# Early life experience and social status in the laboratory rat: Addressing causal questions from social epidemiology

By

Katherine Blair Saxton

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Epidemiology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Ralph A. Catalano, Chair
Professor Alan E. Hubbard
Professor Darlene D. Francis

Fall 2010

Abstract

Early life experience and social status in the laboratory rat:
Addressing causal questions from social epidemiology

By

Katherine Blair Saxton

Doctor of Philosophy in Epidemiology

University of California, Berkeley

Professor Ralph Catalano, Chair

Social position and early life environment consistently correlate with health and disease in human populations and animal models. Social epidemiology has succeeded at describing relationships between social factors and health outcomes; however, fundamental questions of etiology and causation persist. Studies on the health effects of relative social position face complex challenges including the clustering of risk factors, the inability to adequately control confounders, questions of temporality, and difficulty in measuring complicated social environments.

One central hypothesis concerning the link between social status and health focuses on differential exposure and response to stressors, suggesting that the stress of low social status throughout the lifecourse chronically activates biological stress responses, increasing risk of disease. Insight into the biological underpinnings of these relationships in humans can be furthered by innovative approaches to social epidemiology, including the application of carefully designed animal studies.

In Chapter 1, the relationship between social status and biological and behavioral outcomes is examined in group-housed male laboratory rats. Rats matched on weight and maternal behavior at weaning form a social hierarchy in their homecage without intervention. Results demonstrate a social gradient in endocrine response to acute stress, exploratory behaviors, and cognitive performance. Subordinate rats showed a blunted corticosterone response to acute stress, lowest levels of exploratory behavior, and poorest cognitive performance in the homecage. Stress of social subordination likely causes the observed differences. This study improves on animal models of social hierarchy by fully characterizing and controlling for early life environment. Results demonstrate that laboratory rats can be used as a model for social hierarchy in humans.

Chapter 2 examines the interaction between early life experience and adult social position, comparing laboratory rats and humans. For the human study, college students

reported on their current subjective social status and family home ownership during their childhood. The rat study used the model of social hierarchy described in Chapter 1. An interaction between early life experience and adult social status was identified in both species. Rats and humans who experienced early life adversity (low levels of maternal care in rats, low childhood socioeconomic status in humans) represented both the highest and lowest levels of IL-6 in adulthood, depending on their social status as young adults. Therefore, early adversity may not have a monotonically negative effect on later life health, but appears to alter responsiveness to later exposures.

Chapter 3 examined the formation of social hierarchies within cages of group-housed male laboratory rats. Rats were housed together at weaning and competed for access to chocolate and water within their homecage throughout the juvenile, adolescent, and adult periods. Late adolescence (postnatal day 45) emerged as a crucial time for hierarchy development. All cages showed a distinct hierarchy at 45 days of age, and half of the groups maintained stable hierarchies from postnatal day 45 into adulthood. Results suggest that careful consideration of developmental windows would improve studies of social status in animal models.

This approach improves on translational research by providing an animal model specifically designed to address public health questions. Results of these studies suggest that an animal model of social hierarchy can inform questions from social epidemiology and further the understanding of relationships between social experience and health in human populations.

To Duane, Kody, Shasta, and Joey.

**TABLE OF CONTENTS**

## INTRODUCTION

Despite the common claims that animal research informs questions relevant to human health and disease, researchers rarely conduct translational work. In order to capitalize fully on the advantages offered by animal models, we must better understand their relevance to humans. In this dissertation, I focus on the biological correlates of social structure and use a laboratory rat model to address questions from the field of public health. Social epidemiology has succeeded at describing relationships between social factors and health outcomes; however, fundamental questions of etiology and causation persist. One central hypothesis concerning the link between social status and health focuses on differential exposure and response to stressors. However, creative and novel approaches to causal inference are required to understand how social factors become embodied in an organism to influence health and disease states.

Research on the social gradient in health consistently struggles to establish the direction of causality and does not adequately examine the interactions between early life environment and later social experience. Rather, questions of reverse causation remain, and social experience often becomes reduced to one or two time points. These limitations seem inherent in the work's dependence, as intuitive as its origins may be, on human populations alone. Parallel studies in humans and animals, asking the same questions and using the same measurements, may move the field forward but rarely take place.

The laboratory rat can serve as a model of social hierarchy and provides several advantages over human observational studies, as we can observe the entire lifecourse of the animal, control risk factors, and address questions of causality. In addition, the ability to control the physical environment in the lab, to provide equivalent housing, food, and water for all animals, allows us to isolate the effect of social status in contrast to measures of absolute material deprivation. Because we can observe the entire life of the animal, we can track exposures and behavior over time to create a true lifecourse model, which would take decades in humans. Perhaps most importantly, animal models provide the opportunity to determine causation.

Previously, laboratory models have not been designed to explicitly study social gradients in lab rats. What little data exists has primarily been collected from pairs of unfamiliar animals housed together in adulthood (1, 2). With few exceptions (3, 4), laboratory studies of social status in the rat do not measure dominance relationships using competition for resources.

