Coordination of behavior by division of labor and vocal communication in pair-bonded California mice (*Peromyscus californicus*)

By

Nathaniel S. Rieger

A dissertation in partial fulfillment of

The requirements for the degree of

Doctor of Philosophy

(Psychology)

At the

University of Wisconsin-Madison

2018

Date of final oral examination: 9/14/2018

The dissertation is approved by the following members of the Final Oral Committee

Dr. Catherine A. Marler, Professor, Department of Psychology

Dr. Anthony Auger, Professor, Department of Psychology

Dr. Lauren Riters, Professor, Department of Integrative Biology

Dr. Yuri Saalmann, Assistant Professor, Department of Psychology

Dr. Ken Yasukawa, Professor, Department of Biology, Beloit College

Contents

Acknowledgements

I would first like to thank my adviser Dr. Catherine Marler for her guidance, support and funding throughout my time in graduate school. Because of her I have become the scientist I am today, and I could not have asked for a better mentor to teach me the nuances of scientific research and writing. Her endless curiosity and excitement about animal behavior has been a constant source of inspiration for me, and I am proud to have been a member of her lab.

I would also like to thank my committee Drs. Anthony Auger, Lauren Riters, Yuri Saalmann and Ken Yasukawa for their time and advice over the course of this project. I'd particularly like to thank Dr. Anthony Auger for always being willing to talk over the years and the great conversations that kept me sane throughout graduate school. I would also like to make special note of Dr. Lauren Riters for running her graduate student seminar which provided me a platform to present my data each semester and to gain feedback on my work. This seminar helped me become a much more confident presenter.

I would like to thank my lab mates and fellow graduate students over the years for being there for me when I needed it, whether it is to learn a new technique, be a sounding board for ideas or simply to talk. Dr. Josh Pultorak taught me how to record, analyze and playback ultrasonic vocalizations. Caleigh Guoynes taught me how to administer intranasal oxytocin. Xin Zhao and Frank Castelli helped me to design and write up my experiments. Also, a special thanks to Dr. Devin Merullo, Dr. Jeremy Spool and Liza Chang for constantly motivating me and providing invaluable feedback on my projects.

I'd also like to thank the animal research technicians and undergraduates who have worked with me to make these projects and this dissertation a reality. Animal research technicians John Irwin and Kate Skogen both helped with animal husbandry but were also friends. Without my many undergraduates these projects would not have been completed. I would particularly like to thank Evan Stanton, JB Ramos Hernandez, Juliette Schefelker, Kathryn Hartfield, Alexis Payette and Sage Keyes. I hope all of my undergraduates find great success in their future.

Finally, and most importantly I would like to thank my family and friends. First, my wonderful wife Amanda, without whom I never would have completed the dream of earning my PhD. Her unwavering support and willingness to follow me across the country all the while being the family breadwinner are sacrifices I will never forget. Amanda, I love you forever. To my parents, Bryan and Debra, thank you for always believing in me, supporting me and pushing me to be more. To my sisters, Jennifer and Stephanie, thank you for your support. I would also like to thank my friends Mark Hardee and Kevin Graham for being the best friends I could have ever asked for.

Abstract

Division of labor allows social groups to efficiently complete tasks while minimizing resource expenditure. Vocal communication also plays an important role in coordinating behavior within groups. However, little is known about division of labor or the role of vocal communication in behavioral coordination by monogamous species. California mice (*Peromyscus californicus*) are a monogamous and biparental species that produce a rich set of ultrasonic vocalizations during social interactions. However, to this point, studies of California mouse behavior have focused on pair-bonded males and females in isolation. This dissertation examines division of labor and vocal communication in pair-bonded California mice by testing paired animals while they are together using a variety of techniques. I predicted that pair-bonded California mice would divide labor across tasks related to pup retrievals and territorial defense. I also predicted that ultrasonic vocalizations would be used to coordinate labor divisions. First, I showed that, while alone, males and females displayed equal aggression against same-sex conspecifics and that shortened duration of sustained vocalizations (SV) predicted greater aggression. I then tested for division of labor and vocal coordination by testing pairs while together during pup retrieval and territorial defense. During pup retrievals division of labor was sex-specific with only females retrieving pups with little effect of vocalizations. Conversely, in territorial defense, I found that pairs used one of two strategies: 1) joint or 2) divided which were robust across contexts and that shortened SV durations again predicted greater aggression. Moreover, I found a potential neuropeptide mechanism of division of labor. Oxytocin altered division of labor and communication in response to intruders in a sex specific manner. When administered to females, division of labor increased but when administered to males, SVs were shortened. Finally, I tested how pair-bonding altered the behavioral responses of individuals to aversive vocal playbacks and found that individuals altered their behavior post-pairing to become more similar to their mate. Taken together, these studies show for the first time, that monogamous rodents divide labor, that oxytocin alters division of labor in a sex-specific manner and that USVs play a role in coordinating these responses.

General Introduction

Coordination of behavior by individuals is vital to the success of social groups (Clutton-Brock, 2002; Leimar et al., 2010; Lukas & Clutton-Brock, 2013). In many species, labor is divided amongst individuals in order to efficiently complete tasks while minimizing resource expenditure (Goldsby, Dornhaus, Kerr, & Ofria, 2012; Jeanne, 2016; Robinson, 2003; Robson & Traniello, 2016). However, despite being extensively studied in invertebrates (Gordon, 2016; Jeanne, 2016; Robson & Traniello, 2016), relatively little is known about division of labor in vertebrates, particularly in monogamous species. Moreover, vocal communication between individuals can be important to behavioral coordination in groups (Noe, 2006). Vocalizations can be used to recruit group members (Gouzoules, Gouzoules, & Marler, 1984; Slocombe & Zuberbühler, 2007), alert group members to threats (Litvin, Blanchard, & Blanchard, 2007; Morales et al., 2008; Seyfarth, Cheney, & Marler, 1980) and coordinate group movement (Campbell & Boinski, 1995). As such, vocal communication could play a role in coordinating division of labor in monogamous species, but this role has not yet been tested. This dissertation sought to fill these gaps in our knowledge of behavioral coordination by examining division of labor and vocal communication in the monogamous and biparental California mouse (*Peromyscus californicus*) using a variety of techniques.

Division of labor

Division of labor is a process by which individuals within a social group allocate tasks (Jeanne, 2016). Much of our knowledge on division of labor comes from extensive studies completed on invertebrate species (Page, Scheiner, Erber, & Amdam, 2006; Robson & Traniello, 2016). In many invertebrates, task allocation is fixed such that individuals specialize in a specific task in order to minimize costs (Goldsby et al., 2012). This task allocation can be governed by a number of factors including age (Reim & Scheiner, 2014), animal personality or behavioral syndromes (Wright, Holbrook, & Pruitt, 2014) and monoamines including octopamine and serotonin (Dolezal, Johnson, Hölldobler, & Amdam, 2013; Giraldo, Patel, Gronenberg, & Traniello, 2013; Robinson, 2003). However, despite our knowledge of division of labor in invertebrates, we know much less about the prevalence and underlying mechanisms of division of labor in vertebrates.

In vertebrate species, division of labor has generally been hypothesized to fit into three categories 1) sex-specific, 2) mate guarding and 3) cooperation (Clutton-Brock, 2009; Leimar et al., 2010). First, in sex-specific division of labor, tasks are allocated by sex such that males will always complete one task while females complete another. This form of division of labor has been seen in both cichlid fish (Rogers, 2010) and Kirk's dik dik (*Madoqua kirkii*) (Brotherton, Pemberton, Komers, & Malarky, 1997) wherein males will patrol the edge of territories while females remain close to the nest and care for young. Second, in mate guarding, individuals will alter their behavior in response to intruders based on the sex of the intruder (Mathews, 2002). Specifically, individuals will approach and repel same-sex intruders in order to prevent extrapair copulations (French, Cavanaugh, Mustoe, Carp, & Womack, 2018; Hall, 2000; Koloff & Mennill, 2011; Reburn & Wynne-Edwards, 1999). Finally, in cooperative behavioral coordination individuals will work with other members of their group or pair in order to expel intruders and care for offspring (Clutton-Brock, 2009; Dugatkin, 1997). Specifically, individuals will often recruit group members using vocalizations allowing them to repel intruders together, as has been seen in both primates and duetting birds (Caselli, Mennill, Bicca-Marques, & Setz, 2014; Levin, 1996). However, despite our knowledge of these alternative methods to divide labor, a monogamous rodent experimental system of division of labor has yet to be developed.

Most of our current knowledge on division of labor in monogamous rodents comes from prairie voles (*Microtus ochrogaster*) (Ahern, Hammock, & Young, 2011). In prairie voles division of labor is most commonly associated with parental care (Numan & Young, 2016) with females generally showing a greater amount of parental care than males (Solomon, 1993) indicating a potentially sex-specific mechanism. Moreover, when together, prairie vole parents will alternate time in the nest in order to keep pups protected while allowing for one parent to forage for food (Ahern et al., 2011), which may indicate a cooperative mechanism. However, while some evidence exists that prairie voles mate guard during territorial defense (Insel, Preston, & Winslow, 1995), this possibility has not been extensively studied and pairs have not been challenged with a conspecific intruder while together. As such, we still do not understand the mechanisms by which monogamous rodents divide labor to complete territorial defense or pup care. The role of vocal communication, which is vital in primates and birds, and the mechanisms underlying division of labor have also yet to be tested in monogamous rodents providing a significant gap in our knowledge. To examine this topic, we tested pair-bonded California mice while together during territorial defense and pup retrieval to better understand division of labor in a monogamous rodent.

Vocal communication and coordination of behavior

One method by which individuals can coordinate behavior across a group is vocal communication (Noe, 2006). Within groups, calls by individuals contain information that drive the behavioral responses of the rest of the group (Bradbury & Vehrencamp, 1998) including food calls (Boucaud, Aguirre Smith, Valère, & Vignal, 2016; Seyfarth et al., 2010), alarm calls (Seyfarth et al., 1980; Townsend et al., 2011), recruitment calls (Gouzoules et al., 1984; Slocombe & Zuberbühler, 2007) and calls to coordinate group movement (Campbell & Boinski, 1995). In each of these cases, individuals either provide information about their needs, such as food or support, which recruits other members of the group, or threats, such as a predator, which leads other members of the group to seek cover (Seyfarth et al., 2010; Seyfarth & Cheney, 2010). The ability to coordinate behavior by vocal communication to recruit or warn group members and alter behavior makes vocalizations a prime candidate to coordinate division of labor as well. However, to this point, little is known about how vocalizations by rodents alter the behavior of other members of their social group.

Rodents communicate vocally primarily through the use of ultrasonic vocalizations (USV) (Holy & Guo, 2005; Portfors, 2006). USVs are calls produced at frequencies > 22 kHz and are often produced during social interactions (Arriaga, 2012; Burgdorf et al., 2008). ~~To this point,~~ USVs have been primarily studied either in the context of mating (Mahrt, Perkel, Tong, Rubel, & Portfors, 2013; Seffer, Schwarting, & Wöhr, 2014), with USVs produced by males being attractive to females and increasing the likelihood of mating in both rats and mice (Burgdorf et al., 2008; Pasch, Tokuda, & Riede, 2017; Von Merten, Hoier, Pfeifle, & Tautz, 2014). Alternatively, USVs have been hypothesized to be an external indication of the affective states of individuals (Knutson, Burgdorf, & Panksepp, 2002). Specifically, in rats (*Rattus norvegicus*), 22 kHz calls have been hypothesized to indicate negative affect and are increased in response to adverse situations such as social defeat (Brudzynski & Holland, 2005; Inagaki, Kuwahara, Kikusui, & Tsubone, 2005; Kroes, Burgdorf, Otto, Panksepp, & Moskal, 2007). Conversely, 50 kHz calls are seen as relating to positive affect, with 50 kHz calls increasing in response to actions such as mating (Finton, Keesom, Hood, & Hurley, 2017; Seffer et al., 2014). However, little is known about the function of USVs in rodent behaviors outside of mating, particularly during aggression. As such, a better understanding of USVs and how they may coordinate behavior between individuals is required.

Oxytocin and behavior

Very little is known about the neuropeptide mechanisms that underlie division of labor and communication. Oxytocin is a highly conserved neuropeptide that plays an important role in a number of social behaviors including pair-bonding, affiliation, aggression and communication (Beery, 2015; Insel & Shapiro, 1992). Classically, central oxytocin has been found to be vital to the formation and maintenance of pair bonds in monogamous rodents (Lieberwirth & Wang, 2016). Oxytocin is increased in females following the formation of a pair bond (Insel & Shapiro, 1992; Keebaugh & Young, 2011) and is required in order for pair-bonds to be formed and maintained (Lieberwirth & Wang, 2016). Importantly, whereas oxytocin was once thought to have a strictly prosocial effect on behavior, recent work has shown that oxytocin has context-specific effect on behavior (Shamay-Tsoory & Abu-Akel, 2016). Specifically, the social salience hypothesis states that individuals given oxytocin in a positive social environment will show increased affiliative behavior while oxytocin administered in a negative manner will depress prosocial behavior and increase aggression (Beery, 2015; Shamay-Tsoory & Abu-Akel, 2016). Because of its effects on a wide variety of social behaviors as well as communication, oxytocin is a prime candidate to be involved in to control of division of labor.

We tested the role of oxytocin in division of labor by administering oxytocin or saline intranasally to pair-bonded California mice. Intranasal administration of oxytocin has become prevalent in recent years due to its ease of use, effectiveness and clinical implications (Quintana & Woolley, 2016; Veening & Olivier, 2013). Administering oxytocin intranasally is remarkably easy and can be done in many species including rodents, primates and humans (Quintana, Alvares, Hickie, & Guastella, 2015). Despite the exact mechanism by which oxytocin reaches the brain remaining unknown, it is hypothesized that intranasal oxytocin delivered into the nostrils, travels across the nasal mucosa and enters the brain via the olfactory bulbs (Bales et al., 2013; Quintana & Woolley, 2016). In line with this possibility, recent work has shown that intranasally administered oxytocin reaches cortical regions of the brain, subcortical regions of the brain and the cerebral spinal fluid (Li, Chen, Mascaro, Haroon, & Rilling, 2017), leading to changes in brain activation in under an hour (Galbusera et al., 2017). This method of administration, therefore, has been hypothesized to help alleviate social deficits in humans, particularly children with autism spectrum disorders (Young & Barrett, 2015). However, behavioral results have been mixed in humans with some studies finding prosocial effects and others finding no effect (Veening & Olivier, 2013). As such, the efficacy of intranasal oxytocin has been questioned (Leng & Ludwig, 2016) and more studies are needed to better understand the context specific effects of oxytocin.

In monogamous rodents, oxytocin has been shown to have sex- and context-specific effects. In prairie voles, acute intranasal oxytocin has been shown to increase prosociality in males towards familiar conspecifics, however, chronic treatments lead to a decrease in social behavior (Bales et al., 2013). Conversely, female California mice administered oxytocin show decreased social behavior but increased vigilance in response to a novel female (Duque-Wilckens et al., 2018). As such, sex, acute or chronic treatment, familiarity of conspecifics and context all play an important role in behavioral outcomes following oxytocin administration. However, how oxytocin affects the behavior of pairs while together when given to either the male, female or both partners, remains unknown. Thus, we tested the role of oxytocin in division of labor and communication by pair-bonded California mice during territorial defense.

Emergent properties of pairs

Beyond neuropeptide mechanisms, individual's behavioral syndromes may play an important role in the division of labor of pairs and groups. Behavioral syndromes are a suite of behavioral responses produced by an individual in response to stimuli across contexts (Sih, Bell, & Johnson, 2004; Sih, Bell, Johnson, & Ziemba, 2004). Generally, these behavioral syndromes are thought of as being fixed across the lifespan (Dingemanse, Kazem, Réale, & Wright, 2010; Fürtbauer, Pond, Heistermann, & King, 2015; Sih, Bell, Johnson, et al., 2004). However, both time and life history changes can have effects on individuals behavioral responses (Biro & Stamps, 2008; Sinn, Moltschaniwskyj, Wapstra, & Dall, 2010). Changes to an animal's social environment can also alter individual's behavioral syndromes. The majority of studies on behavioral syndromes examine individual animals in isolation (Webster & Ward, 2011), but for many animals this type of testing lacks ecological validity. Joining a social group can alter an individual's behavior in a number of ways. First, individuals can become more similar to their partner, a process known as social conformity (King, Williams, & Mettke-Hofmann, 2015; Van De Waal, Borgeaud, & Whiten, 2013). Social conformity decreases group differences and increases overall cohesion. Importantly, similarity has also been shown to increase reproductive success (Gabriel & Black, 2012). Conversely, individuals can exaggerate their behavioral differences, a process known as facilitation (King et al., 2015). Facilitation increases group differences and could help to encourage task specialization and division of labor. However, whether monogamous rodents show social conformity or facilitation following pair-bonding has yet to be studied.

A classic example of behavioral syndromes is 'boldness,' the likelihood that an individual will explore novel areas, or quickly return to normal behaviors such as foraging after, or despite, a threat such as a predator (King et al., 2013; Stamps, 2007). Boldness varies within individuals of a species across a continuum from bold to shy, with bold individuals approaching novel situations and areas significantly more than shy individuals (King et al., 2013; Swaney, Cabrera-Álvarez, & Reader, 2015). One way to test boldness is to expose individuals to aversive vocal playbacks, more bold individuals will approach these playbacks while more shy individuals will not (Brumm, 2004; King et al., 2015; Koloff & Mennill, 2011). The differences in boldness are of particular interest in monogamous pair-bonding animals as individuals can pair with an individual who either shares or differs in boldness and navigating these similarities and differences is vital to the success of the pair. Despite this difference, very little is known about how pair-bonding alters behavioral responses. We studied the behavioral responses of California mice to aversive vocal playbacks before and after pair-bonding. Mice were categorized for their response prior to bonding and paired with an individual who either showed a similar or different behavioral response. Pairs were then retested together allowing us to identify whether pairs showed social conformity or facilitation in response to pair bonding.

California mice

We used California mice to study the intersection of division of labor and vocal communication. California mice are a monogamous, biparental and territorial species (Gubernick, 1988; Gubernick & Alberts, 1981.; Ribble, 1991; Ribble & Salvioni, 1990) that produce a rich set of USVs (Briggs & Kalcounis-Rueppell, 2011; Kalcounis-Rueppell et al., 2010; Kalcounis-Rueppell, Pultorak, & Marler, 2018). California mice form strictly monogamous pair-bonds with no evidence of extrapair copulations in the field (Ribble, 1991). Once bonded, paired California mice will hold territories and care for pups as a pair until death (Ribble & Salvioni, 1990). Previous work has shown that both male and female pair-bonded California mice are capable of defending territories against same-sex intruders (Davis & Marler, 2003; Fuxjager & Marler, 2010; Fuxjager, Montgomery, Becker, & Marler, 2010). Moreover, both male and female members of a pair will care for pups with fathers capable of completing all aspects of parental care outside of lactation (Gubernick & Teferi, 2000; Gubernick & Nelson, 1989; Gubernick, Wright, & Brown, 1993) and both males and females retrieving pups (Bester-Meredith, Conley, & Mammarella, 2016; Frazier, Trainor, Cravens, Whitney, & Marler, 2006) while in isolation. However, despite the fact that California mice form pair bonds where both males and females can complete behaviors vital to territorial defense and parental care, the majority of studies on California mice have focused on individuals in isolation. It remains unknown how pairs will complete tasks when together and what role vocal communication may play in coordinating behaviors between individuals.

California mice communicate vocally by the production of a rich set of USVs. To this point, four different calls have been identified and recorded in adult California mice in both the field and the lab: simple sweeps, complex sweeps, sustained vocalizations (SV) and barks (Kalcounis-Rueppell et al., 2018). Simple sweeps are short, upward or downward modulated calls with a peak frequency around 50 kHz. Complex sweeps are similar to simple sweeps but include an inflection point and have a higher peak frequency, near 100 kHz. SVs are long low-bandwidth calls made up of one to five syllables with a mean frequency near 20 kHz. Barks are short, high amplitude calls that begin and end in the audible range (around 12 kHz) and have a peak frequency in the ultrasonic range, near 20 kHz. Despite having extensive recordings of California mouse vocalizations in the field (Briggs & Kalcounis-Rueppell, 2011; Kalcounis-Rueppell et al., 2010; Kalcounis-Rueppell, Metheny, & Vonhof, 2006), we still know relatively little about the functions of USVs across social contexts. It has been found that SVs play an important role in mating (Pultorak, Matusinec, Miller, & Marler, 2017) and mate fidelity (Pultorak, Fuxjager, Kalcounis-Rueppell, & Marler, 2015) in bonded California mice. Barks, on the other hand, have been hypothesized to play a role in aggressive behaviors (Kalcounis-Rueppell et al., 2018) as they have been most often recorded during interactions between pairs that also contained aggressive behaviors (Pultorak et al., 2017). However, the production of USVs by pairs during aggression and pup retrieval has not been studied and, thus, their role in coordinating behavior by pairs remains unknown.

California mice provide an intriguing experimental system to study division of labor and vocal communication. The long-term pair-bonds formed by mice and the ability of both males and females to complete almost all tasks required of the pair leaves open the question of how paired mice will complete tasks while together. Moreover, the rich set of USVs produced by California mice provide a vocal mechanism by which pairs could coordinate behavior. However, California mice have almost always been studied in isolation and the social function of their calls remains understudied. As such, we tested California mice as pairs for the first time to elucidate their behavioral coordination strategies and whether or not they use vocal communication to maintain this coordination.

Dissertation goals and predictions

The goal of this dissertation was to elucidate the role of division of labor and vocal communication in a monogamous rodent. To do this, we completed four studies examining division of labor and ultrasonic vocalization production in the monogamous California mouse. In chapter one, we compared the aggressive behaviors and vocal production of male and female California mice in response to an intruder while alone to determine if sex differences existed in territorial defense or the use of vocalizations during aggressive encounters. In chapter two, we tested how pair-bonded California mice responded to an intruder while together before and after the birth of pups, as well as how pair-bonded California mice retrieved pups while together to determine if division of labor occurred within pairs. In chapter three, we administered intranasal oxytocin to either one or both members of a pair to determine the role of central oxytocin in division of labor by pairs. Finally, in chapter four, we tested individual's responses to aversive vocal playback before and after pair-bonding to determine if division of labor occurred due to pre-existing behavioral differences within pairs or if emergent properties manifested in pairs after bonding. We predicted that California mice would show division of labor after the birth of pups such that one member of the pair would be defend the territory while the other remained near the pups. We also predicted that labor divisions would be affected by oxytocin, as this neuropeptide plays an important role in pair-bond formation and maintenance (Lieberwirth & Wang, 2016). We then predicted that pair-bonding would lead to the formation of emergent properties as previous research has shown that changes to an individual's social environment can alter their behavioral response to stimuli (Webster & Ward, 2011). Finally, we predicted that, throughout these experiments, vocal communication would play a role in the coordination of behavior as seen in many other social species (Noe, 2006). This dissertation, then, provides a new experimental system to understand behavioral coordination in monogamous species and provides a framework by which to better elucidate the mechanisms of division of labor.

References

Ahern, T. H., Hammock, E. A. D., & Young, L. J. (2011). Parental division of labor, coordination, and the effects of family structure on parenting in monogamous prairie voles (Microtus ochrogaster). *Developmental Psychobiology*, *53*(2), 118–31. http://doi.org/10.1002/dev.20498

Arriaga, G. (2012). Of Mice, Birds, and Men: The Mouse Ultrasonic Song System and Vocal Behavior. *Dissertation Abstracts International, B: Sciences and Engineering*, *72*(10), 3859. http://doi.org/10.1371/journal.pone.0046610

Bales, K. L., Perkeybile, A. M., Conley, O. G., Lee, M. H., Guoynes, C. D., Downing, G. M., … Mendoza, S. P. (2013). Chronic intranasal oxytocin causes long-term impairments in partner preference formation in male prairie voles. *Biological Psychiatry*, *74*(3), 180–8. http://doi.org/10.1016/j.biopsych.2012.08.025

Beery, A. K. (2015). Antisocial oxytocin: complex effects on social behavior. *Current Opinion in Behavioral Sciences*, *6*, 174–182. http://doi.org/10.1016/J.COBEHA.2015.11.006

Bester-Meredith, J., Conley, M., & Mammarella, G. (2016). Peromyscus as a Model System for Understanding the Regulation of Maternal Behavior. *Seminars in Cell and Developmental Biology*. http://doi.org/10.1016/j.semcdb.2016.07.001

Biro, P. A., & Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution*, *23*(7), 361–8. http://doi.org/10.1016/j.tree.2008.04.003

Boucaud, I. C. A., Aguirre Smith, M. L. N., Valère, P. A., & Vignal, C. (2016). Incubating females signal their needs during intrapair vocal communication at the nest: a feeding experiment in great tits. *Animal Behaviour*, *122*, 77–86. http://doi.org/10.1016/j.anbehav.2016.09.021

Bradbury, J. W., & Vehrencamp, S. L. (1998). *Principles of Animal Communication*. Sunderland, Ma: Sinauer Associates, Inc. Retrieved from http://efish.fiu.edu/lab_business/training/Reading2.pdf

Briggs, J. R., & Kalcounis-Rueppell, M. C. (2011). Similar acoustic structure and behavioural context of vocalizations produced by male and female California mice in the wild. *Animal Behaviour*, *82*(6), 1263–1273. http://doi.org/10.1016/j.anbehav.2011.09.003

Brotherton, P. N., Pemberton, J. M., Komers, P. E., & Malarky, G. (1997). Genetic and behavioural evidence of monogamy in a mammal, Kirk's dik-dik (Madoqua kirkii). *Proceedings. Biological Sciences*, *264*(1382), 675–81. http://doi.org/10.1098/rspb.1997.0096

Brudzynski, S. M., & Holland, G. (2005). Acoustic characteristics of air puff-induced 22-kHz alarm calls in direct recordings. *Neuroscience & Biobehavioral Reviews*, *29*(8), 1169–1180. http://doi.org/10.1016/j.neubiorev.2005.04.007

Brumm, H. (2004). Male–male vocal interactions and the adjustment of song amplitude in a territorial bird. *Animal Behaviour*, *67*(2), 281–286. http://doi.org/10.1016/j.anbehav.2003.06.006

Burgdorf, J., Kroes, R. A., Moskal, J. R., Pfaus, J. G., Brudzynski, S. M., & Panksepp, J. (2008). Ultrasonic vocalizations of rats (Rattus norvegicus) during mating, play, and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback.. *Journal of Comparative*

Psychology, *122*(4), 357–367. http://doi.org/10.1037/a0012889

Campbell, A. F., & Boinski, S. (1995). Use of Trill Vocalizations To Coordinate Troop Movement Among White-Faced Capuchins: a Second Field Test. *Behaviour*, *132*(11), 875–901. http://doi.org/10.1163/156853995X00054

Caselli, C. B., Mennill, D. J., Bicca-Marques, J. C., & Setz, E. Z. F. (2014). Vocal behavior of black-fronted titi monkeys (*Callicebus nigrifrons*): Acoustic properties and behavioral contexts of loud calls. *American Journal of Primatology*, *76*(8), 788–800. http://doi.org/10.1002/ajp.22270

Clutton-Brock, T. (2009). Cooperation between non-kin in animal societies. *Nature*, *462*(7269), 51–57. http://doi.org/10.1038/nature08366

Clutton-Brock, T. H. (2002). Breeding together: kin selection and mutualism in cooperative vertebrates. *Science*, *296*(5565), 69–72. http://doi.org/10.1126/science.296.5565.69

Davis, E. S., & Marler, C. A. (2003). The progesterone challenge: steroid hormone changes following a simulated territorial intrusion in female Peromyscus californicus. *Hormones and Behavior*, *44*(3), 185–198. http://doi.org/10.1016/S0018-506X(03)00128-4

Dingemanse, N. J., Kazem, A. J. N., Réale, D., & Wright, J. (2010). Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology & Evolution*, *25*(2), 81–89. http://doi.org/10.1016/J.TREE.2009.07.013

Dolezal, A. G., Johnson, J., Hölldobler, B., & Amdam, G. V. (2013). Division of labor is associated with age-independent changes in ovarian activity in Pogonomyrmex californicus harvester ants. *Journal of Insect Physiology*, *59*(4), 519–24. http://doi.org/10.1016/j.jinsphys.2013.02.008

Dugatkin, L. A. (1997). *Cooperation among animals: An evolutionary perspective*. New York: Oxford University Press.

Duque-Wilckens, N., Steinman, M. Q., Busnelli, M., Chini, B., Yokoyama, S., Pham, M., … Trainor, B. C. (2018). Oxytocin Receptors in the Anteromedial Bed Nucleus of the Stria Terminalis Promote Stress-Induced Social Avoidance in Female California Mice. *Biological Psychiatry*, *83*(3), 203–213. http://doi.org/10.1016/J.BIOPSYCH.2017.08.024

Finton, C. J., Keesom, S. M., Hood, K. E., & Hurley, L. M. (2017). What's in a squeak? Female vocal signals predict the sexual behaviour of male house mice during courtship. *Animal Behaviour*, *126*. http://doi.org/10.1016/j.anbehav.2017.01.021

Frazier, C. R. M., Trainor, B. C., Cravens, C. J., Whitney, T. K., & Marler, C. A. (2006). Paternal behavior influences development of aggression and vasopressin expression in male California mouse offspring. *Hormones and Behavior*, *50*(5), 699–707. http://doi.org/10.1016/j.yhbeh.2006.06.035

French, J. A., Cavanaugh, J., Mustoe, A. C., Carp, S. B., & Womack, S. L. (2018). Social Monogamy in Nonhuman Primates: Phylogeny, Phenotype, and Physiology. *The Journal of Sex Research*, *55*(4–5), 410–434. http://doi.org/10.1080/00224499.2017.1339774

Fürtbauer, I., Pond, A., Heistermann, M., & King, A. J. (2015). Personality, plasticity and predation: linking endocrine and behavioural reaction norms in stickleback fish. *Functional Ecology*, *29*(7), 931–940. http://doi.org/10.1111/1365-2435.12400

Fuxjager, M. J., & Marler, C. A. (2010). How and why the winner effect forms: influences of contest environment and species differences. *Behavioral Ecology*, *21*(1), 37–45. http://doi.org/10.1093/beheco/arp148

Fuxjager, M. J., Montgomery, J. L., Becker, E. A., & Marler, C. A. (2010). Deciding to win: interactive effects of residency, resources and ‘boldness’ on contest outcome in white-footed mice. *Animal Behaviour*, *80*(5), 921–927. http://doi.org/10.1016/j.anbehav.2010.08.018

Gabriel, P. O., & Black, J. M. (2012). Behavioural Syndromes, Partner Compatibility and Reproductive Performance in Steller’s Jays. *Ethology*, *118*(1), 76–86. http://doi.org/10.1111/j.1439-0310.2011.01990.x

Galbusera, A., De Felice, A., Girardi, S., Bassetto, G., Maschietto, M., Nishimori, K., … Gozzi, A. (2017). Intranasal Oxytocin and Vasopressin Modulate Divergent Brainwide Functional Substrates. *Neuropsychopharmacology*, *42*(7), 1420–1434. http://doi.org/10.1038/npp.2016.283

Giraldo, Y. M., Patel, E., Gronenberg, W., & Traniello, J. F. A. (2013). Division of labor and structural plasticity in an extrinsic serotonergic mushroom body neuron in the ant Pheidole dentata. *Neuroscience Letters*, *534*, 107–11. http://doi.org/10.1016/j.neulet.2012.11.057

Goldsby, H. J., Dornhaus, A., Kerr, B., & Ofria, C. (2012). Task-switching costs promote the evolution of division of labor and shifts in individuality. *Proceedings of the National Academy of Sciences*, *109*(34), 13686–13691. http://doi.org/10.1073/pnas.1202233109

Gordon, D. M. (2016). From division of labor to the collective behavior of social insects. *Behavioral Ecology and Sociobiology*, *70*(7), 1101–1108. http://doi.org/10.1007/s00265-015-2045-3

Gouzoules, S., Gouzoules, H., & Marler, P. (1984). Rhesus monkey (Macaca mulatta) screams: Representational signalling in the recruitment of agonistic aid. *Animal Behaviour*, *32*(1), 182–193. http://doi.org/10.1016/S0003-3472(84)80336-X

Gubernick, D. J. (1988). Reproduction in the California Mouse, Peromyscus californicus. *Journal of Mammalogy*, *69*(4), 857–860. http://doi.org/10.2307/1381649

Gubernick, D. J., & Alberts, J. R. (n.d.). The Biparental Care System of the California Mouse, Peromyscus californicus. *Journal of Comparative Psychology 19S7*, *101*(2), 169–177. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.597.5446&rep=rep1&type=pdf

Gubernick, D. J., & Nelson, R. J. (1989). Prolactin and paternal behavior in the biparental California mouse, Peromyscus californicus. *Hormones and Behavior*, *23*(2), 203–210. http://doi.org/10.1016/0018-506X(89)90061-5

Gubernick, D. J., & Teferi, T. (2000). Adaptive significance of male parental care in a monogamous mammal. *Proceedings. Biological Sciences*, *267*(1439), 147–50. http://doi.org/10.1098/rspb.2000.0979

Gubernick, D. J., Wright, S. L., & Brown, R. E. (1993). The significance of father’s presence for offspring survival in the monogamous California mouse, Peromyscus californicus. *Animal Behaviour*, *46*(3), 539–546. http://doi.org/10.1006/ANBE.1993.1221

Hall, M. L. (2000). The function of duetting in magpie-larks: conflict, cooperation, or commitment? *Animal Behaviour*, *60*(5), 667–677. http://doi.org/10.1006/anbe.2000.1517

Holy, T. E., & Guo, Z. (2005). Ultrasonic Songs of Male Mice. *PLoS Biology*, *3*(12), e386. http://doi.org/10.1371/journal.pbio.0030386

Inagaki, H., Kuwahara, M., Kikusui, T., & Tsubone, H. (2005). The influence of social environmental condition on the production of stress-induced 22 kHz calls in adult male Wistar rats. *Physiology & Behavior*, *84*(1), 17–22. http://doi.org/10.1016/j.physbeh.2004.10.006

Insel, T. R., Preston, S., & Winslow, J. T. (1995). Mating in the monogamous male: Behavioral consequences. *Physiology & Behavior*, *57*(4), 615–627. http://doi.org/10.1016/0031-9384(94)00362-9

Insel, T. R., & Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(13), 5981–5985. http://doi.org/10.1073/pnas.89.13.5981

Jeanne, R. L. (2016). Division of labor is not a process or a misleading concept. *Behavioral Ecology and Sociobiology*, *70*(7), 1109–1112. http://doi.org/10.1007/s00265-016-2146-7

Kalcounis-Rueppell, M. C., Metheny, J. D., & Vonhof, M. J. (2006). Production of ultrasonic vocalizations by Peromyscus mice in the wild. *Frontiers in Zoology*, *3*(1), 3. http://doi.org/10.1186/1742-9994-3-3

Kalcounis-Rueppell, M. C., Petric, R., Briggs, J. R., Carney, C., Marshall, M. M., Willse, J. T., … Crossland, J. P. (2010). Differences in ultrasonic vocalizations between wild and laboratory California mice (Peromyscus californicus). *PloS One*, *5*(4), e9705. http://doi.org/10.1371/journal.pone.0009705

Kalcounis-Rueppell, M. C., Pultorak, J. D., & Marler, C. A. (2018). Ultrasonic Vocalizations of Mice in the Genus Peromyscus. *Handbook of Behavioral Neuroscience*, *25*, 227–235. http://doi.org/10.1016/B978-0-12-809600-0.00022-6

Keebaugh, A. C., & Young, L. J. (2011). Increasing oxytocin receptor expression in the nucleus accumbens of pre-pubertal female prairie voles enhances alloparental responsiveness and partner preference formation as adults. *Hormones and Behavior*, *60*(5), 498–504. http://doi.org/10.1016/j.yhbeh.2011.07.018

King, A. J., Fürtbauer, I., Mamuneas, D., James, C., Manica, A., Edenbrow, M., … Louca, V. (2013). Sex-Differences and Temporal Consistency in Stickleback Fish Boldness. *PLoS ONE*, *8*(12), e81116. http://doi.org/10.1371/journal.pone.0081116

King, A. J., Williams, L. J., & Mettke-Hofmann, C. (2015). The effects of social conformity on Gouldian finch personality. *Animal Behaviour*, *99*, 25–31. http://doi.org/10.1016/j.anbehav.2014.10.016

Knutson, B., Burgdorf, J., & Panksepp, J. (2002). Ultrasonic vocalizations as indices of affective states in rats. *Psychological Bulletin*, *128*(6), 961–977. http://doi.org/10.1037/0033-2909.128.6.961

Koloff, J., & Mennill, D. (2011). Aggressive responses to playback of solos and duets in a Neotropical antbird. *Animal Behaviour*, *82*(3), 587–593. http://doi.org/10.1016/j.anbehav.2011.06.021

Kroes, R. A., Burgdorf, J., Otto, N. J., Panksepp, J., & Moskal, J. R. (2007). Social defeat, a paradigm of depression in rats that elicits 22-kHz vocalizations, preferentially activates the cholinergic signaling pathway in the periaqueductal gray. *Behavioural Brain Research*, *182*(2), 290–300. http://doi.org/10.1016/j.bbr.2007.03.022

Leimar, O., Hammerstein, P., Agrawal, A. A., Agrawal, A. A., Fordyce, J. A., Atsatt, P. R., … Wakano, J. Y. (2010). Cooperation for direct fitness benefits. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *365*(1553), 2619–26. http://doi.org/10.1098/rstb.2010.0116

Leng, G., & Ludwig, M. (2016). Intranasal Oxytocin: Myths and Delusions. *Biological Psychiatry*. http://doi.org/10.1016/j.biopsych.2015.05.003

Levin, R. N. (1996). Song behaviour and reproductive strategies in a duetting wren,Thryothorus nigricapillus: I. Removal experiments. *Animal Behaviour*, *52*(6), 1093–1106. http://doi.org/10.1006/anbe.1996.0257

Li, T., Chen, X., Mascaro, J., Haroon, E., & Rilling, J. K. (2017). Intranasal oxytocin, but not vasopressin, augments neural responses to toddlers in human fathers. *Hormones and Behavior*, *93*, 193–202. http://doi.org/10.1016/J.YHBEH.2017.01.006

Lieberwirth, C., & Wang, Z. (2016). The neurobiology of pair bond formation, bond disruption, and social buffering. *Current Opinion in Neurobiology*. http://doi.org/10.1016/j.conb.2016.05.006

Litvin, Y., Blanchard, D. C., & Blanchard, R. J. (2007). Rat 22kHz ultrasonic vocalizations as alarm cries. *Behavioural Brain Research*, *182*(2), 166–172. http://doi.org/10.1016/j.bbr.2006.11.038

Lukas, D., & Clutton-Brock, T. H. (2013). The evolution of social monogamy in mammals. *Science (New York, N.Y.)*, *341*(6145), 526–30. http://doi.org/10.1126/science.1238677

Mahrt, E. J., Perkel, D. J., Tong, L., Rubel, E. W., & Portfors, C. V. (2013). Engineered deafness reveals that mouse courtship vocalizations do not require auditory experience. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(13), 5573–83. http://doi.org/10.1523/JNEUROSCI.5054-12.2013

Mathews, L. M. (2002). Territorial cooperation and social monogamy: factors affecting intersexual behaviours in pair-living snapping shrimp. *Animal Behaviour*, *63*(4), 767–777. http://doi.org/10.1006/anbe.2001.1976

Morales, M. A., Barone, J. L., Henry, C. S., Agrawal, A. A., Fordyce, J. A., Axelrod, R., … Wilson, E. (2008). Acoustic alarm signalling facilitates predator protection of treehoppers by mutualist ant bodyguards. *Proceedings. Biological Sciences / The Royal Society*, *275*(1645), 1935–41. http://doi.org/10.1098/rspb.2008.0410

Noe, R. (2006). Cooperation Experiments: coordination through communication versus acting apart together. *Animal Behaviour*, *71*, 1–18. http://doi.org/10.1016/j.anbehav.2005.03.037

Numan, M., & Young, L. J. (2016). Neural mechanisms of mother-infant bonding and pair bonding: Similarities, differences, and broader implications. *Hormones and Behavior*. http://doi.org/10.1016/j.yhbeh.2015.05.015

Page, R. E., Scheiner, R., Erber, J., & Amdam, G. V. (2006). The Development and Evolution of Division of Labor and Foraging Specialization in a Social Insect (Apis mellifera L.). *Current Topics in Developmental Biology*, *74*, 253–286. http://doi.org/10.1016/S0070-2153(06)74008-X

Pasch, B., Tokuda, I. T., & Riede, T. (2017). Grasshopper mice employ distinct vocal production mechanisms in different social contexts. *Proceedings. Biological Sciences*, *284*(1859), 20171158. http://doi.org/10.1098/rspb.2017.1158

Portfors, C. V. (n.d.). Types and Functions of Ultrasonic Vocalizations in Laboratory Rats and Mice.

Pultorak, J. D., Fuxjager, M. J., Kalcounis-Rueppell, M. C., & Marler, C. A. (2015). Male fidelity expressed through rapid testosterone suppression of ultrasonic vocalizations to novel females in the monogamous California mouse. *Hormones and Behavior*, *70*, 47–56. http://doi.org/10.1016/j.yhbeh.2015.02.003

Pultorak, J. D., Matusinec, K. R., Miller, Z. K., & Marler, C. A. (2017). Ultrasonic vocalization production and playback predicts intrapair and extrapair social behaviour in a monogamous mouse. *Animal Behaviour*, *125*, 13–23. http://doi.org/10.1016/j.anbehav.2016.12.023

Quintana, D. S., Alvares, G. A., Hickie, I. B., & Guastella, A. J. (2015). Do delivery routes of intranasally administered oxytocin account for observed effects on social cognition and behavior? A two-level model. *Neuroscience & Biobehavioral Reviews*, *49*, 182–192. http://doi.org/10.1016/J.NEUBIOREV.2014.12.011

Quintana, D. S., & Woolley, J. D. (2016). Intranasal Oxytocin Mechanisms Can Be Better Understood, but Its Effects on Social Cognition and Behavior Are Not to Be Sniffed At. *Biological Psychiatry*, *79*(8), e49–e50. http://doi.org/10.1016/J.BIOPSYCH.2015.06.021

Reburn, C. J., & Wynne-Edwards, K. E. (1999). Hormonal Changes in Males of a Naturally Biparental and a Uniparental Mammal. *Hormones and Behavior*, *35*(2), 163–176. http://doi.org/10.1006/hbeh.1998.1509

Reim, T., & Scheiner, R. (2014). Division of labour in honey bees: age- and task-related changes in the expression of octopamine receptor genes. *Insect Molecular Biology*, *23*(6), 833–841. http://doi.org/10.1111/imb.12130

Ribble, D. O. (1991). The monogamous mating system of Peromyscus californicus as revealed by DNA fingerprinting. *Behavioral Ecology and Sociobiology*, *29*(3), 161–166. http://doi.org/10.1007/BF00166397

Ribble, D., & Salvioni, M. (1990). Social organization and nest co-occupancy in Peromyscus californicus, a monogamous rodent. *Behavioral Ecology and Sociobiology*, *26*(1), 9–15. http://doi.org/10.1007/BF00174020

Robinson, G. E. (2003). Regulation of Division of Labor in Insect Societies. *Http://Dx.Doi.Org/10.1146/Annurev.En.37.010192.003225*.

