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About this Issue

Statement of Purpose

The Rhodes Journal of Biological Science is a student-edited publication that recognizes the scientific achievements of Rhodes students. Volume XXVIII marks the seventh year since Mark Stratton and Dr. David Kesler brought the journal back into regular publication in 2006. Founded as a scholarly forum for student research and scientific ideas, the journal aims to maintain and stimulate the tradition of independent study among Rhodes College students. We hope that in reading the journal, other students will be encouraged to pursue scientific investigations and research.

Acknowledgements

The editorial staff would like to thank Dr. Boyle of the Biology department for her support and guidance in preparing this publication.

Image Credits

The cover image is an anatomical illustration created by Elise Hastings, '13. Elise Hastings is a Political Science major and an Art minor from St. Louis, MO. Elise is involved in various activities on campus including Social Regulation Council, Diplomats, and Chi Omega. She has been a Community Development fellow at Vollintine Evergreen Community Association for the last two years, and plans to continue working for social justice with Jesuit Volunteer Service corps next year. Elise likes to travel, especially in Central America, and loves running and key lime pie. She especially loves to paint and draw, and has a special interest in rendering the human body.

Editorial Staff

Helen Floersh '13 (Senior Editor) is a Neuroscience major from Rutherford, Tennessee. She spends the majority of her time running PCR in the McGargill Immunology lab of St. Jude Children's Research Hospital, but in her few hours away from the bench she is the Rhodes Student Associate for Campus Safety, a member of βββ Biology Honors Society, and loves hanging out with her Chi Omega sisters. She has spent her collegiate career immersed in service work as a part of Rhodes Rebuilds and as a volunteer at Baptist East and the MED. Helen's main goal in life is to somehow magically transform into her hero, Sanjay Gupta. Until then, she plans to obtain an MS in Pharmacology at Tulane before applying to medical school, and intends to become a Neurologist and someday work as a medical correspondent for the BBC.

Liz Karolczuk '14 (Junior Editor) is a Neuroscience major from Crystal Lake, IL. Liz is the Co-President of GlobeMed, an organization on campus that advocates for social justice through global health education and activism. She is a Bonner Community Service Scholar and spends her time as a Labor and Delivery Unit assistant at The MED. She co-founded Science Fridays at Evergreen Aftercare, through which she strives to build a passion for science through experiments and lesson plans with elementary school children. She has held internships at the American Red Cross and the Church Health Center, and is a member of the Order of Omega, Gamma Sigma Epsilon and Delta Epsilon Iota honor societies. Liz has served as the Community Service Coordinator for her sorority, Chi Omega, and she completed her Bonner International Summer of Service in an orphanage in Dodowa, Ghana during the summer of 2012. This summer she will be completing a self-designed fellowship investigating barriers to healthcare access for those suffering from mental illness in Ghana and Nicaragua. While in Nicaragua, she will also be interning with the non-profit AMOS, monitoring and evaluating water filtration systems in rural communities. After graduation, she plans on attending medical school and pursuing a career bridging global health policy and clinical medicine.

Erik Klingbeil '13 is from San Francisco, California and is pursuing a double degree in Biology (B.S.) and Political Science and International Studies (B.A.). Erik is currently a member of Beta Beta Beta, Delta Epsilon Iota, Sigma Iota Rho, Omicron Delta Kappa, Mortar Board, Pi Sigma Alpha and is a Resident Assistant. He has been active in several off campus internships involved in public health and health services that include the Trauma unit at the Memphis Regional Medical Center (MED), Congressman Steve Cohen's district office, and the Frager Law Firm. Erik is actively involved with research for the past two years in the department of Infectious Disease at St. Jude Children's Research Hospital determining risk factors for nosocomial infections in Latin American hospitals. He has also researched with Professor Luque de Johnson in the biology department determining the binding mechanisms of Malaria parasites to Red Blood Cells. This summer he will be researching with the department of Immunology at St. Jude in collaboration with LeBonheur determining the maturation of Influenza infections among infants. During his college career, Erik will have taken health and service based trips to India, Kenya, Namibia, Dominican Republic and Peru. Last summer he volunteered in a hospital and HIV/AIDS clinic in the Kilimanjaro district of Tanzania as a Mertie Buckman Fellow. Currently, Erik is also an Editor for the Modus Vivendi International Relations Journal. He is planning on applying to an MD/MPH program following graduation. Erik's personal contribution to the journal, "Impact of Climate Change on *Bacillus anthracis* Virulence in Southern Africa," is featured on page 4.

Austin Armstrong '14 hails from the metropolis of Searcy, Arkansas. He ultimately pursued a major in history despite an equally intense love for all things science-y. Luckily for him, he was allowed the opportunity to work for the Rhodes Biology Department as a Laboratory Teacher's Assistant, which was an experience of immeasurable joy, camaraderie, and hazardous waste. When he is not diligently (or not-so-diligently) studying in the labs of FJ, he is most likely laboring on something involving public health or simply satiating his daily requirement of NPR reading. Austin's aspirations for fighting for global health equity began in summer of 2012 when he interned with AMOS Salud y Esperanza in Nicaragua. His six weeks working on water filtration in rural communities would later be deemed such cheesy things as "life changing" and "transformative". Upon returning to Memphis he decided to put his passions into practice by working with the Mid-South AIDS Fund. Mid-South AIDS Fund or MAF endeavors to reduce HIV transmission and stigma via community based prevention initiatives. Most recently he was honored by being elected as Internal Co-President of GlobeMed at Rhodes for the 2013-2014 academic year. Aside from his main extra-curricular activities, he has been fortunate to be inducted into Omicron Delta Kappa, Phi Alpha Theta, and Delta Epsilon Iota honor societies. The upcoming future holds great promise as Austin will begin scribing this

May at Le Bonheur Children's Hospital. He is still undecided on his exact trajectory after graduation from Rhodes, but he is certain that it will involve some co-mingling of public health, policy, and medicine.

Caroline Elbaum, '14 is a Neuroscience major from Murfreesboro, TN. She is a research assistant in Dr. David Kabelik's Behavioral Neuroendocrinology Lab, Regional Medical Center at Memphis Labor and Delivery volunteer, Rhodes Student Associate for the Center of Outreach in the Development of the Arts, member of Chi Omega, and an English teacher to Spanish-speakers at Su Casa Family Ministries. As a Buckman Fellow, she studied abroad in the fall in Santiago, Chile where she examined the organization of the Chilean healthcare system and the general health of the population. She plans to attend medical school after she graduates to obtain a joint MD/ Master of Public Health degree.

Stephen Leavelle, '14 is a Biology major and serves as the Treasurer of βββ Biology Honors Society. He is involved with behavioral research at the Memphis Zoo and plans to pursue a career in zoo research and design. His favorite animal is the African elephant. His article on these wonderful creatures, dedicated in the memory of Dr. Rosanna Cappellato, can be found on page 24.

Grace Mosley, '14 is a Neuroscience major and Philosophy minor from St. Louis, Missouri. She is a member of the varsity swim team, Vice President of Communications for the Alpha Omicron Pi fraternity, a volunteer in the Trauma Unit at The MED, and member of the Tri Beta, Gamma Sigma Epsilon, and Phi Sigma Tau honor societies. Grace does research in the Chen Lab in the department of Chemical Biology and Therapeutics at St. Jude Children's Research Hospital during the academic year, and in the Hultgren Lab in the department of Molecular Microbiology at Washington University in St. Louis School of Medicine during the summer. After graduation, she hopes to pursue an MD and a PhD in neuroscience as a part of the Medical Scientist Training Program. Grace's work is also showcased here in the RJBS; her article, "Schizophrenia and Criminal Behavior as a Vulnerability-Stress Model," can be found on page 21.

Matt Cannavo '15 is a Biochemistry and Molecular Biology major from Orlando, FL. As a St. Jude Summer Plus International Fellow, he traveled to Santiago, Chile last summer and worked in a molecular biology lab while taking a medical Spanish class. After returning to Memphis, he began researching in pharmacogenetics implementation at St. Jude Children's Research Hospital, and will continue his work there this summer. He is also a pediatrics intern at LeBonheur Children's Hospital, and enjoys volunteering at St. Jude, the Human Society, a soup kitchen, and in trauma at the MED. He is involved on campus as an International Peer Advisor and on the HOLA leadership board, and next year will be a Resident Assistant and Kinney Coordinator for Healthcare and Wellness. After graduation, Matt hopes to pursue a career in medicine.

Impact of Climate Change on *Bacillus anthracis* Virulence in Southern Africa

Erik Klingbeil
Rhodes College

Impact of Climate Change on Infectious Disease and Wildlife Management in Namibia

International relations theory creates models that protect domestic actors from external predation with the intent of diluting losses in life and productivity. As traditional security threats surrounding the Cold War recede backstage of the international theater, new dilemmas facing global and domestic security have taken priority. As such, definitions of national security have expanded beyond the classical-realism paradigm to encapsulate more normative models and approaches. Multilateral threats that concern the international community in its entirety such as resource scarcity and environmental degradation impact all aspects of social welfare and political stability. One such concern that has taken center stage since the immersion of liberalism and the expansion of transboundary interactions has been the proliferation of infectious agents and communicable diseases.

These emerging and re-emerging infectious diseases (ERIDs) have halted social productivity and crippled socio-political structures. Re-emerging pandemics such as Tuberculosis, Malaria, Dengue and Cholera have expanded in areas of endemicity as a consequence of urbanization and resource over-usage and extraction (Price-Smith,1999). Newly emerging forms of pathogenic microorganisms including Hanta, Ebola and antimicrobial resistant strains of organisms such as methicillin-resistant staphylococcus aureas (MRSA) have spread as a consequence of zoonosis (animal to human disease transmission) and increasing exposure to human communities across the globe (Price-Smith, 1999). In particular, Sub-Saharan Africa has been victimized socially and politically as a consequence of ERIDs. The HIV/AIDS pandemic has continued to expand and appear in regions where not previously endemic. HIV and other debilitating autoimmune diseases have enhanced the susceptibility and pathogenicity of infected hosts to secondary infections (Price-Smith, 1999). Recent studies have strengthened the validity of claims of heightening predispositions to long-term chronic diseases as a consequence of exposure to infectious agents. In recent decades infection-induced mortality has been on the rise in both the developed and developing world. Within the African Sub-continent, the association between infectious disease and socio-political instability is direct and causal (Price-Smith, 1999). However, where treatment for particular diseases is accessible among developed societies, those living within underdeveloped communities are not.

Current research has also revealed a relationship between global climate change and the proliferation of infectious diseases. For example, increasing mean ambient temperatures can expedite the development and virulence of pathogenic agents within mosquitos and other transmission vectors (WHO, 2008). Changing patterns in rainfall can induce flash-floods at one extreme and water scarcity at the other (DRFN, 2008). Namibia is a country that has and is predicted to experience significant changes to its climate in the near future. In a country that maintains a large population of livestock and wildlife zoonotic infections are more common than countries with smaller populations of animals (Nordberg, 1999). Therefore, the consistency of these infections is likely to intensify with anticipated weather extremes.

Communicable diseases amongst wildlife populations are common within Sub-Saharan Africa. Where some infections have devastated species populations and displaced others, communicable agents are a natural cycle of the regional ecosystem (Lindeque & Turnbull, 1994). Dispersal methods, which are necessary for an infectious microbe to exist, are contingent upon a variety of environmental conditions (Nordberg 1999). One such infection that remains prevalent amongst wildlife populations in Namibia is *Bacillus anthracis*, Anthrax.

Etosha National Park in Northern Namibia is a sanctuary to a host of endemic fauna. Social and economic factors have attributed to the existence of the Etosha Park and will continue to be important to its existence and public support in the coming years. Ecotourism in particular is a large industry in Namibia with Etosha being a particularly lucrative attraction. Businesses and surrounding communities can benefit from the influx of tourists the wildlife draws. Hence, they generally supported the park's existence. As a consequence some of the park management strategies have been contingent upon political agendas that are influenced by the demands of locals and Namibians. For example, when cattle farmers faced a scarcity of plain grasses for their livestock, veldt management within the park came under heavy criticism. Political pressure from the Namibian government forced the Etosha Park rangers to eliminate their veldt management program. Within ten years unmaintained grass plains facilitated a large fire within the park (Etosha Ecological Institute, 2012).

Wildlife Population Management with Anthrax in Etosha National Park

In Etosha the persistence of *Bacillus anthracis*, Anthrax, has been permitted in order to regulate wildlife populations though a more natural alternative to methods such as culling. Yet, the transmission of Anthrax spores is conditional on important environmental factors such as wind, temperature and rainfall (Oertzen, 2008). Anthrax transmission from wildlife to livestock and humans within the region has occurred at a minimal level. If the predicted weather extremes across the country occur, then Anthrax infection rates and transmission rates from wildlife to livestock and humans outside the park could increase (Lindeque & Turnbull, 1994). These increases could negatively impact regional support of the park and its methods of population regulation. While wildlife regulation with Anthrax has been successful in the past, park rangers should seek alternative methods that are not a danger to the health and social wellbeing of the surrounding communities.

Anthrax in Etosha was first discovered in 1964 (Lindeque & Turnbull, 1994). Infectious outbreaks of Anthrax occur annually with regular intensity which makes Etosha unique. Consequently, Etosha's fauna remains at a steady standard on a per annum basis. Whereas outbreaks of Anthrax in other regions have significantly impacted wildlife populations and could potentially devastate their overall abundance. Due to the success of Anthrax in regulating population levels of many plain ungulates, Etosha's park management has made no attempt to lessen the proliferative capacity of the bacteria (Etosha Ecological Institute, 2012). This alternative method to population regulation has been more popular than culling because Anthrax is ultimately a natural part of the ecosystem.

In addition to Etosha's uniqueness of annual Anthrax pervasiveness, there is also a seasonal fluctuation in the prevalence of its levels within Etosha's various wildlife populations (Turner & Cizauskas, 2010). On average, elevated incidences of Anthrax emerge during the majority of the dry seasons throughout all of Sub-Saharan Africa (Turner, 2010). However, some regions prove to be the exception like Northern Namibia where Etosha is located. In Etosha, Anthrax prevalence among wildlife is at its highest during the end of the rainy season. This period is when plain ungulates and carnivores are infected, the exception being pachyderms. Elephants more frequently die during the end of the dry season and start of the rainy season (Turner, 2010).

Anthrax Sporulation and Germination

Anthrax is an acute bacterial infection that commonly afflicts grass-eating animals and humans throughout Sub-Saharan Africa. Anthrax infects both livestock (cattle, goats and sheep) and wildlife (zebra, wildebeest, springbok, gemsbok, cheetah and elephant). Anthrax is transmitted in a spore stage that can remain dormant within the soil, feces or corpses of deceased animals and be transmitted following several years (De Vos 1990; Turner and Cizauskas 2010). Plain ungulates and other grazing herbivores can become infected following the consumption of grasses and shrubs with contaminated feces and soil. Carnivores and scavengers that are susceptible acquire the bacterium from consuming other infected carcasses (Bellan, 2009).

Once ingested by the susceptible host, there is an incubation period that lasts an average of two days (Lindeque & Turnbull,1994). This period gives Anthrax ample time to enter a vegetative cell state that induces the degeneration of the spleen and lymph nodes. Shortly thereafter, the rupture of the lymph nodes allows a rapid release of bacillus inducing a septicemia (Nordberg, 1999). Until just a few hours prior to death, the infected host appears asymptomatic. The Anthrax then releases harmful toxins that can disseminate throughout the bloodstream. Shortly prior to death, animals will typically fall to the ground with a strong fever (Nordberg, 1999). Post-mortem, blood hemorrhages from all orifices of the corpse, exposing Anthrax bacilli to the environment. Exposure of the bacterium to oxygen induces sporulation in a process that lasts a few hours (Lindeque et al., 1998). Spores can therefore preserve the microbe long enough to be effectively dispersed and transmitted over a significant period of time (Dragon, 1995).

Causes of Seasonal Anthrax Pervasiveness in Etosha Wildlife

Seasonal fluctuations in infection rates are most likely due to physical stresses placed on the indigenous wildlife as a result of environmental altercations that occur during the wet season. Healthy animals that do not have an acquired immunity against Anthrax can still keep the spores from germinating with simply their innate immunity and other host defenses (Dragon, 1995). Yet lowered immunological success during the wet season can heighten chances of infection amongst these animal populations. For example, immunomodulation as a consequence of environmental change can be stimulated by breeding and birthing periods, nutritional deficiencies and high exposure to various parasites (Turner, 2010). Specifically, gastrointestinal parasites have been known to induce altered immune reactions away from Th1 bacterial responses that would target Anthrax towards Th2 parasitic responses (Kamath & Getz, 2011; Turner, 2010). At the end of the rainy season, dwindling drinking pools create a higher concentration of water-borne parasites. Therefore the prevalence of parasitic attacks and Anthrax infections of wildlife in Etosha is directly proportional.

Annual oscillations in Anthrax related fatalities can also be a consequence of the availability of natural flora for consumption. Declining water levels and general scarcity consolidates wildlife populations within more remediate vicinities; these rising concentrations at the end of the rainy season consequently support closer contact and overgrazing. Decimated grasslands and heavily clipped grass heightens the likelihood of plain ungulates consuming soil contaminated with Anthrax spores (Lindeque & Turnbull, 1994). In addition, researchers have hypothesized that decreasing water availability at watering holes during the end of the rainy season likens them to fecal and sputum contamination (Turner & Cizauskas, 2010). Consolidated wildlife populations are therefore at greater risk of imbibing Anthrax polluted water.

There is also an observed relationship between migratory patterns and occurrences of Anthrax infection. During the wetter seasons Zebras migrate towards the western edge of Etosha where the soil may be more conducive to Anthrax sporulation and density (Kamath & Getz, 2011). Because Zebras travel in large herds, their large concentrations in western Etosha could result in over-clipped grass and a greater likelihood of Anthrax exposure. This slight variation in infection rates and annual prevalence between plain ungulates and elephants is most likely the result of differing migratory and foraging patterns and behaviors.

Despite the successful spread of Anthrax among most of Etosha's grazing wildlife populations, several species of carnivores are readily immune from its infection. Lion, spotted hyena, jackal and vulture are among the few (Bellan, 2009; Bellan et al., 2012). They are predominantly responsible for the removal of vegetative Anthrax spores from carcasses (Bellan, 2009) and the swiftness with which these scavengers consume the Anthrax is important in determining the virulence and transmission of the bacterium. If the carcass is consumed by scavengers before the Anthrax spores have enough time to develop then they will be denatured and removed from the environment (Dragon, 1995). However, if sporulation is given ample time to complete, then scavengers can disperse the developed spores elsewhere through their feces.

Another primary mode of distribution and transmission of Anthrax spores is through wind. Spores can be carried by wind to an average of eighteen meters with other heavy soil particulates (Lindeque et al., 1998). Although it is unlikely for Etosha's wildlife to inhale spores while in the immediate vicinity of the carcass, wind plays an important role in contaminating soil sources. In addition, Northern Namibia experiences a west to east wind coming from the Atlantic Ocean and moving inland (Lindeque et al., 1998). If there are in fact heavier concentrations of Anthrax contaminated soil sources in the western region of Etosha, wind can play an important role in its distribution eastward throughout the park.

Effects of Climate Change on Anthrax Transmission in Etosha

Statistics predict an aggregate increase in temperature, wind intensity and rainfall variability over the course of the next fifty years in Namibia. Trend models from the 1960s to the present highlight changes regarding temperature and weather patterns that are consistent with these predictions (McMichael et al., 2008). Overall data has shown that there has been an increase in the mean values within the maximum and minimum brackets for temperatures, rainfall quantities and wind speeds (Oertzen, 2008).

The wet season has experienced a noticeable increase in the number of consecutive rainy days over the past several years. Due to increased weather variability, there has been a progression in longer dry seasons and shorter wet seasons. As a result, damage and changes to the environment have intensified as a consequence of more concentrated bouts of rainfall (WHO, 2003). Etosha National Park, a habitat that is sensitive to fluctuations in available water sources for wildlife, has been largely impacted by these changes. One of the leading causes of Anthrax transmission has been immunosuppression during the rainy season due to environmental stresses on the wildlife. If periods of rainfall were to intensify this could induce greater stress on the wildlife, making them more vulnerable to Anthrax and other infections (Oertzen, 2008). Alterations in rainfall patterns could also induce changes in vegetation density and habitats. As a result there could be changing migratory patterns within the park. If animals were to concentrate in higher quantities in the west where there is larger Anthrax load in the soil, transmission could be more likely.

The late summer which has been short, has progressively experienced more pronounced periods of rainfall (Oertzen, 2008). In northern Namibia the rainy season has witnesses a later start and early end. Where wildlife has traditionally begun to congregate at the end of the rainy season at more established water holes, shorter rainy seasons will exacerbate this effect. With greater wildlife interaction around the waterholes it is more likely that drinking water can be contaminated by feces and corpses containing Anthrax (Turner & Cizauskas, 2010). Overall, the trends indicate that the Namibian climate is becoming more extreme and drier (Oertzen, 2008). With time this will continue to intensify the infectious spread due to prolonged periods of concentrated wildlife levels.

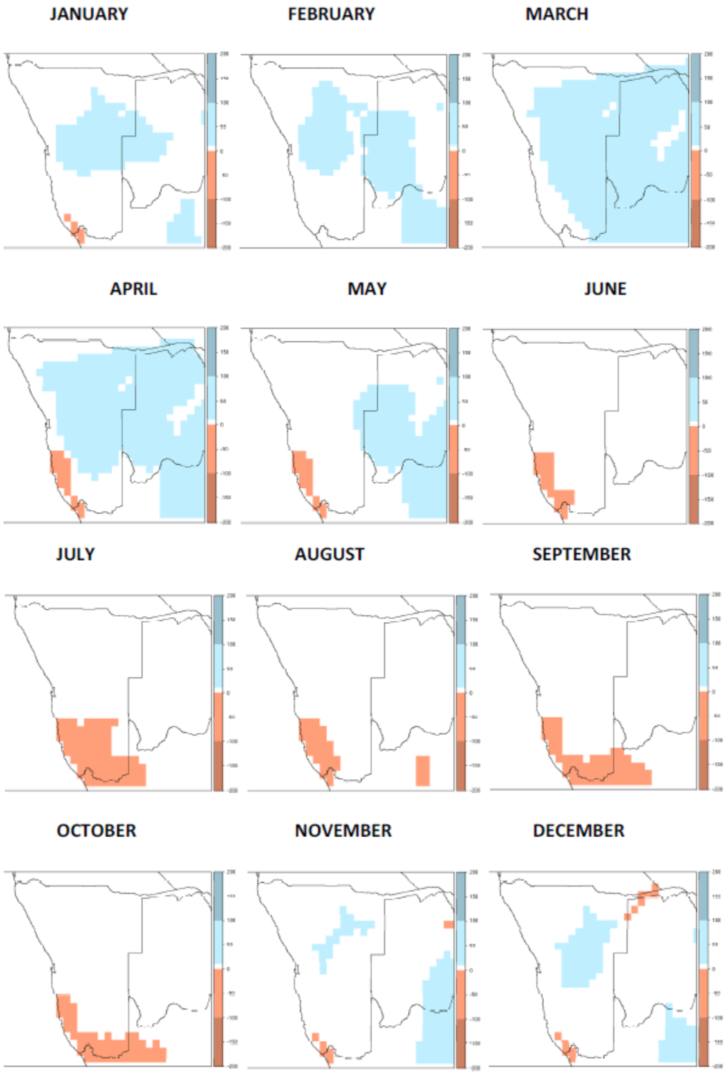

Figure 1: Rainfall projections for 2046 to 2065 show an aggregate increase. It shows there will be roughly a 50 inch increase in annual rainfall. Hightening rainfall patterns increase likelyhood of Anthrax dispersal and transmission during the months of January to April. Low and more severe dry seasons from May to October highten the concentration of Anthrax in common pools of water where animals drink and congregate. (DRFN, 2008)

IPCC reports predict that global mean temperatures to rise by 1.8°C to 4.0°C (Oertzen, 2008). Northern Namibia and the Etosha are predicted to reach a mean temperature increase of 2°C to 3.5°C throughout the full year by 2046-2060 (Oertzen, 2008). There is predicted to be a greater increase in summer rainfall over most of the country followed by a long period of dryness. Increasing temperatures with other weather changes could induce greater stress on the Namibia wildlife that would make them more vulnerable to infection. Moreover, higher temperatures could alter the rate of Anthrax sporulation (De Vos, 1990; Dragon, 1995). If temperature increase induce faster rates of sporulation, then scavengers consuming infected carcasses are more likely to ingest fully developed Anthrax spores. These spores can then be distributed throughout the park through the feces of animals such as vultures (Mundy, 1978).

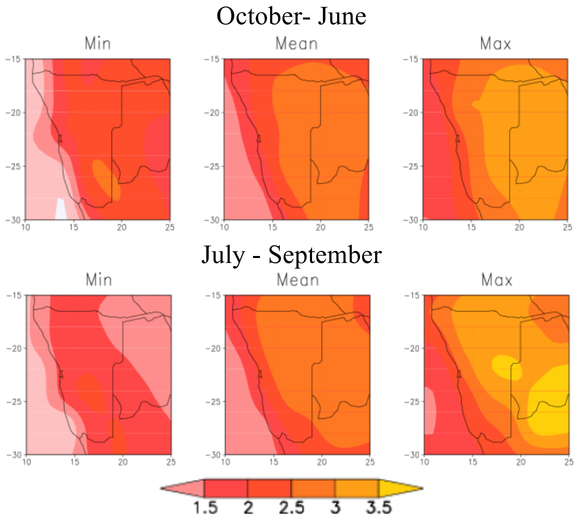

Figure 2: Changing climate patterns over recent years indicate rising temperatures in the Etosha National Park region. (DRFN, 2008)

Wind patterns coming from the ocean moving towards the eastern board of Namibia are predicted to heighten with increasing temperature discrepancies between ambient ocean air temperature and land temperature (Oertzen, 2008). Where the soil in the western portion of the park is known to have greater concentrations of Anthrax, changing wind patterns could expand the area of infectious agents. Stronger winds moving in the west to east direction could blow spores into the proximity of otherwise non-contaminated water holes and habitats in the eastern section of Etosha.

Changing Impacts of Anthrax on Greater Etosha Community

Future altercations to rainfall, temperature and wind patterns are likely to impact the Namibian landscape and environment over the coming century. These physical changes to the country are likely to induce pressure on available water sources. Where water access is concerned, Northern Namibia and in particular Etosha National Park are vulnerable to these changes due to its high concentration of livestock and wildlife. Where water is in lower supply and higher demand, contamination due to overuse and crowding around such sources is likely (Turner & Cizauskas, 2010). Humans, livestock and wildlife are more likely to share water sources and as a result transmit infections between one another. In the past these situations have stimulated outbreaks of cholera and dysentery and other water-borne illnesses (Price-Smith, 1999; Oertzen, 2008).

The potential heightening of human, livestock Anthrax infection rates is risky for the future of wildlife conservation. As the Etosha wildlife becomes at greater risk of contracting anthrax as a result of climate change, so is the livestock and inhabitants in the surrounding communities. Where anthrax mediated wildlife regulation may have been an effective natural form of population control in the past, it may now be a danger to Etosha fauna as a whole. Additionally, environmental conservation and in particular government controlled wildlife regulation (e.g. national parks) is heavily influenced by social and political factors (Etosha Ecological Institute, 2012). If anthrax contaminated water sources and soil were to spread beyond the park into the surrounding community, popular support for conservation would drastically decline.

Future of Wildlife Management in Etosha NP

In recent decades, several countries in Sub-Saharan Africa have moved to Community Based Natural Resource Management, CBNRMs. These organizations have been successful in aligning the needs of the community with overall conservation goals. Where Etosha has functioned well as a government mandated national park for many years, park management may be able to learn from the recent success of CBNRMs. Finding other methods of wildlife regulation such as wildlife relocation or regulated hunting may be prime ways of stimulating the local economy and promoting eco-tourism in other parts of Namibia.

Management methods such as hunting have been successful in other parts of Namibia such as the Waterburg Conservancy. Private industry and the greater community have been given incentives to preserve the regional wildlife becomes of its economic value. Hunting has pumped money into the economy and government through tag costs, lodge expenses, professional hunting guides and other channels. Therefore, liberalizing wildlife management in the Etosha preserve may be the best alternative to preservation.

Where anthrax mediated regulation has worked in naturally selecting and preserving populations, these animals will again be at danger if the park is not popularly supported by Namibian citizenry. Creating the balance between human contentment and wildlife conservation exists in a delicate equilibrium. While park management should continue to seek out nature avenues of wildlife regulation, they should not pursue those opportunities if they threaten the social and political aspects of conservation.

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About the author: Erik Klingbeil, ’13;, also contributed to this publication as an editor. Read about him and the rest of our editorial staff on page 2.

Higher-level Cognitive Abilities in Ants

Matthew Roberts
Rhodes College

As one of the most successful animals on the planet, ants have developed numerous evolutionary adaptations that allow them to execute incredibly complex behaviors in the areas of navigation and nutrient acquisition. The complexity of these behavioral strategies appear to indicate the presence of advanced cognitive abilities, though the physical limitations of the size and complexity of the ant's brain severely restricts any such reasoning. An analysis of these intricate ant behaviors is therefore necessary to identifying what neural mechanisms must be in place to yield complex behavioral expression from relatively simple neural circuits. Thus, the following review is an attempt to begin the process of identifying ant behaviors that seem to exhibit higher-level cognitive processes. Given the current literature on these behaviors, there is extensive research that still has to be completed to gain a better understanding of how simple neural circuitry can translate into complex social behaviors.

Introduction

If asked to name an animal that raises crops, tends to livestock, sets traps to ambush prey, holds slaves, navigates with a celestial compass, and passes down knowledge by teaching, few people would likely think of ants. Considering, however, that ants have successfully colonized every continent but Antarctica and represent 15-25% of the total terrestrial animalian biomass, it is not surprising that ants have developed considerable evolutionary adaptations (Schultz, 2000). Nevertheless, it seems hard to believe that such feats of higher level cognition would be possible for an animal that has a brain averaging 0.1 mg, more than 130 million times smaller than the average human brain (Wehner, 2003). Notwithstanding their small brain size, ants have developed several abilities that seem to indicate higher cognitive capabilities that are seldom found outside primates. Varying across 300 genera and 15,000-22,000 species, these adaptations allow ants to effectively meet their basic needs across the widely variable ecosystems they inhabit (Schultz, 2000).

