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Behavioral convergence in defense behaviors in pair bonded individuals correlates with neuroendocrine receptors in the medial amygdala

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ABSTRACT

Monogamous, pair-bonded animals coordinate intra-pair behavior for spatially separated challenges including territorial defense and nest attendance. Paired California mice, a monogamous, territorial and biparental species, approach intruders together or separately, but often express behavioral convergence across intruder challenges. To gain a more systems-wide perspective of potential mechanisms contributing to behavioral convergence across two conspecific intruder challenges, we conducted an exploratory study correlating behavior and receptor mRNA (Days 10 and 17 post-pairing). We examined associations between convergence variability in pair time for intruder-oriented behaviors with a pair mRNA index for oxytocin (OXTR), androgen (AR), and estrogen alpha (ERα) receptors within the medial amygdala (MeA) and the anterior olfactory nucleus (AON), brain regions associated with social behavior. An intruder behavior index revealed a bimodal distribution of intruder-related behaviors in Challenge 1 and a unimodal distribution in Challenge 2, suggesting population behavioral convergence, but no significant correlations with neuroendocrine measures. However, OXTR, AR, and ERα mRNA in the MeA were positively associated with convergence in individual intruder-related behaviors, suggesting multiple mechanisms may influence convergence. Mice could also occupy the nest during intruder challenges and convergence in nest attendance was positively correlated with MeA OXTR. At an individual level, nest attendance was positively associated with MeA ERα. Vocalizations were positively associated with AR and ERα mRNA. No positive associations were found in the AON. Overall, neuroendocrine receptors were implicated in convergence of a monogamous pair's defense behavior, highlighting the potential importance of the MeA as part of a circuit underlying convergence.

1. Introduction

Animals that rely on cooperation for survival navigate complex social interactions throughout their lives. In pair-bonding species, we expect a male-female dyad to coordinate their individual behaviors to maximize their fitness [1–29,31–56]. In some species, demarcations within a pair bond are rigid, with specific individual roles (e.g. [59,80,89], while in others, bonded partners more fluidly switch between tasks [11,62,90,86]. In species with little sexual dimorphism, such as California mice, pair members adjust behavior relative to their partner in various contexts including territorial defense [85,63]. For monogamous, biparental mammalian species [52] such as the California mouse, in which both sexes are territorial and aggressive [83,84,13,95,27,40,18,24], animals navigate a set of behavioral responses to social/-environmental challenges. Within California mice, two pair mates typically converge in their approach response to simulated intruders while pairing and prior to having young [85,63]. We speculated that the drive to be similar changes both with the intensity of the challenge as

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well as the level of competing demands such as an intruder combined with care of young. Within California mice prior to having pups, but while females are pregnant, we expect a mixture of joint and divided defense (e.g. [86], while maintaining a tendency to converge (e.g. become more similar in behavioral durations) in behavior with the heightened threat of a live intruder.

Neuroendocrine systems play an important role in driving pair bonding and aggression across rodent species, and potentially coordinating these behaviors [2,7,47,66,67]. Oxytocin (OXT) can drive pair bond formation and maintenance across species [49], as well as cooperation such as in humans [105]. OXT can also, however, be associated with aggression. In California mice, OXT increases behavioral convergence in pairs defending a territory [63,86]. This suggests that OXT acts in the brain to drive pair convergence. Here we define convergence as showing the same amount or duration of a behavior between pair mates.

The role of sex steroids in influencing convergence in response to an intruder is exploratory, nonetheless, van Anders et al. (2011) [100] provide a synthesis linking both androgens and neuropeptides with both defensive aggression and pair bonding. Within California mice, androgens and estrogens have been related to male-male aggression in multiple contexts [20,25,33,43,61,97,96] and with proximity between mates [37]. Both androgens and estrogens are also important for aggressive behaviors and specifically territorial defense in many species [20,25,33,43,61,97,96]. In California mice, testosterone spikes in males that win male-male aggressive encounters, leading to increased ability to win future encounters [33,32,71,70,98,95], and is accompanied by increases in neural androgen receptors (ARs) [32, Finally, sex steroid receptors are expected to be positively associated with vocalizations (for review see [58]. It therefore follows that sex steroid receptors could be important to individual aggression, but how these affect coordination of pair aggression towards an intruder remains unknown.

A number of brain regions are likely to be involved in behavioral coordination. We investigated a narrow set of brain regions that are receptive to sex steroids, OXT, and social stimuli. The loci of action for sex steroids and OXT in the brain that impact pair coordination likely include a network of brain regions involving social sensory cue processing. We focus on two sex steroid-sensitive nuclei: the medial amygdala (MeA) and the anterior olfactory nucleus (AON). They are implicated in social sensory cue processing and densely innervated by OXT neurons from the hypothalamus [68]. The MeA is an integrative center for social sensory cues with connections to the AON [82] and is associated with the vomeronasal olfactory system [50]. Moreover, the MeA is a component of the social decision-making network, and well-poised to modulate behavioral strategy [12,29,101]. The MeA is particularly sensitive to circulating steroids [21], review by [39], which act to drive male and female sexual behavior, parenting, and aggression [64], as well as OXT, which plays a key role in social recognition [4,29,106]. The MeA is strongly associated with aggression in both male-male and female-female aggressive encounters in California mice [24,34].

While the effect was expected to be stronger in the MeA, we also explored the AON, a part of the "main" olfactory stream, located between the main olfactory bulb and more caudal regions of the olfactory cortex (i.e., piriform cortex) [16]. The AON contains both ARs and ERs [92] and receives strong projections from OXT neurons in the periventricular nucleus of the hypothalamus, which can enhance social recognition [44,68,102,103,107]. Both brain regions were therefore candidates for coordinating social interactions towards an intruder although many other brain regions in the social decision network are likely involved [66,30].

We addressed several questions based on the gaps emerging from the studies above. Does behavioral convergence occur in already paired mates (prior to having offspring) over two exposures towards a novel, high-level threat (e.g. a live intruder)? This encompasses the additional challenge of a resource to defend in the form of a nest site (igloo) within the territory. Pair members could therefore converge on approaching the intruder and/or defending the nest site, or simply not converge. A second question is whether a pair index of ERα, AR and/or OXTR mRNA in the MeA and/or the AON correlates with variation in pair behavioral convergence or an aggregate measure of convergence in response to two territorial temporally-separated intrusions? The third question is whether individual receptor measurements correlate with behaviors at the nest and/or towards the intruder on an individual level in each challenge, but potentially contributing to an overall behavioral response during an intruder challenge. Answers to these questions provide us with insight into the complex behaviors and their neuroendocrine underpinnings required for coordinated responses to an environmental challenge.

2. Methods and materials

2.1. Animals

We used 20 male (10 paired, 10 naive intruders) and 10 female (all paired) adult California mice aged 6–12 months from a long term colony at the University of Wisconsin-Madison. Male intruders were used because intruder sex does not appear to influence pair intruder behavior [86]. Mice were maintained in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All individuals were randomly paired to an opposite-sex partner that was unrelated for at least two generations. Pairs were housed for 10 days prior to testing in standard cages (48 ×27 x 16 cm) lined with aspen bedding and provided with a nestlet and food (Purina 5015™ Mouse Chow) and water ad libitum. During these 10 days, pairs displayed affiliative behaviors, including huddling and shared nest attendance, indicative of pair bonding [79]. Naive intruders were housed with 1–2 same-sex conspecifics under the same conditions. The housing room was maintained at 20–23 ℃ and ~30% humidity on a reversed 14:10 h light:dark cycle (lights off at 14:00 CST). All testing occurred 1–3 hrs after the onset of the dark cycle under dim red light. Pairs were tested at two time points, 10 and 17 days post pairing following the procedure outlined below [86]. These are time points at which pairs are likely pregnant, as the average colony birth latency is 31 days [37]. Furthermore, this study also compiled colony data for our lab and discerned that 70% of females were pregnant within 3 weeks of pairing. Pregnancy does not seem to impact OXT receptor concentrations in rabbits in the MeA [46], but pregnancy may impact sex steroid concentrations in various brain areas [57]. We can not rule out the possibility that the state of pregnancy influenced our measurements. Pairs are expected to be pair bonded at both time points [79] and were huddling together in the same nest.

2.2. Testing apparatus

Pairs were moved into the testing chamber 24 h prior to behavioral tests for establishment of residency on Day 10 and Day 17 of their pair establishment. The testing chamber was a glass aquarium (50 ×30 x 30 cm) lined with aspen bedding and containing a red tube (15 cm length) and an igloo (10 ×10 x 8 cm) for enrichment and nesting. A Plexiglas lid with four 2.5 cm diameter holes in the corners was affixed to the chamber to allow for placement of USV microphones into the chamber during testing (Fig. 1).