Robson, S. K. A., & Traniello, J. F. A. (2016). Division of labor in complex societies: a new age of conceptual expansion and integrative analysis. *Behavioral Ecology and Sociobiology*, *70*(7), 995–998. http://doi.org/10.1007/s00265-016-2147-6

Rogers, W. (2010). Parental Investment and Division of Labor in the Midas Cichlid (Cichlasoma citrinellum). *Ethology*, *79*(2), 126–142. http://doi.org/10.1111/j.1439-0310.1988.tb00706.x

Seffer, D., Schwarting, R. K. W., & Wöhr, M. (2014). Pro-social ultrasonic communication in rats: Insights from playback studies. *Journal of Neuroscience Methods*, *234*, 73–81. http://doi.org/10.1016/j.jneumeth.2014.01.023

Seyfarth, R. M., & Cheney, D. L. (2010). Production, usage, and comprehension in animal vocalizations. *Brain and Language*, *115*(1), 92–100. http://doi.org/10.1016/j.bandl.2009.10.003

Seyfarth, R. M., Cheney, D. L., Bergman, T., Fischer, J., Zuberbühler, K., & Hammerschmidt, K. (2010). The central importance of information in studies of animal communication. *Animal Behaviour*, *80*(1), 3–8. http://doi.org/10.1016/j.anbehav.2010.04.012

Seyfarth, R. M., Cheney, D. L., & Marler, P. (1980). Vervet monkey alarm calls: Semantic communication in a free-ranging primate. *Animal Behaviour*, *28*(4), 1070–1094. http://doi.org/10.1016/S0003-3472(80)80097-2

Shamay-Tsoory, S. G., & Abu-Akel, A. (2016). The Social Salience Hypothesis of Oxytocin. *Biological Psychiatry*. http://doi.org/10.1016/j.biopsych.2015.07.020

Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution*, *19*(7), 372–378. http://doi.org/10.1016/j.tree.2004.04.009

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral Syndromes: An Integrative Overview. *The Quarterly Review of Biology*, *79*(3), 241–277. http://doi.org/10.1086/422893

Sinn, D. L., Moltschaniwskyj, N. A., Wapstra, E., & Dall, S. R. X. (2010). Are behavioral syndromes invariant? Spatiotemporal variation in shy/bold behavior in squid. *Behavioral Ecology and Sociobiology*, *64*(4), 693–702. http://doi.org/10.1007/s00265-009-0887-2

Slocombe, K. E., & Zuberbühler, K. (2007). Chimpanzees modify recruitment screams as a function of audience composition. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(43), 17228–33. http://doi.org/10.1073/pnas.0706741104

Solomon, N. G. (1993). Comparison of parental behavior in male and female prairie voles (*Microtus ochrogaster*). *Canadian Journal of Zoology*, *71*(2), 434–437. http://doi.org/10.1139/z93-061

Stamps, J. A. (2007). Growth-mortality tradeoffs and "personality traits" in animals. *Ecology Letters*, *10*(5), 355–63. http://doi.org/10.1111/j.1461-0248.2007.01034.x

Swaney, W. T., Cabrera-Álvarez, M. J., & Reader, S. M. (2015). *Behavioural responses of feral and domestic guppies (Poecilia reticulata) to predators and their cues*. *Behavioural Processes* (Vol. 118). http://doi.org/10.1016/j.beproc.2015.05.010

Townsend, S. W., Manser, M. B., Fitch, W. T., Neubauer, J., Herzel, H., Herzel, H., … Fitch, W. T. (2011). The function of nonlinear phenomena in meerkat alarm calls. *Biology Letters*, *7*(1), 47–9. http://doi.org/10.1098/rsbl.2010.0537

Van De Waal, E., Borgeaud, C., & Whiten, A. (n.d.). *Potent Social Learning and Conformity Shape a Wild Primate's Foraging Decisions*. Retrieved from www.sciencemag.org/cgi/content/full/science.1233675/DC1

Veening, J. G., & Olivier, B. (2013). Intranasal administration of oxytocin: Behavioral and clinical effects, a review. *Neuroscience & Biobehavioral Reviews*, *37*(8), 1445–1465. http://doi.org/10.1016/J.NEUBIOREV.2013.04.012

Von Merten, S., Hoier, S., Pfeifle, C., & Tautz, D. (2014). A role for ultrasonic vocalisation in social communication and divergence of natural populations of the house mouse (Mus musculus domesticus). *PLoS ONE*, *9*(5). http://doi.org/10.1371/journal.pone.0097244

Webster, M. M., & Ward, A. J. W. (2011). Personality and social context. *Biological Reviews*, *86*(4), 759–773. http://doi.org/10.1111/j.1469-185X.2010.00169.x

Wright, C. M., Holbrook, C. T., & Pruitt, J. N. (2014). Animal personality aligns task specialization and task proficiency in a spider society. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(26), 9533–7. http://doi.org/10.1073/pnas.1400850111

Young, L. J., & Barrett, C. E. (2015). Neuroscience. Can oxytocin treat autism? *Science (New York, N.Y.)*, *347*(6224). http://doi.org/10.1126/science.aaa8120

Chapter 1

The function of ultrasonic vocalizations during territorial defense by pair-bonded male and female California mice

Originally published as:
Rieger, N. S., & Marler, C. A. (2018). The function of ultrasonic vocalizations during territorial defence by pair-bonded male and female California mice. *Animal Behaviour*, *135*. http://doi.org/10.1016/j.anbehav.2017.11.008

Abstract

Acoustic communication is vital to complex social behaviors such as territorial defense. The use of ultrasonic vocalizations (USVs), particularly in territorial defense by monogamous species and females, remains understudied. We studied USV production and associated aggression in the monogamous, biparental, and territorial California mouse (*Peromyscus californicus*) in which both males and females were found to display similar levels of physical aggression against same-sex intruders. We identified specific USV calls that are modulated based on social context. Calls included 1) sustained vocalizations (SV): long, low bandwidth calls ranging from 22-25 kHz and 2) barks: short, high intensity calls beginning and ending in the audible range. Despite similarities in physical aggression, sex differences emerged in vocal communication. Only resident males, and not females, produced SVs prior to the onset of physical aggression, and were found to shorten the duration of individual SV calls over both the course of the pre-encounter phase and from the pre-encounter to encounter phase. In addition, the degree of SV shortening in males predicted offensive aggression of the resident. Males exhibited shorter SV calls during encounters than females. Barks occurred more frequently during female-female physical aggression than in male-male encounters and correlated highly with defensive aggression by intruders. Finally, a newly identified highly complex call, sweep-phrases, was recorded in a subset of both sexes in the pre- and post-encounter phases. The overall results indicate that USVs may play an important role in territorial defense during both territorial advertisement and aggression in a monogamous rodent. Overall, this monogamous species showed sex similarities in physical aggression but sex differences in vocal communication and a more sophisticated function for SVs than previously recognized.

Keywords: Ultrasonic vocalizations, aggression, agonistic, territoriality, animal communication, social behavior, monogamy, pair bond, *Peromyscus californicus,* sex difference

Highlights

- No sex differences were displayed in physical aggression in this monogamous rodent.
- Males produced more and longer sustained vocalizations (SV) before contact.
- Females produced more barks during encounters.
- Low-frequency barks correlated highly with defensive aggression in both sexes.
- We recorded a new complex call type, sweep phrases.

Introduction

Vocalizations play a distinct role in the expression and escalation of aggressive behaviors in many species (van Staaden, Searcy, & Hanlon, 2011). These signals can provide information to conspecifics about the resource holding potential (RHP) and/or fighting ability of the signaler, thus determining whether contests will escalate (Maynard Smith, Price, Smith, & Parker, 1973). Vocal signals during aggression can differ or be modulated in several ways including their amplitude, frequency, complexity and duration (Seyfarth & Cheney, 2010; van Staaden, Searcy & Hanlon 2011). High amplitude calls are indicators of aggression in many species (Brumm, 2004; Brumm & Ritschard, 2011; but see Maddison, Anderson, Prior, Taves et al., 2012) and low frequency calls often signal larger body size and greater fighting ability (Reby, McComb, Cargnelutti, Darwin, et al., 2005; Reichert & Gerhardt, 2014). Complexity of calls, such as bird trills, indicate fighting quality and playback studies reveal that producing more trills by an "intruder" reduces the aggressive response of territory holders (Cramer & Jordan Price, 2007; Illes, Hall, & Vehrencamp, 2006). Alterations in call duration can signal aggression in some species; for example, black redstarts (*Phoenicurus ochruros*) shorten call duration to produce a greater number of calls in the same time frame (Apfelbeck, Kiefer, Mortega, Goymann et al., 2012), while others such as European starlings (*Sturnus vulgaris*) increase song length during territorial defense (Alger, Larget, & Riters, 2016). Taken together, this information on non-rodent species indicates that modulation of call types and spectral properties is of particular importance to aggression.

In rodents, the role of vocalizations in both aggressive behavior and territorial defense remains understudied with limited overlap with the species just described. Generally, rodents produce ultrasonic vocalizations (USVs) at frequencies >22 kHz as a means of vocal communication during social behavior (Arriaga, Zhou, & Jarvis, 2012; Holy & Guo, 2005; Sales, 2010a). Rats (*Rattus norvegicus*), for instance, produce USV calls in two major frequency bands, 22 kHz and 50 kHz, (Burgdorf, Kroes, Moskal, Pfaus, et al., 2008; Kroes, Burgdorf, Otto, Panksepp, et al,, 2007; Sales, 1972; Sirotin, Costa, & Laplagne, 2014), whereas mice (*Mus musculus*) produce a variety of USV calls at a frequency of > 45 kHz

(Hoffman, Musolf, & Penn, 2012; von Merten, Hoier, Pfeifle, Tautz, et al. 2014, Portfors, 2007). However, lower frequency calls do not uniformly indicate increased aggression or body size across rodent species (Hoffman, Musolf, & Penn, 2012). Further, increased call amplitude, a signal of aggression or territoriality in many non-rodent species, has instead been linked to submissive behavior in rodents (Constantini & D'amato, 2006; Portfors, 2007). Other call attributes, therefore, may be more important to rodent aggression. Calls are altered during aggression with species such as the Turkish spiny mouse (*Acomys cilicicus*) in which males increase number of USVs produced and alter the proportion of call types in response to agonistic encounters (Griffiths, Dow, & Burman, 2010). In contrast, house mice (*Mus musculus*) and Syrian hamsters (*Mesocricetus auratus*) decrease USV calling and increase lower frequency broadband calls (< 20 kHz) during aggression (Arriaga, Zhou, & Jarvis, 2012; Fernández-Vargas, & Johnston, 2015; Keesom, Rendon, Demas, & Hurley, 2015). Call duration and complexity have been tied to aggressive output in rodents. For example, castrated Alston's singing mice (*Scotinomys tequina*) produce fewer trills with shorter durations while expressing less aggression compared to testosterone implanted controls (Alger, Larget, & Riters, 2016; Pasch, George, Hamlin, Guillette, et al., 2011). Further exploration of the role of USVs in aggression in rodents is warranted, particularly in females and monogamous species which to this point remain understudied.

The vast majority of studies of vocal communication within aggressive contexts have focused on males (Cain, Cockburn, & Langmore, 2015; Cain & Langmore, 2015). In many rodent and non-rodent species, males are both more aggressive and more vocal than females (Reby, McComb, Cargnelutti, Darwin, et al., 2005; Reichert & Gerhardt, 2013a). However, across taxa, in species where females are territorial, and in monogamous species, aggressive behavior is more similar between sexes, with both males and females displaying aggression towards novel intruders (Langmore, 1998). Monogamous species that share territorial defense responsibilities often do so in a sex-specific manner defending territories against same-sex intruders as a form of mate guarding with both males and females using similar vocal repertoires during aggression (Cross, Zedrosser, Nevin, & Rosell, 2014; Fedy & Stutchbury, 2005; Koloff & Mennill, 2011; Levin, 1996; Levin & Wingfield, 1992; but see Yang, Zhang, Cai, Stokke, et al., 2011). While there is evidence in both duetting birds (Colombelli-Négrel, 2016; Quinard & Cézilly, 2012), and California mice (*Peromyscus californicus*) (Rieger & Marler, unpublished) for coordinated territorial defense that is not sex-specific, same-sex intruders were used to induce reliable aggression. Here we expand our understanding of the role of vocal communication, particularly USVs, across different phases of an aggressive encounter by studying male-male and female-female aggression and the associated vocalizations of the monogamous California mouse.

We worked with the California mouse, a strictly monogamous biparental species that forms lifelong pair bonds (Gubernick, 1988; Ribble, 1991). Both male and female California mice exhibit reliable aggression against intruders, especially in the context of territorial defense (Ribble & Salvioni, 1990). California mice have a rich and distinct set of USV call types that have been categorized both in the field and laboratory (Kalcounis-Rueppell, Petric, Briggs, Carney et al., 2010; Kalcounis-Rueppell, Metheny, & Vonhof, 2006), that vary based on social context (Pultorak, Fuxjager, Kalcounis-Rueppell, & Marler, 2015; Pultorak, Matusinec, Miller, & Marler, 2017) and do not appear to differ spectrally between sexes (Briggs & Kalcounis-Rueppell, 2011). In this study, we focused on three major call types, sustained vocalizations (SVs), barks and, described here for the first time, complex sweep-phrases (phrases of complex and simple sweeps; see methods). Briefly, SVs have been hypothesized to act as long-distance communication between individuals and can be modulated both in the duration and total number of calls produced (previously referred to as syllables) and the number of calls produced within a bout (previously referred to as a phrase) (Kalcounis-Rueppell, Pultorak & Marler in press). Barks are hypothesized to be aggressive in nature. Sweep-phrase functions are to this point unknown. Individual sweeps that make up these phrases, however, are simple frequency modulated calls whose production can be altered in response to changing social context (Kalcounis-Rueppell, Pultorak & Marler, in press).

To understand the role of USVs in intra-sexual territorial defense of monogamous pairs we used a resident-intruder paradigm encompassing three main objectives. The first was to directly compare the aggressive behaviors and territorial defense of male and female California mice using same-sex encounters. We hypothesized that, as a monogamous species in which both sexes display aggression (Davis & Marler, 2004; Davis & Marler, 2003; Fuxjager, Oyegbile, & Marler, 2011; Oyegbile & Marler, 2005; Trainor, Pride, Villalon Landeros, Knoblauch, et al., 2011), males and females would exhibit few sex differences in aggression when compared directly. Second, we sought to characterize the USV call types produced by male and female residents at different stages of a territorial intrusion: baseline (prior to the introduction of an intruder), pre-encounter (before any physical aggression but after initial visual, olfactory and auditory contact), encounter and post encounter (after contest resolution). We were able to isolate the vocalizations of individuals both prior to and after physical aggression but not during physical encounters. We predicted that SVs would be the predominant vocalization for males and females prior to physical aggression as Kalcounis-Rueppell and colleagues speculate that these calls function in long distance communication based on field research (i.e. communication with the mate) (Kalcounis-Rueppell, Matheny, & Vonhoff, 2006). We further explored both the duration of single SV calls and the number of SV calls in a bout across social contexts to determine if SV characteristics were modulated based on physical aggression. We also predicted that barks would be the predominant call during aggression due to their previously found role in male-female interactions, where after a period of separation reunited mates produced barks only when aggression also occurred (Pultorak, Matusinec, Miller, & Marler, 2017). Finally, we tested whether these vocalizations were predictive of aggressive behavior. Overall, we sought to bring a greater understanding of the role of USVs in the dynamic aggressive interactions of rodents.

Methods

Animals

Twenty-four male (12 bonded and 12 sexually naïve age 4-6 months) and 24 female California mice (12 bonded and 12 sexually naïve; age 4-6 months) were obtained from a laboratory colony at the University of Wisconsin-Madison. Mice were housed either in opposite sex pairs or with 1-2 same sex conspecifics in standard cages (48x27x16cm) lined with aspen bedding, a nestlet and water and food

(Purina 5015^{TM} mouse chow) available *ad libitum*. The colony room was maintained between 20-23ºC on a 14:10 light/dark cycle (Lights on at 21:00 CST), with behavioral testing occurring 1-4 h after the onset of the dark cycle under dim red light. Animals used in dyads (either for pairing or aggressive encounters) were unrelated for at least two generations.

Ethical Note

Animals were maintained in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals*. Animal treatment and research protocols were approved by the University of Wisconsin, Madison College of Letters and Sciences Institutional Animal Care and Use Committee (IACUC-L00547). No animals were injured by any of the behavioral manipulations and/or assays.

Testing Apparatus

Testing occurred in a glass aquarium (50x30x30 cm) retrofitted with metal tracks to allow for the introduction and removal of a Plexiglas divider. The Plexiglas divider included two mesh cutouts that allowed for visual, olfactory and acoustic interactions between conspecifics but prevented physical contact. With the divider present, the testing chamber was split into two even compartments (25x30x30 cm). The lid of the chamber included five cm diameter holes placed 1 inch from the corners of the arena for the placement of microphones into opposite sides of the chamber to record USVs. With the divider in place, we recorded USVs in the early stages of an aggressive encounter prior to physical contact and were able to assign USVs to a single individual.

Experimental procedure

Males and females were randomly assigned to be either bonded residents or sexually naïve intruders with residents cohabitating for two weeks with their pair-bond mate prior to testing, while intruders remained with their original same sex cage-mates. Bonding was verified by the observation of side-by-side contact between mates during cohabitation as seen in other monogamous rodents (Insel, Preston, & Winslow, 1995; Williams, Catania, & Carter, 1992). Twenty-four hours prior to aggression trials, residents were moved from their standard cage to the testing chamber (divider not present) lined with aspen bedding, a nestlet and containing food and water *ad libitum*. This 24 h period allowed for the formation of the residency effect creating a home field advantage for individuals where the arena becomes their territory, increasing the likelihood of winning (Bester-Meredith & Marler, 2001; Fuxjager & Marler, 2009; Fuxjager, Mast, Becker, & Marler, 2009, Fuxjager, Xhao, Rieger & Marler, 2017). Intruders were randomly assigned to a same-sex resident and had a small patch of fur shaved from their right flank to allow for experimenters to recognize individuals. All intruders were socially naïve except for exposure to cage mates with no sexual or aggression testing experience.

We used a resident-intruder paradigm divided into four encounter phases to characterize territorial defense and the accompanying ultrasonic vocalizations. The four encounter phases were (1) baseline (focal animal was isolated with no divider present), (2) pre-encounter (a same-sex intruder was introduced to the arena behind a Plexiglas divider with mesh cutouts), (3) encounter (the divider was removed and physical contact was made possible), and (4) post-encounter (the resident and intruder mice were once again separated behind the divider).

The focal resident remained in the testing chamber, while their mate was removed, and returned to the original standard cage. The focal resident was isolated in the testing chamber for 10 minutes prior to baseline testing. During the baseline phase, one-minute acoustic and video recordings were made of the isolated resident to obtain a baseline level of vocalizations. The divider was introduced and the pre-encounter phase occurred with the resident on the nest side of the testing chamber and the intruder present on the opposite side of the divider. Vocalizations and behavior were recorded for four minutes. The divider was then removed so the mice could interact in the encounter phase for eight minutes, which has previously been shown to be a sufficient amount of time for California mice to win encounters and produce a winner effect (Fuxjager, Oyegbile, & Marler, 2011). During this interaction phase, however, calls could not be conclusively localized to an individual and, therefore, resident and intruder calls were combined for analysis. The mice were then separated by the divider on their original sides and the post-encounter phase occurred for four minutes, allowing for discrimination of each individual's calls. Differences in phase length were based on pilot studies used to determine how long individuals were likely to call. Following testing, animals were removed from the arenas and returned to their standard cage.

Behavior Analysis

Video recordings were scored for aggressive behaviors by an observer blind to the sex of the animal. The number of aggressive behaviors were counted and parsed out into either offensive (bites to the flank, chases and wrestling bouts), or defensive (bites to the neck, rearing up and boxing/jabbing) behaviors (Blanchard, Wall, & Blanchard, 2003) and scored as frequency of occurrence for each specific behavior. Submissive behaviors (retreats, jumps away and freezes) were also counted as frequency of occurrence (Pultorak, Fuxjager, Kalcounis-Reuppell, & Marler, 2015). A winner was designated as a mouse that conducted three consecutive attacks that elicited losing behaviors by its opponent (Fuxjager & Marler, 2009; Fuxjager, Montgomery, Becker, & Marler, 2010; Oyegbile & Marler, 2006). A winner index was also calculated to assess the difference between aggressive and submissive behaviors as a function of total behavior for each individual: $\frac{Aggressive\ Behavior - Submissive\ Behavior}{Aggressive\ behavior + Submissive\ behavior}$ (Fuxjager, Montgomery, Becker, & Marler, 2010). The winner index allows for a finer grain analysis of the efficiency by which an individual wins an aggressive encounter and better reveals individual variation across winners.

Ultrasonic vocalization analysis

Ultrasonic vocalizations were recorded using two Emkay/Knowles FG series microphones (detection range 10-120kHz) placed in opposite corners of the arena 55 cm apart and 20 cm from the arena floor, with one microphone in each of the resident and intruder compartments. To control for potential differences in microphone sensitivity, microphone placement was randomized across trials between the resident and intruder sides of the arena. Microphone channels were calibrated to equal gain (-60 dB noise floor). RECORDER software (Avisoft Bioacoustics, Berlin, Germany) was used to produce WAV file recordings for each of the four encounter stages in all trials. Recordings were made using a 250-kHz sampling rate with 16-bit resolution. Spectrograms were produced with a 512 Fast Fourier Transform (FFT) using Avisoft-SASLab Pro sound analysis software (Avisoft Bioacoustics). Ultrasonic vocalizationss were differentiated by visual and auditory inspection of spectrograms and WAV files with the sampling rate reduced to 4% of real-time speed for auditory inspection (11,025 kHz). All calls were attributed to either the resident or the intruder based on visual amplitude differences seen in the spectrogram during phases when the divider was in place. In the rare case that there was ambiguity in call origin, time of arrival of the start of the USV was compared between channels (accuracy up to 0.0001 s); this occurred in <4% of all calls analyzed. In cases where no divider was present (encounter phase) and interacting animals were close together (generally in the center of the arena) calls were analyzed as a dyad. All calls were analyzed by an experienced observer blind to the sex and residency status of the focal mouse.

USV Terminology and Structure

Three categories of USV calls were analyzed in this study (figure 1), two of which, sustained vocalizations (SVs) and barks, were defined based on previous work focusing on California mouse USVs (Kalcounis-Rueppell, Petric, Briggs, Carney, et al., 2010; Pultorak, Fuxjager, Kalcounis-Reuppell, & Marler, 2015). SVs are low bandwidth calls that feature low modulation with a peak frequency around 20 kHz and a duration of 100-500 ms for each individual syllable. SVs included up to 13 calls in our laboratory making up a singular bout, using an inter-syllable interval of 150 ms (a duration longer than this denoted the start of a new bout) (Kalcounis-Rueppell, Pultorak, & Marler, in press). In this study, we analyzed SVs in two ways, first by counting each individual call and second by counting the number of calls per bout produced by an individual. The length of individual calls was measured to determine if differences correspond with different aspects of social behavior as described in both field and laboratory studies (Kalcounis-Rueppell, Petric, Briggs, Carney et al., 2010; Pultorak, Fuxjager, Kalcounis-Rueppell, & Marler, 2015). Barks are relatively short, high amplitude calls, with an upside down chevron shape that begins and ends in the audible range, usually occurring in phrases of more than one bark (Kalcounis-Rueppell, Metheny, & Vonhoff, 2006; Kalcounis-Reuppell, Pultorak, Marler, In Press). Sweep-phrase calls are previously undescribed calls with durations of 30-100 ms, made up of continuous and overlapping frequency modulated calls including upward and downwardly modulated sweeps, similar to frequency jump calls in mice (Kalcounis-Reuppell, Pultorak, & Marler, in press; Hoffman, Musolf, & Penn 2012, Chabout, Sarkar, Dunson, & Jarvis, 2015, Arriaga, & Jarvis, 2013). Compared to other California mouse calls sweep-phrases are highly complex with a wide bandwidth from 25-100 kHz, with multiple inflection points over a short duration with periods of silence of at least 30 ms both before and after the call (see supplemental audio for examples of each call type).

Statistics

All analyses were performed using SPSS (SPSS version 22, IBM Corp., NY). One male and two females were excluded from analyses because they did not perform any aggressive or submissive behaviors and produced no vocalizations throughout the paradigm, giving a final sample size of 11 male and 10 female resident-intruder dyads. Aggressive and submissive behaviors and USV calls per minute were log(x+1) transformed to correct for non-normal data. Offensive and defensive aggression, measured as frequencies, and attack latencies were analyzed using two-way ANOVA with sex and residency as factors. Winner index between males and females was compared using a student's t-test. SVs and bark calls were first analyzed across phase at the level of the dyad, to control for the inability to triangulate calls during the encounter phase, as calls produced per minute using a repeated measures ANOVA with sex and phase as factors. Where calls could be assigned to an individual, call production was then compared between resident and intruder males and females using a two-way repeated measures ANOVA with sex and residency as between subject's factors and phase as a within subject's factor. SV calls per bout were analyzed using a chi-square test on pooled calls within a dyad. Sweep-phrases were compared both within and between sexes as proportions of total calls produced during each phase using Chi-square and Fisher's exact tests because of the small sample sizes. Tukey post-hoc tests were used to correct for multiple statistical tests. Correlations between USV calls and aggressive behaviors were calculated using linear regression on log(x+1) normalized data. The alpha level for all analyses was set at 0.05.

Results

Male and female resident aggressive behavior

Overall, male (n = 11) and female (n = 10) residents were remarkably similar in their aggression levels. Resident males and females were equally likely to win a given same-sex encounter (11 of 11 males, 9 of 10 females, Fisher's exact test, p = 0.48), and showed no difference in overall winner index (males: 0.88 ± 0.08, females: 0.61 ± .13, student's t-test, t = 1.86, df = 19, p = 0.08). There was no sex difference in resident latency to attack (males: 66.55 ± 18.53 sec, females: 120.84 ± 38.21 sec, ANOVA, $F_{1, 20}$ = 0.91, p = 0.35), offensive aggression (males: 10.0 ± 2.47, females: 6.50 ± 1.21, ANOVA, $F_{1, 20}$ = .973 p = 0.33) or defensive aggression (males: 0.09 ± 0.08, females: 0.40 ± 0.18, ANOVA, $F_{1, 20}$= 1.32 p = 0.26) towards same-sex conspecifics. There was also no significant sex difference in resident submissive behaviors (males: 0.36 ± 0.28, females: 1.40 ± 0.50, ANOVA, $F_{1, 20}$ = 0.02, p = 0.89). Moreover, there were no differences in size between male (40.65 ± 1.71 g) and female residents (44.87 ± 3.42 g) (ANOVA, $F_{3, 34}$ = 1.08, p = 0.29) or between residents (n=21) (42.76 ± 1.93 g) and intruders (n=21) (43.49 ± 2.46 g) (ANOVA, $F_{1, 41}$ = 0.22, p = 0.83).

There were no sex differences exhibited by intruder males (n = 11) or females (n = 10) in the display of offensive aggression (males: 2.91 ± 0.65, females: 3.5 ± 0.54, ANOVA, $F_{3, 34}$ = 0.01, p = 0.9), defensive aggression (males: 6.45 ± 2.88, females: 13.7 ± 5.23, $F_{3, 34}$ = 0.87, p = 0.39) or submissive behaviors (males: 11.75 ± 3.53, females: 8.50 ± 1.95, ANOVA, $F_{3, 34}$ = 0.37, p = 0.72) exhibited during the encounter.

When comparing all residents (n = 21) and intruders (n = 21) and controlling for sex, residents displayed significantly more offensive aggression than intruders (residents, 8.33 ± 1.40, intruders, 3.19 ± 0.42, ANOVA, $F_{1,41}$ = 11.70, $p < 0.01$), while intruders displayed significantly more defensive aggression (residents: 0.24 ± 0.11, intruders: 9.90 ± 3.06, $F_{1,41}$ = 8.96, $p < 0.01$) and more submissive behaviors than residents (residents: 0.86 ± 0.25, intruders: 9.24 ± 1.73, , $F_{1,41}$ = 35.99, $p < 0.01$).

USV analysis

Sustained vocalizations (SV)

When analyzing calls at the level of the dyad, we found changes in SV production both between and within sexes over the four encounter phases: baseline, pre-encounter, encounter and post-encounter. There was a significant change in SV production across phases by sex (ANOVA, $F_{1,17}$ = 5.26, $p < 0.01$, partial-eta squared = 0.454). Specifically, post-hoc tests showed that male dyads (n = 11) produced significantly more SVs than female dyads (n = 10) in the pre-encounter stage (males: 9.84 ± 4.15, females: 0.15 ± 0.15, ANOVA, $F_{1,18}$ = 5.26, $p < 0.01$). This sex difference disappeared in the encounter phase when female SV production increased to male levels (males: 3.01 ± 1.35, females: 7.36 ± 2.5, ANOVA, $F_{1,18}$ = 0.18, p = 0.976).

Within sexes, both males and females showed changes across the four phases ($F_{1,18}$ = 9.15, $p < 0.01$, partial eta^2 = 0.591, figure 2A). Using post-hoc tests we found that, for male dyads (n = 11 pairs), the presence of a same-sex intruder behind a divider elicited a significant increase in SVs compared to baseline (baseline: 0 ± 0, pre-encounter: 9.84 ± 4.15, p = 0.004). Male SV production remained at pre-encounter levels during the encounter phase (pre-encounter: 9.84 ± 4.15, encounter: 3.01 ± 1.35, p = 0.266). In contrast, female dyads (n = 10) did not differ from baseline in pre-encounter SV production, with only one female producing SV calls during this phase (baseline: 0 ± 0, pre-encounter: 0.15 ±0.15, p = 0.339). Females significantly increased SV production during the encounter phase compared to the pre-encounter phase (encounter: 7.36 ± 2.50, pre-encounter: 0.54 ± 0.50, p = 0.015), equaling the SV production of males. During the post-encounter stage, male SV production decreased to baseline levels (post-encounter 0.05 ± 0.05, $p = 0.31$). Vocalizations were then tested at the level of the individual for the two phases, pre-encounter and post-encounter in which both individuals were present and calls could be assigned to a specific individual. Resident males ($n = 11$) were significantly more likely to produce SV calls pre-encounter than resident females ($n = 10$) (7 males to 1 female, Fisher's exact test, $p < 0.01$). Resident males also produced significantly more SVs per minute than any other group at either time point (Resident males: Pre-encounter 9.31 ± 4.12, post-encounter 1.43 ± 1.41; Intruder males ($n = 11$): Pre-encounter 0.53 ± 0.21 post-encounter 0.05 ± 0.05; Resident females: Pre-encounter 0.15 ± 0.15, post-encounter 0.05 ± 0.05; Intruder females ($n=10$): Pre-encounter: 0.00 ± 0.00, post-encounter 0.03 ± 0.03, figure 2B).

SV calls and aggressive behavior

Several lines of evidence suggest that the number of SV calls produced pre-encounter by male residents is associated with greater aggression in the ensuing encounters. Most notably, the number of pre-encounter SV calls produced by resident males positively correlated with increased defensive aggression (rearing and boxing) in intruders (linear regression $F_{1, 9} = 19.5$, $R^2 = 0.68$, $p < 0.01$, Supplemental figure 1) but not resident offensive aggression ($F_{1,9} = 0.31$, $R^2 = 0.04$, $p = 0.59$). Intruders who were not exposed to SV calls during the pre-encounter stage ($n = 4$) did not display any defensive aggression (i.e. no boxing or rearing). There was a non-significant trend for a weak correlation between pre-encounter SV calling and a faster latency to aggression in males (Linear regression, $F_{1, 7} = R^2 = 0.17$, $p = 0.15$), hinting at a role for SV calling in territorial advertisement or in the resident's motivation to fight. Finally, pre-encounter SV calls correlated with number of barks produced during the subsequent encounter phase (Linear regression, $F_{1,10} = 5.097$, $R^2 = 0.384$, $p = 0.04$). In sum, pre-encounter SV calling behavior by residents was associated with greater levels of defensive behavior in the intruders.

SV Call Duration

Significant changes in SV call duration were found both between phases and between sexes. In resident males, pre-encounter SV call duration was analyzed within subjects such that the first 50% of calls produced by the resident were compared to the second 50%. The magnitude of durational change correlated with total aggression displayed by residents (linear regression, $F_{1,5} = 12.95$ n = 7, $R^2 = 0.7215$, p = 0.012), such that the more calls were shortened during the pre-encounter stage; the more aggression was displayed during the encounter. Pre-encounter SV call duration was not different between male residents and intruders (residents (7 males produced a total of 403 calls): 184.83 ± 18.5 ms, intruders (5 males produced a total of 30 calls): 166.3 ± 72.0, student's t-test, t = 0.29, df = 430, p = 0.81). Only one female resident produced SV calls pre-encounter (6 total calls) with the same average duration as male residents (males: (n = 403 total calls): 183.54 ± 20.5 ms, female (n = 6 total calls): 137.3 ± 56.1 ms, student's t-test, t = 0.90, df = 414, p = 0.71). SV call durations were significantly decreased from the pre-encounter to the encounter phase in male dyads (pre-encounter (n = 433 calls): 183.54 ± 20.5 ms, encounter (n = 282 calls): 104 ± 4.10 ms, student's t-test, t = 4.85, df = 690 $p < 0.01$). Males dyads also had significantly shorter SV call durations during encounters than female dyads (males (n= 282 calls): 104 ± 4 ms, females (n = 587 calls): 125 ± 3 ms, student's t-test, t = 4.07, df = 867 p = 0.013). Further, linear regression shows that the magnitude of the change in SV call duration in dyads, pre-encounter to encounter, predicted total aggression during the encounter phase (linear regression, $F_{1,12} = 6.794$, $R^2 = 0.3818$, p = 0.024, figure 3).

SV Bouts

Analysis of SV bout length across phases at the level of the dyad found that phrases of up to 11 syllables were produced in the pre-encounter phase with the most common bout length being one call bouts (33%, X^2, p = 0.03) followed by two and three call bouts (21% each) and four call bouts (10%) with all other bouts comprising less than 15% of the total bouts. Sex differences could not be examined pre-encounter because only one female produced any SV calls.

During the encounter phase, one call bouts were also the most common bout length for both sexes (81% of bouts X^2, $p < 0.01$). However, the proportion of one call bouts produced by a dyad in the encounter phase (81%) was significantly increased when compared to pre-encounter proportions (33%)

(X^2, $p < 0.01$, figure 4). The next most common bout length by proportion was two call bouts (12%), with all other bout lengths making up only 7% of the total SV vocalizations. The maximum SV bout length observed in both the pre-encounter and encounter phase was 11 calls and no sex differences were detected in either length or proportion of SV bouts during encounters (X^2 test, $p = 0.41$).

Barks

A sex difference was found in the production of barks during the encounter phase such that female dyads ($n = 10$) produced more barks than male dyads ($n = 11$) (males: 2.68 ± 1.15, females: 9.22 ± 3.47, $F_{1, 21} = 1.79$, $p = 0.046$, partial $eta^2 = 0.194$, Figure 5). Controlling for sex, bark production changed across phases for both male and female dyads such that barks were produced most often during the encounter phase in comparison to all other phases. (encounter ($n = 21$): 5.79 ± 1.26, all other phases 0.02 ± 0.02, ANOVA, $F_{1, 21}= 32.127$, $p < 0.001$, partial $eta^2 = 0.594$).

Barks and aggressive behavior

Barks only occurred following the onset of physical aggression, indicating that barks are intrinsically tied to physical aggression. Barks correlated strongly with frequency of occurrence of intruder boxing behavior, a form of defensive aggression, (linear regression, $F_{1,21} = 48.07$, $R^2 = 0.6923$, $p < 0.01$, supplemental figure 2) occurring most often when intruder mice were in the reared-up position and boxing at the resident. Barks were visually confirmed to be produced by intruders in this position in 8 of 17 dyads on video by hearing the bark while being able to observe the mouse opening its mouth while expelling air (see supplemental video). In the other cases, animals were generally blocked from view by the resident mouse or too far away from the camera to see this behavior clearly.

Sweep-phrases

Sweep-phrases were produced by 6 of 11 male residents and 3 of 10 female residents. Sweep-phrases occurred as standalone calls with distinct periods of silence both before and after the call for a minimum of 30 ms. The frequency range for these calls was between 25-100 kHz and had a duration of at least 30 ms. Over this time course, sweep phrases included a minimum of three inflection points compared to individual sweeps which include no more than one inflection points (Kalcounis-Reuppell, Pultorak, & Marler, 2015). Due to the small sample size, statistics for sweep-phrases were calculated based on their relative proportion of total calls and calls per phase (see supplemental table 1). Likelihood of producing sweep-phrases did not differ between resident male and female mice (6 of 11 males and 3 of 10 females, Fisher's exact test, $p = 0.21$). When calculated across all phases at the level of the dyad, males produced a significantly greater proportion of sweep-phrases than females (males: 6.24% of all calls, females 1.24% of all calls, Fisher's exact test, $p < 0.01$), However, within the pre-encounter (males: 9.03% of calls, females: 66.66% of calls, Fisher's exact test, $p < 0.01$) and post-encounter phases (males: 26.14% of calls, females: 62.5% of calls, Fisher's exact test, $p < 0.01$) females produced a greater proportion of sweep-phrases than males. The number of sweep-phrases produced pre-encounter and post-encounter were highly correlated (Pearson's correlation 0.718, $p = 0.012$). There were no significant differences in either offensive or defensive aggression or submissive behaviors based on whether residents produced sweep-phrase calls (ANOVA, $F_{1,21} = 0.29$, $p > 0.81$).

Discussion

Our findings reveal the repertoire of USVs used by the monogamous California mouse during same-sex resident-intruder encounters including SVs, barks and a newly identified complex call type, sweep phrases. In addition, relatively similar levels of aggressive behavior were expressed between the sexes as would be predicted in a monogamous species. In contrast, however, sex differences were revealed in detailed analyses of vocalizations. Only males gave SVs earlier during the encounters prior to physical aggression and produced shorter SVs than females during the physical encounters. Females on the other hand, produced more barks during physical aggression than males. Finally, our results suggest that modulation of the duration of SV calls is predictive of increased escalation of aggression later in a male-male encounter, while barks appear to play a role in the defensive behaviors of intruders during same sex encounters (Supplemental Figure 2) and infrequent sweep phrases occur primarily during the pre-encounter.

Vocalizations across the different phases of a same-sex encounter

All USV calls were produced in greater numbers when a conspecific was present. SV calls increased in males when acoustic, chemical and visual contact were possible during the pre-encounter. With the addition of physical contact, SV call production remained steady in males and was increased in females. SV calls then dropped to baseline levels during the post encounter phase. For barks, almost no calls were produced until physical contact had been made during the encounter period and bark calling ceased during the post-encounter phase. Sweep phrases were present in a subset of males and females pre-encounter, were reduced as a proportion of total calls during the encounter, and then increased as a proportion of total calls during post-encounter (Supplementary Table 1).

Of these calls, SVs (Briggs & Kalcounis-Rueppell, 2011; Kalcounis-Rueppell, Methany, & Vonhoff 2006; Pultorak, Fuxjager, Kalcounis-Reuppell, & Marler, 2015) and barks (Briggs & Kalcounis-Rueppell, 2011; Kalcounis-Rueppell, Petric, Briggs, Carney, et al., 2010; Pultorak, Fuxjager, Kalcounis-Reuppell, & Marler, 2015) have previously been identified in both the laboratory and field. SVs have been recorded from isolated individuals in the field (Briggs & Kalcounis-Rueppell, 2011; Petric & Kalcounis-Rueppell, 2013) and also during social interactions in the field (Kalcounis-Reuppell, Metheny, & Vonhoff, 2011) and laboratory, indicating that these calls likely have multiple functions. Specifically, this laboratory study found that SVs are used in aggression while previous laboratory studies show that SVs also occur frequently in affiliative interactions and when a pair is briefly separated and then reunited (Pultorak, Fuxjager, Kalcounis-Reuppell, & Marler, 2015; Pultorak and Marler unpublished data). Barks have been limited to aggressive encounters in the laboratory studies conducted thus far including the current study and others (Pultorak, Fuxjager, Kalcounis-Reuppell, & Marler., 2015; Pultorak Matusinec, Miller, & Marler 2017). The function of barks appears to be restricted to aggressive interactions, and here we find that these calls are related to defensive rather than offensive aggression. Finally, the production of sweep phrases before and after the encounters may suggest a function associated with either general territorial advertisement but are only produced by a subset of individuals.

Function of vocalizations and levels of aggression

We can add to our speculation about the function of these different calls by examining physical interactions. Both male and female residents showed a similar aptitude for winning fights against same-sex intruders as would be expected of a strictly monogamous species (Colombelli-Négrel, 2016; Fedy & Stutchbury, 2005; Koloff & Mennill, 2011). The number of aggressive behaviors produced by males and females were not significantly different suggesting a general similarity in aggression levels.

There were also no sex differences between the social interactions of residents and intruders, but there were differences in types of physical aggression between residents and intruders. Residents displayed more overall aggression and, specifically, more offensive attacks than intruders including chasing, wrestling and bites to the flank or belly (Blanchard, Wall, & Blanchard, 2003). In male dyads, SV duration shortened during the pre-encounter stage, and the change in duration from the pre-encounter stage to the encounter stage correlated with increased offensive aggression by residents. This provides evidence that modulation of SV calls may play a role in the escalation of aggression, or territorial or motivational displays prior to fighting. In contrast, intruders showed little overall aggression, mainly using defensive aggression including rearing up and boxing which act to protect the flank and other vital areas of an individual being aggressed against (Blanchard & Blanchard, 1977). Barks seem to play an important role in defensive aggression as these vocalizations were highly correlated with boxing, were audible during the display of defensive behaviors during which the mouth was visibly open.

SV calls: Sex differences, modulation and function

Neither males or females residents produced SV calls while isolated in this paradigm, indicating that, similar to previous findings in the laboratory (Pultorak, Fuxjager, Kalcounis-Reuppell, & Marler, 2015), the presence of another individual helps to elicit SV calls. Additional functions of SV calls are likely to be established because male and female residents in the field produce calls in isolation

(Kalcounis-Reuppell, Petric, Briggs, & Carney, et al. 2010). The onset of SV calling differed based on sex, with males producing SV calls in the pre-encounter phase, while females did not begin producing SV calls until physical interaction occurred. We speculate that overall pre-encounter SV calling acts as a territorial advertisement or as a signal of motivation to fight towards an intruder. This would indicate a sex difference similar to aggressive advertisement calls seen in other species such as red deer (Reby, McComb, Darwin, Fitch, et al., 2005) and frogs and toads (Reichert & Gerhardt, 2013a). Alternatively, pre-encounter SV calls could be a signal to the non-present mate that an intruder is present. As in primates (Gouzoules, Gouzoules, & Marler, 1984; Slocombe & Zuberbühler, 2007) these calls could recruit the mate to help defend the territory or act as a warning for the mate to stay in the nest. Like birds, pre-encounter SV calls would then be expected to increase as intruders move closer to the nest (Hau, Wikelski, Soma, & Wingfield, 2000; Levin & Wingfield, 1992); however, we did not test for this function.

These changes in SV duration and bout length prior to and during aggression indicates that, like other species (Alger, Larget, & Riters, 2016; Pasch, George, Hamlin, Guillette, et al., 2011; Weerts, Miczek, & Miczek, 1996), male California mice modulate the duration of their calls based on social context. Previously it has been shown that male California mouse SV duration is positively correlated with female approach behavior (Pultorak, Matusinec, Miller, & Marler 2017). In the current study, the shortening of SV calls within male dyads correlated with increased resident aggression. This shortening of SV calls in male dyads may indicate greater motivation to aggress if these calls are being produced by residents, as greater shortening of calls indicated both increased offensive aggression by residents and increased defensive behaviors by intruders. Similarly, squirrel monkeys (*Saimiri sciureus*) shorten the duration of their peep calls to display dominance (Weerts, Miczek, & Miczek, 1996). Conversely, neotropical singing mice add trills to their song and produce longer overall songs that correlate with greater aggression. This is controlled by testosterone with castrated mice producing shorter calls and displaying less aggression than testosterone implanted controls (Pasch, George, Campbell, & Phelps, 2011; Pasch, George, Hamlin, Guillette, et al., 2011). We speculate, but have not tested whether testosterone may also shorten SV call duration since it increases aggression in California mice (Fuxjager, Oyegbile, & Marler, 2011; Oyegbile & Marler, 2005; Trainor, Bird, & Marler, 2004). Overall, we have evidence that duration of individual calls and bout length are important spectral parameters in California mouse behavior, likely more so than total calls alone.

Barks act as aggressive signals

Barks are the lowest frequency call produced by California mice and start in the audible range, making them similar to broadband calls produced by Siberian hamsters during aggression (Keesom, Rendon, Demas, & Hurley, 2015). Like barks in California mice, Siberian hamsters increase their proportion of broadband calls during aggression, but, unlike hamsters, female California mouse dyads showed an increase in bark calls during aggressive encounters compared to males. The reason for this sex difference remains unknown, as females did not show increased defensive aggression compared to males. Similar to rats (Knutson, Burgdorf, & Panksepp, 2002; Riede, 2013), we speculate that low frequency barks of California mice are produced more by intruders rather than residents because intruders performed more defensive aggression than residents and defensive aggression correlated highly with barks (Supplementary figure 2). Moreover, anecdotal visual and auditory inspection of dyads during aggressive encounters showed intruders producing barks while engaged in boxing behavior (see supplementary video). This likely indicates a stress response or subordinate behavior to being aggressed against, which would be consistent with the calls only occurring after the onset of physical aggression. Overall, this corroborates previous findings that low frequency calls in rodents are closely tied to aggressive behaviors.

Sweep-phrases

We discovered a new, complex call type for California mice that we termed sweep-phrases. These calls show a level of complexity in frequency modulation and structure that has yet to be seen in this species. Sweep phrases were mostly seen in the pre- and post-encounter phases making up 9-67% of the total calls produced by males and females within the pre-encounter and post-encounter phases, but were produced less during aggressive interactions and not at all in isolation. Females produced a greater proportion of sweep phrases in the pre-encounter and post-encounter phases than males. As such, sweep phrases appear to be induced by the presence of a conspecific. These calls may function as advertisements of motivation to defend territory by a resident, similar to songbirds (Cain & Langmore, 2015; Wacker, Coverdill, Bauer, & Wingfield, 2010). This is further supported by the fact that only resident mice produced these calls during the pre- and post- encounter phases. Thus, this may indicate that life history (i.e. territory ownership or bonding) is important to the production of these calls.

These calls seem to be similar in complexity to those produced by songbirds (Nowicki & Searcy, 2004), the trill based calls of rats (Riede, 2013), trilled songs of neotropical singing mice (Pasch, George, Campbell, Guillette, et al., 2011) and frequency jump calls of house mice (Hammerschmidt, Radyushkin, Ehrenreich, & Fischer, 2012;Hoffman, Musolf, & Penn, 2012; von Merten, Hoier, Pfeifle, Tautz, et al. 2014). Similar to rat 22 kHz trill calls and neotropical singing mouse songs, sweep-phrases show constant upward and downward modulation, however the duration of rat trills are much shorter than sweep phrases and both rat trills and singing mouse songs are of a much lower frequency (Riede, 2013; Pasch, George, Hamlin, Guillette, et al., 2011). Sweep phrases are more similar in frequency to mouse frequency jumps that occur at frequencies greater than 45 kHz (von Merten, Hoier, Pfeifle, Tautz, et al., 2014). However, much still remains unknown about California mouse sweep phrases (as with SVs) including whether sweep-phrases include repeatable motifs or if there is a learning component of these calls as in bird song (Arriaga & Jarvis, 2013; Chabout, Sarkar, Dunson, & Jarvis, 2015). Currently, similar to mice, it appears that these high frequency USV calls do not play a major role in aggression. However, further study in multiple social contexts is ongoing to elucidate the function of the call as well as their importance to factors such as individual recognition, territorial defense and mate acquisition.