One of the oldest and most studied aspects of ant behavior is their remarkable ability to determine both their own location and the location of their nest with great accuracy. This adaption allows ants to forage for food a great distance from their nest site, and take a direct path back to the nest without retracing their steps, enabling them to efficiently minimize energy expenditures and the amount of time spent most vulnerable to predation. Ants accomplish this by a utilizing an extensive array of sensory modules that include visual landmark recognition, a polarized skylight compass, magnetic compass, olfactory trails, vibration sensors, and an internal odometer, each of which are fed into a path integration system. (Banks & Srygley, 2003; Wehner, 2003; Buehlmann et al., 2012). Each sensory module functions independently and is fed into the path integration system to inform the ant of its location. As such, even if one or several modules are incapable of helping the ant find its location, it can still reliably navigate with the others (Wehner, 2003). For example, if an ant is underground, where the it cannot use visible landmarks or its celestial compass, it can still determine its relative position to the nest by means of its odometer, vibration sensors, magnetic compass, and path integration system.

Ants' sophisticated cognitive abilities, however, are not merely limited to advanced navigational strategies. Though the cold efficiency with which ants capture prey and subsequently divide it evenly among the colony is impressive, this feat is by no means unique to ants, nor is it indicative of any higher level neural processing. Ants' employ several complex strategies for obtaining nutrients that extend far beyond mindless foraging methods. One such strategy is employed by leafcutter ants (*Atta* spp.), which lead a semi-agrarian lifestyle based upon fungiculture (Schultz, 2000). These ants raise crops of *Attamyces* fungi for a dependable food source, one which they carefully attend, controlling for temperature, humidity, fertilization, and propagation (Schultz, 1999; Mueller et al., 2011). Another mutualistic strategy employed by ants involves not the tending of fungal crops, but rather honeydew-producing insects. Insects such as aphids and mealy bugs extract cellulose from the phloem of plant stems, and excrete a substance extremely rich in carbohydrates known as honeydew (Styrsky & Eubanks, 2007). In order to make this honeydew accessible at a scale large enough to support an entire colony, ants assume the care of these honeydew-producing insects. Ants feed and defend them from predation, assume the care of their young, and harvest the honeydew (Helms & Vinson, 2003). A wholly different strategy is employed by arboreal ants, whose insect prey has the ability to evade capture by either quickly jumping, flying, or dropping out range of the ants (Dejean et al., 2010). Thus, to satisfy their need for dietary proteins, some arboreal ants coordinate en masse, building elaborate traps to catch their prey. Once the trap is set, the ants then lie in wait by the hundreds or thousands, ready to ambush their victim the second it lands and tie it down with leaf fibers. This method allows them to capture prey tens of thousands times the weight of a single ant (Dejean et al., 2005; Dejean et al., 2010).

Each of the above described behaviors seem to defy classification as simple fixed action patterns and indicate the presence of considerable cognitive abilities; yet, the minute size of the ant brain appears to belie the possibility of any such conclusion. Nonetheless, the extremely complex repertoire of behaviors that ants exhibit challenges our notion of the link between neural complexity and cognitive ability. Thus, the scrutiny of these behaviors is incredibly important to further our understanding of how simple neural networks can accomplish such complicated behavioral feats. The following review therefore highlights research on various ant behaviors that illustrate the presence of what seem to be higher cognitive abilities.

Navigational Abilities

Calculation of distance

Navigational and cartographical data allow ants to navigate to a known food source, then find their way back to the nest site. At the most basic level, ants can navigate as long as they have two different types of information: distance and direction traveled, the two necessary ingredients for path integration. (Sommer & Wehner, 2003; Wehner, 2003). Many different hypotheses have been proposed to explain how ants estimate the distance they traveled with such accuracy, but recent research has conclusively demonstrated that ants calculate distance by the use of an internal pedometer, or step counter (Wittlinger et al., 2006). By modifying the leg lengths of ants- via stilts or cutting their legs short- Wittlinger et al. (2006) found that ants with longer legs overshot the distance to their nest, while ants with stunted legs underestimated the distance to the nest. Starting from a food source 10 meters away from their trained nest location, the ants on stilts traveled on average 15.3 meters before they started to search around for their nest, while the ants with shortened legs only traveled a mean of 5.75 meters until they began searching for where they expected the nest to be (Wittlinger et al., 2006). No current research has yet that ants form a cognitive map of their environment that includes distances between objects, the odometer appears to be the only means by which an ant determines the distance it has traveled (Ronacher, 2008). Further support for this hypothesis comes from the discovery that as the foraging trips increased in distance, the more error prone ants' step counting mechanism became, as small variations in height compounded over time in confounding the ants' distance calculation (Sommer & Wehner, 2003).

Modular Detection of Direction

Compared to an ant's calculation of distance, which is informed by a single pedometric module, the navigational clues that inform an ant of its direction come from a wide array of sensory modules. Like bees, ants have the ability to sense polarized light, and they use this information to inform a celestial compass (Wehner, 2003). This was confirmed in another, which found that certain "compass neurons" in the visual center of ants' brains respond to different orientations of polarized light, thereby allowing an ant to determine its direction based on what orientations of polarized light excite certain compass neurons that correspond to the direction they are moving (Akesson & Wehner, 2002). Polarized light is not the only visual information that ants can utilize to determine their direction above ground, however.

Another module that informs ants' of their direction is their magnetic compass, which helps inform the ant based off their own orientation in relation to earth's magnetic poles. The existence of this module was long debated, but separate work on both desert ants and leaf-cutter ants have independently confirmed that, while it is not their primary directional module, ants do indeed have the ability to orient themselves by the use of an internal magnetic compass (Banks & Srygley, 2003; Buehlmann et al., 2012). In the absence of any other directional clues, by applying a magnetic field that effectively reversed the polarity of the magnetic field within a test arena, ants that normally left a food source at a 162° angle to return to the nest instead left the food source at a 49° angle (Banks & Srygley 2003). While not a 180° turn around, the presence of the magnetic field significantly altered the course the ants took in trying to return home.

Once a foraging ant has found food, it uses the information of distance it has traveled in each direction to inform a path integration system that calculates the most direct route back to the nest, even if such a route has never been traveled before (Wehner, 2003; Ronacher, 2008). Path integration allows the ant to conserve energy while carrying food, and instead of having to retrace its steps, it can always travel a direct, novel route home. In Saharan desert ant species, for example, this ability is incredibly important, as over-exposure to extreme heat caused by any inefficiency drastically increases the risk of dehydration (Wehner, 2003). Though scientists are clear that ants benefits from path integration, exactly how ants are able to compute this integral vector back to the nest is yet unknown (Wehner, 2003; Ronacher, 2008). How then ants are able to calculate their route home with such limited neural machinery is an area that warrents extensive further research.

Associative Learning

Ants use visual landmarks as one of the most primary identifiers of their location, based on what landmarks are present within their visual field when oriented in a certain way (Akesson & Wehner, 2002; Wehner, 2003). Not only are ants able to use visual information that towers above their visual field and can be used from great distances away, but they can also learn to use visual information on the ground to determine their direction (Seidl & Wehner, 2006). This was demonstrated in a lab by painting the ground in a linear channel with brown paint everywhere except one spot with black paint at a specific point between a food source and the nest. The researchers subsequently moved the ants to a new test channel identical in every aspect except that the location of the black spot was placed closer to the food source than before. In the new channel, ants began their nest search earlier, in such a way that corresponded to the black paint's relative distance from the food source, thereby confirming that the ground landmark location influenced their estimation of the nest's location (Siedl & Wehner 2002).

Further research has also demonstrated that ants have another sensory module that informs them of their location based on tactile and vibrational landmarks. In two studies with the same channel procedure as described above, ants were trained in a linear channel with either sandpaper or a vibrating landmark was placed in front of the nest. In both cases, when the sandpaper or vibrating mechanism was moved closer to the food source up the channel, ants prematurely began to search for their nest (Siedl & Wehner, 2006; Buehlmann et al., 2012). In addition to using magnetic fields to determine direction, ants can learn to associate a magnetic field with nest location, and when tested in a new area identical to their learned area in every way except for the position of the magnetic field, ants would ignore their odometer and start searching for their nest prematurely when the magnetic field was moved closer to the food source.

Taken together, these three studies illustrate that ants are able to form memories based off various (visual, tactile, vibrational, and magnetic) elements of their environment and associate them with their nest location. Furthermore, the recall of these memories was enough to actually overpower their internal odometer's calculation of distance back to the nest site, which points to the fact that ants' cognitive network performs a heuristic that makes inferences based off of the strongest evidence as to where there nest is actually located when there is a disconnect between sensory modules. Whether or not this associative learning is accomplished by merely conditioning has yet to be established.

Teaching

An even more stunning feat of navigation that ants display has been recently demonstrated by a study which found that ants employ teaching to help one ant show another ant the location of a food source, the only non-primate documented case (Franks & Richardson, 2006). Teaching here is defined as one experienced individual that changes its behavior to show another inexperienced individual a novel concept and that necessarily includes feedback between the learner and teacher throughout the process (Franks & Richardson, 2006). This inclusion of bidirectional feedback, therefore, is what separates teaching of ants and humans from, say the waggle dance bees perform to inform other individuals the location of food, which is only uni-directional. Franks & Richardson (2006) documented that leading ants slow down their pace to allow the following ant to run after it, constantly maintaining a tactile connection between the antennae of the follower and the abdomen of the leader, and if the leader no longer senses antennae on its abdomen, it will slow down until the follower has regained connection. This phenomenon of tandem running is then passed along, as once the naïve learner has obtained the knowledge of the food source. The learner then goes back to the colony and becomes a teacher, a process that is then repeated until all the food at the site has been obtained (Franks & Richardson, 2006). What neural mechanisms ants possess to elicit such behavior are currently unknown. Here again, neural complexity does not appear to be a prerequisite for such advanced levels of social behavior.

Nutrient Acquisition Strategies

Mutualism

Leafcutter ants were originally thought to cut and carry leaves back to the colony for food. Recent research has revealed that this behavior is actually a part of the mutualistic relationship between leafcutter ants and their *Attamyces* fungal gardens (Schultz, 2000). Leafcutter ants bring these leaf cuttings back to their colony as a food source for the *Attamyces* fungus to decompose which breaks down the plant cellulose into a substance that the ants then consume, providing the ant with essential dietary carbohydrates and nitrogen (Schultz, 1999). If that were the extent of the ants' symbiotic relationship with fungi, then this would hardly qualify as seemingly higher-level cognitive processes. The ants, however, monitor their fungal colony with the utmost attention to detail, in terms of temperature and humidity (Mueller et al., 2010). Ants' antennae are more sensitive to temperature than any others in the insect kingdom, and they use these antennae to make sure their fungus gardens are as close to their optimum temperature of 20-25 °C as possible (Mueller et al., 2010). In order to achieve the thermoregulation of their fungus gardens, ants will vertically relocate their garden to different depths varying between a few centimeters and tens of meters underground, depending on ambient temperature associated with a given depth and season. This allows the ants to maintain their crops throughout both summer high temperatures and winter low temperatures (Mueller et al., 2010). The ants also control for low humidity levels by transferring moisture to the gardens by drinking excessive amounts of water, then defecating a liquid fecal excretion on the fungus to prevent desiccation, or, on the other end of the spectrum, the ants will siphon water off the fungi if flooded, and excrete the liquid refuse elsewhere (Mueller et al., 2010). Then, when a new queen moves to a novel location, she carries off a mouthful of the fungus from her old garden to the area she will start a new colony, thereby clonally propagating the fungus in a new location (Schultz, 1999). The proximate neural correlates that could explain such a specific set of behaviors are as of yet completely unknown (Mueller et al., 2010).

Another strange method that ants use to obtain their nutritional needs is by tending to hemipteran honeydew-producing insects (Styrsky & Eubanks, 2007). The mutualistic relationship between ants and these honeydew-producing insects extends beyond simple defense of the insects by the ants, as the honeydew producers are transported into a colony's nest, where they are corralled and fed (Styrsky & Eubanks, 2007). Moreover, the presence of honeydew cultivating ants in proximity of aphids caused an 82-fold increase in aphid population size (Styrsky & Eubanks, 2007)..

Trap Setting

Because neither methods of fungiculture nor hemipteran tending provide a good source of protein for the ants' diets, arboreal ants have developed a strategy of trap setting to capture large prey (Dejean et al., 2010). Using the fibers of plant trichomes, the ants weave a dense furry network on the bottom side of a leaf, held in place by a regurgitated substance that serves as the mortar (Dejean et al., 2005). Once the foundation of the trap is laid, the ants then weave in the mycelium of a mold species to add further strength (Dejean et al., 2005). Then, the ants saw tiny holes all throughout the leaf, only large enough for their mandibles to fit through, completing the trap construction (Dejean et al., 2005). Now, the ants lie and wait along the both the periphery of the underside of the leaf, as well as directly below the holes that were chewed in the center of the leaf; once the prey lands on the leaf, the ants immediately jump out and grab on to the prey, holding it down and stretching it out (Dejean et al., 2005). Simply lying in ambush, however, is neither the secret to the ants' success, nor an impressive cognitive feat. What makes this strategy so effective is that the ants dig in and anchor their spiny legs to tiny fibers and loops of the trichomes and mycelium. In the same manner that makes Velcro stick, this tactic gives ants incredible mechanical advantage (Dejean et al., 2010). Field measurements found that a single worker ant could reliably hold onto a weight of 8.0 grams for over 5 seconds, a load nearly 6,000 times their body mass (Dejean et al., 2010). To put that in perspective, this would be the equivalent of a 70 kg man holding a 444 ton weight. When working as a group, the ants can reliably catch locusts up to 13,350 times the weight of a single worker, stretch it out, cut it up, and transport the pieces back to the colony within a span of 4-10 minutes (Dejean et al., 2010). The neural mechanisms behind such organized and highly specific social behavior cannot be simply explained by fixed action patterns, yet without conscious executive function, it remains a mystery how the simple neural network of ants can trigger such behavior.

Conclusion

A review of the literature on ant behaviors that seem to be driven by higher level cognitive abilities yields two distinct conclusions. First, we know a great deal about what physiological adaptations ants possess to allow the execution of these complex behaviors, be they modular neural systems that give ants a celestial compass, the antennae that allow ants to maintain the optimum temperature of their fungus cultures, or combination of threadlike woven networks with the leg spines that allow arboreal ants to trap prey thousands of times their own body mass. What we don't understand is how ants, with such a miniscule brain, are able to execute such complex social behaviors that vary from trap setting, crop raising, selective assimilation and prioritization of several parallel streams of navigational information, path integration, and bi-directional teaching. The existence of just one of these behaviors by itself would be hard to explain in terms of the neural complexity present in ants, but the fact that such a widely ranging array of extraordinary behaviors can be accomplished in a brain over 100 million times smaller than our own defies our current understanding of the balance between neural capability and complexity. The second conclusion, therefore, that can be drawn from this account, is that there is dire need for further research into the neural systems that allow ants to accomplish these feats.

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About the Author: Matthew Roberts, '14, is a Neuroscience major. He is currently pursuing an honors research project with Dr. Klatzkin on social support, stress, and nociception. Matthew is the recipient of the Margaret Ruffin Hyde Award for research in psychology and a neuroscience summer 2013 research fellow. His research and quest for understanding why the world behaves the way it does are driven by an unbridled and passionate sense of curiosity that often gets him into trouble.

Aggression in Siamese Fighting Fish, *Betta splendens*

Adiha Khan
Rhodes College

Aggression is a conventional behavior of the Siamese fighting fish, Betta splendens. This behavior is so well recognized that B. splendens has become the most widely studied lab model for aggression. This aggressive behavior is highly modulated based on reproductive status, environmental variables, and observation of conspecifics. Female B. splendens do not usually engage in aggressive contact with other females or males, but male B. splendens can become aggressive towards females. Different physical actions constitute the male aggressive response, and a unique right-eye and left-hemisphere lateralization of the response is seen in B. splendens and other teleost fish. These behaviors are capable of changing in intensity towards different sexes with relation to reproductive status, reflected through courtship and bubble nest building. Aggressive B. splendens behavior is resistant to change by certain environmental variables, which is indicative of the hardiness of the aggressive response, even when exposed to adverse conditions. The aggressive behavior of B. splendens has been shown to be additionally resistant to change even after repeated aggressive interactions. However, behavior of B. splendens is subject to alterations when assessing the coloration, body condition, body size, and fighting success of a conspecific rival male as decisions on making aggressive contact are being made. Although aggressive behavior has been extensively studied in B. splendens, there is a scarcity of information on the early experiential effects of the exposure to aggressive and courtship behaviors. There is also a definite lack of information on the physiological origins and mechanisms driving the aggressive response.

Betta splendens is native to the muddy, flooded rice paddies of Southeast Asia (Jaroensutasinee & Jaroensutasinee 2003). *Betta splendens* (suborder Anbantoidei) have a unique feature: a lung-like labyrinth organ that allows oxygen to be directly extracted from the air and subsequently absorbed into the bloodstream (Rnic 1975). This ability to access oxygen contributes to the general resilience of *B. splendens*, as these animals are capable of handling the following situations: environmental fluctuations (e.g., pH: Rnic 1975; hypoxic water conditions: Kuperberg et al. 2009), as well as invasive trauma brought about by gonadectomies conducted for experimental purposes (Weiss & Coughlin 1979). The hardiness of *B. splendens* in response to adverse conditions, potentially death-inducing for other fish and animal species, obviously increases their chances of survival under many environmental conditions. This increased survivability is substantially important for *B. splendens*, considering the physical and metabolic costs associated with their highly aggressive lifestyle (Castro et al. 2006).

Aggression is present, but to a lesser degree, in some other teleost species (e.g., the Redtail splitfin, *Xenotoca eiseni*; the Eastern mosquitofish, *Gambusia holbrooki*) (Bisazza & de Santi 2003). *Xenotoca eiseni* live in small streams in Central America; these fish are not normally territorial, but can become aggressive when competing for females (Bisazza & de Santi 2003). *Xenotoca eiseni*, unlike *B. splendens*, is capable of coexisting in mixed-sex groupings in the same tank without engaging in such highly aggressive confrontations that would result in death (Bisazza & de Santi 2003). *Gambusia holbrooki* males become competitive with other males in order to defend their reproductive privileges; because they are not highly aggressive, males can be housed in mixed-sex groupings in the same space under the same conditions as *X. eiseni* (Bisazza & de Santi 2003). *Betta splendens* display a great degree of sexual dimorphism; males are larger, have longer plumage, and have brighter coloration than females (Jaroenustasinee & Jaroensutasinee 2003). In the wild, male *B. splendens* have short fins and dull green or brown coloration, but artificially selected fighting fish have long, flowing plumage and vibrant, elaborate coloration (Jaroensutasinee & Jaroensutasinee 2003). Although wild male *B. splendens* are naturally aggressive, undergoing intense artificial selection for coloration while being bred for commercial sale has caused the forms of *B. splendens* sold in local pet stores to be highly aggressive, in addition to being brightly colored (Dzieweczynski et al. 2009). Wild and captive-bred male *B. splendens* exhibit agonistic behaviors as they establish territorial boundaries through the production and defense of bubble nests, a collection of mucous-coated air bubbles, and they defend these nests, to which males attract females and subsequently raise two to three broods per breeding season. This agonistic behavior ensures that an individual male *B. splendens* has exclusive access to the resources found within the territorial boundaries, like mates and food (Jaroensutasinee & Jaroensutasinee 2003). Female *B. splendens* do not exhibit agonistic behavior towards other female conspecifics, and these females also do not usually exhibit aggressive behaviors towards males (Snekser et al. 2006). However, males will become aggressive towards females, especially after copulation, because the female may eat her own eggs; female consumption of the eggs would be a detriment to the male's reproductive fitness (Jaroensutasinee & Jaroensutasinee 2003). The male *B. splendens* expression of agonistic behavior through territoriality, competition for females, and rival interactions has caused this species to become the most widely used model in laboratories for the study of aggressive behavior (Weiss & Coughlin 1979; Bisazza & de Santi 2003; Lynn et al. 2007). This aggressive behavior in male *B. splendens* will be reviewed here, including its origin, physical aspects, response to environmental changes, and implications in interaction dynamics with conspecifics.

Origins of Aggressive Behavior

Weiss & Couglin (1979) investigated the importance of gonads in the aggressive behavior of *B. splendens.* It was predicted that because the *B. splendens* testes housed live, active sperm, sex hormones (e.g. testosterone) that mediate sexual and aggressive behavioral responses would also be located there (Weiss & Couglin 1979). Even after gonadectomy, *B. splendens* males continued to produce sufficient amounts of testosterone to maintain the production of agonistic responses, and they continued to engage in normal sexual behaviors (Weiss & Coughlin 1979). Because the effects of testes regeneration were minimized by conducting experimentation before full testes regeneration could be allowed, Weiss & Coughlin (1979) concluded that the production of testosterone that maintains secondary sexual characteristics and normal sexual behaviors occurs elsewhere in the body in addition to the gonads. The ability of *B. splendens* to produce testosterone in other regions of the body suggests that aggressive responses form an essential, conserved component of *B. splendens* behavior. However, a scarcity of literature exists to describe the origins of the production and release of these hormones, and their implications in aggressive *B. splendens* interactions.

Physical Aspects of Aggression

Actions involved in aggression

The aggressive interactions, primarily occurring among male *B. splendens*, consist of various behaviors, designed to ward off the male conspecifics (McGregor et al. 2001; Lynn et al. 2007; Kuperberg et al. 2009). The most prominent feature of the aggressive display is the extension of the fins and the branchiostegal membranes covering the gills, causing erection of the operculae (Kuperberg et al. 2009). Another facet of the display is vigorous swimming, including swimming with the lateral side of the body facing the opponent (Lynn et al. 2007; Kuperberg et al. 2009). While swimming in this manner, the males twist into an S-shape, beating currents of water against each other with their tails, an action termed tail-beating (McGregor et al. 2001). *Betta splendens* are also capable of brightening their scale coloration during aggressive encounters (Lynn et al. 2007). The mechanism for this color intensification has not yet been described by any published literature. If the encounter escalates in aggression, biting, where males lock jaws and engage in violent jerking and wrestling motions may occur (McGregor et al. 2001; Lynn et al. 2007). The loser of the interaction will either die from mortal wounds, or leave the fight with clamped fins and the submissive head-up posture (Castro et al. 2006).

Lateralization of aggression

Betta splendens and teleost fishes exhibit a unique direction of lateralization of the aggressive display, in comparison to other vertebrates, during these physical responses (Bisazza & de Santi 2003). A left-eye and right-hemisphere dominance is a marked component of lateralization in all classes of vertebrates, except teleosts, which show a marked right-eye and left-hemisphere dominance, measured during induced aggressive interactions in Bisazza & de Santi (2003). The difference in lateralization of aggression in *B. splendens* and teleosts suggests that there is a differential suite of cognitive functions that power the aggressive response mechanisms of these fish compared to other vertebrates (Bisazza & de Santi 2003). It was further suggested in Bisazza de Santi (2003) that the left-eye and right-hemisphere is specialized for spontaneous responses associated with emotions, and the right-eye and left-hemisphere is specialized for decision-making and the weighing of costs and benefits with a particular interaction. Bisazza & de Santi (2003) concluded that because *B. splendens* exhibits an asymmetry for the right-eye and left-hemisphere, costs and benefits are continuously being assessed before engaging in aggressive interactions in order to determine the most beneficial choice based on the opponent's physical appearance before bringing potential harm onto oneself.

Aggressive response serving as honest indicator of fighting fish quality

The behaviors that characterize the complete aggressive response of *B. splendens* are metabolicallycostly (Castro et al. 2006). After measuring the duration of operculae displays during an induced aggressive encounter between two male *B. splendens* separated by a transparent barrier, a winner, the individual maintaining the display for the longest period of time, was determined; the next day metabolic analyses of the winning and losing fish determined that the winning male experienced an increase in metabolism to compensate for used-up glycogen and energy stores, and to prepare the male for subsequent challenges (Castro et al. 2006). Because the agonistic display of *B. splendens* is difficult and metabolically costly to sustain during aggressive encounters, Castro et al. (2006) concluded that the erection of opeculae was an honest signal of a quality male, armed with fighting ability and good physical condition (Castro et al. 2006). However, under the hypoxic conditions studied in Kuperberg et al. (2009), it was found that despite differing body conditions, male *B. splendens* showed no significant difference in display rates. It was hypothesized that females prefer the male capable of longer displays during hypoxic water conditions, but this hypothesis was not supported, causing the researchers to conclude that the labyrinth, an air-breathing organ, makes opercular display an unreliable indicator of male quality (Kuperberg et al. 2009). Dzieweczynski et al. (2009) stated that male courtship behavior and agonistic displays (e.g. opercular gill flaring, fin-spreading) were found to increase when a rival male came into view. Because the signal served as a dual indicator to attract the female as well as to repel the intruder, the same way a blue tit sings a dawn chorus for the same dual purpose (Poesel et al. 2001), Dzieweczynski et al. (2009) concluded that this unique increase in courtship behaviors could serve as a reliable indicator of male quality in *B. splendens.*

Aggression and reproduction

Aggressive behaviors during courtship

Some of the same displays employed during agonistic interactions with another male are also exhibited when courting females (Dzieweczynski et al. 2006, 2009). However, what differentiates male-directed behavior and female-directed behavior by male *B. splendens* is associated with the frequency and intensity of the male display (Dzieweczynski et al. 2006). Males are more aggressive to rival males than to females, and males have lower 11-ketotestosterone levels after exposure to females; this difference in testosterone and aggression levels could be because highly aggressive behaviors associated with high levels of testosterone deter female mates (Dzieweczynski et al. 2006; Dzieweczynski et al. 2009). The suite of behaviors performed specifically for female attention include circling, leading, and zigzagging (Dzieweczynski 2009). These behaviors were shown to serve as an effective reproductive strategy in securing female mates, facilitating communication with multiple females simultaneously (Dzieweczynski 2009). This strategy also allows the male to alter his behavior to increase aggression if a rival male approaches (Dzieweczynski 2009). Unlike animal species that decrease or conceal courtship behaviors in the presence of a rival male (e.g. the Alpine newt, *Triturus alpestris*; the Italian agile frog, *Rana latastei*; the Satin bowerbird, *Ptilonorhynchus violaceus*; the Beaugregory damselfish, *Stegastest leucostictus*; the guppy, *Poecilia reticulata*; the common garter snake, *Thamnophis sirtalis parietalis*), B. *splendens* was found to court most vigorously when a screen was lowered so that a rival male was in view (Dzieweczynski et al. 2009). As described before, this increase in aggressive courtship behavior was found to serve as a dual indicator, courting and attracting females while repelling male intruders (Dzieweczynski et al. 2009). A possible explanation for this response was that becoming more vigorous in display could ensure that the focal male will keep the female's attention diverted away from the presence of the new male (Dzieweczynski et al. 2009).

Timing of aggression in correlation with nest building

The male *B. splendens* invests a large amount of energy into taking care of young and building and maintaining a bubble nest (Dzieweczynski et al. 2009). After the female lays the eggs, the male will fertilize the eggs and then implant them into the nest, where they will be constantly aerated by the male *B. splendens* until the eggs hatch (Jaroensutasinee & Jaroensutasinee 2003). Males with nests had lower 11-ketotestosterone levels, a hormone implicated in the aggressive responses in *B. splendens*, than did males without nests (Dzieweczynski et al. 2006). One possible explanation for why these males are less likely to engage in aggressive contact with rivals while building and maintaining nests could be that nest-building is energetically costly, so these males simply do not have the energy to engage in fighting (Dzieweczynski et al. 2006). Another explanation for this behavior is that in this particular moment the male is in a courtship phase and is more invested in reproduction than aggression, so he will not display extremely aggressive behavior in order to prevent deterring females (Dzieweczynski et al. 2006). One more explanation for this behavior could be that because territory has been established by the male, and he has invested energy in forming the nest, the risk of engaging in an aggressive encounter and losing this territory is too great (Dzieweczynski et al. 2006). It was also discovered that male *B. splendens* was more aggressive when the eggs were hatching from the nest than when they were laid in the nest; this escalation of aggressive behavior and paternal care could be because the young increase in reproductive value to the male as they age (Jaroensutasinee & Jaroensutasinee 2003). In the same study, male *B. splendens* were found to react aggressively, in escalating degree, to the following conspecifics: egg-laying females, gravid females, males; the male *B. splendens* reacted most aggressively to other males because these males presented the greatest danger to his nesting territory and to the well-being of his progeny (Jaroensutasinee & Jaroensutasinee 2003).

Impact of environmental variables on aggression

Weiss & Coughlin (1979) explained that *B. splendens* has been used in many studies with changes in environmental variables to observe changes in the aggressive response; these environmental variables have included water temperature, food, and the addition of chemical agents. The resilience of the aggressive response in *B. splendens* is apparent because of its continued expression despite manipulations with certain environmental variables (Rnic 1975; Lynn et al. 2007; Kuperberg et al. 2009).

pH changes

After exposure to water of pH ranging from 5.0 to 9.0, *B. splendens* males did not vary in aggressive display rate in response to a mirror image (Rnic 1975). The optimal pH range for *B. splendens* and some tropical species is between 6.9 to 7.0, so it is unusual for *B. splendens* to continue on with normal aggressive displays without a decrease in agonistic behavior or ill health effects from the pH imbalance (Rnic 1975). Possible explanations for the failure of pH change to reduce the frequency of aggressive displays include that *B. splendens* are protected from pH-induced coagulation of gill secretions due to an endogenous-pH-control system located in gill tissues, but this system has not been confirmed yet through experimental study; also, the presence of a labyrinth organ for air-breathing allows *B. splendens* to not have to rely solely on the gill system for respiration (Rnic 1975).

