Ants: A model system to study the molecular and neurobiological basis of complex social behavior and phenotypic/behavioral plasticity.

One of the major challenges of neuroscience is to unravel the mechanisms underlying complex behaviors, such as an animal’s ability to communicate and to form and maintain social relationships. However, many aspects of the molecular and neurobiological basis of social behavior remain poorly understood. Social insects, like ants and bees, display complex behavior characterized by division of labor, cooperative broodcare and sophisticated communication that is paralleled in complexity only by humans (Hölldobler & Wilson 2009) and therefore constitute ideal novel systems to further our understanding of social behavior. Complex behavior is modulated by a host of factors, and interactions between genes, the environment, and previous experiences are thought to determine behavioral responses (Jaenisch & Bird 2003). However, how these factors interact to generate behaviors remains the subject of intense investigation. Because of the adaptive nature of behavior, a leading hypothesis is that epigenetic mechanisms that lead to changes in gene expression are at the core of behavioral adaptations (Simola et al. 2013; Yan et al. 2014). In addition, changes in neuromodulatory/neuroendocrine signaling are also known to be involved in the regulation of physiology and behavior in rapidly changing environments (Beets et al. 2013). Identification of the epigenetic and neurohormonal mechanisms that modulate neuronal circuit function in ants could provide the basis for developing models for understanding social behavior in response to changes in the environment.

Research in my lab will focus on identifying the molecular and cellular mechanisms underlying phenotypic plasticity and complex social behavior. We will use comparative transcriptomics to identify genes common to multiple social insect species that may be involved in the regulation of social behavior, and we will use the clonal raider ant Ooceraea biroi as a model system to study the underlying molecular and cellular mechanisms. O. biroi has recently emerged as an ideal model organism by combining complex social behavior with unparalleled experimental accessibility, to study the interplay between the environmental, genetic, and social components of behavior. O. biroi ants are totipotent and reproduce clonally, they undergo synchronized behavioral and reproductive cycles, alternating between a “queen-like” reproductive phase and a “worker-like” foraging phase, during which individuals perform specialized behavioral tasks such as nursing or foraging, and individuals eclose in synchronized age cohorts. In addition, workers show variability in reproductive potential (some individuals have two ovarioles whereas others have four or more) (Tsuji et al. 1995; Ravary & Jaisson 2002; Ravary & Jaisson 2004; Ravary et al. 2006; Lecoutey et al. 2011; Kronauer et al. 2012; Oxley et al. 2014). These features will allow me to easily and precisely control the genetic background, reproductive state, age structure, and social environment in lab-reared colonies making this species a truly tractable model system to study the molecular and cellular mechanisms underlying behavior. My lab will use this organism in conjunction with recently developed techniques such as CRISPR-based gene editing and automated behavioral tracking (Trible et al. 2017; Yan et al. 2017) to investigate the relationship between social behavior and the physical and social environment focusing on the projects outlined below.
1. Identification of the neuronal substrates involved in complex behaviors in the clonal raider ant.

Assigning behavioral functions to neural structures has long been a central goal in neuroscience and is a necessary first step toward a circuit-level/cellular understanding of how the brain generates behavior. Immediate early genes (IEGs), such as c-fos, whose expression is transiently and rapidly upregulated upon neural activity, are classic/powerful tools for detecting behavior-related neural activity in vertebrates (Jarvis et al. 1998; Guzowski et al. 1999; Fujita et al. 2013). In insects, finding conserved and reliable IEGs has been a challenging task. However, two genes have recently been identified as promising candidates that work as neural activity markers across insects, c-jun (McNeill et al. 2015) and Hr38 (Fujita et al. 2013). We will map regions of neuronal activity that correlate with the expression of certain behaviors or castes in ants by using the expression of these immediate early genes as a readout of neuronal activity through an already established method of whole brain fluorescence in situ hybridization in O. biroi. In parallel we will also test existing and/or develop new antibodies against these IEGs to be used in ants. The mapping of neural activity correlated with complex behaviors in ants will provide significant insights into the functional modalities involved in the display of specific social behaviors such as nursing tasks vs. foraging tasks or ants in the queen-like phase vs. the worker-like phase, and will open new avenues to dissect circuits underlying these behaviors and their modulation by the environment and social experience. To my knowledge, this will constitute the first and most complete brain activity map in any social insect and will significantly increase our understanding of the neural basis of complex social behavior in ants.