Integration of SVs and Barks with rodent aggressive vocalizations

In rat literature there has been an emphasis placed on interpreting 22 kHz calls as signifying negative affect (Brudzynski & Holland, 2005; Burgdorf et al., 2008; Kim, Kim, Covey, & Kim, 2010;

Knutson et al., 2002). In particular 22 kHz calls are produced by rats in response to aversive stressful events, including male-male aggression (Burgdorf, Kroes, Moskal, Pfaus et al., 2008; Sales, 1972b), social defeat (Kroes, Burgdorf, Otto, Panksepp, et al., 2007), predator exposure (Blanchard, Blanchard, Agullana, & Weiss, 1991; Litvin, Blanchard, & Blanchard, 2007) and fear response (Choi & Brown, 2003; Kim, Kim, Covey, & Kim, 2010). While California mice have calls within their vocal repertoire that are similar to calls of both rats and house mice, the majority of call types described to this point are more similar to rat vocalizations. This vocal similarity is consistent with the knowledge that *Peromyscus maniculatus* share more similarities at the genome level to rats than to mice (Ramsdell, Lewandowski, Glenn, & Vrana, 2008). This is particularly true of SV calls and barks which play a role during aggressive encounters and are more similar to the 22 kHz calls in rats than USV calls in mice (Brudzynski, 2013; Briggs & Kalcounis-Reuppell, 2011).

SV calls in particular share similar spectral properties with rat 22 kHz calls including a long duration and low modulation (Briggs & Kalcounis-Rueppell, 2011; Brudzynski & Holland, 2005). Rats exhibit only slight variations in frequency (Brudzynski & Holland, 2005; Wöhr & Schwarting, 2013) and duration is bimodally distributed between short and long 22 kHz calls (Vivian, & Miczek, 1993). Similarly, California mice within this study are showing little change in SV frequency and long and short duration SV calls. While California mouse SVs are similar to rat 22 kHz calls during encounters (likely indicating stress or fear), prior to an interaction, changes in SV duration are predictive of future aggressive behavior. This greater complexity in function is further illustrated by longer duration SVs being produced during non-aversive social contexts including courtship (Pultorak, Fuxjager, Kalcounis-Rueppell, & Marler, 2015) and reunion (Pultorak, Matusinec, Miller, & Marler, 2017). As such, SVs likely convey more information to the receiver than simply the affective state of the signaler as they occur prior to and predict aggressive behavior.

Barks have similarities with both hamster broadband calls and rat 22 kHz calls. Functionally both broadband calls and bark production are increased during aggression. In California mice, barks were produced more by females than males, but only occurred after the onset of physical aggression and were closely tied to defensive behavior. This is similar to hamsters, which increase broadband calling during aggressive encounters but hamsters do not show a sex difference in broadband call production (Keesom Rendon, Demas, & Hurley, 2015). In rats, intruders who have been previously aggressed against will increase 22 kHz calls, resulting in a decrease in aggressive behaviors expressed by the resident (Kroes, Burgdorf, Otto, Panksepp, et al., 2007; Lore, Flannelly, & Farina, 1976; Wöhr & Schwarting, 2013). We therefore speculate that coupling barks with jabbing may help the intruder prevent the resident from attacking. This indicates that barks occur as a reaction to an aversive stimulus and therefore may, as in rats (Portfors, 2007) indicate a negative affective state of the signaler.

Overall, we studied the production and function of USVs during territorial defense in a monogamous species. We saw considerable similarities between the sexes in both the expression of aggression towards a same sex intruder and the overall vocal repertoire, however, several sex differences were found in vocal communication. Females produced more barks that are tightly associated with defensive aggression for both sexes (See results and supplemental figure 2). Furthermore, females produced a greater proportion of phrase sweeps in the pre-encounter and post-encounter stage. Males produce more SV calls than females prior to physical aggression which may indicate that males play a greater role in territorial advertisement than females. Moreover, males have shorter SV calls than females during physical encounters and the shortening of SV calls predicts future escalation of aggression. This study expands the hypothesized functions for SVs beyond long distance communication and courtship to include aggression. (Briggs & Kalcounis-Rueppell, 2011; Pultorak, Fuxjager, Kalcounis-Reuppell, & Marler, 2015). It is, as yet, unknown what factors may control SV plasticity, however, testosterone (Inagaki & Mori, 2014) and the ascending mesolimbic cholinergic system (Brudzynski, 2014) play important roles in 22 kHz call production and conveying affective state, making them likely candidates. Further exploring this plasticity in other social contexts such as male-female parent-offspring interactions is warranted.

Acknowledgments

We thank Evan Stanton for his role in identifying and quantifying sweep phrase calls, Joshua Pultorak, Allison Hare, Emily Cierzan and Eliza Van Dixhorn for their help with collecting and analysing data for this project, Xin Zhao, Frank Castelli and two anonymous referees for comments on the manuscript and the University of Wisconsin animal research technicians. Finally, we thank the National Science Foundation for funding on this project IOS1132419.

References

Alger, S. J., Larget, B. R., & Riters, L. V. (2016). A novel statistical method for behaviour sequence analysis and its application to birdsong. *Animal Behaviour*, *116*, 181–193. http://doi.org/10.1016/j.anbehav.2016.04.001

Apfelbeck, B., Kiefer, S., Mortega, K. G., Goymann, W., & Kipper, S (2012). Testosterone Affects Song Modulation during Simulated Territorial Intrusions in Male Black Redstarts (Phoenicurus ochruros). *PLoS ONE*, *7*(12), e52009. http://doi.org/10.1371/journal.pone.0052009

Arriaga, G., & Jarvis, E. D. (2013). Mouse vocal communication system: are ultrasounds learned or innate? *Brain and Language*, *124*(1), 96–116. http://doi.org/10.1016/j.bandl.2012.10.002

Arriaga, G., Zhou, E. P., Jarvis, E. D., Fischer, J., Hammerschmidt, K., Holy, T., … Jarvis, E. (2012). Of Mice, Birds, and Men: The Mouse Ultrasonic Song System Has Some Features Similar to Humans and Song-Learning Birds. *PLoS ONE*, *7*(10), e46610. http://doi.org/10.1371/journal.pone.0046610

Bester-Meredith, J. K., & Marler, C. A. (2001). Vasopressin and Aggression in Cross-Fostered California Mice (Peromyscus californicus) and White-Footed Mice (Peromyscus leucopus). *Hormones and Behavior*, *40*(1), 51–64. http://doi.org/10.1006/hbeh.2001.1666

Blanchard, R. J., Blanchard, D. C., Agullana, R., & Weiss, S. M. (1991). Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. *Physiology & Behavior*, *50*(5), 967–972. http://doi.org/10.1016/0031-9384(91)90423-L

Blanchard, R. J., & Blanchard, D.C. (1977). Aggressive behavior in the rat. *Behavioral Biology*, *21*(2), 197–224. http://doi.org/10.1016/S0091-6773(77)90308-X

Blanchard, R. J., Wall, P. M., & Blanchard, D. C. (2003). Problems in the study of rodent aggression. *Hormones and Behavior*, *44*(3), 161–170. http://doi.org/10.1016/S0018-506X(03)00127-2

Briggs, J. R., & Kalcounis-Rueppell, M. C. (2011). Similar acoustic structure and behavioural context of vocalizations produced by male and female California mice in the wild. *Animal Behaviour*, *82*(6), 1263–1273. http://doi.org/10.1016/j.anbehav.2011.09.003

Brudzynski, S. M. (2013). Ethotransmission: communication of emotional states through ultrasonic vocalization in rats. *Current Opinion in Neurobiology*, *23*(3), 310–317. http://doi.org/10.1016/j.conb.2013.01.014

Brudzynski, S. M. (2014). The ascending mesolimbic cholinergic system--a specific division of the reticular activating system involved in the initiation of negative emotional states. *Journal of Molecular Neuroscience : MN*, *53*(3), 436–45. http://doi.org/10.1007/s12031-013-0179-1

Brudzynski, S. M., & Holland, G. (2005). Acoustic characteristics of air puff-induced 22-kHz alarm calls in direct recordings. *Neuroscience & Biobehavioral Reviews*, *29*(8), 1169–1180. http://doi.org/10.1016/j.neubiorev.2005.04.007

Brumm, H. (2004). Male–male vocal interactions and the adjustment of song amplitude in a territorial bird. *Animal Behaviour*, *67*(2), 281–286. http://doi.org/10.1016/j.anbehav.2003.06.006

Brumm, H., & Ritschard, M. (2011). Song amplitude affects territorial aggression of male receivers in chaffinches. *Behavioral Ecology*, *22*(2), 310–316. http://doi.org/10.1093/beheco/arq205

Burgdorf, J., Kroes, R. A., Moskal, J. R., Pfaus, J. G., Brudzynski, S. M., & Panksepp, J. (2008). Ultrasonic vocalizations of rats (Rattus norvegicus) during mating, play, and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback.. *Journal of Comparative Psychology*, *122*(4), 357–367. http://doi.org/10.1037/a0012889

Cain, K. E., Cockburn, A., & Langmore, N. E. (2015). Female song rates in response to simulated intruder are positively related to reproductive success. *Frontiers in Ecology and Evolution*, *3*, 119. http://doi.org/10.3389/fevo.2015.00119

Cain, K. E., & Langmore, N. E. (2015). Female and male song rates across breeding stage: testing for sexual and nonsexual functions of female song. *Animal Behaviour*, *109*, 65–71. http://doi.org/10.1016/j.anbehav.2015.07.034

Chabout, J., Sarkar, A., Dunson, D. B., & Jarvis, E. D. (2015). Male mice song syntax depends on social contexts and influences female preferences. *Frontiers in Behavioral Neuroscience*, *9*(April), 1–16. http://doi.org/10.3389/fnbeh.2015.00076

Choi, J.S., & Brown, T. H. (2003). Central Amygdala Lesions Block Ultrasonic Vocalization and Freezing as Conditional But Not Unconditional Responses. *Journal of Neuroscience*, *23*(25). Retrieved from http://www.jneurosci.org/content/23/25/8713

Colombelli-Négrel, D. (2016). Female splendid and variegated fairy-wrens display different strategies during territory defence. *Animal Behaviour*, *119*, 99–110. http://doi.org/10.1016/j.anbehav.2016.07.001

Constantini, F., & D'amato, F. R. (2006). Ultrasonic vocalizations in mice and rats : social contexts and functions. *Acta Zoologica Sinica*, *52*(4), 619–633. Retrieved from http://europepmc.org/abstract/cba/623593

Cramer, E. R. A., & Jordan Price, J. (2007). Red-winged blackbirds Ageliaus phoeniceus respond differently to song types with different performance levels. *Journal of Avian Biology*, *38*(1), 122–127. http://doi.org/10.1111/j.2006.0908-8857.03839.x

Cross, H. B., Zedrosser, A., Nevin, O., & Rosell, F. (2014). Sex Discrimination via Anal Gland Secretion in a Territorial Monogamous Mammal. *Ethology*, *120*(10), 1044–1052. http://doi.org/10.1111/eth.12277

Davis, E.S., & Marler, C.A. (2004). C-FOS changes following an aggressive encounter in female California mice: A synthesis of behavior, hormone changes and neural activity. *Neuroscience*, *127*(3), 611–624. http://doi.org/10.1016/j.neuroscience.2004.05.034

Davis, E. S., & Marler, C. A. (2003). The progesterone challenge: steroid hormone changes following a simulated territorial intrusion in female Peromyscus californicus. *Hormones and Behavior*, *44*(3), 185–198. http://doi.org/10.1016/S0018-506X(03)00128-4

Fedy, B. C., & Stutchbury, B. J. M. (2005). Territory defence in tropical birds: are females as aggressive as males? *Behavioral Ecology and Sociobiology*, *58*(4), 414–422. http://doi.org/10.1007/s00265-005-0928-4

Fernández-Vargas, M., Johnston, R. E. (2015). Ultrasonic Vocalizations in Golden Hamsters (Mesocricetus auratus) Reveal Modest Sex Differences and Nonlinear Signals of Sexual Motivation. *PLOS ONE*, *10*(2), e0116789. http://doi.org/10.1371/journal.pone.0116789

Fuxjager, M. J., & Marler, C. A. (2009). How and why the winner effect forms: influences of contest environment and species differences. *Behavioral Ecology*, *21*(1), 37–45. http://doi.org/10.1093/beheco/arp148

Fuxjager, M. J., Mast, G., Becker, E. A., & Marler, C. A. (2009). The “home advantage” is necessary for a full winner effect and changes in post-encounter testosterone. *Hormones and Behavior*, *56*(2), 214–219. http://doi.org/10.1016/j.yhbeh.2009.04.009

Fuxjager, M. J., Montgomery, J. L., Becker, E. A., & Marler, C. A. (2010). Deciding to win: interactive effects of residency, resources and “boldness” on contest outcome in white-footed mice. *Animal Behaviour*, *80*(5), 921–927. http://doi.org/10.1016/j.anbehav.2010.08.018

Fuxjager, M. J., Oyegbile, T. O., & Marler, C. A. (2011). Independent and Additive Contributions of Postvictory Testosterone and Social Experience to the Development of the Winner Effect. *http://dx.doi.org/10.1210/en.2011-1099*.

Fuxjager M.J., Xhao, X., Rieger N.S., and Marler, C.A.. (2017). Why animals fight: uncovering the function and mechanisms of territorial aggression. *American Psychological Association Handbook of Comparative Psychology*. American Psychological Association: Washington DC., pg. 853-875.

Gouzoules, S., Gouzoules, H., & Marler, P. (1984). Rhesus monkey (Macaca mulatta) screams: Representational signalling in the recruitment of agonistic aid. *Animal Behaviour*, *32*(1), 182–193. http://doi.org/10.1016/S0003-3472(84)80336-X

Griffiths, S., Dow, S., & Burman, O. (2010). Ultrasonic vocalizations and their associations with the non vocaluzation behaviour of the endangered Turkish spiny mouse (*Acomys cilicius spitzenberger*) in a captive population. *Bioacoustics*, *19*(3), 143–157. http://doi.org/10.1080/09524622.2010.9753621

Gubernick, D. J. (1988). Reproduction in the California Mouse, Peromyscus californicus. *Journal of Mammalogy*, *69*(4), 857–860. http://doi.org/10.2307/1381649

Hammerschmidt K, Radyushkin K, Ehrenreich H, Fischer J (2012) The Structure and Usage of Female and Male Mouse Ultrasonic Vocalizations Reveal only Minor Differences. PLoS ONE 7(7): e41133. https://doi.org/10.1371/journal.pone.0041133

Hau, M., Wikelski, M., Soma, K. K., & Wingfield, J. C. (2000). Testosterone and Year-Round Territorial Aggression in a Tropical Bird. *General and Comparative Endocrinology*, *117*(1), 20–33. http://doi.org/10.1006/gcen.1999.7390

Holy, T. E., & Guo, Z. (2005). Ultrasonic Songs of Male Mice. *PLoS Biology*, *3*(12), e386. http://doi.org/10.1371/journal.pbio.0030386

Hoffman, F., Musolf, K., & Penn, D.J. (2012) Ultrasonic courtship vocalizations in wild house mice: spectrographic analyses. *Journal of Ethology*. 30(1), 173-180

Illes, A. E., Hall, M. L., & Vehrencamp, S. L. (2006). Vocal performance influences male receiver response in the banded wren. *Proceedings of the Royal Society of London B: Biological Sciences*, *273*(1596).

Inagaki, H., & Mori, Y. (2014). Relationship between 22-kHz calls and testosterone in male rats. *Hormones and Behavior*, *65*(1), 42–46. http://doi.org/10.1016/j.yhbeh.2013.11.003

Insel, T. R., Preston, S., & Winslow, J. T. (1995). Mating in the monogamous male: Behavioral consequences. *Physiology & Behavior*, *57*(4), 615–627. http://doi.org/10.1016/0031-9384(94)00362-9

Kalcounis-Rueppell, M. C., Metheny, J. D., & Vonhof, M. J. (2006). Production of ultrasonic vocalizations by Peromyscus mice in the wild. *Frontiers in Zoology*, *3*(1), 3. http://doi.org/10.1186/1742-9994-3-3

Kalcounis-Rueppell, M. C., Petric, R., Briggs, J. R., Carney, C., Marshall, M. M., & Willse, J. T., (2010). Differences in ultrasonic vocalizations between wild and laboratory California mice (Peromyscus californicus). *PloS One*, *5*(4), e9705. http://doi.org/10.1371/journal.pone.0009705

Kalcounis-Reuppell, M.C., Pultorak, J.D. & Marler, C.A. (2017). Ultrasonic vocalization of mice in the genus *Peromyscus*. *The Handbook of Ultrasonic Vocalizations*. Elsevier.

Keesom, S. M., Rendon, N. M., Demas, G. E., & Hurley, L. M. (2015). Vocal behaviour during aggressive encounters between Siberian hamsters, Phodopus sungorus. *Animal Behaviour*, *102*, 85–93. http://doi.org/10.1016/j.anbehav.2015.01.014

Kim, E. J., Kim, E. S., Covey, E., & Kim, J. J. (2010). Social Transmission of Fear in Rats: The Role of 22-kHz Ultrasonic Distress Vocalization. *PLoS ONE*, *5*(12), e15077. http://doi.org/10.1371/journal.pone.0015077

Knutson, B., Burgdorf, J., & Panksepp, J. (2002). Ultrasonic vocalizations as indices of affective states in rats. *Psychological Bulletin*, *128*(6), 961–977. http://doi.org/10.1037/0033-2909.128.6.961

Koloff, J., & Mennill, D. (2011). Aggressive responses to playback of solos and duets in a Neotropical antbird. *Animal Behaviour*, *82*(3), 587–593. http://doi.org/10.1016/j.anbehav.2011.06.021

Kroes, R. A., Burgdorf, J., Otto, N. J., Panksepp, J., & Moskal, J. R. (2007). Social defeat, a paradigm of depression in rats that elicits 22-kHz vocalizations, preferentially activates the cholinergic signaling pathway in the periaqueductal gray. *Behavioural Brain Research*, *182*(2), 290–300. http://doi.org/10.1016/j.bbr.2007.03.022

Langmore, N. (1998). Functions of duet and solo songs of female birds. *Trends in Ecology & Evolution*, *13*(4), 136–140. http://doi.org/10.1016/S0169-5347(97)01241-X

Levin, R.N. (1996). Song behaviour and reproductive strategies in a duetting wren,Thryothorus nigricapillus: I. Removal experiments. *Animal Behaviour*, *52*(6), 1093–1106. http://doi.org/10.1006/anbe.1996.0257

Levin, R. N., & Wingfield, J. C. (1992). The Hormonal Control of Territorial Aggression in Tropical Birds. *Ornis Scandinavica*, *23*(3), 284. http://doi.org/10.2307/3676651

Litvin, Y., Blanchard, D. C., & Blanchard, R. J. (2007). Rat 22kHz ultrasonic vocalizations as alarm cries. *Behavioural Brain Research*, *182*(2), 166–172. http://doi.org/10.1016/j.bbr.2006.11.038

Lore, R., Flannelly, K., & Farina, P. (1976). Ultrasounds produced by rats accompany decreases in intraspecific fighting. *Aggressive Behavior*, *2*(3), 175–181. http://doi.org/10.1002/1098-2337(1976)2:3<175::AID-AB2480020302>3.0.CO;2-7

Maddison, C. J., Anderson, R. C., Prior, N. H., Taves, M. D., & Soma, K. K. (2012). Soft song during aggressive interactions: Seasonal changes and endocrine correlates in song sparrows. *Hormones and Behavior*. http://doi.org/10.1016/j.yhbeh.2012.08.002

Maynard Smith, J. Price, G. R., Smith, J. M., & Parker, G. A. (1973). The logic of animal conflict. *Evaluation*, *246*(1), 15–18. http://doi.org/10.1016/S0003-3472(76)80110-8

Musolf, K., Meindl, S., Larsen, A. L., Kalcounis-Rueppell, M. C., Penn, D. J., & Perez-Diaz, F. (2015). Ultrasonic Vocalizations of Male Mice Differ among Species and Females Show Assortative Preferences for Male Calls. *PLOS ONE*, *10*(8), e0134123. http://doi.org/10.1371/journal.pone.0134123

Nowicki, S., & Searcy, W. A. (2004). Song function and the evolution of female preferences: why birds sing, why brains matter. *Annals of the New York Academy of Sciences*, *1016*, 704–23. http://doi.org/10.1196/annals.1298.012

Oyegbile, T. O., & Marler, C. A. (2005). Winning fights elevates testosterone levels in California mice and enhances future ability to win fights. *Hormones and Behavior*, *48*(3), 259–267. http://doi.org/10.1016/j.yhbeh.2005.04.007

Oyegbile, T. O., & Marler, C. A. (2006). Weak winner effect in a less aggressive mammal: Correlations with corticosterone but not testosterone. *Physiology & Behavior*, *89*(2), 171–179. http://doi.org/10.1016/j.physbeh.2006.05.044

Pasch, B., George, A. S., Campbell, P., & Phelps, S. M. (2011). Androgen-dependent male vocal performance influences female preference in Neotropical singing mice. *Animal Behaviour*, *82*(2), 177–183. http://doi.org/10.1016/j.anbehav.2011.04.018

Pasch, B., George, A. S., Hamlin, H. J., Guillette, L. J., & Phelps, S. M. (2011). Androgens modulate song effort and aggression in Neotropical singing mice. *Hormones and Behavior*, *59*(1), 90–97. http://doi.org/10.1016/j.yhbeh.2010.10.011

Petric, R., & Kalcounis-Rueppell, M. C. (2013). Female and male adult brush mice (Peromyscus boylii) use ultrasonic vocalizations in the wild. *Behaviour*, *150*(14), 1747–1766. http://doi.org/10.1163/1568539X-00003118

Portfors, C. V. (2007). Types and Functions of Ultrasonic Vocalizations in Laboratory Rats and Mice. *Journal for the American Association of Laboratory Animal Science*, *46*(1), 28-34.

Pultorak, J. D., Fuxjager, M. J., Kalcounis-Rueppell, M. C., & Marler, C. A. (2015). Male fidelity expressed through rapid testosterone suppression of ultrasonic vocalizations to novel females in the monogamous California mouse. *Hormones and Behavior*, *70*, 47–56. http://doi.org/10.1016/j.yhbeh.2015.02.003

Pultorak, J. D., Matusinec, K. R., Miller, Z. K., & Marler, C. A. (2017). Ultrasonic vocalization production and playback predicts intrapair and extrapair social behaviour in a monogamous mouse.

Animal Behaviour, *125*, 13–23. http://doi.org/10.1016/j.anbehav.2016.12.023

Quinard, A., & Cézilly, F. (2012). Sex roles during conspecific territorial defence in the Zenaida dove, Zenaida aurita. *Animal Behaviour*, *83*(1), 47–54. http://doi.org/10.1016/j.anbehav.2011.09.032

Ramsdell, C.M., Lewanowski, A.A., Weston Glenn, J.L., Vrana, P.B., O'Neill, R.J. & Dewey, M.J. (2008). Comparative genome mapping of the deer mouse (*Peromyscus maniculatus*) reveals greater similarity to rat (*Rattus norvegicus*) that to the lab mouse (*Mus musculus*). *BMC Evolutionary Biology,* 8(1) 65. http://doi.org/10.1186/1471-2148-8-65.

Reby, D., McComb, K., Cargnelutti, B., Darwin, C., Fitch, W. T., & Clutton-Brock, T. (2005). Red deer stags use formants as assessment cues during intrasexual agonistic interactions. *Proceedings of the Royal Society of London B: Biological Sciences*, *272*(1566).

Reichert, M. S., & Gerhardt, H. C. (2013). Gray tree frogs, Hyla versicolor, give lower-frequency aggressive calls in more escalated contests. *Behavioral Ecology and Sociobiology*, *67*(5), 795–804. http://doi.org/10.1007/s00265-013-1503-z

Reichert, M. S., & Gerhardt, H. C. (2014). Behavioral strategies and signaling in interspecific aggressive interactions in gray tree frogs. *Behavioral Ecology*, *25*(3), 520–530. http://doi.org/10.1093/beheco/aru016

Ribble, D. O. (1991). The monogamous mating system of Peromyscus californicus as revealed by DNA fingerprinting. *Behavioral Ecology and Sociobiology*, *29*(3), 161–166. http://doi.org/10.1007/BF00166397

Ribble, D., & Salvioni, M. (1990a). Social organization and nest co-occupancy in Peromyscus californicus, a monogamous rodent. *Behavioral Ecology and Sociobiology*, *26*(1), 9–15. http://doi.org/10.1007/BF00174020

Ribble, D., & Salvioni, M. (1990b). Social organization and nest co-occupancy in Peromyscus californicus, a monogamous rodent. *Behavioral Ecology and Sociobiology*, *26*(1), 9–15. http://doi.org/10.1007/BF00174020

Riede, T. (2013). Stereotypic laryngeal and respiratory motor patterns generate different call types in rat ultrasound vocalization. *Journal of Experimental Zoology. Part A, Ecological Genetics and Physiology*, *319*(4), 213–24. http://doi.org/10.1002/jez.1785

Sales, G. D. (1972a). Ultrasound and aggressive behaviour in rats and other small mammals. *Animal Behaviour*, *20*(1), 88–100. http://doi.org/10.1016/S0003-3472(72)80177-5

Sales, G. D. (2010). *Ultrasonic calls of wild and wild-type rodents*. *Handbook of Behavioral Neuroscience*. http://doi.org/10.1016/B978-0-12-374593-4.00009-7

Seyfarth, R. M., & Cheney, D. L. (2010). Production, usage, and comprehension in animal vocalizations. *Brain and Language*, *115*(1), 92–100. http://doi.org/10.1016/j.bandl.2009.10.003

Sirotin, Y. B., Costa, M. E., & Laplagne, D. A. (2014). Rodent ultrasonic vocalizations are bound to active sniffing behavior. *Frontiers in Behavioral Neuroscience*, *8*, 399. http://doi.org/10.3389/fnbeh.2014.00399

Slocombe, K. E., & Zuberbühler, K. (2007). Chimpanzees modify recruitment screams as a function of audience composition. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(43), 17228–33. http://doi.org/10.1073/pnas.0706741104

Trainor, B. C., Bird, I. M., & Marler, C. A. (2004). Opposing hormonal mechanisms of aggression revealed through short-lived testosterone manipulations and multiple winning experiences. *Hormones and Behavior*, *45*(2), 115–121. http://doi.org/10.1016/j.yhbeh.2003.09.006

Trainor, B. C., Pride, M. C., Villalon Landeros, R., Knoblauch, N. W., Takahashi, E. Y., Silva, A. L., & Crean, K. K. (2011). Sex Differences in Social Interaction Behavior Following Social Defeat Stress in the Monogamous California Mouse (Peromyscus californicus). *PLoS ONE*, *6*(2), e17405. http://doi.org/10.1371/journal.pone.0017405

van Staaden, M. J., Searcy, W. A., & Hanlon, R. T. (2011). Signaling Aggression. In *Aggression*.

Vivian, J. A., & Miczek, K. A. (1993). Morphine attenuates ultrasonic vocalization during agonistic encounters in adult male rats. *Psychopharmacology*, *111*(3), 367–375. http://doi.org/10.1007/BF02244954

von Merten, S., Hoier, S., Pfeifle, C., Tautz, D., Branchi, I., Santucci, D., … Hu, M. (2014). A Role for Ultrasonic Vocalisation in Social Communication and Divergence of Natural Populations of the House Mouse (Mus musculus domesticus). *PLoS ONE*, *9*(5), e97244. http://doi.org/10.1371/journal.pone.0097244

Wacker, D. W., Coverdill, A. J., Bauer, C. M., & Wingfield, J. C. (2010). Male territorial aggression and androgen modulation in high latitude populations of the Sooty, Passerella iliaca sinuosa, and Red Fox Sparrow, Passerella iliaca zaboria. *Journal of Ornithology*, *151*(1), 79–86. http://doi.org/10.1007/s10336-009-0428-9

Weerts, E. M., Miczek, K. A., & Miczek, K. A. (1996). Primate vocalizations during social separation and aggression: effects of alcohol and benzodiazepines. *Psychopharmacology*, *127*(3), 255–264. http://doi.org/10.1007/BF02246134

Williams, J. R., Catania, K. C., & Carter, C. S. (1992). Development of partner preferences in female prairie voles (Microtus ochrogaster): The role of social and sexual experience. *Hormones and Behavior*, *26*(3), 339–349. http://doi.org/10.1016/0018-506X(92)90004-F

Wöhr, M., & Schwarting, R. K. W. (2013). Affective communication in rodents: ultrasonic vocalizations as a tool for research on emotion and motivation. *Cell and Tissue Research*, *354*(1), 81–97. http://doi.org/10.1007/s00441-013-1607-9

Yang, C., Zhang, Y., Cai, Y., Stokke, B. G., & Liang, W. (2011). Female Crowing and Differential Responses to Simulated Conspecific Intrusion in Male and Female Hainan Partridge (*Arborophila ardens*). *Zoological Science*, *28*(4), 249–253. http://doi.org/10.2108/zsj.28.249

Figures

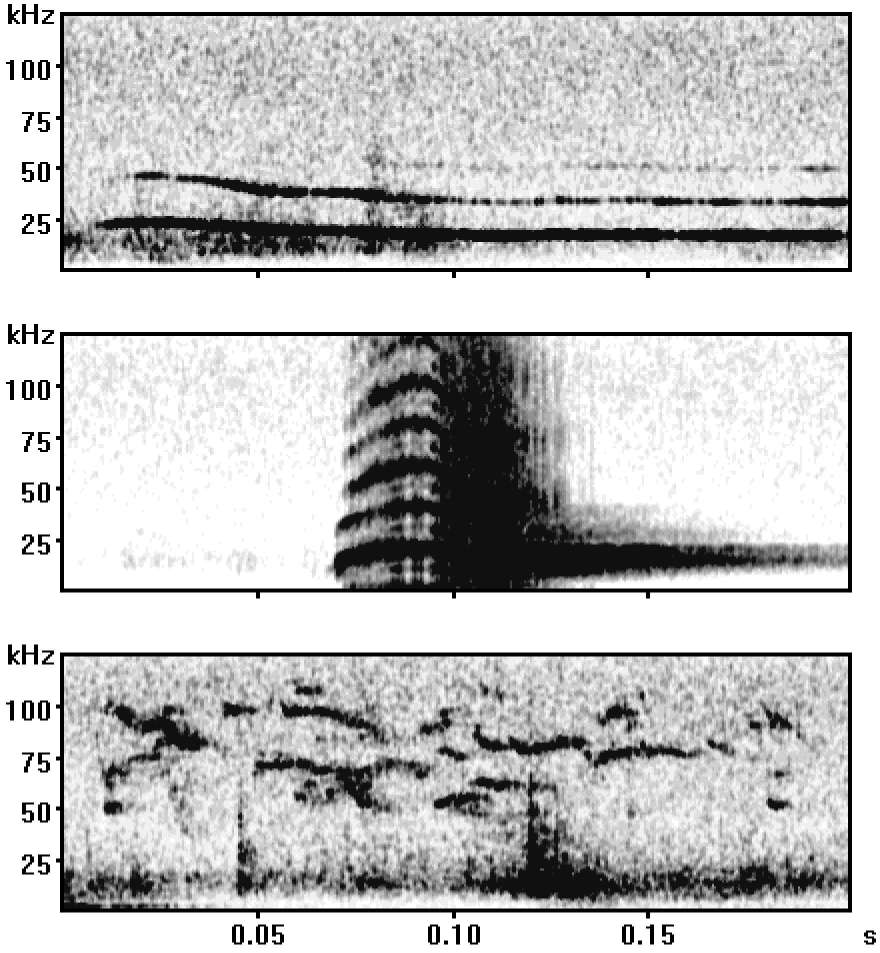
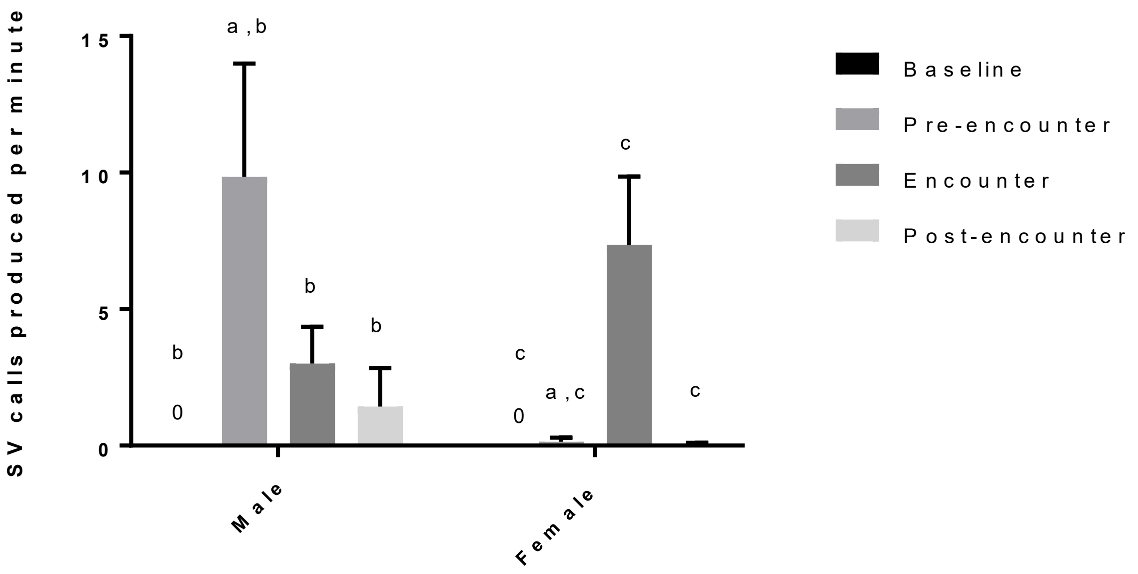


Figure 1. Representative spectrograms of three USV calls. Top: Sustained vocalization (SV), Middle: Bark, Bottom: Sweep-phrase. Frequency in kHz is shown on the Y axis and duration in seconds is shown along the X axis.'

A.


SV calls produced per minute
15
10
5
0
a,b
b
b
b
0
c
c
c
a,c
c
0
Male
Female
Baseline
Pre-encounter
Encounter
Post-encounter

B.

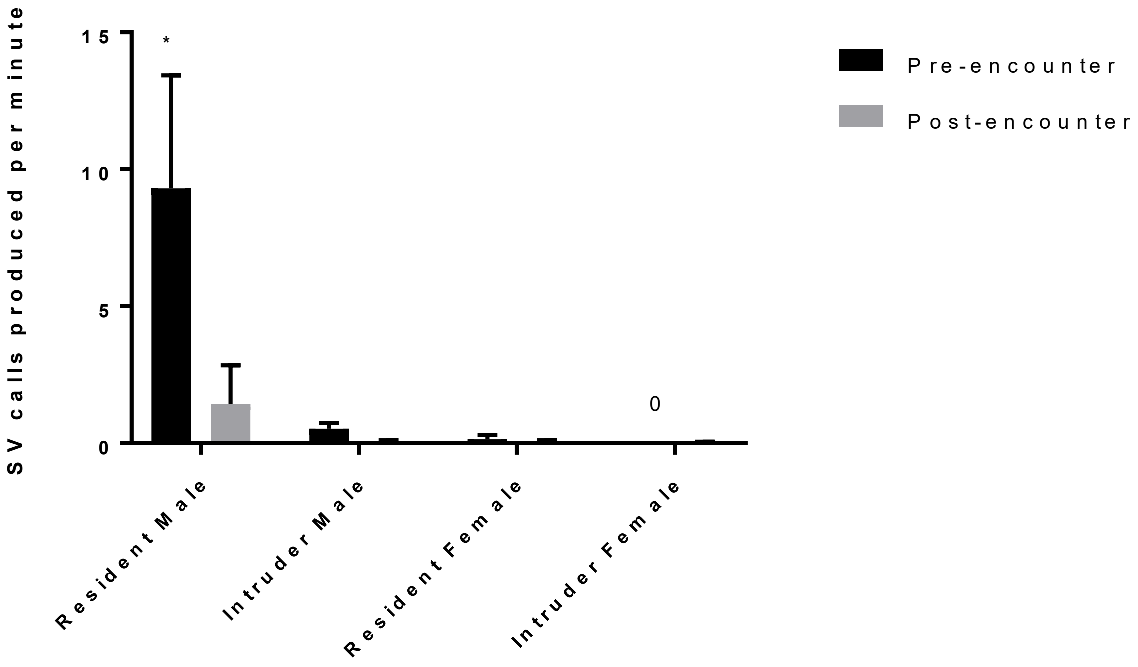

SV calls produced per minute
15
10
5
0
*
0
Resident Male
Intruder Male
Resident Female
Intruder Female
Pre-encounter
Post-encounter

Figure 2. All analyses were completed on log(x+1) transformed calls per minute to control for unequal testing times. Bars represent raw means and SEM **A**. Production of SV calls across phases within a dyad. SV calls within each phase were pooled by dyad (n = 11 male dyads and n = 10 female dyads). Male dyads produced significantly more SVs than female dyads in the pre-encounter stage (a, $p < 0.01$). Male SV calling was also significantly increased in the pre-encounter and encounter stage compared to the baseline and post-encounter stage (b, $p < 0.01$). Female dyads showed significantly increased calling in the encounter phase compared to all other phases (c, $p < 0.01$). **B**. Male resident (n = 11) SV call production was significantly greater than male resident post-encounter SV call production, and the pre- and post-encounter SV call production of intruder males (n = 11), resident females (n = 10) and intruder females (n = 10) (*$p < 0.01$). Statistical significance is denoted by letters in panel A (a, b & c, $p < 0.01$) and asterisk in panel B (*$p < 0.01$).

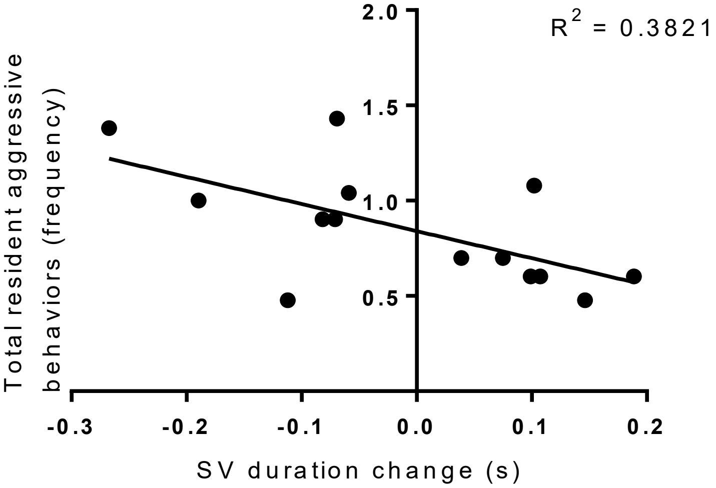


Figure 3. SV call duration changes from the pre-encounter to encounter stage within a dyad (n=14) correlated with total aggressive behavior shown by residents. Shorter SVs during the encounter stage, as denoted by negative values, indicated more frequency of occurrence of aggressive behavior by residents, while longer SV calls indicated lesser aggression.

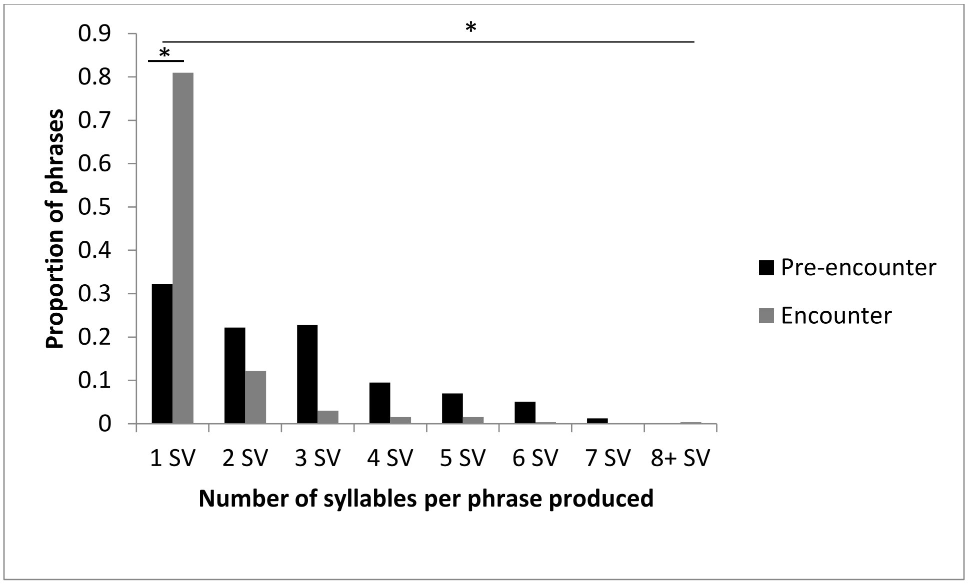


Figure 4. Proportion of SV bout lengths across phases. Data within dyads was pooled across sexes for pre-encounter (n = 8 dyads) and encounter (n = 18 dyads) bout lengths. One call bouts were produced in significantly greater proportions than any other bout length (*, $p = 0.03$). However, one call bouts significantly increased during the encounter phase versus the pre-encounter phase (*, $p < 0.01$). Bars represent the proportion of bout lengths compared to the whole (* = $p < 0.05$).

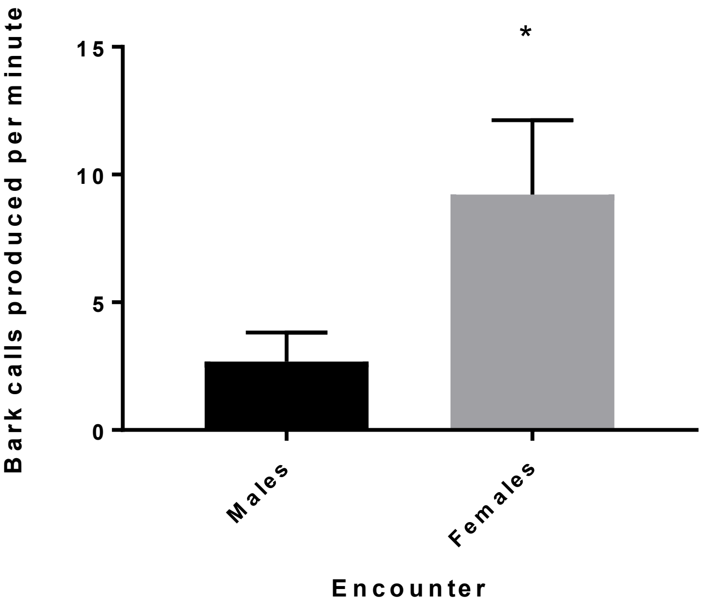


Figure 5. Female dyads (n = 10) produced a greater number of barks per minute than male dyads (n = 11). Analyses were done on log(x+1) transformed data and raw means and SEM are shown (*p = 0.046)

Chapter 2

Division of labor in territorial defense and pup retrieval by pair-bonded California mice (*Peromyscus californicus*)

Abstract

Division of labor allows group living species to efficiently complete tasks while minimizing resource expenditure. This generally involves task allocation between individuals within a group. In territorial defense and parental care specifically, division of labor can be completed using different strategies, but often involves one or more individuals defending the territory while others care for offspring. Little is known, however, about division of labor strategies employed by monogamous and biparental mammals. In this study, we investigated division-of-labor strategies across territorial defense and pup retrievals of the monogamous, biparental and territorial California mouse (*Peromyscus californicus*) as well as the role of vocal communication in coordinating these behaviors. It is known that both male and female pair-bonded California mice will display aggression towards same-sex intruders and retrieve pups while alone, but not how pairs complete these tasks while together. We found that California mouse pairs used one of two pair-specific strategies during territorial defense: 1) joint defense or 2) divided defense. Overall, these strategies were not altered by the sex of the intruder or the birth of pups. However, following the birth of pups, individuals spent more time in the nest and pairs spent less time together investigating intruders. Pup retrievals, conversely, followed a sex specific strategy where mothers retrieved pups in 89% of pairs. This study shows for the first time a monogamous and biparental rodent that uses different strategies to divide labor during the vital tasks of territorial defense and pup retrieval. Importantly, we found that vocal communication via sustained vocalizations were predictive of aggressive behavior in males but not retrieval behavior, indicating that vocalizations may only play a role in coordinating specific behaviors. Moreover, these strategies are task-dependent and robust across multiple contexts, providing a framework for better understanding division of labor in mammals.

Keywords: Division of labor, Coordination, Aggression, Ultrasonic vocalizations, Monogamy, parental care, pup retrieval, territoriality

Highlights

- Pairs defend territories across contexts using a joint or divided defense strategy.

- A sex difference exists in pup retrievals such that females predominately retrieve.

- Male sustained vocalizations predict defense behavior but not retrieval behavior.