Effect of hypoxia on reduced body size and body condition

Opercular displays are known to increase oxygen consumption (Kuperberg et al. 2009). Whereas the control group for the study reported with male *B. splendens* in Kuperberg et al. (2009) received food every day, the experimental group only received food once a week, causing a reduction in body size, and presumably body condition. It was predicted that those males in poorer body condition would be most affected by hypoxia and exhibit less frequent aggressive displays, but this prediction could not be supported because there was no significant difference in the amount of time control males and experimental males engaged in opercular displays (Kuperberg et al. 2009). The researchers concluded, based on these findings that because of the presence of a labyrinth organ, the opercular displays of *B. splendens* do not serve as a reliable indicator of a male's quality and body condition (Kuperberg et al. 2009). However, the metabolic use of energy in an operculae-erecting action is no doubt costly and dependent to a degree to on energy reserves; thus energy should be more abundant, given that the animal is less stressed and well-nourished (Castro et al. 2006).

Impacts of fluoxetine

SSRIs (selective serotonin reuptake inhibitors) impact moods by preventing the neuronal reuptake of a hormone associated with happiness, serotonin, which consequently provides the body with excess serotonin; SSRIs are used for the treatment of major depression in humans, making this class of drugs very popular with commercial brand names like Prozac (Lynn et al. 2007). Fluoxetine is an antidepressant of the SSRI class, and can be used to treat depression in humans and also to reduce aggressive behaviors in animals; an epithelial form of uptake for fluoxetine was designed for the experimental study in Lynn et al. (2007) investigating the effects of this chemical on *B. splendens* aggressive display. Addition of fluoxetine was indeed capable of lessening certain aggressive behaviors in *B. splendens* (e.g. broadside displays, 90° turns) by approximately one-third (Lynn et al. 2007). However, other aspects of the display (e.g. total time with operculae extended, latency to first aggressive response) were not significantly different between the control and fluoxetine-treated groups. Dzieweczynski & Herbert (2012) recently concluded that fluoxetine alters behavioral consistency of aggression and courtship in *B. splendens.* Male behaviors towards females stayed relatively similar after treatment with fluoxetine, but male behaviors towards other males (e.g. aggression) were affected; this disruption of behavioral consistency with differing levels of influence for different males, underlines the impact of pharmaceutical products to the reproductive success and lifetime success of *B. splendens* (Dzieweczynski & Herbert 2012).

Conspecific interaction dynamics

Behavioral consistency of B. splendens

Even though *B. splendens* are normally characterized as aggressive, these fish can be separated into three further groupings: fighters, lovers, or dividers (Dzieweczynski et al. 2012). Fighters were defined as males that spent the greatest proportion of their time in aggressive interactions with other males; lovers were males that spent the greatest proportionof time courting and copulating with females; dividers were those males that spent relatively equal proportions of time courting, copulating, and fighting (Dzieweczynski et al. 2012).

Even after exposing *B. splendens* to repeated wins or losses in trials with rival male dummies (made from the casts of dead males), the behavior of the individual towards the male and female dummies (made from the casts of dead females) remained relatively the same as it had been prior to testing (Dzieweczysnski et al. 2012). It was concluded that fighters stayed fighters, lovers stayed lovers, and dividers remained dividers in most trials (Dzieweczynski et al. 2012). Although the number of tail beats and bites increased in all three *B. splendens* personalities after aggressive encounters and winning aggressive encounters, the fighting fish still seemed to retain the general character traits of their personality groupings (Dzieweczynski et al. 2012). This concept of behavioral consistency reiterates the hardiness of the *B. splendens* aggressive response. Behavioral consistency among the described *B. splendens* personality groupings also displays the complexities of aggression within the species, being exhibited in unique amounts with other behaviors by *B. splendens* individuals. To better understand how these behaviors and personality types arise differently in individual *B. splendens*, further study will need to take place concerning early influences with courtship and aggression (Dzieweczynski et al. 2012).

Response to conspecifics

Male *B. splendens* use information about themselves (e.g. coloration) to determine how their interactions will be with similarly or differently colored rivals (Thompson & Sturm 1965). When a red fish, blue fish, and blue-green fish were introduced to differently colored dummies (made from balsa wood and colored cellophane), the fish displayed in higher frequency towards those fish with coloration most unlike their own, and they displayed in lowest frequency towards those fish with coloration most similar to their own (Thompson & Sturm 1965). I postulate that a kin-preservation strategy could be an explanation for the low-frequency display and attack behaviors towards similarly colored fish; similar coloration may serve as an indication of shared genetic material, and thus relation. It was also found that *B. splendens* displayed the most to a mirror image even though their reflection would obviously display coloration identical to their own; it was concluded here that the mirror is a highly complex stimulus and thus does not elicit the normal, expected behavior from *B. splendens* (Thompson & Sturm 1965). No further study on *B. splendens* aggressive interactions based on coloration have been conducted. In addition to deciding confrontation based on conspecific coloration, male *B. splendens* may also base behaviors on relative extracted information from an interaction between two other males (McGregor et al. 2001). *Betta splendens* subjects that had seen a male win a particular interaction tended to respond to that male with more displays, more tail beats, more bites, and shorter latencies; this increase in aggression could indicate that the focal male considers the observed winning male to be a potential threat (McGregor et al. 2001). These findings provide support to the idea that *B. splendens* perceive the fighting ability, body condition, and body size of their conspecifics and that they use this information to make the most beneficial decisions about the exhibition of their own aggressive behaviors (McGregor et al. 2001).

Conclusions

The aggressive response exhibited by *B. splendens* is complex, highly modulated, extensively studied, yet still elusive. The different physical actions implicated in a full aggressive response have been well-defined and characterized. Additionally, the relationships of aggressive behaviors and 11-ketotestosterone levels with courtship and nesting have been well investigated. Probably most investigated, have been the effects of changing environmental variables on the aggressive response of *B. splendens* (Weiss & Couglin 1979). Furthermore, *B. splendens* exhibit behavioral consistency with their unique personality types, and these individual personalities and physical characteristics (e.g. fighting success, body size, body condition, coloration) and their influence on conspecific interaction dynamics have also been studied. Assessment of conspecifics and their fighting ability and body condition correlates with the lateralization of a right-eye and left-hemisphere dominance in *B. splendens*, where consequences and benefits are consistently assessed about a potential rival interaction before engaging in that interaction (Bisazza de Santi 2003). However, assessment of rivals is only a potential mechanism at work in the brains of *B. splendens*; this assessment mechanism is of course not limited to right-eye and left-hemisphere dominant teleosts, because this behavior is also exhibited by left-eye and right-hemisphere dominant vertebrates (e.g. Green anole, *Anolis carolinensis*). Future directions for research would involve investigating the components of the aggressive *B. splendens* response for which a scarcity of published scientific literature exists. The actual hormonal and physiological mechanisms of the aggressive response need study. To better understand how *B. splendens* personality types arise and how individuals exhibit differing amounts of courtship and aggressive behaviors, further study will need to take place concerning early influences with courtship and aggression (Dzieweczynski et al. 2012). The study of the physiological origins of aggressive behavior and early influences on behavior would work towards better describing behavioral beginnings and the full implications of these behaviors for the *B. splendens* lifestyle of the present.

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About the author: Adiha Khan, '13, is Biology major who will be starting with dental school at the University of Tennessee this July. She had been actively involved during her four years with a study entitled "An Integrative Assessment of Snake Parasitism in an Urban Old-growth Forest" with Dr. Luque de Johnson. She has served as president of the American Chemical Society, vice president of the Omicron Delta Kappa honors society, and president of the Tri Beta biology honors society. She loves animals and has had the chance to better explore this interest through her biology classes.

Schizophrenia and Criminal Behavior as a Vulnerability-stress Model

Grace Mosley
Rhodes College

Although pleading "not guilty by reason of insanity" is a common variety of not guilty plea on popular television crime dramas, in reality it is much less frequently invoked in criminal hearings. But despite the rarity of the usage of the insanity plea, the relationship between mental illness and criminal behavior have been studied extensively in a variety of different societies. Schizophrenia has been of particular interest forensically because of its relatively strong symptomology, strong genetic associations, and historical link to being associated with antisocial and criminal behavior in popular culture. But while some subtypes of schizophrenia may correlate with certain varieties of crime, a fatalistic view of this relationship is inappropriate. Instead, the links between schizophrenia and criminality should be viewed as a vulnerability-stress model, in which schizophrenia serves as a vulnerability to behaving criminally and such behavior is substantially more likely occur when a secondary stressor agitates the schizophrenic. Thus, in order to reduce convictions among schizophrenics, mental health screenings should be performed in early life to identify at-risk individuals.

There have been a variety of data regarding whether or not schizophrenics show a greater crime rate than the general population (Modestin & Ammann 1996). These differences are largely due to large methodological errors. For example, some studies' results were likely more of a product of when data was collected than the actual impact of schizophrenia. With patients upon admission to treatment being compared to when they were discharged, the discharged patients act as a selected group, for their rates of violence must be lower than at admission, or they would not be discharged (Walsh et al. 2002). Others failed to account for many schizophrenics' extended time in hospital, which limits the time during which they are at risk of committing a crime, and mistakenly concluded that differences in criminality between schizophrenics and the general population were not significant (Wessely et al. 1994). Some studies did not differentiate between schizophrenia and other forms of mental illness, making it difficult to determine a clear relationship between the condition and crime, as varying rates of diverse mental disorders caused the data to be inconsistent (Modestin & Ammann 1996). Often, inappropriate controls were used, with neurotypical individuals not controlled for socio-demographic data, and few studies comparing criminality or violence in schizophrenia within the group of those with mental disorders or distinguishing between different types of schizophrenia (Walsh et al 2002, Modestin & Ammann 1996).

Different mental disorders exhibit a variety of effects on those they affect, and as a result can be predicted to produce differing rates of criminal conviction. A study which was controlled for sex, age, time at risk for crime, and socio-demographic measures and in which controls were diagnosed with a non-schizophrenic, non-psychotic mental disorder, showed an increase in total crime in female schizophrenics, but only an increase only violent crime in male schizophrenics, with schizophrenia leading to the highest increase in convictions over controls in affected Afro-Caribbean men (Wessely et al. 1994). However, in examining predictors of first conviction, although schizophrenia exerted a significant effect, it had a weaker association than ethnicity, gender, substance abuse, and age of onset of mental disorder (Wessely et al 1994). Thus, while schizophrenia significantly effects criminal convictions, environmental factors likely exert an additive effect within the mentally ill classification. This suggests that while schizophrenia has a greater risk for female criminality and male violent criminality, it still requires additional stress from substance abuse, longer suffering from illness (a result of early onset), or possibly ethnic disparities in treatment to lead to greater rates of criminal behavior.

Schizophrenia not only has a varied effect from other mental disorders, but it is also a spectrum disorder, so it is not unrealistic to expect different types and rates of crime enacted by different sub-groups of schizophrenics. When highly-matched non-mentally ill controls and schizophrenics as a whole were compared, there was no significant difference in percent with criminal records, however, schizophrenics were five times more likely to commit violent crime and 2.5 times more likely to commit property crime than controls (Modestin & Ammann 1996). Additionally, when broken down into schizophreniform disorder, acute schizophrenia, and chronic schizophrenia, further differences were seen. Those with schizophreniform disorder were more likely than controls to commit property and drug crimes, acute schizophrenics more likely to commit property and violent crimes, and chronic schizophrenics showed only a reduction in traffic crimes from controls (Modestin & Ammann 1996). In addition, while risk of aggression in the six months following discharge was not increased by past substance abuse or manifestation of antisocial personality disorder, positive symptoms (such as psychosis) led schizophrenic individuals to be five times more likely to be violent during this time frame (Hodgins et al 2003). Further, in schizophrenics, those that were psychotic as measured by the PCL-R test were convicted of more crimes and more violent crimes, and first convicted at earlier ages than non-psychotic schizophrenics, and psychosis was strongly associated with violent recidivism (Tengström et al 2000, Tengström et al 2004). Not only does psychotic schizophrenia correlate with crime, but it also aligns with particular personality traits. Psychotic schizophrenics have less self-control, and are more aggressive, coercive, and hostile than their non-psychotic counterparts (Fullam & Dolan 2006). Interestingly, an in-group effect of increase in total and violent crime was seen in non-mentally ill psychotic individuals as well (Tengström et al 2004). This suggests that psychosis plays a strong role in criminality, and can have an additive effect with schizophrenia, indicating that strong positive symptomology can perhaps be interpreted as a "secondary stressor" to the schizophrenic. Therefore, when assessing risk for criminal behavior in schizophrenics, the specifics of the particular schizophrenia must be taken into account.

In addition to personally having schizophrenia, a family history can also put individuals at risk for criminal behavior. A potential genetic link between schizophrenia and criminality was observed in a study of 47 offspring of schizophrenic mothers raised by foster parents, in which 5 developed schizophrenia, and 23% were imprisoned for violent crimes (Tehrani et al 1998). It should be noted that as more offspring developed violent behaviors than schizophrenia, individuals should not be considered at risk of committing such crimes only after presentation of schizophrenia symptoms, but from birth if determined a genetic risk. Recidivism was also impacted by status of parents, as among adoptees with a violent conviction before age 18, 45% had biological parents admitted to a mental hospital, and 68% of those convicted of two or more violent offences had mentally ill biological parents (Tehrani et al 1998). Thus brain dysfunction observed in schizophrenia is likely to serve as a source of violent criminal behavior, with more violent offenders having stronger genetic sensitivities to exhibiting mental illness and violent behavior.

An important environmental factor that has been suggested as a cause of criminality is substance abuse. Substance abuse has been shown to increase risk of acquiring a criminal record for the mentally ill in general (Wessely et al 1994), and specifically schizophrenics, with 68.1% of schizophrenics with a substance abuse disorder convicted of crimes (Wallace et al 2004). It appears possible that what seems to be the affect of schizophrenia on crime rates could in fact be due to substance abuse, as schizophrenics show relatively high rates of substance abuse. However, in schizophrenics with and without substance use disorders, no difference in total crime or violent crime was observed (Tengström et al 2004). Despite the lack of independent effect of substance abuse on criminality, in schizophrenics with psychosis and substance abuse disorders that started before age 18, the average number of crimes committed per year at risk was more than double that of psychotic schizophrenics whose substance abuse disorder began after age 18 (Tengström et al 2004). Additionally, schizophrenic fire setters who were also alcoholics had the longest criminal records among schizophrenic fire setters, and repeat offences were common (Repo & Virkkunen 1997). This indicates that for substance abuse to act as a strong secondary environmental factor in criminal behavior in schizophrenics, it must occur while the brain is still developing, but that it can meaningfully affect the criminality of schizophrenics.

In recognition of the effects of genetic predispositions, subtypes of schizophrenia, personality traits, and environmental factors in the risk of criminal behavior (specifically violent criminal behavior) in schizophrenics, the way in which such cases are treated must be modified. Because the interaction of schizophrenia and these variables fit a vulnerability-stress model, recognition of risk factors and prevention must be emphasized over mass-institutionalization and post-conviction treatments. A drastic reduction in institutionalization has taken place over the last several years, and while schizophrenics convicted of crimes increased significantly, so did the over all crime rate (Wallace et al 2004). Therefore, the substantial changes in institutionalization practices had no significant effect on conviction rates for schizophrenics, and it can be concluded that mass-institutionalization of schizophrenics is not an effective means of reducing violent behavior. Additionally, higher recidivism was seen in schizophrenics, particularly in schizophreniform disorder patients (Modestin & Ammann 1996). This suggests that because these individuals do not have DSM-classified schizophrenia, they may not have received appropriate treatment for their mental illness. Left untreated, it is not surprising that many of these individuals turn to substance abuse or develop criminal behavior. Finally, early expression of impulsivity, sensation seeking, and antisocial behavior and an underactive autonomic nervous system can be seen as early as two years old, and remain stable throughout life (Tengström et al 2004). Thus, personality screening could easily be implemented in childhood, especially for at-risk groups, and appropriate pre-emptive treatment for mental illness provided, in an effort to prevent manifestation of violent behavior.

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About the author: Grace Mosley, '14, also contributed to this publication as an editor. Read about her and the rest of our editorial staff on page 2.

Culture Wars: Elephant Behavior and the Consequences of Human Interaction

Dedicated in the memory of Dr. Rosanna Cappellato

Stephen Leavelle
Rhodes College

Humans have long lived in awe of the elephants and not without cause. The African elephant (*Loxodonta africana*) is the largest of all land animals, with adult males reaching three meters at the shoulder and 5000 kilograms (Estes 1991). Equally striking about elephants is their acute intelligence, unparalleled amongst digitigrade herbivores. Elephants have shown evidence of extreme long-term memory, complex social relationships, anticipatory planning, insight, self-recognition, and a concept of death (Poole and Moss 2008). These traits allow elephants to live without fear of any natural predators. However, the increasing human population of Africa (currently about 500 million, five times the population in 1900) (Kiiru 2008) and the continuing conversion of natural habitat into cultivated land is increasing the amount of contact between elephants and humans. Wild elephants in protected areas continue to practice natural behaviors but do so now in a very different ecological context than in the past. The current trends in human-elephant interaction threaten to disrupt and eliminate the behavior patterns and culture of wild elephants.

Elephant Behavior in the Wild

Physiology and Behavior

The behavior of elephants is centered on eating. In order to achieve their characteristic size, elephants are adapted to consume a wide range of plant material. They have one molar in each quadrant of the jaw, which moves with a forward and backward action rather than the side-to-side motion characteristic of ruminants. This shearing action allows elephants to grind woody plants as well as grasses, making their diet the most catholic of any herbivore (Estes 1991). Consequently elephants are able to live in any environment with sufficient food and water, varying from montane forests and low-lying swamps to wooded grasslands and even subdesert ecosystems. This adaptation also allows them to be seasonally selective in choosing the most palatable foods, grasses and herbs in the dry season and woody plants in the wet season (Estes 1991). However, despite the diversity of their diet, elephants receive very little nutritional value from their food, assimilating only 44% of their intake (compared with 66% in ruminants) (Estes 1991; Fraser 2009). Moreover, immense body size requires each elephant to consume 4-6% of its body mass every day, a task that requires sixteen hours of feeding (Estes 1991).

Besides their molars the only other teeth elephants have are their tusks, modified incisors that are present in both males and females and which grow continuously throughout an elephant's life. By the age of sixty an elephant's tusks can weigh, on average, 61 kilograms in males and 9.2 kilograms in females (Estes 1991; Shorrocks 2007). Elephants use their tusks for stripping bark and, amongst males, for intimidating sexual competitors (Estes 1991). Other physical traits such as the trunk also factor heavily in elephant behavior. Acting effectively as an elephant's 'fifth limb', the trunk is composed of more than one hundred thousand muscles and is both immensely strong and yet capable of precise movements using the two finger-like "lips" at the end (O'Connell 2007). It is used as a siphon while drinking, allowing elephants to consume up to 100 liters of water at a time and up to 227 liters in a single day (Estes 1991). The trunk, along with the elephant's large ears, account for its acute senses of smell and hearing (Estes 1991; Hendrick Munembone, personal communication).

Social Behavior

As highly social animals, elephants communicate through various means. Tactile communication manifests in acts such as greeting, caressing, and slapping (Estes 1991). Elephants also communicate vocally—trumpeting when excited, growling contently while feeding, roaring when threatening a predator, and rumbling at frequencies below human hearing (10 hertz) that can be felt as vibrations miles away (Estes 1991). Additionally, elephants gain information about one another chemically by smelling urine, feces, and gland secretions (Sukumar 2008).

Elephants have a strong sexual dimorphism (Sukumar 2008). Consequently, males and females operate in separate herds (Shorrocks 2007). Females form larger groups, the most common of which are matriarchal herds. The matriarch is almost always the oldest and most experienced female, and the herd consists of related females and their offspring (Estes 1991). The matriarch has experiential knowledge of where to find food and water and leads the herd between feeding sites (Estes 1991; Fraser 2009; Shorrocks 2007). The maximum lifespan of elephants is around sixty years (Estes 1991), which in females extends well past reproductive age.

Although this is unusual for animals, it befits a species for which, like humans, experience is a valuable asset (Estes 1991; Fraser 2009; Shorrocks 2007). When a matriarch is too weak or sick to lead, she will leave or be abandoned by the herd, and the next-oldest female will take her place (Estes 1991). However, if the matriarch is injured unexpectedly, the group will not abandon her but will remain until she is able to continue, sometimes placing themselves at great risk. This is evidence of altruism amongst elephants (Estes 1991). Although herds tend to range 2-24 in number (9-11 on average), under conditions of range compression or hunting pressure, multiple herds have been known to coalesce into larger aggregations, which historically have been migratory (Estes 1991; Sukumar 2008). Even when not aggregated, herds travel great distances to seek food and water, covering ranges of up to five hundred square miles (Fraser 2009).

Bull elephants operate under very different social circumstances than females. By 12 or 13, male calves are pushed out of their maternal herd. From this point on they are largely solitary but may join a herd of other males (Estes 1991). Like most sexually dimorphic species, elephants have a polygynous sexual model (Sukumar 2008). Bulls undergo a period of heightened hormonal activity called "musth" during which they seek out receptive females for breeding (Estes 1991; Fraser 2009; Shorrocks 2007). Elephants are capable of determining the reproductive status of females by transferring scent particles from the genitalia to the vomeronasal organ in the roof of the mouth (Estes 1991; Hendrick Munembone, personal communication). The agitated activity of the females during estrus often attracts additional males, which leads to (rarely lethal) sparring amongst competitors (Estes 1991).

Learning Behavior

The most important function of the matriarchal herds is the protection of calves, which are more vulnerable to predation (5-15% mortality annually compared to 2-3% amongst adult females) (Sukumar 2008). Cows generally first conceive at 10-11 years old, calving every 4-9 years based on population density and nutrition (Estes 1991; Sukumar 2008). After a gestation of twenty-two months, cows bear one calf, sometimes two (Estes 1991; Shorrocks 2007). A calf remains within a few meters of its mother for up to ten years (Estes 1991). During this time the calf learns valuable survival skills from its mother and other adult females. The most important of these is learning which food sources are the most palatable, a knowledge the calf gains by sampling food from the mouths of other elephants and even eating fresh feces. In fact, through their first five years calves spend 15.8% of their time exploring the food intake of other elephants (Poole and Moss 2008). One skill that surprisingly does not come naturally to a calf is the ability to use its trunk. It learns the proper motor technique over years of practice, but until that point, the trunk can more often be an impediment to eating and drinking (O'Connell 2007; Poole and Moss 2008).

As elephants reach sexual maturity, they continue to learn new skills. Young females learn caretaking skills by helping to raise calves. The importance of learning these skills through experience is apparent in the fact that the highest infant mortality is among first-born calves (Poole and Moss 2008). When young females first become estrous, older females demonstrate estrous positions to them, even when not in estrus themselves (Poole and Moss 2008). Similarly, young bulls engage in play with other males, learning skills for later challenges of sexual dominance. Young males are also often incapable of differentiating the odors of females in estrus from those who have recently given birth, so an experienced male will occasionally allow young males to come as close as five meters from the receptive female in order to learn the correct scent (Poole and Moss 2008).

The impact of elephant behavior is not limited to only within the species. Elephants are second only to humans in the capacity they have to alter their environment (Estes 1991). In their search for food and water, they create trails to water sources and wells that bring ground water to the surface. Their foraging also leaves leftovers for animals that cannot normally access browse (Estes 1991). They are also responsible for the spreading of seeds through their dung (Fraser 2009; Sukumar 2008). The most dramatic environmental effect is among bulls that have mastered the skill of knocking over trees to reach preferable browse. This habit causes whole patches of woodland to be transformed into grassland, altering the dynamics of the ecosystem (Estes 1991; Fraser 2009; Sukumar 2008; Varner 2008). Because of this capacity, some experts consider elephants a keystone species of their ecosystem (Fraser 2009; Wemmer and Christen 2008).

Human-Elephant Conflict

Reserves and Systemized Culling

The destruction of trees is a normal behavior amongst elephants, and across a large area it increases habitat diversity by introducing mild disturbance (Estes 1991). However, as human expansion has led to the cultivation of more and more land in Africa, elephants are increasingly cut off from their historical range and migratory routes (Kiiru

2008). At the same time, elephant populations have decreased due to poaching and the ivory trade (approximately 1.3 million elephants in the early 1980s that had declined by 80% in 1990) (Estes 1991). Nonetheless, the elephants that remain are increasingly concentrated in fenced reserves, where their natural capacity for habitat conversion is magnified (Estes 1991; Varner 2008).

Because reserves house a variety of endangered plants and animals in addition to elephants, the natural behavior of wild elephants becomes a threat to the survival of other species (Wemmer and Christen 2008). In Kruger National Park in South Africa the threat posed by elephants towards endangered tree species was among the justifications given for culling much of the Kruger elephant population (Fraser 2009). An additional reason was that the South African elephant population has been increasing rapidly (250,000 elephants in 2009, projected to be 400,000 by 2020) (Fraser 2009). However, Kruger's specific problem was largely the park management's own fault. The creation of artificial water sources in the park to increase tourism prompted an elephant baby boom in the 1950s, as the plentitude of resources encouraged higher reproductive rates (Fraser 2009). Consequently 14,000 elephants were culled from 1967 to 1994 in order to maintain a population of 7000 in the park, but studies showed that the survivors had an even higher birth rate than before (Fraser 2009; Varner 2008).

The failure of culling to control elephant overpopulation in Kruger was a factor in the creation of the Great Limpopo Transfrontier Park between South Africa, Mozambique, and Zimbabwe (Fraser 2009). Such transboundary parks remove physical barriers that restrict the movement of wide-ranging species such as elephants (Wasser et. al. 2009). In 2001 a group of seven elephants was ceremonially allowed to walk from Kruger in South Africa across the border into Coutada 16, a former hunting concession in Mozambique. However the elephants had strong memory and, recalling the danger of the area, quickly migrated back into Kruger. Since then the flow of elephants out of Kruger has been slow, prompting some management to want to reinstate culling, but ecologists argue that the system will balance out in time (Fraser 2009).

Poaching for Ivory

The elephants' fear of areas like Coutada 16 is no doubt ingrained through the long history of elephant hunting, specifically for the highly valued ivory in their tusks. Tusks have been a trait of elephants and their ancestors since at least the Oligocene (Sukumar 2008), and ivory figurines have been found dating to the Paleolithic era (Walker 2009), making ivory one of the oldest substances sought solely for decorative value. The colonization of Africa by Europeans from the sixteenth century onward opened up a huge market for tusks. From 1850 to 1910 Britain alone imported five hundred tons of raw ivory annually (Walker 2009). Despite the invention of alternative plastics in the early twentieth century, the legal ivory trade continued at a high rate even with dwindling elephant populations, so that by 1979 populations were being removed at a rate of 7.4% annually, exceeding the optimal birth rate of 6% (Walker 2009; Wasser et. al. 2009). Consequently in 1990 the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) listed the African elephant in Appendix I, prohibiting the international trade of elephants or any of their parts (Shorrocks 2007).

The ivory ban, despite its intent, has been far less successful than anticipated. In fact, the demand for ivory from East Asian countries and the subsequent illegal poaching is removing elephants at a rate of 8% as of 2006—higher than the removal rate that incited the ban (Wasser et. al. 2009). In the same year 25,000-29,000 kilograms of ivory were seized while leaving Africa en route to Asian destinations. The legalization of the sale of ivory in China in 2008 has opened up a legal channel for illegally obtained ivory to be sold (Colin Craig, personal communication). The issue is complicated by the desire of southern African countries to sell their ivory stockpiles legally. As of August 2011 the African elephant populations of Botswana, Namibia, South Africa, and Zimbabwe have been placed in Appendix II, permitting the legal trade of hide, hair, and, particularly, tusks (personal lecture notes, 2 February 2012).

The continued killing of elephants through culling, poaching, and trophy hunting has severe effects on the behavior of wild elephant populations. The victims of poaching are most often matriarchal herds, which are larger and easier to find than solitary males, even though the males carry more ivory (Wasser et. al. 2009). Poachers manipulate the elephants' altruism and target the matriarch first. Without their leader, the other females fall into disarray and will often allow themselves to be shot rather than leave their fallen companions (Estes 1991; Wasser et. al. 2009). The unfortunate survivors of such attacks are the calves, who are spared because they carry no ivory. Without the familiar social structure to nurture and teach, orphaned calves grow up without learning vital skills for survival and reproduction. Poole describes the effect in calves orphaned by culling: "Early disruption of attachment such as occurred during culls where adults were killed and infants were spared can result in social trauma that may affect the physiology, behavior, and culture of elephants over generations" (Poole and Moss 2008).

Increasing Human Population

Perhaps the simplest but most significant source of human-elephant conflict is the increase of human population and the consequent habitat loss for elephants. As elephants' natural habitat is increasingly fragmented, elephants are brought more and more frequently into contact with cultivated land and the farmers that tend it (Sukumar 2008). Driven perhaps by territorial fidelity or long memory of the routes to water sources, elephant traverse farmland that was previously free range (Sukumar 2008). The mere presence of an elephant on cultivated land is often enough to incite hostility on the part of the farmers, who perceive elephants as pests (personal lecture notes, 2 February 2012). Due to their size, unpredictability and capacity to cause severe damage on raids, elephants are often targeted and blamed for the work of other pests. One survey at Kasungu National Park in Malawi between January and June 1990 found that there were fifteen complaints about elephant raids while the mean frequency of raids was 3.01 per month. By comparison, there were only five complaints about bushpigs, who had a mean raid frequency of 9.63 (Shorrocks 2007).

Although elephants are often excessively vilified by rural farmers, their fears are not wholly unfounded. Elephants have been known to destroy homes, eat crops, and demolish water pumps (Fraser 2009). Elephants' predilection for crops is understandable, since cultivated plants have higher nutritional value than an elephant's natural diet, and elephants select crops that are analogous to their wild diet (Sukumar 2008). The propensity of elephants to damage human water sources is most problematic in arid areas where very few reliable water sources exist, and both elephants and humans put high demand on the same limited supply of water. The Kunene region of Namibia, part of the Namib Desert, is one such area. The desert elephants that inhabit the region have been known to destroy pumps and dig up buried reservoirs in order to reach the water supply. EHRA (Elephant-Human Relations Aid), an NGO operating in the region, has garnered international support to construct stone walls around farmers' water tanks to prevent destruction and alleviate conflict (Hendrick Munembone, personal communication).