This approach improves on translational research by providing an animal model specifically designed to address public health questions. Historically, researchers have over-interpreted animal research to draw conclusions about human health, usually starting from an animal model designed to answer mechanistic questions from another field such as neuroscience or psychology. However, using a more appropriate animal model allows for bi-directional investigation, testing relationships in parallel in both animal models and human populations. The animal model described in this dissertation provides an innovative approach to the examination of the ways in which early life experience may impart resilience or vulnerability to later-life stress and offers distinct advantages for isolating exposures, manipulating social place, and for designing studies in parallel with human work.

*Social gradient, stress, and health*

Social place, or socioeconomic status (SES), has long been known to predict health outcomes. More recently, researchers have demonstrated a social gradient in health, with higher prevalence of disease at each step lower in social status (5), although not monotonic or linear for all outcomes. Prospective studies demonstrate that both social selection and social causation co-occur (6-8). The social gradient in health seems to emerge early, as children display differences by socioeconomic status (SES) in stress reactivity (9), physical health (10), neurocognitive function (11), and inflammatory gene expression (12). Even the best longitudinal human studies, however, cannot definitively determine which component of SES causes these disparities, because health behaviors, poor physical environments, and psychosocial factors are socially patterned and often cluster together (13, 14), making it difficult to identify which of these exposures cause disease.

Individuals lower on the social ladder experience more stressful life events and report higher levels of distress than those higher in social status (15, 16), while having fewer resources to cope with that stress (17). One explanation of the SES-health gradient suggests that exposure to daily stressors associated with low SES has direct biological effects and also shapes later responses to new stressful experiences (17). This hypothesis gains support from the repeated observation that stress hormone secretion, both throughout the day and in response to stressors, differs by SES (9, 18, 19). However, existing research has not followed individuals over time to track the temporal change in stress reactivity in relation to social status (or vice versa), and the questions of temporality and plasticity cannot be resolved through cross-sectional studies alone.

Social rank in animals in many ways parallels social status in humans as a potent stressor (20). Rank in animals correlates with multiple physiological measures and most often involves exposure to increased social stress, few coping resources, and lack of control over stressful encounters (21). However, much of the research into social hierarchy in animals has stemmed from observational studies of primates in the wild, without the ability to control material resources or manipulate the social group.

Social status may have different effects, depending on the early life experience of an organism. Early life environment plays an important role in determining health trajectories; early social experiences may program biological systems (e.g. via epigenetic mechanisms) shaping an individual's response to later exposures. Studies in rodent models provide convincing evidence that the stress-axis is calibrated by parental behavior towards offspring. Naturally occurring differences in maternal licking and grooming affect the development of the hypothalamic-pituitary-adrenal (HPA) axis and the stress response in offspring (22, 23). Offspring of high licking and grooming mothers exhibit a more modest endocrine and behavioral response to stress in adulthood, whereas offspring of low licking and grooming mothers show an elevated response to stress (22). These differences emerge in part due to differential regulation of the glucocorticoid receptor gene in the hippocampus (24). Such plasticity may lead to increased vulnerability to later exposures or may provide resilience to buffer the effects of later challenges. How such early environment influences and interacts with later social experience is unknown.

*Using animal models to address public health questions*

Social position and early life environment consistently correlate with health and disease in humans and animal models. Insight into the biological underpinnings of these relationships in humans can be furthered by innovative approaches to social epidemiology, including the application of carefully designed animal studies. Animal models allow for the isolation of risk factors to address the effects of social experiences, independent from other socially-patterned exposures. In addition, the ability to observe the entire lifecourse in the animal model allows us to determine directionality of observed associations, and to investigate the questions of causality vs. selection. The model of social status presented in this dissertation was designed to represent the constant exposure to social hierarchy experienced by human populations and to explicitly consider early life experience.

The first chapter describes the behavioral and endocrine correlates of relative social status in group-housed laboratory rats. Among rats of similar early life experience, a gradient emerged in social status, such that rats housed together formed linear hierarchies. Social

gradients emerged in exploratory behavior, cognitive ability, and corticosterone response to stress. At each increasing level of social position, rats displayed more exploratory behavior (lower anxiety), higher cognitive performance, and a more appropriate endocrine response to acute stress. These results demonstrate that relative social status correlates with multiple outcomes in laboratory rats, just as in human populations.

Chapter two examines the interaction between early life experience and adult social position, comparing laboratory rats and humans. The study identified an interaction between early life experience and adult social status in relation to interleukin 6 (IL-6) production, in which early life stress increased the responsiveness to adult social status. We found parallel results in college students and the laboratory rat model of social hierarchy. Early life conditions seem to influence the plasticity of the inflammatory response to later social experiences. Rats and humans who experienced low levels of maternal care (rats) or low childhood socioeconomic status (humans) represented both the highest and lowest levels of IL-6 in adulthood, depending on their social status as young adults. Therefore, adversity in childhood may not have a monotonically negative effect on later life health, but may alter responsiveness to later exposures.