Introduction

A major advantage of group living is the ability to allocate tasks and divide labor amongst individuals that allows groups to efficiently complete tasks while minimizing costs (Jeanne, 2016; Page, Scheiner, Erber, & Amdam, 2006; Robson & Traniello, 2016; Giraldo, Patel, Gronenberg, & Traniello, 2013; Goldsby, Dornhaus, Kerr, & Ofria, 2012). By coordinating behavior to divide labor, groups can more successfully defend territories (Hall, 2000), gather food and move locations (Campbell & Boinski, 1995; King & Sueur, 2011) and raise offspring (Ahern, Hammock, & Young, 2011). However, most of our knowledge on behavioral coordination and division of labor comes from invertebrates (G. E. Robinson, 2003). How mammals, particularly monogamous mammals, leverage their unique social structure into successfully completing important tasks such as territorial defense and pup retrieval remains relatively unknown.

Vocal communication is an important contributor to the coordination of behaviors by pairs in a number of species and serves to organize individuals to complete complex tasks (King & Sueur, 2011). Vocal communication can mediate coordination (Noe, 2006) via calls or songs produced by individuals within the group or pair. In particular, these calls help to coordinate territorial defense efforts in primates (Noe, 2006) and birds (Brenowitz, Arnold, & Levin, 1985; Hall, 2000). However, vocalizations do not need to be produced by group members in order to drive coordination. In downy woodpeckers (*Picoides pubescens)*, the vocalizations of intruders indicate the level of threat to resident pairs and as this threat level increases it leads to pairs approaching the intruder together instead of separately (Schuppe, Sanin, & Fuxjager, 2016). However, while we know that vocalizations can influence coordinated behaviors of birds, we do not know what role, if any, vocalizations play in coordinating the behavior of monogamous mammals.

Monogamous species have been shown to divide labor in service of territorial defense and parental care (Bendesky et al., 2017; Mathews, 2002; Mitani, 1984; Yang, Zhang, Cai, Stokke, & Liang, 2011). In many cases division of labor by monogamous species is sex specific (Mathews, 2002; Quinard

& Cézilly, 2012) and is measured primarily when pairs have offspring (Ahern et al., 2011; Rogers, 2010). There is evidence in monogamous species for both mate guarding, where individuals defend their territory from same sex intruders to prevent extrapair copulations as in birds (Koloff & Mennill, 2011; Mathews, 2002; Reburn & Wynne-Edwards, 1999), and for sex-specific territorial defense where one sex predominately defends the territory while the other engages in direct parental care as in cichlid fish (Rogers, 2010). However, little is known about division of labor in monogamous rodents, outside of the fact that they will jointly care for pups (Ahern et al., 2011; Bester-Meredith, Conley, & Mammarella, 2016; Bester-Meredith & Marler, 2001). Division of labor during territorial defense and pup retrievals to this point remains untested.

To better understand division of labor, we studied the monogamous, biparental and territorial California mouse (Ribble, 1991; Ribble & Salvioni, 1990). California mice are a strictly monogamous species (Ribble, 1991) that care for pups and hold territories together from the time they bond until death (Ribble & Salvioni, 1990). Moreover, both male and female bonded California mice can successfully defend territories against same-sex intruders (Rieger & Marler, 2018), and retrieve pups (Bester-Meredith et al., 2016; Bester-Meredith & Marler, 2007; Frazier, Trainor, Cravens, Whitney, & Marler, 2006; Marler, Trainor, Gleason, Bester-Meredith, & Becker, 2008) while alone. California mouse fathers are capable of all aspects of parental care excluding lactation (Gubernick, Wright, & Brown, 1993). California mice can also adjust their level of parental care in order to compensate for their partner under certain conditions (review by Bester-Meredith, Conley, & Mammarella, 2016). Despite this, the majority of work on California mice has been done with males or females in isolation and, as such, it is yet unknown how pairs will divide labor to defend territories and retrieve pups when both pair members are present.

California mice also produce a rich variety of ultrasonic vocalizations (USV) calls that may be important for coordinating behavior between bonded pairs (Kalcounis-Rueppell, Metheny, & Vonhof, 2006; Pultorak, Matusinec, Miller, & Marler, 2017; Rieger & Marler, 2018). One particularly important call in the social behavior of California mice is sustained vocalizations (SV). SVs are long low-bandwidth calls with relatively low frequencies (~22 kHz) that are produced across a wide variety of social interactions including courtship (Kalcounis-Rueppell et al., 2010; Pultorak, Fuxjager, Kalcounis-Rueppell, & Marler, 2015) and aggression (Rieger & Marler, 2018). Moreover, SV duration can be modified to alter the meaning of the signal such that shorter SVs indicate aggression (Rieger & Marler, 2018) and longer SVs are more likely to be approached by an opposite sex individual (Pultorak et al., 2017). The ability to modify these calls, and their broad use across social context (Kalcounis-Rueppell, Pultorak & Marler, 2018) make SVs a prime candidate to function in the coordination of behavior between mates (Hurley and Kalcounis-Rueppell, 2018).

Our goal was to study division of labor in California mice across two different behavioral tasks. In experiment 1 we used a modified resident-intruder paradigm to test if one member of a pair would consistently defend against an intruder. We predicted that prior to the birth of pups pairs would defend territories together as previous research has shown both males and females show similar aggression and are equally capable of winning fights against same sex intruders (Rieger & Marler, 2018). We also tested whether pairs use a mate guarding strategy by presenting each pair with a male and female intruder in a counterbalanced order. If a mate guarding strategy is used males would defend against male intruders while females would defend against female intruders. Following the birth of pups, we predicted pairs would divide labor with males being the predominant defender, as previous research in other species has shown that pairs with offspring generally have one member that patrols to edge of the territory while the other remains close to the nest (Ahern et al., 2011; Rogers, 2010). In experiment 2 we tested for division of labor in pup retrievals. We predicted that both male and female pair bond members would retrieve pups as previous studies have shown that both males and female California mice can retrieve pups when alone (Bester-Meredith & Marler, 2003; Frazier et al., 2006; Gubernick et al., 1993).

Methods

Animals

Thirty-eight male (22 paired and 16 naïve intruders) and 34 female (22 paired and 12 naïve intruders) California mice were used to test division of labor in territorial defense and pup retrieval. Individual males and females were randomly paired with an opposite-sex partner unrelated for at least two generations and cohabitated in a standard cage (48 x 27 x 16 cm) lined with aspen bedding, a nestlet, food (Purina 5015^{TM} mouse chow) and water available *ad libitum* for 17 days prior to testing. Naïve male and female mice lived with 2–3 same sex conspecifics. The housing room was maintained at 20–23ºC on a 14:10 h light:dark cycle (lights on at 23:00 central standard time) with all testing occurring 1–3 hours after the onset of the dark cycle in dim red light. All individuals were ear tagged for individual identification. Pairs were tested for division of labor during territorial defense up to three times. 1) 17 days post-pairing (22 pairs), 2) 24 days post-pairing (22 pairs) and 3) 7 days post parturition (12 pairs) following the method outlined below (supplemental fig 1). The 12 pairs tested post-parturition were also tested on pup retrieval behavior 3–4 days post-parturition following the method outlined below.

Ethical statement

All animals were maintained according to the National Institute of Health *Guide for the care and use of laboratory animals*. All procedures were approved by the University of Wisconsin – Madison College of Letters and Sciences Institutional Animal Care and Use Committee (Protocol L005447). No animals were injured by any of the behavioral manipulations or assays.

Testing apparatus

Testing occurred in a glass aquarium (50 x 30 x 30 cm) lined with aspen bedding and containing a red tube (15 cm length) for enrichment and a plastic igloo (10 x 10 x 8 cm) to be used as a nest. The aquarium had a Plexiglas lid with 5-cm diameter holes placed 2.5 cm from the corners of the apparatus to allow for microphones to be placed inside of the apparatus to record USVs. Intruders were placed in a 10 x 10 x 10 cm wire mesh cage that was inserted into the testing apparatus in the corner opposite of the igloo for the duration of the resident-intruder encounter.

Experimental Procedure

Experiment 1: Territorial defense

Twenty-two male-female pairs were used to test territorial defense against either a male or female conspecific acting as an intruder. Pairs cohabitated with each other for 17 days before testing. At 7 days post bonding, pairs show side to side contact, decreased aversive vocalizations towards their mate and decreased investigation of novel individuals compared to their mate indicative of bonding (Becker, Castelli, Yohn, Spencer, & Marler, 2018). At 17 days post-pairing pairs were likely pregnant because pregnancy last 28 days and all 12 pairs used had litters within 18 days after the initial test. Twenty-four hours prior to testing, pairs were moved from their standard cage to the testing apparatus. This 24-h period allowed for the formation of the residency effect, which increases the likelihood that residents will defend a territory and win male-male encounters (Bester-Meredith & Marler, 2001; Fuxjager, Mast, Becker, & Marler, 2009; Fuxjager, Zhao, Rieger, & Marler, 2017). Residents had a small patch of fur shaved from either their left or right flank for identification purposes. Male and female intruders were sexually naïve and did not have social experience outside of interactions with their cage mates.

We tested pairs using a modified version of a resident-intruder paradigm previously used in our lab (Rieger & Marler, 2018) at three different time points 1) 17 days post pairing (22 pairs), 2) 24 days post pairing (22 pairs) and 3) 1 week post-parturition (12 pairs). At time points one and two, pairs were nonparental and challenged with either a male or female intruder in a randomized counterbalanced order. At time point three a male intruder was used. Each resident intruder test was made up of three phases 1) pre-encounter, pairs were alone in the arena for 2 minutes, 2) encounter, a randomly assigned male or female intruder was placed in the arena within a wire mesh cage for 6 minutes and 3) post-encounter, the intruder was removed and the pair was alone in the cage for 4 minutes. All times were selected following pilot studies that showed that behaviors of interest and vocalizations for each phase occurred within these time frames. Ultrasonic vocalizations (USVs) were recorded at all three time points, with vocal analyses conducted at the level of the dyad as assigning calls to individuals was often not possible.

Video recordings were scored for time spent in contact with the intruder cage (defined as a resident being within 2.5 cm of the cage as demarcated by a black rubber base under the intruder's cage) versus time spent at the nest (defined as time spent inside or on top of the igloo) for both members of the resident pair. We defined pairs as either defending in a 1) joint (see supplemental video) or 2) divided (see supplemental video) manner dependent on difference in time spent near the intruder by the individual members of the pair. The difference in time spent near the intruder by all pairs was pooled and a distribution was formed. It was found that pairs distributed bimodally around an average of 130 seconds such that pairs with a difference greater than 130 seconds were categorized as defending in a divided manner, while pairs with a difference of less than 130 seconds were defined as defending jointly. Latency to approach the intruder's cage, aggressive behavior (number of bites and jabs towards the cage) and investigatory behavior (time spent sniffing the anogenital region or nose of the intruder) were also scored. Following the removal of the intruder, we scored patrolling behavior, defined as time spent outside of the nest investigating the site where the caged intruder had been or the perimeter 2.5 cm of the apparatus. All behaviors were scored by an observer blind to both treatment and sex of the residents and intruder.

Ultrasonic vocalization analysis

We recorded USVs with two Emkay/Knowles FG series microphones (detection range 10–120 kHz). Microphones were placed 55 cm apart at opposite corners of the apparatus, 20 cm from the apparatus floor with one microphone placed over the resident nest and one placed over the intruder cage. Microphone placement was randomized across trials to control for potential differences in sensitivity. Microphone channels were calibrated to equal gain (-60 dB noise floor) and WAV files for each of the three stages of the encounter were produced using RECORDR software (Avisoft Bioacoustics, Berlin, Germany). Recordings were made using a 250-kHz sampling rate with 16-bit resolution and spectrograms were produced with a 512 fast Fourier transform made using Avisoft SASLab Pro (Avisoft bioacoustics). USVs were differentiated by visual and auditory inspection of WAV files with sampling rates reduced to 4% of normal speed (11025 kHz). Due to an extremely low number of sweep-phrases and barks, which were each only produced by one pair, we focused our analyses on sustained vocalizations (SV) that have been previously defined (Pultorak, Matusinec, Miller, & Marler, 2017; Rieger & Marler, 2018; Kalcounnis-Reuppell, Pultorak, & Marler, 2018). In the resident-intruder test, all calls were analyzed at the level of the dyad by an observer blind to treatment.

Experiment 2: Pup Retrieval

We video recorded retrieval behavior by bonded pairs 3–4 days post-parturition for 12 total pairs. 24 hours prior to testing, pairs and their pups (average 2.25 ± 0.25, range 1–3 pups per pair) were moved from the standard cage into a testing arena as in experiment 1, described above. After 24 hours the arena was divided into two equal sized compartments by a Plexiglas divider with two 5-cm diameter mesh cutouts to allow for visual, auditory and chemical (but not physical) signaling between chambers. Pups were taken from their parents and moved to the side of the apparatus opposite the igloo that served as the pairs' nest (termed the separation phase). Behavior of the pair and USVs were recorded for 4 minutes. After 4 minutes, we removed the Plexiglas divider and videotaped pup retrievals (termed the retrieval phase). Based on pilot studies, pairs were only allowed up to 8 minutes to retrieve pups as parents who did not retrieve during this time frame generally failed to retrieve pups at all. Latency to first reach the pups was recorded for both the mother and father. We recorded whether the mother or father retrieved pups and how long retrievals took and how many pups were retrieved. Retrieval was defined as a parent picking up a pup, usually by the scruff of the neck, and transporting them back into the igloo. Time to retrieval was recorded as the time from when the divider was removed until the pup was fully inside the nest.

Vocalization analysis for pup retrievals

Vocalizations were recorded and analyzed following the same procedure as described above in experiment 1. Pup calls and parental USVs were analyzed for both the separation and retrieval phases. Pup calls were categorized as being similar to adult SVs but also include a sharp downward inflection, termed a whine (Rieger & Marler, unpublished data). For parents, as in experiment 1, SV calls were analyzed. During the separation phase, determining whether calls originated from pups or parents was done via differences in amplitude created by the Plexiglas divider as well as visual inspection. During the retrieval phase, pup calls were differentiated from parental SVs due to the sharp downward inflection present in whine calls which can be categorized by visual inspection.

Statistics

All statistics were run on SPSS v 22 (IBM Corp, Armonk, NY, USA). We compared strategies used across timepoints in intruder and pup retrieval tests using Fisher's exact tests and Chi-square tests of independence. Mixed ANOVAs were used to measure the time in seconds of multiple behavioral variables including latency to approach intruders or pups, investigation of the intruder, presence in or on the nest, being together with a mate, retrieving pups and huddling over and grooming pups. The number of aggressive behaviors towards the intruder and the mate was analyzed across timepoints using a mixed ANOVA. Pairs were used as covariates within all analyses including both members of a pair. Linear regressions were completed to analyze the relationship between SV duration and behaviors across timepoints as well as behaviors across intruder and pup retrieval tests.

Results

Experiment 1: Resident-Intruder Paradigm

Defense Strategies in non-parental pairs

Pairs (N = 22) showed two major territorial defense strategies across the first two time points in response to male or female intruders: 1) joint and 2) divided. Overall, there was no difference in the use of joint or divided defensive strategies by pairs regardless of whether the intruder was male (joint: n = 14 pairs, divided: n = 8 pairs; Fisher's exact test, p = 0.13, Fig. 1A) or female (joint: n = 11 pairs; divided: n = 11 pairs; Fisher's exact test, p = 0.76; Fig. 1A). Overall, only four pairs switched strategies between the two tests, which was not statistically significant (Chi-Square $\chi^2_1 = 0.371$, p =0.54). In three of the four cases the pairs that switched changed from a joint to a divided strategy. Within pairs that used divided defense, there was also no difference in the number of individual defenders who were male versus female regardless of whether the intruder was male (male defenders: n = 5; female defenders: n = 3; Fisher's exact test, p = 0.61, Fig. 1B), or female (male defenders: n = 7; female defenders: n = 4, Fisher's exact test; p = 0.66, Fig. 1B). In only one case did a pair originally using a divided strategy change from a female to a male defender (Chi-square, $\chi^2_1 = 0.003$, p = 0.95). Importantly, pairs did not differ significantly in size (males: 42.12 ± 1.40 g; females: 44.34 ± 2.21 g; Students t-test, $t_{42} = 0.85$, p = 0.40).

There was no sex difference in time spent near the intruder (Male: 163.5 ± 24.5 s; Female: 120.23 ± 24.23 s) or at the nest (Male: 112.05 ± 21.54 s; Females: 156.18 ± 29.16 s; ANOVA, $F_{2, 41} = 0.810$, p = 0.452) between males and females regardless of the sex of the intruder (ANOVA, $F_{1,21} = 0.085$, p = 0.774). We found that time spent in the nest in the pre-encounter stage was predictive of time spent in the nest during the encounter for both males and females, regardless of the sex of the intruder (Linear regression, $F_{1, 36} = 5.713$, p= 0.022, $r^2 = 0.137$).

Individuals who approached the intruder showed a suite of aggressive behaviors including jabbing and biting the cage that were added together to make an aggression score. The frequency of aggressive behaviors did not differ by sex of the pair mate (males: 1.429 ± 0.42 aggressive behaviors; females: 0.57 ± 0.23 aggressive behaviors, ANOVA, $F_{1, 41} = 2.155$, p = 0.15). Individuals also showed aggression towards their mates, regardless of strategy type, which may act as displaced aggression, but which did not differ based on sex of the aggressor (Males: 0.81 ± 0.35 aggressive behaviors; Females: 0.57 ± 0.33 aggressive behaviors, ANOVA $F_{1,41} = 0.233$, p = 0.632). The number of total aggressive behaviors towards both the intruder and the mate also did not differ based on the sex of the intruder (ANOVA, $F_{1,21} = 0.377$, p = 0.549).

Defense strategies in parental pairs

Twelve random pairs were retested following the birth of pups. Overall, the strategies used by pairs were not different from preparturition testing with seven pairs using a joint strategy and five pairs using a divided strategy (Chi-square, $\chi^2 = 0.92$, p = 0.76, figure 1A). Within the five pairs showing divided defense, three of the defenders were female while two were male, similar to the preparturiation levels (Chi-square, $\chi^2 = 0.043$, $p = 0.83$, figure 1B). Within all 12 pairs, only three showed changes in strategy from preparturition to postparturition which was not a significant proportion (25% change Fisher's exact test, $p = 0.67$). Of the three pairs that changed strategies, all changed from a joint strategy to a divided strategy. In two of the three cases, the female became the predominant defender, and in one case the male became the predominant defender. Combined with changes from the first two time points it was found that 6 out of 7 strategy changes were from joint to divided strategies (Fisher's exact test, $p = 0.029$)

Individual members of parental pairs spent significantly more time at the nest following the birth of pups compared to preparturition (preparturition: 71.81 ± 14.38 s; postparturition: 146.62 ± 34.10 s; ANOVA, $F_{1, 22} = 6.48$, $p = 0.023$, figure 2A). However, the time spent together at the nest by pairs, as defined by cohabitation by both members of the pair in the nest, did not change (preparturition: 20.75 ± 6.5 s; postparturition: 45.88 ± 18.45 s; ANOVA, $F_{1,22} = 1.98$, $p = 0.18$, figure 2B). Moreover, parental pairs did not change either the amount of time they spent near the intruder (preparturition 167.31 ± 30.7 s; postparturition 166.5 ± 27.2 s; ANOVA, $F_{1,22} = .001$, $p = 0.98$, figure 2A) or the total number of aggressive behaviors displayed towards the intruder or towards the mate (preparturition: 2.19 ± 0.64 aggressive behaviors; postparturition: 3.13 ± 0.89 aggressive behaviors; ANOVA, $F_{1, 22} = 1.05$, $p = 0.323$). However, parental pairs did spend significantly less time together near the intruder, defined as when both members of the pair were within 2.5 cm of the intruder's cage (preparturition: 104.38 ± 26.15; postparturition: 61.38 ± 24.62; ANOVA, $F_{1,22} = 4.86$, $p = 0.043$, figure 2B).

Ultrasonic vocalizations

Vocalizations in non-parental pairs

Call production and duration were calculated at the level of the dyad. In nonparental pairs the overall production of SV calls per minute did not differ across phases (pre-encounter: 2.14 ± 0.59; encounter: 1.85 ± 0.50; postencounter: 1.39 ± 0.40; ANOVA, $F_{2, 40} = 1.06$, $p = 0.36$). The number of calls produced also did not correlate with any behavioral measures across phases. Call duration was significantly reduced during the encounter stage (pre-encounter: 191.74 ± 14.39 ms; encounter: 129.21 ± 15.81 ms; postencounter: 150.63 ± 9.49 ms, ANOVA, $F_{2,40} = 4.98$, $p = 0.03$) across pairs. However, the number of calls produced (joint: 15.15 ± 8.60; divided: 11 ± 5.66, ANOVA $F_{1,13} = 0.89$, $p = 0.39$) and the duration of calls (joint: 144.87 ± 24.82; divided: 173.98 ± 21.96, ANOVA, $F_{1,13} = 0.27$, $p = 0.79$) did not differ based on the strategy of the pair.

While the number of calls produced did not predict strategy or other behavioral measures, the duration of calls was associated with a number of behavioral measures across phases. Pre-encounter SV duration significantly predicted the latency to approach the intruder in males but not females, such that longer calls indicated a quicker latency to approach the intruder (linear regression, $R^2 = 0.6453$, $F_{1,6} = 10.92$, $p = 0.0163$, figure 3A). Longer calls pre-encounter also predicted less time spent in the nest by males but not females (Linear regression, $R^2 = 0.4965$, $F_{1,6} = 5.916$, $p = 0.0510$, figure 3B). Interestingly, shorter calls during the encounter predicted greater aggression towards the intruder by males (linear regression: $R^2 = 0.37$, $F_{1,11} = 6.49$, $p = 0.027$, figure 4A). Conversely, a nonsignificant trend was seen such that longer calls during the encounter predicted individuals spending a greater amount of time spent sniffing and interacting with their mate for both males and females (linear regression. $R^2 = 0.1587$, $F_{1,20} = 3.77$, $p = 0.056$, figure 4B).

Vocalizations in parental pairs

Parental pairs showed no difference in their production of calls per minute across phases (pre-encounter: 3.35 ± 1.56; encounter: 2.65 ± 0.77; 0.49; post-encounter: 1.10 ± 0.56, ANOVA, $F_{2,9} = 1.75$, $p = 0.23$). There was also no difference between pre-parturition call production and post-parturition call production within pairs (ANOVA, $F_{2,9} = 1.13$, $p = 0.31$). Moreover, there was also no change in the number of pairs that produced calls from preparturition to postparturition (Fisher's exact test, $p = 0.67$). Unlike in the preparturition stage, however, there was no change in the duration of calls across phases in parental pairs (pre-encounter: 153.10 ± 40.0 s; encounter: 156.43 ± 24.9 s; post-duration: 195.79 ± 21.99

s; ANOVA: $F_{2,6} = 0.01$, $p = 0.98$). There was also no difference in call duration from pre-parturition to post-parturition (ANOVA, $F_{1,6} = 1.36$, $p = 0.22$). Unlike during the pre-parturition tests, pre-encounter duration did not predict latency to attack (linear regression, $R^2 = 0.01$, $F_{1,6} = 0.067$, $p = 0.80$) or time spent near the nest (linear regression, $R^2 = 0.01$, $F_{1,6} = 0.01$, $p = 0.95$) in males or females. Similar to pre-parturition, however, there was a trend for shorter SV calls to predict greater aggression in males (linear regression, $R^2 = 0.63$, $F_{1,4} = 6.84$, $p = 0.059$) as well as a nonsignificant trend for fewer total calls during the encounter to predict more time spent near the intruder in males (linear aggression, $R^2 = 0.48$, $F_{1,6} = 5.60$, $p = 0.056$).

Experiment 2: Pup retrieval

Pup retrieval

Pup retrieval tests revealed a sex difference in behavior. Pups were successfully retrieved in nine out of 12 pairs. Within those nine pairs females retrieved all pups in eight out of nine pairs, which was significantly more often than males (Fisher's exact test: $p = 0.0034$, figure 5). However, males were first to approach the pups in seven of the nine pairs where retrieval occurred (Fisher's exact test, $p = 0.056$, figure 5) and overall had a trend for approaching pups more quickly (Student's t-test, $t_{12} = 1.84$ $p = 0.089$). Across the intruder and pup retrieval tests, the latency to approach pups was highly correlated with the latency to approach intruders (linear regression, $R^2 = 0.53$, $F_{1,10} = 11.08$, $p = 0.008$). Moreover, there was a trend for time spent huddling with pups during pup retrievals to correlate with time spent in the nest during intruder trials, although it was not significant (linear regression, $R^2 = 0.36$, $F_{1,9} = 4.94$, $p = 0.053$).

Vocalizations during pup retrieval

During the separation phase, pups produced a greater number of calls than their parents (pups: 25 ± 8.00 calls; parents: 3.13 ± 1.50 calls, paired t-test, $t_{15} = 2.98$, $p = 0.009$). When combined, the total calls produced by both pups and parents did not differ between the separation and the retrieval phases (separation: 31.0 ± 17.03 calls; retrieval: 18.5 ± 8.33 calls, paired t-test, $t_{11} = 1.26$, $p = 0.25$). Call production by pups was not correlated with the total number of pups in the litter (linear regression, $R^2 < 0.01$, $F_{1, 14} < 0.01$, $p = 0.98$).

Unlike during territorial defense, call production and call duration did not correlate with behavioral measures during pup retrieval. Pup call production in the separation phase did not correlate with parent's latency to approach pups (linear regression, $R^2 = 0.05$, $F_{1,11} = 0.46$, $p = 0.51$), parents huddling and grooming time with pups (linear regression, $R^2 = 0.06$, $F_{1,11} = 0.65$, $p = 0.45$) or the time to retrieval of pups (linear regression, $R^2 = 0.04$, $F_{1,11} = 0.16$, $p = 0.7$). Parental calls and duration during the separation phase also did not correlate with latency to approach (linear regression, $R^2 = 0.04$, $F_{1,11} = 0.46$, $p = 0.51$), huddling and grooming (linear regression, $R^2 = 0.05$, $F_{1,11} = 0.54$, $p = 0.47$), or time to retrieval (linear regression, $R^2 = 0.003$, $F_{1,11} = 0.01$, $p = 0.92$). Moreover, parental calls during the retrieval phase did not correlate with latency to approach (linear regression, $R^2 = 0.02$, $F_{1,11} = 0.23$, $p = 0.64$), huddling and grooming (linear regression, $R^2 = 0.07$, $F_{1,11} = 0.81$, $p = 0.38$) or time to retrieval (linear regression, $R^2 = 0.10$, $F_{1,11} = 0.44$, $p = 0.54$). However, combined pup and parental calls decreased after pups were retrieved. Analyzing calls before and after the final pup was retrieved showed an almost complete cessation of calling (pre-retrieval 15.78 ± 6.22 calls; post-retrieval: 1.11 ± 0.75 calls; paired t-test, $t_8 = 2.60$, $p = 0.03$).

Discussion

Monogamous species across taxa divide labor to complete tasks (Mathews, 2002) and these behavioral strategies become particularly important with the birth of offspring (Boucaud, Aguirre Smith, Valère, & Vignal, 2016; Hanson, O'Connor, Van Der Kraak, & Cooke, 2009). Previous research in birds (Hall, 2004; Quinard & Cézilly, 2012), fish (Rogers, 2010) and primates (Caselli, Mennill, Bicca-Marques, & Setz, 2014; French & Snowdon, 1981) has shown that species use different strategies in order to defend territories and care for offspring. Despite this research, little is known about how monogamous and biparental rodents divide labor in order to complete these tasks. We studied the California mouse to better answer these questions and found that division of labor in California mice is task specific, with territorial defense and pup retrieval following different systems. Territorial defense was completed by pairs using one of two strategies, 1) joint defense or 2) divided defense that are pair specific. Generally, pairs maintained one strategy across contexts with little behavioral change. Pup retrievals, on the other hand, followed a sex-specific strategy, such that males approached pups first, but females retrieved pups to the nest. Moreover, in other species including birds and primates we know that vocal communication plays an important role in coordinating behavior in division of labor (French & Snowdon, 1981; Haimoff, 1986; Hall, 2000, 2004; Koloff & Mennill, 2011). As such, we tested whether vocal communication, via the production of USVs, has the potential to mediate behavioral coordination in California mice. We found that, while SV duration predicted a number of behavioral outcomes during territorial defense, USV production seemed to play no role in coordinating pup retrieval. These findings indicate that California mouse division of labor is task specific and that vocal communication plays a role in coordinating territorial defense but not pup retrieval.

Division of labor in territorial defense

Division of labor and behavioral coordination are used to efficiently defend and maintain territories against intruders (Mathews, 2002). Previous research has found that pairs of different species generally use one of three strategies to defend their territories from intruders (French & Snowdon, 1981; Hall, 2004; Koloff & Mennill, 2011; Sandell, 1998). Sex-specific strategies, as is seen in cichlid fish (Rogers, 2010) and dik diks (*Madoqua kirkii*) (Brotherton, Pemberton, Komers, & Malarky, 1997), involve one sex defending the territory against all intruders while the other remains closer to the nest. Mate guarding strategies, which have been seen in birds and voles involve members of a pair defending against same sex intruders (Koloff & Mennill, 2011; Reburn & Wynne-Edwards, 1999). Finally, cooperative strategies, often seen in primates, involve members of the pair working together in order to fend off intruders (Clutton-Brock, 2009; French & Snowdon, 1981; J. G. Robinson, 1981). In this study we tested whether California mice would also follow one of these common strategies. We found that, unlike many species tested to this point (Bales & Carter, 2003; Koloff & Mennill, 2011; Mathews, 2002;

Reburn & Wynne-Edwards, 1999; Rogers, 2010), California mice follow neither a mate guarding nor a sex-specific strategy of territorial defense. Instead California mice followed a pair-specific strategy that was maintained regardless of the sex of the intruder presented to the pair. To our knowledge, this is the first time pairs within a species have been shown to follow differing strategies to complete the same task. It is possible that this reflects that relatively strict level of monogamy within this species. Importantly, these strategies were maintained regardless of the sex of the intruder and even after the birth of pups. That these strategies are maintained indicates that there is little behavioral plasticity in labor divisions during territorial defense and may provide insight, through future studies, on behavioral syndromes and how they affect coordination within pair bonded individuals. It would also be interesting to investigate whether mate choice is based on behavioral strategy used.

In many species, the birth of offspring alters division of labor such that pairs spend more time completing separate tasks (Bester-Meredith et al., 2016; Goymann & Wingfield, 2004; Numan & Young, 2016). In both fish (Rogers, 2010) and prairie voles (Ahern et al., 2011), one member of the pair spends more time patrolling the edge of the territory for potential threats while the other remains close to the nest. Similarly in California mice, pairs show differences in scent marking with males marking more at the edge of territories and females marking more in the middle (Becker et al., 2018; Becker, Petruno, & Marler, 2012)Although California mouse pairs did not show a change in overall strategy, they did show similar changes in time allocation following the birth of pups. Individuals spent more overall time in the nest. Along with this, pairs spent less time together near the intruder. Similar to prairie voles (Ahern et al., 2011), this subtle change in behavior likely helps to prevent pups from being exposed while both parents are away. Separating tasks in this manner would seemingly increase efficiency of the pair. By altering the time allocation of individuals within the pair, the pair can optimize behavior to complete multiple tasks at one time.

Vocal communication as a mediator of behavioral coordination during territorial defense

Previous work has shown that vocal communication plays a vital role in the coordination of behavior across species (Noe, 2006). This is particularly true of territorial defense. For example, black fronted titi monkeys (genus *Callicebus*) produce loud calls that can be directed both within and outside of an individual's group to warn away intruders or recruit help in defending a territory (Caselli, Mennill, Bicca-Marques, & Setz, 2014; Robinson, 1981). Similarly, duetting in song birds functions to help protect territorial boundaries and keep intruders at bay (Brenowitz et al., 1985; Hall, 2000; Langmore, 1998). However, to this point it remained unknown what role, if any, vocalizations played in the coordination of behaviors by rodents. The majority of studies on ultrasonic vocalizations in rodents have focused on courtship and aggression (Burgdorf et al., 2008; Hammerschmidt et al., 2012), but we sought to understand how these calls may affect the coordination of behavior of our monogamous species.

We predicted that SVs, due to their dynamic role across social interactions in both the lab and the field (Kalcounis-Rueppell et al., 2010, 2006; Pultorak et al., 2015), would function in the coordination of territorial defense. We found that the duration of SV calls was generally predictive of territorial defense behaviors by males, but not females. That SV calls broadly predict male but not female behavior likely means that many of these calls were produced by males as has been seen in previous studies (Rieger & Marler, 2018). This may mean that vocalizations play a greater role in the behavioral repertoire of male California mic. We initially found that shorter SV calls during an encounter predicted greater aggression by males replicating previous findings (Rieger & Marler, 2018), whereas longer SV calls predicted more affiliative behavior between mates. However, these patterns were reversed in calls produced during the pre-encounter stage such that longer calls predicted a shorter latency to approach intruders as well as less time spent in the nest during the encounter. This would indicate that, in the absence of an intruder, longer calls, which travel further (Kalcounis-Rueppell et al., 2006; von Merten et al., 2014; Wagner, 1989), may act as an expression of territoriality. In support of this suggestion, two males were seen to stand on their igloos and produce long SV calls prior to the introduction of an intruder. These observations are consistent with findings in other species including primates (Caselli et al., 2014) birds (Große Ruse, Hasselquist, Hansson, Tarka, & Sandsten, 2016) and rock hyraxes (*Procavia capensis*) (Goll, Demartsev, Koren, & Geffen, 2017), where longer calls are used to express ownership of a territory and maintain territorial boundaries. This study provides further evidence that SVs are context dependent, consistent with previous work in this and other rodent species (Ma, Resendez, & Aragona, 2014; Hurley and Kalcounis-Reuppell, 2018) in that their duration has different meaning depending on the social situation experienced by the pair. This is similar to studies in primates, which show that the same call can be used in different contexts, thus having different meanings to group members (Snowdon, 2017). Overall, call duration is an important predictor of future behavior in males and is context specific, which may help to coordinate the behavior of the pair in advance of the presence of an intruder.

Pup retrievals

Monogamous species generally show biparental care where both the mother and father provide vital care to ensure the wellbeing and survival of the offspring (Lukas & Clutton-Brock, 2013). Depending on the species, the overall input to parental care by the mother and father varies (Bales & Saltzman, 2016; Gubernick & Alberts, 1981; Reburn & Wynne-Edwards, 1999). Paternal care can range from patrolling the edge of territories (Dey, O'Connor, Gilmour, Van Der Kraak, & Cooke, 2010; Lynn, 2008) to more hands on care including feeding, grooming and retrieval (Gubernick & Teferi, 2000; Insel, Wang, & Ferris, 1994). How species divide labor in parental care is vital to the success of the offspring, while also allowing for individuals to alternate between caring for pups and other tasks such as foraging (Clutton-Brock et al., 2001). In California mice, a biparental species, both paternal and maternal care are important to the survival of pups (Bester-Meredith et al., 2016; Gubernick & Teferi, 2000; Gubernick et al., 1993) and the pups behavioral outcomes as adults (Bester-Meredith & Marler, 2003, 2007). However, despite knowing that both mothers and fathers will retrieve pups while alone (Bester-Meredith & Marler, 2012; Frazier et al., 2006; Marler, Bester-Meredith, & Trainor, 2003) we did not yet know how pairs would complete this task while together. We predicted that both males and females would retrieve pups. This prediction was not supported as a sex difference was found in pup retrievals where males were the first to approach pups but females acted as the predominant retriever. This may be due to the young age of the pups used in this study as previous research has found that fathers play more often retrieve older, more mobile pups (Bester-Meredith, Young, & Marler, 1999; Dulac, O'Connell, & Wu, 2014; Frazier et al., 2006; Gubernick & Nelson, 1989, Marler et al., 2008). Overall, this study showed a robust sex difference in pup retrieval.

Vocalizations do not play a major role in coordinating pup retrievals

Vocalizations by offspring are important to directing parental care including driving feeding (Ricklefs, 1992) and retrieval (Bowers, Perez-Pouchoulen, Edwards, & McCarthy, 2013; Marlin, Mitre, D'amour, Chao, & Froemke, 2015; Portfors, 2007) across species. In many rodent species, increasing pup vocalizations leads to quicker retrieval by mothers (Bowers et al., 2013; Marlin et al., 2015), however, this relationship has not been seen in California mice (Wright & Brown, 2004). We tested the role of USVs in pup retrievals, predicting that increased pup calling would speed retrieval and that pairs would use USV calls to coordinate their response. These predictions were not supported and findings were consistent with Wright & Brown (2004). SV calling by both pups and parents did not correlate with measures of pup retrieval. This result seems to indicate that vocalizations are less important to pup retrieval and behavioral coordination by parents. One important caveat to this, though, was that pup vocalizations ceased after the completion of retrievals. This cessation to calling is at least ancillary evidence that pup calls play some role in retrievals, though this connection should be investigated further. Currently, studies are being conducted to directly test whether playbacks of pup calls influence the approach behavior and time spent together by parental pairs.

Conclusion

These experiments show that California mice divide labor to complete tasks and that different strategies are employed depending on the tasks employ. Territorial defense strategies were robust across multiple tests and life history points, indicating limited plasticity. Importantly, the mechanisms by which these strategies are chosen and how they are maintained remains unknown. We found that vocal communication predicted multiple territorial defense behaviors, but not pup retrieval. This result indicates that vocal communication likely only helps to coordinate behaviors in specific tasks. This, as well as the different strategies in general, provides evidence that territorial defense and pup retrievals may work through separate mechanisms. Overall, division of labor in pair bonded California mice provides a unique opportunity to investigate the processes that contribute to the coordination of behavior that allows for division of labor in different tasks.

Acknowledgments

We thank Eliza van Dixhorn, Sage Keyes, Juliette Schefelker, JB Hernandez and Hannah Poessehl for their help with collecting and analyzing data for this project and the University of Wisconsin Madison animal research technicians. We also thank Dr. Charles Snowdon for valuable comments and discussion on this project and Dr. Frank Castelli for comments on this manuscript. Finally we thank the National Science Foundation for funding this project IOS11312419.

References

Ahern, T. H., Hammock, E. A. D., & Young, L. J. (2011). Parental division of labor, coordination, and the effects of family structure on parenting in monogamous prairie voles (Microtus ochrogaster). *Developmental Psychobiology*, *53*(2), 118–31. http://doi.org/10.1002/dev.20498

Bales, K. L., & Carter, C. S. (2003). Sex differences and developmental effects of oxytocin on aggression and social behavior in prairie voles (Microtus ochrogaster). *Hormones and Behavior*, *44*(3), 178–184. http://doi.org/10.1016/S0018-506X(03)00154-5

Bales, K. L., & Saltzman, W. (2016). Fathering in rodents: Neurobiological substrates and consequences for offspring. *Hormones and Behavior*, *77*. http://doi.org/10.1016/j.yhbeh.2015.05.021

Becker, E. A., Castelli, F. R., Yohn, C. N., Spencer, L., & Marler, C. A. (2018). Species differences in urine scent-marking and counter-marking in Peromyscus. *Behavioural Processes*, *146*, 1–9. http://doi.org/10.1016/J.BEPROC.2017.10.011

Becker, E. A., Petruno, S., & Marler, C. A. (2012). A Comparison of Scent Marking between a Monogamous and Promiscuous Species of Peromyscus: Pair Bonded Males Do Not Advertise to Novel Females. *PLoS ONE*, *7*(2), e32002. http://doi.org/10.1371/journal.pone.0032002

Bendesky, A., Kwon, Y.-M., Lassance, J.-M., Lewarch, C. L., Yao, S., Peterson, B. K., … Hoekstra, H. E. (2017). The genetic basis of parental care evolution in monogamous mice. *Nature*. http://doi.org/10.1038/nature22074

Bester-Meredith, J., Conley, M., & Mammarella, G. (2016). Peromyscus as a Model System for

Understanding the Regulation of Maternal Behavior. *Seminars in Cell and Developmental Biology*. http://doi.org/10.1016/j.semcdb.2016.07.001

Bester-Meredith, J. K., & Marler, C. A. (2001). Vasopressin and Aggression in Cross-Fostered California Mice (Peromyscus californicus) and White-Footed Mice (Peromyscus leucopus). *Hormones and Behavior*, *40*(1), 51–64. http://doi.org/10.1006/hbeh.2001.1666

Bester-Meredith, J. K., & Marler, C. A. (2003). The Association Between Male Offspring Aggression and Paternal and Maternal Behavior of Peromyscus Mice. *Ethology*, *109*(10), 797–808. http://doi.org/10.1046/j.0179-1613.2003.00917.x

Bester-Meredith, J. K., & Marler, C. A. (2007). Social Experience During Development and Female Offspring Aggression in Peromyscus Mice. *Ethology*, *113*(9), 889–900. http://doi.org/10.1111/j.1439-0310.2007.01393.x

Bester-Meredith, J. K., & Marler, C. A. (2012). Naturally occurring variation in vasopressin immunoreactivity is associated with maternal behavior in female Peromyscus mice. *Brain, Behavior and Evolution*, *80*(4), 244–53. http://doi.org/10.1159/000341899

Bester-Meredith, J. K., Young, L. J., & Marler, C. A. (1999). Species Differences in Paternal Behavior and Aggression in Peromyscus and Their Associations with Vasopressin Immunoreactivity and Receptors. *Hormones and Behavior*, *36*(1), 25–38. http://doi.org/10.1006/hbeh.1999.1522

Boucaud, I. C. A., Aguirre Smith, M. L. N., Valère, P. A., & Vignal, C. (2016). Incubating females signal their needs during intrapair vocal communication at the nest: a feeding experiment in great tits. *Animal Behaviour*, *122*, 77–86. http://doi.org/10.1016/j.anbehav.2016.09.021

Bowers, J. M., Perez-Pouchoulen, M., Edwards, N. S., & McCarthy, M. M. (2013). Foxp2 Mediates Sex Differences in Ultrasonic Vocalization by Rat Pups and Directs Order of Maternal Retrieval. *Journal of Neuroscience*, *33*(8), 3276–3283. http://doi.org/10.1523/JNEUROSCI.0425-12.2013

Brenowitz, E. A., Arnold, A. P., & Levin, R. N. (1985). Neural correlates of female song in tropical duetting birds. *Brain Research*, *343*(1), 104–112. http://doi.org/10.1016/0006-8993(85)91163-1

Brotherton, P. N., Pemberton, J. M., Komers, P. E., & Malarky, G. (1997). Genetic and behavioural evidence of monogamy in a mammal, Kirk's dik-dik (Madoqua kirkii). *Proceedings. Biological Sciences*, *264*(1382), 675–81. http://doi.org/10.1098/rspb.1997.0096

Burgdorf, J., Kroes, R. A., Moskal, J. R., Pfaus, J. G., Brudzynski, S. M., & Panksepp, J. (2008). Ultrasonic vocalizations of rats (Rattus norvegicus) during mating, play, and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback.. *Journal of Comparative Psychology*, *122*(4), 357–367. http://doi.org/10.1037/a0012889

Campbell, A. F., & Boinski, S. (1995). Use of Trill Vocalizations To Coordinate Troop Movement Among White-Faced Capuchins: a Second Field Test. *Behaviour*, *132*(11), 875–901. http://doi.org/10.1163/156853995X00054

Caselli, C. B., Mennill, D. J., Bicca-Marques, J. C., & Setz, E. Z. F. (2014). Vocal behavior of black-fronted titi monkeys (*Callicebus nigrifrons*): Acoustic properties and behavioral contexts of loud calls. *American Journal of Primatology*, *76*(8), 788–800. http://doi.org/10.1002/ajp.22270

Clutton-Brock, T. (2009). Cooperation between non-kin in animal societies. *Nature*, *462*(7269), 51–57. http://doi.org/10.1038/nature08366

Clutton-Brock, T. H., Brotherton, P. N., Russell, A. F., O'Riain, M. J., Gaynor, D., Kansky, R., … Monfort, S. (2001). Cooperation, control, and concession in meerkat groups. *Science (New York, N.Y.)*, *291*(5503), 478–81. http://doi.org/10.1126/science.291.5503.478

Dey, C. J., O'Connor, C. M., Gilmour, K. M., Van Der Kraak, G., & Cooke, S. J. (2010). Behavioral and physiological responses of a wild teleost fish to cortisol and androgen manipulation during parental care. *Hormones and Behavior*, *58*(4), 599–605. http://doi.org/10.1016/j.yhbeh.2010.06.016

Dulac, C., O'Connell, L. A., & Wu, Z. (2014). Neural control of maternal and paternal behaviors. *Science (New York, N.Y.)*, *345*(6198), 765–70. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4230532&tool=pmcentrez&rendertype=abstract

Frazier, C. R. M., Trainor, B. C., Cravens, C. J., Whitney, T. K., & Marler, C. A. (2006). Paternal behavior influences development of aggression and vasopressin expression in male California mouse offspring. *Hormones and Behavior*, *50*(5), 699–707. http://doi.org/10.1016/j.yhbeh.2006.06.035

French, J. A., & Snowdon, C. T. (1981). Sexual dimorphism in responses to unfamiliar intruders in the tamarin, Saguinus oedipus. *Animal Behaviour*, *29*(3), 822–829. http://doi.org/10.1016/S0003-3472(81)80016-4

Fuxjager, M. J., Mast, G., Becker, E. A., & Marler, C. A. (2009). The 'home advantage' is necessary for a full winner effect and changes in post-encounter testosterone. *Hormones and Behavior*, *56*(2), 214–219. http://doi.org/10.1016/j.yhbeh.2009.04.009

Fuxjager, M. J., Zhao, X., Rieger, N. S., & Marler, C. A. (n.d.). Why animals fight: uncovering the function and mechanisms of territorial aggression. In *American Psychological Association Handbook of Comparative Psychology*.