Similar measures have been taken elsewhere in Africa to counter elephant raids on crops. In Kenya the elephant population had been reduced from 167,000 to 20,000 over the course of fifteen years, and the remaining populations were kept in small protected areas. Following the 1989 ivory ban, elephants began to venture outside of their reserves and came into conflict with neighboring farmers. The Kenyan Wildlife Service (KWS) encouraged villagers to dig moats around their field. Unfortunately the elephants were able to refill the moats and cross them, requiring constant surveillance and maintenance. Over 1000 kilometers of electric fencing were installed with variable success. In some cases adult elephants were able to push apart the live wires with their tusks and force young elephants through the gap. Moreover, farmers were not equipped to perform maintenance on damaged fences. The most effective method has been to border fields with rows of chili pepper plants or to build barriers coated in capsicum (the spicy chemical of peppers) because elephants are repelled by it (Kiiru 2008). In addition to preventive measure, organizations such as EHRA have counteracted human-elephant conflict through education, and they have successfully prevented the culling of perceived 'problem animals' by purchasing hunting licenses from communities (Hendrick Munembone, personal communication).

Despite all of these efforts, the severity and frequency of elephant conflicts seems to be worsening (Fraser 2009). This may be due, in part, to the effects of poaching. Herds that have lost their matriarchs have been observed to become disoriented and wander out of protected areas into farmland. When they come into contact with farmers, they assume them to be poachers, and conflict ensues, usually resulting in the deaths of the elephants (Wasser et. al. 2009). In situations such as these, all of the forms of interaction between humans and elephants are operating simultaneously to disrupt the behavior of remaining wild elephants.

Conclusions

The long history of humans and elephants has been characterized by conflict, as both species exhibit advanced intelligence, place great demand on resources, and have a strong capacity to alter their environment. Because humans outweigh elephants in all three qualities, the 'culture war' between human expansion and what Poole and Moss call the "culture of elephants" (2008) has thus far been one-sided. The invention of acceptable alternatives to ivory leaves no justification for the continued treatment of elephants as a natural resource. Yet the most common ways in which humans directly interact with elephants—displacing, culling, poaching, orphaning—serve only to accelerate the demise of wild populations and to disrupt the fragile social structure that comprises elephant culture. Without the maternal social structure, young elephants fail to learn vital skills and become increasingly dependent on humans for their survival.

The continued existence of wild elephant populations will only be possible as long as experienced generations are able to teach skills to their progeny, requiring ample space, sufficient water and food, and freedom of social interaction without disruption. Transboundary parks allow elephants and other wildlife to move freely through large areas, practicing natural behaviors without coming into conflict with humans or other elephants. The further expansion of conservation areas will alleviate the problems of overpopulation in reserves and the damage to social structure that inevitably results from culling or conflict with local human populations. The further education of rural farmers is also necessary to create successful barriers around farmland, because conflict will be unavoidable as long as elephants are able to move freely in cultivated areas. It is clear from the current trends that human and elephant populations will never be able to successfully co-habitate without conflict. Therefore only if barriers remain between the two species and if humans are content to remain as distant observers will it be possible for elephants in the future to still practice natural behaviors.

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About the author: Stephen Leavelle, ’14, also contributed to this publication as an editor. Read about him and the rest of our editorial staff on page 2.

Trypanosoma-induced Behavioral Changes in the Tsetse Fly, Mammals, and Humans

Lan Tran
Rhodes College

Human African trypanosomiasis, or sleeping sickness, is a debilitating disease that is one of the most common among the world's poorest populations. Classified as a tropical neglected disease, sleeping sickness mostly affects African sub-Sahara, but also affects those in South America and India. The causal agent of the disease is a single-celled, flagellate protozoa in the Trypanosoma *genus. The genus contains two subspecies capable of infecting humans*, Trypanosoma brucei gambiense *and* T. b. rhodesiense. *This protozoa is able to infect its vector, the tsetse fly, and a wide range of livestock and humans. Research has supported mechanical and biochemical alterations of vector and host as a means to further enhance virulence. To increase transmission and thus reproductive success, these parasites have acquired mechanisms to induce lethargy, changes in feeding behavior, weight loss, sleep disturbances, and manic behavior within their hosts. The manipulation hypothesis is not a novel concept, as other species of parasites exhibit the ability to alter host behavior, and such manipulation is thought to be an example of convergent evolution. This review aims to take a closer look at the manipulative behaviors associated with* Trypanosoma *infection in the parasite's vector, the tsetse fly, and its multiple hosts. Examining the distinct features of manipulation may provide clinicians with more effective resources to diagnose and properly treat trypanosomiasis in its early stages as opposed to the more fatal late-stage.*

Introduction

Trypanosoma brucei is a species of flagellate protozoan and are vector-borne parasites responsible for human African trypanosomiasis, or sleeping sickness (Kennedy 2004). Trypanosomiasis occurs worldwide, but most cases occur in sub-Sahara Africa (Kennedy 2004). All species of this genus infect mammals, but only two subspecies are responsible for the human infection—*Trypanosoma brucei rhodiense* and *T. b. gambinese* (Fevre et al. 2006). Tsetse flies belong to the *Glossina* genus and are the blood-feeding vectors that infect and transfer protozoa from one host to the next through their saliva (Van den Abbeele et al. 2010). Within the tsetse fly, trypanosomes undergo a complex reproductive cycle that begins in the fly's midgut and eventually ends in the salivary glands in which the protozoan's metacyclic form waits to infect mammals during the fly's next blood meal, (Fig. 1, Vickerman et al. 1988). The timeframe from the tsetse fly's initial infection to the fly's ability to infect mammals is approximately 3-5 weeks (Vickerman et al. 1988).

The manipulation of animals that carry and transmit *Trypanosoma* is of medical and economic importance, as trypanosomiasis is a tropical neglected disease that adversely affects more than 300,000 lives a year and places over 60 million at risk for contracting the disease (Kennedy 2004). These tropical neglected diseases consist of major debilitating and devastating illnesses that infect the world's poorest populations, often times causing chronic infection (see review by Hotez et al. 2007).

The two infectious subspecies of *Trypanosoma brucei* (*T. b. gambiense* and *T. b. rhodiense*) cause the human Gambian sleeping sickness and Rhodesian sleeping sickness, respectively, and the bodily disease is not contagious due to lack of an infected host (Knight 1971). Different geographical patterns exist between the occurrences of the two infectious subspecies of African trypanosomiasis (Knight 1971). The Rhodesian form is more localized in eastern Africa, whereas the Gambian form is more prevalent in western Africa (Kennedy 2004). Differences in clinical manifestations between the two forms have also been cited, with prolonged and chronic infection seen only with the *T. b. gambiense* subspecies (Kennedy 2004; Kuepfer 2011). In contrast to the Gambian infection, the Rhodesian course is more rapid with less pronounced sleeping involved (Knight 1971). Unfortunagely, only a few trypanosomes are necessary to initiate infection, with *T. b. gambinese* more easily contracted but less lethal compared to *T. b. rhodiense* (Knight 1971).

General trypanosomiasis transmission includes four main players: 1) infected host 2) potential host 3) reservoir, and 4) vector (Fig. 2, Knight 1971). In addition, the distribution and frequency of sleeping sickness is not based solely on vector presence, but also on the interrelationships between the disease, environmental conditions, and the behavioral patterns of the vector, reservoir, and host itself (Knight 1971). The Rhodesian subspecies is a zoonotic form of *T. brucei*, as it is capable of infecting livestock and wildlife (Fevre et al. 2008). These animals serve as both a reservoir and a food source for the protozoan (Knight 1971; Fevre et al 2008). Due to heavy reliance on livestock as means of livelihood, the zoonotic form has devastating impact on economic and social costs, significantly in poorer countries such as Africa and India (see review by Stitch et al. 2002).

In humans, the clinical manifestations of trypanosomiasis include two distinct stages: early and late (Kennedy 2004; Chevrier 2005). Early-stage complications include skin lesions, chancre, and multi-system problems, whereas late-stage symptoms include tremor, motor weakness, and sleep disorders that usually end in coma (Fevre et al. 2008).

Host manipulation is not a novel phenomenon only present in the *Trypanosoma* genus; species manipulation is thought to be a form of convergent evolution. This is supported by observations from malaria-infected mosquitoes as well as trematode and acanthocephalan-infected vectors that participate in host manipulation to enhance parasitic transmission (Hurd 2003; Lefevre et al. 2007). One of the most pronounced changes in the tsetse fly is its feeding behavior after *Trypanosoma brucei* infection (Abbeele 2010; Hurd 2003). This review paper will examine the supporting evidence of *Trypanosoma* manipulation of vector feeding and host physiologic behavioral modifications as a result of parasitic infection.

Feeding Behavior in Tsetse Flies

Tsetse flies belong to the genus *Glossina* and are characterized as obligate blood feeders. As primary vectors of sleeping sickness, their behaviors are medically important due to their role in transmission of the parasite to the potential host. To determine whether or not feeding behavior of the tsetse fly was altered due to trypanosome infection in the salivary glands, Van den Abbeele et al. (2010) compared the feeding efficiency of infected and non-infected flies and found that those infected spent more time feeding than non-infected flies. To further explain the phenomena, Van den Abbeele et al. (2010) attributed the significant difference in feeding time to prolonged probing behavior demonstrated by infected flies before feeding. The prolongation of feeding in infected insects may not be unique to T. brucei infections, as other protozoan infections appear to have a similar effect (Botto-Mahan et al. 2006). Chagas disease, a protozoan-induced sickness caused by *Trypanosoma cruzi*, is transmitted from vector to host via the blood-feeding kissing bug *Mepraia spinolai* (Botto-Mahan et al. 2006). Researchers have observed that infected bugs were able to detect and position themselves towards a potential host twice as quickly as uninfected bugs, and the bite rate was 45% above the rate of those uninfected bugs (Botto-Mahan et al. 2006).

Further research as been conducted to analyze the salivary composition of *Trypanosoma brucei*-infected and non-infected tsetse flies (Van den Abbeele et al. 2010). Researchers used tricine-SDS-PAGE to break down components of the flies' saliva into distinct groups (Van den Abbeele et al. 2010). They found that saliva of infected flies had 70% lower protein products than did saliva of non-infected flies, and some proteins were completely absent from the infected group (Van den Abbeele et al. 2010). As a result of these findings, Van den Abbeele et al. (2010) proposed that the high density of active trypanosomes may have caused physiological stress and thus, suppressed the production of those salivary proteins.

Lefevre et al. (2007) also examined tsetse flies and their biochemical changes upon infection with *Trypanosoma*, and the researchers' results were in line with Van den Abbeele et al. (2010). Using different laboratory techniques, Lefevre et al. (2007) analyzed the head proteome, an organism's set of expressed proteins, of their experimental tsetse flies and reported significant alterations in key metabolic and signaling pathways protein levels. Lefevre et al. (2007) suggested that the parasites were manipulating these metabolic pathways to induce hunger with aims to provoke additional feedings (Lefevre et al. 2007).

The species of *Trypanosoma* also seemed to affect feeding behaviors of infected livestock animals as well. Moloo et al. (2000) infected cattle with either *T. congolense* or *T. vivax,* two zoonotic species, to determine whether tsetse flies displayed a feeding preference. While all the tsetse flies were able to feed successfully, as measured by swollen guts, the flies that fed on the cattle infected with *T. congolense* had a greater increase in gut blood volume than did flies that fed on cattle infected with *T. vivax* (Moloo et al. 2000). The researchers' results were in line with the previously published work of Baylis & Nambiro (1993) who saw increases in feeding success rates of *G. pallidipes*, a species of tsetse flies, in cattle infected with *T. congolense* and no significant increase in feeding rates in flies that fed on uninfected cattle. These two studies suggest that the feeding rate and success of tsetse flies are affected by both the species of *Trypanosoma* that is present and whether or not infection is present (Baylis & Nambiro 1993; Moloo et al. 2000).

Feeding behavior is controlled mechanically, but tsetse fly's saliva components could also be altered by *Trypanosoma* presence to enhance transmission. A study by Caljon et al. (2006) revealed that tsetse fly saliva enhanced the *Trypanosoma* infection in mice that received the parasite-saliva mixture compared to mice injected with parasites only and no saliva. Salivary factors that influence transmission were also observed by Moloo et al. (2000) who found that haematophagous insects produced vasodilators in their saliva that aided in increasing the blood supply to the insect during feedings. Thus, the saliva composition aids in dispersion of parasites from vector to host (Caljon et al. 2006). In addition, Caljon et al. (2006) evaluated the blood serum levels of mice infected in the presence or absence of tsetse fly saliva and suggested that the trypanosomes may be exploiting the suppressed host defense system as a way to initiate infection.

Sleep Disruptions

Trypanosoma species affect the behaviors of their vectors, the tsetse flies, as well as the behaviors of their hosts. Contrary to what the name implies, trypanosomes do not cause excessive sleep or hypersomnia (Buguet et al. 1993; Grassi-Zucconi et al. 1995). Using electroencephalogram (EEG) recordings, it was shown that mice infected with *T. b. brucei* displayed more episodes of daytime wakefulness compared to the control mice, whereas slow-wave sleep was significantly lower in infected rats (Grassi-Zucconi et al. 1995; Chianella 1999). Those same mice were also shown to display overall unsynchronized sleep patterns with significant reduction in rapid-eye movement (REM) latency (Grassi-Zucconi et al. 1995; Chianella 1999). An earlier study by Bentivoglio et al. (1994) demonstrated that infected mice had increased wakefulness and decreased synchronized rest. Also, Chianella et al. (1999) reported more advanced and pronounced sleep fragmentation at the terminal stage of infection in mice compared to control mice.

Because the *T. b. brucei* strain is not pathogenic to humans, it is used as a model organism to infect laboratory animals (Darsaud et al. 2004). Researchers have observed *T. b. brucei*-infected rats using electrocorticogram to observe the brains and an electromyogram to monitor neck muscles (Darsaud et al. 2004). The results revealed disruption in 24-hour sleep cycles, with wakefulness in the night and sleep predominately in the day (Darsaud et al. 2004). The sleep-cycle changes and associated symptoms in *Typanosoma*-infected humans manifest similarly to the *T. b. brucei*-infected rats (Darsaud et al. 2004).

Sleeping disorders have been documented in human patients with trypanosomiasis. Blum et al. (2006) reported irregular sleep in 74.4% of the infected patients, the second-most-frequent symptom after headache, increasing with infection duration and amount of white blood cells in 2541 patients as the disease progressed. In children, the combination of drowsiness and the development of convulsions during sleep usually correlates with the severity of the trypanosomiasis (Buyst 1977).

Lethargy and weight loss

Trypanosomiasis dates back to slave traders, who wouldoften call the disease “negro lethargy” because infected individuals would display lethargic behavior (Knight 1971). This induced laziness could be attributed to the 24-hour-sleep-cycle interruptions of wakefulness and sleepiness (Bentivoglio et al. 1994; Grassi-Zucconi et al. 1995; Chianella 1999; Darsaud et al. 2004; Blum et al. 2006).

Using acrylic cages with photocell beams connected to computers and printers to collect activity data, Grassi-Zucconi et al. (1995) reported that locomotion was significantly reduced in *T. b. brucei*-infected rats compared to controls. Trypanosome-derived lymphocyte triggering factors were released by the injected parasites and were cited to be the cause of the observed lethargic behavior in mice (Grassi-Zucconi et al. 1995). Darsaud et al. (2003) used an open field test that evaluated motor reactivity, measured the paths taken by infected mice, and found significant differences between activity in the *T. b. brucei*-infected mice and controls. The exploratory activity of the infected rats began to decline as trypanosome-ridden mice started to remain at the corner of their wooden open-field boxes—similar to patients observed to become less motile and more lethargic during late-stage trypanosomiasis (Darsaud et al. 2003).

Similarly, Bentivoglio et al. (1994) observed hypokinesia in *T. b. brucei*-infected mice as well, but especially during the hours of darkness. *Trypanosoma vivax* has also been reported to cause progressive weakness, along with loss of physical condition and appetite and eventually lethargy and emaciation in cattle (Silva et al. 1998). Two studies have observed that vervet monkeys inoculated with *T. b. rhodesiense* by a single bite display progressive weakness, lethargy, fever, and weight loss (Kagira et al. 2006; Thuita et al. 2008). Transmissions via a single bite from the tsetse fly and the clinical manifestations of lethargy and physical weakness have been used to establish a model that mimics human sleeping sickness (Kagira et al. 2006; Thuita et al. 2008).

A reduction in food intake in mammals has also been cited as a consequence of *Trypanosoma* infestation. Toth et al. (1994) reported that *T. brucei*-infected rabbits exhibited reduced food intake just four days after subcutaneous inoculation. In Darsaud et al. (2003), all infected rats lost substantial weight as a consequence of reduced eating while the control rats steadily increased body weight throughout the experiment. The same held true for *Trypanosoma*-infected rats and vervet monkeys that started to lose weight at day 39 post-infection and whose daily food consumption decreased compared to pre-inoculation period, respectively (Kristensoon et al. 1998; Thuita et al. 2008).

Other behavioral alterations

In addition to loss of sleep rhythms and lethargy, *Trypanosoma*-infected animals also have exhibited manic episodes of behavior. Upon entrance into the spine and brain, trypanosomes have been reported to cause nervous system complications (Knight 1971; Darsaud 2003; Thuita et al. 2008). In *Trypanosoma brucei*-infected dogs, neurological changes were evident just 6 days after infection (Morrison et al. 1980). The experimentally induced trypanosomiasis caused unexpected aggression, barking, ataxia, convulsions and trembling (Morrison et al. 1980). Ataxia and trembling have also been reported in vervet monkeys with *T. b. rhodesiense* (Kagira et al. 2006; Thuita et al. 2008).

Conclusion

With 60 million people at risk of contracting human trypanosomiasis a year, it is vital to understand the mechanism by which the parasite is able to induce changes in vector behavior to enhance transmission (World 2012). It is equally important to discuss the clinical alterations of host behavior as a result of *Trypanosoma* infection. To ensure proper and efficient transmission from vector to host, it would be advantageous for the parasite to manipulate transmission-related behaviors.
Subspecies within the *Trypanosoma* genus have the power to manipulate not only the tsetse fly, but also their human and mammalian hosts. These extracellular protozoans are capable of altering feeding behaviors in both its vector and hosts as well as inducing sleep abnormalities, causing lethargic behaviors, and inducing manic episodes within host organisms. By inducing both behavioral and biochemical modifications, *Trypanosoma* ensures its reproduction and successful parasitic transmission to the next host, and thus virulence.

Additional research should be focused on psychological behavioral changes in humans in response to *Trypanosoma* infection, as the literature is sparse. By understanding the psychological clinical manifestations, physicians will be able to differentiate trypanosomiasis from other tropical diseases, such as malaria. Because the symptoms of malaria and trypanosomiasis are fairly similar, trypanosomiasis is frequently misdiagnosed as malaria (see the review by Simarro et al. 2011). Given the prevalence of the disease, more research should aim at uncovering more precise and exclusive clinical, physical, and psychological manifestations of trypanosomiasis. Also, future research should be directed at differences in the response to primary versus secondary infections. By distinguishing between primary and secondary infections, clinicians will have a better opportunity to utilize the limited resources in treating patients who are not contracting the disease for the first time.

Acknowledgements

The author would like to thank Dr. Sarah Boyle for her editing this review and Alex Yu for his aid in accessing literature.

Appendix

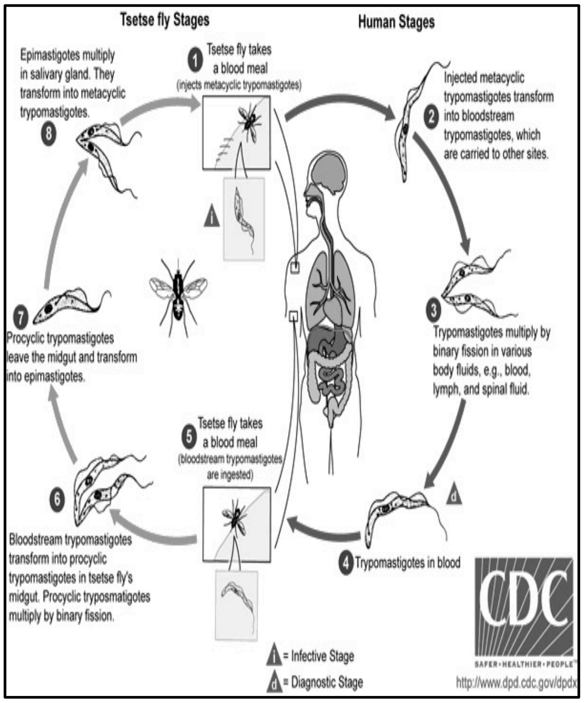

Figure 1. Life cycle of *Trypanosoma brucei* involving tsetse fly and human stages. Image from: Alexander J. da Silva and Melanie Moser, Centers for Disease Control Public Health Image Library

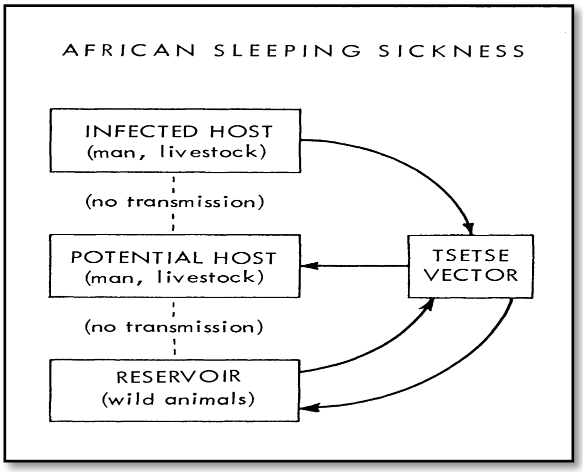

Figure 2. Pathogenic transmission of trypanosomes. Adapted from Knight (1971).

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About the author: Lan Tran, '13, is a Biology major from Memphis, Tennessee. She is a member of βββ Biological Honor Society and a Rhodes/ St. Jude Summer Plus Fellow. Her research project at St. Jude involved assessing and analyzing the physical function among childhood brain tumor survivors. After Rhodes, Lan will be attending the University of Tennessee Health Science Center and pursuing a Masters in Cytopathology and Histotechnology. She enjoys spending time and playing soccer with her family.

The Investigation of AF9, a Potential Regulation of HSC Specification, in Definitive Hematopoeisis

Harrison Daniel
Rhodes College

Introduction

Embryonic stem cells (ESCs) have two distinguishing characteristics: the ability to self-renew to maintain stem cell identity and the ability to differentiate into the various cell lineages. The capability of ESCs and induced pluripotent stem cells (iPSCs), which are cells with stem cell identity that are reprogrammed from the somatic cells, to differentiate into various progeny has powerful implications clinically.[1] Currently, only 30% of bone marrow transplant recipients have access to a matching donor in their family.[8] The generation of hematopoietic stem cells (HSCs) from ESCs could greatly enhance the donor pool for bone marrow transplants by increasing the availability of HSCs for clinical use. In order to effectively derive transplantable HSCs from ESCs, key aspects of hematopoietic development must be recapitulated in culture.

Hematopoiesis occurs in two sequential phases: primitive and definitive hematopoiesis. Primitive hematopoiesis produces a transient population of progenitors that differentiate into macrophages, primitive erythrocytes, and unique primitive megakaryocytes. The establishment of this transient hematopoietic population occurs in the yolk sac around embryonic day 7.5 (E7.5).[2] Definitive hematopoiesis gives rise to long term HSCs, which have the capacity to reconstitute all the lineages of the peripheral blood for the lifetime of the organism.[3] The long term HSCs originate in the aorta-gonad-mesonephros (AGM) at approximately E10.5. In culture, primitive hematopoiesis has successfully been recapitulated with ESC differentiation through the enforced expression of either *Hoxb4* or the combination of *Cdx4* and *Hoxb4*.[1] Definitive hematopoiesis with long term HSCs has not been successfully recapitulated in culture.

To be able to generate long term HSCs from ESCs, it is necessary to isolate and recapitulate definitive hematopoiesis. To accomplish recapitulation requires the establishment of a serum free differentiation protocol conducted similarly to Irion's previous study consisting of two separate waves of Flk1 populations. Flk1 is the receptor tyrosine kinase for vascular endothelial growth factor (VEGF), which is the key morphogen that is required for proper vascular genesis and angiogenesis. Thus, Flk1 and VEGF are known key regulators of endothelial development and function. Flk1 may mark the putative hematopoietic precursor the hemangioblast and may be involved in early hematopoiesis.[6] The second Flk1 population presumably represents precursors of definitive hematopoiesis. After establishing this systematic differentiation protocol, we will evaluate the role of AF9 in definitive hematopoiesis through overexpression at different developmental steps. AF9 is known to be a regulator of human erythroid and megakaryocytic differentiation. More recent studiess have also suggested that AF9 may be a novel regulator of HSC specification. AF9 is expressed in the AGM. When AF9 was knocked down in developing zebrafish embryos, there was a loss of emerging HSCs in the zebrafish AGM.[4] The tool we will use to overexpress AF9 is the Tet-On inducible system, which uses a murine parental Ainv15 ESC line. This line has been modified by inserting the tetracycline transactivator into the ubiquitously expressed Rosa26 locus. Additionally, the HPRT locus was engineered to contain a tetracycline response element (TRE) and human AF9 downstream of the TRE.[7] When doxycycline is added, it binds to the tetracycline transactivator. The transactivator then undergoes a conformational change, which allows it to bind to the TRE and induce transcription of AF9. We will use this tool to test the hypothesis that AF9 is a novel regulator of HSC specification.

Methods

ESC Cell Culture. The iAF9 ESC line was maintained in ESC medium (DMEM) supplemented with 15% prescreened Fetal Calf Serum, 1% Glutamax, 2mM Penicillin Streptomycin, 1% HEPES, 0.1mM Non-Essential Amino Acids, 1% Sodium Pyruvate, 0.055mM beta-mercaptoethanol, and 1000U/mL leukemia inhibitory factor. ESCs were cultured on an irradiated mouse embryonic fibroblast feeder layer and refreshed with ESC media daily. The cultures were split every two to three days with trypsin.

Genomic DNA Prep. Confluent ESC cultures were collected by centrifugation at 136 g for 10 minutes. The genomic DNA was prepared according to the manufacturer instructions (Qiagen).

RNA/cDNA Prep. iAF9 ESCs cultured either with or without doxycycline were harvested in TRIZOL Reagent (Invitrogen). RNA was isolated according to manufacturer instructions and then treated with the DNAse I, Amplification Grade kit (Invitrogen). The samples were then split into four groups: Doxycycline + Reverse Transcriptase (RT) +, Dox+ RT-, Dox- RT+, and Dox- RT- and cDNA was prepared according to manufacturer instruction (SuperScript III First-Strand Synthesis System for RT-PCR; Invitrogen).

Serum Free Differentiation (SFD). The ESCs were harvested when 80%-confluent, and cultured at 75,000 cells/ml in suspension in the serum-free differentiation media as described in Irion et al. 2010. 48 hours later, Activin A (2 ng/mL), BMP 4 (8 ng/mL), and VEGF (5 ng/mL) were added. The cells were incubated for 36 hours. On Day 3.5, the embryoid bodies (EBs) were disassociated with enzyme-free cell dissociation buffer (Gibco, Cat# 13151). The generation of first wave of Flk1+ cell population was confirmed by flow cytometry using ant-Flk1 antibody conjugated with PE (Biolegend, 89B3A5). Flk1-cells were negatively sorted by magnetic-activated cell sorting (MACS) and replated at 500,000 cells per mL in a 48-well non-tissue culture treated plate with Activin A (3 ng/ml). On Day 4.5, the cells were treated with BMP4 (60 ng/ml) and VEGF (30 ng/ml) and incubated for another 36 hours. The day-6 EBs were disassociated, and the second wave of Flk1+ cells were collected by flow cytometry. The hematopoietic induction of the second wave of Flk1-positive population was further completed as described in Irion's et al 2010 (Figure 1).

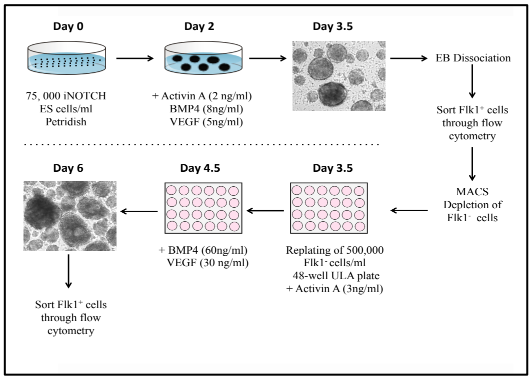

Figure 1. Targeting long term HSCs through SFD. A schematic representation of the tool used to evaluate AF9's role in definitive hematopoiesis.

***PCR*.** PCR was performed with 10X Standard Taq Buffer (NEB), Taq Polymerase (NEB), and 10μM dNTPs. PCR was performed on the Biorad MyCycler thermocycler.

The amplification conditions for AF9 were:

Initial Denaturation	2min	94°C
Denaturation	15sec	94°C
Annealing	15sec	57°C
Extension	45sec	72°C
Final Extension	5min	72°C
Hold	∞	4°C

The sequence of the hAF9 forward primer was: aacaacccagtcctgccag.

The sequence of the hAF9 reverse primer was: gtggttttgtccagcgagc.

The concentration of the primers were 10μM.

Redesign the Tet-On System. The DNA was purified using the Wizard SV Gel and PCR Clean-Up System (Promega). The samples were prepared according to the manufacturer instructions. The ligation was performed with 10X Buffer (NEB) and T4 ligase (NEB).

