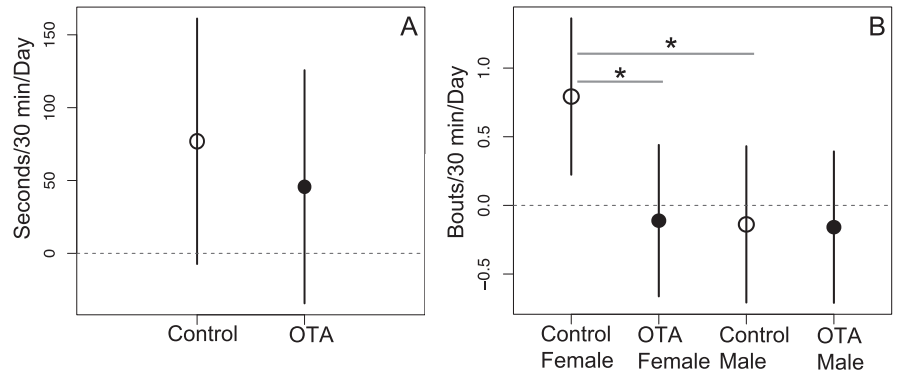

Fig. 2. AB. Estimates for the slope of the time spent clumping (A) and frequency of allopreening (B) vs. day using a linear mixed effect model for control and oxytocin antagonist (OTA) treatment zebra finch pairs. The error bars span the 95% confidence intervals of the slope estimates. A significant difference ($P < 0.05$) between groups is indicated by *.

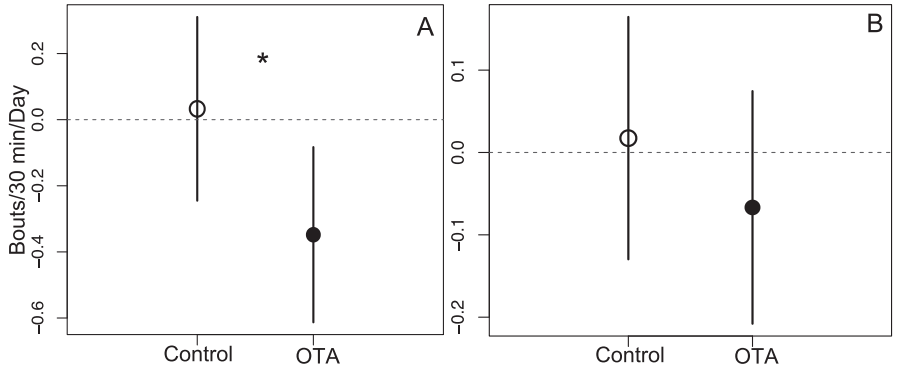

Fig. 3. AB. Estimates for the slope of the frequency spent following (A) and pecking (B) vs. day using a linear mixed effect model for control and oxytocin antagonist (OTA) treatment zebra finch pairs. The error bars span the 95% confidence intervals of the slope estimates. A significant difference ($P < 0.05$) between groups is indicated by *.

males was not significantly different ($F_{24.889} = 2.986$, $P = 0.096$) (Fig. 4B).

3.4. Confidence intervals

For allopreening bouts, the difference in slopes between OTA and control females was estimated −0.904 (95% confidence interval, CI, range: −1.815 to −0.007 bouts/day) and the difference in slopes between control females and males was 0.931 (95% CI: 0.151 to 1.712). For following bouts, the difference in slopes is estimated as −0.381 (95% CI range: −0.709 to −0.053 bouts/day). For song bouts the difference in slopes is estimated as −1.111 (95% CI range: −2.215 to −0.007 bouts/day). The differences in slopes and 95% CI for non-significant results (clumping, pecking, total song) are found in Table 3.

4. Discussion

I tested whether nonapeptides regulate pair maintenance by administering a general OTA to established zebra finch pairs and measuring its effects on pair maintenance behaviors. Administration of OTA significantly affected some pair maintenance behaviors (following for both sexes, allopreening for females, song bouts for males), but had no effect on others (clumping and pecking for both sexes). These results suggest that the nonapeptides and the OT-like VT3 receptor, which have been shown to regulate pair formation in zebra finches, continue to regulate some pair-maintenance behaviors in long-term zebra finch pairs.