Lastly, the third chapter explores the emergence of the linear hierarchy in this animal model. Results indicate that adolescence is a critical developmental window for hierarchy emergence and stabilization. Early life experience did not affect hierarchy development. The results demonstrate the importance of characterizing social experience over the entire lifespan and considering developmental time points in studies of social experience in animal models.

In conclusion, I use a novel approach to the study of social epidemiologic questions, namely, modeling social hierarchy in the laboratory rat. I present studies that use this animal model to investigate effects of the social environment throughout development on stress reactivity, behavioral outcomes, and inflammatory processes. Results of these studies suggest that an animal model of social hierarchy can inform questions from social epidemiology and further the understanding of relationships between social experience and health in human populations.

## REFERENCES


1. Pohorecky LA, Blakley GG, Kubovcakova L, et al. Social hierarchy affects gene expression for catecholamine biosynthetic enzymes in rat adrenal glands. Neuroendocrinology 2004;80:42-51.
2. Hoshaw B, Evans J, Mueller B, et al. Social competition in rats: cell proliferation and behavior. Behav Brain Res 2006;175:343-51.
3. Cordero MI, Sandi C. Stress amplifies memory for social hierarchy. Frontiers in Neuroscience 2007;1:175-84.
4. Barnum CJ, Blandino P, Deak T. Social status modulates basal IL-1 concentrations in the hypothalamus of pair-housed rats and influences certain features of stress reactivity. Brain, Behavior, and Immunity 2008;22:517-27.
5. Pincus T, Callahan LF, Burkhauser RV. Most chronic diseases are reported more frequently by individuals with fewer than 12 years of formal education in the age 18-64 United States population. J Chronic Dis 1987;40:865-74.
6. Johnson JG, Cohen P, Dohrenwend BP, et al. A longitudinal investigation of social causation and social selection processes involved in the association between socioeconomic status and psychiatric disorders. J Abnorm Psychol 1999;108:490-9.
7. Adler NE, Ostrove JM. Socioeconomic status and health: what we know and what we don't. Annals of the New York Academy of Sciences 1999;896:3-15.
8. Chandola T, Bartley M, Sacker A, et al. Health selection in the Whitehall II study, UK. Soc Sci Med 2003;56:2059-72.
9. Lupien SJ, King S, Meaney MJ, et al. Child's stress hormone levels correlate with mother's socioeconomic status and depressive state. Biol Psychiatry 2000;48:976-80.
10. Spencer N. Social, Economic, and Political Determinants of Child Health. Pediatrics 2003;112:704-6.
11. Hackman DA, Farah MJ. Socioeconomic status and the developing brain. 2009;13:65-73.
12. Miller G, Chen E. Unfavorable socioeconomic conditions in early life presage expression of proinflammatory phenotype in adolescence. Psychosom Med 2007;69:402-9.
13. Lynch JW, Kaplan GA, Salonen JT. Why do poor people behave poorly? Variation in adult health behaviours and psychosocial characteristics by stages of the socioeconomic lifecourse. Soc Sci Med 1997;44:809-19.
14. Adler NE, Newman K. Socioeconomic disparities in health: Pathways and policies. Health Aff 2002;21:60-76.
15. Dohrenwend BS. Social status and stressful life events. J Pers Soc Psychol 1973;28:225-35.
16. Kessler RC. Stress, social status, and psychological distress. Journal of Health and Social Behavior 1979;20:259-72.

17. Baum A, Garofalo JP, Yali AM. Socioeconomic status and chronic stress: does stress account for SES effects on health? Annals of the New York Academy of Sciences 1999;896:131-44.
18. Cohen S, Doyle WJ, Baum A. Socioeconomic status is associated with stress hormones. Psychosom Med 2006;68:414-20.
19. Sloan RP, Huang M-H, Sidney S, et al. Socioeconomic status and health: is parasympathetic nervous system activity an intervening mechanism? Int J Epidemiol 2005;34:309-15.
20. McEwen BS, Seeman T. Protective and damaging effects of mediators of stress: elaborating and testing the concepts of allostasis and allostatic load. Annals of the New York Academy of Sciences 1999;896:30-47.
21. Sapolsky RM. The influence of social hierarchy on primate health. Science 2005;308:648-52.
22. Liu D, Diorio J, Tannenbaum B, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 1997;277:1659-62.
23. Caldji C, Tannenbaum B, Sharma S, et al. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proceedings of the National Academy of Sciences 1998;95:5335-40.
24. Francis DD, Champagne FA, Liu D, et al. Maternal care, gene expression, and the development of individual differences in stress reactivity. Annals of the New York Academy of Sciences 1999;896:66-84.

**ACKNOWLEDGEMENTS**

I would like to express my gratitude to the faculty, friends, and family who have helped me through the dissertation process.

I appreciate the guidance, support, and patience of my dissertation committee members, Ray Catalano, Darlene Francis, and Alan Hubbard, over the last several years. Thank you for allowing me to write an epidemiology dissertation on lab rats!