Results

Amplification of human AF9 genomic DNA

To verify that the TRE-AF9 construct is present in the iAF9 ESC line before testing induction efficiency with doxycycline, we did genomic PCR with primers against the TRE and AF9 cDNA. We first isolated genomic DNA using the plasmid DNA miniprep purification kit (Qiagen). No bands of expected size were amplified after attempting to troubleshoot PCR conditions by optimizing the annealing temperature, varying the Mg^{2+} concentration, and varying the DMSO concentration. Optimizing the annealing temperature ensures the primers anneal properly to the single stranded DNA. If the annealing temperature is too low, the primers may anneal nonspecifically. If the temperature is too high, the primers will not anneal efficiently. Varying the Mg^{2+} concentration controls the specificity of primer binding. A higher concentration allows base pairing in the annealing step to be more permissive but also increases the chance of errors. A low concentration requires more stringent base pairing in the annealing step. DMSO is used to relieve secondary structures during amplification.

Amplification of human AF9 cDNA

Since we could not successfully detect the presence of human iAF9 construct in our electroporated AinV15 ESC line, we decided to shift our focus to testing the expression level of the human iAF9 from the ESC line with doxycycline treatment. This new approach is more promising because it was uncertain if the TRE and AF9 were adjacent in the construct, which would explain our issues with the genomic AF9 amplification. The RNA was extracted with the addition of Trizol following doxycycline exposure in culture. The experiment consisted of four samples: Doxycycline (Dox) + Reverse Transcriptase (RT) +, Dox+ RT-, Dox- RT+, and Dox- RT-. In the experiment, RT- samples control for possible contamination from genomic DNA. The AF9 cDNA was obtained by using reverse transcriptase PCR. To avoid possible genomic DNA contamination from the parental AinV15 ESC line, primers were designed to cross the introns of the parental line. We obtained bands of the expected size for two out of the four samples: Dox+ RT+ and Dox- RT+ (Figure 2). The results suggest basal leakiness in the Tet-On inducible system since the amplification of the Dox- RT+ sample may suggest doxycycline induction was not necessary for AF9 expression. Our results prompted us to recreate the inducible ESC line. This new Tet-On system uses the A2lox.cre ESC line and the p2lox plasmid. Currently, we are in the process of extracting the DNA from the ligations. Next, we will analyze the results and then proceed to introduce the recombinant plasmid into bacterial cells through electroporation.

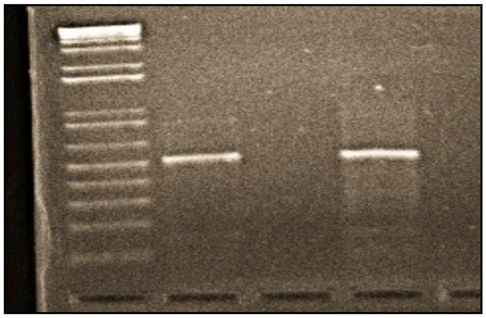

Figure 2. The Tet-On inducible system may contain basal leakiness. The bands from left to right are: Dox+ RT+ and Dox- RT+. The figure indicates that doxycycline induction was not necessary for AF9 expression since the Dox- RT+ sample has arguably a brighter band than the Dox+ RT+ sample. This suggests that the Dox- RT+ sample has a higher expression for AF9 than the Dox- RT+ sample.

Discussion

In this study, we used the Tet-On inducible system to evaluate whether AF9 could play a role in hematopoietic development. Prior to evaluating AF9's role as a novel regulator of HSC specification, we first tried to confirm the presence human AF9 cDNA in the targeted iAF9 ESC cell line by genomic PCR and also by examining doxycycline induced mRNA expression of human AF9 in iAF9 cells. To determine the origin of the issues for amplifying iAF9 was difficult since we did not have a positive control. One possibility is that the mouse genome of the Ainv15 parental ESC line was being amplified instead of human iAF9. Even though the mouse and human cDNA are more than 80% homologous, it is unlikely that murine AF9 is being amplified since the primers cross two introns for the mouse genome. The best explanation for our inability to amplify human AF9 successfully is basal leakiness in the Tet-On system. High basal expression is most likely due to the site of chromosomal integration as it has been proven to be an important factor in the regulation of the Tet promoter. Leaky expression can also be contributed to false promoters or cryptic initiation signals.[4] Based on our issues with the Ainv15 ES line, we redesigned the Tet-On system. The new Tet-on system utilizes the A2lox.cre ESC line and the p2lox plasmid. This system provides enhanced targeting efficiency in comparison to the Ainv15 ES line since the cre cDNA excises itself after recombination and placement of the cDNA of interest downstream of the TRE (Figure 3).

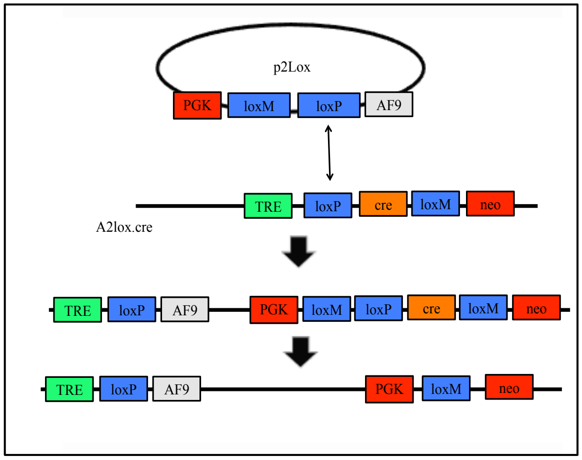

Figure 3. The new Tet-On system makes use of the A2lox.cre ESC line and the p2lox plasmid. The figure indicates AF9 is under the control of the TRE, and cre is under the control of the PGK promoter following recombination at the loxP sites. Recombination at the loxM sites allows AF9 to still be under the control of TRE and neomyosin resistant gene to be under the control of the PGK promoter, which will be used to select the right colonies in culture.

AF9 holds promise in our efforts to achieve our goal of manipulating the differentiation of ESCs to long term HSCs. AF9 is a regulator of human erythroid and megakaryotic differentiation and has a potential role in regulating HSC specification.[4] Therefore, we intend on using a systematic differentiation protocol based on Irion's previous study to evaluate when AF9 is crucial in hematopoietic development. We are in the process of redesigning the Tet-On system for our inducible ESC line and have extracted the DNA from the ligations in preparation for electroporation. When we have completed the redesign of the Tet-On system, we will determine if AF9 affects hematopoietic specification during ESC differentiation by inducing AF9 expression during embryoid body formation. We will also determine if AF9 affects the expansion and differentiation of hematopoietic progenitors by inducing its expression in Flk-1+ cells plated into hematopoietic induction culture. We will perform hematopoietic progenitor colony assays on OP9 stroma and colony forming units (CFUs) in semisolid media supplemented with cytokines to assess the emergence of hematopoietic progenitors from these cultures. I can also use quantitative real-time RT PCR to examine what effect the induction of AF9 has on the expression of hematopoietic genes during ESC differentiation. I expect AF9 will promote hematopoietic specification when ectopically expressed during EB differentiation. I also expect the results will indicate AF9 will enhance hematopoietic potential when induced in assays of hematopoietic colony formation. Although a further study is required, evaluating candidate genes as potential regulators of hematopoietic commitment in ESCs could allow us to more efficiently derive transplantable HSCs that could be used in bone marrow transplants.

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About the author: Harrison Daniel, '13, is a Biology major. During his time at Rhodes, he has participated in notable activities, such as a research fellowship at St. Jude through the Summer Plus program, volunteering in the trauma unit at the Med, and an internship at Campbell Clinic Orthopedics. He will be attending medical school at the University of Tennessee Health Science Center starting this fall.

The Link Between Anxiety and Cardiovascular Disease

Lauren Miller
Rhodes College

Cardiovascular disease is the leading cause of mortality in the United States, as well as other developed countries, in both men and women. Despite the increase in the number of programs offered for early detection and prevention, it is expected that the number of cardiovascular disease diagnoses will continue to climb as the population ages (Olivo et al., 2009). Along with the widely known biological risk factors and components, there is strong and consistent research evidence for psychological risk factors and their role in the development of cardiovascular disease (Martens et al., 2010). While the high comorbidity between mental disorders and the development of cardiovascular disease is clear, it often is under recognized by health professionals and many individuals are not aware of the link between the two. Mental disorders such as depressive disorders and anxiety disorders are not only among the most commonly diagnosed mental disorders, but they also have some of the strongest evidence suggesting their increase in one's risk for cardiovascular disease (Shen et al., 2008). In fact, Generalized Anxiety Disorder presents a stronger risk factor than any other psychological problem (Shen et al., 2008). Anxiety disorders can be combated by a variety of methods, and while they make up the largest psychological risk factor for cardiovascular disease, steps can be taken to lessen the risk they present. In this paper I will examine the role of anxiety disorders, focusing mainly on generalized anxiety disorder, as a risk factor for cardiovascular disease, to try to further illustrate the importance of a biopsychosocial approach to cardiovascular health.

Biological and Physiological Aspects of Cardiovascular Disease

Biological risk factors contributing to the development of cardiovascular disease are well known, well researched, and include: age, gender, family history, and ethnic background. Those with a family history of cardiovascular disease are more likely to develop a form of cardiovascular disease than those without. Men have a higher risk of cardiovascular disease than do women, although risk increases with age in both sexes. Those of African American ethnicity have a higher rate of cardiovascular disease development than do European Americans. However, Native Americans, Asian Americans, and Hispanic Americans have an even lower rate of cardiovascular disease than do European Americans.

Physiological risk factors help explain the biological basis of cardiovascular disease. Hypertension is perhaps the most important physiological risk factor that many are not aware of; followed by blood serum cholesterol levels, low-density lipoproteins (LDL, or "bad cholesterol"), and glucose metabolism. Glucose metabolism is particularly an issue in individuals with diabetes and individuals who suffer from obesity. All of these physiological components of cardiovascular disease lead to excess abdominal fat, elevated and irregular blood pressure, and problems with cholesterol levels, which put excess stress on the heart on a consistent basis.

Psychosocial Risk Factors

Psychosocial stress has been shown to correlate directly with a higher prevalence, as well as clustering, of cardiovascular risk factors according to a study done in 2002 (Merz et al., 2002). Most psychosocial risk factors are well known by the general public and include marital status, employment, and behavioral factors (such as smoking, diet, physical activity) and socioeconomic status. However, mental health is the biggest component of psychosocial risk factors for cardiovascular disease, yet health professionals often overlook it and the general public is often unaware of the link between the two (Ai et al., 2010). In one study, researchers found that medical doctors asked only 38% of anxious patients being treated for cardiovascular disease about their psychological symptoms and psychotherapeutic treatment (Ai et al., 2010). This could present a very serious problem; in fact by 2020 the World Health Organization estimates that cardiovascular disease and major depression will be the two strongest factors in not only American disease, but also the contribution to global disease (Ai et al., 2010). Clearly the link between mental health and cardiovascular health should not be ignored. Depression and anxiety are two of the most prevalent mental disturbances; and while there is an abundance of research on depression as a risk factor, there are very few studies examining the role of anxiety in cardiovascular health (Merz et al., 2002). This is somewhat surprising because in cardiovascular disease, symptoms of anxiety are very common, with prevalence rates ranging from 24% to 31% (Martens et al., 2010).

Chronic Anxiety as a Risk Factor

Although very little research is available on anxiety as a risk factor for cardiovascular disease, what research is available is very clear: chronic anxiety is a stronger risk factor than any other psychological problem (Shen et al., 2008). Highly anxious individuals are more likely to experience higher levels of repeated stress on a day-to-day basis creating, over time, a chronic problem (Shen et al., 2008). Experiencing this repeated stress consistently puts one at a higher risk of myocardial infarction, or heart attack, due to the continuous stress on the heart, which is logical. The under recognition of the link between anxiety and cardiovascular disease may be due in part to the overlap of symptoms, and lack of awareness about the overlap (Ai et al., 2010). Often times symptoms such as shortness of breath, high blood pressure and irregular heart beat can be seen in both cardiovascular diseases and anxiety. On the level of medical professionals, often times psychological disorders are not brought up as part of the medial examination; and on an individual level, the general population does not think to relate their physical symptoms to a psychological disorder. This can be a major problem not only in the development of cardiovascular disease, but chronic anxiety left untreated can be predictive of disability, increased physical symptoms, and a worse quality of life should cardiovascular disease develop. Psychological disorders associated with chronic anxiety include: generalized anxiety disorder, posttraumatic stress disorder, panic disorder, phobic anxiety, and obsessive compulsive disorder (Player et al., 2011). Generalized anxiety disorder and phobic anxiety have been found to be the most important in increasing the risk of developing cardiovascular disease (Kawachi et al., 1997).

Generalized Anxiety Disorder

Generalized Anxiety Disorder is not only one of the most common anxiety disorders, with a lifetime prevalence rate of 5.7%, but it also is indicative of a higher risk for cardiovascular disease than any other type of anxiety (Lydiard et al., 2010). Generalized anxiety disorder in one study was associated with a rate of 74% of cardiac events (Martens et al., 2008). Symptom severity of generalized anxiety disorder (or GAD) has also been shown to predict further cardiac events after the onset of cardiovascular disease (Schienle et al., 2011). GAD interferes with day-to-day living, causing an anxiousness so overcoming, individuals' thoughts are consumed and daily interactions no longer occur in "normalcy." Anger, fear, and helplessness are often suddenly and explosively felt in addition to the crippling anxiety (Schienle et al., 2011). This continual experience of strong emotions results in the over activation of the sympathetic nervous system and "fight or flight" response causing continual, extreme stress on the cardiovascular system (Vale, 2004). This physiological impact will be discussed in detail later.

Individuals with GAD have also been shown to have lower levels of omega-3-fatty acid, are more likely to smoke, are less active, and are less likely to adhere to strict medication regimens; all of which are strong risk factors predicting the development of cardiovascular disease in and of themselves (Martens et al., 2010). In the same study, researchers found that of participants with GAD, 9.6% developed some type of cardiovascular disease; where as only 6.6% of participants without GAD developed some type of cardiovascular disease (Martens et al., 2010). Perhaps one of the keys to GAD as a risk factor is that it is easily modifiable through treatment, thus cutting down on the risk factor presented (Ai et al., 2010).

Physiological Mechanisms linking Chronic Anxiety and Cardiovascular disease

Because of the limited amount of research linking anxiety and cardiovascular disease, it is not completely understood what physiological mechanisms are responsible for the increased risk. However several links to pathological mechanisms involved in cardiovascular disease have been proposed (Martens et al., 2008). Chronic anxiety is often an indicator of autonomic dysfunction, specifically in reduced baroflex cardiac control and reduced heart rate variability, and indicates deregulation of the serotengeric system (Martens et al., 2008). Psychological stressors, such as chronic anxiety, can also raise arousal of the sympathetic nervous system via the hypothalamic-pituitary axis (HPA axis), which causes a surge in catecholamine circulation (Player et al., 2011). The infusion of catecholamines, like norepinephrine and epinephrine, which are agonists, cause excess stress to the heart through spasms and vasodilatation (Vale, 2004). They also induce a higher rate of activity in blood clotting factors. A rapid increase in atrial pressure and heart rate by the sympathetic activation causes a higher demand for oxygen in the myocardium. A common symptom of anxiety is difficulty breathing, or the shortness of breath, which in combination with this higher demand for oxygen can pose a serious problem.

These "stress responses" are thought to cause cardiovascular disease when linked with the perception of psychosocial problems. The ventromedial and ventrolateral prefrontal cortex regions are important in this link between emotional experiences and behaviors in response to outside stimuli perceived by those with GAD (Vale, 2004).

Perhaps the most important link between physiological mechanisms and the development of cardiovascular disease however are seen through the release of previously mentioned catecholamines, as well as glucocorticoids by the HPA axis and sympathetic nervous system (Vale, 2004). When stimulated by an emotional response, the hypothalamus influences the pituitary gland to release corticotrophin releasing factor, or CRF, which eventually causes rapid increase in the initiation of cardiovascular damage (Vale, 2004). When CRF is released, the anterior pituitary gland releases corticotrophin, or ACTH, which then stimulates the adrenal cortex to release glucocorticoids. Glucocoriticoids, like the stress hormone cortisol, have important immunosuppressive effects on the cardiovascular system, as well as decrease the amount of cytokines and other molecules that mediate the inflammatory response. Cortisol is also known to affect the preservation abdominal fat, as well as cause salt retention, insulin resistance, and increase levels of LDL, all of which are known to be risk factors for cardiovascular disease (Vale, 2004). Cortisol levels are also spiked by panic attacks, which are often associated with chronic anxiety disorders, as well as a surge in blood pressure (Martens et al., 2008). Other mechanisms through which anxiety could possibly increase the risk of cardiovascular disease include: hyperventilation, which could induce a coronary spasm, or ventricular arrhythmias triggered by an acute anxiety attack (Kawachi et al., 1994).

Treating Anxiety to Reduce Risk of Cardiovascular Disease

Pharmacological treatment is effective in reducing chronic anxiety, however it can be difficult given the complex pathophysiology of cardiovascular disease (Ai et al., 2010). For instance, tricyclic antidepressants and MAOIs have cardio toxic effects. SSRIs, or selective serotonin reuptake inhibitors, are often used to combat generalized anxiety disorder. However, most SSRIs interact with medications commonly used to treat cardiovascular disease. There are two exceptions with along with cognitive behavioral therapy, or CBT, are safe and effective: sertraline and citalopram (Ai et al., 2010). A long term study of sertraline showed the reduction of anxiety symptoms without affecting ventricular arrhythmia. A separate study showed after a post-hoc analysis that the use of sertraline for anxiety in patients who had previously experienced a heart attack reduced the rate of death or a recurrent heart attack by 42% (Ai et al., 2010). Thus the efficacy of safe SSRIs is an important breakthrough in treating anxiety with consideration to the development of cardiovascular disease.

Along with traditional pharmacological treatment, there are also several alternative treatments used to reduce anxiety, thus reducing the risk of developing cardiovascular disease. Perhaps one of the simplest ways to reduce anxiety along with reducing the risk of cardiovascular disease is to incorporate a healthier lifestyle. One study showed the efficacy of adding a serving of fish everyday to increase the bodies level of omega-3-fatty acids (Martens et al., 2008). Other lifestyle changes include the reduction of salt/sodium intake, more exercise, the reduction of “unhealthy cholesterol.” It has been suggested that by simply increasing the amount of exercise, both mental health and cardiovascular health have been shown to improve in women but not men (Ai et al., 2010).

Mindfulness-Based Stress Reduction Programs, which include yoga and psychological education discussions about stress and coping, as well as take home relaxation assignments, were initially thought to be one alternate way to reduce the symptoms of chronic anxiety. However, much to researchers surprise, there were no significant differences between baseline anxiety readings and anxiety readings after the completion of the program (Olivo et al., 2009). Deep breathing and relaxation have been found to be helpful in coping with the physical symptoms of generalized anxiety disorder on the surface (Olivo et al., 2009). Perhaps what will be most beneficial in the long run are educational programs for therapists, medical physicians, and communities in order to increase awareness of the link between mental health and cardiovascular health.

Behavioral research has found a link between optimism, hope, and adaptive coping and improved outcomes of cardiovascular disease when anxiety is present (Ai et al., 2010). Optimism was found to have resulted in fewer hospitalization readmissions, whereas pessimism was a predictor of death. After cardiac surgery, those who were optimistic were likely to have a faster recovery time with fewer complications. Positive spiritual coping was associated with hope and the alleviation of not only cardiovascular disease symptoms but GAD symptoms as well. Patients were also less likely to have further cardiac events if they had a positive attitude about the future as well as their own health (Ai et al., 2010).

Along similar lines, an expert panel from the National Institute of Health conducted research that showed the “faith effect” and its effectiveness on cardiovascular disease patients, which surprisingly was more evident than in cancer patients. According to the study, strong spirituality, either of religious nature or secular nature, predicted better medical outcomes among patients, along with shorter hospital stays and the absence of complications (Ai et al., 2010). Another study with similar goals, found a link between prayer better recoveries in psychosocial terms (Ai et al., 2010). Perhaps prayer is helpful when dealing with large levels of acute stress, such as hospitalization, because optimism and a positive attitude counteract each other. The interesting aspect here is that it did not matter what religion the individual subscribed to. As long as there was a strong belief, there were better results. This shows just how important psychological beliefs are in the role of disease and dealing with diseases. This further illustrates that health is not simply comprised of biological factors, but rather a biopsychosoical approach is further supported.

Critiques

There were quite a few issues I encountered in researching chronic anxiety disorders as a risk factor for cardiovascular disease. First, almost all of the studies focused on white men, over the age of 60. There were very few examining cardiovascular disease in women. While men are more likely to suffer from cardiovascular disease than women, women are far more likely to suffer from anxiety disorders (Barger et al., 2005). The age discrepancies also present a bit of a problem. Generalized anxiety disorder is typically diagnosed before the age of 25, where as cardiovascular disease has a much later onset.

Depression is well studied and understood as a risk factor for cardiovascular disease, however anxiety disorders require that much more research be done in order to better understand the link. Many of the studies took place over a span of several years; very few were actually “long term.” Its safe to say that anxiety as a risk factor for cardiovascular disease has a future in terms of research potential.

Personal Relevance

I wanted to research cardiovascular disease because I have a strong family history of heart attack, stroke, and hypertension. Two years ago, my grandfather who I was very close to, died of cardiovascular disease. Ever since then I have been interested in risk factors for the development of cardiovascular disease and ways to reduce those risk factors. I didn’t know very much about cardiovascular disease before not only researching this paper, but also taking this class. Psychological factors were something I never really thought of, as a contributing to the onset of disease, which is part of the reason why I think community education of biopsychosocial contributions to disease, is important for the future.

I chose to narrow my focus to chronic anxiety as a risk factor for cardiovascular disease, specifically looking at research talking about generalized anxiety disorder and cardiovascular disease because I myself have GAD. I never realized the strong correlation between cardiovascular disease and GAD, and now I am aware that I have that much more of an increased likelihood of developing cardiovascular disease at some point in my life. I found the research surrounding the pharmacological treatment of GAD and its impact on cardiovascular disease particularly interesting because I actually take the SSRI, sertraline, as well as participate in CBT. It was encouraging to know that the efficacy of this particular drug is well studied and supported in terms of cardiovascular health. Researching this topic has been incredibly beneficial in knowing what steps to take to minimize my risk, as well as helping my family minimize their risk also.

Conclusion

As early as 1628, English physician William Harvery proposed a link between the mind and mental emotions and the heart (Ai et al., 2010). Researchers today continue to explore the impact the mind, and mental health, has on diseases such as cardiovascular disease. While mental disorders have a strong incidence of comorbitidy with heart health, generalized anxiety disorder presents the strongest risk factor, proving idea of a biopsychosocial interaction in the development of cardiovascular disease.

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About the author: Lauren Miller, ’14, is a Neuroscience major from El Paso, Illinois. In addition to being a Resident Assistant, Lauren is a member of Chi Omega, volunteers as a baby hugger at Le Bonheur, works as a Scribe in the Baptist East Emergency Department, is involved with Compassion International as child sponsor, and has her own online Etsy shop. In her free time she can usually be found crafting or baking. After graduation Lauren plans to combine her passion for healthcare and children by attending nursing school and working as a neonatal nurse, eventually hoping to serve on medical mission trips to orphanages in third world countries.

Prenatal Stress and the Effect on Offspring Health

Alex Yu
Rhodes College

Prenatal stress can have a variety of effects on the health of offspring. This review aims to define prenatal stress based on the literature available as well as examine studies in both human and non-human subjects. Prenatal stress in many cases was defined by a stressful event that occurred during gestation, with the exception of maternal depression or gestational diabetes. Within the human studies examined, prenatal stress was found to have gestational, physiological, and psychological effects. Within the non-human studies examined, effects were seen more in the physiology rather than psychology of offspring. The damaging agent of prenatal stress in many of the studies was maternal cortisol levels. In addition to examining the various effects, this review also discusses a possible approach for preventing and reversing the damage caused by prenatal stress. There is a clear need for stress reduction techniques for females who are pregnant, either through the use of stress reduction programs for humans, or environmental enrichment for animals.

Introduction

Prenatal stress is found to affect offspring during gestation (Fenster et al. 1995; Neugebauer et al. 1996; Kurki et al. 2000; Heaman et al. 2005), infancy (Shang et al. 2010), childhood (Laplante et al. 2004), and even adulthood (Malaspina et al. 2008). The effects of prenatal stress are broad, ranging from physiological (Hansen et al. 2000; Coe et al. 2002; Chang et al. 2003; Bowman et al. 2004; Li et al. 2005; Shang et al. 2009; Nielsen et al. 2011; Shi et al. 2012; Buchwald et al. 2012; Dancause et al. 2012) to psychological effects (Laplante et al. 2004; Malaspina et al. 2008; Mueller & Bale 2008). The effects of prenatal stress have been studied in primates, both human (Fenster et al. 1995; Neugebauer et al. 1996; Franklin et al. 2000; Kurki et al. 2000; Hansen et al. 2000; Laplante et al. 2004; Heaman et al. 2005; Malaspina et al. 2008; Shang et al. 2010; Nielsen et al. 2011; Shi et al. 2012) and non-human (Coe et al. 2002; Buchwald et al. 2012), as well as in rodents (Chang et al. 2003; Bowman et al. 2004; Li et al. 2005; Mueller & Bale 2008; Dancause et al. 2012).

However, before reviewing the effects of prenatal stress, it is necessary to define what constitutes a prenatal stressor. In human studies, prenatal stress is often defined as stressful life events such as death of a family member (Nielsen et al. 2011), natural disasters (Laplante et al. 2004), financial troubles (Shang et al. 2010), or stress from maternal health issues (Li et al. 2005). Prenatal stress in non-human animals is often in relation to habitat conditions such as temperature or danger of harm (Jensen & Toates 1997), and is quantified by measuring maternal glucocorticoid levels (Coe et al. 2002; Bowman et al. 2004).

Exposure of the fetus to elevated levels of cortisol, a stress response hormone, appears to be the causal agent of prenatal stress effects in both human and non-human animals according to a review prepared by Field & Diego (2008). Within their review, Field & Diego (2008) described how maternal cortisol is able to cross the placenta and cause effects such as miscarriage, premature delivery, and impaired development among a host of other effects examined. Mulder et al. (2002) also reviewed the effects of prenatal stress on gestation and offspring, and emphasized that further studies should be conducted due to increasing amounts of stress placed upon pregnant women in the modern world.

This review of prenatal stress aims to examine both human and non-human studies in order to reveal the diverse effects of prenatal stress, bring attention to the issue, and examine ways through which prenatal stress might be alleviated. While other reviews of the topic were conducted within the past decade, this review includes more recent studies and hopes to serve as a progress report on the research being conducted within the topics of prenatal stress.

Effects of Prenatal Stress in Human Studies

Gestational Effects

The relationship between miscarriage and prenatal stress has been examined in terms of stressful life events (Neugebauer et al. 1996) and workplace stress (Fenster et al. 1995). In the stressful life event study, women who recently experienced a chromosomally normal miscarriage were interviewed to determine if the stressful event occurred during gestation or just before conception (Neugebauer et al. 1996). Chromosomal abnormalities were not considered in relation to maternal stress, thus miscarriages resulting from such abnormalities were excluded (Neugebauer et al. 1996). Of the women studied, 54% experienced one or more stressful life events, with legal woes and deaths of friends or family members most frequently involved with miscarriage (Neugebauer et al. 1996). However, the relationship between stress and miscarriage was only significant if the stress occurred during gestation

(Neugebauer et al. 1996). In the study that examined workplace stress and miscarriage, pregnant women within the first 13 weeks of gestation were interviewed and evaluated based on job stress (hours worked, physical exertion, social support) and life stress (anxiety about pregnancy, alcohol consumption, cigarette use, caffeine consumption), with follow-up interviews conducted after pregnancy (Fenster et al. 1995). Results indicated that workplace stress did not increase the risk of miscarriage in all working females; however, women with a first-time pregnancy, women smokers, and women over the age of 32 did appear more at risk for miscarriage (Fenster et al. 1995).

Pre-term birth has also been associated with prenatal stress (Heaman et al. 2005). High levels of personal stress (defined using a modified version of the Perceived Stress Scale from Cohen et al. 1983) during pregancy more than doubled the chances of pre-term (<37 weeks) birth (Heaman et al. 2005). In contrast to Neugebauer et al. (1996), life event stress did not have a significant effect on the gestational length of the pregnancy; however, low self-esteem and lack of support from peers, both considered as stress-enhancers, appeared to increase the risk for pre-term birth (Heaman et al. 2005).

Preeclampsia and growth retardation are also identified as effects of prenatal stress (Franklin et al. 2000; Kurki et al. 2000). Stress in the form of depression and/or anxiety during pregnancy was associated with more than a three-fold increased risk for preeclampsia (Kurki et al. 2000). Snoring induces preeclampsia according to a study by Franklin et al. (2000) which also found that preeclampsia may cause fetal growth retardation. Thus, by combining the findings of Franking et al. (2000) and Kurki et al. (2000), one can conclude that if prenatal stress leads to preeclampsia, and preeclampsia to growth retardation, then prenatal stress can indirectly lead to growth retardation.

Physiological Effects

The relationship between prenatal stress and congenital malformations was studied by Hansen et al. (2000), who predicted that stress would increase the occurrence of malformations involving the cranial neural crest based on studies linking cortisone exposure to cleft palate/lip malformation. Hansen et al. (2000) examined severe stressors that occurred either during gestation or within the 17 months prior to gestation, and found that offspring who were exposed to severe stressors during gestation were nearly ten percent more likely to develop a congenital malformation (cleft palate/lip or heart malformation) than the unexposed control group. Hansen et al. (2000) also found that offspring from mothers who experienced the loss of a pre-existing child during the first trimester of the pregnancy had nearly a two-fold increase in the risk of congenital malformations. In addition, prenatal stress is also linked to neurological impairments (Shang et al. 2010).