2.3. Territorial defense tests and pair-bonding

We tested territorial defense of 10 California mouse pairs at two time points using a previously defined paradigm of behavioral convergence [86]. Briefly, pairs were moved from their standard cages into the testing chamber 24 h prior to testing and had a small patch of fur shaved from the flank for individual recognition. 24 h allows for the formation of a 'residency effect' (e.g. [34] during which pairs create nests in the igloos and display side by side contact, an index of pair bond formation [9,86,78]. Pairs were tested for 6 min on territorial defense against one novel male intruder on Day 10 and a second novel intruder on Day 17

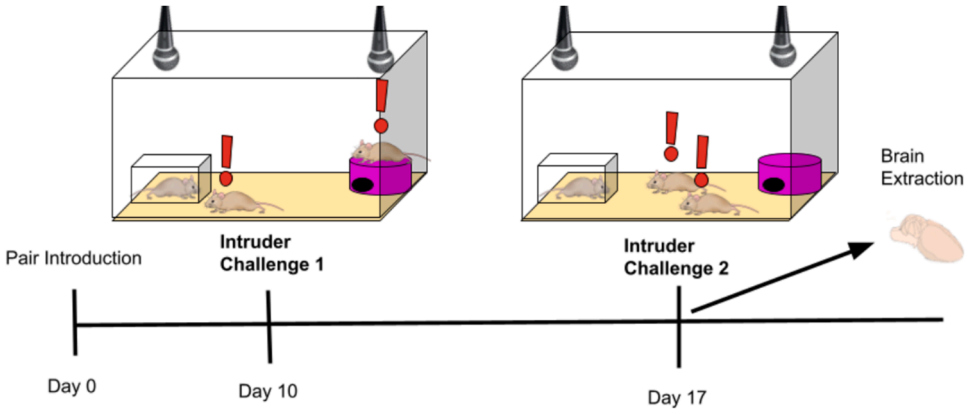

Fig. 1. Timeline for examining variability in behavioral convergence over two intruder challenges to the residential pairs (identified by the red exclamation points). Brains were extracted 90 min after Challenge 2. Two ultrasonic microphones were placed at both ends of the apparatus.

post-pairing. Each unfamiliar and sexually naive intruder had no social experience outside of their cage mates. An intruder was placed in a 10 ×10 x 10 cm wire mesh cage and introduced into the testing chamber at the onset of the territorial challenge. No pair encountered the same intruder more than once. Following testing, mice were returned to their standard cages.

Challenge tests at 10 and 17 days post-pairing (Fig. 1) were video recorded and hand-scored independently for behavior as defined in Rieger et al. [86] by two trained observers naive to treatment conditions (see ethogram below). We ensured that the primary and secondary scorers were in > 90% agreement and then deferred to the primary scorer for analysis. Vocalizations were analyzed at the level of the dyad, as assigning calls to individuals was not possible due to close proximity of individuals and little individual visual change during vocalization. Following the first test, pairs were returned to their standard cage in the housing room. After the second test, pairs remained in the testing chamber for 90 min prior to brain extraction for mRNA.

We recorded USVs for 6 min during each of the two challenges while the behaviors were videotaped and analyzed using previously validated methods [86,78,63]. Briefly, we used two Emkay/Knowles FG series microphones placed 55 cm apart at opposite ends of the testing apparatus 20 cm above the apparatus floor. One microphone was placed over the resident's nest and one was placed above the intruder cage. Placement was randomized to account for potential differences in sensitivity. Microphone channels were calibrated for equal gain (−60 dB noise floor) and WAV files were recorded for each of the three segments of the test using RECORDR (Avisoft Bioacoustics, Berlin, Germany). Recordings were made with 150 kHz sampling rate at 16 bit resolution and a 512 fast Fourier transform was used to generate spectrograms via Avisoft SASLab Pro (Avisoft Bioacoustics). As in Rieger et al. [86], there were a limited number of sweeps and barks generated in this paradigm and thus we focused our analyses only on sustained vocalizations (SVs). SVs are long, low-frequency vocalizations (~20 kHz) that are associated with social behaviors in California mice [15,48,77,79]. Shorter SVs are associated with and predict aggression [85] and longer SVs are associated with affiliation and long-term bonding [79]. SVs were detected visually and auditorily by an observer unaware of treatment using spectrograms and audio files reduced to 4% of normal speed (11, 025 kHz).

2.4. Sample preparation and quantitative real-time PCR (qPCR)

90 min after the second test, pair-bonded animals were anesthetized with isoflurane and rapidly decapitated. Brains were rapidly extracted, flash frozen on dry ice and stored at − 80 ℃ until processed for qPCR. Brains were sectioned on a cryostat at − 15 °C to obtain 200 μm coronal sections. Sections were transferred onto subbed microscope slides and moved to a petri dish filled with dry ice, where the AON and MeA were dissected using a Fine Science Tools Sample Corer (Item No. 18035–02, Foster City, CA, USA). For each individual, punches on each section were 1 mm in diameter and punches for all sections for a given region (two bilateral punches) were stored together in capped 1.8 ml microcentrifuge tubes at − 80 °C. Multiple non-target brain regions were also punched for a separate study and used for the creation of standards, including the main olfactory bulb, insular cortex, bed nucleus of the stria terminalis, and ventral hippocampus.

We extracted RNA from punch samples using a Bio-Rad Aurum Total RNA Fatty and Fibrous Tissue Kit (Catalog No. 73206830, Bio-Rad, Hercules, CA, USA), following instructions by the manufacturer. RNA concentration and integrity were measured with a Nanodrop system (Catalog No. ND-2000, Thermo-Scientific, Wilmington, DE, USA). To stabilize samples for qPCR, RNA was then converted into single-stranded cDNA with an Invitrogen SuperScript III First-Strand Synthesis System (catalog #18080–051, Life Technologies, Carlsbad, CA, USA). Tissue punches from non-target brain regions were pooled and processed as above to serve as standards for quantitative real-time polymerase chain reaction (qPCR) runs (see below). The reference gene beta-2-microglobulin mRNA was chosen, as opposed to the typical use of beta-actin, because sex differences have been noted in beta-actin expression [93]. For both the MeA and the AON, qPCR was used to measure relative mRNA expression for androgen receptor (AR), estrogen receptor alpha (ERα), and oxytocin receptor (OXTR). Primers for each gene were designed using the NCBI Gene Database Primer-Blast. Netprimer (Premier Biosoft, Palo Alto, CA, USA) was then used to examine any secondary structures of primers. Primers were ordered from Integrated DNA Technologies (Coralville, IA). Products of successful runs were sent for Sanger sequencing with both forward and reverse primers at the University of Wisconsin – Madison Biotechnology Center, and sequences matched their expected targets. All primer details for qPCR are presented in Table 1.

Samples were always run together with five standards prepared in a 1:10 dilution series, and a negative control (i.e., water only) in triplicate as in previous studies (Spool et al., 2016). Briefly, samples, standards, and controls were mixed with primers, nuclease-free water, and SsoFast EvaGreen Supermix (Catalog No. 172–5201, Bio-Rad, Hercules, CA), plated, and run in a Bio-Rad CFX96 Touch Real-Time PCR Detection System (Catalog No. 185–5195, Bio-Rad). Runs consisted of a 30 s initiation step at 95 °C, 40 cycles at 95 °C for 5 s, a 30 s annealing step set at a melting temperature specific to the primer set (retrieved from NCBI Gene Database Primer-Blast) and a 20 s elongation step at 72 °C, followed by a 60–88 °C melt curve (5 s for each 0.5 °C). Only runs that met the listed MIQE guidelines were used (Bustin et al., 2009). All successful runs contained single melt peaks. qPCR raw data were transformed according to the Pfaffl Method to obtain expression level

Table 1
Ethogram of behaviors recorded and measured during the intruder challenges.

Behavior	Description
Sustained Vocalizations (SVs)	Long (100–500 ms), low-frequency vocalizations (~20 kHz) with little frequency modulation that are associated with both affiliative (long form, 74) and aggressive (short form, 84) behaviors. Vocalizations are considered pair variables, as individual identity of vocalizers within pairs cannot be discerned
Latency	Time to approach the intruder for individual pair mate (sec)
In Nest	Time in the nest for individual pair mate (sec)
Time spent with intruder	Time individuals spent investigating the intruder cage (sec)
Olfactory investigation	Time individuals or pair spends sniffing (nose to nose or nose to anogenital) the intruder
Behaviors calculated using above measures:	
Nest Convergence	The increase in similarity in time each individual spent in the nest (together or apart) (see Methods for calculation)
Olfactory Convergence	The increase in similarity of time each pair member spent investigating the intruder together and/or apart (see Methods for calculation)
Intruder Convergence	The increase in similarity of time each individual within a pair spent near the intruder together and/or apart (see Methods for calculation)
Latency Convergence	The increase in similarity of the latency to approach the intruder between pair mates (see Methods for calculation)
Intruder-oriented Behavior (IOB) convergence	An aggregated and normalized measure of the increased similarity within a pair on all intruder-associated behaviors examined (Olfactory Convergence, Intruder Convergence, and Latency Convergence) (see Methods for calculation)

values relative to beta-2 microglobulin expression [22,74,94].