2. The role of epigenetics and transcriptional regulation in social behavior in ants.

The epigenetic mechanisms that underlie polyethism in ants can easily be studied by analyzing natural and induced variation in behavior among individuals in experimental colonies. Behavioral repertoires in O. biroi are highly dynamic and sensitive to the social context, nutrition and physical environment. Individuals of O. biroi, despite being genetically identical, display task specialization. In colonies of age matched individuals, some specialize in nursing behavior whereas others specialize in foraging behavior (Figure 1A and B). In colonies of mixed ages, task allocation highly correlates with age, with older individuals foraging more than younger ones. Moreover, O. biroi ants switch from behavioral reproductive states to broodcare states and back every month in a cycle regulated by the presence or absence of larvae (Tsuji et al. 1995; Ravary & Jaisson 2002; Ravary & Jaisson 2004; Ravary et al. 2006; Lecoutey et al. 2011; Kronauer et al. 2012; Oxley et al. 2014). Because the behavioral transitions in O. biroi occur in fully developed adult individuals, this species offers a unique opportunity to explore the role of gene regulation and epigenetic pathways in brain function and behavioral plasticity. For example, removing nurses from a colony induces foragers to switch to nursing behavior and adding larvae to colonies or removing them can rapidly change their behavioral reproductive state. Epigenetic mechanisms underlying these transitions can then be easily studied in controlled experimental colonies. Through RNA-seq, ChIP-seq and bisulfite sequencing we will identify which non-coding RNAs, histone post-translational
modifications and chromatin states are important for these behavioral transitions or states. Patterns of DNA methylation have been analyzed in a few species including O. biroi and no differences between the reproductive vs. the worker state could be detected when analyzing global levels of DNA methylation in whole brains (Libbrecht et al. 2016). Shifting the focus from whole body and whole brain gene expression studies to more specific brain regions will improve our understanding of the cellular and molecular basis of social behavior in ants. We will conduct these experiments in samples obtained from whole brains and from specific parts of the brain previously identified as important for specific behaviors (see number 1) and including a larger set of behavioral comparisons such as (nurses vs. foragers, young vs. old, and higher reproductive individuals vs. low reproductive individuals). Along with this molecular characterization we will also analyze staining patterns for different histone post-transcriptional modifications and chromatin structure related proteins (such as chromatin remodeling complexes, enzymes and the histone acetyl transferase and transcriptional coactivator CREB binding protein CBP) in specific brain areas using expansion microscopy protocols that I have already developed for whole ant brains to increase spatial resolution. To address the functional significance of the genes and pathways identified, we will use already established experimental approaches in ants such as CRISPR cas9 genome editing, RNAi as well as pharmacological and hormonal treatments. These studies have the potential to uncover key epigenetic processes regulating phenotypic plasticity and complex social behavior in animals.

3. The role of candidate neuropeptides in regulating social behavior in ants.

There is great interest in dissecting the genetic regulation of behavior in animals. Nevertheless, few genes have been directly linked to complex behavioral traits (e.g. Bendesky et al. 2017; Gospocic et al. 2017). Neuropeptides are interesting in this context because they are known to have important effects on social behavior across a wide range of taxa (Donaldson & Young 2008; Brockman et al. 2009; Nässel & Winther 2010; Han et al. 2015). In ants, the neuropeptide corazonin has recently been shown to be a key regulator of foraging behavior and worker caste identity (Gospocic et al. 2017). Moreover during my postdoc, I have uncovered the roles of two evolutionary conserved neuropeptides in ants. My results show that the neuropeptides inotocin (oxytocin/vasopressin ortholog) and insulin-like peptide 2 (ILP2) are involved in the regulation of social and reproductive behavior in ants, respectively. I have developed custom made antibodies for these peptides and characterized their sites of expression and projections in the ant brain (Figure 1C and D). I have specifically shown that higher levels of inotocin peptide correlate with age and foraging propensity in ants and that pharmacologically increasing inotocin levels in ants increases foraging behavior. I have also shown, through an unbiased transcriptomic screen of seven distantly related species of ants, that ILP2 is upregulated in queens across ant species and in O. biroi ants during the reproductive phase and in higher reproductive individuals (Chandra and Fetter-Pruneda et al. 2018). However, it remains unknown when, where and how these neuropeptides exert their effects in the brain and other tissues in the ant to modulate social behavior. We currently don't know which neurons, neuronal circuits or target tissues express the inotocin and insulin receptors and how these are regulated
Research Statement

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throughout the behavioral cycle and between behavioral castes. In mammals, the distribution of oxytocin receptors in the brain is crucial for determining behavioral phenotypes, such as monogamy or polygamy in prairie voles (Insel & Shapiro 1992). To understand the role that these two neuropeptides play in the regulation of behavior in ants it is instrumental to characterize the transcriptional dynamics and expression pattern of the receptor in different behavioral castes and behavioral paradigms. To this end we will develop antibodies and in situ hybridization protocols to study the expression site of the receptors of these neuropeptides. Moreover, to functionally characterize these peptides we will generate mutant lines and perform RNAi experiments. This strategy will focus on the oxytocin/vasopressin and insulin systems initially. However, this will become a general experimental approach in my lab to understand the role of neuromodulators and neurohormones in social behavior in ants. This work will significantly increase our understanding of the role of two very important and conserved neuropeptides in complex social behavior.