Infantile spasms are the result of an epileptic syndrome thought to be caused by an interruption in the development of the brain (Shang et al. 2010). Based on the background information that epileptic medications were not effective in treating infantile spasms while ACTH could effectively treat the symptom, Shang et al. (2010) conducted a study where women with children experiencing infantile spasms were evaluated for prenatal stress. Shang et al. (2010) found that increased prenatal stress was positively correlated with infantile spasms. In addition to the Shang et al. (2010) study, a study by Shi et al (2012) hypothesized that prenatal stress effects hormone levels, expression of neurotransmitter receptors, and damages neuron ultrastructure, all of which possibly contribute to infantile spasms.

Nielsen et al. (2011) examined groups of Danish children who experienced either serious infectious diseases (SID) or less severe acute infectious diseases (LID) for approximately 14 years after birth, along with family records, in order to see if contraction of infectious disease was related to prenatal stress. Nielsen et al. (2011) defined stressful life events as death of spouse, child, or divorce. Nielsen et al. (2011) found that the loss of another child was the most-common prenatal stressor, and that exposure to prenatal stress resulted in a 25% increase in the chances of developing a SID or LSID, and needing hospitalization. Nielsen et al. (2011) found that stress exposure during pregnancy resulted in a 71% increased chance of SID with hospitalization as compared to exposure to stress one to two years before conception.

Psychological Effects

Prenatal stress has also been linked to diminished intellectual and language functioning based on a study involving the toddlers of women who had experienced stress related to a natural disaster while pregnant (Laplante et al. 2004). Stress from a natural disaster, as defined by Laplante et al. (2004), involves four variables: scope (amount of exposure), loss (death of people or destruction of property), threat (danger to the life of the individual), and change (disruption in normal life and relocation). Laplante et al. (2004) found that higher levels of prenatal stress resulted in diminished intellectual and language abilities in the toddlers who were exposed to the prenatal stress of the natural disaster during the third trimester of the pregnancy. Another finding of Laplante et al. (2004) was that toddlers with higher birth weights tended to have lower scores on intellectual tests; however, this finding is speculative because according to background provided, higher birth weights are often related to birth traumas such as perinatal anoxia, a condition associated with diminished cognitive skills.

Acute prenatal stress and increased rates of schizophrenia was studied by examining a group of pregnant women who were exposed to a brief wartime experience (Malaspina et al. 2008). According to Malaspina et al. (2008), the acute window of stress would allow for a close approximation of gestational month, rather than trimester, since the stressful war experience lasted around twenty-four days (from mid to late May to early June). Malaspina et al. (2008) found that the occurrence of schizophrenia was twice as high in the offspring who were exposed to the acute prenatal stress during the second month of gestation; in addition, there was also a host of other mental illnesses (anxiety disorders, eating disorders, drug abuse) in the offspring who were exposed during the third month of gestation.

Effects of Prenatal Stress in Animal Studies

Physiological Effects

Gestational diabetes also serves as a form of prenatal stress because oxidative stress is placed upon the developing fetus (Chang et al. 2003; Li et al. 2005). Both studies show that oxidants resulting from diabetic conditions inhibit the expression of the *Pax3* gene, a gene involved in the proper closure of the neural tube (Chang et al. 2003; Li et al. 2005). In addition, both studies show that the use of anti-oxidants such as glutathione ethyl ester (Li et al. 2005) and alpha-tocopherol (Chang et al. 2003) were able to combat the decreased *Pax3* gene expression and thus the risk of neural tube defects.

Buchwald et al. (2012) investigated whether or not prenatal stress, simulated with the use of dexamethasone, had an effect on lipid metabolism in the common marmoset (*Callithrix jachus*). Buchwald et al. (2012) found that prenatal stress increased blood cholesterol levels and thus cardiovascular risk. The application of dexamethasone resulted in higher mean levels of blood cholesterol in the second and third offspring generations (F2: 3.95 mM, F3: 4.40 mM) compared to the young aged control group (3.23 mM) (Buchwald et al. 2012). The mean blood cholesterol levels in the first generation were not significantly different from the old aged or young aged control group (Buchwald et al. 2012). In addition to the higher levels of cholesterol, Buchwald et al. (2012) also found that there was a decrease in the amount of healthy triglycerides. Due to the differences seen between age and generation in terms of blood cholesterol levels, Buchwald et al. (2012) concluded that effects of prenatal stress from the first generation may be passed on to subsequent generations.

Dancause et al. (2012) examined the effect of prenatal stress on the length of long bones in rats (*Rattus norvegicus)*. Dancause et al. (2012) exposed their rats to both prenatal and postnatal stressors such as sleep and food deprivation, unstable and uncomfortable housing situations, exposure to strobe lighting, periods of restraint and forced swimming, and wet bedding. Dancause et al. (2012) found that long bone length was shortened with the use of prenatal stressors, indicating that prenatal stress may contribute to growth retardation.

Coe et al. (2002) conducted a study where pregnant rhesus monkeys (*Macaca mulatta*) were subjected to stress, and the offspring were then examined for proper immune response. Prenatal stressors in the study involved daily relocation to a dark room within a small cage where acoustical disturbance took place for six week periods in both early and late gestation (Coe et al. 2002). Analysis of offspring immune response was completed by stimulating collected blood samples with endotoxin (Coe et al. 2004). Results indicated that prenatal stress reduced (by around 40% according to figures) the amount of cytokines produced with levels of cytokines falling even more (a 50% reduction) in the presence of dexamethasone, with reductions being dose dependent (Coe et al. 2004). In the conclusion of their study, Coe et al. (2004) suggested that the reduction of the cytokine response in younger animals due to prenatal stress could result in an overactive response when the animals become adults, based on previous studies of bidirectional shifts, thus indicating the potential harmful effects of prenatal stress.

Sexually Specific and Dimorphic Effects

Prenatal stress appears to effect mental health in male mice depending on the timing of the exposure (Mueller & Bale 2008). Pregnant mice (*Mus musculus*) were exposed to stress during early (days 1-7), middle (days 8-14), and late gestation (days 15-21) and grouped according to week of exposure (Mueller & Bale 2008). Prenatal stressors used involved prolonged periods of light, exposure to predator scents, introduction of new object, restraints, sleep deprivation, cage changes, and wet bedding (Mueller & Bale 2008). Resulting offspring were analyzed with three tests: tail suspension test, forced swim test, and an open field test (Mueller & Bale 2008). Males exposed to prenatal stress in early gestation were found to display depressive behaviors in both the tail suspension test and the forced swim test, and additional tests were run to confirm the depressive phenotype (Mueller & Bale 2008). It was also noted by Mueller & Bale (2008) that prenatally-stressed males from the early-gestation exposure group had higher levels of corticosterone following restraint stress than the control group (Figure 1). Mueller & Bale (2008) noted that the fetal brain is absent during the early gestational period and thus the effects seen in the study may be due to interruptions in placental functioning.

Bowman et al. (2004) indicated that the effects of prenatal stress could be sexually dimorphic as exhibited by rats. The first evidence of sexually-dimorphic effects appeared in tests relating to corticosterone habituation in prenatally-stressed groups (Bowman et al. 2004). Prenatally-stressed males showed greater habituation to corticosterone than did females (Bowman et al. 2004). When examining the data, Bowman et al. (2004) discovered that the main reason behind indifferences in several of the tests was due to female masculinization and male feminization. Because females became more masculine, both their behavior and performance increased to the normal male levels, while the feminized males behavior and performance fell towards female levels; thus, the rise of the females and the fall of the males led to lack of differences in test results (Bowman et al. 2004).

Potential Solution to the Effects of Prenatal Stress

Li et al (2012) aimed to provide evidence for a preventative measure against the effects of prenatal stress. Li et al. (2012) suggested that enrichment within the environment for the pregnant rats during gestation may help prevent the damaging effects of prenatal stress. Pregnant rats were divided into treatment groups with variations in enrichment during different periods of gestation, and the resulting offspring were subjected to various behavioral tests and then killed for brain physiology analysis (Li et al. 2012). The results of behavioral tests and brain histology indicated that gestational enrichment for the pregnant rats appeared to inhibit anxious behavior as well as learning and memory disabilities in the offspring (Li et al. 2012). In addition, Li et al. (2012) also found that gestational enrichment corrected neurological impairments (e.g. decreases in spine density of several neural cells). Thus, Li et al. (2012) concluded that enrichment of the environment during gestation may be a viable preventative measure to the effects of prenatal stress.

Field & Diego (2008) included preventative measures of prenatal stress in a review. Field & Diego (2008) discussed the need for stress reduction that does not involve anti-depressants and reviewed a study in which pregnant women either received stress reduction (in the form of engaging in activities the women thought were relaxing) or did not, revealing that women within the stress reduction group had lower levels of cortisol. Another study within their review involved pregnant women who received massage therapy, who at the end of the study had lower levels of cortisol as well (Field & Diego 2008).

Conclusions and Future Research

As shown in this review, the effects of prenatal stress on the health of offspring can be detrimental, starting from infancy and lasting through adulthood. While prenatal stress can have serious effects on the pregnancy itself, the effects on the offspring, assuming the fetus survives the pregnancy, could lead to a lifetime of bad health. These effects pose a serious concern to public health due to the potential physiological and psychological effects induced by prenatal stress. Future research is needed, as indicated in a review by Mulder et al. (2002), because among the causes and effects of pregnancy issues, prenatal stress may be the agent in cases where the cause is unknown. Future research should also focus more on preventative measures against the effects prenatal stress like the study conducted by Li et al. (2012). Eliminating stress during pregnancy completely would be the logical solution to the issue at hand; however, anti-anxiety medications carry their own risk on pregnancy and fetal development, and animals are not always able to regulate their situation, especially in the zoo environment where stress can be high, or in the wild where stress comes in the form of predation or potential starvation. Therefore, finding alternative solutions to stress such as enrichment or stress-reduction therapies may be the most successful way to prevent the harmful effects of prenatal stress on offspring.

Acknowledgements

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Appendix

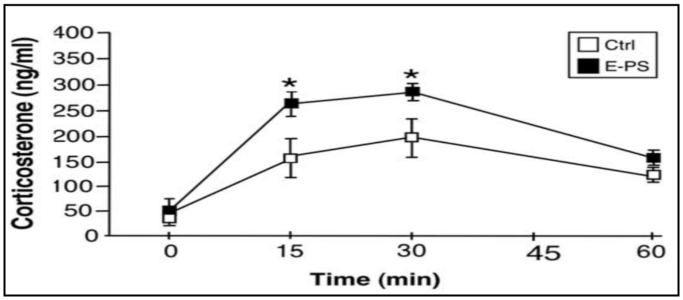

Figure 1. Corticosterone levels in males after restraint stress in control verses early gestation exposure group. Males exposed to prenatal stress during early gestational period experience higher levels of corticosterone than control males. From Mueller & Bale (2008)

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About the author: Alex Yu, '13, is a Biology major with plans to attend medical school. Alex conducts research on campus with Dr. Laura Luque de Johnson, as well as a recent project with Dr. Carolyn Jaslow. In addition, he has also worked at St. Jude Children's Research Hospital in both the research and clinical settings, and is a Kinney Site Coordinator for the MED volunteer program. In his spare time, Alex enjoys cooking, reading, painting, and binging on television seasons.

The Effect of Weaning Methods on Behavior and Stress in Foals (*Equus caballus*)

Sarah Ferguson
Rhodes College

The weaning process in many species is known to be a highly stressful time that can impact the overall welfare of the animal. Foal weaning can be performed using various methods, resulting in varying levels of stress-related behaviors. Variables shown to decrease stress and increase overall welfare during the weaning period include: introducing a supplemented diet prior to weaning to allow familiarity to food the foal will eat after weaning, gradual weaning with partial contact of either the mother or an unrelated adult, and group weaning in a paddock to facilitate social interactions among peers. Weaning is also a critical period for the introduction of positive human handling. Early, forced human contact after weaning helps familiarize foals with the human-horse relationship by making the foal easier to handle and less afraid of humans, which is important for better welfare later in the horse's life. The short-term effects of these weaning practices are reviewed, but more information is needed to determine the effects of different weaning methods on the long-term welfare of the foal and their temperament later in life.

Introduction

Weaning in domestic horses (*Equus caballus*) often utilizes sudden and complete separation of mother and foal, usually resulting in the foal entering social isolation from peers as well. This complete separation causes an increase in stress related behaviors and abnormal behaviors (McCall et al. 1985; Hoffman et al. 1995; Visser et al. 2008). The goal of many studies has been to decrease this stress and allow behavior post-weaning to be as natural as possible. Increased maternal investment causes increased playing behavior between wild foals, which has been linked to better body condition and increased survival rate (Cameron et al. 2008). Play behavior is a good indicator of future reproductive success, and thereby fitness, due to an increased body condition and social interactions (Cameron et al. 2008). Reproducing this positive play behavior and decreasing stress increases the overall welfare of foals during weaning and should be looked for when choosing a weaning method.

Stress related to weaning can be quantified using many different behavioral indicators. Increased vocalizations, defecations, and locomotion have been shown to be signs of distress during weaning (Hoffman et al. 1995; Heleski et al. 2002; Nicol et al. 2005). A study by McCall et al. (1985) found that during weaning vocalizations were negatively related to time spent standing but were positively related to time spent in locomotion. This positive correlation suggests that increased vocalizations and locomotion are indicators of high stress levels in foals. Stress is also indicated by an increase in stereotypic and abnormal behaviors (Heleski et al. 2002; Visser et al. 2008).

Various methods have been studied to increase the welfare of foals during weaning. Group weaning instead of individual weaning has shown positive impacts on behavior due to social interaction (Hoffman et al. 1995; Visser et al. 2008). During group weaning social relationships and adequate space for the foals need to be taken into account to decrease the amount of aggressive interactions (Hoffman et al. 1995). Group weaning can also utilize the addition of unrelated adults or partial maternal contact to decrease stress (McCall et al. 1985; Henry et al. 2012). Paddock weaning has also shown to decrease stress during weaning, due in part to its use of group weaning (Heleski et al. 2002; Nicol et al. 2005). Besides separation during weaning, stress is also caused by the change in nourishment from maternal milk to outside food sources (McCall et al. 1985). Providing supplement before weaning allows foals to become accustomed to outside food and thereby decreases stress related to a change in diet during weaning (McCall et al. 1985; Hoffman et al. 1995; Nicol et al. 2005). Human handling early after weaning has also been shown to increase welfare due to ease of handling in human-horse interactions as well as decrease stress due to the positive perception of human touch by foals (Lansade et al. 2004; Ligout et al. 2008).

No research has shown that any of these differing methods of weaning result in unhealthy welfare of the foal. Instead, research has been done to better foal welfare and decrease stress during weaning. This review aims to examine various weaning methods and provide the current practices for the best future welfare of foals after weaning.

Weaning method

Most research on weaning method has focused on individual versus group weaning, stall versus paddock weaning, and partial versus full maternal separation (McGall et al. 1985; Heleski et al. 2002; Visser et al. 2008). Studies have also looked into the effect of differing preweaning diets on the stress of weaning as well as introducing unrelated adults during weaning to reduce stress (Hoffman et al. 1995; Nicol et al. 2005; Henry et al. 2012). All studies were performed over less than a year period, showing only the short-term responses to the various weaning methods (McGall et al. 1985; Hoffman et al. 1995; Heleski et al. 2002; Nicol et al. 2005; Visser et al. 2008; Henry et al. 2012). More research must be done on the long-term effects of these weaning methods on foal behavior and welfare later in life.

Individual versus group weaning

The effects of individual versus group weaning on behavior have been studied to determine which method produces the least-stressful environment for the foal (Hoffman et al. 1995; Visser et al. 2008). Foals weaned individually spent more time standing and showed more signs of distress (vocalizations, defecations, pawing, and biting wood in the stall) than foals weaned in pairs (Hoffman et al. 1995). Visser et al. (2008) found that 67% of individually weaned foals developed a stereotypy, while none of the pair weaned foals developed a stereotypy, adding support to the idea that sudden individual weaning results in many unwanted, stressful behaviors that are not seen in pair weaned foals. Some foals weaned in pairs have shown significantly more aggressive behavior, causing more stress from aggressive interactions rather than isolation, as paired foals had higher cortisol levels when compared to individually weaned foals (Hoffman et al. 1995). However, a study done by Visser et al. (2008) did not find this increase in aggressive behavior in foals weaned in pairs, which could be due to the larger stalls used in their study to house paired foals (Visser et al. 2008: 48 m^2; Hoffman et al. 1995: 19 m^2).

An increase in agonistic behavior during group weaning is also seen in other livestock. Pigs show an increase in aggression towards unknown litter-mates versus familiar litter-mates to determine social ranking (Puppe et al. 1997). Taking social relationship into account when weaning foals in groups could help eliminate agonistic behavior and thereby reduce stress. When pair weaning, however, the amount of space given to the foals needs to be adequate as well as social relationships in order to create the least stressful environment.

Stall versus paddock weaning

Research has shown a difference in stall weaning versus paddock weaning in the amount of stress-related behavior exhibited (Heleski et al. 2002; Nicol et al. 2005). In a study done by Nicol et al. (2005) it was found that stall-weaned foals exhibited more stress-related behaviors (defecation, less foraging, more walking, and more pawing) than paddock-weaned foals. Paddock-weaned foals showed less stress-related behaviors and more grazing and foraging behavior, closer to the amount seen in wild horses (Heleski et al. 2002). Besides stress-related behaviors, Heleski et al. (2002) also found that stall-weaned foals engaged in more unwanted behaviors (licking or chewing wood in the stall, kicking the stall wall, pawing, and bucking/rearing). The frequency of these behaviors increased over time during the study (Heleski et al. 2002). Paddock weaned foals had the opportunity to perform these behaviors because there was an open, three-sided shelter in the paddock (Heleski et al. 2002). However, none of these unwanted behaviors were observed in the paddock-weaned foals (Heleski et al. 2002). Paddock weaning is also correlated to group weaning, as more than one foal is often placed in the paddock. Heleski et al. (2002) found that the paddock-weaned foals stood close to other foals, less than 10 meters, 89.1% of the time and engaged with another foal an average of 3 times an hour. Like group or paired stall weaning, paddock-weaned foals exhibit fewer stressful behaviors. Another advantage of paddock weaning over group stall weaning is the increase in grazing and foraging behaviors (Heleski et al. 2002). Group, paddock weaning is shown to produce the least-stressful environment as well as the most positive, natural behaviors (Heleski et al. 2002; Nicol et al. 2005).

Partial versus full maternal separation

Traditional weaning uses full maternal separation, which can be extremely stressful on both the mother and the foal (McCall et al. 1985). Partial maternal separation, which allowed fence-line contact between mare and foal for one week following weaning, caused less stress-related behaviors than full maternal separation (McCall et al. 1985). Stress from isolation was not a factor in this study because weaning was done in groups to allow the focus to be on stress from maternal separation (McCall et al. 1985). Partial maternal separation foals also showed locomotion patterns that were not different from control foals (who were never separated from their mother), suggesting that their locomotion was similar to that of preweaning due to a less stressful environment (McCall et al. 1985). The locomotion pattern involved less walking, which is known to be an indicator of stress during weaning (McCall et al. 1985; Nicol et al. 2005). Partial maternal separation foals were also observed in lateral recumbency, a sign of relaxation in horses, while full maternal separation foals were observed only in sternal recumbency (McCall et al. 1985). This difference may have been due to the higher stress of full maternal separation foals, which felt too anxious to lie down laterally (McCall et al. 1985).

Increased vocalizations are another indicator of stress during weaning (Hoffman et al. 1995). Foals weaned using partial maternal separation vocalize less than those weaned using full maternal separation, but do not differ from control foals, again indicating a less stressful environment similar to preweaning (McCall et al. 1995). A similar idea of partial maternal separation was used in cow weaning, where calves that were prevented from nursing for 2 weeks prior to weaning were less distressed after weaning than those that underwent the traditional, sudden separation (Haley et al. 2005).

Partial separation during preweaning for ten-minute periods caused more maternal attention upon reunion (Moons et al. 2005). Unlike other species this increase in maternal attention did not reduce the stress response later in life, particularly during weaning (Moons et al. 2005). Rat pups are known to have a better stress response (less intense response and shorter duration of response) later in life if they are handled more by their mothers at a young age, particularly after separation (Moons et al. 2005). This better stress response could be present in foals for other events later in life, but the sudden, complete separation of weaning may be too stressful a period to determine normal stress response. This study by Moons et al. (2005) used the individual stall weaning method, which could have induced even more stress on the foals. A better stress response may have been seen if a different weaning method had been used.

Preweaning diet

The sudden absence of maternal nourishment during weaning can add to the stress exhibited by foals after weaning, particularly when the foal has not had previous access to outside nourishment (McCall et al. 1985). This stress can be alleviated by an additional preweaning diet given to foals to help the transition from milk from the mother to other food sources (McCall et al. 1985; Hoffman et al. 1995; Nicol et al. 2005). Foals with a diet of pasture grazing supplemented with hay and a pellet concentrate vocalized less and spent more time eating than foals with a diet of pasture grazing supplemented with hay only (Hoffman et al. 1995). The foals without the pellet supplement also had higher cortisol levels after weaning than foals fed the pellet supplement, indicating that foals without this supplement are more stressed (Hoffman et al. 1995).

Locomotion induced by stress also differed due to preweaning diet, as foals fed a preweaning diet supplemented with creep feed high in protein had lower activity levels than those without a preweaning supplement of creep feed (McCall et al. 1985). A study done by Nicol et al. (2005) found a difference in fearfulness of foals to novel objects and people due to differing preweaning diets. Foals with diets high in fiber and fat versus diets high in starch and sugar were less afraid of novel interactions, spending less time looking at a novel object, more time investigating a novel object, and were less likely to walk away from a novel person (Nicol et al. 2005). This difference in behavior was seen only during times of stress as no difference in behavior was observed under normal circumstances, suggesting that diet affects stress response in foals (Nicol et al. 2005). The study done by Nicol et al. (2005) also compared the differing diets to stall versus paddock-weaned foals. Foals fed a diet of fat and fiber performed less locomotion than foals fed starch and sugar when stall weaned, while when paddock weaned the foals fed a diet of fat and fiber grazed more (Nicol et al. 2005). Supplementing the preweaning diet of foals helps alleviate stress after weaning by familiarizing the foal to a diet other than maternal milk (McCall et al. 1985; Hoffman et al. 1995; Nicol et al. 2005).

Unrelated adult interaction

Stress from the sudden separation of foal and mother may be alleviated with the addition of unrelated adults into a group of weaned foals (Henry et al. 2012). In this study two unrelated adult horses were introduced into an experimental group of foals 15 minutes after weaning (Henry et al. 2012). Over the course of the three-week study the foals spent time watching the adults but never displayed mother-foal behaviors (Henry et al. 2012). Foals with and without unrelated adults performed increased levels of vocalizations, increased walking, and decreased grazing directly after weaning (Henry et al. 2012). Foals without unrelated adults vocalized significantly longer after weaning than foals with unrelated adults (Henry et al. 2012). Salivary cortisol levels of foals without unrelated adults were also elevated longer than foals with unrelated adults, showing a decrease in stress when adult horses were present (Henry et al. 2012). Lower levels of aggression were also seen in foals weaned with unrelated adults, with no chasing or kicking behaviors displayed (Henry et al. 2012). This trend was consistent immediately after weaning as well as in the three weeks observed after weaning (Henry et al. 2012). Abnormal behaviors were seen more frequently in foals weaned with no unrelated adults, particularly wood-chewing and redirected suckling, suggesting that weaning with unrelated adults present helps maintain behavior similar to that before weaning (Henry et al. 2012). More research must be done on the effects of the presence of unrelated adults during weaning, but Henry et al. (2012) found that the least stressful method of weaning to date is group, paddock-weaning with the presence of unrelated adults.

Human handling after weaning

Many studies have been performed to determine the effects of human handling after weaning, an important time period to produce positive human-horse interactions later in life (Lansade et al. 2004; Ligout et al. 2008). Differences have been found in early versus late handling after weaning as well as forced versus unforced human handling (Lansade et al. 2004; Ligout et al. 2008). However, more research must be done to determine why this time period following maternal separation is so pivotal for encouraging positive human handling throughout life (Lansade et al. 2004). Handling during weaning has positive short-term effects, but more research must be done on the long-term effects (Ligout et al. 2008).

Early versus late handling

Reducing the fear of humans in foals is important for both foal welfare and human-horse interactions later in life (Lansade et al. 2004; Ligout et al. 2008). Foals handled early after weaning were easier to handle (less time to halter and pick up all feet) versus foals handled late after weaning (Lansade et al. 2004). Both early and late handled foals were significantly easier to handle than the control foals, which had no human handling at all, strengthening the idea that human handling in general results in better welfare for the foal than no handling at all (Lansade et al. 2004). When exposed to the surprise opening of an umbrella, foals that were handled early after weaning had a smaller increase in heart rate and were less frightened by the surprise than late handled foals (Lansade et al. 2004). Early handled foals were easier to handle in the short-term, suggesting that this is the more effective method of human handling, but more research must be done to see if early handled foals remain easier to handle later in life (Lansade et al. 2004).

Forced versus unforced handling

In the study by Ligout et al. (2008) foals were either under forced human contact, which consisted of stroking while restrained, or unforced human contact, with no restraint so that the foal could avoid human contact if desired. Foals that had forced contact were easier to handle than those with unforced contact due to the researchers ability to fit a halter quicker as well as fewer defensive behaviors exhibited by the foal (Ligout et al. 2005). The human-horse relationship appeared stronger in forced contact foals because the foals initiated contact with a human sooner, maintained contact for a longer period of time, and allowed the human to touch them sooner and handle them more easily (Ligout et al. 2005). When the same tests were performed in an unknown environment, though, the behavior of forced versus unforced contact did not differ except that the forced contact foals were caught faster, suggesting that the type of human contact does not matter in stressful situations (Ligout et al. 2005). Because foals with forced human contact were easier to handle, it may be that horses perceive human touch as positive; however, more research much be done on this to determine how positive this interaction is for the foal and how human handling can have a positive impact during stressful situations like a novel environment (Ligout et al. 2008).

Conclusion

Based on the current research available, the weaning method that appears to provide the best welfare for foals during the weaning period is group, paddock weaning, with a supplemental diet before weaning (McCall et al. 1985; Hoffman et al. 1995; Heleski et al. 2002; Nicol et al. 2005). The presence of unrelated adults after weaning or partial maternal contact is also most effective in alleviating stress (McCall et al. 1985; Henry et al. 2012). This weaning method provides the least stressful environment based on the lower amounts of stress-related behaviors. Group, paddock weaning allows positive social interactions with other foals to encourage the most natural behaviors, guarded there is enough space for all individuals and social compatibility between individuals is taken into account to decrease aggression (Heleski et al. 2002; Nicol et al. 2005; Visser et al. 2008). Introducing a supplemental diet before weaning allows foals to adjust more adequately to the loss of maternal nourishment during weaning, decreasing stress due to the familiarity of the food (McCall et al. 1985; Nicol et al. 2005). Having either an unrelated adult present or partial maternal contact show decreased stress-related behaviors and help the transition of weaning become less sudden for the foals (McCall et al. 1985; Henry et al. 2012). Much research has been done into the short-term effects of various weaning methods on stress and stress-related behaviors, but much more needs to be done on the long-term effects to see how the varying weaning methods affect a horse's temperament and stress response later in life. The studies reviewed all used different ages of weaning as well as varying gender and breeds.

Further research should look into whether or not age of weaning, gender, and breed show consistent results with the findings shown here. Most research focuses on the foal stress response to weaning, but research must also be done into the mothers' stress response, as weaning can be a stressful time for mothers as well as foals. None of the weaning methods report bad welfare for foals, so the goal of weaning research is create the least stressful environment for foals to better their welfare during and after weaning.

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About the author: Sarah Ferguson is a senior biology major and history minor from St. Louis, MO. She is a member of Chi Omega sorority and Rhodes varsity Field Hockey team. Sarah is also a member of the biology, history, and athletic honor societies. Upon graduation, Sarah will be returning to St. Louis to begin work in the animal industry.

Arguments for a More Complicated Nervous System in Cnidarians with Special Reference to Cubozoa (Box Jellyfish)

Allison Julien
Rhodes College

Cnidarians have often been portrayed as simple, primitive organisms with equally primitive nervous systems consisting of a simple neural net with randomly dispersed neurons. Recent studies, however, provide evidence that some cnidarians, specifically, cubozoa, the box jellyfish, have more complex, polarized nervous systems that contain more than one neural net. A more complex nervous system is necessary for utilizing the complex cubozoan rhopalian, or visual, system. A proposed structure of the cubozoan nervous system includes a motor nerve net (MNN) for transmitting motor output and a diffuse nerve net (DNN) for transmitting sensory information. Between these two systems is the rhopalia system that converts sensory inputs from the DNN into motor outputs from the MNN. This organization allows for the active hunting and escape responses in cubozoa that are not found in other cnidarians. Here, I present evidence for the theory that cubozoa have at the very least two neural nets that allow for a complex visual system and the ability to process sensory stimuli and produce appropriate motor response.

Introduction

The box jellyfish, or cubozoa, possesses a complicated visual system (Garm et al. 2011), and what was thought to be a neural net that served as a simple nervous system (Watanabe et al. 2009; Satterlie 2011), making it unique among jellyfish. The visual system of cubozoa consists of four rhopalia containing six eyes of four types: upper and lower lenses and pit and slit eyes (Garm et al. 2011). Although the eyes appear to be very simple and primitive, a large degree of regionalization of the neural structures of cubozoa has been discovered (Watanabe et al. 2009). Cubozoa have complex visual systems for cnidarians consisting of photoreceptors, allowing for them to detect changes in light to differentiate between lighter and darker spaces in water (Nordstrom et al. 2003). This ability to detect differences in light is useful to species such as *Tripedalia cystophera*, a species of cubozoa native to the dense mangrove swamps of the Caribbean (Garm et al. 2011). It is imperative that *T. cystophera* navigate with accuracy to avoid injury to their delicate bodies and to hunt for prey (Garm et al. 2011).

T. cystophera are active hunters of small crustaceans called copepods that gather in light shafts between mangrove branches, unlike other species of cnidarians that hunt by passively-drifting (Garm et al. 2011). *T. cystophera* detects the differences in light shafts and the darkness of the mangrove branches, and navigates towards them (Garm et al. 2011).