2.5. Analyses and statistics

We first calculated convergence (Fig. 1), defined here as pair similarity in behavior between Challenge 1 and Challenge 2. This measure was calculated using the equation: $abs(P1_1 - P2_1) - abs(P1_2 - P2_2)$, where abs = absolute value, P1 = Pair member 1, P2 = Pair member 2, and subscripts represent Challenge 1 and 2 respectively. All individual convergence scores were then normalized by converting to a z score, allowing for comparisons of behaviors with different sample parameters. The use of z scores has been used to compare correlated behaviors in previous studies [37,13]. The z-score of convergence for each individual behavior was created with the following equation for each pair: $Normalized\ convergence = ((x - \mu)/\sigma)$, where x = a pair's convergence value for a behavior (see above), μ = mean of convergence values for pair sample, and σ is the standard deviation of the convergence values for that behavior. An aggregated measure of intruder-oriented behavior (IOB) was created by adding the z score convergence values for time spent with intruder, olfactory investigation, and latency to approach which are three correlated behavioral variables (Intruder to Olfactory: $F(1,18) = 21.07$, $p < 0.001$, adj $R^2 = 0.51$, Olfactory to Latency: $F(1,18) = 3.438$, $p = 0.08$, adj. $R^2 = 0.11$, Latency to Intruder: $F(1,18) = 6.65$, $p = 0.019$, adj $R^2 = 0.28$) to create a normalized measure of IOB convergence. Positive values reflect an increase in the similarity in the time spent displaying a behavior between pair members from Challenge 1 to Challenge 2 (behavioral pair convergence) (Fig. 2). Negative values reflect an increase in the difference in behavior between pair members from Challenge 1 to Challenge 2 (behavioral pair divergence).

To investigate relationships between individual behaviors and mRNA, we conducted separate linear mixed effects models with each mRNA variable as a predictor variable and each behavior as a dependent variable (see Supplemental Table 1-8 for models and results of all analyses conducted). A random effect of pair identity was added when dyadic observations were considered because pair behavior is both a collective and an individual behavior [55]. Degrees of freedom and p-values for these models are from a Kenward-Roger approximation. As noted later, the aggregate measure (IOB) did not correlate with any neuroendocrine mRNA measurement and was then reduced to its components to investigate further mRNA associations. This breakdown of the IOB measure was completed after noting the bimodality change between challenges (Fig. 1) because bimodality distributions can indicate multiple influences to a distribution [31]. We were too underpowered to detect interactions between sex and each mRNA transcript, therefore statistical analyses (simple linear regressions) were run separately for males and females. For each simple linear model run, each model consisted of different combinations of dependent and independent variables. Due to the exploratory nature of the study, we did not correct for multiple comparisons [10,72] but present our analyses with effect sizes. Effect size measures used are adjusted R^2 for simple linear models and adjusted omega^2 for linear mixed effects models. The ranges used for determining medium and large effect sizes are as follows: omega^2 and R^2: medium range = .09 − .25, large range = .25 - 1.00. We do not report findings as statistically significant when effect sizes were in the small range (i.e., < 0.09). Vocalizations occurred in Challenge 1, but too few occurred in Challenge 2 to analyze (see Supplemental Fig. 1, see below for details on SVs in Challenge 1 and 2). For a table of all statistics (including predictor model estimates) see Supplemental Table 1-6.

Analyses were conducted in R v. 3.6.2 with RStudio v. 1.2.5033 (citations: R Core Team, RStudio Team) and graphs were created with the GraphPad Prism graphing software. Statistical assumptions were checked by examining residuals by fitted values plots. Normality was tested using the Shapiro-Wilks test and visual examination of histogram distributions of all variables. Highly influential data points were identified visually and by Cook's distance. A significant effect between time spent in the nest and OXTR mRNA values in the AON was disregarded because the effect was driven by one high influence outlier data point, no other high influence data points were detected statistically. R packages used include tidyverse, effect size, and lme4 (see references for package citations). Behaviors and mRNA were analyzed at a pair level, as a collective trait, and at an individual level. air convergence across challenges were compared to a pair index of mRNA (the average between pair mates) to obtain a dyadic measure of mRNA. This approach was also used because mRNA is extracted at one time point, but behaviors were measured twice over the two challenges. Pair convergence

Table 2
Details for primers used to quantify relative mRNA for genes of interest.

Gene	Direction	Sequence (5' -> 3')	Annealing Temperature (°c)	Product (Base Pairs)	Reference
beta-2 microglobulin	**F**	TCTAGTGGGAGGTCCTGTGG	61.7	**106**	[93]
	R	TGCGTTAGACCAGCAGAAGG			
oxytocin receptor	**F**	TCAGGGCTGGAGGTTGTATT	58.3	**166**	N/A
	R	TACCAAAAGGAGACCACGGA			
estrogen receptor alpha	**F**	GAACAGCCCCGCCTTGT	61.9	**57**	[97]
	R	GCATCCAGCAAGGCACTGA			
androgen receptor	**F**	GTGGTGTGTGCTGGACATGAC	62.6	**61**	N/A
	R	GGCTAGATAACAGGGCAGCAA			

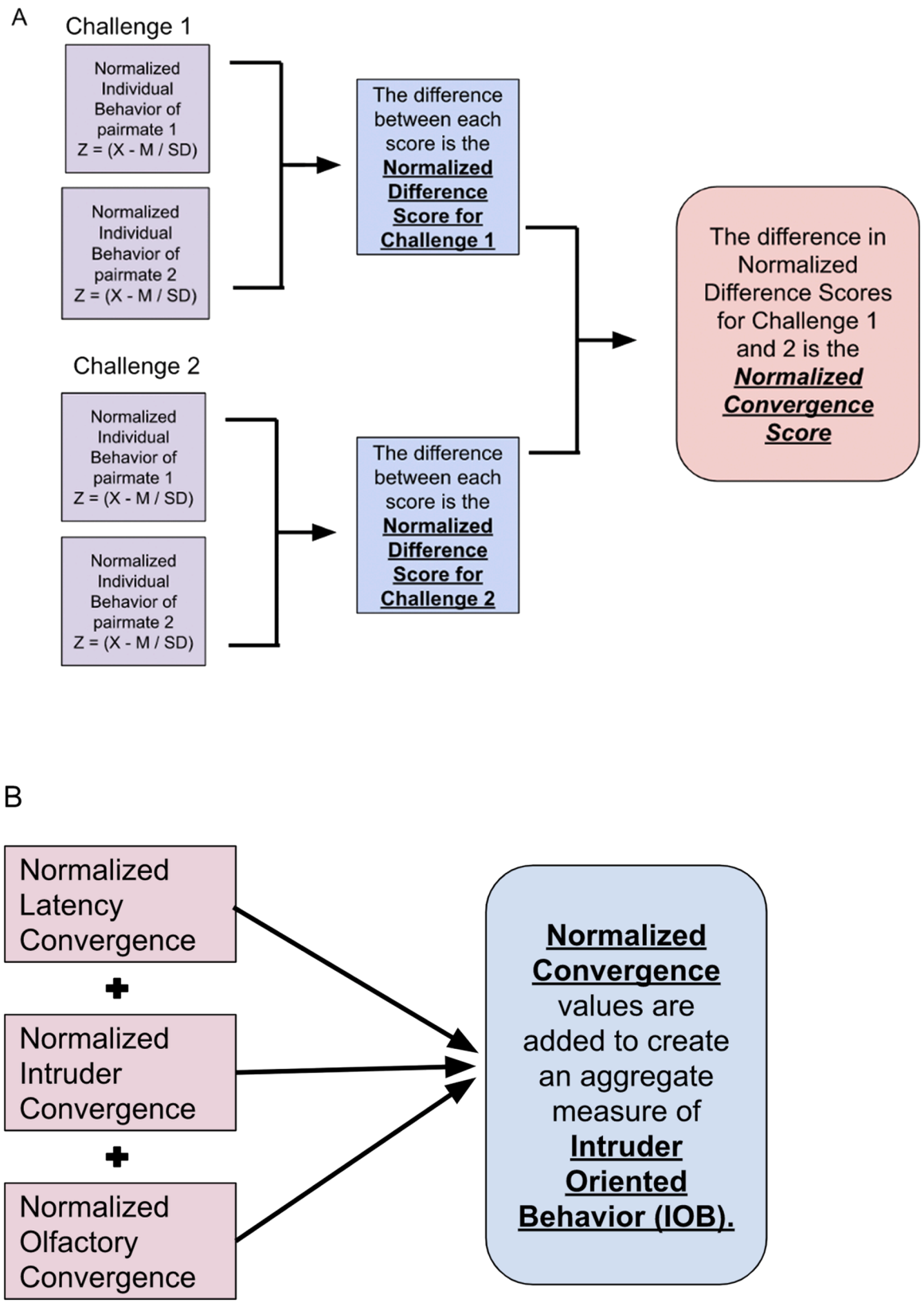

Fig. 2. Creation of convergence values for later statistical analyses of intruder-oriented behavior (IOB) variables is presented using the above flowchart. Panel A) Normalized convergence values (purple), are created by standardizing (z-score = Behavioral Value (X) - sample mean (M) / standard deviation (SD)) individual behavior. The pair differences (blue) are then calculated for latency to approach the intruder, time spent olfactorily investigating the intruder, and time spent with the intruder (see ethogram in Table 1). The standardized difference between pairs and between challenges is convergence (pink). Panel B) After normalized convergence values are created for each individual behavior (pink), they are added together to create an aggregate variable of intruder-oriented behavior (IOB) (light blue).

measures of pair behavior and average mRNA were then compared (n = 10). Individual male and female behaviors were then correlated with individual mRNA levels (n = 20), with the exception of vocalizations that were recorded from pairs (n = 10). Effect size is reported for both non-significant trends and significant findings.