Figure 1: A) Ooceraea biroi colony. Ants are individually tagged for automated behavioral tracking; some individuals cluster around the brood (nurses) while others leave the nest to explore or forage. B) Automated tracking setup. Hundreds of colonies (in petri dishes) can be recorded simultaneously by webcams to identify variation in task performance. C) Dorsal view of an O. biroi brain stained for insulin-like peptide 2 (ILP2) (green). Note that there is a single cluster of ~14 insulin producing cells (IPCs) D) Ventral view of an O. biroi brain stained for inotocin (green). Note that there are two cell bodies in the subesophageal zone (SEZ) and dense core vesicles throughout the SEZ. Cell nuclei are stained in blue and actin in magenta.

4. Identification of novel genes involved in lifespan and aging in ants through comparative transcriptomics.

One of the most striking examples of phenotypic plasticity in ants is variation in lifespan due to reproductive status, with workers living short lives and reproducitives having very long lives. My laboratory will employ a comparative transcriptomics approach using RNAseq to identify genes involved in senescence and lifespan in different species of ants with varying aging phenotypes. To this end, we will use three species that vary in their longevity and life history. The first is O. biroi, described above, in which I can measure expression differences between ants kept in either worker-like or queen-like states, and also between high and low reproductive individuals, where different phenotypes are expected to display differences in longevity. The second is Harpegnathos saltator, a species in which some workers have a unique ability to
transition into a queen-like state, gaining both reproductive potential and increasing their lifespan up to five-fold (Yan et al. 2014). The third is Lasius niger, a species in which the worker and queen castes are fixed and queens are extraordinarily long lived, often living more than 20 years (Hölldobler & Wilson 2009). Identifying common differentially expressed genes in these three systems will allow robust identification of strong, novel candidate genes involved in aging and lifespan which can be further explored using the genetic tools available in O. biroi.

Moreover, since the underlying mechanisms of lifespan variation are unknown and may in principle also be epigenetic and neurohormonal in nature, my lab will perform a comprehensive study of aging in O. biroi focusing on these mechanisms. I will measure the differences in lifespan between ants permanently kept in worker-like or queen-like states, between high-reproductive and low-reproductive individuals, and between ants embedded in different social environments. Through transcriptomic analyses I will identify novel candidate genes involved in senescence and lifespan in the clonal raider ant. Moreover, as mentioned in number 3, in my previous work on the molecular mechanisms underlying social behavior in ants I have found significant changes in the expression of neuropeptides and molecules that correlate with age related changes in behavior. Interestingly, these molecules have also been previously implicated as important players in healthspan and aging in model organisms. These include inotocin, peptide levels of which increase in older ants, and insulin-like peptide 2 and the histone acetyl transferase CREB binding protein (CBP), that are expressed at higher levels in the queen-like phase and in higher reproductive individuals, respectively (in prep).

Remarkably, oxytocin and CBP have been shown to reverse and prevent age-associated muscle degeneration and loss of brain plasticity, respectively, and have been proposed as rejuvenating agents in mammals (Alarcon et al. 2004; Korzus et al. 2004; Zhang et al. 2009; Elabd et al. 2014). Using pharmacology and functional genetics, I will identify the specific roles of these genes in aging. Studying their causal role in aging ants that exhibit natural lifespan polymorphisms will allow me to identify mechanisms that induce queen-like phenotypes (i.e. extended lifespan compared to workers) despite having identical genetic backgrounds. Experiments proposed in this application represent a truly novel approach to the study of aging, and will provide important information for translational studies in higher organisms.

**Long-term research plan:**

In the long term, knowledge gathered in my lab could lead to the development of two important applications:

1. Due to my expertise working also in mammalian systems (both rodents and human cell lines) my long-term research goal is to bridge the knowledge generated by my group in ants and test it in mammalian systems. This approach has proven to be fruitful in the discovery of new modulatory drugs for human receptors by using insect peptides. For example a very potent antagonist of a human vasopressin receptor has been developed from the ant oxytocin/vasopressin ortholog gene (Di Giglio et al. 2017). Similarly, ant orthologs of other neuromodulators may affect a response in human receptors and could lead to the development of new pharmacological tools.
Furthermore, genes important for aging or longevity in ants may play similar roles in rodents and humans.

2. Ants are important agricultural and domestic pests throughout the world, and insights gathered in my lab on the molecular basis of insect reproduction and foraging behavior could lead to the development of novel and environmentally friendly pest control mechanisms. Through comparative genomics it may be possible to identify genes or pathways that would affect social behavior in pest ants but leave beneficial insects such as bees unaffected. Similar efforts are currently being investigated by groups studying diverse species of insect pests such as flies, beetles, aphids and moths (See for instance http://www.neurostresspep.eu/home). Ants being one of the most abundant insect species on earth could become an important target for such efforts and knowledge generated in my lab could instruct such efforts.

References