I found that OTA significantly reduced the number of times birds followed their partners. These results suggest that OTA may reduce an individual bird's motivation to be near their partner, causing them to follow their partner to fewer perches. Similarly, unpaired female zebra finches receiving OTA have been shown to be less motivated to pair (Pedersen and Tomaszycki, 2012). This lack of motivation may be more general than pair maintenance behaviors and may instead be a symptom of decreased gregariousness. Knockdown of MT in the paraventricular nucleus of the hypothalamus (PVN) of zebra finches reduced side-by-side perching and vasotocin (AVT) knockdown in the PVN significantly reduced gregariousness in both sexes (Kelly and Goodson, 2014). Though I administered an oxytocin receptor antagonist, the OT-like VT3 receptor may bind both MT and AVT (Leung et al., 2009), such that the observed reduction in following may be due to blocking MT, AVT, or both. Therefore, in zebra finches, MT and AVT may work in

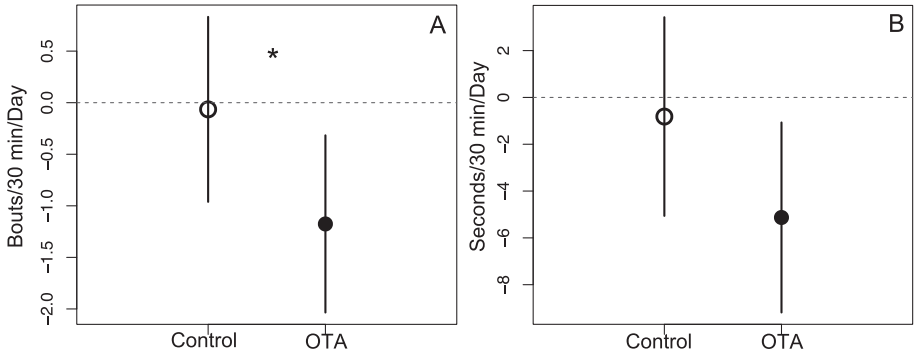

Fig. 4. AB. Estimates for the slope of the frequency of song bouts (A) and time spent singing (B) vs. day using a linear mixed effect model for control and oxytocin antagonist (OTA) treatment zebra finch males. The error bars span the 95% confidence intervals of the slope estimates. A significant difference ($P < 0.05$) between groups is indicated by *.

tandem in the PVN to regulate gregariousness.

Decreased gregariousness may also be responsible for the effect of OTA on female allopreening. Though control and OTA males did not significantly differ in the amount of allopreening they performed, control females performed significantly more allopreening than both OTA females and control males. These results support one study on pair formation in zebra finches, where ICV infusions of OTA significantly reduced allopreening in females but not males (Klatt and Goodson, 2013a). By contrast, our results are inconsistent with another study that found peripheral injections of OTA during pair formation had no effect on allopreening in zebra finches, although variability was high within the groups (Pedersen and Tomaszycki, 2012). These differences may be methodological, as this study injected birds over three days and the Pedersen and Tomaszycki (2012) study only injected birds over two days. Alternatively, the inconsistency in how OTA effected allopreening between this study and Pedersen and Tomaszycki (2012) may be due to differences in the importance of allopreening for zebra finches in established pairs and zebra finches forming pairs. Based on this study and the results from Klatt and Goodson (2013a), nonapeptide regulation of allopreening during pair formation and maintenance in zebra finches may be sex-specific.

Other studies of social behaviors in zebra finches have found sex specific effects of nonapeptides. Peripheral administration of OTA significantly reduced the amount of time females but not males spent in close proximity with familiar cage mates (Goodson et al., 2009). Knockdown of MT in the PVN also reduced gregariousness in females but not males (Kelly and Goodson, 2014). Similarly, sex-specific regulation of social behaviors by nonapeptides has been found in a number of mammals. Sex-specific roles for nonapeptides are found in prairie voles, where OTA disrupts pairing in females (Liu and Wang, 2003) and vasopressin receptor antagonists disrupt pairing in males (Liu et al., 2001). Prairie voles also display a sex- and dose-dependent response to administration of nonapeptides in partner preference tests. Virgin males display significant partner preference after receiving low (1 μg), medium (10 μg) and high (100 μg) doses of arginine vasopressin (mammalian homolog of AVT) and medium (10 μg) and high (100 μg) doses of OT whereas virgin females only display partner preference after receiving high doses of AVP or OT (Cho et al., 1999). AVP has also been shown to influence affiliative and antisocial behaviors in humans in a sex-specific manner (Thompson et al., 2006). Thus, the observed sex-specific effects of OTA on allopreening are consistent with a range of studies suggesting that nonapeptides regulate social behaviors in a sex-dependent manner.

Though I found OTA to significantly effect allopreening and following, the size of the effect, as indicated by the difference in the slopes describing how behavioral duration or frequency changed over the 5 days of observation, were relatively small for both behaviors. Although the changes in these behaviors between treatment groups were statistically significant, it remains unclear if effects of this magnitude are sufficient to disrupt established ZF pairs. Future studies should focus on increasing study length or sample size to determine whether administration of OTA and its subsequent behavioral effects would lead to dissolution of the pair.