Goll, Y., Demartsev, V., Koren, L., & Geffen, E. (2017). Male hyraxes increase countersinging as strangers become 'nasty neighbours.' *Animal Behaviour*, *134*, 9–14. http://doi.org/10.1016/J.ANBEHAV.2017.10.002

Goymann, W., & Wingfield, J. C. (2004). Competing females and caring males. Sex steroids in African black coucals, Centropus grillii. *Animal Behaviour*, *68*(4), 733–740. http://doi.org/10.1016/j.anbehav.2003.12.012

Große Ruse, M., Hasselquist, D., Hansson, B., Tarka, M., & Sandsten, M. (2016). Automated analysis of song structure in complex birdsongs. *Animal Behaviour*, *112*, 39–51. http://doi.org/10.1016/j.anbehav.2015.11.013

Gubernick, D. J., & Alberts, J. R. (1981). The Biparental Care System of the California Mouse, Peromyscus californicus. *Journal of Comparative Psychology 19S7*, *101*(2), 169–177. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.597.5446&rep=rep1&type=pdf

Gubernick, D. J., & Nelson, R. J. (1989). Prolactin and paternal behavior in the biparental California mouse, Peromyscus californicus. *Hormones and Behavior*, *23*(2), 203–210. http://doi.org/10.1016/0018-506X(89)90061-5

Gubernick, D. J., & Teferi, T. (2000). Adaptive significance of male parental care in a monogamous mammal. *Proceedings. Biological Sciences*, *267*(1439), 147–50. http://doi.org/10.1098/rspb.2000.0979

Gubernick, D. J., Wright, S. L., & Brown, R. E. (1993). The significance of father's presence for offspring survival in the monogamous California mouse, Peromyscus californicus. *Animal Behaviour*, *46*(3), 539–546. http://doi.org/10.1006/ANBE.1993.1221

Haimoff, E. H. (1986). Convergence in the duetting of monogamous Old World primates. *Journal of Human Evolution*, *15*(1), 51–59. http://doi.org/10.1016/S0047-2484(86)80065-3

Hall, M. L. (2000). The function of duetting in magpie-larks: conflict, cooperation, or commitment? *Animal Behaviour*, *60*(5), 667–677. http://doi.org/10.1006/anbe.2000.1517

Hall, M. L. (2004). A review of hypotheses for the functions of avian duetting. *Behavioral Ecology and Sociobiology*, *55*(5), 415–430. http://doi.org/10.1007/s00265-003-0741-x

Hammerschmidt, K., Radyushkin, K., Ehrenreich, H., Fischer, J., Scattoni, M., Crawley, J., … Janik, V. (2012). The Structure and Usage of Female and Male Mouse Ultrasonic Vocalizations Reveal only Minor Differences. *PLoS ONE*, *7*(7), e41133. http://doi.org/10.1371/journal.pone.0041133

Hanson, K. C., O'Connor, C. M., Van Der Kraak, G., & Cooke, S. J. (2009). Paternal aggression towards a brood predator during parental care in wild smallmouth bass is not correlated with circulating testosterone and cortisol concentrations. *Hormones and Behavior*, *55*(4), 495–499. http://doi.org/10.1016/j.yhbeh.2009.02.001

Hurley, L.M., & Kalcounis-Rueppell, M.C., In press. State and context in vocal communication in rodents.

Insel, T. R., Wang, Z. X., & Ferris, C. F. (1994). Patterns of brain vasopressin receptor distribution associated with social organization in microtine rodents. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *14*(9), 5381–92. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8083743

Jeanne, R. L. (2016). Division of labor is not a process or a misleading concept. *Behavioral Ecology and Sociobiology*, *70*(7), 1109–1112. http://doi.org/10.1007/s00265-016-2146-7

Kalcounis-Rueppell, M. C., Metheny, J. D., & Vonhof, M. J. (2006). Production of ultrasonic vocalizations by Peromyscus mice in the wild. *Frontiers in Zoology*, *3*(1), 3. http://doi.org/10.1186/1742-9994-3-3

Kalcounis-Rueppell, M. C., Petric, R., Briggs, J. R., Carney, C., Marshall, M. M., Willse, J. T., … Crossland, J. P. (2010). Differences in ultrasonic vocalizations between wild and laboratory California mice (Peromyscus californicus). *PloS One*, *5*(4), e9705. http://doi.org/10.1371/journal.pone.0009705

Kalcounis-Rueppell, M.C., Pultorak, J.D. Marler, C.A. Ultrasonic Vocalizations of Mice in the Genus Peromyscus . In: Stefan M. Brudzynski, editor, Handbook of Behavioral Neuroscience, Vol. 25 , Amsterdam: Academic Press, 2018, pp. 227-235.

King, A. J., & Sueur, C. (2011). Where Next? Group Coordination and Collective Decision Making by Primates. *International Journal of Primatology*, *32*(6), 1245–1267. http://doi.org/10.1007/s10764-011-9526-7

Koloff, J., & Mennill, D. (2011). Aggressive responses to playback of solos and duets in a Neotropical antbird. *Animal Behaviour*, *82*(3), 587–593. http://doi.org/10.1016/j.anbehav.2011.06.021

Langmore, N. . (1998). Functions of duet and solo songs of female birds. *Trends in Ecology & Evolution*, *13*(4), 136–140. http://doi.org/10.1016/S0169-5347(97)01241-X

Lukas, D., & Clutton-Brock, T. H. (2013). The evolution of social monogamy in mammals. *Science (New York, N.Y.)*, *341*(6145), 526–30. http://doi.org/10.1126/science.1238677

Lynn, S. E. (2008). Behavioral insensitivity to testosterone: Why and how does testosterone alter paternal and aggressive behavior in some avian species but not others? *General and Comparative Endocrinology*, *157*(3), 233–240. http://doi.org/10.1016/j.ygcen.2008.05.009

Ma, S. T., Resendez, S. L., & Aragona, B. J. (2014). Sex differences in the influence of social context, salient social stimulation and amphetamine on ultrasonic vocalizations in prairie voles. *Integrative Zoology*, *9*(3). http://doi.org/10.1111/1749-4877.12071

Marler, C. A., Bester-Meredith, J. K., & Trainor, B. C. (2003). Paternal Behavior and Aggression: Endocrine Mechanisms and Nongenomic Transmission of Behavior. *Advances in the Study of Behavior*, *32*, 263–323. http://doi.org/10.1016/S0065-3454(03)01006-4

Marler, C. A., Trainor, B. C., Gleason, E. D., Bester-Meredith, J. K., & Becker, E. A. (2008). The effects of paternal behavior on offspring aggression and hormones in the biparental California mouse. In R. S. Bridges (Ed.), *Neurobiology of the parental brain* (pp. 435–448). Academic. Retrieved from https://books.google.com/books?hl=en&lr=&id=5kGGAE90nmYC&oi=fnd&pg=PA435&dq=info:_RV_HYZXFOcJ:scholar.google.com&ots=yO5HMsbabG&sig=sj05oKkJxN4uJQTet7QrRkUsk9k#v=onepage&q&f=false

Marlin, B. J., Mitre, M., D'amour, J. A., Chao, M. V., & Froemke, R. C. (2015). Oxytocin enables maternal behaviour by balancing cortical inhibition. *Nature*, *520*(7548), 499–504. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4409554&tool=pmcentrez&rendertype=abstract

Mathews, L. M. (2002). Territorial cooperation and social monogamy: factors affecting intersexual behaviours in pair-living snapping shrimp. *Animal Behaviour*, *63*(4), 767–777. http://doi.org/10.1006/anbe.2001.1976

Mitani, J. C. (1984). The behavioral regulation of monogamy in gibbons (Hylobates muelleri). *Behavioral Ecology and Sociobiology*, *15*(3), 225–229. http://doi.org/10.1007/BF00292979

Noe, R. (2006). Cooperation Experiments: coordination through communication versus acting apart together. *Animal Behaviour*, *71*, 1–18. http://doi.org/10.1016/j.anbehav.2005.03.037

Numan, M., & Young, L. J. (2016). Neural mechanisms of mother-infant bonding and pair bonding: Similarities, differences, and broader implications. *Hormones and Behavior*. http://doi.org/10.1016/j.yhbeh.2015.05.015

Page, R. E., Scheiner, R., Erber, J., & Amdam, G. V. (2006). The Development and Evolution of Division of Labor and Foraging Specialization in a Social Insect (Apis mellifera L.). *Current Topics in Developmental Biology*, *74*, 253–286. http://doi.org/10.1016/S0070-2153(06)74008-X

Portfors, C. V. (2007). Types and Functions of Ultrasonic Vocalizations in Laboratory Rats and Mice.

Pultorak, J. D., Fuxjager, M. J., Kalcounis-Rueppell, M. C., & Marler, C. A. (2015). Male fidelity expressed through rapid testosterone suppression of ultrasonic vocalizations to novel females in the monogamous California mouse. *Hormones and Behavior*, *70*, 47–56. http://doi.org/10.1016/j.yhbeh.2015.02.003

Pultorak, J. D., Matusinec, K. R., Miller, Z. K., & Marler, C. A. (2017). Ultrasonic vocalization production and playback predicts intrapair and extrapair social behaviour in a monogamous mouse. *Animal Behaviour*, *125*, 13–23. http://doi.org/10.1016/j.anbehav.2016.12.023

Quinard, A., & Cézilly, F. (2012). Sex roles during conspecific territorial defence in the Zenaida dove, Zenaida aurita. *Animal Behaviour*, *83*(1), 47–54. http://doi.org/10.1016/j.anbehav.2011.09.032

Reburn, C. J., & Wynne-Edwards, K. E. (1999). Hormonal Changes in Males of a Naturally Biparental and a Uniparental Mammal. *Hormones and Behavior*, *35*(2), 163–176. http://doi.org/10.1006/hbeh.1998.1509

Ribble, D. O. (1991). The monogamous mating system of Peromyscus californicus as revealed by DNA fingerprinting. *Behavioral Ecology and Sociobiology*, *29*(3), 161–166. http://doi.org/10.1007/BF00166397

Ribble, D., & Salvioni, M. (1990). Social organization and nest co-occupancy in Peromyscus californicus, a monogamous rodent. *Behavioral Ecology and Sociobiology*, *26*(1), 9–15. http://doi.org/10.1007/BF00174020

Ricklefs, R. E. (1992). The roles of parent and chick in determining feeding rates in Leach's storm-petrel. *Animal Behaviour*, *43*(6), 895–906. http://doi.org/10.1016/S0003-3472(06)80003-5

Rieger, N. S., & Marler, C. A. (2018). The function of ultrasonic vocalizations during territorial defence by pair-bonded male and female California mice. *Animal Behaviour*, *135*. http://doi.org/10.1016/j.anbehav.2017.11.008

Rieger, N.S., & Marler, C.A. Unpublished data. Parental response to pup ultrasonic vocalizations.

Robinson, G. E. (2003). Regulation of Division of Labor in Insect Societies. *Http://Dx.Doi.Org/10.1146/Annurev.En.37.010192.003225*.

Robinson, J. G. (1981). Vocal regulation of inter- and intragroup spacing during boundary encounters in the titi monkey,Callicebus moloch. *Primates*, *22*(2), 161–172. http://doi.org/10.1007/BF02382607

Robson, S. K. A., & Traniello, J. F. A. (2016). Division of labor in complex societies: a new age of conceptual expansion and integrative analysis. *Behavioral Ecology and Sociobiology*, *70*(7), 995–998. http://doi.org/10.1007/s00265-016-2147-6

Rogers, W. (2010). Parental Investment and Division of Labor in the Midas Cichlid (Cichlasoma citrinellum). *Ethology*, *79*(2), 126–142. http://doi.org/10.1111/j.1439-0310.1988.tb00706.x

Sandell, M. I. (1998). Female aggression and the maintenance of monogamy: female behaviour predicts male mating status in European starlings. *Proceedings of the Royal Society B: Biological Sciences*, *265*(1403), 1307–1311. http://doi.org/10.1098/rspb.1998.0434

Schuppe, E. R., Sanin, G. D., & Fuxjager, M. J. (2016). The social context of a territorial dispute differentially influences the way individuals in breeding pairs coordinate their aggressive tactics. *Behavioral Ecology and Sociobiology*, *70*(5), 673–682. http://doi.org/10.1007/s00265-016-2088-0

von Merten, S., Hoier, S., Pfeifle, C., Tautz, D., Branchi, I., Santucci, D., … Hu, M. (2014). A Role for Ultrasonic Vocalisation in Social Communication and Divergence of Natural Populations of the House Mouse (Mus musculus domesticus). *PLoS ONE*, *9*(5), e97244. http://doi.org/10.1371/journal.pone.0097244

Wagner, W. E. (1989). Graded aggressive signals in Blanchard's cricket frog: vocal responses to opponent proximity and size. *Animal Behaviour*, *38*(6), 1025–1038. http://doi.org/10.1016/S0003-3472(89)80141-1

Wright, S. L., & Brown, R. E. (2004). Sex differences in ultrasonic vocalizations and coordinated movement in the California mouse (Peromyscus californicus). *Behavioural Processes*, *65*(2), 155–162. http://doi.org/10.1016/j.beproc.2003.09.004

Yang, C., Zhang, Y., Cai, Y., Stokke, B. G., & Liang, W. (2011). Female Crowing and Differential Responses to Simulated Conspecific Intrusion in Male and Female Hainan Partridge (*Arborophila ardens*). *Zoological Science*, *28*(4), 249–253. http://doi.org/10.2108/zsj.28.249

Figures

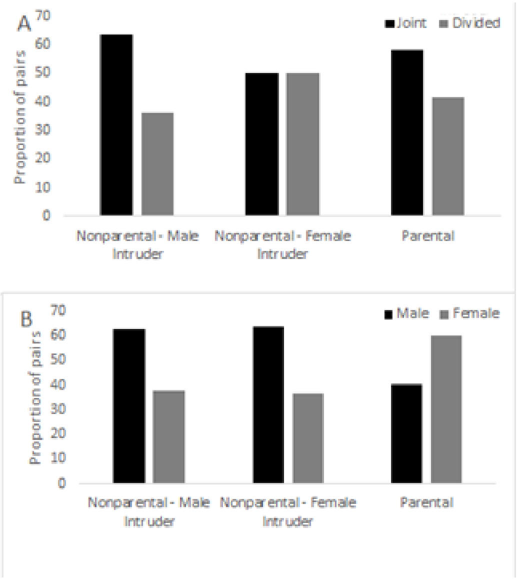


Figure 1. A) The proportions of pairs displaying a divided versus joint defensive strategy. There was no difference in the defensive strategies displayed regardless of context, with only 25% of pairs altering strategies across context (Chi-square, $\chi^2_2 = 0.371$, $p = 0.54$). B) The proportions of defenders that were male or female within pairs that used a divided defense strategy. There were no sex differences in defense. Parental pairs had the highest proportion of female defenders but the sample size was too small to reach significance (Chi-square, $\chi^2_1 = 0.003$, $p = 0.95$).

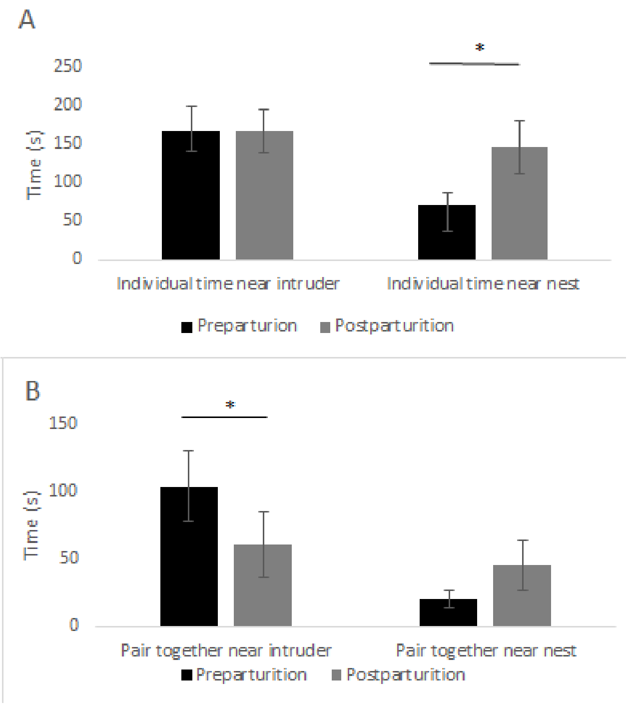


Figure 2. A) Changes in time spent near the intruder and near the nest within pairs pre= and post-parturition. Individual males and females did not alter their total time spent near the intruder, but did spend significantly more time in the nest post parturition (* = $p < 0.05$). B) Time spent together near the nest and near the intruder pre- and post-parturition. Pairs spent significantly less time together near the intruder following parturition but did not differ in their time spent together in the nest (* = $p < 0.05$).

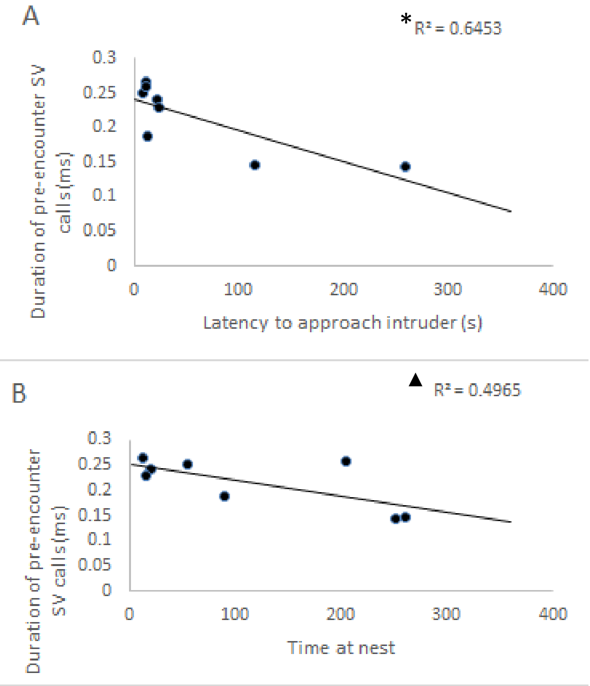


Figure 3. Pre-encounter SV duration by pairs predicted the latency to approach an intruder and the time spent at the nest during encounter. A) Shorter calls were associated with longer latencies to approach the intruder (p = 0.016). B) Shorter calls showed a nonsignificant trend for more time at the nest during encounter (p = 0.051).

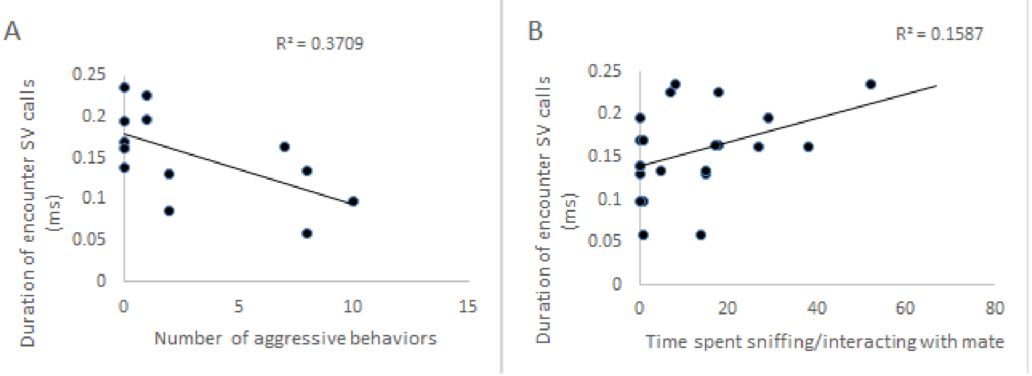


Figure 4. Linear regression of encounter SV calls predicting behavioral outcomes by males. A) Shorter calls during the encounter predicted more aggression (jabs, bites) directed towards the intruder (p = 0.02). B) A nonsignificant trend was found that longer calls by pairs predicted more time spent interacting between mates (p = 0.056).

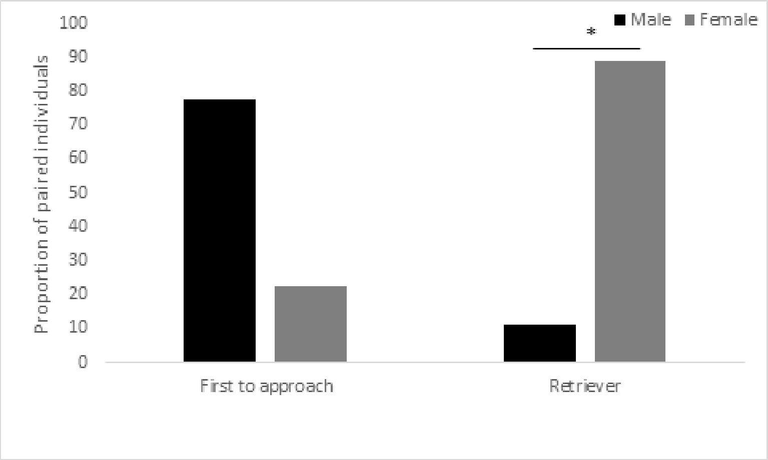


Figure 5. The proportion of individuals, male versus female, that were the first to approach the pups, and the retriever of the pups. Overall, males had a nonsignificant trend to first approach pups (p = 0.056) but did not retrieve them. Females acted as the predominant pup retrievers (p = 0.03).

Chapter 3

Oxytocin alters division of labor and communication by pair-bonded California mice in a sex specific manner

Abstract

Monogamous pair bonding allows for division of labor and coordination of complex social behaviors within a pair. Although oxytocin (OT) influences formation and maintenance of pair bonds in monogamous species, its role in division of labor and territorial defense by pair-bonded individuals is unknown. We studied OT's role in territorial defense by pair-bonded California mice (*Peromyscus californicus*). Previously we found that resident pairs of California mice use either divided defense or joint defense against territorial intruders, with pairs maintaining a single strategy across varied conditions. To elucidate the role of OT, we administered an intranasal dose (0.8 IU) of OT either to both members of the pair or only the male or female of the pair 5 minutes prior to a territorial intrusion. OT revealed sex-specific differences in defense coordination and vocal communication (ultrasonic vocalizations) during territorial intrusions. When OT was administered to females or both members of a pair, division of labor was increased as shown by pairs spending less time together investigating an intruder. When females, but not males, received OT, pairs were more likely to divide defense (Female OT: 69%, Saline: 46%), and females were more likely to approach intruders first than in any other condition (Female OT: 85%, Saline: 31%). When males, but not females, received OT, call duration was shortened (Male OT: 112 ± 9.9 ms, Saline: 298 ± 31 ms). An additive effect of OT was observed when both individuals received OT, with pairs increasing divided defense (Both OT: 69%, Saline: 46%) and shortening calls (Both OT: 197 ± 27 ms, Saline: 298 ± 31 ms). Overall, OT administration revealed that behavioral plasticity in defense strategy and approach behavior was greatest in females, while plasticity in ultrasonic vocalizations was greatest in males, illustrating a sex specific role for oxytocin in territorial defense.

Keywords: Division of labor, Oxytocin, Intranasal, Aggression, Vocal Communication, Ultrasonic vocalizations, Pair-bond

Highlights

- Oxytocin revealed sex-specific changes in division of labor by pair-bonded California mice
- Females being administered oxytocin led to increased division of labor by pairs
- Males being administered oxytocin led to decreased duration of sustained vocalizations
- California mice administered oxytocin approached intruders quicker than their saline administered mates

Introduction

Coordination of behavior by group living species allows for individuals to divide labor and efficiently complete tasks (Clutton-Brock, 2009; Goldsby, Dornhaus, Kerr, & Ofria, 2012; King & Sueur, 2011; Kokko, Johnstone, & Clutton-Brock, 2001; Page, Scheiner, Erber, & Amdam, 2006). Territorial defense is one task that is benefitted by division of labor (Hall, 2000; Rieger, Stanton & Marler, under review). In many cases, such as Kirk's dik dik (*Madoqua kirkii*; Brotherton, Pemberton, Komers, & Malarky, 1997) and cichlid fish (Rogers, 2010a), these divisions are sex specific where one sex (usually males) defend and patrol territories while the other remains closer to the nest or center of the territory. However, in other species such as California mice (*Peromyscus californicus*) pairs use different strategies to complete the same tasks (Rieger, Stanton & Marler, under review). However, despite our behavioral knowledge of division of labor and coordination across species, we know relatively little about the neuropeptide mechanisms and neuromodulators underlying these behaviors.

One potential neuropeptide that could play an important role in division of labor is Oxytocin (OT). OT is a highly conserved nonapeptide that plays a context specific role in a number of social behaviors (Caldwell & Albers, 2015; O'Connor, Marsh-Rollo, Ghio, Balshine, & Aubin-Horth, 2015) including pair-bond formation and maintenance (Insel & Shapiro, 1992; Lieberwirth & Wang, 2016), aggression (Bosch, Meddle, Beiderbeck, Douglas, & Neumann, 2005; Veenema, 2008), social communication (Song et al., 2014), and vigilance (Duque-Wilckens et al., 2018). Intranasal (IN) administration of OT has become a popular delivery method for OT, administering the compound through the nasal mucosa and into the central nervous system and the brain (Bales et al., 2013; Leng & Ludwig, 2016; Quintana & Woolley, 2016). Both fMRI studies of rodents and cerebral spinal fluid (CSF) measures in Rhesus macaques (*Macaca mulatta*) have validated that OT administered via IN reach cortical and subcortical regions of the brain and the CSF on a scale of minutes to hours (Galbusera et al., 2017; Lee et al., 2018). Moreover, Behavioral studies using IN OT have found both acute and chronic changes in social behavior in animals (Bales et al., 2013; Duque-Wilckens et al., 2018) and humans

(Calcagnoli, Kreutzmann, de Boer, Althaus, & Koolhaas, 2015; Li, Chen, Mascaro, Haroon, & Rilling, 2017; Rilling et al., 2014; Veening & Olivier, 2013) generally leading to increased sociality. Due to this technique's ease of use, fast action and behavioral effects, it has been postulated that there is great clinical potential of IN OT for disorders such as autism (Young & Barrett, 2015). However, the context of IN administration appears to be important to behavioral outcomes (Bales et al., 2013; Duque-Wilckens et al., 2018; Johnson, Walum, Xiao, Riefkohl, & Young, 2017) and this is yet to be fully understood in either territorial defense or division of labor. Based on OT's sex- and context-specific effects on pair-bonding and aggression we were interested in how IN OT would affect division of labor by pairs in response to an intruder.

We tested the role of OT in division of labor by pair-bonded California mice, a strictly monogamous species (Gubernick, 1988; Ribble, 1991). Both male and female California mice will defend territories and win encounters against same sex intruders (Rieger & Marler, 2018). Moreover, when together California mouse pairs can divide labor using different strategies when faced with an intruder (Rieger, Stanton & Marler, under review). Specifically, pairs will defend territories one of two ways: 1) divided, where one member of the pair (either the male or female) will approach the intruder while the other remains close to the nest, and 2) joint, where both members of the pair will approach the intruder together (Rieger, Stanton & Marler, under review). Along with this, California mice produce a rich set of ultrasonic vocalizations (Briggs & Kalcounis-Rueppell, 2011; Kalcounis-Rueppell, Metheny, & Vonhof, 2006; Kalcounis-Rueppell, Pultorak, & Marler, 2018) that can be used to coordinate behavior by pairs. In particular, sustained vocalizations (SV), long low bandwidth calls between 22–25 kHz, are important to coordinating division of labor between pairs. Specifically, total SV calling by pairs predicts more similar responses to aversive playbacks (Rieger, Hartfield & Marler, unpublished), whereas shorter SV duration predicts more aggression by pairs during resident-intruder paradigms (Rieger, Stanton & Marler, under review). However, the neuropeptide mechanisms including the role of OT, that underlie division of labor by monogamous pairs has not been studied.

Our goal in this study was to elucidate the role of OT in division of labor of a monogamous mammal. To test this, we administered either saline or OT intranasally to pair-bonded California mice. Following administration, pairs were tested in a modified resident-intruder paradigm where an intruder was placed in their residence while the pair was together. We predicted that OT would have a sex-specific effect on members of the pair such that males administered OT would investigate intruders more and females administered OT would spend more time at the nest. This is in line with unpublished work from our lab which shows increased investigation of a novel same-sex individual by males but not females (Guoynes & Marler, personal communication). We further predicted that if pairs showed a sex-specific effect of OT, then those pairs administered OT would show greater division of labor than those given only saline. Finally, we predicted increased calling in pairs where there was greater investigation of the intruder, indicating increased aggressive behaviors (Rieger & Marler, 2018).

Methods

Animals

Ninety-six male (56 paired and 40 sexually naïve) and 56 female (all paired) California mice were used to test the role of OT in division of labor. Individuals were randomly paired with an opposite-sex individual unrelated for at least two generations. Pairs cohabitated in a standard cage (48 x 27 x 16 cm) lined with aspen bedding, a nestlet, food (Purina 5015^{TM} mouse chow) and water available *ad libitum* for 7 days prior to testing. Sexually naïve males lived with 1–2 same sex conspecifics. The housing room was maintained at 20–23º C on a 14:10 h light:dark cycle (lights on at 23:00 central standard time) with all tests occurring between 1 and 3 hours after the start of the dark cycle under dim red light. All mice were ear tagged for recognition and pairs had a small patch of fur shaved from their flank for individual recognition during testing. Pairs were randomly assigned to a treatment group and tested for division of labor seven days after pairing following the method outlined below.

Ethical statement

All animals were maintained according to the National Institute of Health *Guide for the care and use of laboratory animals*. All procedures were approved by the University of Wisconsin – Madison College of Letters and Sciences Institutional Animal Care and Use Committee (Protocol L005447). No animals were injured by any of the behavioral manipulations or assays.

Apparatus

Testing occurred in a 50 x 30 x 30 cm glass aquarium lined with aspen bedding containing a red tube (15 cm) as enrichment and a plastic igloo (10 x 8 x 8 cm) to act as a nest. The aquarium was fitted with a Plexiglas lid with 5-cm holes 2.5 cm from each corner to allow for microphones to be placed inside the apparatus for USV recording. Intruders were placed in the apparatus in the corner opposite the igloo in a 10 x 10 x 10 cm wire mesh cage.

Intranasal OT administration

California mice were administered a dose of either sterile saline or OT (Bachem, Torrence, Ca). OT was administered in a dose of 0.8 IU/kg in line with previous studies on prairie voles (Bales et al., 2013) and California mice (Duque-Wilckens et al., 2018) and roughly equivalent to weight-adjusted doses given in human studies. Intranasal doses were administered once in the testing apparatus 5 minutes prior to division of labor testing which has been shown to be a sufficient time scale for inducing behavioral effects (Bales et al., 2013). To administer OT and saline a blunt cannula needle (33-gauge, 2.8 mm length, Plastics One, Roanoke, Va.) was attached to cannula tubing affixed to an airtight Hamilton syringe which was then flushed and filled with compound. The animal was then held still and 25 µl of compound was administered and allowed to absorb across the nasal mucosa, evenly distributed between both nostrils. After administration animals were returned to the testing apparatus. Overall, administration was completed in under 30 seconds, handling was consistent across animals and the order of administration was randomized across pairs.

Division of labor procedure

Fifty-six male-female pairs were used to test the role of OT in division of labor by pair-bonded California mice. Pairs cohabitated for seven days prior to testing at which point pairs show side-to-side contact, decreased aversive vocalizations towards their mate and decreased investigation of novel individuals, all hallmarks of pair-bonding (Becker, Castelli, Yohn, Spencer, & Marler, 2018). Pairs were randomly assigned to one of four groups prior to testing: 1) Both pair members administered saline, 2) male pair members administered OT and female pair members administered saline, 3) male pair members administered saline and female pair members administered OT and 4) both pair members administered OT. Twenty-four hours prior to testing pairs had a patch of fur shaved from their flank for recognition purposes and moved from their standard housing cage to a testing apparatus. Twenty-four hours has been previously shown to provide pairs with a residency effect, which makes them more likely to defend a territory and to win same-sex encounters (Bester-Meredith & Marler, 2001; Matthew J. Fuxjager, Mast, Becker, & Marler, 2009; Matthew J. Fuxjager, Montgomery, Becker, & Marler, 2010; Matthew J. Fuxjager, Zhao, Rieger, & Marler, n.d.; Rieger & Marler, 2018).

After 24 hours in the testing apparatus, pairs were tested for division of labor using a modified resident-intruder paradigm (Rieger & Marler, 2018; Rieger, Stanton & Marler, under review). First pairs were administered OT and/or saline based on their treatment group and allowed 5 minutes for the OT to take effect. After the 5 minutes pairs completed a three-phase encounter: 1) pre-encounter, pairs were alone in the chamber for 2 minutes, 2) encounter, a male intruder was placed in the arena for 6 minutes, 3) post-encounter, the intruder was removed, and pairs were alone in the chamber for 4 minutes. All times were based on prior studies which showed that the behaviors and vocalizations of interest occurred within these time frames (Rieger & Marler, 2018; Rieger, Stanton & Marler, under review). Only male intruders were used as a previous study showed no difference in pairs responses towards an intruder based on sex (Rieger, Stanton & Marler, under review). Both behavior and USVs were recorded for all three time points.

Videos were analyzed for each pair’s strategy in response to an intruder, based on the time spent near the intruder by each member of the pair. In a previous study, and replicated in this study, we found a bimodal distribution of pairs with the modes separated at around 130 s difference in time spent near the intruder, as defined by being within 2.5 cm of the intruder (Rieger, Stanton & Marler, under review). As such, we categorized pairs where one member spent 130 s more than their mate near the intruder as employing a divided defense strategy while those with less than a 130 s difference were categorized as using a joint strategy. Latency to approach an intruders cage, and number of aggressive behaviors (bites and jabs towards and intruders cage) were also scored. Sustained vocalization production and duration were scored at the level of the dyad. All behavioral analyses were completed by observers blind to the treatment of the pairs.

Ultrasonic vocalization analysis

Ultrasonic vocalizations were recorded with two Emkay/Knowles FG series microphones (detection range 10–120 kHz). Microphones were placed 55 cm apart at opposite corners of the apparatus, 20 cm from the apparatus floor with one microphone placed over the resident nest and one placed over the intruder cage with microphone placement randomized across trials to control for potential differences in sensitivity. Microphone channels were calibrated to equal gain (-60 dB noise floor) and WAV files for each test were created using RECORDR software (Avisoft Bioacoustics, Berlin, Germany). A 250-kHz sampling rate and 16-bit resolution was used to record USVs and a 512 fast Fourier transform was used to generate spectrograms in Avisoft SASLab Pro (Avisoft bioacoustics). USVs were differentiated by visual and auditory inspection of WAV files with sampling rates reduced to 4% of normal speed (11025 kHz) by an observer blind to treatment at the level of the dyad. We focused our analyses on sustained vocalizations (SV) that have been previously defined (Pultorak, Matusinec, Miller, & Marler, 2017; Rieger & Marler, 2018; Kalcounnis-Reuppell, Pultorak, & Marler, 2018) due to pairs not producing barks or sweep phrases.

Statistics

All statistics were run on SPSS v 22 (IBM Corp, Armonk, NY, USA). We tested for a treatment effect of OT overall on behavioral measures using a multivariate analysis of variance (MANOVA). To compare differences in overall strategy use in response to an intruder we used Chi-square tests of independence. We then completed follow up ANOVAs and used Tukey's post-hoc tests to look for specific changes in behaviors including, time spent together, latency to approach the intruder, time spent investigating the intruder, aggression and vocal production due to treatment group.

Results

We found that OT had significant effects on division of labor in pair-bonded California mice in response to an intruder (MANOVA, $F_{12,282} = 2.025$, $p = 0.022$). First, animals administered OT spent less time together with their mate investigating the intruder than those that were administered saline, regardless of sex (OT: 22.87 ± 4.63%; Saline: 41.83 ± 5.56%; ANOVA: $F_{1,100} = 6.776$, $p = 0.011$, Fig. 1). Moreover, group differences were found in both proportion of time spent together near the intruder (ANOVA: $F_{3,96} = 4.065$, $p = 0.009$) as well as total time spent near the intruder (ANOVA: $F_{3,96} = 2.73$, $p = 0.048$). Specifically, it was found that pairs where both members were administered OT and those where females only were administered OT spent less total time together than pairs where both members were administered saline or only males were administered OT (Both Saline: 87.54 ± 18.24 s Male OT: 76.54 ± 17.74 s Female OT: 46.92 ± 13.6 s, Both OT: 29.92 ± 12.54 s; Tukey's post hoc test, $p < 0.05$, figure 1 A). Pairs where both members were administered OT also had the lowest proportion of time spent together near intruders compared to all other groups with pairs where one member was administered OT showing an intermediate response between saline and OT controls (Both Saline: 50.27 ± 7.99%, Male OT: 32.66 ± 7.09%, Female OT: 31.84 ± 7.22% Both OT: 14.63 ± 6.04%, Tukey's post hoc test, $p < 0.05$, figure 1 B).

In conjunction with these results, pairs where females were administered OT and pairs where both members were administered OT showed nonsignificant increases in the proportion of pairs using a divided defense strategy compared to pairs administered saline and pairs where males were administered

OT (Both saline: 46% divided, Male OT: 38% divided, Female OT: 69% divided, Both OT: 69% divided, $X^2_3 = 4.71$, $p = 0.19$, figure 2 A). Moreover, there was a nonsignificant trend for females to be more likely to act as the defender if either or both members of the pair were administered OT versus those where they received only saline (OT: 14 out of 21 divided pairs, Saline: 1 out of 6 divided pairs, Fisher's exact test, $p = 0.08$, figure 2 B). While these results were not significant due to small sample size, taken together with the decreased time spent together by pairs receiving OT they show an overall increase in division of labor by pairs.

Despite the increases in division of labor, there was no significant change in the total amount of time spent near the intruder by individual members of the pair (ANOVA: $F_{3,96} = 1.24$, $p = 0.03$) or the total combined time spent near the intruder by pairs (ANOVA: $F_{3,48} = 0.295$, $p = 0.83$) based on OT treatment. There was a nonsignificant trend for pairs where females There was also no significant increase in aggression against the intruder (ANOVA $F_{3,96} = 0.28$, $p = 0.84$) or towards their own mate (ANOVA: $F_{3,96} = 0.88$, $p = 0.46$) across treatment groups.

OT did show a significant effect on the latency to approach an intruder ($F_{3,96} = 3.80$, $p = 0.013$). In pairs where one member was administered OT, regardless of sex, the member that received OT was quicker to approach the intruder than their saline administered mate (OT: 49.58 ± 19.47 s, Saline: 137.43 ± 30.77, ANOVA: $F_{1,48} = 5.604$, $p = 0.022$, figure 3 A). However, this pattern is reversed in pairs where both members receive either saline or OT, with pairs where both members receive saline showing significantly faster latencies than pairs where both members receive OT (Both Saline: 54.54 ± 18.40, Both OT: 139.27 ± 30.90, ANOVA: $F_{1,50} = 5.55$, $p = 0.022$, figure 3 B). Moreover, we found that in pairs where females received OT, the likelihood of the female being the first to approach the intruder was significantly increased compared to all other groups (Both Saline: 30.77% female first, Male OT: 30.77% female first, Female OT: 84.62% female first, Both OT, 53.85% female first, $X^2_3 = 10.15$, $p = 0.017$, figure 4.)

Although differences existed during the encounter phase based on OT treatment, no changes were found in behavior in the pre or post phase of the resident-intruder paradigm. Pre-encounter we found that time spent in the nest was not different based on administration of OT or saline (Saline: 77.7 ± 15.74, OT: 80 ± 13.10, Student's t-test, $t_{103} = 1.01$, $p = 0.34$). There was also no difference post encounter in time spent patrolling the territory based on OT administration (Saline: 126.75 ± 13.1, OT: 124.33 ± 29.04, Student's t-test, $t_{103} = 0.06$, $p = 0.95$).

OT treatment had significant effects on the duration of SV calls produced by pairs during all stages of the resident-intruder paradigm (ANOVA: $F_{3,48} = 103.90$, $p < 0.001$). Specifically, SV duration was significantly decreased in pairs where the male was administered OT compared to all other groups (Both Saline: 298 ± 31 ms, Male OT: 112 ± 9.9 ms, Female OT: 260 ± 39 ms, Both OT: 197 ± 27 ms, Tukey's post hoc test $p < 0.01$, figure 4). Pairs where both members were administered OT also showed significantly decreased SV durations compared to pairs where both members were administered saline (Tukey's post hoc test $p < 0.05$) but not pairs where females were administered OT (Tukey's post hoc test $p = 0.19$). Pairs where females were administered OT did not show SV duration differences compared to pairs where both members were administered saline (Tukey's post hoc test, $p = 0.45$). Despite changes in OT duration, there were no changes in total call production of SVs across treatment groups (ANOVA: $F_{3,48} = 0.349$, $p = 0.45$). Barks and sweeps were only recorded during two encounters with intruders and thus were not analyzed.

Discussion

In this study we wanted to elucidate the role of OT in division of labor in response to an intruder in the monogamous California mouse. We found that OT had both global and sex-specific effects on behavior throughout a pairs interaction with an intruder. Globally, we found that OT led to pairs spending less total time and less time proportionally with their partner investigating the intruder, indicating increased division of labor. Moreover, we found that in pairs that both received OT and used a divided defense strategy females became more likely to be the pair member who investigated the intruder. We also found that in pairs where one member was administered OT and the other was administered saline, the animal that received OT showed a quicker latency to approach the intruder. We further found that OT had sex-specific effects on division of labor. When OT was administered to females, but not males, it became more likely that females would be quicker to approach the intruder. However, when OT was administered to males, but not females, we found changes in vocal communication such that SV duration was significantly shortened. Taken together this data provides evidence that OT, administered intranasally has significant effects on the coordination of behavior and division of labor in a monogamous species.

One advantage to group living and monogamy is the ability to divide labor across individuals to efficiently complete tasks (Mathews, 2002). Although task division is usually considered to be sex-specific, with one sex defending territory while the other remains close to the nest (Brotherton et al., 1997; Rogers, 2010), in other cases, such as birds (Hall, 2000) or primates (French & Snowdon, 1981), pairs can also cooperative strategies (Colombelli-Négrel, 2016; Koloff & Mennill, 2011). We previously found that in a monogamous rodent, the California mouse, pairs can use one of two strategies, a joint defensive strategy where the pair approaches an intruder together, or a divided strategy where one member approaches the intruder while the other remains close to the nest that is independent of mate-guarding (Rieger, Stanton & Marler, under review). However, despite our knowledge of these different strategies, relatively little was known about the neuropeptide underpinnings of division of labor prior to this study.

We predicted that OT would play a role in division of labor because of its sex-specific (Kelly & Goodson, 2014; Rilling et al., 2014; Taylor, Intorre, & French, 2017) and context-specific roles in social behavior (Cavanaugh, Mustoe, & French, 2018; Duque-Wilckens et al., 2018; Johnson et al., 2017; Koch et al., 2014; Oettl et al., 2016). OT has been shown to have a vital role in many social behaviors including pair-bonding (Insel, Preston, & Winslow, 1995; Winslow & Insel, 2002), affiliation (Beery, 2015; Calcagnoli et al., 2015), and aggression (Kelly & Goodson, 2014; Ragnauth et al., 2005). Specifically, OT plays an important role in bond formation and affiliation in females (Lieberwirth & Wang, 2016) as well as increasing vigilance in females presented with a novel individual (Duque-Wilckens et al., 2018). In males, OT has been shown to play a role in aggression (Campbell, 2008) and investigation of novel intruders (Guoynes & Marler, unpublished). To test the role of OT in division of labor, we administered OT intranasally. Intranasal OT has become more common recently as a method for administering OT (Veening & Olivier, 2013). Importantly, intranasal OT leads to behavioral effects in minutes (Bales et al., 2013) while also having clinical relevance as it can be easily given to human patients (Alvares, Hickie, & Guastella, 2010; Domes et al., 2013; Koch et al., 2014; Quintana, Alvares, Hickie, & Guastella, 2015) and is hypothesized to help alleviate social disorders such as autism (Young & Barrett, 2015). Despite this hypothesis, the efficacy of intranasal administration has been questioned (Leng & Ludwig, 2016); recent work has shown that intranasal OT reaches both cortical and subcortical areas of the brain (Galbusera et al., 2017) as well as the CSF (Lee et al., 2018). However, the context-dependent effects of intranasal OT are still poorly understood (Quintana et al., 2015). However, it has been shown that OT administration is not strictly prosocial as was once believed (Alvares et al., 2010; Bales et al., 2013; Duque-Wilckens & Trainor, 2017). And we predicted that intranasal OT would lead to sex-specific differences in response to intruders. In line with the social salience hypothesis (Shamay-Tsoory & Abu-Akel, 2016), we believed that individuals administered OT would show increased aggression when faced with an intruder.

Consistent with our predictions, we found that OT played a role in division of labor, with pairs that were administered OT spending less total time and proportionally less time together when faced with an intruder. Importantly, the total time spent investigating the intruder and the amount of aggression shown towards the intruder did not change in response to OT treatment or treatment group. This result indicates that OT is affecting the synchronicity of pair behavior, increasing division of labor. This synchrony allows for pairs to both assess the threat of an intruder while allowing one pair member to remain closer to the nest, potentially increasing efficiency. It is interesting to note that this division was strongest when both members of the pair received OT and intermediate effects were seen when only one member of the pair received OT, indicating that OT may have an additive effect on pairs. We also found sex-specific changes, but they were not in the direction we predicted. Whereas we expected males to become more aggressive when administered OT, it was females who were quicker to approach intruders and more likely to become defenders following OT administration. One possibility is that OT may be activating areas of the brain associated with maternal aggression (Lonstein & Gammie, 2002; Veenema, 2008), even though these pairs were nonparental. However, we did not find an increase in actual aggressive behaviors by mice given OT, though this could be because physical contact with the intruder was prevented.