3. Results

3.1. Behavioral convergence (Challenge 1 to Challenge 2)

We first tested whether established pairs exhibit convergence in behavioral responses when exposed to a new intruder at Days 10 and 17 after pairing.

3.1.1. IOB convergence

The z-scores of the aggregate intruder-oriented behavior (IOB) measurements from Challenges 1 and 2 after pairing were significantly correlated (*F(1,8) = 5.468, p = .048, adj R^2 = .33)*, indicating repeatability of the IOB index (for correlations of component IOB measures, see Supplemental Table 8). While individual IOB behaviors were correlated, there was not a significant increase in the correlation strengths from Challenge 1–2 (see Supplemental Fig. 3).

We tested for modality changes in the distribution of the standardized IOB pair differences across the two challenges (Fig. 3). Bimodality can be evaluated by calculating a bimodality coefficient such that any bimodality coefficient exceeding a 0.555 cutoff is considered bimodal [75]. We found that the distribution of pair difference scores on the IOB measure in Challenge 1 was bimodal (bimodality coefficient =.67), whereas the distribution of IOB values were unimodal in Challenge 2 (bimodality coefficient =.51). This change in modality may signify an overall population strategy to increase similarity within pairs in response to an intruder event, as their pair differences converge to the mean. Note that when investigating modality changes in the component IOB behaviors (pair differences in olfactory, intruder, and latency behavior), no changes in modality were detected (for bimodality coefficients, see Supplemental Table 7). As such, this is a relatively unique feature of the aggregate IOB measures between challenges. Overall, we have evidence for variability in convergence within pairs over the two challenges. We note that these pairs were already bonded and were not originally mismatched in the intruder approach as in a previous study [63]. We therefore proceeded to investigate if the variability in this IOB behavioral convergence measure could be explained by or associated with mRNA.

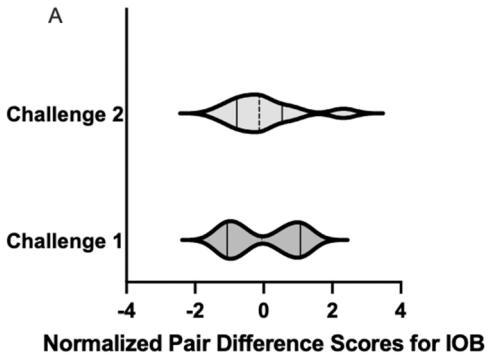

Fig. 3. *Pair behavior changes modality from Challenge 1 to Challenge 2.* The empirical value of a bimodality coefficient greater than 0.555 indicates that the modality of IOB values changes across challenges from a bimodal shape (bimodality coefficient =.67) to a unimodal distribution (bimodality coefficient =.51) in Challenge 2. This suggests convergence around the mean for the IOB index.

3.1.2. Nest convergence

We did not find convergence for time at the nest over the two challenges or a correlation between nest convergence and IOB convergence when conducting a simple linear regression in which nest convergence values for each pair were regressed on IOB convergence values (F(1,8) = .49, p = .506, adj R^2 = −.06). It is interesting, however, that nest convergence had a trending association with latency convergence, with a notable effect size (F(1,8) = 3.89, p = .084, adj R^2 = .24).

3.2. Convergent behavior variability and mRNA

We next explored relationships between pair receptor mRNA and convergence variability. Using simple linear regressions, a number of significant positive associations were found in the MeA as described below. The AON provides a negative contrast to the MeA. After conducting a regression in which pair AR mRNA was regressed on IOB convergence, a single negative association was found in the AON (F(1,8) = 7.85, p = .023, adj R^2 = .43).

3.2.1. IOB convergence

There was not a significant association between IOB convergence and any receptor measurements after conducting separate simple linear regressions in which IOB convergence values were regressed on a single pair mRNA variable (see Supplemental Table 1 for nonsignificant statistics). However, when this complex pair behavior was broken down into its individual components, we found that each individual measure of IOB convergence variation was associated with a different pair receptor mRNA measure. These unique associations were determined via separate simple linear regressions in which the convergence behavior measure was regressed on an mRNA variable. Specifically, convergence variability in time spent with the intruder was positively associated with both pair ERa and AR mRNA indexes as measured by assessing both P values and effect sizes (ERa: F(1,8) = 8.874, p = .018, adj R^2 = .47, AR: F(1,8) = 4.474, p = .067, adj R^2 = .28, Fig. 4B). Olfactory convergence had a nonsignificant trending association with pair AR mRNA and a notable effect size in the MeA (F(1,8) = 3.57, p = .095, adj R^2 = .22, Fig. 4A), while the association between pair AR mRNA and time with intruder was a nonsignificant trend but with a large effect size. The third intruder-related behavior, pair convergence variability in latency to approach, was positively associated with the pair OXTR mRNA index in the MeA (F(1,8) = 10.04, p = .013, adj R^2 = .50, Fig. 4D). Additionally, intruder convergence variability had a trending association with pair SV number, and a large effect size, which is associated with affiliative behavior (F(1,8)= 5.05, p = .055, adj R^2 = .31, not depicted) in Challenge 1.

3.2.2. Nest convergence

When nest convergence was regressed on the OXTR mRNA index, we found that nest convergence variability was positively associated with the OXTR mRNA index in the MeA, similar to latency convergence to the intruder, raising the speculation that the OXT system may influence both convergence at the nest and one measure of convergence in intruder-directed behaviors (F(1,8) = 5.93, p = .041, adj R^2 = .35, Fig. 2C). Cook's distance analysis on data for panels in Figs. 2C and 2D revealed no outliers in the relationship between nest convergence and pair OXTR mRNA. One statistical outlier was detected in the relationship between latency convergence and the pair OXTR mRNA index, but the effect remained significant with the outlier removed (F(1,7)= 9.73, p = .017, adj R^2 = .52). No other pair index of mRNA receptors was significantly associated with nest convergence variability (see Supplemental Table 1).

3.3. Correlations between behaviors in Challenges 1 and 2 and mRNA

We examined variable associations from an individual perspective followed by sex specific effects. Several separate linear mixed effects models were conducted in which individual behavior variables were