OTA also negatively affected singing, as OTA males significantly reduced the number of song bouts they performed across the experiment, whereas the number of song bouts performed by control males remained relatively constant. These results support previous work showing that, during pair formation in zebra finches, OTA males decreased singing (Pedersen and Tomaszycki, 2012), and that males treated with an arginine vasotocin receptor antagonist (V1aR) as juveniles sang significantly less as adults than controls during a pair reunion trial (Baran et al., 2016). However, when zebra finch males received chronic intracerebroventricular (ICV) infusions of OTA, they increased song production compared to controls (Klatt and Goodson, 2013a). These differences may be due to peripheral vs. central binding sites of the OTA, as this study and Pedersen and Tomaszycki (2012) both found similar results with respect to song using a peripheral injection of OTA, whereas Klatt and Goodson (2013a) found the opposite result using ICV administered OTA. Nonapeptide receptors are involved in a number of peripheral processes that may influence behavior including osmoregulation though the kidney (Goldstein, 2006), stress response through the pituitary (Cornett et al., 2013), and egg-laying and nesting through the oviduct uterus (Gubrij et al., 2005; Srivastava et al., 2007; Klatt and Goodson, 2013b). By injecting the OTA into the pectoral muscle, the OTA could influence behavior by binding to both peripheral and central receptors (since it can cross the blood–brain barrier). Indeed, peripheral but not central injections of an OTA into female zebra finches significantly reduced nesting behaviors (Klatt and Goodson, 2013b). Regardless of whether OTA significantly decreased or increased singing, song was affected across all of these studies, supporting the hypothesis that nonapeptides regulate song in male zebra finches.

Though I found OTA to significantly affect some pair maintenance behaviors, pecking and clumping were unaffected by the antagonist. As there was no trend for either behavior across the treatment days, these behaviors may be outside of nonapeptide regulation after pair bonds form. Aggression was also unaffected by OTA in similar studies of pair formation in zebra finches (Pedersen and Tomaszycki, 2012; Klatt and Goodson, 2013a). For example, in one experimental manipulation of pair formation, zebra finch females reduced clumping after the first day after peripheral injection of OTA, though the same effect was not observed in males and clumping on subsequent days was unaffected by the antagonist for either sex (Pedersen and Tomaszycki, 2012). Therefore, the nonapeptides may regulate clumping during pair formation (Pedersen and Tomaszycki, 2012) but not pair maintenance. Clumping and pecking may also be under regulation by the nonapeptides but through a different receptor (such as V1a or VT1), as the OTA used in this study is specific to the OT-like VT3 receptor (Manning et al., 2008).

Though I found no effect of OTA on clumping or pecking, the OTA may be effective at higher or lower doses as nonapeptide regulation of behavior tends to be dose-dependent. In rats, low but not high doses of OT facilitate social recognition (Popik et al., 1992; Benelli et al., 1995). When pairing OT with an OT receptor antagonist (OTA), low doses of OT and OTA eliminated any positive OT effects on social recognition, but high doses of OT and OTA had a positive effect on social recognition (Benelli et al., 1995). In female prairie voles, administration of OT had dose-dependent effects on pair bonding, with high but not low doses reducing female partner preference and likelihood to form pair bonds (Bales et al., 2007). Both males and female prairie voles displayed dose-dependent responses to administration of OT and AVP in partner-preference testing, with virgin males displaying significant partner preference at low, medium, and high doses of AVP and medium and high doses of OT whereas only high doses of either AVP or OT elicited significant partner preference in virgin females (Cho et al., 1999).

Pair formation behaviors in zebra finches were also differentially affected depending on the size of the OTA dose. In females, the medium (5 μg) but not low (1 μg) or high (10 μg) OTA dose significantly reduced the time individuals spent in the nest-box with males (Pedersen and Tomaszycki, 2012). In males, the low and medium but not high doses significantly reduced song on the first day, though all doses reduced song on subsequent days. Likelihood to pair was also dose-dependent in males, as males treated with the medium but not low or high dose of OTA were significantly more likely to remain unpaired compared with control males (Pedersen and Tomaszycki, 2012). However, regardless of the dose, there was no effect of the OTA on aggression for females or males and clumping for males (Pedersen and Tomaszycki, 2012). For females, clumping was negatively affected by all doses on the first day but none of the doses had any effect on clumping on the subsequent days of the experiment. Therefore, though possible that a larger or smaller dose of OTA could affect clumping and aggression, it seems more likely that these behaviors are outside of nonapeptide regulation through the OT-like VT3 receptor during pair maintenance.

5. Conclusions

This study demonstrated the importance of the nonapeptides for some, but not all, pair maintenance behaviors in long-term pairs. Relative to control birds, the OTA treated male zebra finches sang less to their partners, female zebra finches spent less time allopreening their partners, and both sexes performed fewer following bouts. However, neither clumping nor pecking were affected by the OTA, indicating these behaviors may be independent of nonapeptides during pair maintenance. These results, when contrasted with previous studies on nonapeptide regulation during pair bonding, illustrate how the regulation of different pair behaviors can either change or be conserved within an individual depending on life history stage. Overall, this research supports the hypothesis that nonapeptides, which are known to regulate a suite of behaviors involved in pair-formation, continue to play an important role in regulating affiliative behaviors during pair maintenance.

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Appendix A. Supplementary data

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