We also found interesting patterns in the latency to approach intruders in response to OT administration by pairs. First, we found that in pairs where one member received OT and the other received saline, OT quickened approach behavior in the member receiving OT, particularly in females. This result could be similar to increased vigilance that has been seen in female California mice (Duque-Wilckens et al., 2018) that is driven by OT in the bed nucleus of the stria terminalis (BNST) an area also important to aggression (Fuxjager & Marler, 2009; Trainor, Bird, & Marler, 2004). However, this pattern was reversed in pairs where both members received OT or saline. In pairs where both members received the same treatment, those that received saline showed a shorter latency to approach than those that both received OT. This result could indicate that, when both members of a pair receive OT, it slows down territorial defense. The mechanisms underlying these seemingly conflicting results should be studied in greater detail in future studies.

We were also interested in the role that OT would have on vocal communication between pairs. Vocal communication is vital to the coordination of behavior across species (Noe, 2006) and we have found previously that ultrasonic vocalizations by California mice are correlated with behaviors across division of labor. While OT has been shown to have an effect on the perception of vocalizations by mothers (Marlin, Mitre, D'amour, Chao, & Froemke, 2015; Winslow et al., 2000) and OT knockout mice have been shown to produce fewer vocalizations (Winslow & Insel, 2002), the role it plays in communication between mates remains unknown. We did find that OT affected vocal communication in a sex-specific manner. Specifically, we found that in pairs where males were given OT the duration of SV calls was significantly reduced compared to other groups. SV calls in general are social calls that can indicate affiliation or aggression (Kalcounis-Rueppell et al., 2018), with shorter calls having been shown to be more indicative of aggression (Rieger & Marler, 2018). However, in this case SV call duration did not correlate with greater aggression, likely because total aggression was depressed across groups. This is possibly, again, because the intruder mouse was in a cage making physical aggression difficult. It is possible then that a free ranging intruder would provide us with a greater understanding of how pairs would aggress in this context.

For the first time we show that OT, administered intranasally, alters division of labor and communication in a monogamous species. However, it remains unknown what brain areas OT is acting upon in the brain as well as whether or not OT is necessary for division of labor. It is likely that both cortical and subcortical areas are important to division of labor. Possible candidates including the BNST, amygdala, hypothalamus and insula, prefrontal cortex and anterior cingulate due to their role in aggression affiliation and cooperation (Davidson & Irwin, 1999; Delville, De Vries, & Ferris, 2000; Duque-Wilckens et al., 2016, 2018; Goldstein, Rasmusson, Bunney, & Roth, 1996; Haroush & Williams, 2015; Ophir, Gessel, Zheng, & Phelps, 2012). Further studies should determine the brain areas and receptors that intranasal OT is acting upon in this paradigm. This finding expands the importance of OT to social behavior by showing that it plays a role in behavioral coordination by increasing labor divisions between pairs and altering their communication in a sex-specific manner.

Acknowledgements

We thank Kathryn Hartfield, JB Ramos Hernandez, Alexis Payette and Sage Keyes for their help collecting and analyzing data for this experiment. We also thank the animal research technicians for their help with animal care. Finally, we thank our funding source the National Science Foundation IOS11312419.

References

Alvares, G. A., Hickie, I. B., & Guastella, A. J. (2010). Acute effects of intranasal oxytocin on subjective and behavioral responses to social rejection. *Experimental and Clinical Psychopharmacology*, *18*(4), 316–321. http://doi.org/10.1037/a0019719

Bales, K. L., Perkeybile, A. M., Conley, O. G., Lee, M. H., Guoynes, C. D., Downing, G. M., … Mendoza, S. P. (2013). Chronic intranasal oxytocin causes long-term impairments in partner preference formation in male prairie voles. *Biological Psychiatry*, *74*(3), 180–8. http://doi.org/10.1016/j.biopsych.2012.08.025

Becker, E. A., Castelli, F. R., Yohn, C. N., Spencer, L., & Marler, C. A. (2018). Species differences in urine scent-marking and counter-marking in Peromyscus. *Behavioural Processes*, *146*, 1–9. http://doi.org/10.1016/J.BEPROC.2017.10.011

Beery, A. K. (2015). Antisocial oxytocin: complex effects on social behavior. *Current Opinion in Behavioral Sciences*, *6*, 174–182. http://doi.org/10.1016/J.COBEHA.2015.11.006

Bester-Meredith, J. K., & Marler, C. A. (2001). Vasopressin and Aggression in Cross-Fostered California Mice (Peromyscus californicus) and White-Footed Mice (Peromyscus leucopus). *Hormones and Behavior*, *40*(1), 51–64. http://doi.org/10.1006/hbeh.2001.1666

Bosch, O. J., Meddle, S. L., Beiderbeck, D. I., Douglas, A. J., & Neumann, I. D. (2005). Brain Oxytocin Correlates with Maternal Aggression: Link to Anxiety. *Journal of Neuroscience*, *25*(29), 6807–6815. http://doi.org/10.1523/JNEUROSCI.1342-05.2005

Briggs, J. R., & Kalcounis-Rueppell, M. C. (2011). Similar acoustic structure and behavioural context of vocalizations produced by male and female California mice in the wild. *Animal Behaviour*, *82*(6), 1263–1273. http://doi.org/10.1016/j.anbehav.2011.09.003

Brotherton, P. N., Pemberton, J. M., Komers, P. E., & Malarky, G. (1997). Genetic and behavioural evidence of monogamy in a mammal, Kirk's dik-dik (Madoqua kirkii). *Proceedings. Biological Sciences*, *264*(1382), 675–81. http://doi.org/10.1098/rspb.1997.0096

Calcagnoli, F., Kreutzmann, J. C., de Boer, S. F., Althaus, M., & Koolhaas, J. M. (2015). Acute and repeated intranasal oxytocin administration exerts anti-aggressive and pro-affiliative effects in male rats. *Psychoneuroendocrinology*, *51*. http://doi.org/10.1016/j.psyneuen.2014.09.019

Caldwell, H. K., & Albers, H. E. (2015). Oxytocin, Vasopressin, and the Motivational Forces that Drive Social Behaviors (pp. 51–103). Springer International Publishing. http://doi.org/10.1007/7854_2015_390

Campbell, A. (2008). Attachment, aggression and affiliation: The role of oxytocin in female social behavior. *Biological Psychology*, *77*(1), 1–10. http://doi.org/10.1016/j.biopsycho.2007.09.001

Cavanaugh, J., Mustoe, A., & French, J. A. (2018). Oxytocin regulates reunion affiliation with a pairmate following social separation in marmosets. *American Journal of Primatology*, e22750. http://doi.org/10.1002/ajp.22750

Clutton-Brock, T. (2009). Cooperation between non-kin in animal societies. *Nature*, *462*(7269), 51–57. http://doi.org/10.1038/nature08366

Colombelli-Négrel, D. (2016). Female splendid and variegated fairy-wrens display different strategies during territory defence. *Animal Behaviour*, *119*, 99–110. http://doi.org/10.1016/j.anbehav.2016.07.001

Davidson, R. J., & Irwin, W. (1999). The functional neuroanatomy of emotion and affective style. *Trends in Cognitive Sciences*, *3*(1), 11–21. http://doi.org/10.1016/S1364-6613(98)01265-0

Delville, Y., De Vries, G. J., & Ferris, C. F. (2000). Neural Connections of the Anterior Hypothalamus and Agonistic Behavior in Golden Hamsters. *Brain, Behavior and Evolution*, *55*(2), 53–76. http://doi.org/10.1159/000006642

Domes, G., Heinrichs, M., Kumbier, E., Grossmann, A., Hauenstein, K., & Herpertz, S. C. (2013). Effects of Intranasal Oxytocin on the Neural Basis of Face Processing in Autism Spectrum Disorder. *Biological Psychiatry*, *74*(3), 164–171. http://doi.org/10.1016/j.biopsych.2013.02.007

Duque-Wilckens, N., Steinman, M. Q., Busnelli, M., Chini, B., Yokoyama, S., Pham, M., … Trainor, B. C. (2018). Oxytocin Receptors in the Anteromedial Bed Nucleus of the Stria Terminalis Promote Stress-Induced Social Avoidance in Female California Mice. *Biological Psychiatry*, *83*(3), 203–213. http://doi.org/10.1016/J.BIOPSYCH.2017.08.024

Duque-Wilckens, N., Steinman, M. Q., Laredo, S. A., Hao, R., Perkeybile, A. M., Bales, K. L., & Trainor, B. C. (2016). Inhibition of vasopressin V1a receptors in the medioventral bed nucleus of the stria terminalis has sex- and context-specific anxiogenic effects. *Neuropharmacology*, *110*. http://doi.org/10.1016/j.neuropharm.2016.07.018

Duque-Wilckens, N., & Trainor, B. C. (2017). *Behavioral Neuroendocrinology of Female Aggression* (Vol. 1). Oxford University Press. http://doi.org/10.1093/acrefore/9780190264086.013.11

French, J. A., & Snowdon, C. T. (1981). Sexual dimorphism in responses to unfamiliar intruders in the tamarin, Saguinus oedipus. *Animal Behaviour*, *29*(3), 822–829. http://doi.org/10.1016/S0003-3472(81)80016-4

Fuxjager, M. J., & Marler, C. A. (2009). How and why the winner effect forms: influences of contest environment and species differences. *Behavioral Ecology*, *21*(1), 37–45. http://doi.org/10.1093/beheco/arp148

Fuxjager, M. J., Mast, G., Becker, E. A., & Marler, C. A. (2009). The ‘home advantage’ is necessary for a full winner effect and changes in post-encounter testosterone. *Hormones and Behavior*, *56*(2), 214–219. http://doi.org/10.1016/j.yhbeh.2009.04.009

Fuxjager, M. J., Montgomery, J. L., Becker, E. A., & Marler, C. A. (2010). Deciding to win: interactive effects of residency, resources and ‘boldness’ on contest outcome in white-footed mice. *Animal Behaviour*, *80*(5), 921–927. http://doi.org/10.1016/j.anbehav.2010.08.018

Fuxjager, M. J., Zhao, X., Rieger, N. S., & Marler, C. A. (n.d.). Why animals fight: uncovering the function and mechanisms of territorial aggression. In *American Psychological Association Handbook of Comparative Psychology*.

Galbusera, A., De Felice, A., Girardi, S., Bassetto, G., Maschietto, M., Nishimori, K., … Gozzi, A. (2017). Intranasal Oxytocin and Vasopressin Modulate Divergent Brainwide Functional Substrates. *Neuropsychopharmacology*, *42*(7), 1420–1434. http://doi.org/10.1038/npp.2016.283

Goldsby, H. J., Dornhaus, A., Kerr, B., & Ofria, C. (2012). Task-switching costs promote the evolution of division of labor and shifts in individuality. *Proceedings of the National Academy of Sciences*, *109*(34), 13686–13691. http://doi.org/10.1073/pnas.1202233109

Goldstein, L. E., Rasmusson, A. M., Bunney, B. S., & Roth, R. H. (1996). Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *16*(15), 4787–98. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8764665

Gubernick, D. J. (1988). Reproduction in the California Mouse, Peromyscus californicus. *Journal of Mammalogy*, *69*(4), 857–860. http://doi.org/10.2307/1381649

Hall, M. L. (2000). The function of duetting in magpie-larks: conflict, cooperation, or commitment?

Animal Behaviour, *60*(5), 667–677. http://doi.org/10.1006/anbe.2000.1517

Haroush, K., & Williams, Z. M. (2015). Neuronal Prediction of Opponent's Behavior during Cooperative Social Interchange in Primates. *Cell*, *160*(6), 1233–1245. http://doi.org/10.1016/j.cell.2015.01.045

Insel, T. R., Preston, S., & Winslow, J. T. (1995). Mating in the monogamous male: Behavioral consequences. *Physiology & Behavior*, *57*(4), 615–627. http://doi.org/10.1016/0031-9384(94)00362-9

Insel, T. R., & Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(13), 5981–5985. http://doi.org/10.1073/pnas.89.13.5981

Johnson, Z. V., Walum, H., Xiao, Y., Riefkohl, P. C., & Young, L. J. (2017). Oxytocin receptors modulate a social salience neural network in male prairie voles. *Hormones and Behavior*. http://doi.org/10.1016/j.yhbeh.2016.10.009

Kalcounis-Rueppell, M. C., Metheny, J. D., & Vonhof, M. J. (2006). Production of ultrasonic vocalizations by Peromyscus mice in the wild. *Frontiers in Zoology*, *3*(1), 3. http://doi.org/10.1186/1742-9994-3-3

Kalcounis-Rueppell, M. C., Pultorak, J. D., & Marler, C. A. (2018). Ultrasonic Vocalizations of Mice in the Genus Peromyscus. *Handbook of Behavioral Neuroscience*, *25*, 227–235. http://doi.org/10.1016/B978-0-12-809600-0.00022-6

Kelly, A. M., & Goodson, J. L. (2014). Hypothalamic oxytocin and vasopressin neurons exert sex-specific effects on pair bonding, gregariousness, and aggression in finches. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(16). http://doi.org/10.1073/pnas.1322554111

King, A. J., & Sueur, C. (2011). Where Next? Group Coordination and Collective Decision Making by Primates. *International Journal of Primatology*, *32*(6), 1245–1267. http://doi.org/10.1007/s10764-011-9526-7

Koch, S. B. J., Van Zuiden, M., Nawijn, L., Frijling, J. L., Veltman, D. J., & Olff, M. (2014). Intranasal oxytocin as strategy for medication-enhanced psychotherapy of PTSD: Salience processing and fear inhibition processes. *Psychoneuroendocrinology*. http://doi.org/10.1016/j.psyneuen.2013.11.018

Kokko, H., Johnstone, R. A., & Clutton-Brock, T. H. (2001). The evolution of cooperative breeding through group augmentation. *Proceedings. Biological Sciences / The Royal Society*, *268*(1463), 187–96. http://doi.org/10.1098/rspb.2000.1349

Koloff, J., & Mennill, D. (2011). Aggressive responses to playback of solos and duets in a Neotropical antbird. *Animal Behaviour*, *82*(3), 587–593. http://doi.org/10.1016/j.anbehav.2011.06.021

Lee, M. R., Scheidweiler, K. B., Diao, X. X., Akhlaghi, F., Cummins, A., Huestis, M. A., … Averbeck, B. B. (2018). Oxytocin by intranasal and intravenous routes reaches the cerebrospinal fluid in rhesus macaques: determination using a novel oxytocin assay. *Molecular Psychiatry*, *23*(1), 115–122. http://doi.org/10.1038/mp.2017.27

Leng, G., & Ludwig, M. (2016). Intranasal Oxytocin: Myths and Delusions. *Biological Psychiatry*. http://doi.org/10.1016/j.biopsych.2015.05.003

Li, T., Chen, X., Mascaro, J., Haroon, E., & Rilling, J. K. (2017). Intranasal oxytocin, but not vasopressin, augments neural responses to toddlers in human fathers. *Hormones and Behavior*, *93*, 193–202. http://doi.org/10.1016/J.YHBEH.2017.01.006

Lieberwirth, C., & Wang, Z. (2016). The neurobiology of pair bond formation, bond disruption, and social buffering. *Current Opinion in Neurobiology*. http://doi.org/10.1016/j.conb.2016.05.006

Lonstein, J. S., & Gammie, S. C. (2002). Sensory, hormonal, and neural control of maternal aggression in laboratory rodents. *Neuroscience & Biobehavioral Reviews*, *26*(8), 869–888. http://doi.org/10.1016/S0149-7634(02)00087-8

Marlin, B. J., Mitre, M., D'amour, J. A., Chao, M. V., & Froemke, R. C. (2015). Oxytocin enables maternal behaviour by balancing cortical inhibition. *Nature*, *520*(7548), 499–504. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4409554&tool=pmcentrez&rendertype=abstract

Mathews, L. M. (2002). Territorial cooperation and social monogamy: factors affecting intersexual behaviours in pair-living snapping shrimp. *Animal Behaviour*, *63*(4), 767–777. http://doi.org/10.1006/anbe.2001.1976

Noe, R. (2006). Cooperation Experiments: coordination through communication versus acting apart together. *Animal Behaviour*, *71*, 1–18. http://doi.org/10.1016/j.anbehav.2005.03.037

O'Connor, C. M., Marsh-Rollo, S. E., Ghio, S. C., Balshine, S., & Aubin-Horth, N. (2015). Is there convergence in the molecular pathways underlying the repeated evolution of sociality in African cichlids? *Hormones and Behavior*, *75*. http://doi.org/10.1016/j.yhbeh.2015.07.008

Oettl, L. L., Ravi, N., Schneider, M., Scheller, M. F., Schneider, P., Mitre, M., … Kelsch, W. (2016). Oxytocin Enhances Social Recognition by Modulating Cortical Control of Early Olfactory Processing. *Neuron*, *90*(3). http://doi.org/10.1016/j.neuron.2016.03.033

Ophir, A. G., Gessel, A., Zheng, D.-J., & Phelps, S. M. (2012). Oxytocin receptor density is associated with male mating tactics and social monogamy. *Hormones and Behavior*, *61*(3), 445–453. http://doi.org/10.1016/J.YHBEH.2012.01.007

Page, R. E., Scheiner, R., Erber, J., & Amdam, G. V. (2006). The Development and Evolution of Division of Labor and Foraging Specialization in a Social Insect (Apis mellifera L.). *Current Topics in Developmental Biology*, *74*, 253–286. http://doi.org/10.1016/S0070-2153(06)74008-X

Pultorak, J. D., Matusinec, K. R., Miller, Z. K., & Marler, C. A. (2017). Ultrasonic vocalization production and playback predicts intrapair and extrapair social behaviour in a monogamous mouse. *Animal Behaviour*, *125*, 13–23. http://doi.org/10.1016/j.anbehav.2016.12.023

Quintana, D. S., Alvares, G. A., Hickie, I. B., & Guastella, A. J. (2015). Do delivery routes of intranasally administered oxytocin account for observed effects on social cognition and behavior? A two-level model. *Neuroscience & Biobehavioral Reviews*, *49*, 182–192. http://doi.org/10.1016/J.NEUBIOREV.2014.12.011

Quintana, D. S., & Woolley, J. D. (2016). Intranasal Oxytocin Mechanisms Can Be Better Understood, but Its Effects on Social Cognition and Behavior Are Not to Be Sniffed At. *Biological Psychiatry*, *79*(8), e49–e50. http://doi.org/10.1016/J.BIOPSYCH.2015.06.021

Ragnauth, A. K., Devidze, N., Moy, V., Finley, K., Goodwillie, A., Kow, L.-M., … Pfaff, D. W. (2005). Female oxytocin gene-knockout mice, in a semi-natural environment, display exaggerated aggressive behavior. *Genes, Brain and Behavior*, *4*(4), 229–239. http://doi.org/10.1111/j.1601-183X.2005.00118.x

Ribble, D. O. (1991). The monogamous mating system of Peromyscus californicus as revealed by DNA fingerprinting. *Behavioral Ecology and Sociobiology*, *29*(3), 161–166. http://doi.org/10.1007/BF00166397

Rieger, N. S., & Marler, C. A. (2018). The function of ultrasonic vocalizations during territorial defence by pair-bonded male and female California mice. *Animal Behaviour*, *135*. http://doi.org/10.1016/j.anbehav.2017.11.008

Rilling, J. K., DeMarco, A. C., Hackett, P. D., Chen, X., Gautam, P., Stair, S., … Pagnoni, G. (2014). Sex differences in the neural and behavioral response to intranasal oxytocin and vasopressin during human social interaction. *Psychoneuroendocrinology*, *39*, 237–248. http://doi.org/10.1016/j.psyneuen.2013.09.022

Rogers, W. (2010a). Parental Investment and Division of Labor in the Midas Cichlid (Cichlasoma citrinellum). *Ethology*, *79*(2), 126–142. http://doi.org/10.1111/j.1439-0310.1988.tb00706.x

Rogers, W. (2010b). Parental Investment and Division of Labor in the Midas Cichlid (Cichlasoma citrinellum). *Ethology*, *79*(2), 126–142. http://doi.org/10.1111/j.1439-0310.1988.tb00706.x

Shamay-Tsoory, S. G., & Abu-Akel, A. (2016). The Social Salience Hypothesis of Oxytocin. *Biological Psychiatry*, *79*(3), 194–202. http://doi.org/10.1016/j.biopsych.2015.07.020

Song, Z., McCann, K. E., McNeill, J. K., Larkin, T. E., Huhman, K. L., & Albers, H. E. (2014). Oxytocin induces social communication by activating arginine-vasopressin V1a receptors and not oxytocin receptors. *Psychoneuroendocrinology*, *50*, 14–19. http://doi.org/10.1016/j.psyneuen.2014.08.005

Taylor, J. H., Intorre, A. A., & French, J. A. (2017). Vasopressin and Oxytocin Reduce Food Sharing Behavior in Male, but Not Female Marmosets in Family Groups. *Frontiers in Endocrinology*, *8*, 181. http://doi.org/10.3389/fendo.2017.00181

Trainor, B. C., Bird, I. M., & Marler, C. A. (2004). Opposing hormonal mechanisms of aggression revealed through short-lived testosterone manipulations and multiple winning experiences. *Hormones and Behavior*, *45*(2), 115–121. http://doi.org/10.1016/j.yhbeh.2003.09.006

Veenema, A. H. (2008). Central vasopressin and oxytocin release: regulation of complex social behaviours. *Progress in Brain Research*, *170*, 261–276. http://doi.org/10.1016/S0079-6123(08)00422-6

Veening, J. G., & Olivier, B. (2013). Intranasal administration of oxytocin: Behavioral and clinical effects, a review. *Neuroscience & Biobehavioral Reviews*, *37*(8), 1445–1465. http://doi.org/10.1016/J.NEUBIOREV.2013.04.012

Winslow, J. T., Hearn, E. F., Ferguson, J., Young, L. J., Matzuk, M. M., & Insel, T. R. (2000). Infant Vocalization, Adult Aggression, and Fear Behavior of an Oxytocin Null Mutant Mouse. *Hormones and Behavior*, *37*(2), 145–155. http://doi.org/10.1006/hbeh.1999.1566

Winslow, J. T., & Insel, T. R. (2002). The social deficits of the oxytocin knockout mouse. *Neuropeptides*, *36*(2), 221–229. http://doi.org/10.1054/npep.2002.0909

Young, L. J., & Barrett, C. E. (2015). Neuroscience. Can oxytocin treat autism? *Science (New York, N.Y.)*, *347*(6224). http://doi.org/10.1126/science.aaa8120

Figures

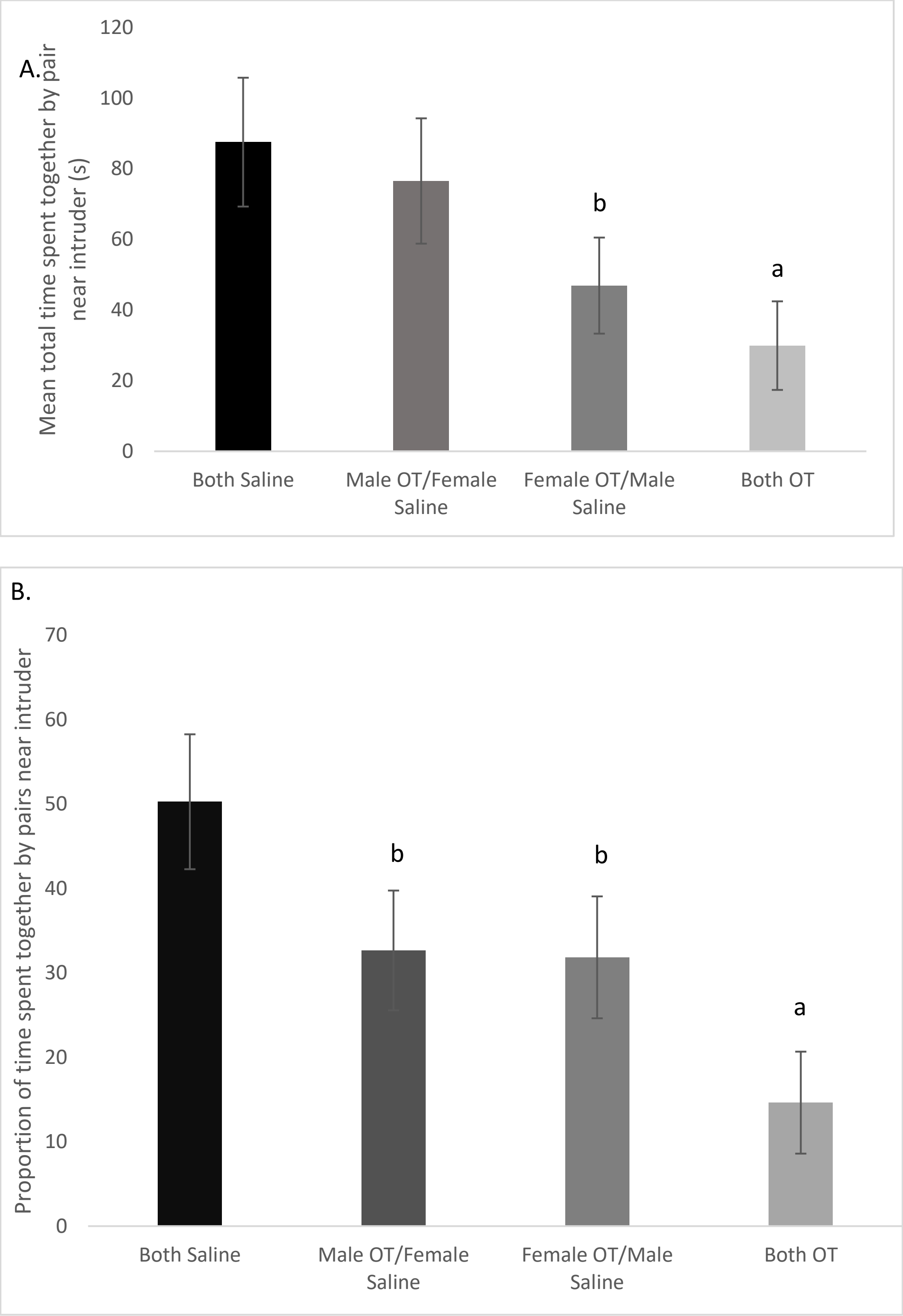


Figure 1. Oxytocin increased division of labor by pair-bonded California mice in response to an intruder. A. Total time spent near the intruder together by the pair was decreased in pairs where either both members received OT compared to pairs where only the male received OT or both members received

saline (a, $p < 0.05$). Pairs where only the female received OT also spent less time together than pairs where only the male received OT or both members received saline (b, $p < 0.05$). B. Proportion of time spent together near the intruder was decreased in all pairs that were administered OT compared to saline controls. The greatest decrease was in pairs where both members were administered OT which showed decreased time together compared to all other groups (a, $p < 0.05$). Pairs where one member received OT showed decreases in proportional time spent together compared to saline controls (b, $p < 0.05$).

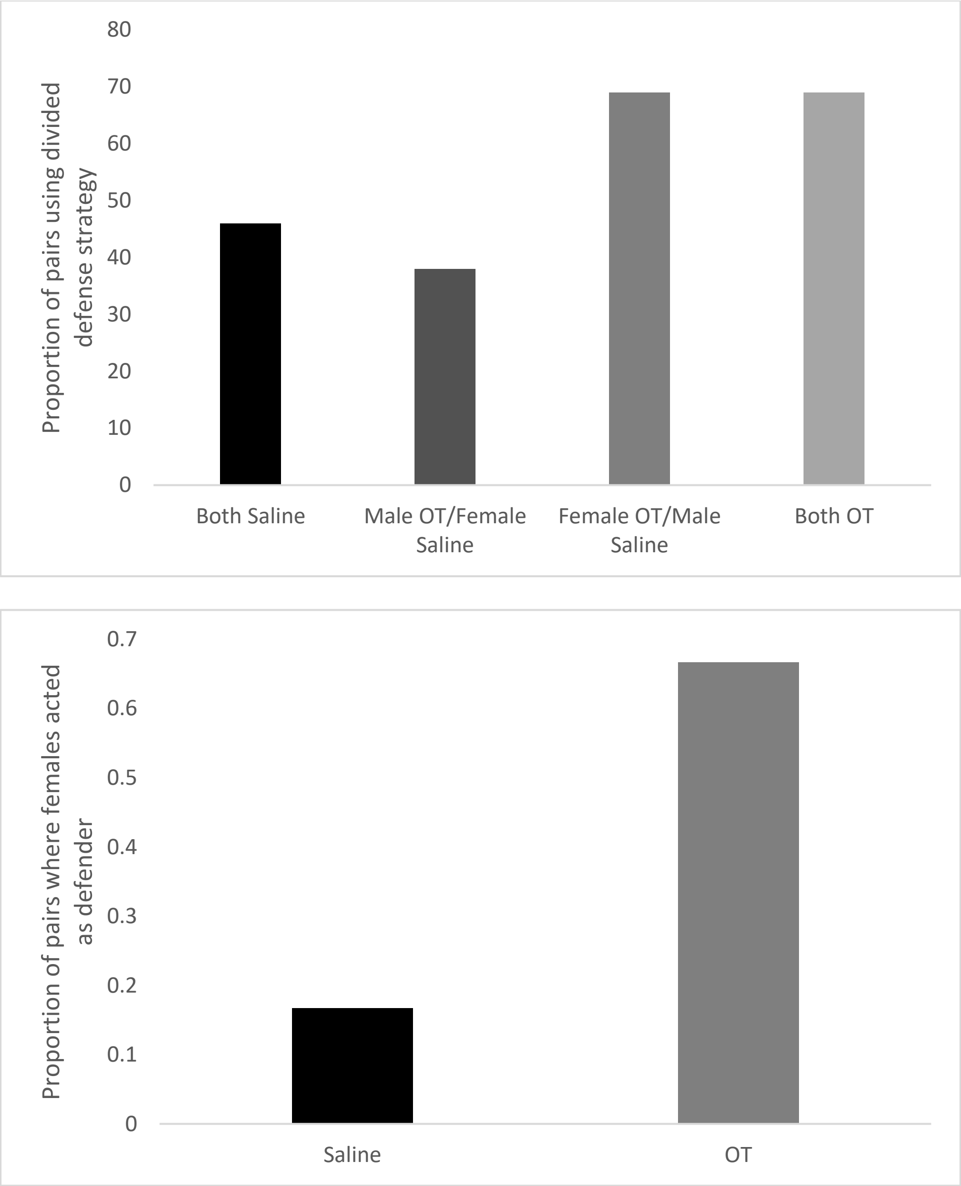


Figure 2. Pairs that received oxytocin showed a nonsignificant trend to increase the use of divided defense. A. Pairs where either females only or both members were administered OT had higher proportions of the use of a divided defense strategy than pairs where only males or neither member receives OT though this was not significant (p = 0.19). B. In all pairs where at least one member received OT and a divided defense strategy was used there was a nonsignificant trend for more females to act as the defender within the pair than males (p = 0.08)

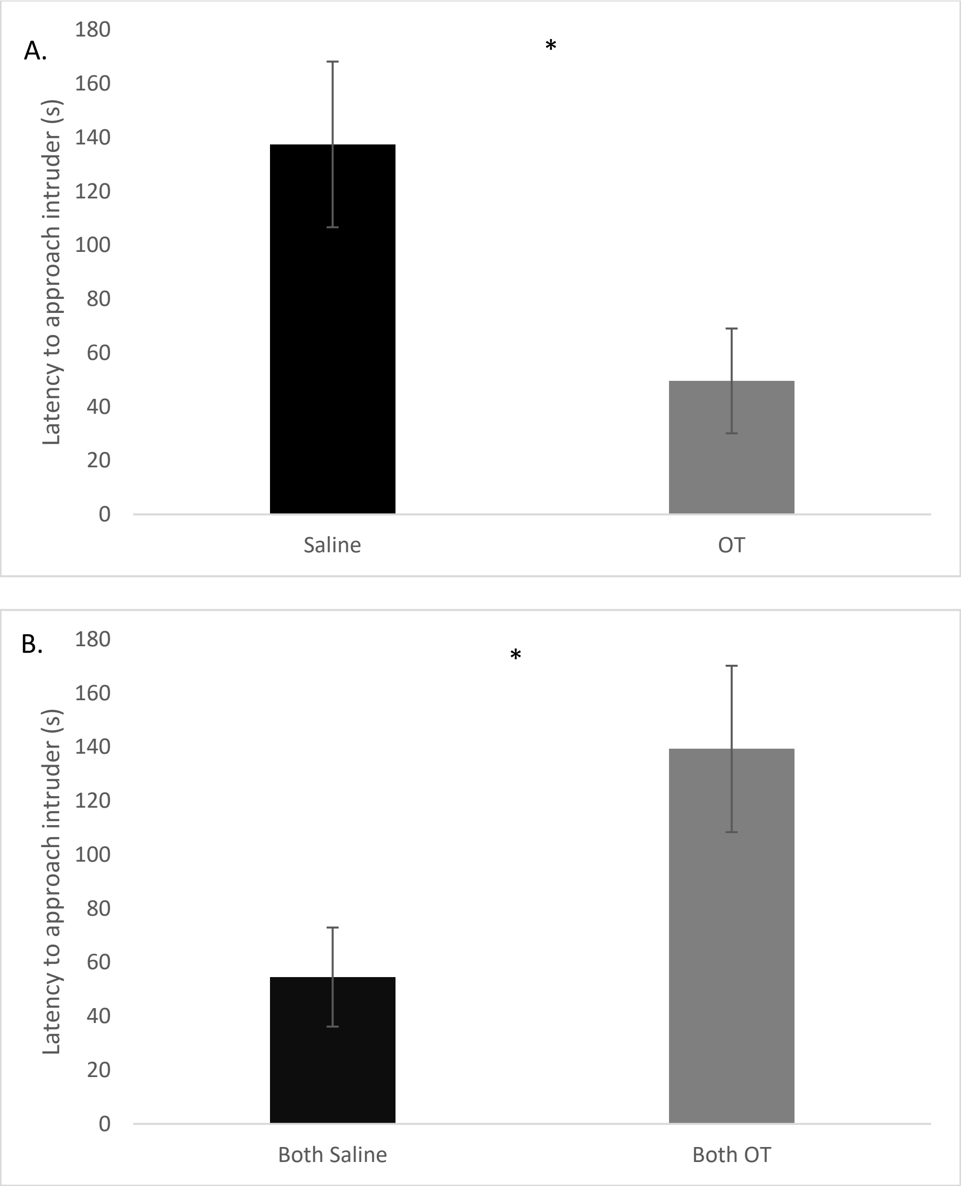


Figure 3. Latency to approach an intruder was altered by oxytocin dependent on whether one or both members of the pair is administered OT. A. When only one member of a pair is administered OT, the latency to approach an intruder was quickened in the member of the pair who received OT compared to their saline administered partner (* = $p < 0.05$). B. However in pairs where both members are administered OT the average latency to approach the intruder was increased from pairs where both members are administered saline (* = $p < 0.05$).

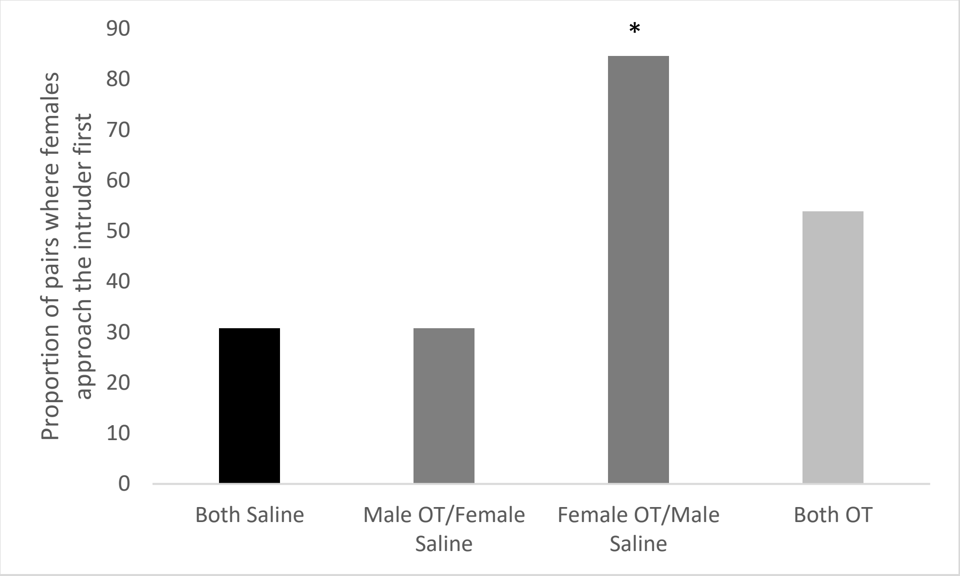


Figure 4. OT administration to females made it more likely for females to approach an intruder first. In pairs where females only receive OT the female partner was significantly more likley to make contact with the intruder first after introduction (* = $p < 0.05$)

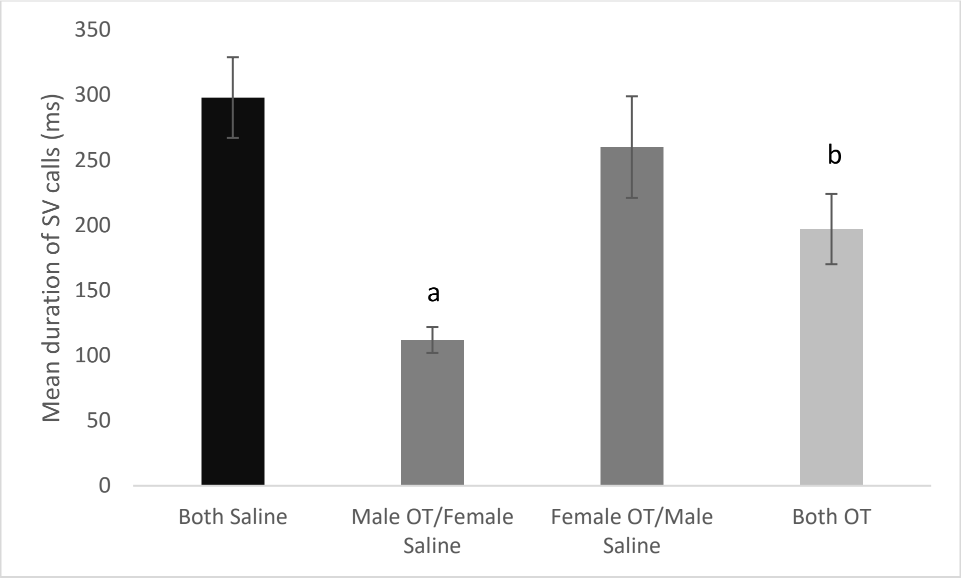


Figure 5. Pairs where males received OT showed changes in vocal communication in response to intruders. Duration of SV calls were significantly shortened in pairs where only males received OT compared to all other groups (a, $p < 0.05$). Pairs where both members were administered OT showed shortened SV durations compared to pairs where both membered were administered saline (b, $p < 0.05$).

Chapter 4

California mice show increased behavioral similarity following pair-bonding in response to aversive vocal playbacks

Abstract

While behavioral syndromes of individual animals are generally fixed in social isolation, providing an individual with a social partner or group can lead to alterations in behavioral responses. In general, if social groups alter individual behavior, they do so in one of two ways: 1) the individual's behavior becomes more similar to that of their group, known as social conformity, or 2) the individual exaggerates its previously held behavioral type, known as facilitation. Despite knowing that social groupings can alter behavior, little is known about how pair-bonding affects the behavioral reactions of individuals to stimuli, particularly in monogamous mammals. In this study, we examined the change in approach behavior to an aversive conspecific vocal stimulus, bark ultrasonic vocalizations (USVs), in California mice before and after pair-bonding. After an initial test to determine the behavioral phenotype of each individual, animals were either paired with a partner that shared or differed from their phenotypes or remained unpaired as sexually naive controls. All animals were then retested for approach behavior and USV production in response to bark playbacks. We found that unpaired individuals did not change in their response to bark calls, but that pair-bonded individuals altered their approach behavior to become more similar to their partner. We also found that pairs, analyzed as a dyad, produced a greater number of sustained vocalizations (SV) than nonpaired controls in response to bark playbacks. Moreover, increased SV calling by pairs was correlated with increased similarity in approach behavior by pairs. Overall, this study shows that following pair-bonding individuals alter their behavior to become more similar to their mate and that vocal communication may play an important role in coordinating this change.

Keywords: Behavioral syndrome, emergent property, similarity, ultrasonic vocalizations, approach/avoidance

Highlights

- Pairs increase similarity in behavior following pair bonding

- Ultrasonic vocalizations are increased in pairs compared to individuals in response to playbacks

- Increased similarity pairs correlates with greater sustained vocalization production

Introduction

Behavioral syndromes manifest within animal populations as a suite of behaviors in response to different external stimuli or events (Sih, Bell, & Johnson, 2004; Sih, Bell, Johnson, & Ziemba, 2004). Individuals within species and populations have behavioral types that dictate their specific responses to particular situations such as defending territories, responding to challenges from conspecifics, or exploring new areas (Biro & Stamps, 2008; Briffa, Rundle, & Fryer, 2008; Kralj-Fišer & Schuett, 2014; Stamps, 2007). However, while we know that animals in isolation will show generally fixed responses to many stimuli over the course of their lives (Bergmüller & Taborsky, 2010; Dingemanse, Kazem, Réale, & Wright, 2010; Wright, Holbrook, & Pruitt, 2014), we know much less about how major life events such as pair-bonding alter these behavioral types. Pair-bonding is particularly interesting in this context as individuals can bond with others who are either share or differ in behavioral type and navigating these behavioral similarities and differences is vital to the success of the pair.

A classic example of behavioral syndromes across taxa is ‘boldness,’ which can be defined as a willingness to explore novel situations, or to return quickly to a behavior after, or in spite of, a threat (Mamuneas, Spence, Manica, & King, 2015; Stamps, 2007; Swaney, Cabrera-Álvarez, & Reader, 2015). While these traits are often thought of as being fixed (King et al., 2013; Laskowski & Bell, 2014), research has shown different factors including social partners or groups can either change or enhance an individual’s behavioral type (Webster & Ward, 2011). The social conformity hypothesis, for example, states that individuals will alter their behavior in order to match their social group, thus becoming more similar to their peers and increasing group cohesion (Dávid-Barrett & Dunbar, 2012; King & Cowlishaw, 2009; King, Williams, & Mettke-Hofmann, 2015). With this hypothesis, there is evidence that breeding pairs who are more similar in their behavioral outputs will have greater reproductive success than those that are different (Gabriel & Black, 2012). Conversely, the facilitation hypothesis states that individual’s behavioral types will become more exaggerated in the presence of a social partner with a different behavioral type. This enhancement of behavior can help to facilitate social coordination or division of labor (Conradt & Roper, 2009; Hemelrijk & Wantia, 2005). However, it is not known if members of a pair will maintain their initial individual behavior, constituting a fixed behavioral syndrome, or alter their behavior based on their mates behavioral type, constituting an emergent property of the pair.

The behavioral plasticity of pair bonding individuals is of particular interest because of the effect this plasticity likely has on their success as a pair. Pair-bonding species have been shown to coordinate their behavior in order to protect their territory, care for offspring, and forage for food (Ahern, Hammock, & Young, 2011). In some cases, such as cichlid fish (Rogers, 2010) and Kirk's dik-dik (*Madoqua kirkii*) (Brotherton, Pemberton, Komers, & Malarky, 1997), pairs show rigid sex-specific coordination of behavior such that males always defend territories while females care for offspring. However, in monogamous rodents such as prairie voles (*Microtus ochrogaster*) (Ahern et al., 2011) and California mice (Bester-Meredith, Conley, & Mammarella, 2016; Frazier, Trainor, Cravens, Whitney, & Marler, 2006; Rieger, Stanton & Marler, under review), both members of the pair can complete any necessary task making coordination between pair-bond members of the utmost importance. Despite this flexibility, whether this coordination is simply pairs maintaining their individual behavioral phenotypes from prior to pair bonding or altering their behavior to match their mate and maximize efficiency has not been tested.

To understand better how pair-bonding alters behavioral responses we studied the approach behavior and vocalizations of California mice (*Peromyscus californicus*) in response to aversive vocal playbacks. California mice form pair-bonds that last for life (Gubernick & Alberts, 1981; Ribble, 1991; Ribble & Salvioni, 1990) and individuals show differences in boldness (Fuxjager, Montgomery, Becker, & Marler, 2010). Importantly, California mice can also show division of labor in their response to different tasks (Rieger, Stanton & Marler, under review). For example, when challenged by an intruder in their home territory, paired California mice will either investigate this intruder jointly, with both the male and the female approaching, or separately, with one of the male or female approaching the intruder while the other remains near the nest (Rieger, Stanton & Marler, under review). These strategies are robust across contexts and as such show evidence for behavioral types within California mice that may be altered following bonding.

California mice also produce a rich set of ultrasonic vocalizations (USV) (Kalcounis-Rueppell et al., 2010; Kalcounis-Rueppell, Metheny, & Vonhof, 2006; Kalcounis-Rueppell, Pultorak, & Marler, 2018) that have been shown to be important across social contexts (Pultorak, Fuxjager, Kalcounis-Rueppell, & Marler, 2015; Pultorak, Matusinec, Miller, & Marler, 2017; Rieger & Marler, 2018). Of particular interest to this study are barks, which are a short, loud aversive call that begin and end in the audible range (Kalcounis-Rueppell et al., 2018). These calls are most commonly used during defensive aggression and are aversive, facilitating aggressors moving away from the individual producing them (Rieger & Marler, 2018). We also tracked the production of two other call types: sweeps and sustained vocalizations (SV). Sweeps are short contact calls in the 50–100 kHz range (Kalcounis-Rueppell et al., 2018) and SVs are long, low bandwidth calls that can be used to signal affiliation (Kalcounis-Rueppell et al., 2018; Pultorak et al., 2017) and aggression (Rieger & Marler, 2018).