My doctoral work would not have been possible without the support of Ray Catalano and Darlene Francis. Ray pushed me to think more deeply about the implications of epidemiologic results, took a chance on me by enabling me to pursue a non-traditional line of research, and put up with me spending as much time in the lab as working for him. I have enjoyed and looked forward to our conversations, and I am inspired by his commitment to the science of public health research. A big thank you to Ray's research group (Sidra Goldman-Mellor, Claire Margerison-Zilko, Mina Subbaraman, and Beth Anderson) for tirelessly "pushing back the frontiers of ignorance!"

I want to thank Darlene Francis for introducing me to the rats, for providing the intellectual and physical space to pursue my research questions, and for treating me as a colleague. I look forward to future collaborations and continued friendship. I appreciate all I have learned from and shared with the other members of Darlene's lab (especially Matt Reid, Neha John-Henderson, Sam Sakhai, Lisa Margerum, and Filomene Morrison) from 6am observations to our latest research findings.

I can never thank my family enough for their unconditional support. I am continually inspired by their accomplishments, commitment, and love. I would like to thank my parents, Ruth and Paul Saxton, for the many meals, words of wisdom, and hours of dog-sitting that helped me survive the last few months of writing. Kirsten, David, Karl, and Nicky have made this journey worthwhile and reminded me that there is life outside of graduate school.

Most of all, I am forever grateful to my husband Duane for keeping me sane, for having unending faith in me, and for being a wonderful best friend. Thanks!

# Chapter 1

## Behavioral and biological correlates of social position in the laboratory rat: A model for addressing causal questions from social epidemiology

Katherine B. Saxton[1], Matt Reid[2], Darlene D. Francis[1,2,3]

[1]School of Public Health, University of California, Berkeley
[2]Psychology Department, University of California, Berkeley
[3]Helen Wills Neuroscience Institute, University of California, Berkeley

## INTRODUCTION

Social position inversely correlates with many physical and mental health outcomes in human populations in a graded fashion, and has garnered increasing scientific, media, and political interest over the past several decades. However, studies on the health effects of relative social position face complex challenges including the clustering of risk factors, the inability to adequately control confounders, questions of temporality, and difficulty in measuring complicated social environments. Due to the observational and non-experimental nature of social epidemiologic studies, causal relationships between social factors and biology remain difficult to determine. Researchers have suggested adopting new methods to address these challenges (1, 2). We believe that better integration of human and animal research provides one approach to tackling the causal relationship conundrum and can greatly contribute to moving the field forward. Investigations into the biological correlates of social structure could be improved by using laboratory animals to directly model questions of human health. True interdisciplinary work would allow us to explore the complex questions regarding the biological embedding of social experiences. In this paper, we describe a laboratory animal model of social hierarchy, which can help to elucidate biological antecedents and effects of social position, thereby informing questions of human health.

The value of various measures of social position (income, education, occupation, etc.) has been debated repeatedly for over a century, and there remains no consensus on the most meaningful or appropriate measures (3). Regardless of study design, observational studies face the challenge that humans inhabit complicated environments, making the identification and measurement of the relevant social exposures difficult. For instance, in a study of social position, a person inhabits multiple hierarchies – at work, at home, in the local community, in the national community. Confounders in human studies can range from racial discrimination to early life environmental factors (i.e., parenting styles, maternal stress), and can threaten the interpretation of observational studies when not

fully identified, measured, or controlled. For instance, residual confounding can bias results when social variables are poorly measured, broken into limited categories, or otherwise imprecisely operationalized or identified (4). Experimental treatment assignment (ETA) or positivity describes a situation in which for each combination of confounders, a study includes the full range of exposure possibilities. When this assumption is violated, but goes unrecognized, analyses often extrapolate to create statistical estimates which do not reflect people actually studied. As described by Oakes, social stratification separates people with respect to "realized and potential resources" and can create a lack of positivity, presenting analytical challenges for observational studies in social epidemiology (5, 6).

Much debate exists concerning the ability of epidemiologic studies to differentiate between the social selection (i.e. downward social mobility due to poor health) and social causation (poor health due to adversity) hypotheses, and to accurately measure temporality between social exposures and health. Social selection can operate through either direct mechanisms, in which health causes low social position, or indirectly, through accumulation of advantage or disadvantage over the lifecourse (7). In part because of the political controversy over selection processes, social selection has often been viewed as contributing only slightly to health inequities. However, this may be because research on selection has most often focused primarily on major childhood diseases or mental health issues such as schizophrenia (8, 9). Less clear is how common health states influence social position later in life.

To address these limitations of human observational studies, we propose a novel approach to interdisciplinary work, namely the use of a laboratory animal model to explicitly address and test questions and hypotheses from social epidemiology. A laboratory model offers the ability for refined measurement or control of environment, the ability to ensure positivity, the ability to eliminate or account for confounders, and the ability to accurately measure temporality. In addition, it allows us to study selection vs. causation while measuring and controlling the early life environment of the individuals in the hierarchy.

Animal models of social stress are not new, nor are inferences from the animal literature to human populations (10). Animal models have contributed greatly to our current understanding of a stress response; however, use of these models has been primarily contained to identifying and characterizing biological mechanisms. To study biological mechanisms, environmental conditions are typically standardized or controlled for. For example, standard laboratory housing conditions typically dictate that rats are pair-housed in homogeneous cages. However, such standardization often ignores the dominance relationships that emerge between rats housed together.