It was previously assumed that cubozoa have only a neural net, or a network of neurons that are diffusely dispersed and interact with each other seemingly at random (Satterlie 2011), with which to process these sensory signals. Other studies suggest that cnidarians have polarized nervous systems with defined synapses (Nakanishi et al. 2009). Nakanishi et al. (2009) propose that cnidarians have a motor nerve net (MNN) and diffuse nerve net (DNN). The DNN transmits sensory information to the rhopalia nervous system where the marginal center of the rhopalium converts the sensory input into motor output that is sent to the MNN to change motor behavior (Nakanishi et al. 2009). Studying the nervous systems of cnidarians is especially useful in discovering evolutionary origins or nervous systems in both invertebrates and vertebrates, as jellyfish seem to represent a stage of development between the primitive and the complex (Watanabe et al. 2009). While cnidarians have been labeled as simple, primitive creatures in the past, the theory of a simple nerve net in place of a more complicated nervous system seems too simple when considering that these creatures are capable of sensing stimuli, processing the information, and producing appropriate motor outputs. This processing ability is a trait of actively hunting cubozoa. The visual system, motor behavior and the physiology of cubozoa suggest that the model for a more complicated, polarized nervous system in the cnidarian is more accurate than a simple nerve net.

Eye Structure and Visual System

At the most primitive level, eyes take in light information and transduce the light energy into chemical signals (Serb 2008). These chemical signals are then interpreted by either a brain or cerebral ganglia (Serb 2008). The eyes of cubozoa are no different. Located along the bottom the bell of cubozoa, the eyes are structured in rhopalia sensory systems (Garm et al. 2011). Each medusa has four rhopalia, and each rhopalium contains six eyes for a grand total of twenty-four eyes per medusa (Coates 2003). The visual fields of the upper lens eyes suggest that they are used to detect cues from above the water (Garm et al. 2011). These upper lens eyes are situated in such an area on the cubozoan bell that they remain looking constantly looking up regardless of body orientation, providing the organism with information such as the presence of mangrove canopies (Garm et al. 2011). It was found that cubozoa could only detect underwater objects at a distance of 1-2 cm, implying that there must be other eyes to detect objects that are further away (Garm et al. 2007), such as the upper lens eyes (Garm et al. 2011).

The eyes of the cubozoa contain corneas, cellular lenses, a retina with pigmented cells, and possibly vitreous spaces, making them very similar in structure and function to the camera-type eyes of some vertebrates and cephalopods (Coates 2003). Recent studies have found genetic similarities between components used in the development of the visual systems of both vertebrates and cubozoa, such as melanogenic pathways, ciliary photoreceptor cells, and transduction cascades (Kozmik et al. 2008). The similarities between the eyes of cubozoa and those of vertebrates imply a greater level of complexity than has been previously attributed to simple organisms such as cnidarians. Additionally, the functions of the eyes of cubozoa , such as *T. cystophora,* imply further complexity than those of passively floating cnidarians (Garm et al. 2011).

Motor Behavior

T. cystophora live between mangrove roots in Caribbean mangrove swamps, residing near the surface of the water (Garm et al. 2011). The cubozoan's diet consists of copepods that cluster in light shafts between the mangrove branches (Garm et al. 2011). *T. cystophora* is able to detect the mangrove canopy using a specialized eye that is designed to look straight up through the water's surface at all times, regardless of the orientation of its body (Garm et al. 2011). These different eyes allow *T. cystophora* to take in sensory information from many areas of its environment at one time (Garm et al. 2011). Due to their delicate bodies, *T. cystophora* require a visual system capable of detecting obstacles such as the dark mangrove branches they swim through with ease (Piete et al. 2011). In an experiment performed by Piete et al. (2011), when an area of water is darkened, cubozoans increased their swim pulse frequency and orient themselves to turn and swim away from the darkened area, a behavior not found when *T. cystophora* was in constant light. Garm et al (2007) found that when a cubozoan comes into direct contact with a colored obstacle, it will display a very strong escape response.

Cubozoan vision is limited to the differences between light and dark areas (Garm et al. 2011); they cannot see the copepods themselves (Piete et al. 2011). Once a light shaft is located, the cubozoa will swim through it repeatedly, unaffected by the presence or absence of copepod clusters (Buskey 2002). In the presence of copepods, the *T. cystophora* will ensnare the prey passively as it swims through the light shaft (Buskey 2002). It should be noted that while *T. cystophora* may passively collect prey by drifting once it has reached a light shaft, it is quite active in the pursuit of the light shafts (Garm et al. 2011). In open water, where a cubozoan cannot detect light differences, it would starve because it would not be able to locate copepod clusters (Garm et al. 2011). When *T. cystophora* is removed from the mangrove branches, it will rapidly swim back towards them (Garm et al. 2011). Despite the fact that *T. cystophora's* vision is limited to differences in light, this does make its visual system less complicated. In order to exhibit hunting or escaping behavior, *T. cystophora* would need a nervous system complicated enough to transduce outside stimuli and translate the electrical information into motor output (Satterlie 2011).

Nervous System Structure

Cnidarians have radial symmetry, not the bilateral symmetry of invertebrates (Satterlie 2011). Central nervous systems are associated with vertebrates with bilateral symmetry (Satterlie 2011). Cnidarians, on the other hand, are radially symmetrical and thus would seem to be incapable of a central nervous system (Satterlie 2011). The common conception of the cnidarian nervous system is that of a neural net (Watanabe et al. 2009). A neural net is a simple and primitive system wherein neurons are randomly dispersed among epithelial cells (Watanabe et al. 2009) rather than being grouped into nerves (Satterlie 2011). Since the neurons are dispersed in such a way, they interact with each other wherever they cross each other; the conduction is unpolarized, running in any direction (Satterlie 2011).

Cubozoans do, however, possess the ability to produce appropriate motor output and behavior based on sensory stimuli through use of integrative centers (Satterlie 2011), which does not fit the neural net model. Some early life stage forms of cnidarians, or polypoid forms, have ring-like neural tissue on the oral disc region of their bodies that connects to their tentacular networks which may suggest a certain level of centralization (Satterlie 2011). The rhopalia, or cubozoan ganglia, suggest development of integrating centers that are more complex than simple nerve nets, which have more in common with sensory specializations than with effector systems and provide possible evidence of bilateral organization rather than radial organization (Satterlie 2011).

In receptor-effector nervous systems, signal transmission is spread throughout the diffuse nerve nets of the body (Moroz 2009). In order to become closer to a true central nervous system, polarized transmission between neurons is necessary (Moroz 2009). Anderson and Mackie (1977) found that cnidarians have neurons that communicate with each other in the form of synaptic input from other systems within themselves, and that these synapses lead to control of swimming behavior necessitating a more complex, polarized nervous system. Additional studies have shown that some cnidarian nervous systems are indeed polarized and have defined synapses consisting of several nerve nets that communicate with one another to facilitate complex behavior (Moroz 2009), such as those observed in cubozoa.

Some studies show that cubozoan nervous systems contain two nerve nets, the motor nerve net (MNN) and the diffuse nerve net (DNN) that interact at the rhopalia systems (Nakanishi et al. 2009). The MNN consists, for the most part, of bipolar ganglion cells and controls swimming behavior by conducting electrical impulses (Nakanishi et al. 2009). The DNN transmits sensory information to a "marginal center" in the rhopalium, or small sensory structures (Nakanishi et al. 2009). The marginal center, a pacemaker in the rhopalium, communicates electrical impulses to the MNN in order to control swimming behavior Nakanishi et al. 2009). The exact location of the marginal center is unknown, however (Nakanishi et al. 2009). There are at least seven sensory cell groups in the rhopalium which, at their base, have neuronal processes that form neural networks with bilateral symmetry that connect to the nervous system outside of the rhopalium (Nakanishi et al. 2009). It is likely, then, that the marginal center receives neuronal input from the rhopalium and the DNN, sending output information to the MNN (Nakanishi et al. 2009).

Nakanishi et al. (2009) found that the marginal center must communicate with neurons originating from surrounding nervous systems, and connections between rhopalium and the MNN develop from lateral projections of axons from the rhopalium's base (Nakanishi et al. 2009). If this is true, then the marginal center must reside in the cells of the basal region of the rhopalium. This allows for communication between the DNN and sensory cells in the rhopalium in order to transmit electrical impulses to the MNN (Nakanishi et al. 2009). While this model is less complicated than a vertebrate nervous system, it is much more complicated than a simple diffuse nervous system and does have certain similarities to vertebrate nervous systems. The DNN and MNN display functions similar to the peripheral nervous system and the rhopalium functioning like a central nervous system, processing information from the periphery.

Conclusion

The complicated visual system of cubozoa, structured into rhopalia systems, sets it apart from other cnidarians (Garm et al. 2011). The structure of the eyes and visual systems of cubozoa are both structurally and genetically similar to those of cephalopods or vertebrates (Coates 2003). While the cubozoan visual system is limited to determining difference in light, the behavior towards these differences is of extreme importance (Satterlie 2011). Cubozoa are capable of exhibiting complicated hunting and escape behaviors as a result of the processing of sensory stimuli and output of appropriate motor responses (Satterlie 2011). These behaviors suggest that there is a greater complexity to the structure of the cubozoan visual system and, by extension the cubozoan nervous system. While the common model of a cnidarian nervous system is a primitive nerve net containing randomly dispersed neurons (Watanabe et al. 2009), the nervous systems of some cnidarians are much more complicated. Some cnidarian nervous systems are polarized and employ synaptic communication between several nerve nets (Moroz 2009). The most supported model of the cnidarian nervous system is that of two neural nets, a motor nerve net and a diffuse nerve net (Nakanishi et al. 2009). The DNN transfers sensory information to the rhopalium and the rhopalium sends motor impulses to the MNN for motor output (Nakanishi et al. 2009).

The study of the nervous systems of various simple organisms provides a window into the possible evolution of the more complex nervous systems of invertebrates and vertebrates alike. Cnidarians represent a stepping stone in the development of complex nervous systems (Watanabe et al. 2009) and research into the exact mechanisms of the cnidarian nervous systems, especially those of cubozoans, could provide further insight into understanding the evolution of the nervous system. A future direction of research regarding cubozoans could be a comparison between cubozoa and other genuses of cnidarians such as bilateria and ctenophores to determine where cubozoa lies in relation to the two. While many cnidarians are more closely related to ctenophores (Garm et al. 2007), it is very possible that the more polarized nervous system of cubozoa puts it in closer relation to bilateria in complexity. It would also be beneficial to continue to look into the evolutionary development of the cubozoan nervous system specifically to determine an exact location of the marginal center, which remains elusive (Watanabe et al. 2009). It is very possible that there are more aspects of the cubozoan nervous system that have yet to be discovered that may provide further evidence of greater complexity.

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The Extent to which Enrichment Activities Involving Show Behaviors Influence California Sea Lion Behavior (*Zalophus californianus*)

Madison Marullo and Roberta Moore
Rhodes College

Captivity of animals such as California sea lions (Zalophus californianus) sometimes results in the occurrence of stereotypical behaviors. Thus, there is a need for environmental enrichment to deter these unwanted behaviors. We conducted our research on two of the five California sea lions at the Memphis Zoo to determine how enrichment activities impacted "normal" sea lion behavior and to determine areas of locational concentration in the exhibit before, during, and after shows. We created a gridded map of the enclosure, in addition to constructing an ethogram in order to collect both activity budget and locational data. Although we were unable to conduct any statistical analyses, it was still important that we analyze these data visually because for captive research, especially with zoo animals, it is important for showing descriptive, individual behaviors. These data revealed that before a show Chloe and Callie spent the majority of their time swimming below the water. Chloe also spent a significant amount of her time swimming and looking around, perhaps in anticipation for the show. During the shows, both sea lions spent most of their time participating in show behaviors. Locationally, before and during the show, both Chloe and Callie spent the majority of their time in the area where the show would take place. After the show, Chloe spent the majority of her time in the left half of the exhibit, while Callie spent the majority of her time in the right half. No stereotypical or abnormal behaviors were encountered over the course of this study, which suggests that these animals are well taken care of and are stimulated by their environment.

Introduction

California sea lions (*Zalophus californianus*) belong to the pinniped family, meaning mammals with flippers, and they live in large colonies or rookeries along the shores of the Pacific Ocean extending from western North America to the area near Ecuador's Galapagos Islands (Weise et al., 2006; National Geographic, 2012; *Fig. 1*). This species of sea lion is highly polygynous throughout its range with males accumulating harems of females in order to compete to sire offspring (Ono et al., 1987; National Geographic, 2012). California sea lions are very well adapted to both aquatic and terrestrial habitats. They have a streamlined body and powerful front flippers, which allow them to propel through the water at up to 25 miles per hour in addition to the superb ability of hunting for fish due to their extreme range of flexibility (Feldkamp, 1987; National Geographic, 2012). California sea lions have a thick layer of blubber, keeping them warm in the chilly Pacific waters, and are able to slow down their heart rate in order to dive for up to ten minutes at a time before resurfacing for air (Weise et al., 2006; National Geographic, 2012). Adult California sea lions are approximately 5 - 8 feet in length and generally live less than 30 years out in the wild; however, due to the absence of predators such as sharks and killer whales, and the abundant availability of resources in captivity, these California sea lions can live to be much older (Chilvers et al., 2010; National Geographic, 2012). Unfortunately, behavior of California sea lions have been studied little in comparison to other members of the pinniped family, such as walruses and seals. The relatively small amount of research on California sea lions is unusual in that California sea lions are good study subjects because they are very sociable, have phenotypically different attributes distinguishing sex, they are relatively inactive on land, abundant on the west coast of the United States, and are generally indifferent to the presence of humans (Bartholomew and Peterson, 1967).

http://www.pwlf.org/californiasealion/d
istribution.htm

Fig. 1. Natural Range of the California Sea

Captivity of wild animals such as the California sea lion can result in the occurrence of stereotypical behaviors: repetitive behaviors that are consistently displayed by the animal that do not seem to serve a particular purpose (Kastelein and Wiepkema, 1988). Numerous studies have been conducted in order to determine the best method of eradication of these unwanted behaviors across a wide variety of taxa, and thus far, the best method for the suppression of stereotypical behaviors is the use of environmental enrichment (Shepherdson and Swaisgood, 2005; Clubb et al., 2007; Kastelein and Wiepkema, 1988). Environmental enrichment involves items such as food, objects that stimulate the senses, or habitat items in order to enhance the well being of the species'. Keepers provide environmental enrichment to the animals in order to reduce the presence of stereotypical behaviors and stress, in addition to training the animals to perform certain behaviors important in conducting husbandry procedures (Brando 2010). Positive reinforcement is used to support desired behaviors by means of operant conditioning, allowing the keepers to build bonds with the animals in addition to creating a safe learning environment (Brando, 2010). When done correctly, keepers are able to see an increase in frequency of desired behaviors through application of positive reinforcement (Brando, 2010). Such desired behaviors consist of behaviors that sea lions would generally use in the wild such as porpoising, and behaviors that are beneficial to the keepers in husbandry practices such as checking whiskers, eyes, and teeth (Brando, 2010).

Clubb et al. (2007) stated that approximately 10,000 captive animals of a variety of different species were affected by stereotypical behavior worldwide. In addition, Clubb et al. (2007) argued for 'zero tolerance' of these repetitive behaviors, which meant the inclusion of environmental enrichment in zoo and aquarium programs because the stress that arises from these repetitive behaviors are ethically questionable. Kastelein and Wiepkema (1988) also argued that deficiencies in food, housing, interaction with other species and keepers, care-taking, and health could lead to the presence of stereotypical behavior. Kastelein and Wiepkema (1988) found that during weeks when training was not performed, Steller sea lions (*Eumetopias jubata*) spent 7.2% of the time engaging in stereotypic behaviors, such as swimming repeatedly in circles around the enclosure; however, when training was included in the animal's day-to-day activities, presence of stereotypic behaviors was reduced to 0.5%. Kuczaj et al. (2002) noted that it was important to have variable presentation of enrichment items because it was found that the longer animals had access to particular enrichment items, the less enriching the items became after a certain amount of time elapsed. Today, environmental enrichment has become a necessary program in institutions accredited by the Association of Zoos and Aquariums (Association of Zoos and Aquariums 2012). One type of environmental enrichment used specifically for sea lions are the sea lion shows, which incorporate a number of "show" behaviors in addition to the use of enrichment items such as balls and frisbees.

Our research question was: To what extent does the sea lion show influence "normal" California sea lion (*Zalophus californianus*) behavior? We have defined "normal" behavior as being any type of behavior displayed in captivity in the absence of any form of enrichment, whether it be activities or items. Also, we have defined "show" behavior as any type of behavior displayed by sea lions in captivity during enrichment activities such as shows. Our hypotheses and predictions were: (H_1) activity budgets differ based on the presence of a show; (P_1) activity budgets for sea lions would differ before, during, and after a show; (H_2) exhibit use differs based on the presence of a show; (P_2) exhibit use for sea lions would differ before, during, and after a show; and finally, (H_0) there is no difference in behavior or exhibit use before, during, or after a show.

Conduction of this behavioral and locational study was important for a number of reasons. Although zookeepers spend a lot of time with the animals that they care for, they do not have the time to conduct formal research pertaining to topics that interest them. Therefore, by conducting this study on behavior of the California sea lions and taking the keepers interests into account we may relay invaluable information to the keepers. In addition, through data collected during this study we can determine if certain activities influenced positive or negative behaviors in the sea lions. If there was an increase in negative or agonistic behavior at any certain point during the study, we would be able to determine what might be causing this unwanted behavior and hypothesize ways to remedy it. Lastly, there is an abundance of research on how enrichment affects stereotypical behaviors in other members of the pinniped family; however, only a small amount of this information is directed specifically towards California sea lions, especially in the area of wild behaviors. Conduction of this research will contribute to the amount of research available dealing with the extent to which enrichment activities affect behavior of captive California sea lions, allowing new research questions to be formed about California sea lions both in captivity and in the wild.

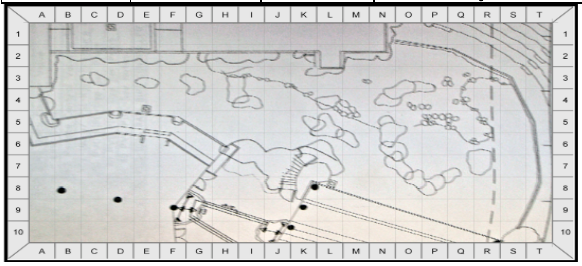

Fig. 2. A gridded aerial view of the layout of the California sea lion (*Zalophus californianus*) exhibit in the Northwest Passage at the Memphis Zoo.

Methods

Study Subjects and Location

Our study site was the California Sea Lion exhibit located in the Northwest Passage area of the Memphis Zoo. The California sea lion exhibit is divided into two major areas: the area that can be seen by visitors from the stands outdoors, comprising the right side of the exhibit, and the area of the exhibit which can be seen from the indoor viewing area, comprising the left side of the exhibit. Our study subjects included the five California sea lions housed in this exhibit: Skimmer, Chloe, Andre, Callie, and Teva. We distinguished the five sea lions through use of defining characteristics provided by the keepers (Tammy Hill, pers. comm.; *Table 1*). However, Chloe and Callie were the only two sea lions on exhibit for the duration of this study.

Name	**Age**	**Sex**	**Defining Characteristics**
Teva	Almost 5 months old	Female	Smallest of the group, not available for this study
Callie*	10 years old	Female	Teva's mother, weighs approximately 200 lbs, darker in coloration, larger than Chloe
Andre	18 years old	Male	Teva's father, weighs approximately 600 lbs, biggest sea lion, survivor of Hurricane Katrina, not available for this study
Chloe*	24 years old	Female	Skimmer's younger sister, weighs approximately 200 lbs, 2 ring impressions around her neck, muted brown in coloration, smaller in size than Callie
Skimmer	26 years old	Female	Chloe's older sister, oldest member of the sea lions in this group, not available for this study

Table 1: Names, Ages, and Defining Characteristics for the Five California Sea Lions Housed in the Northwest Passage at the Memphis Zoo (* = on exhibit)

Data Collection

We constructed an ethogram throughout one, three-hour observation of the sea lions, noting behaviors that they performed in the presence and the absence of a show, and divided the ethogram accordingly between non-show behaviors (*Table 2*) and show (*Table 3*) behaviors. We defined "show" behaviors as any type of behavior exhibited while participating in enrichment activities such as shows. In addition, we overlaid a grid over the blueprints of the sea lion enclosure in order to record locational data of the sea lions at two-minute intervals (*Fig. 2*; Martin and Bateson, 2007). By accompanying the activity budget data that we collected with our locational data, we were able to determine which behaviors they participated in which parts of the exhibit. Behavioral and locational data were collected 30 minutes before the show, 16 minutes during the show, and 30 minutes after the 1:30pm and 3:00pm shows. Unfortunately, the 1:30 pm show was cancelled because of the shift to the winter schedule in October; therefore, we adjusted our schedule to incorporate two shows per week, resulting in an equal amount of data collection each week. The duration of this study spanned from October 22, 2012 to November 8, 2012. We both collected behavioral and locational data of the sea lions at two-minute intervals using scan sampling (Martin and Bateson, 2007) from left to right of the enclosure because the study site was divided into two large sections, making it impossible for one person to survey the entire enclosure. Therefore, one researcher sat in the stands in front of the pool in order to survey the right half of the enclosure, while the other researcher surveyed from the enclosed indoor area, the left section of the exhibit.

Table 2: California Sea Lion (*Zalophus californianus*) Ethogram for Non-show Behaviors.

Routine Sea Lion Behaviors (Non-show)	**Description of behavior**
Swimming Below Water (SB)	Actively moving below the surface of the water
Swimming Above Water (SA)	Actively moving in the water with its head above the surface for >2 seconds
Swimming Above Water & Looking (SAL)	Actively moving in the water with its head above the surface for any amount of time and displays noticeable head movement
Resting (R)	Laying or sitting on rocks with eyes opened or closed
Resting on Bottom (RB)	Laying at the bottom of the pool, no movement
Floating (F)	In water, breathing in and out resulting in a bobbing motion or animal is stationary in the water
Itching (I)	Rubbing part of body with head or flippers or against an inanimate object
Pencil Float (PF)	Floating in water, back flippers are point up while head is down, body perpendicular to bottom of the pool
Agonistic (A)	Any aggressive behavior (biting, vocalization, etc.). Specify behavior.
Vocalizing (V)	Non-aggressive communication
Interacting with Crowd (IC)	Sea lion is interacting with the crowd through the glass (following them around, playing with them, etc.)
Out of Site (OOS)	Researcher is unable to see the sea lion in the enclosure

Table 3: California Sea Lion (*Zalophus californianus*) Ethogram for Show Behaviors.

Show Behaviors	**Description of Behavior**
Wave (W)	One front flipper extended. "Waving" by shaking flipper.
Barking (B)	Vocalizing at the request of the keeper.
Playing Frisbee (PF)	Catching, holding, throwing, or returning to keeper.
Playing With Ball (PB)	Balancing ball on nose or returning to keeper.
Spanking (Sp)	Slapping one of front flipper against back end.
Porpoising (Po)	Jumping out of water in an arch and diving back in while swimming. Belly is facing the water.
Shark Fin (SF)	Swimming in a circle with one front flipper out of the water to resemble a shark fin.
Blowing Kisses (BK)	Bringing one of front flipper to mouth and then away from mouth repeatedly.
Spin Around (SpA)	Out of the water, using all four flippers to turn in a circular motion.
Vocalizing (V)	Non-aggressive communication.
Balancing on Front Flippers (BF)	Out of water, supporting all of weight on front two flippers while raising torso and back flippers into the air.
Showing Whiskers (Wh)	Nose in the air, whiskers splayed out.
Flipping (F)	Jumping all or partly out of water, turning in a tighter circle/arch than porpoising, and diving back into water. Belly is facing up.
Showing Ears (E)	Out of the water, animal bends at the side towards their back flippers to point at their ear with one of their back flippers.
Bending Head Back/Displaying Flexibility (BHB)	Out of the water, animal bends head backward so nose or forehead touches spine.
Nodding (N)	Shaking head vigorously.
Playing Dead (PD)	Out of the water, animal lays flat on its back OR animal lays flat and then rolls over onto back.
Showing Teeth (T)	Animal opens mouth to display teeth.
Seal Scooch (SS)	Animal lays flat and curls back to pull back flippers forward and releases, creating an inching motion.
Eating (EAT)	Animal catches and

	swallows fish given by keeper.
Rocking (Rk)	Animal sits on land, shifting weight from side to side in anticipation for a command.
Jumping (J)	Lifting front half of body and front flippers off of the ground repeatedly.
Shading Eyes (SE)	Animal bends at the side towards their back and brings one front flipper forward to cover their face.
Show Flippers, No Shaking (Sfn)	Animal shifts weight to one front flipper to raise their other front flipper into the air; no shaking of flipper.
Sticking Head in Water (SW)	Body is out of the water; animal bends towards the water in order to put head underwater; underwater sight test.
Racing (Rc)	Swimming around the pool at a high speed causing a large wake.
Clap (C)	Animal puts weight on one of front flippers, brings other front flipper towards the other one holding itself up in a clapping motion.
Sitting (St)	Out of the water, torso off of ground and supported by front flippers, little movement.
Kissing (K)	Two animals bring noses together to touch.
Twirling Above Water (TA)	Sea lion has its head above the water and is swimming in a twirling motion
Twirling Below Water (TB)	Sea lion is underwater and is swimming in a twirling motion

Analysis

To make our data more feasible we grouped certain behaviors into broader categories (*Table 4*). We were unable to run any statistical analyses because of our small sample size, so we relied on descriptive statistics to compare the proportion of time that Chloe and Callie spent conducting certain behaviors before, during, and after a show. Averages were calculated using data from specified groupings of time to denote before, during, and after shows over a period of days. ArcGIS 10.1 was used to create kernel density for our locational data in order to determine the areas in which Chloe and Callie spent most of their time before, during, and after shows.

Table 4: Groupings of California Sea Lion Behaviors

Groupings	**Behaviors Within Groupings**
Show	See *Table 3* for a list of show behaviors
Swimming Below	Swimming Below
Swimming Above	Swimming Above
Swimming Above Looking	Swimming Above and Looking
Twirling	Twirling Above, Twirling Below
Resting	Resting, Resting on Bottom
Sitting	Sitting
Other	Floating, Itching, Pencil Float, Agonistic, Vocalizing, Interacting with Crowd

Results

Before a show, Chloe spent the majority of her time (mean ± standard error) swimming below the water (0.52 ± 0.04; *Fig. 3*). In addition, she spent a portion of her time swimming above the water and looking (0.23 ± 0.042). During the show, Chloe primarily performed show behaviors (0.53 ± 0.01); however, she also exhibited behaviors such as swimming below the water (0.19 ± 0.05) and sitting (0.23 ± 0.08; *Fig. 3*). After the show, Chloe primarily swam below the water (0.44 ± 0.05), and this is also the only time she rested (0.08 ± 0.04). Overall activity budget data (mean ± standard error) computed for Chloe revealed that she spent the highest proportion of time swimming below the water (0.41 ± 0.08), then swimming above (0.17 ± 0.06), exhibiting show behaviors (0.15 ± 0.10), swimming above and looking (0.13 ± 0.03), sitting (0.08 ± 0.00), twirling (0.03 ± 0.02), resting (0.02 ± 0.07), and finally, other (0.01 ± 0.17; *Table 5*). Before a show, Callie spent most of her time swimming below the water (0.53 ± 0.10; *Fig. 4*). During the show, Callie spread her time almost equally between performing show behaviors (0.30 ± 0.01), swimming below the water (0.25 ± 0.05), and sitting (0.27 ± 0.09; *Fig. 4*). After the show, Callie swam below the water the greatest proportion of the time (0.66 ± 0.05; *Fig. 4*). Computed overall activity budget data (mean ± standard error) for Callie revealed that she spent the greatest proportion of her time swimming below the water (0.51 ± 0.01), followed by twirling (0.09 ± 0.04), sitting (0.08 ± 0.02), exhibiting show behaviors (0.07 ± 0.12) and swimming above the water (0.07 ± 0.02), and the lowest proportion of time swimming above and looking (0.06 ± 0.03) and resting (0.06 ± 0.08; *Table 5*).

Table 5: Overall Activity Budget

	Show	*Swimming Below*	*Swimming Above*	*Swimming Above Looking*	*Twirling*	*Resting*	*Sitting*	*Other*
CHLOE	0.15	0.41	0.17	0.13	0.03	0.02	0.08	0.01
CALLIE	0.07	0.51	0.07	0.06	0.09	0.06	0.08	0.06

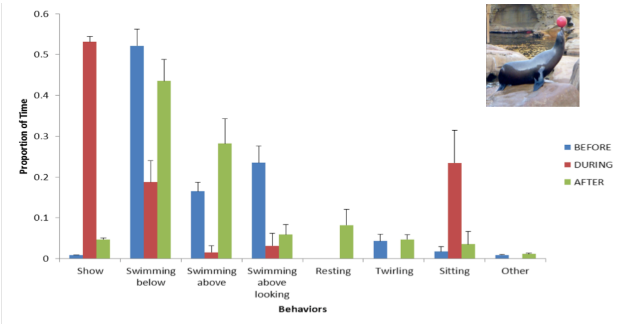

Fig. 3. Activity budgets (mean ± standard error) for the proportion of time that Chloe spent displaying certain behaviors before, during, and after a sea lion show.

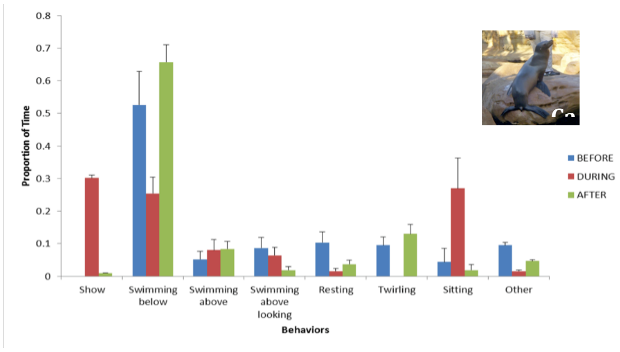

Fig. 4. Activity budgets (mean ± standard error) for the proportion of time that Callie spent displaying certain behaviors before, during, and after a sea lion show.