Our goal was to determine if emergent properties in their response to aversive bark playback tracks manifest in California mice after pair-bonding. To do this we first tested male and female California mice as individuals to determine their initial behavioral response to bark playbacks and categorized individuals as either approachers or avoiders based on how much time they spent near the bark playback speaker. We then either selectively paired individuals with an opposite sex partner who shared or differed in their behavioral response or kept the individual as an unpaired control. Approach behavior to bark playbacks was then retested with pairs tested together and unpaired individuals tested alone. For both tests we scored the approach and avoidance behavior of individuals and pairs as well as the production of USV calls, specifically sweeps and SVs. We predicted changes in paired California mouse behavior would be consistent with facilitation. First, we predicted that pairs that were initially different in their responses would remain different which would allow them to maintain division of labor. Second, we predicted that pairs that were initially similar in their behavioral response would become more different upon retesting increasing division of labor and maximizing efficiency in investigating the aversive stimulus. Finally, we predicted that pairs would produce more USV calls than individuals as these are generally social calls and can help coordinate behavior between partners (Briggs & Kalcounis-Rueppell, 2011; Kalcounis-Rueppell, Pultorak, & Marler, 2018; Pultorak, Fuxjager, Kalcounis-Rueppell, & Marler, 2015; Pultorak, Matusinec, Miller, & Marler, 2017; Rieger, Stanton & Marler, under review).

Methods

Animals

Forty-one male and 41 female California mice (age 3–6 months) were tested for their responses to conspecific bark vocalization playbacks. Individuals were housed in standard cages (48 x 27 x 16 cm) lined with aspen bedding and a nestlet with food (Purina 5015^{TM} mouse chow) and water available *ad libitum* with two to three same-sex conspecifics. The housing room was maintained at 20–23ºC on a 14:10 h light:dark cycle (lights on at 16:00 central standard time) with all testing occurring 1–3 hours after the onset of the dark cycle in dim red light. All individuals were ear tagged for individual identification. Of the 82 total mice, 62 (31 males and 31 females) were selective paired between 3 and 7 days after their initial playback test with an opposite sex partner and were housed together in a standard cage identical to that described above. Pairs did not share common ancestry for at least two generations. The 20 remaining individuals continued to be housed with their original cage mates. Pairs and individuals were retested for their response to bark call playbacks between 10–12 days after pairing.

Ethical statement

All animals were maintained according to the National Institute of Health *Guide for the care and use of laboratory animals*. All procedures were approved by the University of Wisconsin – Madison College of Letters and Sciences Institutional Animal Care and Use Committee (Protocol L005447). No animals were injured by any of the behavioral manipulations or assays.

Apparatus

Testing occurred in Plexiglass apparatus (90 x 30 x 30 cm) lined with aspen bedding equally divided into three chambers (each 30 x 30 x 30 cm) with centrally located openings (11.5 x 11.5 cm) between chambers to allow for free movement between chambers. Two playback speakers (Vifa Dynamic Ultrasound, 1–120 kHz range, Avisoft Bioacoustics, Berlin, Germany) were used in this study with one placed at each end of the three-chambered apparatus 45 cm from the center. Speakers were positioned outside of the apparatus against a closed mesh gate allowing for sound to travel into the chamber.

Playback tracks

Barks were recorded using two Emkay/Knowles FG series microphones (detection range 10–120 kHz) during male-male and female-female aggressive interactions (Rieger & Marler, 2018) with a 250-kHz sampling rate and 16-bit resolution. Spectograms were produced using a 512 fast Fourier transform in Avisoft SASlab pro (Avisoft Bioacoustics, Berlin, Germany). Eight total playback tracks were created using these spectrograms by selecting male and female bark calls while removing all other USV call types. Playback tracks were 2 minutes in duration and contained 120 ± 5 bark calls and no other USV call types. Tracks were assigned to individuals randomly with each track being used between 15 and 17 times. Importantly, no individual heard the same track twice over the course of the two tests. Amplitude of the playbacks was matched to the original amplitude of the barks produced by placing an Emkay/Knowles FG series microphone 30 cm away from the playback speaker and adjusting the output gain/volume until it matched the original sample (as described in Pultorak, Matusinec, Miller, & Marler, 2017). The output gain/volume was then maintained across playback tracks. Ambient noise tracks were used as a control in this study. Ambient noise playback tracks were also 2 minutes in duration and contained no USV calls or other mouse related sounds, such as movement. Ambient noise tracks were composed of a recording of the testing room with all lights off and no mice present using the same microphone described above.

Initial playback test

All animals were first tested for their response to conspecific bark playbacks as nonbonded, sexually naïve individuals. To begin, nonbonded individual animals were placed in the center of the apparatus and allowed a minimum of 5 minutes to habituate to the chamber. Prior to testing animals had to enter each of the three chambers and return to the center chamber and cease rapid movements between the chambers. Once habituated testing started with the animal in the center chamber. Tests were set up as 2-minute playback preference tests with playback speakers placed at opposite ends of the apparatus behind a wire mesh with one playback speaker playing a bark track while the other played an ambient noise track concurrently. Video and audio recordings of individuals were made to track their behavior. Specifically, time spent in the chamber closest to the bark playback speaker ("bark chamber") termed an approach score, and in the chamber closest to the ambient noise playback speaker ("ambient noise chamber") termed an avoidance score, were scored. USV production by focal animals was also recorded using two Emkay/Knowles FG series microphones placed at opposite ends of the apparatus 30 cm from the apparatus floor (see supplementary figure 1 for diagram).

Behavioral type and pairing

Following testing, animals were categorized as being either approachers or avoiders. To define these categories, we created a distribution of all individual responses to bark playbacks based on their time spent in the bark chamber (supplementary figure 2). We then used a median split on time spent in the bark chamber (median = 30 s) to define these two categories. This criterion created two behavioral types, such that animals above the median are more likely to approach, while animals below the median are less likely to approach. Using this criterion, approachers were defined as individuals that spent more than 30 seconds in the bark chamber while avoiders were defined as individuals that spent less than 29 seconds in the bark chamber.

Following categorization, individuals were randomly assigned to be paired (62 total mice) or to remain unpaired (20 total mice). Paired individuals were selectively paired 3 to 7 days after their initial test into one of four groups based on their behavioral classification: 1) male approacher with female avoider (n = 11), 2) female approacher with male avoider (n = 7), 3) male approacher with female approacher (n =5) and 4) male avoider with female avoider (n = 8). These groups could also be collapsed into two overarching types to help offset potential issues with sample size: 1) Pairs that were initially different in their response to bark playbacks, made up of groups 1 and 2, with > 30-second difference in approach score between individuals (n = 18) and 2) pairs that were initially similar in their response to bark playbacks, made up of groups 3 and 4, with < 10-second difference in approach score between individuals (n = 13). Along with behavioral classification, the total difference in time spent in the bark chamber within a pair was used for statistical analyses as it gave a continuous gradient of the pairs similarity or difference.

Second playback tests

All animals underwent a second playback test to determine if responses to bark calls were altered by pairing. Pairs were retested 10–11 days after pairing (13–17 days after the initial test) and individuals were retested between 13 and 17 days after their initial test. At 7 days post pairing, pairs show hallmarks of pair bonding including side by side contact, reduced aggression, increased affiliation and increased affiliative USV calls (Becker, Castelli, Yohn, Spencer, & Marler, 2018) indicating that 10–11 days is sufficient for California mice to form a pair bond. The playback procedure was the same as that described above with the only difference being that paired animals were tested together as a pair, while nonpaired individuals were tested alone. For paired animals, both individuals were placed into the center chamber and allowed to habituate for a minimum of 5 minutes. Both individuals were required to enter all three chambers prior to testing and had to start the test in the middle chamber. Time spent in the bark chamber and the ambient noise chamber was scored for each individual, pairs were also scored for the time they spent together or separate. Time together was defined as anytime during the test when both members of the pair were in the same chamber, while time separate was defined as anytime the two members of the pair were in different chambers. The procedure for nonpaired individuals was the same as the initial test. USV production was scored for both pairs and nonpaired individuals.

Ultrasonic vocalization analysis

We recorded USVs with two Emkay/Knowles FG series microphones (detection range 10–120 kHz). Microphones were placed 85 cm apart at opposite corners of the apparatus, 30 cm from the apparatus floor with one microphone placed in the bark chamber and one microphone placed in the ambient noise chamber. Microphone channels were calibrated to equal gain (-60 dB noise floor) and WAV files were produced using RECORDR software (Avisoft Bioacoustics, Berlin, Germany). Recordings were made using a 250-kHz sampling rate with 16-bit resolution and spectrograms were produced with a 512 fast Fourier transform made using Avisoft SASLab Pro (Avisoft bioacoustics). USVs were differentiated by visual and auditory inspection of WAV files with sampling rates reduced to 4% of normal speed (11025 kHz). Analysis of USV production was focused on sustained vocalizations (SV) and sweeps (as defined in Kalcounis-Rueppell, Pultorak, & Marler, 2018) as these were the only call types produced by focal animals. Both the total number of USV calls produced and the proportion of each USV individual call type produced compared to all calls produced were analyzed within this dataset.

Statistics

All statistics were completed using SPSS v 22 (IBM Corp, Armonk, NY, USA). We analyzed changes between the initial test and post pairing test using a mixed ANOVA with group, sex and initial similarity of pairs as factors. We analyzed the production of USV calls by pairs and individuals using group, paired status and similarity of pairs as factors. Tukey post hoc tests were used to determine differences between groups. Linear regressions were used to determine if changes in behavior were predictive of USV call production and USV call type proportion. Pairs were used as a covariate in all appropriate analyses.

Results

Initial response by individuals to bark playbacks

During the initial testing phase individual male and female California mice showed a wide range of responses to bark call playbacks. The range of time spent in the bark chamber was 0–115 seconds for all individuals combined (see supplementary figure 1). Time spent in the bark chamber did not differ between males and females (males: 29.71 ± 4.28 s; females: 39.76 ± 3.87 s, ANOVA, $F_{1,80} = 3.032$, $p = 0.085$). Similarly, the range of time spent in the ambient noise chamber ranged from 0–115 seconds and the average time spent in the ambient noise chamber did not differ between males and females (males: 35.55 ± 3.994 s; females: 37.07 ± 4.196 s, ANOVA, $F_{1,72} = 0.066$, $p = 0.798$). Overall, individuals did not show a preference for either the bark chamber or the ambient noise chamber (ambient noise chamber: 36.42 ± 2.926 s, bark chamber: 36.19 ± 3.21, $t_{71} = 0.041$, $p = 0.967$). During the initial test no SV calls were produced by individuals and the number of sweeps produced was not statistically different from zero (total sweeps 0.11 ± 0.08, $t_{81} = 1.362$, $p = 0.184$).

Based on these results animals were selectively paired with individuals who showed either similar or different approach patterns creating four groups 1) male approacher and female avoider, 2) female approacher and male avoider, 3) male approacher and female approacher, 4) male avoider and female avoider. As expected, within groups there was a significant group by sex interaction in approach scores (ANOVA, $F_{4,72} = 16.604$, $p < 0.001$, figure 1) with significant approach score differences between males and females in group 1 (male: 71.29 ± 8.67 s, female 20.14 ± 2.68 s, Students t-test, $t_{12} = 5.64$, $p < 0.001$) and group 2 (male: 11.91 ± 3.17 s, female: 55.45 ± 7.41 s, $t_{20} = 5.40$, $p < 0.001$) but not for group 3 (male: 51.6 s ± 8.58, female: 57.40 ± 5.73 s , $t_8 = 0.56$, $p = 0.59$), group 4 (male: 11.00 ± 2.70 s, female: 16.38 ± 3.68 s, $t_{14} = 1.17$, $p = 0.26$) or unpaired controls (male: 24.2 ± 5.56, female: 35.15 ± 7.54, $t_{18} = 1.19$, $p = 0.25$) validating our groups. These groups were also categorized by the difference in approach score within pairs. The approach score difference was significantly higher in groups one and two than groups three and four (group 1: 51.14 ± 8.02 s, group 2: 43.64 ± 7.98, group 3: 9.07 ± 4.06, group 4: 5.88 ± 1.70: ANOVA, $F_{4, 46} = 6.87$, $p < 0.001$). Moreover, this analysis showed that our groups could be split into two homogenous subsets with groups 1 and 2 making up one subset of pairs (the “initially different” group) and groups 3 and 4 making up the second homogenous subset (the “initially similar” group).

Post-pairing response to playbacks

Following pairing, significant three-way and two-way interactions were found. First, a significant three-way interaction was found between sex, group and pairing status on approach score such that males and females in groups 1 and 2 altered their approach behavior to be more similar to their partner. Specifically, individuals who initially were categorized as approachers decreased their approach behavior while those who were initially categorized as avoiders increased their approach behavior (ANOVA, $F_{4,72} = 4.327$, $p = 0.003$, figure 2). There was also a significant two-way interaction between group and pairing status on approach score such that groups one and two, which were initially more different in their approach scores became more similar, and groups 3, 4 and 5 who were generally more similar in their approach scores prior to pair bonding remained similar ($F_{4.,72} = 2.63$, $p = 0.041$, figure 3).

As in the initial test, no preference was found for the bark chamber or the ambient noise chamber for individual animals (bark chamber: 26.71 ± 2.89 s, ambient noise chamber 39 ± 3.089, $t_{61} = 0.443$, $p = 0.66$). Moreover, there was also no difference in preference score based on pair group ($F_{3,54} = 0.281$, $p = 0.839$). However, it was found that initially similar pairs, groups 3 and 4, spent proportionally more time together in the bark chamber than those that were initially different, groups 1 and 2 (similar: 36.36 ± 7.2%, different: 22.67 ± 6.12%, ANOVA, $F_{1,58} = 4.14$, $p = 0.046$).

Pairs, analyzed as dyads, were significantly more likely to produce sweeps (26 out of 31 pairs, 3 out of 20 individuals, $\chi^2_1 = 20.78$, $p < 0.001$) and SV calls (15 out of 31 pairs versus 0 out of 20 individuals, $\chi^2_1 = 11.478$, $p < 0.001$) than unpaired individuals in response to bark playbacks. Total USV call production was increased in both initially different and initially similar pairs compared to unpaired individuals in response to bark playbacks (different: 87.0 ± 16.37 calls, similar 66.08 ± 26.30 calls, individuals 0.8 ± 0.65 calls, ANOVA, $F_{2,48} = 9.78$, $p < 0.001$, figure 4a). By specific call type, sweep production was increased in both initially different and initially similar pairs compared to nonpaired individuals (different: 80 ± 15.73, similar: 66.08 ± 25.81, individual: 0.80 ± 0.65, ANOVA, $F_{2,48} = 9.038$, $p < 0.001$, figure 4b), and pairs that were initially different produced the greatest total number of SV calls when compared to pairs that were initially similar and nonpaired individuals (different: 7 ± 2.38, similar:

2.23 ± 1.06, individual 0 ± 0, ANOVA, $F_{2,48} = 5.99$, $p = 0.005$ figure 4c). Moreover, pairs that were initially different produced the greatest proportion of SV calls as a function of total calls when compared to initially similar pairs and nonpaired individuals (different: 9.25 ± 0.28%, similar: 2.57 ± 1.27%, individual: 0 ± 0%, figure 4d).

Total USV calling and proportion of SV calling correlated with a number of behavioral measures. First, time spent together by pairs in the ambient noise chamber positively predicted total USV calling (sweeps + SVs; Linear regression, $F_{1,29} = 4.253$, $R^2 = 0.1279$, $p = 0.048$, figure 5). Moreover, increased time spent together by mates was predictive of increased sweeps in males ($F_{1,29} = 6.918$, $R^2 = 0.1926$, $p = 0.014$) but not females ($F_{1,29} = 1.58$, $R^2 = 0.0517$, $p = 0.22$). Importantly, increased similarity in approach score by pairs following pair-bonding was predictive of pairs producing more total SVs as a dyad ($F_{1,29} = 4.198$, $R^2 = 0.1265$, $p = 0.049$ figure 6a) and a greater proportion of SV calls compared to all calls ($F_{1,29} = 5.872$, $R^2 = 0.1966$, $p = 0.023$, figure 6b)

Discussion

In this study we tested whether pair-bonding would alter the approach behavior of individuals towards an aversive stimulus. We found that pair-bonded California mice alter their approach behavior after pair-bonding to become more similar to their partner. As such, the greatest changes in behavior were seen in pairs made up of individuals who were initially different in their approach scores prior to pairing, while pairs that were initially similar and those who remained unpaired maintained their initial behavioral strategy. We also found that pairs produced more USVs than individuals. Moreover, after pair-bonding, we found that increasing similarity in approach behavior was correlated with increased SV calling in pairs. These results do not support our initial prediction that pair-bonding would a facilitative effect on behavior, but instead provide evidence for social conformity within a monogamous rodent.

Behavioral syndromes are often fixed in socially isolated animals (Sih, Bell, & Johnson, 2004; Sinn, Moltschaniwskyj, Wapstra, & Dall, 2010), however, these behavioral responses can be altered by changes to an individual's social environment (Webster & Ward, 2011). When placing individuals in a social context, one of three behavioral outcomes can occur. First, individuals can maintain their previous behavioral responses despite being in a group, as seen in three-spined sticklebacks (*Gasterosteus aculeatus*). Second, animals can alter their behavior to become more similar to their group members, as is seen in Gouldian finches (King et al., 2015), vervet monkeys (*Chlorocebus pygerythrus*) (Van De Waal, Borgeaud, & Whiten, 2013) and zebra finches (*Taeniopygia guttata*) (Schuett & Dall, 2009), a process known as social conformity. Finally, individuals can enhance their original behaviors, making them stronger, a process known as facilitation (Hemelrijk & Wantia, 2005; King & Sueur, 2011). Prior to this study little was known about how pair-bonding and social partners affect individual behavioral responses in monogamous rodents.

As such, we tested the behavioral responses of California mice to aversive vocal playbacks before and after pairing. Because California mice can divide labor to complete tasks (Rieger, Stanton & Marler, under review), we predicted that they would follow the facilitation hypothesis, which would lead to increased labor division and help to coordinate behavior between individuals. Specifically, the facilitation hypothesis would predict that a pair consisting of an approacher and an avoider would see those individuals maintain their initial behavioral responses. This would then maximize division of labor within the pair and potentially act to increase efficiency in responding to an aversive stimulus. However, we instead saw results consistent with social conformity. In pairs with initially different behavioral responses, both members of the pair altered their response to be more similar to their partner.

While increased similarity was unexpected it does fit with previous research that has found that breeding pairs that are more similar are more likely to be reproductively successful (Gabriel & Black, 2012). Thus, paired California mice may act to increase similarity to better their chances of successfully reproducing. Moreover, increased similarity may provide greater flexibility within the pair-bond to complete tasks related to territorial defense and parental care. If pairs are more similar it would allow the tactical flexibility of both parents being capable of foraging, caring for pups and retrieving pups when necessary. This would fit with data in voles, which show that both parents will trade off care (Ahern et al., 2011) and in California mice where both parents can complete all aspects of parental care and pup retrieval (Bester-Meredith et al., 2016; Frazier et al., 2006; Gubernick & Teferi, 2000). However, it is important to note that the pairs in this study were both nonparental and nonresidents. As such in a novel environment pairs may prefer to investigate their environment together as there is no nest present to act as a central, safe, home location. Pairs may also become less willing to act together when pups are present as this would likely lead to the pups being exposed and, as seen in prairie voles, biparental pairs tend to act to reduce the time pups are left alone (Ahern et al., 2011).

Across species, communication is important to the coordination of social behaviors (Noe, 2006). Animals use vocal signals to coordinate responses to threats (Litvin, Blanchard, & Blanchard, 2007; Seyfarth, Cheney, & Marler, 1980; Townsend et al., 2011), to defend territories (Rieger & Marler, 2018; Wiewandt, 1969), and to share information (Brudzynski, 2013; Owen, Swaisgood, Zhou, & Blumstein, 2016). As such, communication may play an important role in coordinating behavioral similarities within pairs. We found anecdotal data in this study that both males and females are producing calls, namely that overlapping SV calls and overlapping sweep and SV calls were detected across behavioral trials in pairs. Despite this, we were not able to localize these calls to individuals and thus analyzed pairs as dyads to determine changes in vocal production and the relationship of these calls to behavioral similarity in approach behavior.

We first predicted that pairs would produce more USVs than individuals as California mice tend to produce more calls in social situations than they do in isolation (Rieger & Marler, 2018). We supported this prediction as both pairs that were made up of initially similar and initially different individuals showed increased production of sweeps and SVs compared to individuals. Importantly, the number of calls and proportion of calls produced was correlated with similarities in behavior. First, total USV calling, including both sweeps and SVs produced was correlated with total time spent together in the ambient noise chamber. Moreover, it was shown that pairs that showed increased similarity in approach behaviors produced both more total SV calls and a greater proportion of SV calls as a dyad. These data provide evidence that vocal communication in a monogamous mammal may be important to the coordination of behavior as has been seen in other species (Noe, 2006). Specifically, as USV production was greatest in pairs that had initially different behavioral responses it could be that these vocalizations are necessary to help increase behavioral similarity. One important question that remains is whether pairs call back and forth in response to a stimulus. If pairs call and respond in response to a stimulus and then show greater behavioral similarity this would be evidence that vocal communication is vital to driving behavioral similarities within pairs.

Overall, we found that nonparental, pair-bonded California mice show changes in their responses to aversive stimuli leading to greater similarity in behavior. Furthermore, this increase in similarity of approach behaviors corresponded with increased USV production. Specifically, the more pairs increased similarity in approach behavior after pair-bonding the more SV calls that were produced by the dyad. This result indicates for the first time in a monogamous rodent that vocal communication may be important to coordinating behavior between mates in order to increase similarity in behavior. Moreover, this study provides evidence that in monogamous rodents, as in other species, social partners lead to social conformity. However, what underlies these behavioral changes at a neural or mechanistic level remains unknown and should be a focus of future studies. With this study, we provide a monogamous mammalian model of behavioral changes due to pair-bonding which can provide unique insights into how and why individuals become more similar in their behavior following pair-bonding.

Acknowledgements

We thank Sage Keyes, Juliette Schefelker, Alexis Payette and JB Ramos Hernandez for their help with collecting and analyzing data for this study. We also thank the animal research technicians of the University of Wisconsin-Madison. Finally, we thank our funding source, the National Science Foundation IOS11312419.

References

Ahern, T. H., Hammock, E. A. D., & Young, L. J. (2011). Parental division of labor, coordination, and the effects of family structure on parenting in monogamous prairie voles (Microtus ochrogaster). *Developmental Psychobiology*, *53*(2), 118–31. http://doi.org/10.1002/dev.20498

Becker, E. A., Castelli, F. R., Yohn, C. N., Spencer, L., & Marler, C. A. (2018). Species differences in urine scent-marking and counter-marking in Peromyscus. *Behavioural Processes*, *146*, 1–9. http://doi.org/10.1016/J.BEPROC.2017.10.011

Bergmüller, R., & Taborsky, M. (2010). Animal personality due to social niche specialisation. *Trends in Ecology & Evolution*, *25*(9), 504–511. http://doi.org/10.1016/j.tree.2010.06.012

Bester-Meredith, J., Conley, M., & Mammarella, G. (2016). Peromyscus as a Model System for Understanding the Regulation of Maternal Behavior. *Seminars in Cell and Developmental Biology*. http://doi.org/10.1016/j.semcdb.2016.07.001

Biro, P. A., & Stamps, J. A. (2008). Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution*, *23*(7), 361–8. http://doi.org/10.1016/j.tree.2008.04.003

Briffa, M., Rundle, S. D., & Fryer, A. (2008). Comparing the strength of behavioural plasticity and consistency across situations: animal personalities in the hermit crab Pagurus bernhardus. *Proceedings. Biological Sciences*, *275*(1640), 1305–11. http://doi.org/10.1098/rspb.2008.0025

Briggs, J. R., & Kalcounis-Rueppell, M. C. (2011). Similar acoustic structure and behavioural context of vocalizations produced by male and female California mice in the wild. *Animal Behaviour*, *82*(6), 1263–1273. http://doi.org/10.1016/j.anbehav.2011.09.003

Brotherton, P. N., Pemberton, J. M., Komers, P. E., & Malarky, G. (1997). Genetic and behavioural evidence of monogamy in a mammal, Kirk's dik-dik (Madoqua kirkii). *Proceedings. Biological Sciences*, *264*(1382), 675–81. http://doi.org/10.1098/rspb.1997.0096

Brudzynski, S. M. (2013). Ethotransmission: communication of emotional states through ultrasonic vocalization in rats. *Current Opinion in Neurobiology*, *23*(3), 310–317. http://doi.org/10.1016/j.conb.2013.01.014

Conradt, L., & Roper, T. J. (2009). Conflicts of interest and the evolution of decision sharing. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *364*(1518), 807–19. http://doi.org/10.1098/rstb.2008.0257

Dávid-Barrett, T., & Dunbar, R. I. M. (2012). Cooperation, behavioural synchrony and status in social networks. *Journal of Theoretical Biology*, *308*, 88–95. http://doi.org/10.1016/J.JTBI.2012.05.007

Dingemanse, N. J., Kazem, A. J. N., Réale, D., & Wright, J. (2010). Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology & Evolution*, *25*(2), 81–89. http://doi.org/10.1016/J.TREE.2009.07.013

Frazier, C. R. M., Trainor, B. C., Cravens, C. J., Whitney, T. K., & Marler, C. A. (2006). Paternal behavior influences development of aggression and vasopressin expression in male California mouse offspring. *Hormones and Behavior*, *50*(5), 699–707. http://doi.org/10.1016/j.yhbeh.2006.06.035

Fuxjager, M. J., Montgomery, J. L., Becker, E. A., & Marler, C. A. (2010). Deciding to win: interactive effects of residency, resources and 'boldness' on contest outcome in white-footed mice. *Animal Behaviour*, *80*(5), 921–927. http://doi.org/10.1016/j.anbehav.2010.08.018

Gabriel, P. O., & Black, J. M. (2012). Behavioural Syndromes, Partner Compatibility and Reproductive Performance in Steller's Jays. *Ethology*, *118*(1), 76–86. http://doi.org/10.1111/j.1439-0310.2011.01990.x

Gubernick, D. J., & Alberts, J. R. (n.d.). The Biparental Care System of the California Mouse, Peromyscus californicus. *Journal of Comparative Psychology 19S7*, *101*(2), 169–177. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.597.5446&rep=rep1&type=pdf

Gubernick, D. J., & Teferi, T. (2000). Adaptive significance of male parental care in a monogamous mammal. *Proceedings. Biological Sciences*, *267*(1439), 147–50. http://doi.org/10.1098/rspb.2000.0979

Hemelrijk, C. K., & Wantia, J. (2005). Individual variation by self-organisation. *Neuroscience & Biobehavioral Reviews*, *29*(1), 125–136. http://doi.org/10.1016/J.NEUBIOREV.2004.07.003

Kalcounis-Rueppell, M. C., Metheny, J. D., & Vonhof, M. J. (2006). Production of ultrasonic vocalizations by Peromyscus mice in the wild. *Frontiers in Zoology*, *3*(1), 3. http://doi.org/10.1186/1742-9994-3-3

Kalcounis-Rueppell, M. C., Petric, R., Briggs, J. R., Carney, C., Marshall, M. M., Willse, J. T., … Crossland, J. P. (2010). Differences in ultrasonic vocalizations between wild and laboratory California mice (Peromyscus californicus). *PloS One*, *5*(4), e9705. http://doi.org/10.1371/journal.pone.0009705

Kalcounis-Rueppell, M. C., Pultorak, J. D., & Marler, C. A. (2018). Ultrasonic Vocalizations of Mice in the Genus Peromyscus. *Handbook of Behavioral Neuroscience*, *25*, 227–235. http://doi.org/10.1016/B978-0-12-809600-0.00022-6

King, A. J., & Cowlishaw, G. (2009). All together now: behavioural synchrony in baboons. *Animal Behaviour*, *78*(6), 1381–1387. http://doi.org/10.1016/J.ANBEHAV.2009.09.009

King, A. J., Fürtbauer, I., Mamuneas, D., James, C., Manica, A., Edenbrow, M., … Louca, V. (2013). Sex-Differences and Temporal Consistency in Stickleback Fish Boldness. *PLoS ONE*, *8*(12), e81116. http://doi.org/10.1371/journal.pone.0081116

King, A. J., & Sueur, C. (2011). Where Next? Group Coordination and Collective Decision Making by Primates. *International Journal of Primatology*, *32*(6), 1245–1267. http://doi.org/10.1007/s10764-011-9526-7

King, A. J., Williams, L. J., & Mettke-Hofmann, C. (2015). The effects of social conformity on Gouldian finch personality. *Animal Behaviour*, *99*, 25–31. http://doi.org/10.1016/j.anbehav.2014.10.016

Kralj-Fišer, S., & Schuett, W. (2014). Studying personality variation in invertebrates: why bother? *Animal Behaviour*, *91*, 41–52. http://doi.org/10.1016/j.anbehav.2014.02.016

Laskowski, K. L., & Bell, A. M. (2014). Strong personalities, not social niches, drive individual differences in social behaviours in sticklebacks. *Animal Behaviour*, *90*, 287–295. http://doi.org/10.1016/J.ANBEHAV.2014.02.010

Litvin, Y., Blanchard, D. C., & Blanchard, R. J. (2007). Rat 22kHz ultrasonic vocalizations as alarm cries. *Behavioural Brain Research*, *182*(2), 166–172. http://doi.org/10.1016/j.bbr.2006.11.038

Mamuneas, D., Spence, A. J., Manica, A., & King, A. J. (2015). Bolder stickleback fish make faster decisions, but they are not less accurate. *Behavioral Ecology*, *26*(1), 91–96.

http://doi.org/10.1093/beheco/aru160

Noe, R. (2006). Cooperation Experiments: coordination through communication versus acting apart together. *Animal Behaviour*, *71*, 1–18. http://doi.org/10.1016/j.anbehav.2005.03.037

Owen, M. A., Swaisgood, R. R., Zhou, X., & Blumstein, D. T. (2016). Signalling behaviour is influenced by transient social context in a spontaneously ovulating mammal. *Animal Behaviour*, *111*, 157–165. http://doi.org/10.1016/j.anbehav.2015.10.008

Pultorak, J. D., Fuxjager, M. J., Kalcounis-Rueppell, M. C., & Marler, C. A. (2015). Male fidelity expressed through rapid testosterone suppression of ultrasonic vocalizations to novel females in the monogamous California mouse. *Hormones and Behavior*, *70*, 47–56. http://doi.org/10.1016/j.yhbeh.2015.02.003

Pultorak, J. D., Matusinec, K. R., Miller, Z. K., & Marler, C. A. (2017). Ultrasonic vocalization production and playback predicts intrapair and extrapair social behaviour in a monogamous mouse. *Animal Behaviour*, *125*, 13–23. http://doi.org/10.1016/j.anbehav.2016.12.023

Ribble, D. O. (1991). The monogamous mating system of Peromyscus californicus as revealed by DNA fingerprinting. *Behavioral Ecology and Sociobiology*, *29*(3), 161–166. http://doi.org/10.1007/BF00166397

Ribble, D., & Salvioni, M. (1990). Social organization and nest co-occupancy in Peromyscus californicus, a monogamous rodent. *Behavioral Ecology and Sociobiology*, *26*(1), 9–15. http://doi.org/10.1007/BF00174020

Rieger, N. S., & Marler, C. A. (2018). The function of ultrasonic vocalizations during territorial defence by pair-bonded male and female California mice. *Animal Behaviour*, *135*. http://doi.org/10.1016/j.anbehav.2017.11.008

Rogers, W. (2010). Parental Investment and Division of Labor in the Midas Cichlid (Cichlasoma citrinellum). *Ethology*, *79*(2), 126–142. http://doi.org/10.1111/j.1439-0310.1988.tb00706.x

Schuett, W., & Dall, S. R. X. (2009). Sex differences, social context and personality in zebra finches, Taeniopygia guttata. *Animal Behaviour*, *77*(5), 1041–1050. http://doi.org/10.1016/J.ANBEHAV.2008.12.024

Seyfarth, R. M., Cheney, D. L., & Marler, P. (1980). Vervet monkey alarm calls: Semantic communication in a free-ranging primate. *Animal Behaviour*, *28*(4), 1070–1094. http://doi.org/10.1016/S0003-3472(80)80097-2

Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution*, *19*(7), 372–378. http://doi.org/10.1016/J.TREE.2004.04.009

Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral Syndromes: An Integrative Overview. *The Quarterly Review of Biology*, *79*(3), 241–277. http://doi.org/10.1086/422893

Sinn, D. L., Moltschaniwskyj, N. A., Wapstra, E., & Dall, S. R. X. (2010). Are behavioral syndromes invariant? Spatiotemporal variation in shy/bold behavior in squid. *Behavioral Ecology and Sociobiology*, *64*(4), 693–702. http://doi.org/10.1007/s00265-009-0887-2

Stamps, J. A. (2007). Growth-mortality tradeoffs and “personality traits” in animals. *Ecology Letters*, *10*(5), 355–63. http://doi.org/10.1111/j.1461-0248.2007.01034.x

Swaney, W. T., Cabrera-Álvarez, M. J., & Reader, S. M. (2015). *Behavioural responses of feral and domestic guppies (Poecilia reticulata) to predators and their cues*. *Behavioural Processes* (Vol. 118). http://doi.org/10.1016/j.beproc.2015.05.010

Townsend, S. W., Manser, M. B., Fitch, W. T., Neubauer, J., Herzel, H., Herzel, H., … Fitch, W. T. (2011). The function of nonlinear phenomena in meerkat alarm calls. *Biology Letters*, *7*(1), 47–9. http://doi.org/10.1098/rsbl.2010.0537

Van De Waal, E., Borgeaud, C., & Whiten, A. (n.d.). *Potent Social Learning and Conformity Shape a Wild Primate's Foraging Decisions*. Retrieved from www.sciencemag.org/cgi/content/full/science.1233675/DC1

Webster, M. M., & Ward, A. J. W. (2011). Personality and social context. *Biological Reviews*, *86*(4), 759–773. http://doi.org/10.1111/j.1469-185X.2010.00169.x

Wiewandt, T. A. (1969). Vocalization, Aggressive Behavior, and Territoriality in the Bullfrog, Rana catesbeiana. *Copeia*, *1969*(2), 276. http://doi.org/10.2307/1442074

Wright, C. M., Holbrook, C. T., & Pruitt, J. N. (2014). Animal personality aligns task specialization and task proficiency in a spider society. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(26), 9533–7. http://doi.org/10.1073/pnas.1400850111

Figures

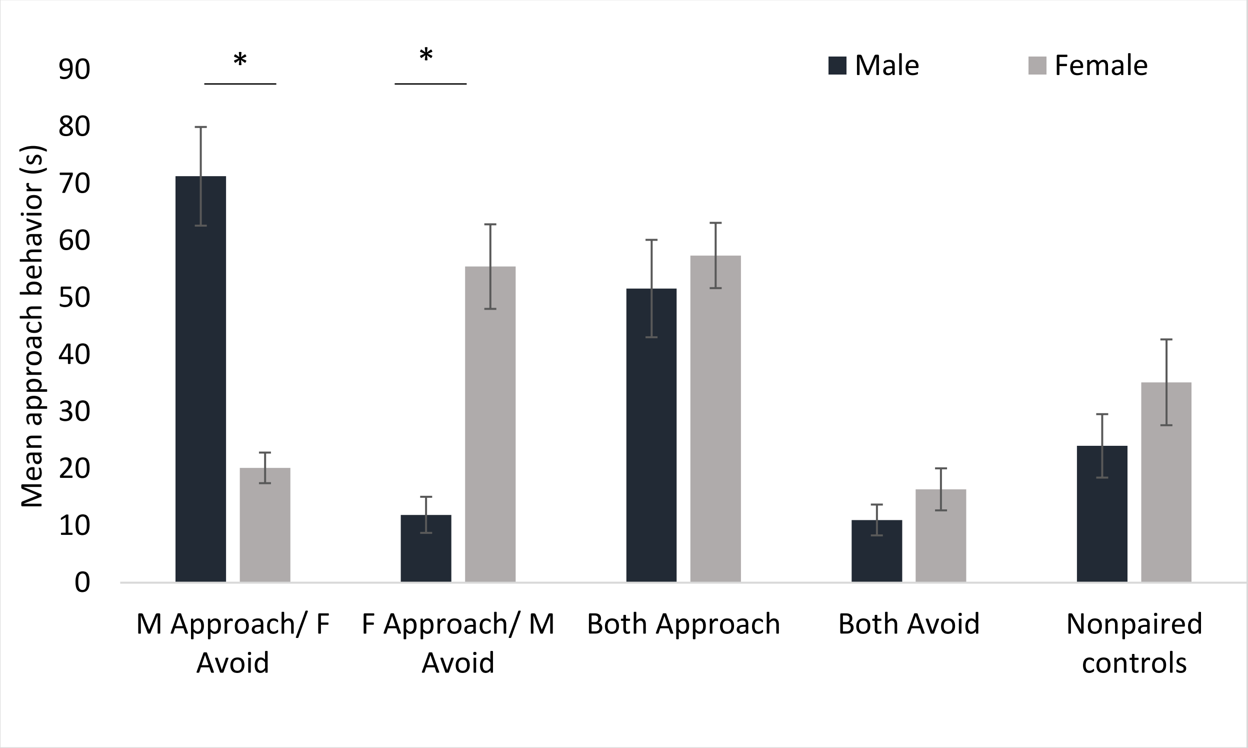


Figure 1. Mean approach behaviors of males (black) and females (grey) for each of the four paired groups and nonpaired controls. Significant differences were seen in approach behavior of groups 1 (male approach, female avoid) and group 2 (female approach, male avoid) but in no other groups (* = $p < 0.05$)

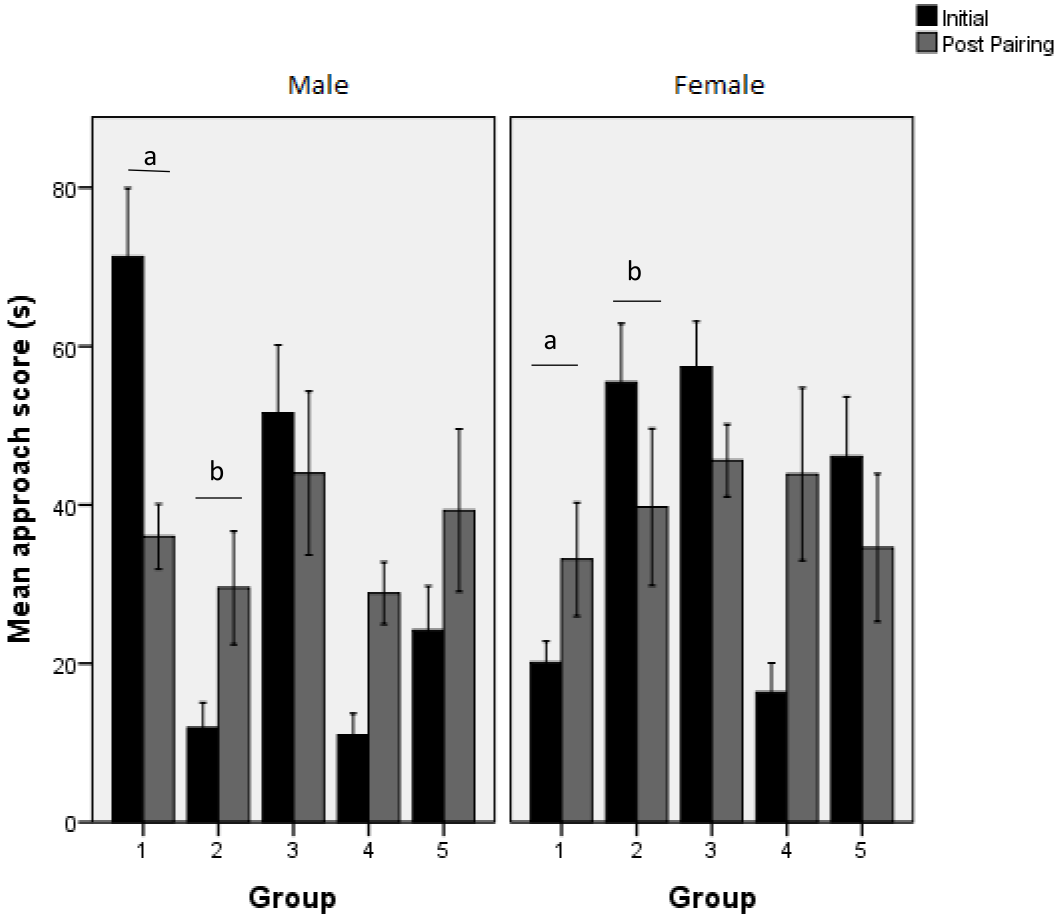


Figure 2. A three-way interaction between sex, group and approach score before and after pairing was found. In general, sex differences within groups occurred when pairs were initially different prior to pairing. Approacher males (left, group 1) that were paired with an avoider female decreased their time spent in the bark chamber from the initial test (black bars) to the post-pairing test (grey bars) while their female mate (right, group 1) increased their time spent in the bark chamber in the post-pairing test creating a significant sex difference (a = $p < 0.05$). Conversely, avoider males, paired with approacher females (group 2) were seen to increase their time spent in the bark chamber following pairing, while their female mate decreased their time in the bark chamber (b = $p <0.05$). Males and females of all other groups show no sex difference in their behavior from the initial test to post pairing (a, b = $p < 0.05$)

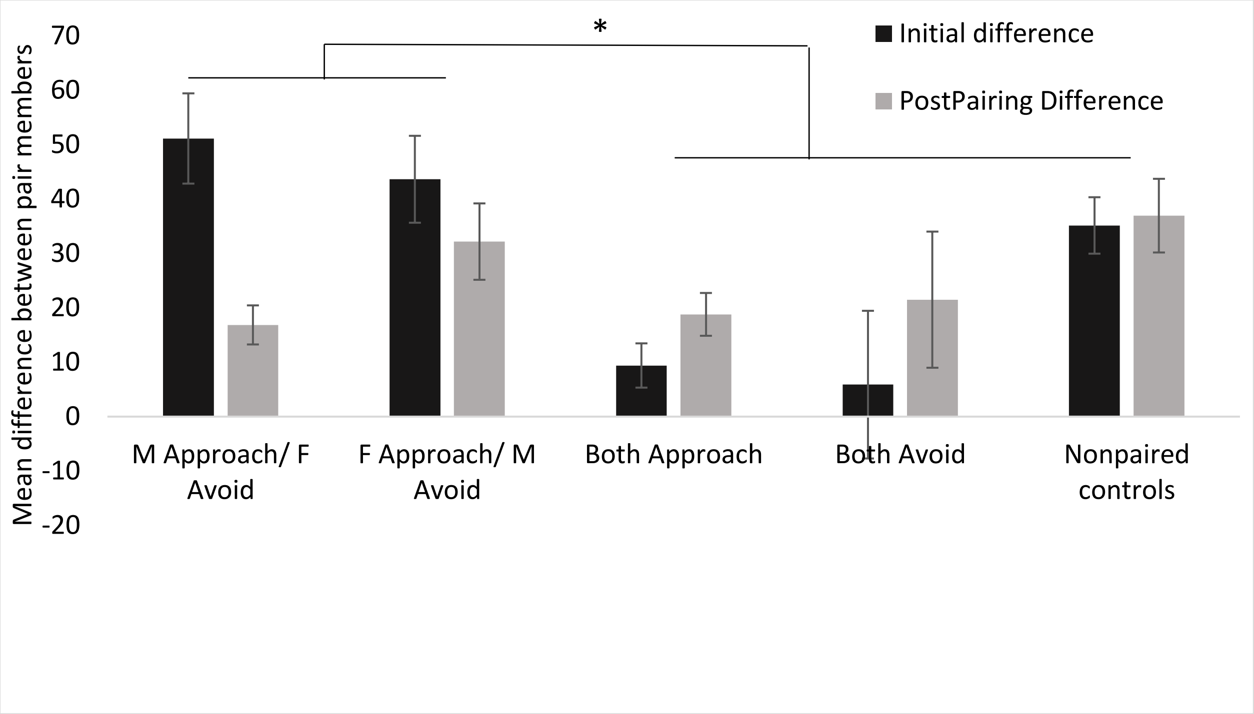


Figure 3. A two-way interaction was found such that pairs with one approacher and one avoider decreased their difference in approach score from the initial test (black bar) to the post-pairing test (grey bar). Overall, initially different pairs became more similar, and initially similar pairs and nonpaired individuals remained similar (* = $p < 0.05$).