Much like humans, rodents living in social groups establish and maintain hierarchies in natural, semi-natural, and laboratory conditions (11-16). Also similar to humans, social rank relates to a diverse number of outcomes including behavioral, endocrine and

immune measures. One rodent model designed to explicitly study social stress in a semi-natural environment has documented that significant differences exist between dominant and subordinate rats with respect to brain, behavioral, and physiological parameters (17-20).

Social 'stressors' are well documented to perturb behavioral, hormonal and endocrine profiles in basic rodent models; however, the actual nature of social relationships in these models, in particular relative social status or rank, has not been fully explored. Laboratory rodent models of social stress most often consist of resident-intruder models or group housing (21). Only a few studies have used competition for resources as a measure of hierarchy position (22-24). Because the animals traditionally began these studies as adults, there is no control over or measurement of early life variables and no developmental context for the emergence of social relationships. Despite limitations of existing models, the laboratory rat provides an opportunity to study social position in a controlled environment, while providing the social structure and interactions we seek to explore.

Primates would appear to be the best model for studying social hierarchy, because of their social and biological similarities to humans. Indeed, much research has elucidated the relationships between social position and health in primate populations, suggesting that there is a relationship between social rank and stress-related disease, although the nature of that relationship depends on characteristics of the species and population (25). However, much of this research has been conducted in the field, using observations of wild primate populations. These studies, while informative and important, suffer from the same limitations as human epidemiological studies, because they do not control the physical environment. Several primate laboratories have projects focused on the biological effects of social hierarchy, but their models include individual housing animals until adulthood (26, 27) or limited social contact (28). In addition, due to the long lifespan of primates and the associated cost and difficulty of laboratory studies, laboratory rodent models would provide advantages as a complement to existing models of social hierarchy. Rodent models provide the ability to manipulate the environment, have short gestational and developmental periods, and have biological systems homologous to human biology.

Social position and the relative stress associated with it provide a promising target for interdisciplinary research. The stress response system is highly conserved across vertebrates (29), making it a logical target for translational research. The hypothalamic-pituitary-adrenal (HPA) axis involves the same circuits and comparable hormones in rodents, primates, and humans. The stress response allows for the transfer of energy from long-term processes to immediate needs. Initially, within seconds of stressor onset, the sympathetic nervous system releases epinephrine and norepinephrine. In the brain, the hypothalamus initiates an endocrine cascade, resulting in secretion of glucocorticoids from the adrenal glands within minutes. Inhibition of the parasympathetic nervous system slows digestion, decreases the secretion of insulin, and inhibits the release of

reproductive hormones. Over the short-term, the stress response enhances cognition, immunity, and increases blood sugar (30). However, chronic activation of the stress response can lead to dysregulation of the HPA axis and can consequently compromise health (31-34). Such changes in regulation and control of the HPA axis may be adaptive for a particular context, but may have additional consequences as well.

Social position correlates with outcomes in multiple/diverse domains in humans, ranging from endocrine profiles to long-term health and academic achievement. The social gradient in health has been observed for many outcomes, including mortality (35), coronary heart disease (36), physical health (37), and inflammatory gene expression (38, 39). A socio-economic gradient has also been identified in children for specific neurocognitive systems. The authors suggest that SES might represent physical and or/social environments that influence brain development (40, 41). In adults, working memory is strongly and inversely correlated with childhood poverty however, this relationship is mediated by allostatic load during childhood (42). While it does not appear for all health conditions (and can take on non-linear shapes), the relative graded relationship between social status and health has been replicated repeatedly and has been linked to a variety of social factors including SES, income, subjective social status, and education in a number of different populations (43).

Psychosocial stress and its biological sequelae provide a plausible mechanism underlying the social gradient in health (44-47). Individuals lower in social status experience more stressful life events, report higher levels of distress than those of higher status (48, 49) and posess fewer resources to cope with that stress (45). Biologically, SES-differences in health may be caused by alterations in the HPA axis response to stress, resulting from chronic or repeated stress and sustained activation (33).

In the current paper we aim to inform biological processes in humans using an animal model of social experience. We provide a more relevant model of the effects of social structure in rats that has been informed by both existing rat models of stress as well as by fundamental findings in human social epidemiology. A better understanding of the biological effects of social context and social experience in an animal model will shed light on the possible mechanistic connections in humans. We hypothesized that a graded social hierarchy will emerge in laboratory rats housed together at weaning. Further, we hypothesized that social place within the hierarchy will correlate with adult endocrine stress reactivity and behavioral measures of anxiety. Finally, we compared the cognitive performance of rats under low- and high-stress conditions and hypothesized that context would influence the relationship between social position and cognitive performance.