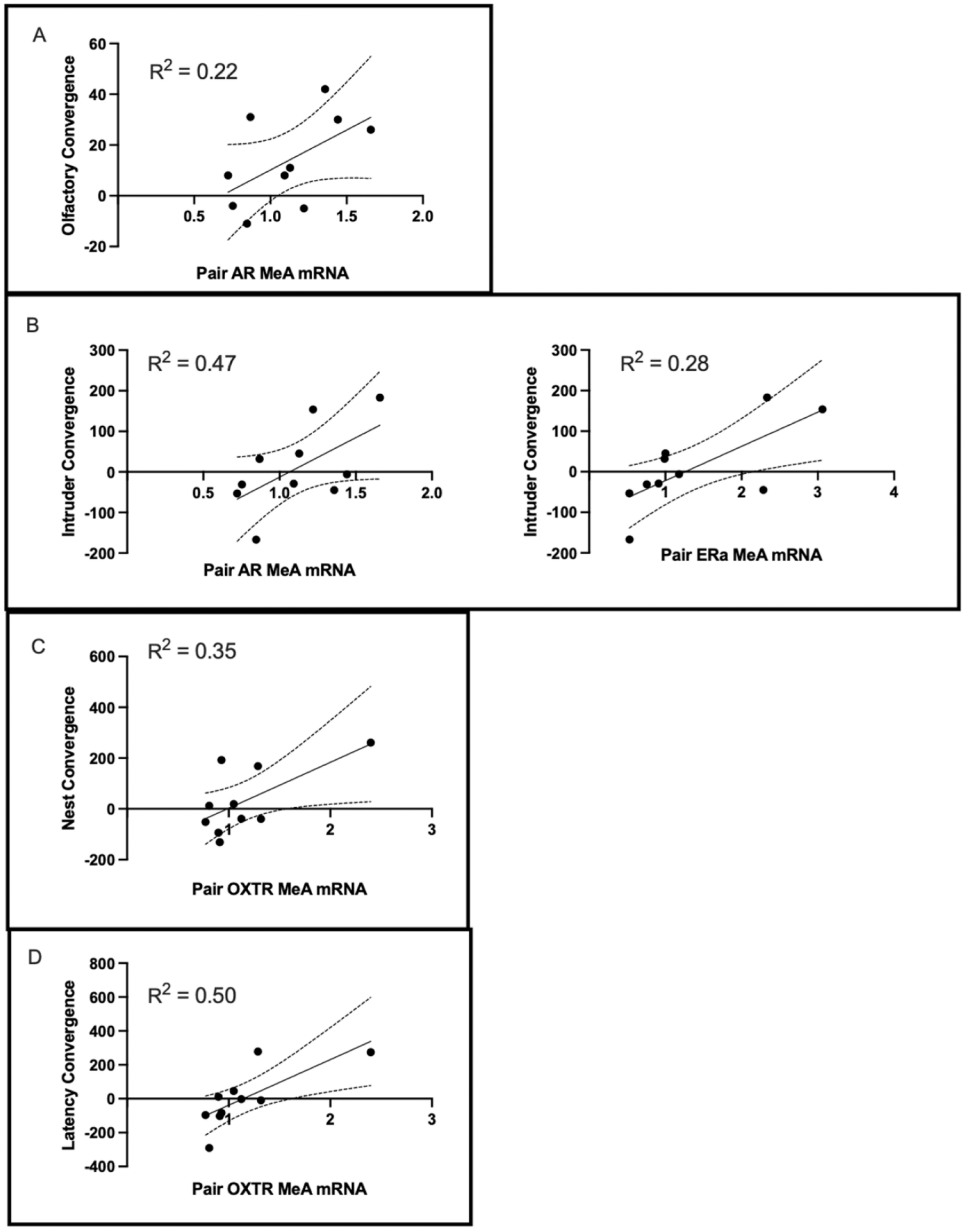

Fig. 4. *Each convergence behavior's variability had a unique relationship with pair mRNA indexes.* A) Olfactory convergence variation was positively associated with the AR mRNA index (nonsignificant trend with a medium effect size, p = .095, adj R^2 =.22). B) Intruder convergence was associated with both the AR mRNA index (nonsignificant trend but with a large effect size, p = .069, adj R^2 =.28) and the ERa mRNA index, (p = .018, adj R^2 =.47). C-D) Latency (to approach the intruder) convergence and nest convergence variation were both positively associated with a pair OXTR mRNA index, respectively (p = .041, adj R^2 = 0.35, p = .013, adj R^2 =.50).

regressed upon individual mRNA values. In addition, a random effect of pair number was added to control for any influence of pair assignment. Measures of mRNA were taken after the second challenge, however, some receptor mRNA measures correlated with Challenge 1 behaviors, while others with Challenge 2 behaviors. For differences in overall behavioral changes between Challenges see Supplemental Fig. 2. As a reminder, USVs remained a pair behavior in these analyses because of difficulty separating USVs between individuals. For nonsignificant effects of individual-specific investigations see Supplemental Table 2.

3.3.1. Challenge 1 behaviors and mRNA

When controlling for pairs, individual ERα mRNA in the MeA, but not OXT or AR system measurements, were associated with Challenge 1 behaviors. There was no association between individual OXTR mRNA and individual Challenge 1 behaviors (Supplemental Table 2). Interestingly, however, a simple linear regression in which pair latency was regressed on pair OXTR mRNA revealed that the more different the intruder approach latency of pairs in Challenge 1, the more pair OXTR MeA was noted within each pair ($F(1,8) = 6.783$, $p = .0316$, adj R^2 = .39) as measured after Challenge 2.

For the sex steroid receptors, individual ERα mRNA associations in the MeA dominated the relationships. Individual ERα mRNA quantities were negatively associated with the individual amount of time investigating the intruder via olfaction and time spent with the intruder in Challenge 1 ($F(1, 13.09) = 13.93$, $p < .003$, partial omega^2 = .92, Fig. 5C, $F(1, 10.71) = 7.87$, $p = .018$, partial omega^2 = .89, Fig. 5B). As expected from less time interacting with the intruder, a positive relationship between individual ERα mRNA quantities and individual time spent in the nest was detected in a linear mixed effects model where time spent at the nest was regressed on ERa mRNA, with an added random effect of pair ($F(1, 10.85) = 5.149$, $p = .045$, partial omega^2 = .78, Fig. 5A.). There were no additional significant associations between individual AR mRNA with individual Challenge 1 behaviors (see Supplemental Table 2). ERα was therefore the main sex steroid receptor associated with the earlier nonvocal baseline behaviors. No sex specific effects were found in any of the associations between individual Challenge 1 behaviors and individual mRNA levels (see Supplemental Table 2).

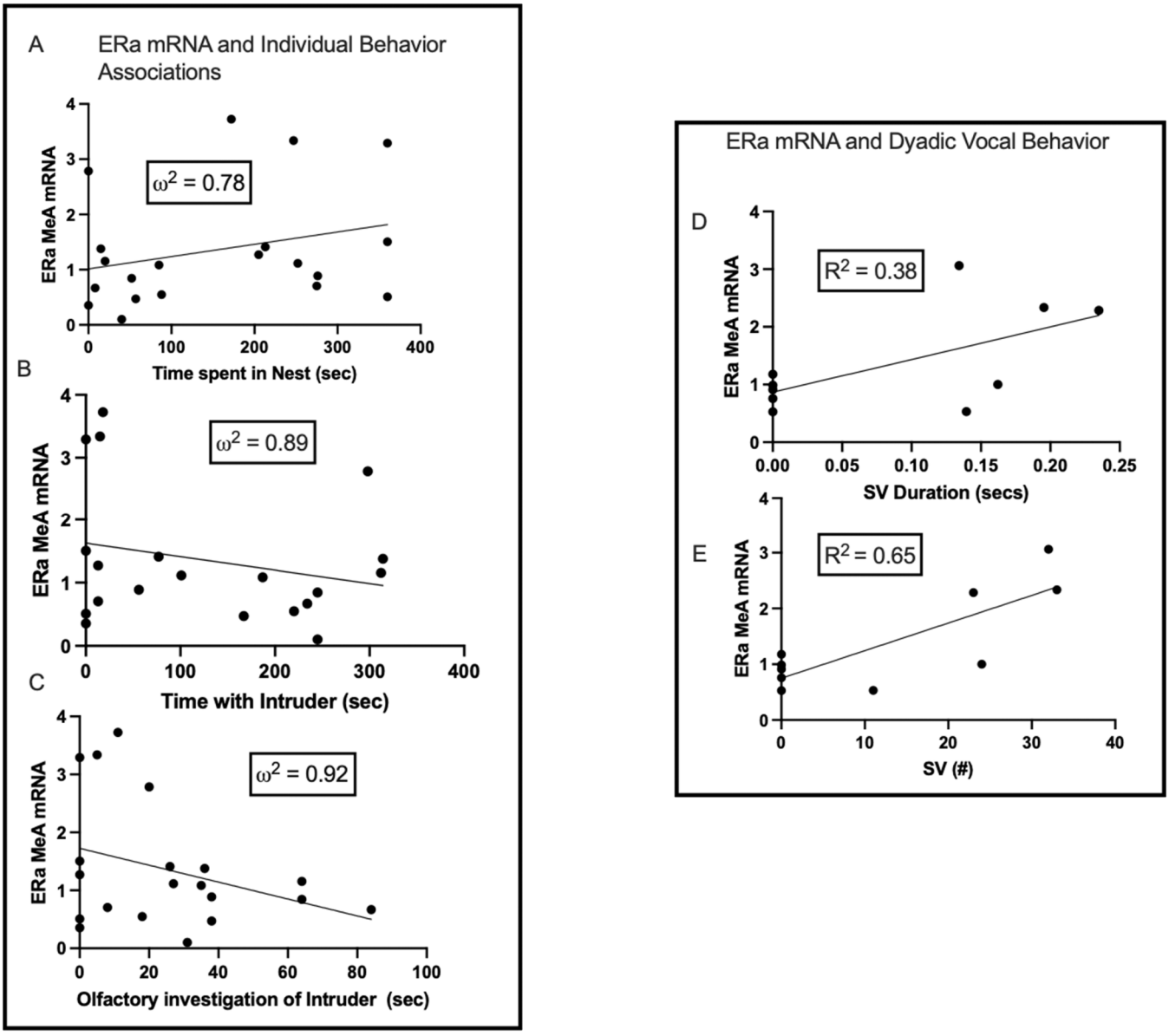

Fig. 5. *Individual ERα mRNA amounts were significantly associated with individual nonvocal behaviors* (A-C) and pair ERa mRNA amounts were associated with Challenge 1 dyadic vocal behaviors (D and E). A-C) Using linear mixed effects models, with a random effect of pair identity, (A) ERa was positively correlated with the amount of time an individual spent within the nest ($p = .045$, omega^2 =.78). B,C) ERa mRNA, expectedly, was negatively associated with time spent near the intruder with its partner and negatively correlated with olfactory investigation of this intruder (with a partner) in Challenge 1 ($p = .018$, omega^2 0.89, $p = .003$, omega^2 =.92). D and E) Two simple linear models detected that a pair ERa mRNA index correlated positively with both measured SV characteristics in Challenge 1 (SV#: $p = .005$, R^2 =.65, SV duration: $p = .058$, R^2 =.38). All effect sizes were large.

3.3.2. Challenge 1 vocalizations and pair mRNA indexes

Because there were too few vocalizations to analyze for Challenge 2, we only include Challenge 1 vocalizations. There was weak to moderate support for associations between SVs (of the pairs) and pair sex steroid receptor mRNA indexes. Results differed when non-vocalizers were included or excluded from our analyses, thus we present our findings both ways. A significant positive relationship with a large effect size was found using simple linear regression between a pair ERα mRNA index and the pair number of SVs ($F(1,8) = 14.53$, $p = .005$, partial $R^2 = .65$, Fig. 5E). In addition, a nonsignificant positive trend with a notable effect size was observed between pair SV duration and pair ERα mRNA in the MeA ($F(1,8) = 4.87$, $p = .058$, $R^2 = .38$, Fig. 5D). Higher ERα mRNA for a pair was therefore associated with less time interacting with the intruder, more time at the nest, and more SVs with longer durations as a dyad.