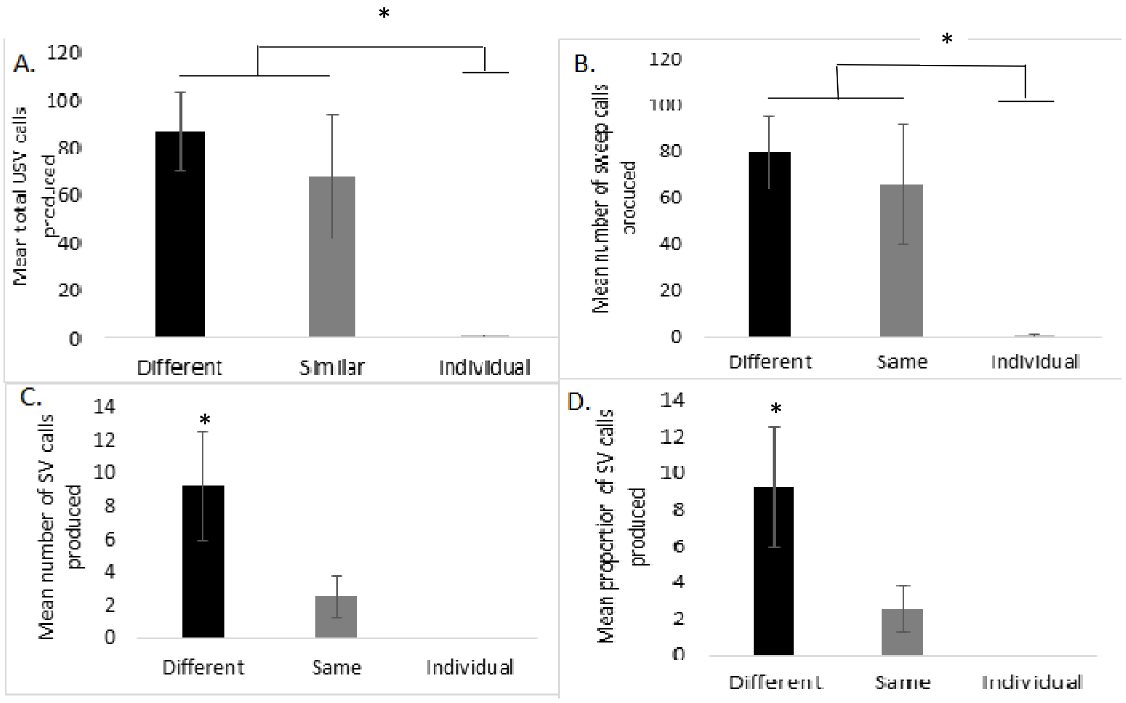


Figure 4. Ultrasonic vocalizations were produced more by pairs when compared to individuals. A. Total SV calling was increased in both pairs with initially different approach scores (black) and initially similar approach scores (grey) compared to individuals. B. The total number of sweeps produced was increased in both initially different and initially similar pairs compared to individuals. C. The total mean number of SV calls produced was increased in pairs that were initially different compared to all other groups. D. Similarly the proportion of SV calls as a function of total calls was also increased in pairs which were initially different compared to all other groups. For all panels * = $p < 0.05$

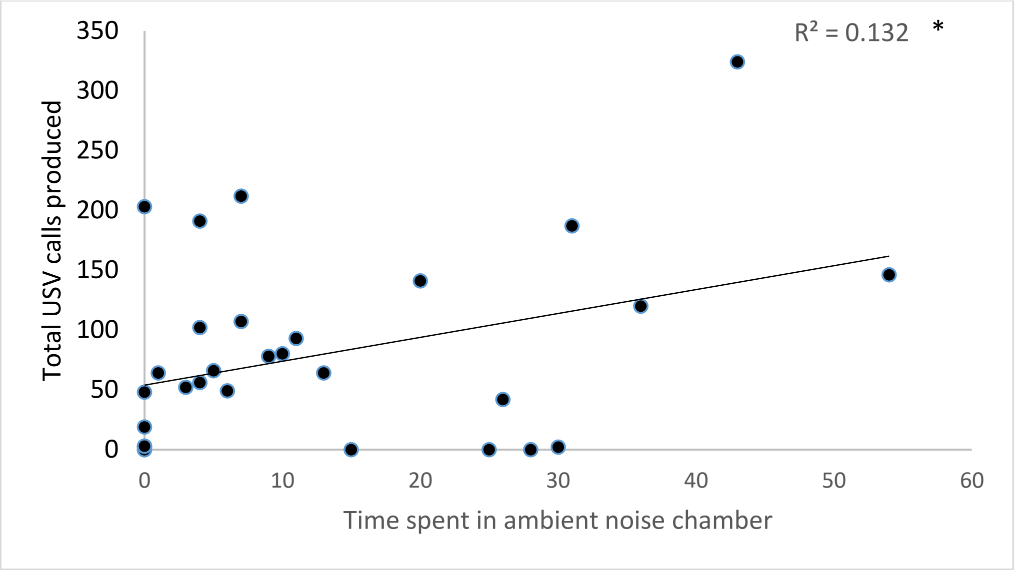


Figure 5. Pairs that spent more total time together in the ambient noise chamber in response to bark playbacks produced a greater total number of USV calls including both sweeps and SVs (* = $p < 0.05$).

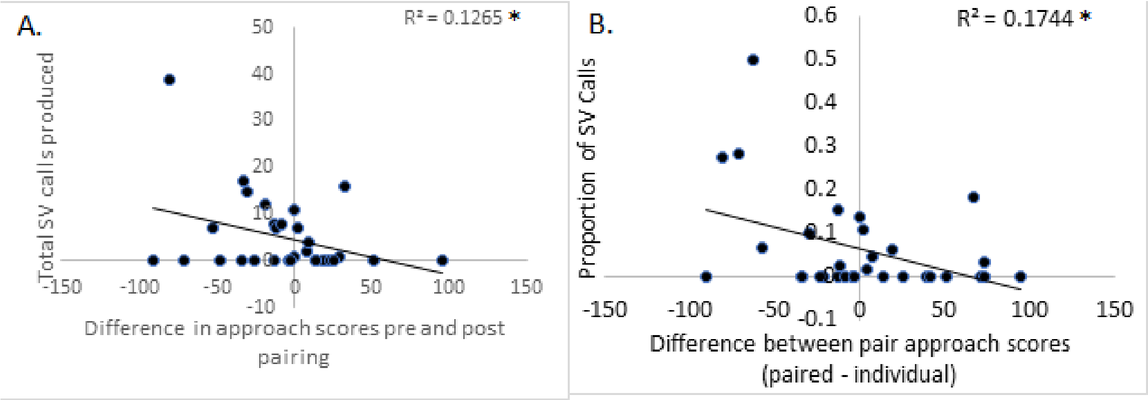


Figure 6. Pairs that showed greater similarity in approach scores after pairing showed greater production of SV calls as a dyad. Pairs that became more similar (post-pairing approach score difference – initial approach score difference) as denoted by negative numbers on the x axis, produced A) Significantly more total SV calls and B) A significantly greater proportion of SV calls to total USV calls produced (* = $p < 0.05$).

Dissertation Discussion

There were four main goals of this dissertation. First, we wanted to test whether California mice divided labor to defend territories and care for pups. While both members of a pair are capable of retrieving pups (Bester-Meredith, Conley, & Mammarella, 2016; Frazier, Trainor, Cravens, Whitney, & Marler, 2006) and defending territory (Davis & Marler, 2003; Fuxjager et al., 2010), when pairs are together they divide labor in a task dependent manner. During pup retrievals, division of labor is sex-specific with females acting as the retriever. However, during territorial defense, pairs use one of two strategies: 1) joint or 2) divided that are robust across contexts. Second, we tested whether oxytocin plays a role in division of labor in a pair-bonded species. We found that oxytocin played a sex specific role in division of labor, such that, when administered intranasally to females, division of labor was increased in pairs, but vocal communication remained unchanged, but when administered to males, however, SV duration was shortened, whereas division of labor remained unchanged. Third, we wanted to determine if labor divisions were due to pre-existing behavioral syndromes of individuals or if individuals altered their behavior in response to their mate. We found that, following pairing, California mice altered their approach behavior to an aversive stimulus in order to become more similar to their mate. Throughout all of these studies we wanted to elucidate the role of vocal communication by USVs in coordinating labor divisions. SVs in particular were important to signaling aggression, with shorter calls predicting greater aggression. Moreover, SV calling was increased in pairs that spent more time together across contexts. Taken together, the studies in this dissertation provide evidence, for the first time, of division of labor in a monogamous rodent that is task specific, altered by central oxytocin and potentially coordinated by ultrasonic vocalizations.

Division of labor in California mice is task specific

Division of labor is a strategy used across taxa to complete tasks and minimize resource expenditure (Gordon, 2016; Jeanne, 2016). While, division of labor has been extensively studied in invertebrates (Page, Scheiner, Erber, & Amdam, 2006; Robinson, 2003; Robson & Traniello, 2016) revealing effects of age (Reim & Scheiner, 2014), behavioral syndromes (Wright, Holbrook, & Pruitt, 2014), and monoamines (Dolezal, Johnson, Hölldobler, & Amdam, 2013; Giraldo, Patel, Gronenberg, & Traniello, 2013) on task specialization, to this point relatively little is known about division of labor or the underlying mechanisms that control these behaviors in vertebrates. Classically, studies on division of labor in vertebrate species have found that tasks are completed in one of three ways: 1) sex-specific, where each sex is responsible for completing a specific task such as males defending territories while females care for offspring (Brotherton, Pemberton, Komers, & Malarky, 1997; Clutton-Brock et al., 2001; Rogers, 2010), 2) mate guarding, where individuals will repel same-sex intruders to prevent extrapair copulations (Hall, 2000; Koloff & Mennill, 2011; Mathews, 2002; Reburn & Wynne-Edwards, 1999), or 3) cooperation, where pairs work together to complete tasks such as expelling intruders and foraging for food (Clutton-Brock et al., 2001; Dávid-Barrett & Dunbar, 2012; Scheel & Packer, 1991; Stevens, Cushman, & Hauser, 2005). Despite this research, we had yet to develop a monogamous rodent experimental system of division of labor and no little about how pair-bonded species allocate tasks across territorial defense and pup care.

In this dissertation we developed California mice as an experimental system of division of labor across tasks related to territoriality and parental care. To do this, we first showed that resident nonparental pair-bonded male and female California mice were capable of winning encounters against same sex intruders and displayed equal levels of aggression while alone. Across rodents, this lack of sex difference in aggression towards an intruder is relatively rare with females in most species being less aggressive than their male counterparts (Beatty, 1979; Floody, 1983; Heiming et al., 2013) outside of maternal aggression (Gammie & Nelson, 1999; Lonstein & Gammie, 2002; Olivier, Mos, & van Oorschot, 1985). Moreover, previous research has shown that both males and females are capable of and will complete all aspects of parental care, with the exception of lactation in males (Gubernick & Teferi, 2000; Gubernick & Nelson, 1989). Importantly, both males and females will retrieving pups while alone (Bester-Meredith et al., 2016; Frazier et al., 2006). However, to this point, studies of social behavior in California mice have focused on individuals in isolation and not how pairs complete tasks while together. Therefore, we developed paradigms in which we could test how pairs would compete pup retrievals, respond to pup calls and defend territories while together.

We first predicted that both male and female California mice would retrieve pups as previous research has shown that they are both capable of doing so (Bester-Meredith et al., 2016). Moreover, by working together to retrieve pups, efficiency of returning the pups would be increased and pup's exposure to possible threats would be minimized. Contrary to this prediction, however, we found that division of labor in pup retrievals was sex-specific. While males were often the first to approach pups, females were almost always the ones to return the pups to the nest. This could be because we tested pup retrieval within the first week of life. Previous studies have shown that males increase their care of pups, particularly retrievals, when pups grow fur, become more mobile and range farther from the nest (Cantoni & Brown, 1997; Frazier et al., 2006; Gubernick & Nelson, 1989; Trainor & Marler, 2001). As such, if we had tested pairs for retrieval when pups were older we may have seen a different pattern of labor division with fathers completing more retrievals. This pattern of division, however, was not maintained during territorial defense.

In the case of territorial defense, we predicted that, prior to the birth of pups, pairs would defend territories together, as both are equally capable of winning aggressive encounters. We also predicted that, after the birth of pups, pairs would divide labor such that one member of the pair would approach the intruder while the other remained close to the pups in line with previous research that has shown that the presence of offspring increases division within pairs (Rogers, 2010). However, we found that division of labor in California mice did not fit into any of the previously defined categories for division of labor in vertebrate species. Rather than all pairs consistently using one strategy as is seen in most species (Dávid-Barrett & Dunbar, 2012), California mice strategies were pair specific with pairs either using 1) Joint or 2) divided defense. In joint defense pairs approached the intruder together, while in divided defense one member of the pair, either male or female approached the intruder while the other remained close to the nest, indicating that these roles are not sex-specific. Moreover, these strategies were robust across contexts. First, pairs maintained their strategy regardless of the sex of the intruder, meaning that defensive strategies were not consistent with mate guarding. Finally, pair strategies were not altered by the birth of pups indicating that labor divisions were maintained even post-parturition.

As such, we found task specific division of labor in monogamous California mice. While pup retrievals were sex-specific, similar to strategies seen in other species (Lonstein & De Vries, 2000) territorial defense was pair specific and did not follow previously defined labor division strategies (Ahern, Hammock, & Young, 2011; Clutton-Brock et al., 2001; King & Sueur, 2011; Rogers, 2010). However, despite knowing that pairs divide labor in territorial defense the neuroendocrine underpinnings of these behaviors were not well understood, but these studies show that California mice provide a strong experimental system through which to test these mechanisms. To elucidate these mechanisms we tested the role of central oxytocin, a nonapeptide important to pair-bond formation and maintenance (Lieberwirth & Wang, 2016), on division of labor during territorial defense.

Vocal communication may help coordinate behavior

Vocal communication occurs across species and is often vital to social interactions (Bradbury & Vehrencamp, 1998). Moreover, in many species, vocalizations are important to the coordination of behavior by eliciting responses in conspecifics (Noe, 2006). Specifically, calls can be used to recruit group members in response to an intruder (Caselli, Mennill, Bicca-Marques, & Setz, 2014; Robinson, 1981), warn fellow group members of threats (Pollard et al., 2012; Seyfarth, Cheney, & Marler, 1980; Townsend et al., 2011), and coordinate feeding behavior and group movement (Boucaud, Aguirre Smith, Valère, & Vignal, 2016; Campbell & Boinski, 1995; D 'vincent, Nilson, & Hanna, 1995). As such, because vocalizations can be used to coordinate behavior in a variety of ways, it follows that vocal communication could be used to coordinate division of labor. However, to this point the role of vocalizations in labor division has yet to be studied particularly in monogamous rodents.

Rodents communicate vocally primarily through the use of USVs (Holy & Guo, 2005; Portfors, 2006). To this point, the majority of studies on rodent USVs have focused on their role in courtship and mating (Burgdorf et al., 2008). However, USVs have also been hypothesized to convey the emotional affect of individuals (Chabout et al., 2012; Constantini & D'amato, 2006) such as when individuals produce low frequency calls in response to aversive stimuli (Brudzynski & Holland, 2005; Kroes, Burgdorf, Otto, Panksepp, & Moskal, 2007). California mice produce a rich set of USVs (Briggs & Kalcounis-Rueppell, 2011; Kalcounis-Rueppell, Metheny, & Vonhof, 2006) that are used across social contexts (Kalcounis-Rueppell, Pultorak, & Marler, 2018; Pultorak, Fuxjager, Kalcounis-Rueppell, & Marler, 2015; Pultorak, Matusinec, Miller, & Marler, 2017). However, prior to this dissertation we had not examined their role in coordinating behavior between pairs. We predicted that sustained vocalizations, SVs, would play a particularly important role in coordinating behavior as these calls have been found to be produced during social behavior with individuals being able to modulate their duration based on context (Kalcounis-Rueppell et al., 2018).

We found that during aggression individuals displayed shorter SV calls and during affiliative interactions pairs displayed longer duration SV calls. This correlation between duration and aggression/affiliation was found across studies in this dissertation, both when individuals were completing tasks alone and when completing tasks with their mate. We further found that SV calls were increased in pairs compared to individuals and, specifically, were increased in pairs while they were together during tasks compared to when they were separate. Thus, we found that SV's spectral properties are altered based on context and that call production was altered based on whether pairs were together or separate. As such, this provides evidence that vocal communication plays a role in not only social behavior but also in the coordination of behavior between mates.

The role of oxytocin in division of labor and vocal communication by pair-bonded California mice

Oxytocin is a highly conserved neuropeptide that plays a role in a number of important social behaviors (Carter, 2014; Feldman & Bakermans-Kranenburg, 2017). Most notably, oxytocin is important to the formation and maintenance of pair-bonds in monogamous species, particularly females (Insel, 2010; Lieberwirth & Wang, 2016), while also playing sex-specific roles in aggressive and affiliative behaviors (Kelly & Goodson, 2014; Winslow et al., 2000) as well as communication (Calcagnoli, Kreutzmann, de Boer, Althaus, & Koolhaas, 2015; Marlin, Mitre, D'amour, Chao, & Froemke, 2015) across species including rodents (Bales et al., 2013; Duque-Wilckens et al., 2018), primates (Cavanaugh, Mustoe, & French, 2018; Taylor, Intorre, & French, 2017), and humans (Calcagnoli et al., 2015; Veening & Olivier, 2013). Importantly, while administration of central oxytocin to individuals has been hypothesized to be prosocial (Domes et al., 2013; Veening & Olivier, 2013), recent work has shown that oxytocin has context-specific effects (Beery, 2015; Shamay-Tsoory & Abu-Akel, 2016) that may increase or decrease sociality and affiliation based on the salience of the situation. Specifically, oxytocin is hypothesized to increase aggression towards novel individuals while increasing affiliation towards group members (Beery, 2015; Harari-Dahan & Bernstein, 2014; Shamay-Tsoory & Abu-Akel, 2016). As such we were interested in testing the role of oxytocin in division of labor to elucidate whether it would lead pairs to increase aggression towards an intruder or increase time spent together by the pair.

To test the role of oxytocin in division of labor by California mice, we administered oxytocin to either one or both members of a pair-bond intranasally. Intranasal oxytocin has been shown to have fairly rapid behavioral effects in both nonhuman animals (Bales et al., 2013; Duque-Wilckens et al., 2018) and humans (Quintana, Alvares, Hickie, & Guastella, 2015; Veening & Olivier, 2013). Moreover, intranasal oxytocin has been shown to reach cortical and subcortical regions of the brain (Galbusera et al., 2017) as well as the CSF (Lee et al., 2018). However, despite being currently used to treat humans with social deficits (Neumann, Maloumby, Beiderbeck, Lukas, & Landgraf, 2013), little is known about how context and intranasal oxytocin interact to affect behavioral outcomes. We predicted, in line with previous work in the lab (Guoynes & Marler, unpublished), that males administered oxytocin would increase aggression towards an intruder and females would increase their time near the nest. Moreover, we predicted that this would lead to an increase in division of labor by pairs. However, while we did find that oxytocin had sex-specific effects on behavior, they were not in the pattern we expected. We found that oxytocin administered to females increased division of labor in pairs and led to females being more likely to act as the sole defender within a pair and approaching intruders first. However, when administered to males, division of labor was not affected. We also found sex-specific effects of oxytocin on vocal communication such that, when oxytocin was administered to males but not females, SV durations were shortened.

Despite these changes, oxytocin did not affect the overall aggression displayed by pairs towards an intruder, or the total time spent investigating the intruder by pairs. As such, oxytocin's effects on the behavior of pairs seem to be specific to the strategies employed by pairs and communication during division of labor. This result expands our understanding of oxytocin's role in pair-bonds beyond its vital function in formation and maintenance of bonds. Based on this study, we have evidence that oxytocin is also important to how pairs choose to divide labor to complete tasks related to territoriality. However, we do not yet know where in the brain this oxytocin is acting. Therefore, future studies need to focus on the brain regions and circuits being activated by oxytocin. This will provide us with a greater understanding of the mechanistic role of oxytocin in social behavior, decision making and vocal communication in monogamous species.

Behavioral responses are altered by pair-bonding

Animals have been shown to have behavioral syndromes, consistent responses to stimuli across contexts (Sih, Bell, & Johnson, 2004). However, in most cases these behavioral syndromes are tested in isolation, and the addition of a social group can alter individuals behavioral responses (Webster & Ward, 2011). The most common alterations to these behavioral responses are 1) social conformity, where individuals become more like their social group, and 2) facilitation, where pairs behavioral responses are exaggerated in comparison to their social group (King, Williams, & Mettke-Hofmann, 2015). However, how pair-bonding effects behavioral responses has not been tested in monogamous mammals. The effect of pair-bonds on behavioral responses of individuals are of particular interest because an individual can bond with another who is either like or unlike them and navigating these behavioral similarities and differences is vital to the success of the pair.

We tested this effect by exposing individuals to aversive vocal playbacks before and after pair-bonding. We predicted that pairs that were initially different in their approach response to aversive playbacks would show facilitation, which would increase their division of labor consistent with our previous results. However, we found that individuals' approach behavior became more similar to their mates after pair-bonding, consistent with social conformity. This result is in line with research that has shown that breeding pairs that are more similar will have greater reproductive success than those that are different (Gabriel & Black, 2012). As such it is possible that pairs become more similar in their behavior in order to increase their likelihood of being successful, however, whether or not individuals prefer mates who are similar remains unknown. Moreover, we predicted that pairs would produce more calls than individuals as California mice produce more calls with a social partner than they do in isolation (Briggs & Kalcounis-Rueppell, 2011; Pultorak et al., 2017). We found that this difference was true with pairs producing both more sweeps and more SVs than unpaired controls. Moreover, we found that SV calling was greatest in pairs that initially had differing responses, but became more similar after pairing. Along with this result, pairs produced more calls while they were in the same chamber than while they were separated indicating that calling could be leading pairs to remain closer together. We therefore provide the first evidence that a monogamous rodent uses vocalizations to coordinate behavior in response to an aversive stimulus.

We showed that monogamous individuals behavioral responses are not fixed across the lifespan. Instead, the alteration of an individual's social environment by the formation of a pair bond leads to behavioral plasticity. Specifically, mates alter their approach behavior to become more like their partners. However, the mechanisms that underlie this behavioral plasticity remain unknown. It is possible, and perhaps likely, that the nonapeptides oxytocin and vasopressin play a major role in these behavioral changes as they are vital to the formation and maintenance of pair bonds (Insel & Shapiro, 1992; Insel, 2010; Insel, Preston, & Winslow, 1995) and that oxytocin plays a sex-specific role in division of labor, as we have shown in this dissertation. Overall, we found that the formation of monogamous pair bonds reveals emergent properties in pairs.

Conclusion

The studies in this dissertation show not only that the California mouse, a monogamous and biparental species, divides labor during both territorial defense and pup retrievals, but also that oxytocin plays a critical role in division of labor. Moreover, individuals alter their behavior after pair-bonding to become more similar to their mates. Throughout our studies we were also able to show that vocalizations played a role in coordinating pair behavior across contexts. These studies provide the framework to expand our knowledge of division of labor and behavioral plasticity, particularly in a monogamous species. California mice provide a new and interesting experimental system to study and understand the mechanisms and circuitry underlying these behaviors and future studies should focus on identifying the brain regions that control these labor divisions. This dissertation, overall, provides the building blocks necessary to understand division of labor and to more fully understand social behavior and vocal communication in monogamous mammals.

References

Ahern, T. H., Hammock, E. A. D., & Young, L. J. (2011). Parental division of labor, coordination, and the effects of family structure on parenting in monogamous prairie voles (Microtus ochrogaster). *Developmental Psychobiology*, *53*(2), 118–31. http://doi.org/10.1002/dev.20498

Bales, K. L., Perkeybile, A. M., Conley, O. G., Lee, M. H., Guoynes, C. D., Downing, G. M., … Mendoza, S. P. (2013). Chronic intranasal oxytocin causes long-term impairments in partner preference formation in male prairie voles. *Biological Psychiatry*, *74*(3), 180–8. http://doi.org/10.1016/j.biopsych.2012.08.025

Beatty, W. W. (1979). Gonadal hormones and sex differences in nonreproductive behaviors in rodents: Organizational and activational influences. *Hormones and Behavior*, *12*(2), 112–163. http://doi.org/10.1016/0018-506X(79)90017-5

Beery, A. K. (2015). Antisocial oxytocin: complex effects on social behavior. *Current Opinion in Behavioral Sciences*, *6*, 174–182. http://doi.org/10.1016/J.COBEHA.2015.11.006

Bester-Meredith, J., Conley, M., & Mammarella, G. (2016). Peromyscus as a Model System for Understanding the Regulation of Maternal Behavior. *Seminars in Cell and Developmental Biology*.

http://doi.org/10.1016/j.semcdb.2016.07.001

Boucaud, I. C. A., Aguirre Smith, M. L. N., Valère, P. A., & Vignal, C. (2016). Incubating females signal their needs during intrapair vocal communication at the nest: a feeding experiment in great tits. *Animal Behaviour*, *122*, 77–86. http://doi.org/10.1016/j.anbehav.2016.09.021

Bradbury, J. W., & Vehrencamp, S. L. (1998). *Principles of Animal Communication*. Sunderland, Ma: Sinauer Associates, Inc. Retrieved from http://efish.fiu.edu/lab_business/training/Reading2.pdf

Briggs, J. R., & Kalcounis-Rueppell, M. C. (2011). Similar acoustic structure and behavioural context of vocalizations produced by male and female California mice in the wild. *Animal Behaviour*, *82*(6), 1263–1273. http://doi.org/10.1016/j.anbehav.2011.09.003

Brotherton, P. N., Pemberton, J. M., Komers, P. E., & Malarky, G. (1997). Genetic and behavioural evidence of monogamy in a mammal, Kirk's dik-dik (Madoqua kirkii). *Proceedings. Biological Sciences*, *264*(1382), 675–81. http://doi.org/10.1098/rspb.1997.0096

Brudzynski, S. M., & Holland, G. (2005). Acoustic characteristics of air puff-induced 22-kHz alarm calls in direct recordings. *Neuroscience & Biobehavioral Reviews*, *29*(8), 1169–1180. http://doi.org/10.1016/j.neubiorev.2005.04.007

Burgdorf, J., Kroes, R. A., Moskal, J. R., Pfaus, J. G., Brudzynski, S. M., & Panksepp, J. (2008). Ultrasonic vocalizations of rats (Rattus norvegicus) during mating, play, and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback.. *Journal of Comparative Psychology*, *122*(4), 357–367. http://doi.org/10.1037/a0012889

Calcagnoli, F., Kreutzmann, J. C., de Boer, S. F., Althaus, M., & Koolhaas, J. M. (2015). Acute and repeated intranasal oxytocin administration exerts anti-aggressive and pro-affiliative effects in male rats. *Psychoneuroendocrinology*, *51*. http://doi.org/10.1016/j.psyneuen.2014.09.019

Campbell, A. F., & Boinski, S. (1995). Use of Trill Vocalizations To Coordinate Troop Movement Among White-Faced Capuchins: a Second Field Test. *Behaviour*, *132*(11), 875–901. http://doi.org/10.1163/156853995X00054

CANTONI, D., & BROWN, R. E. (1997). Paternal investment and reproductive success in the California mouse,Peromyscus californicus. *Animal Behaviour*, *54*(2), 377–386. http://doi.org/10.1006/ANBE.1996.0583

Carter, C. S. (2014). Oxytocin Pathways and the Evolution of Human Behavior. *Annual Review of Psychology*, *65*(1). http://doi.org/10.1146/annurev-psych-010213-115110

Caselli, C. B., Mennill, D. J., Bicca-Marques, J. C., & Setz, E. Z. F. (2014). Vocal behavior of black-fronted titi monkeys (*Callicebus nigrifrons*): Acoustic properties and behavioral contexts of loud calls. *American Journal of Primatology*, *76*(8), 788–800. http://doi.org/10.1002/ajp.22270

Cavanaugh, J., Mustoe, A., & French, J. A. (2018). Oxytocin regulates reunion affiliation with a pairmate following social separation in marmosets. *American Journal of Primatology*, e22750. http://doi.org/10.1002/ajp.22750

Chabout, J., Serreau, P., Ey, E., Bellier, L., Aubin, T., Bourgeron, T., & Granon, S. (2012). Adult male mice emit context-specific ultrasonic vocalizations that are modulated by prior isolation or group rearing environment. *PloS One*, *7*(1), e29401. http://doi.org/10.1371/journal.pone.0029401

Clutton-Brock, T. H., Brotherton, P. N., Russell, A. F., O'Riain, M. J., Gaynor, D., Kansky, R., …

Monfort, S. (2001). Cooperation, control, and concession in meerkat groups. *Science (New York, N.Y.)*, *291*(5503), 478–81. http://doi.org/10.1126/science.291.5503.478

Constantini, F., & D'amato, F. R. (2006). Ultrasonic vocalizations in mice and rats : social contexts and functions. *Acta Zoologica Sinica*, *52*(4), 619–633. Retrieved from http://europepmc.org/abstract/cba/623593

D 'vincent, C. G., Nilson, R. M., & Hanna, R. E. (n.d.). VOCALIZATION AND COORDINATED FEEDING BEHAVIOR OF THE HUMPBACK WHALE IN SOUTHEASTERN ALASKA. Retrieved from http://www.icrwhale.org/pdf/SC03641-47.pdf

Dávid-Barrett, T., & Dunbar, R. I. M. (2012). Cooperation, behavioural synchrony and status in social networks. *Journal of Theoretical Biology*, *308*, 88–95. http://doi.org/10.1016/J.JTBI.2012.05.007

Davis, E. S., & Marler, C. A. (2003). The progesterone challenge: steroid hormone changes following a simulated territorial intrusion in female Peromyscus californicus. *Hormones and Behavior*, *44*(3), 185–198. http://doi.org/10.1016/S0018-506X(03)00128-4

Dolezal, A. G., Johnson, J., Hölldobler, B., & Amdam, G. V. (2013). Division of labor is associated with age-independent changes in ovarian activity in Pogonomyrmex californicus harvester ants. *Journal of Insect Physiology*, *59*(4), 519–24. http://doi.org/10.1016/j.jinsphys.2013.02.008

Domes, G., Heinrichs, M., Kumbier, E., Grossmann, A., Hauenstein, K., & Herpertz, S. C. (2013). Effects of Intranasal Oxytocin on the Neural Basis of Face Processing in Autism Spectrum Disorder. *Biological Psychiatry*, *74*(3), 164–171. http://doi.org/10.1016/j.biopsych.2013.02.007

Duque-Wilckens, N., Steinman, M. Q., Busnelli, M., Chini, B., Yokoyama, S., Pham, M., … Trainor, B. C. (2018). Oxytocin Receptors in the Anteromedial Bed Nucleus of the Stria Terminalis Promote Stress-Induced Social Avoidance in Female California Mice. *Biological Psychiatry*, *83*(3), 203–213. http://doi.org/10.1016/J.BIOPSYCH.2017.08.024

Feldman, R., & Bakermans-Kranenburg, M. J. (2017). Oxytocin: a parenting hormone. *Current Opinion in Psychology*, *15*, 13–18. http://doi.org/10.1016/J.COPSYC.2017.02.011

Floody, O. R. (1983). Hormones and Aggression in Female Mammals. In *Hormones and Aggressive Behavior* (pp. 39–89). Boston, MA: Springer US. http://doi.org/10.1007/978-1-4613-3521-4_3

Frazier, C. R. M., Trainor, B. C., Cravens, C. J., Whitney, T. K., & Marler, C. A. (2006). Paternal behavior influences development of aggression and vasopressin expression in male California mouse offspring. *Hormones and Behavior*, *50*(5), 699–707. http://doi.org/10.1016/j.yhbeh.2006.06.035

Fuxjager, M. J., Forbes-Lorman, R. M., Coss, D. J., Auger, C. J., Auger, A. P., & Marler, C. A. (2010). Winning territorial disputes selectively enhances androgen sensitivity in neural pathways related to motivation and social aggression. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(27), 12393–8. http://doi.org/10.1073/pnas.1001394107

Gabriel, P. O., & Black, J. M. (2012). Behavioural Syndromes, Partner Compatibility and Reproductive Performance in Steller's Jays. *Ethology*, *118*(1), 76–86. http://doi.org/10.1111/j.1439-0310.2011.01990.x

Galbusera, A., De Felice, A., Girardi, S., Bassetto, G., Maschietto, M., Nishimori, K., … Gozzi, A. (2017). Intranasal Oxytocin and Vasopressin Modulate Divergent Brainwide Functional Substrates.

Neuropsychopharmacology, *42*(7), 1420–1434. http://doi.org/10.1038/npp.2016.283

Gammie, S. C., & Nelson, R. J. (1999). Maternal aggression is reduced in neuronal nitric oxide synthase-deficient mice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *19*(18), 8027–35. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10479702

Giraldo, Y. M., Patel, E., Gronenberg, W., & Traniello, J. F. A. (2013). Division of labor and structural plasticity in an extrinsic serotonergic mushroom body neuron in the ant Pheidole dentata. *Neuroscience Letters*, *534*, 107–11. http://doi.org/10.1016/j.neulet.2012.11.057

Gordon, D. M. (2016). From division of labor to the collective behavior of social insects. *Behavioral Ecology and Sociobiology*, *70*(7), 1101–1108. http://doi.org/10.1007/s00265-015-2045-3

Gubernick, D. J., & Nelson, R. J. (1989). Prolactin and paternal behavior in the biparental California mouse, Peromyscus californicus. *Hormones and Behavior*, *23*(2), 203–210. http://doi.org/10.1016/0018-506X(89)90061-5

Gubernick, D. J., & Teferi, T. (2000). Adaptive significance of male parental care in a monogamous mammal. *Proceedings. Biological Sciences*, *267*(1439), 147–50. http://doi.org/10.1098/rspb.2000.0979

Hall, M. L. (2000). The function of duetting in magpie-larks: conflict, cooperation, or commitment? *Animal Behaviour*, *60*(5), 667–677. http://doi.org/10.1006/anbe.2000.1517

Harari-Dahan, O., & Bernstein, A. (2014). A general approach - avoidance hypothesis of Oxytocin: Accounting for social and non-social effects of oxytocin. *Neuroscience and Biobehavioral Reviews*. http://doi.org/10.1016/j.neubiorev.2014.10.007

Heiming, R. S., Mönning, A., Jansen, F., Kloke, V., Lesch, K.-P., & Sachser, N. (2013). To attack, or not to attack? The role of serotonin transporter genotype in the display of maternal aggression. *Behavioural Brain Research*, *242*, 135–41. http://doi.org/10.1016/j.bbr.2012.12.045

Holy, T. E., & Guo, Z. (2005). Ultrasonic Songs of Male Mice. *PLoS Biology*, *3*(12), e386. http://doi.org/10.1371/journal.pbio.0030386

Insel, T. R. (2010). The Challenge of Translation in Social Neuroscience: A Review of Oxytocin, Vasopressin, and Affiliative Behavior. *Neuron*, *65*(6), 768–779. http://doi.org/10.1016/J.NEURON.2010.03.005

Insel, T. R., Preston, S., & Winslow, J. T. (1995). Mating in the monogamous male: Behavioral consequences. *Physiology & Behavior*, *57*(4), 615–627. http://doi.org/10.1016/0031-9384(94)00362-9

Insel, T. R., & Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(13), 5981–5985. http://doi.org/10.1073/pnas.89.13.5981

Jeanne, R. L. (2016). Division of labor is not a process or a misleading concept. *Behavioral Ecology and Sociobiology*, *70*(7), 1109–1112. http://doi.org/10.1007/s00265-016-2146-7

Kalcounis-Rueppell, M. C., Metheny, J. D., & Vonhof, M. J. (2006). Production of ultrasonic vocalizations by Peromyscus mice in the wild. *Frontiers in Zoology*, *3*(1), 3. http://doi.org/10.1186/1742-9994-3-3

Kalcounis-Rueppell, M. C., Pultorak, J. D., & Marler, C. A. (2018). Ultrasonic Vocalizations of Mice in the Genus Peromyscus. *Handbook of Behavioral Neuroscience*, *25*, 227–235. http://doi.org/10.1016/B978-0-12-809600-0.00022-6

Kelly, A. M., & Goodson, J. L. (2014). Hypothalamic oxytocin and vasopressin neurons exert sex-specific effects on pair bonding, gregariousness, and aggression in finches. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(16). http://doi.org/10.1073/pnas.1322554111

King, A. J., & Sueur, C. (2011). Where Next? Group Coordination and Collective Decision Making by Primates. *International Journal of Primatology*, *32*(6), 1245–1267. http://doi.org/10.1007/s10764-011-9526-7

King, A. J., Williams, L. J., & Mettke-Hofmann, C. (2015). The effects of social conformity on Gouldian finch personality. *Animal Behaviour*, *99*, 25–31. http://doi.org/10.1016/j.anbehav.2014.10.016

Koloff, J., & Mennill, D. (2011). Aggressive responses to playback of solos and duets in a Neotropical antbird. *Animal Behaviour*, *82*(3), 587–593. http://doi.org/10.1016/j.anbehav.2011.06.021

Kroes, R. A., Burgdorf, J., Otto, N. J., Panksepp, J., & Moskal, J. R. (2007). Social defeat, a paradigm of depression in rats that elicits 22-kHz vocalizations, preferentially activates the cholinergic signaling pathway in the periaqueductal gray. *Behavioural Brain Research*, *182*(2), 290–300. http://doi.org/10.1016/j.bbr.2007.03.022

Lee, M. R., Scheidweiler, K. B., Diao, X. X., Akhlaghi, F., Cummins, A., Huestis, M. A., … Averbeck, B. B. (2018). Oxytocin by intranasal and intravenous routes reaches the cerebrospinal fluid in rhesus macaques: determination using a novel oxytocin assay. *Molecular Psychiatry*, *23*(1), 115–122. http://doi.org/10.1038/mp.2017.27

Lieberwirth, C., & Wang, Z. (2016). The neurobiology of pair bond formation, bond disruption, and social buffering. *Current Opinion in Neurobiology*. http://doi.org/10.1016/j.conb.2016.05.006

Lonstein, J. S., & De Vries, G. J. (2000). Sex differences in the parental behavior of rodents. *Neuroscience & Biobehavioral Reviews*, *24*(6), 669–686. http://doi.org/10.1016/S0149-7634(00)00036-1

Lonstein, J. S., & Gammie, S. C. (2002). Sensory, hormonal, and neural control of maternal aggression in laboratory rodents. *Neuroscience & Biobehavioral Reviews*, *26*(8), 869–888. http://doi.org/10.1016/S0149-7634(02)00087-8

Marlin, B. J., Mitre, M., D'amour, J. A., Chao, M. V., & Froemke, R. C. (2015). Oxytocin enables maternal behaviour by balancing cortical inhibition. *Nature*, *520*(7548), 499–504. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4409554&tool=pmcentrez&rendertype=abstract

Mathews, L. M. (2002). Territorial cooperation and social monogamy: factors affecting intersexual behaviours in pair-living snapping shrimp. *Animal Behaviour*, *63*(4), 767–777. http://doi.org/10.1006/anbe.2001.1976

Neumann, I. D., Maloumby, R., Beiderbeck, D. I., Lukas, M., & Landgraf, R. (2013). Increased brain and plasma oxytocin after nasal and peripheral administration in rats and mice. *Psychoneuroendocrinology*, *38*(10), 1985–1993. http://doi.org/10.1016/J.PSYNEUEN.2013.03.003

Noe, R. (2006). Cooperation Experiments: coordination through communication versus acting apart together. *Animal Behaviour*, *71*, 1–18. http://doi.org/10.1016/j.anbehav.2005.03.037

Olivier, B., Mos, J., & van Oorschot, R. (1985). Maternal aggression in rats: Effects of chlordiazepoxide and fluprazine. *Psychopharmacology*, *86*(1–2), 68–76. http://doi.org/10.1007/BF00431686

Page, R. E., Scheiner, R., Erber, J., & Amdam, G. V. (2006). The Development and Evolution of Division of Labor and Foraging Specialization in a Social Insect (Apis mellifera L.). *Current Topics in Developmental Biology*, *74*, 253–286. http://doi.org/10.1016/S0070-2153(06)74008-X

Pollard, K. A., Blumstein, D. T., Edmonds, B., Shannon, C. E., Weaver, W., Kolmogorov, A., … Voelkl, B. (2012). Evolving communicative complexity: insights from rodents and beyond. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *367*(1597), 1869–78. http://doi.org/10.1098/rstb.2011.0221

Portfors, C. V. (n.d.). Types and Functions of Ultrasonic Vocalizations in Laboratory Rats and Mice. Retrieved from http://www.ingentaconnect.com/content/aalas/jaalas/2007/00000046/00000001/art00005

Pultorak, J. D., Fuxjager, M. J., Kalcounis-Rueppell, M. C., & Marler, C. A. (2015). Male fidelity expressed through rapid testosterone suppression of ultrasonic vocalizations to novel females in the monogamous California mouse. *Hormones and Behavior*, *70*, 47–56. http://doi.org/10.1016/j.yhbeh.2015.02.003

Pultorak, J. D., Matusinec, K. R., Miller, Z. K., & Marler, C. A. (2017). Ultrasonic vocalization production and playback predicts intrapair and extrapair social behaviour in a monogamous mouse. *Animal Behaviour*, *125*, 13–23. http://doi.org/10.1016/j.anbehav.2016.12.023

Quintana, D. S., Alvares, G. A., Hickie, I. B., & Guastella, A. J. (2015). Do delivery routes of intranasally administered oxytocin account for observed effects on social cognition and behavior? A two-level model. *Neuroscience & Biobehavioral Reviews*, *49*, 182–192. http://doi.org/10.1016/J.NEUBIOREV.2014.12.011

Reburn, C. J., & Wynne-Edwards, K. E. (1999). Hormonal Changes in Males of a Naturally Biparental and a Uniparental Mammal. *Hormones and Behavior*, *35*(2), 163–176. http://doi.org/10.1006/hbeh.1998.1509

Reim, T., & Scheiner, R. (2014). Division of labour in honey bees: age- and task-related changes in the expression of octopamine receptor genes. *Insect Molecular Biology*, *23*(6), 833–841. http://doi.org/10.1111/imb.12130

Robinson, G. E. (2003). Regulation of Division of Labor in Insect Societies. *Http://Dx.Doi.Org/10.1146/Annurev.En.37.010192.003225*.

Robinson, J. G. (1981). Vocal regulation of inter- and intragroup spacing during boundary encounters in the titi monkey,Callicebus moloch. *Primates*, *22*(2), 161–172. http://doi.org/10.1007/BF02382607

Robson, S. K. A., & Traniello, J. F. A. (2016). Division of labor in complex societies: a new age of conceptual expansion and integrative analysis. *Behavioral Ecology and Sociobiology*, *70*(7), 995–998. http://doi.org/10.1007/s00265-016-2147-6

Rogers, W. (2010). Parental Investment and Division of Labor in the Midas Cichlid (Cichlasoma citrinellum). *Ethology*, *79*(2), 126–142. http://doi.org/10.1111/j.1439-0310.1988.tb00706.x

Scheel, D., & Packer, C. (1991). Group hunting behaviour of lions: a search for cooperation. *Animal Behaviour*, *41*(4), 697–709. http://doi.org/10.1016/S0003-3472(05)80907-8

Seyfarth, R. M., Cheney, D. L., & Marler, P. (1980). Vervet monkey alarm calls: Semantic communication in a free-ranging primate. *Animal Behaviour*, *28*(4), 1070–1094. http://doi.org/10.1016/S0003-3472(80)80097-2

Shamay-Tsoory, S. G., & Abu-Akel, A. (2016). The Social Salience Hypothesis of Oxytocin. *Biological Psychiatry*. http://doi.org/10.1016/j.biopsych.2015.07.020

Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution*, *19*(7), 372–378. http://doi.org/10.1016/J.TREE.2004.04.009

Stevens, J. R., Cushman, F. A., & Hauser, M. D. (2005). Evolving the Psychological Mechanisms for Cooperation. *Http://Dx.Doi.Org/10.1146/Annurev.Ecolsys.36.113004.083814*.

Taylor, J. H., Intorre, A. A., & French, J. A. (2017). Vasopressin and Oxytocin Reduce Food Sharing Behavior in Male, but Not Female Marmosets in Family Groups. *Frontiers in Endocrinology*, *8*, 181. http://doi.org/10.3389/fendo.2017.00181

Townsend, S. W., Manser, M. B., Fitch, W. T., Neubauer, J., Herzel, H., Herzel, H., … Fitch, W. T. (2011). The function of nonlinear phenomena in meerkat alarm calls. *Biology Letters*, *7*(1), 47–9. http://doi.org/10.1098/rsbl.2010.0537

Trainor, B. C., & Marler, C. A. (2001). Testosterone, Paternal Behavior, and Aggression in the Monogamous California Mouse (Peromyscus californicus). *Hormones and Behavior*, *40*(1), 32–42. http://doi.org/10.1006/hbeh.2001.1652

Veening, J. G., & Olivier, B. (2013). Intranasal administration of oxytocin: Behavioral and clinical effects, a review. *Neuroscience & Biobehavioral Reviews*, *37*(8), 1445–1465. http://doi.org/10.1016/J.NEUBIOREV.2013.04.012

Webster, M. M., & Ward, A. J. W. (2011). Personality and social context. *Biological Reviews*, *86*(4), 759–773. http://doi.org/10.1111/j.1469-185X.2010.00169.x

Winslow, J. T., Hearn, E. F., Ferguson, J., Young, L. J., Matzuk, M. M., & Insel, T. R. (2000). Infant Vocalization, Adult Aggression, and Fear Behavior of an Oxytocin Null Mutant Mouse. *Hormones and Behavior*, *37*(2), 145–155. http://doi.org/10.1006/hbeh.1999.1566

Wright, C. M., Holbrook, C. T., & Pruitt, J. N. (2014). Animal personality aligns task specialization and task proficiency in a spider society. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(26), 9533–7. http://doi.org/10.1073/pnas.1400850111