## METHODS

### Animals

Rats included in the study were born in our colony from Long Evans rats originally purchased from Charles River. For all animals, temperature was kept constant at 20 ± 2 °C and relative humidity was maintained 50 ± 5%. Rats were maintained on a 12-h

light–dark cycle (lights on 0700 h to 1900 h) and allowed access to food (Purina Rat Chow, Purina Mills, St. Louis, Missouri) and tap water *ad libitum*. Housing and care of the rats were carried out in accordance with the standards and practices of the UC Berkeley Animal Care and Use Committee.

Maternal behavior (frequency of licking and grooming of pups) was observed and scored for the first five days following birth. We selected male pups from litters within one standard deviation of the mean of the maternal behavior distribution. We housed group-housed animals in cages of four (10 cages), and control animals in cages of two (5 cages), at weaning, post-natal day (PND) 23. Group housed animals (four rats per cage) were housed in guinea pig cages (20in x 16in x 8.5in). Pair housed animals were housed in standard rat cages (10.5in x 19x8in). In each of two group housing cages one rat was removed at the start of the study; those cages continued with three rats and were included as group-housed in all analyses. Within each cage, rats were from different litters and were matched on weight and maternal behavior. All animals had access to food and water *ad libitum*. Environmental temperature and relative humidity were maintained at 18-26°C and 30%-70%, respectively. A 12:12 hour light cycle, with lights on at 0700 and lights off at 1900, was used.

Social Hierarchy Measurement

*Food competition task:* Before testing, we habituated animals to chocolate. On the first day of training, we smeared a small amount of melted chocolate onto the inside of each home cage. The next day, we dropped mini chocolate chips into each cage. We continued providing rats with mini chocolate chips daily for five days, until all rats quickly approached and ate the chocolate. During the food competition task, rats competed for access to a small container of chocolate secured vertically to the cage wall. The chocolate was melted and allowed to cool in a small glass dish before testing, so the rats could not remove pieces of the chocolate, but were required to eat at the dish. We video recorded the rats' behavior following chocolate provision, and a blinded investigator scored the amount of time each rat spent eating the chocolate over the first minute. Ranks were assigned based on relative amount of time eating, compared to cage mates. The food competition task was repeated at PND 67, 79, and 166.

*Water competition task:* We deprived rats of water for eight hours prior to testing, by removing water bottles from the cages. Upon return of the water bottles, we video recorded rats' behavior, and a blinded investigator scored the amount of time each rat spent drinking over the first two minutes. Ranks were assigned based on relative amount of time eating, compared to cage mates. The water competition task was repeated at PND 76, and 164.

*Final rank assignment:* Final ranks were found by averaging the rankings from concurrent food and water competition tests. If two rats received identical average ranks, the tie was broken using the ranking from the food competition task. We used the average ranks based on testing at PND 164 and 166 for statistical testing.

Outcomes

*Weight:* We weighed each rat twice per week throughout the study.

*Light-dark box task:* Rats were tested on a 5 minute light-dark box task at weaning (PND 23), in the juvenile period (PND 35), adolescence (PND 48) and early adulthood (PND 73). The light-dark box consists of a box with two compartments – a dark, enclosed chamber and an open, brightly lit chamber. Behavior was recorded and scored by investigators blinded to the social ranking of animals. Behavioral measures included latency to emerge from the dark box and total time spent exposed in the light box.

*Stress testing and blood sampling:* We measured rats' neuroendocrine response to stress in adulthood. Animals were tail-bled within 2 min of removal from the homecage (basal), following a 15 min restraint stress (peak), and then during the recovery period (30, 60, and 90 min). Plasma corticosterone (CORT) concentration was calculated using ELISA. Integrated plasma corticosterone was calculated using the trapezoid method for finding area under the curve.

*Cognitive performance:* We tested cognitive performance using a syringe puzzle task (50). On the first day, rats received one training trial in their home cage. For each trial, a 5ml plastic syringe with chocolate melted to the plunger was taped to the floor of the cage, 4cm from the front. For the 90 second training trials, the plunger was pulled out, so the chocolate was exposed. Rats were placed at the opposite end of the cage and allowed to explore and eat the chocolate. After a 2 hour interval, rats were underwent a puzzle trial individually in their home cage. For the puzzle trial, the plunger was pushed into the syringe so that the chocolate was approximately 2cm inside the tube, and rats were tested on their latency to pull out the plunger and begin eating. Each puzzle trial was limited to 120 seconds. The next day, rats received one training trial and one puzzle trial in a novel environment, using the same protocol as day 1, but in a clear plastic box.

Statistical analysis

All statistical analyses were performed using Stata 10.1 (StataCorp 2009. College Station, TX). We compared the baseline characteristics of the rats based on their adult ranks using one way ANOVAs. To determine the effect of rank on our outcome measures (latency to emerge in the light/dark box test and integrated corticosterone in response to acute stress), we created separate regression models. We used linear regression, including an indicator variable for housing condition (group- vs. pair-housed) and adjusting for clustering by cage. In all statistical models, group-housed animals were ranked 1-4, with 4 indicating the highest rank, and control animals were ranked 1-2, with a rank of 2 indicating dominance.