3.3.3. Sex specific associations between vocalizations in Challenge 1 and mRNA measures

When non-vocalizers were included in separate simple linear regressions but including only one sex for the mRNA measure, we identified a positive relationship between pair SV number and male ERα and AR mRNA in the MeA ($F(1,8) = 5.60$, $p = .046$, $R^2 = .41$, $F(1,8) = 5.58$, $p = .046$, $R^2 = .41$, Fig. 7A-B). For females, there was a significant sex-specific relationship between female ERα mRNA expression amounts and pair SV number when non-vocalizers were included ($F(1,8) = 5.73$, $p = .044$, $R^2 = .42$, Fig. 7E). Pair averaged SV duration was associated with male AR and ERα mRNA quantities, while female mRNA values failed to reveal such associations with pair SV duration (however, see results when non-vocalizers are excluded below). While there was an overall association between ERα and SV qualities in both sexes, sex specific analyses indicated the same positive associations, with one

exception being a nonsignificant effect between pair SV duration and female ERα mRNA quantities ($F(1,8) = .81$, $p = .396$, Fig. 6D). For nonsignificant effects of sex-specific investigations see Supplemental Tables 4, 5 and 6. Additionally, sex comparisons between mRNA measures are located in Supplemental Fig. 1.

We also analyzed the data by excluding non-vocalizers when investigating SVs and mRNA measures because there appears to be a dimorphic distribution between vocalizers and non-vocalizers in females (Figs. 6E and 6F). When non-vocalizers are excluded, the overall pattern for positive associations between SV qualities and neuroendocrine measures are further supported. With these points excluded, the positive associations between SV number and duration and AR mRNA remained significant (respectively, $(F(1,3) = 11.85$, $p = .041$, $R^2 = .80$, $F(1,3) = 67.70$, $p = .004$, $R^2 = .96$, not depicted) The positive association between the ERα mRNA index and SV number in females in the MeA also remained ($F(1,3) = 11.37$, $p = .043$, $R^2 = .79$, not depicted). However, with non-vocalizers excluded, there was an additional positive association between female MeA OXTR mRNA and pair SV duration ($F(1,3) = 20.76$, $p = .020$, $R^2 = .87$). In general, prior to excluding non-vocalizers, positive associations between pair SV duration and male sex-steroid receptor mRNA were only seen in males, but after excluding non-vocalizers, positive relationships between pair SV number and

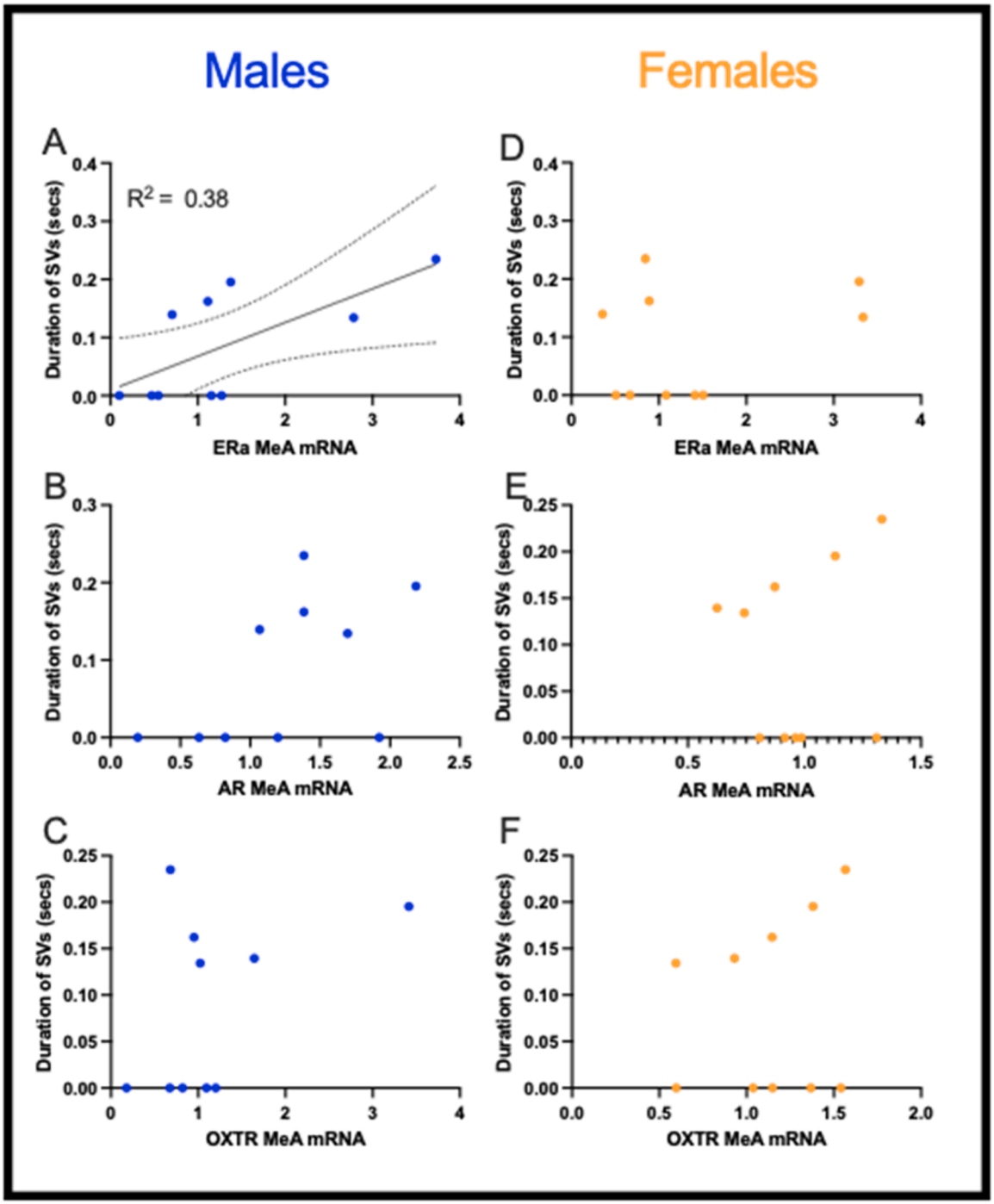

Fig. 6. *One positive association was seen between male sex steroid receptor mRNA (ERα) and pair SV duration.* A-B) Pair SV duration was significantly and positively associated with male ERα mRNA in the MeA ($p = .046$, $R^2 = .38$). B-F) No other relationships were found between neuroendocrine measures and SV duration. See text for analysis of vocalizers only.

duration and female sex steroid and OXTR mRNA were detected in females. However, because of the small sample size resulting from removal of non-vocalizers (n = 5), we refer to this as weak support for any conclusions.

3.3.4. AON Associations

For the AON, SV duration was positively associated with male ERa mRNA values (F(1,8) = −8.16, p = .021, R^2 = .51). Two trending but non-significant findings with notable effect sizes revealed a positive link between SVs and male AR and ERα mRNA quantities in vocalizers (F(1,8) = 4.45, p = .068, R^2 = .36, F(1,8) = −3.76, p = .089, R^2 = .32). In both analyses, SV number was regressed on either ERa or AR Fig. 7.

3.3.5. Challenge 2 behaviors and mRNA

Because Challenge 2 is closer in time to brain collection than Challenge 1, we expected different correlations, but had no a priori predictions. Only linear mixed effects models between OXTR mRNA and olfactory investigation and individual time spent with the intruder were