Chloe spent the most of her time before shows in the right side of the enclosure (*Fig. 5*). During the show, Chloe spent most of her time in the area where the show took place (*Fig. 6*). Finally, after the show, Chloe spent most of her time on the left side of the sea lion enclosure by the underwater viewing area (*Fig. 7*). Callie distributed her location throughout the enclosure before the occurrence of a show (*Fig. 5*), during the show, Callie, like Chloe, seems to have spent most of her time in the area in which the show took place (*Fig. 6*), and after the show, Callie seems to have spent the most time along the glass portion of the exhibit (*Fig. 7*).

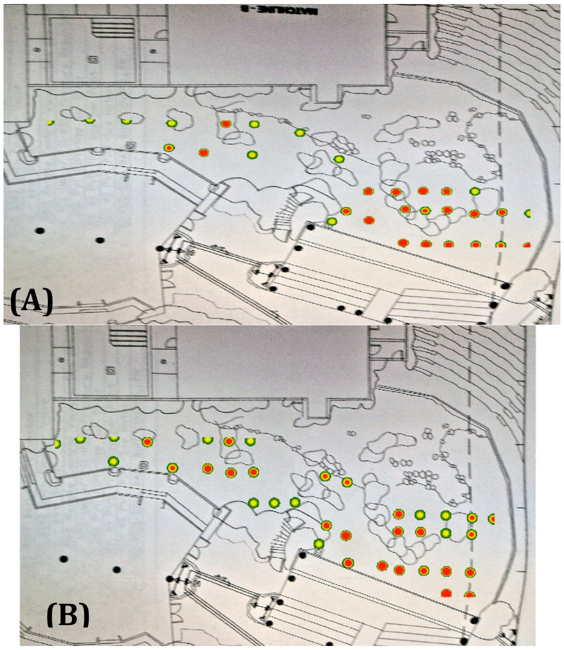

Fig. 5. The more time that Chloe (A) and Callie (B) spent in a particular grid of our map is denoted with a redder spot, while the less time spent in a particular area is denoted by a yellowish green spot.

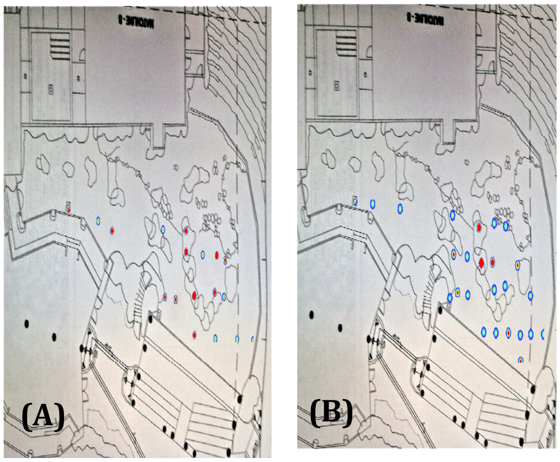

Fig. 6. The more time that Chloe (A) and Callie (B) spent in a particular grid of our map is denoted with a redder spot, while the less time spent in a particular area is denoted by a light blue spot.

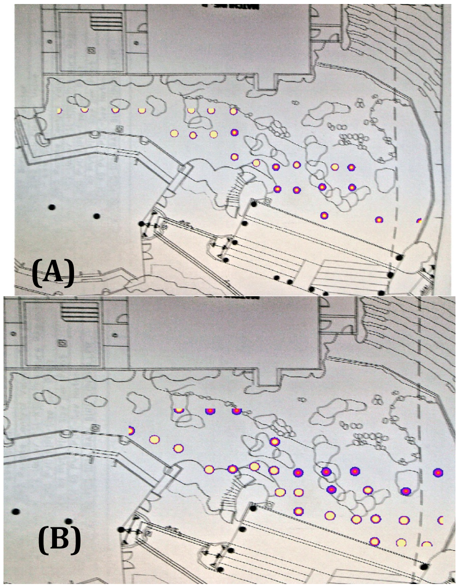

Fig. 7. The more time that Chloe (A) and Callie (B) spent in a particular grid of our map is denoted with a more yellow spot, while the less time spent in a particular area is denoted by a more purple spot.

Discussion

Although we were unable to conduct any statistical analyses, and therefore were unable to accept or reject our null hypothesis, visually analyzing the data revealed much information about Chloe and Callie as individuals. One aspect of the activity budget that was interesting was that Chloe spent a proportion of time before shows swimming above and looking around, as if in anticipation for the show that was to come. Although we are unable to prove this, my partner and I both noticed that approximately fifteen minutes before the occurrence of a show Chloe began to exhibit this behavior. In the future it may be interesting to use this observation and conduct a study on how sea lion behavior changes approximately 15 minutes before a show. Another aspect of the activity budget that revealed individual information about the sea lions was behavior exhibited during a show. Chloe appeared to be more invested in the show, spending the majority of the time participating in show behaviors. Callie, however, split her time almost evenly between show behaviors, swimming below the water and sitting. The difference in behaviors between the two female sea lions could partially be due to the age of the sea lions. Chloe is older than Callie and perhaps is more attuned to the shows because she has been participating in shows for a longer period of time. It also could be that because Chloe is older than Callie, she has less energy than Callie does and therefore does not move around as much. Another aspect that was interesting for both Chloe and Callie pertaining to the shows was that both sea lions displayed some show behaviors, though very little, beyond the length of the show time. Although some of the shows only lasted for the 16-minute period that we observed behaviors, the keepers would continue to train the sea lions until all of the left over fish were gone – as long as the sea lions cooperated. Also, the behavior seen before the show that Chloe exhibited was rocking, which we observed mostly when Chloe was waiting for a command from the keeper, so we viewed this behavior as an anticipatory behavior.

For the location data, both Chloe and Callie spent the greatest proportion of time around the area in which the show would take place before and during a show. Although Callie did use a greater area of the exhibit during the show, she still spent the greatest amount of time in the proximate area to where the show was taking place. After a show, Chloe spent most of her time in the left half of the exhibit near the underwater viewing area. This may be because after a show, the sea lions were allowed inside to visit with Teva, Andre and Callie's daughter that was born in June 2012. There happens to be a gate that leads to the inside portion of the exhibit on the left side of the exhibit, so Chloe may have been concentrating her time in this area in hopes that she would be let inside. Callie on the other hand, spent most of her time in the deep-water pool located in the right corner of the exhibit. This was not very surprising because we observed that Callie spent a lot of her time floating and resting in the deep-water pool. However, it was surprising that there was such a high concentration in the right half of the exhibit because Callie enjoyed interacting with the crowd via the underwater viewing area.

Our research related to that of Kuczaj et al. (2002) in that it is extremely important that the sea lions do not have access to the same enrichment items all of the time because increased exposure to these items yields less enrichment over time. Over the course of this study, we did not observe any stereotypical behaviors, suggesting that shows can be considered a form of enrichment for these animals and they are engaged in their surrounding environment. Our research also relates to the research of Kastelein and Wiepkema (1988); however, it would need to be compared to other research in which other sea lions were not exposed to environmental enrichment as often. One option for future research would be to track the patterns of the individuals at a shorter time interval and compare the patterns of locomotion in the presence and absence of enrichment. Researchers could then determine if patterns exist in the absence of enrichment and if this could be considered the equivalent to pacing behavior found in terrestrial animals.

One main problem that we encountered in our research was that only two of the five California sea lions housed at the Memphis Zoo were available for the duration of this study. The lack of individuals lead to an extremely small sample size, which made it impossible for us to conduct any statistical analyses, and therefore, our conclusions are limited. Also, although we did our best to denote the location of Chloe and Callie at two-minute intervals, there are many areas of the enclosure where the sea lions are able to hide such as arches and behind boulders. In addition, the enclosure is split into two large chunks and it is very hard to see the area of the enclosure between the two sides, resulting in a blind spot. We did our best to denote location as accurately as possible. Next, the shows at the beginning of this study were an hour and a half apart; however, at the end of the study the shows were more spread out throughout the day. Therefore, it is possible that for the beginning of this study, some of the behaviors from after the first show at 1:30 pm lagged into the behaviors before the next show at 3:00 pm.

In order to address some of these problems, it would be better to continue this research at a time when all five sea lions were on exhibit in order to achieve a larger sample size. There is really no remedy for the blind spot in the enclosure because there is a barrier, making it impossible to determine exactly where a particular sea lion is if they happen to be in that blind spot. Perhaps the grid overlay on the graph could have been made a bit larger in order to encompass that area, so that if they were in that particular area, it would be denoted by one square as opposed to two. Lastly, it might have been better to conduct this study either in the summer or the winter, rather than when the seasons were changing and therefore so were the show times. It would be better to collect data either during the summer hours or the winter hours, not both, to avoid data being skewed.

This research has the potential for numerous directions in the future. It is possible to expand this research across numerous zoos achieving a larger sample size and more likely more accurate conclusions for how enrichment activities such as shows affects sea lion behavior and location. In addition, this research could open multiple new doors into the study of California sea lions in the wild, considering that not many studies exist for this particular species. Also, it would be possible in the future to include not only an aerial grid, but also a grid denoting depth in order to more exactly indicate location of the sea lions during certain time intervals. Another aspect of this research that would be interesting to look at in the future is the sea lion equivalent of pacing. It would be interesting to map the individuals at a shorter time interval to determine if there were patterns, which could possibly be the marine equivalent of pacing. This research not only gives us the opportunity to explore how enrichment activities such as shows affects behavior of sea lions, but also gives us the chance to study how these enrichment activities affect the sea lions individually. By doing so, future research will provide ways in which zoos around the world can better care for and reduce the occurrence of these unwanted stereotypical behaviors.

Conclusions

1. Before the show, Chloe and Callie spent most of their time swimming below the water. Also, Chloe spent a portion of her time before shows swimming and looking above the water, perhaps in anticipation of the show that will occur. Locationally, Chloe spent more time in the area in which the show would take place before the show, while Callie utilized more area in the exhibit.
2. Chloe spent a large proportion of time participating in show behaviors during the show while Callie distributed behaviors equally between show behaviors, swimming below the water, and sitting. Both sea lions spent the majority of their time locationally in the area where the show took place during the occurrence of a show.
3. After the show, both Chloe and Callie spent the majority of their time swimming below the water. Chloe spent most of her time in the left half of the exhibit by the underwater viewing area, while Callie spent the majority of her time in the right

half of the exhibit, near the deep-water pool.

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We would like to thank the Memphis Zoo for allowing us to conduct this research on the California Sea Lions. We would specifically like to thank the sea lion keepers Tammy Hill and Emily Smith for all of the help that they provided to us with regards to background and natural history information for the sea lions. We would also like to thank Professor Boyle for all of the help that she provided to us for this project throughout the duration of this semester.

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About the author: Madison Marullo '13 is a senior Biology major from New Orleans, Louisiana. She is a member of Beta Beta Beta Biology Honor Society, as well as being involved in conducting behavioral research at the Memphis Zoo on the African elephants, Nile hippopotamuses, and California sea lions. Her major areas of interest are conservation and marine biology. Madison interned at the Audubon Aquatic Center rehabilitating a wide range of marine life after the BP oil disaster in addition to her previous internship at the Audubon Aquarium of the Americas. After graduation, she will be interning at Mote Marine Laboratory in Sarasota, Florida conducting behavioral and genetic research on manatees. She plans to ultimately pursue a master's degree in marine biology.

The Rusty Blackbird (*Euphagus carolinus*): A Once Common Species in Mysterious Decline

Kelly Patton
Rhodes College

The Rusty Blackbird (*Euphagus carolinus*), has declined by more than 88% over the past several decades. Although no one knows why the once common North American species has declined at an alarming rate of ~ 5.1% a year, several hypotheses do exist. Hematozoa infections, global climate change, mercury contamination, predation, and habitat loss are all factors that are hypothesized to limit the reproductive fitness and survival of the Rusty Blackbird. Since little information is known on the behavioral ecology and social organization of the Rusty Blackbird, further research in these areas will help researchers evaluate valuable management efforts that will improve the conservation of the species.

Introduction

The Rusty Blackbird (*Euphagus carolinus*) breeds in boreal forests across North America where it is associated with forested wetlands (Matsuoka et al. 2010). During the nonbreeding months of the year, the Rusty Blackbird migrates to the southeastern United States where it can be found nesting in anthropogenic habitats such as orchards, agricultural fields, and urban areas as well as wooded forests, river bottomlands, and flooded woodlands (Greenberg & Droege 1999, Edmonds et al. 2010). This once abundant North American songbird has experienced a population decline of greater than 88% over the last half century at a declining rate of ~5.1% a year (Greenberg & Matsuoka 2010). Although there is no definitive answer behind the Rusty Blackbird's decline, there are several plausible hypotheses. Global climate change, competition, disease, and environmental contaminants have been advanced as causes of accelerated decline. In this paper, I will first present current published hypotheses that assess potential causes for the Rusty Blackbird's decline, with a specific focus on blood parasites, environmental mercury contamination, predation, global climate change, nesting ecology, and habitat loss. I will then examine the importance of future research and address future conservation management designs that will need to be implemented to aid in the conservation of the Rusty Blackbird.

Environmental Contaminants: Mercury

Environmental mercury (Hg) poisoning is a common occurrence in migratory songbirds and is commonly sequestered by the Rusty Blackbird for two primary reasons: diet and high bioavailability, which is the access a chemical or environmental pollutant, has to an organism in the environment (Edmonds et al. 2010). During the breeding season, Rusty Blackbirds forage almost exclusively on terrestrial and aquatic invertebrates such as insects and spiders, while during the nonbreeding season their diet consists mostly of plant matter (Edmonds et al. 2010). Methylmercury (MeHg) has a high bioavailability due largely to aquatic point-source contamination, which makes it highly toxic in bird species (Edmonds et al. 2010). Edmonds et al. (2010) found that in blood samples of Rusty Blackbirds, mercury concentrations were greater in breeding birds than in nonbreeding birds and were 3 to 4 times greater in the Acadian forests of New England and the Canadian Maritimes than in the boreal forests of Alaska. Hg concentrations in the blood are usually associated with a species' food habits; therefore, sampling the Rusty Blackbird's diet resources during the breeding and nonbreeding season would be helpful in determining the trends and sources of Hg bioavailability (Edmonds et al. 2010). Furthermore, the trend of higher Hg concentrations in Acadian forests implies higher local concentrations of Hg in comparison to the other regions studied (Edmonds et al. 2010). Although there is no current study that focuses on the negative effects of mercury blood concentrations on the Rusty Blackbird, Heinz et al. (2009) discovered that blackbirds in particular seem to be more sensitive to MeHg poisoning during embryonic development than other studied species. Furthermore, Heinz et al. (2009) showed that MeHg blood concentrations at 1.8 mg/g killed 100% of the Common Grackle embryos when injected. Hence, Edmonds et al. (2010) found it reasonable to presume that MeHg embryonic levels between 1 to 3 mg/g would cause negative effects on the Rusty Blackbird where 95% of the feathers sampled from the Acadian forests exceeded the 1-3 mg/g level (Edmonds et al. 2010).


Figure 1: Image of Rusty Blackbird (from National Zoo)

Blood Parasitism

Barnard et al. (2010) discovered that from 2007-2009, there was an unusually high prevalence (49%) of hematozoa infections in wintering Rusty Blackbirds found in Mississippi and Arkansas, compared to previous studies on avian hematozoa. Blood parasites during the nonbreeding season generally have lower transmission rates, due to a reduced density of blood parasites and vectors (Garvin et al. 2004). This high prevalence of hematozoa infections of the wintering Rusty Blackbird may be due to a nonseasonal relapse which suggests a lowered immune response due to higher environmental stresses (Barnard et al. 2010). Wintering habitat changes, in particular, drying wetlands may be forcing Rusty Blackbird populations to forage in less suitable habitats where intra- and interspecific competition with other species of blackbird is likely, leading to a lowered immune response and a relapse of hematozoa infections (Klein et al. 2005, Barnard et al. 2010). The unusually high prevalence (49%) of hematozoa infections in wintering Rusty Blackbirds may also be due to a lowered innate immune response due to environmental stressors such as mercury bioaccumulation (Edmonds et al. 2010). Sol et al. (2002) found that high levels of hematozoa infections can result in severe anemia, weight loss, and death in some juvenile birds; therefore, further research on the causes and effects of hematozoa infections in the breeding and wintering range of Rusty Blackbirds should be implemented in order to gain a better understanding on how these infections contribute to the species' decline (Edmonds et al. 2010).

Climate Change

Range retraction is the most common mechanism by which directional climate change negatively affects species populations (McClure et al. 2011). Therefore, McClure et al. (2011) tested for evidence of range retraction in relation to weather observation data in the declining Rusty Blackbird. Statistical analysis was performed on two regions: survey routes east of -100 degrees longitude (eastern region) and survey routes west of -100 degrees longitude (western region) (McClure et al. 2011). In the eastern region, the Rusty Blackbird's southern range has shifted 142.60 kilometers north since 1966 (Figure 2; McClure et al. 2011).

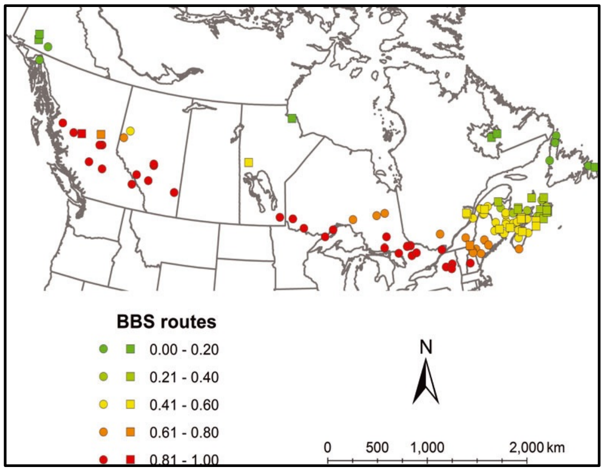

Figure 2: A diagram representation of the probability of extinction of Rusty Blackbirds between the periods 1967-1977 and 1998-2008. Circles represent areas where Rusty Blackbirds no longer existed and squares represent where Rusty Blackbirds still persisted between the two time periods (from McClure et al. 2011).

There is a markedly northward shift of Rusty Blackbird populations since 1967 (Figure 2; McClure et al. 2011). Furthermore, weather observation data of the Pacific Decadal Oscillation (PDO), a fluctuation of oceanic sea surface temperatures over a period of 20-30 years, affects the climate across much of North America (McClure et al. 2011). The PDO significantly corresponded to annual northward shifts of the Rusty Blackbird's breeding range (McClure et al. 2011). McClure et al.'s (2011) results provide evidence that changes in climate negatively affect Rusty Blackbird populations by limiting their distribution range. Although Wilcove and Terborgh (1984) found that some declining species showed evidence of range retraction when climate change was not taken into account, it is important to note that range retractions caused by climate change exhibit different patterns than range retractions caused by the loss of species (McClure et al. 2011). Range retraction due to declining populations shows distribution loss around the periphery of the species' range, while range retraction due to climate change generally shows distribution loss around the southern margin, the latter as found by McClure et al. (2011). McClure et al. (2011) is the first study to provide evidence that climate change has a negative impact on the distribution range of the Rusty Blackbird and is a large contributing factor in its decline

Habitat Loss

Hamel et al. (2009) evaluated the potential effect of habitat loss in the population declines of nonbreeding Rusty Blackbirds by collecting information on population trends of Rusty Blackbirds across the Southeastern region of the United States. Population data collections from years 1953-2001 showed a peak in the number of Rusty Blackbirds, 1.3 million birds, in 1969 (Hamel et al. 2009). Hamel et al. (2009) discovered that the prevailing pattern of Rusty Blackbird populations in the eastern and western nonbreeding regions was a trend of population decline since 1953. Moreover, Hamel et al. (2009) found that there was no significant difference in rate of decline between the eastern and western nonbreeding ranges of the Rusty Blackbird. To quantitatively compare population trends with habitat patterns, Hamel et al. (2009) collected data on forested wetlands and land use trends in the nonbreeding range of Rusty Blackbirds. In the Southeast region of the United States loss of forested wetlands from the 1950s-1970s was attributed to agricultural uses of the land (Hamel et al. 2009). Agricultural land use was more prevalent in the Lower Mississippi Valley (28%), compared to the South Atlantic states (16%) (Hamel et al. 2009). Furthermore, conversion to agricultural land has declined from 1983-2003 with a slower rate in the Lower Mississippi Valley (12%) than in the South Atlantic (25%) with an increase in urbanization (Hamel et al. 2009). Although there is little research on how Rusty Blackbirds use habitat at the landscape scale, Hamel et al. (2009) found that as Rusty Blackbird numbers dramatically decreased, there was not as steep of a decline in abundance of forested wetlands over the same time period in the eastern and western nonbreeding ranges. Population declines of the Rusty Blackbird in the Mississippi Alluvial Valley and the South Atlantic Coastal Plain were similar even though land uses were different in these areas (Hamel et al. 2009). However, Hamel et al. were unable to produce a sophisticated comparison of wetland habitat with Rusty Blackbird population patterns due to the lack of publicly available information. Therefore, it is difficult and premature to conclude that habitat changes in the nonbreeding range of Rusty Blackbirds are causally linked to population declines (Hamel et al. 2009).

Nesting Ecology

Improved knowledge on the Rusty Blackbird's nesting ecology is important for assessing whether low breeding success and other limiting factors such as nest competition, are causally linked to their overall decline (Greenberg et al. 2011). Matsuoka et al. (2010) found that Rusty Blackbirds inAlaska and Canada preferred nesting in dense conifers, primarily spruces (Alaska 100% in conifers, Canada 80% in conifers). The Rusty Blackbird's affinity for dense, coniferous habitats resulted in high rates of nest survival (56%) (Matsuoka et al. 2010). High nest survival rate would subsequently result in a higher number of nests; therefore, predation would likely decrease due to an increase in conifer density (Matsuoka et al. 2010). In contrast, Powell et al. (2010) found that in Maine, Rusty Blackbirds' nest preference for dense spruces resulted in lower nest survival rates (33%). This difference is mainly due to the fact that dense, coniferous habitats in New England had been disturbed within the past 20 years from logging practices, whereas Alaskan and Canadian habitats were not disturbed by anthropogenic factors (Matsuoka et al. 2010). Rusty Blackbird fledglings were 2.3 times more likely to survive in areas of no timber harvest than in areas that had been recently harvested within the past 20 years (Powell et al. 2010). Furthermore, out of the 14 nests that were located in areas of recent timber harvest, 100% failed to survive, with 11 of the nest failures attributed to predation and the remaining 3 from unknown causes (Powell et al. 2010). Although the reproductive success of Rusty Blackbirds in Alaska fluctuated from 2006-2008, average nest survival (56%) is similar to nest survival of Rusty Blackbirds in New England (62%, Powell et al. 2008). Overall patterns from studies in Alaska and New England disprove the hypothesis that the Rusty Blackbird's decline is largely due to low rates of nest success. In fact, nest survival of Rusty Blackbirds was higher than rates of other blackbird species such as Red-winged Blackbirds (30-39%, Martin 1995). However, the relatively low nest survival rates of Rusty Blackbirds in Alaska and New England may still be attributed to timber harvests in forests (Powell et al. 2010).

Future Research

Promoting conservation efforts and the need for more research on Rusty Blackbird populations is critical to the survival. In this section, I will discuss several studies that identify areas needed for future research focusing on nest success, habitat loss, and mercury exposure.

Nest Success:

The relationship between low reproductive success and the overall decline of the Rusty Blackbird needs to be studied over a longer period to provide stronger results (Matsuoka et al. 2010). Future studies should focus on areas in North America where Rusty Blackbirds are affected by human-induced habitat disturbance and low rates of nest survival, specifically in the boreal forests of Canada and New

England (Matsuoka et al. 2010). Moreover, 90% of the eggs in Alaska hatched, which was similar to the viability rate in New England where only 2 out of the 43 Rusty Blackbirds nests did not hatch (Powell et al. 2010). The high viability of Rusty Blackbird eggs in New England was unexpected because blood levels of methylmercury were three to four times higher in New England than in the boreal forests of Alaska (Edmonds et al. 2010). These high levels of methylmercury may lower body condition and survival rates of adult Rusty Blackbirds rather than harming the development of embryos (Matsuoka et al. 2010). The study of avian nest survival rates is valuable, but only measures one aspect of reproduction – future studies need to examine the survival of Rusty Blackbirds at multiple life-history stages (Powell et al. 2010). Because nest survival rates of Rusty Blackbirds are not limited to one causing factor, it is important to study the survival rates of juvenile and adult Rusty Blackbirds to understand the necessary rates of survival to sustain a population (Matsuoka et al. 2010). Furthermore, the comparison of juvenile and adult Rusty Blackbirds along multiple spatial scales and under different habitats of timber harvests would be ideal to understand the relationship between logging and Rusty Blackbird fitness. Greenberg et al. (2011) stress the importance of comparing physiological effects, survival rates, and growth rates in breeding and wintering Rusty Blackbird populations. These cross-seasonal investigations would aid in understanding the mechanisms behind the causes of the Rusty Blackbird's extraordinary decline.

Habitat Loss:

Hamel et al. (2009) were unable to conclude that the loss of nonbreeding habitat is causally linked to the Rusty Blackbird's decline; therefore, there is a need for research on changes in wetland habitats at a finer spatial scale. Knowing habitat patterns and population changes not only from a national scale, but also from a state and regional scale would help studies reach further conclusions on the relationship between habitat loss and population declines (Hamel et al. 2009). Furthermore, areas of wetland reforestation have increased the abundance of Rusty Blackbirds (Hamel et al. 2009). For example, a reforestation project was initiated in 1998 in Mississippi where there were no observations of Rusty Blackbirds in the area (Hamel et al. 2009). Moreover, from 1995-2002, Rusty Blackbirds were observed on a daily basis using 3 out of the 4 original afforestation treatments: natural regeneration, oak seeding, planted oak seedlings, and cottonwood oak inter-planting (Hamel 2003). Therefore, conservation efforts to promote the restoration of additional nonbreeding habitat would be beneficial for Rusty Blackbirds (Hamel et al. 2009).

Mercury Exposure:

Although Edmonds et al. (2010) presumed that MeHg embryonic levels within 1-3 mg/g would have negative effects on the Rusty Blackbird; there is currently no research on these harmful effects in Rusty Blackbirds. Further research should quantify the apparent risks of MeHg exposure in Rusty Blackbirds and analyze these effects across multiple breeding areas such as Quebec, Ontario, and New York (Edmonds et al. 2010). A standardized and continuous collection of Hg samples throughout the Rusty Blackbird's distribution range will also help to determine how the levels of Hg change in the environment (Edmonds et al. 2010). Researchers should also gain an understanding of how Hg exposure contributes to declining populations of the Rusty Blackbird and how exposure at the individual and population level affects reproduction and survival (Edmonds et al. 2010). Analysis of Hg exposure should not only be a study of nesting success, but also of juvenile and adult survival (Edmonds et al. 2010). Hg levels in Rusty Blackbirds should be compared with Hg levels of other bird species with increasing population sizes (Edmonds et al. 2010).

Conservation Management Designs

Understanding avian home ranges and distributions is helpful for creating conservation strategies, especially in areas of habitat destruction and predation. Powell et al. (2010a) determined that mean home ranges and core areas of Rusty Blackbirds in Maine was 37.5 and 11.1 ha. Furthermore, Rusty Blackbirds in loose colonies had home ranges and core areas three times larger than solitary nesting Rusty Blackbirds (Powell et al. 2010). Powell et al. (2010b) was the first study to find evidence of a colonial nesting Rusty Blackbird population in New England – out of the 43 nests found, only one colony was identified in 2007. The daily nest survival rate of Rusty Blackbirds in this colony was only 22.4% while daily nest survival was much higher at 66.2% in noncolonial birds (Powell 2008). This difference in daily survival may be due to the influence of timber management around wetlands (Powell et al. 2010). In other words, most colonial Rusty Blackbirds are located in areas of recent timber harvests and as stated before, fledgling survival is 2.3 times more likely in areas of no recent timber harvest (Powell et al. 2010a, Powell et al. 2010b). Powell et al. (2010b) determined that timber harvests seem to attract Rusty Blackbirds and act as an "ecological trap," providing an increasing number of water bodies as well as a higher density of conifers.

Therefore, it is important for the conservation of Rusty Blackbirds to create an unlogged buffer around foraging wetlands. Powell et al. (2010b) found that because 75 m buffers included 51% of the core areas of Rusty Blackbirds, this would be a suitable measurement in conservation strategies to protect Rusty Blackbird habitats. Creating buffers in areas that are required by the species – large areas with sufficient wetlands and dense conifers, away from high methylmercury exposure and nest predation – would be the most useful in terms of conserving the Rusty Blackbird by maximizing its reproductive success (Edmonds et al. 2010).

Conclusion

The Rusty Blackbird used to be one of the most abundant migratory birds in North America but has suffered a steep population decline greater than 88% (Greenberg and Matsuoka 2010). In 1932, Rosalie Edge's proactive statement has been widely accepted by conservation biologists today, "The time to save a species is while it is still common. The only way to save a species is to never let it become rare" (Furmansky 2009). For the Rusty Blackbird, this proactive effort is unfortunately too late for the now rare species, but measures for future research and conservation efforts are still valuable for the attempt at population recovery (Greenberg and Matsuoka 2010). Recent studies have hypothesized several factors that may be contributing to the Rusty Blackbird's decline: blood parasitism, habitat loss, global climate change, nesting ecology, predation, and mercury contamination. However, little is yet known in regards to its social structure, nesting ecology, use of habitat, and the long term effects of environmental contaminants and blood parasites on the Rusty Blackbird. As studies move forward to produce valuable data on what is less known, there is still hope to take proactive measures to conserve the Rusty Blackbird.

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