**RESULTS**

Hierarchy formation

Rats matched on weight and maternal behavior at weaning form a social hierarchy in their homecage without intervention. No measures taken at weaning (light dark box

behavior, weight, maternal care, litter size) predicted adult rank (see Table 1). Weight did not differ significantly between animals at any point in the study. As shown in Figure 1, the growth curves of the animals are almost identical, regardless of adult social status.

Social rankings became stable in early adulthood and remained stable for the remainder of the study. Among the group-housed animals, social rank in each competition task (food and water tasks from early adulthood forward) was significantly correlated with final rank (r's: 0.37-0.75). The association between competition tasks and final rank were less consistent and non-significant among pair-housed controls.

Light-Dark Box

The light-dark box provides an ethologically relevant measure of approach/avoid and exploratory behavior. We tested all animals on a 5-minute light-dark box task before group-housing, during the juvenile period, in adolescence, and in adulthood.

The effect of social place on exploratory behavior emerged in early adulthood, as adult rank correlated only with the adulthood measure of latency to emerge from the dark compartment, not with latency at PND 22, 35, or 48. In both the group-housed and control animals, we identified a linear social gradient in latency to emerge from the dark box in adulthood (PND 73). An increase of one rank was associated with a decrease in latency of 31.8 seconds, using linear regression adjusting for housing condition and clustering by cage ($B=-31.78$, $p=0.048$). In the group-housed animals, the highest ranking animals entered the light box over 95 seconds sooner than the lowest ranking animals. The same pattern occurred in the control animals, with a smaller difference between dominant and subordinate animals. A similar gradient emerged for total time spent exploring in the light compartment (data available upon request). Examining the light-dark box test in a developmental context indicates that animals' exploratory behavior did not differ by rank until adulthood, and these differences emerged over time. Figure 2 shows the social gradient in latency to emerge from the dark compartment, measured in adulthood.

We tested for selection effects by adding baseline (pre-hierarchy) measures to the regression model, to explore whether differences in early life experience or behavior predicted adult outcomes. We added variables for maternal care, weight at weaning, and pre-weaning light dark box latency to the model. Among the group-housed animals, adult rank remained the only significant predictor of adulthood exploratory behavior. Among pair-housed animals, no predictor variables remained significant in the fully adjusted model.

Endocrine stress reactivity

We identified a social gradient of stress hormone production in response to acute stress and over 90 minutes of recovery in adulthood. Using linear regression, adjusting for clustering by cage, as well as group-housing status, more dominant social status was associated with an increased endocrine response to stress. The highest ranking animals

showed the strongest endocrine response to acute stress, whereas the lowest ranking animals showed a blunted endocrine response. In group-housed animals, the highest ranking rats produced nearly twice the amount of corticosterone in response to acute restraint stress as did the lowest ranking animals. An increase of one place in social position was associated with 272.92 µg/dL higher plasma corticosterone concentration (B=343.99, p=0.005), using linear regression adjusting for housing condition and clustering by cage. Figure 3 shows stress recovery profiles and integrated corticosterone production by social status. Behavioral measures did not correlate with endocrine measures, perhaps because of the timing differences (5 minute task vs. 15 minute stress and 90 minute recovery) of the tasks.

We tested for selection effects by adding baseline (pre-hierarchy) measures to the regression model. In the model adjusted for early life predictors of adult corticosterone output, adult rank remained significant (B=352.86 µg/dL, p=0.020), and weight at weaning emerged as significant (B=43.15, p=0.037). Including adult social position in this model increased the proportion of variance explained from 0.10 to 0.25. Therefore, position in the social hierarchy remains an important correlate of endocrine stress reactivity, independent of early life experiences.

Cognitive performance

Rats attempted a puzzle-solving task for a chocolate reward alone in their home cage and in a novel environment. A social gradient emerged in latency to solve the puzzle in the home cage (low stress condition), but not in the novel cage (high stress environment), as shown in Figure 4. A one-step increase in social position was associated with a 15 second decrease in latency to solve the puzzle in the low stress condition (B=-15.28, p=0.016), using linear regression adjusting for housing condition and clustering by cage. Rats of lower social position took longer to approach the chocolate in the home cage training session, although the effect did not reach statistical significance (B=-5.99, p=0.072), using linear regression adjusting for housing condition and clustering by cage. Adjusting for latency to approach and investigate the chocolate during training trials lessened the effect of social position on latency to solve the puzzle in both conditions (home cage or novel environment) although a trend in the same direction remained. Including both social position and training latency in the regression model, the effect of social position on puzzle-solving latency was lessened (B=-11.0, p=0.057), whereas the effect of training latency emerged as significant (B=0.71, $p<0.0005$). Adding training latency to the model increased the proportion of variance explained from 0.13 to 0.39, compared to including only social position and housing condition. Latency to approach in the training trial may reflect a stress effect.

We tested for selection effects by adding baseline (pre-hierarchy) measures to the regression model. In both low and high stress environments, training latency emerged as the only significant predictor of puzzle-solving latency. In the fully adjusted model for the low stress condition, a trend remained for the effect of social position, such that higher ranking rats solved the puzzle more quickly than lower ranking rats, adjusting for

early life environment (B=-11.73, p=0.114). These results suggest that the effects of adult rank were independent from early life experience.