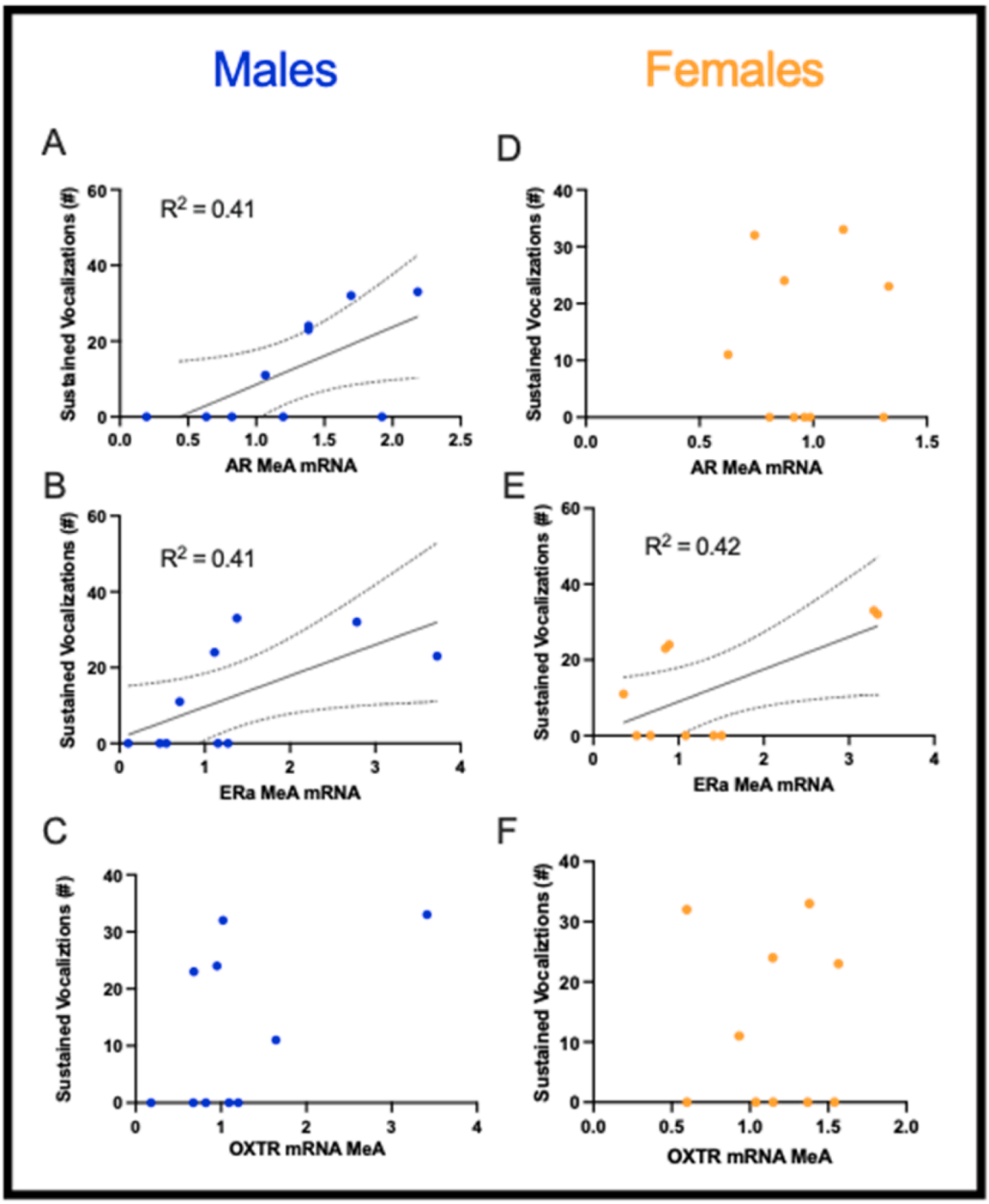

Fig. 7. *Positive associations between sex-steroid hormone mRNA and pair SV # were seen in both males and females in Challenge 1.* Positive associations were found between (A) AR mRNA and pair SV # in the MeA (p = .046, R^2 =.41) and (B) ERα mRNA and pair SV # (p = .046, R^2 =.41) C). No significant associations were found between SV # and OXTR mRNA in males. No significant associations were detected between SV # and AR mRNA in females (p > .05). E) The only significant association detected in females was between ERα mRNA and pair SV # (p = .044, R^2 =.42), F) and no significant associations between OXTR mRNA and SV # was detected in females (p > .05).

positively associated (respectively, F(1, 13.93) = 4.675, p = .049, omega^2 = .90, Fig. 6B, F(1, 17.95) = 5.10, p = .037, omega^2 = .92, Fig. 8A-B). We found no additional associations between behaviors and mRNA quantities (see Supplemental Table 2). There were no significant associations found with the AON when we examined sex differences.

3.4. Correlations among mRNA measurements

The one significant association found within MeA mRNA measurements, after conducting a linear mixed effects model with a fixed effect of individual OXTR and a random effect of pair, was a positive association between individual AR and individual OXTR mRNA (F(1, 17.97) = 6.59, p = .019, partial omega^2 = .83) in the MeA (all others were non-significant, *AR/ERα*: F(1, 15.88) = 2.08, p = .169, ERα/OXTR: F(1, 17.11) = .10, p = .756).

4. Discussion

At a population level, we observed a modality change in the distribution of convergence-related values within the IOB measure from a bimodal to a unimodal distribution. A bimodal distribution is not unexpected because of prior evidence showing pairs and individuals can display proactive versus reactive responses (approachers versus avoiders) to intruder stimuli [86,87]. The bimodal distribution indicates that multiple competing processes were involved in this change, as found in cognitive processes and the data distributions resulting from dual processes impacting a variable [31]. At a pair level, we did not find evidence for pair behavioral convergence after a pair bond is formed, but did find associations between neuroendocrine mRNA correlates and variability in convergence values within a pair, which we attribute to multiple competing processes involved in responding to an intruder stimulus. Specifically, when we reduced the IOB measure into its constituents, we found that convergence variability in intruder-directed behaviors was associated with different combinations of mRNA for OXTR, AR, and ERa. Typically, pair-related behaviors are explored through the lens of the OXT system, however, here we find that convergence variability in three different intruder-directed behaviors are associated with different combinations of mRNA for not only OXTR, but also AR and ERa. Moreover, because we use a more ethologically relevant scenario of both pair residency (territoriality) and the addition of a nesting site (igloo), pairs had choices about whether to stay in the nest or approach the intruder. The time spent together at the nest and away from the intruder in Challenge 1 was associated with ERa in the MeA, suggesting a mechanism that could balance time between approaching a social challenge versus defending the nest or showing avoidant behaviors (possibly along a proactive-reactive continuum, [87, 53]. We note that our experimental design may reflect similar states within a pair rather than an active convergence through communication (e.g. [30]. Past literature has noted similarities in hormonal, neural, and behavioral states between pair bonded individuals across species (e.g. [108,69]. As such, we are unable to disentangle the effect of similar physiological states on behavior from the impact of pair bonding on behavior. However, similar physiological profiles of pair mates dappear to be a feature of pair bonding across the animal kingdom. Finally, although not all pairs vocalized, we have evidence that the sex steroid receptor mRNA in the MeA was positively associated with SV number and duration as described below. This is a first step in teasing apart the different behaviors and neuromodulators that coordinate complex dyadic behavior [76] in dynamic environmental contexts and post-pair bond establishment.

4.1. Convergence variability in post pair bond establishment

The formation of pair bonds between animals involves neural and physiological changes that help initiate and maintain the bond. To be successful, the pair must engage in cooperative behaviors, achieved through either a strict division of behaviors based on sex or a dynamic process that allows for behavioral adjustments as the pair faces new challenges. These dynamic processes rely on the brain's ability to change and adapt via the underlying neural and physiological mechanisms (e.g. receptor densities). We focus on receptor densities in this paper, however, there are many alternative mechanisms that may influence bonding and convergence, including circulating hormone and neurosteroid levels.

Previous studies on California mice found that newly paired monogamous and territorial mice showed similar approach behavior towards playback of an intruder's call. This convergence in behavior was even more rapid when females were administered OXT, a hormone known to influence social bonding, before the encounter [63,86]. This effect is unlikely to be habituation because isolated females that were

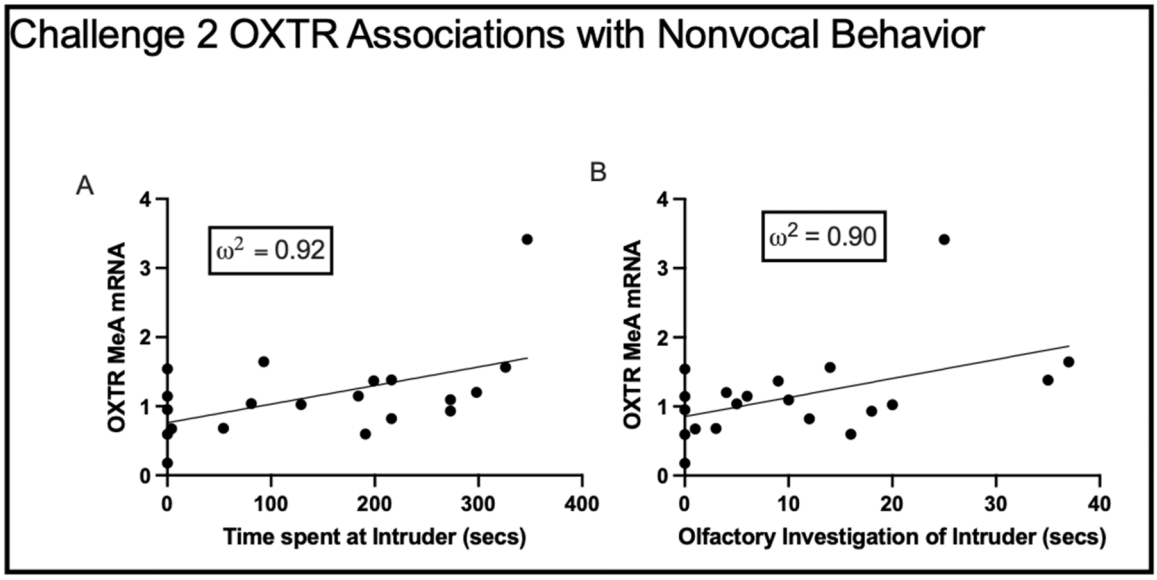

Fig. 8. *Challenge 2 behaviors were positively associated with OXTR mRNA.* A-B) Pair OXTR mRNA was positively associated with the time spent in contact with an intruder (p = .037, omega^2 =.92) and the amount of pair olfactory investigation that occurred (p = .049, omega^2 =.90, all effect size values are large and presented as partial omega^2.

administered OXT did not show changes in approach behavior from one playback challenge to a second challenge [63]. Furthermore, we did not find a difference in the average time it took for pairs to approach the intruder between the first and second challenge. Finally, pairs were only subjected to the defense challenge twice and a novel intruder was used for each challenge.

The results are consistent with the concepts that convergence in pair behavior can be adaptive and that OXT can facilitate behavioral convergence [53,5,1,41,65]. The previous and current studies were conducted prior to having pups, in the current study, however, the pair bond was already formed and most females were likely pregnant on days 10 and 17 post pairing [37,79,86]. While convergence was not as clear as in previous studies [87,63], *our current study, nonetheless, extends this body of research by illustrating that after pair formation, but at a population level there is a change from a bimodal to a unimodal distribution in pair IOB behaviors with repeated intruder challenges, but with pair variability in their convergence tendencies.*

In addition to a lack of convergence in IOB measures, we also did not find nest convergence across these challenges. This may change with larger sample sizes and the presence of pups. Of interest is a positive relationship between nest convergence and latency convergence suggesting that these variables may still be linked but in a complex way dependent on a balance of multiple competing stimuli.

4.2. Associations between convergence variability and OXTR, ERa and AR expression

Recognizing a threat and coordinating a response between two individuals is a complex social behavior, and little is known about how these responses are controlled by the brain [23], especially after pair establishment. Formation and maintenance of pair bonds have been associated with OXT, dopamine and opioids [56]. We hypothesized that the neural OXTR, ERa, and AR act to modulate pair coordination due to their established roles in territorial aggression [35,109,88] and pair bond formation and maintenance [47,49]. In other rodents, these three systems interact in brain regions such as the MeA to influence social recognition [6]. Consistent with this, we found a positive association between pair AR and OXTR mRNA indexes in the MeA.

The aggregate IOB measure did not correlate with any pair mRNA index in the MeA, however, behavioral coordination within a pair is likely to be under complex control involving a combination of both pair cooperation and motivation to drive an intruder from the territory. We found instead that different components of IOB correlated with different receptor mRNA measures in the MeA (and not the AON, see below): olfactory convergence was positively associated with AR mRNA, intruder convergence was positively associated with pair indexes of AR and ERa mRNA (the nonsignificant trend between the AR mRNA index and intruder convergence had a large effect size), and convergence in latency to approach was positively associated with a pair OXTR mRNA index. *These results suggest that convergence in behavior may be conducted through integration of several receptor systems with contributions from both OXT and sex steroids in the MeA.* Our results take a new perspective by integrating several receptors with convergence behavior, albeit on a correlational level.

Unfortunately, we could not assess how receptors changed from Challenge 1 to 2. However, if pair convergence is an ongoing process, we speculate that the OXT system is re-engaged when a pair needs to coordinate behavior, consistent with the social salience hypothesis which argues that OXT helps to attune individuals to the current social context, whether negative or positive [91]. In addition, the greater the difference in latency to approach the intruder by the pair during Challenge 1, possibly representing a greater need to converge in a strategy, the more OXTR mRNA in the MeA of the pair members after Challenge 2.

4.3. Challenge 1 and 2 correlations with OXTR, ERa, and AR

We can move beyond convergence and pair mRNA indexes and ask what individual behaviors correlated with individual receptor mRNA measures in either Challenges 1 or 2. There were no overlapping significant correlations between mRNA measures and behaviors in Challenge 1 versus 2, perhaps because Challenge 1 behaviors represent baseline and in Challenge 2, members of a pair respond to experience, as in a repeated challenge. The caveat with the design is of course that the mRNA measures were taken after the second challenge and can reflect either the baseline behaviors in Challenge 1 and/or the behaviors representing a response to experience.

In Challenge 1, higher ERα was associated with spending more time at the nest and away from the intruder for both males and females. ERα may therefore be an important candidate contributing to expression of a joint or divided (one sex approaches the intruder) intruder approach, as mentioned earlier [86]. The ERα pattern may eventually be modified by the OXT system, as indicated by the bidirectional relationship between OXT and ER expression (For examples, see: [104,60], but the interplay between the OXT and estrogen signaling systems in this behavioral scenario of cooperation remains to be explored.

Also in Challenge 1, we found several positive associations between SV duration, SV number and individual AR and ERα mRNA, but not OXTR. In the MeA, individual ERα mRNA values were associated with both SV number and duration when controlling for the impact of pair non-independence. Male AR and ERα mRNA were positively associated with longer pair average SV durations, while the same associations between AR and SV traits were not present in females. However, when removing non-vocalizers, a strong association between AR mRNA and SV number was found in both sexes, although the sample size is small ($n = 5$). Furthermore, two new positive associations in pair vocalizers were found: female OXTR and ERα mRNA were positively associated with pair SV duration. Despite the significant correlations and medium to high effect sizes, we refer to this as weak support for the concept that sex steroid receptors in the MeA are associated with the production of SVs because of the small sample size. Worth noting is literature supporting the link between rodent vocalizations and sex steroid hormones, however, most studies have focused on males [58]. Within California mice, the number of SVs have been associated with pairing and affiliative behavior [63,79]. Longer durations are also associated with affiliative behavior, and there was a positive association between longer SV lengths and AR and ERa in the MeA (however, note that there was a nonsignificant pattern for SVs to be shorter during the intruder challenges, see Supplemental Table 8). Here we provide evidence that the MeA may be influencing vocalizations through OXTR and ERα, as well as the typical findings associating vocalizations with androgen systems.

The above describes the results for associations in the MeA, however, we also found evidence for a negative association between the AON and SVs. Exclusively in males, ERα mRNA in the AON and SV duration was found to be negatively associated. In addition, two nonsignificant negative trends with large effect sizes were seen between SV number and sex steroid mRNA (ERα and AR) in the AON, but only in males. This indicates that the hormonal receptors in the AON may be important for male USV production, but it is unclear how.

In contrast to Challenge 1 correlations, in Challenge 2 there were no correlations between ERa or AR mRNA, and/or time in the nest or intruder-related behaviors. Instead, OXTR mRNA quantities were associated with two intruder-oriented behaviors: time spent with the intruder and time spent in olfactory investigation of the intruder. This supports the association between OXTR and behavior after convergence. We speculate that the second intruder experience is driven more by mechanisms associated with pair-driven dynamics such as the OXT system. One speculation is that associations between behavior and the OXT system in Challenge 2 would disappear in future challenges, reverting back to associations with sex steroid receptors. If the OXT system is functioning to help transition into a new response this may

disappear once the behavior has been established.

The results for the individual challenges suggest, on a correlational level, that early or novel pair coordination of behavior may be more reliant on sex steroid systems, and that the same behaviors after convergence associated with the pair-driven dynamics may be more linked with the OXT system, at least in the MeA. Such speculation would be interesting to test in future studies.

4.4. We have added to the understanding of potential functions of the MeA and the AON

Our correlational results indicate that slightly new perspectives can be taken on the functions of both the MeA and the AON: two brain regions with high numbers of sex steroid and OXT receptors. We expected a strong involvement of the MeA, but also of the AON because of extensive OXT-related and sex steroid connectivity and expression in both areas [42,19]. The MeA's importance in pheromone communication, fear response/perception, aggression/territoriality, stress, sexual behavior, and parenting in rodents makes it an interesting point of investigation for hormonally-modulated behaviors (for review see [73]. In particular, the MeA possesses steroid receptors and maintains OXT-related connectivity with other social brain areas [51]. The MeA is also a seat of important steroid hormone function and flexibility (e.g. rich in aromatase, etc., [99]. As such, we speculate that the MeA's importance in processing social stimuli is reflected in its numerous associations between both behavioral convergence and pair territory defense with the mRNA measures examined in this experiment (e.g. [73, 81]. The AON, on the other hand, was notably less associated with the social behaviors measured. As mentioned earlier, future studies could explore whether the associations between sex steroid receptors and vocalizations are indicative of a new, unexplored function of the AON. In addition, brain regions such as the lateral septum, ventral anterior cingulate cortex, bed nucleus of the stria terminalis, are future areas to investigate as these regions are enriched for hormone receptors and possess links with social approach and stress [28].

5. Conclusion

Together these studies show that sex steroid hormone receptors and OXT systems in the MeA and AON of pair bonded individuals correlate with both behavioral convergence variability in response to an intruder and a pair's production of ultrasonic vocalizations. These findings indicate that ERa, AR and OXTR may be important to behavioral coordination during complex social interactions and could act as a mechanism by which individuals change their behavior to match their partner and increase the pair's success. At the very least the results indicate that these systems are likely involved in decisions to approach intruders. Overall, our results provide exciting new avenues for research to explore the neural underpinnings of coordinated behavior and provide candidate circuits to manipulate in future studies to test causal roles for these neuromodulators in pair bond maintenance and behavior.

CRediT authorship contribution statement

Candice L. Malone: Formal analysis, Visualization, Methodology, Conceptualization, Writing. **Nathaniel S. Rieger:** Conceptualization, Methodology, Investigation, Data Curation, Writing – review & editing. **Jeremy A. Spool:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing. **Alexis Payette:** Investigation. **Lauren V. Riters:** Resources, Funding acquisition, Writing – review & editing. **Catherine A. Marler:** Conceptualization, Resources, Funding acquisition, Writing, Supervision, Project administration.

Declaration of Competing Interest

none.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2023.114556.

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