We also compared puzzle-solving latency between conditions for individual rats. Rats with higher social position took significantly longer to solve the puzzle in the novel environment, compared to the home cage, both among the group-housed and control animals. Lower ranking rats did not differ in puzzle-solving latency between conditions. The rats of low social position performed similarly in both conditions, suggesting that they experience stress even in their home cage. In contrast, the rats of higher social position exhibited a difference in performance between the low- and high-stress environments, suggesting that to them, the home cage represents a lower stress environment. Therefore, it is possible that the effect we see is not truly a cognitive difference, but rather reflects differential stress experiences for the rats of different social position. Conducting the test in two different settings allows us to identify such contextual effects.

**DISCUSSION**

We identified endocrine, behavioral, and cognitive correlates of social position in the laboratory rat; a social gradient emerged for each outcome. Latency to emerge in the light-dark box task showed that differences by social position emerged over time, and rats with higher social position exhibited reduced anxiety in adulthood. Lowest-ranking rats exhibited a blunted corticosterone response to an acute stress, whereas dominant rats showed higher reactivity. We identified a social gradient in cognitive performance in the low-stress environment, an effect which did not appear under stressful conditions.

Epidemiologists have debated the importance of absolute levels of resources versus relative social position. Because all rats in our study had free access to food and water throughout the study, and because all rats in each social group experienced identical housing conditions, the results of this study cannot be attributed to differential material resources. Rats of all social positions gained weight at equivalent rates, indicating sufficient access to nutrition for all animals, regardless of rank.

Blunted reactivity was also found among a subset of subordinates in the visible burrow system (18). In humans, decreased HPA response to stress has been identified among healthy adults with a history of childhood trauma (51). This stress reactivity data indicates the danger of attaching valence to high or low reactivity, adaptive for individual animals, depending on their own social context. HPA stress reactivity has been identified as an important correlate of social structure, one which may predict later health states in both humans and animal models (31). Alterations in the stress response, including both heightened and blunted cortisol responses, represent forms of allostatic load (52). Therefore, we caution against interpretations of high or low reactivity as “bad” or “good,” and rather frame the conversation in terms of biological sensitivity to context (53).

Differences in outcomes by social position were likely not due to differences in early life, because we matched on maternal care and weight. Studies in humans (38) and animals (54) suggest that early life matters for stress reactivity and anxiety behavior, independent of later life experience. This study demonstrates that social experiences throughout life, holding constant early life experience, can also affect biological systems. Previous studies of social position in the laboratory rat have not considered maternal care. Although social position remained a significant predictor of adult outcomes, controlling for early life environment, a substantial portion of the variance in outcomes remained unexplained. Therefore, we expect that early life experiences or characteristics that we did not measure also influenced the outcomes. Selection may have acted through unmeasured factors to influence both social position and adult outcomes.

The biological implications of social ranking depend on the environmental context and species studied (30). Social position does not mean the same thing, or have the same effects, in animal populations that differ in stability or level of affiliative behaviors. Such dependence on context appears in humans as well, as social position may be more or less meaningful depending on characteristics of the society or culture an individual inhabits. With this understanding, we sought to explore the correlates of social position in a laboratory rat model.

Our study of social hierarchy in the laboratory rat includes several important limitations. Because this was intended as an exploratory and descriptive study, we were unable to determine what causes social rank. We do not have information on baseline measurement of stress reactivity, so it remains possible that baseline endocrine reactivity determines both rank and future reactivity. Although we matched on prior weight and maternal care to prevent those individual characteristics from affecting social position, we cannot determine whether other prior attributes such as baseline cognitive ability may have influenced eventual social status of our animals. It is possible that the same characteristic determined social status, stress reactivity, and light dark box behavior. However, as evidenced by the light dark box data, behavioral changes emerge after social place stabilizes. In addition, primate studies suggest directionality of the stress reactivity – social status relationship, namely that rank determines reactivity, and that baseline levels of reactivity do not predict future social status (28).

There has been much discussion of whether social hierarchies emerge because of preexisting characteristics of individuals, or because of social dynamics between individuals within a group. A well-controlled experimental study found that both prior attributes of individuals and social dynamics of the group influence the formation of dominance hierarchies in animal models; groups form linear social hierarchies, but an individual's social place depended on both prior characteristics and social interactions (55). Future studies are needed to determine causation in our rodent model.

Despite its limitations, this study demonstrates that the laboratory rat can be used as an experimental animal model of social hierarchy. This model can be manipulated and

refined to study lifecourse questions and address questions of selection and causality. We suggest that this model can help answer causal questions from social epidemiology, which cannot be answered by human studies alone. This model provides an opportunity to study a conserved biological system in an animal model with a complex social structure, apply it to human research, and help us better understand the biological effects of social experience in humans. This research will allow us to make statements about how social experiences affect biological pathways applicable to human studies.

Integrating a laboratory rat model into the study of the social gradient in health provides a new approach to interdisciplinary collaboration in epidemiology. More specifically, the rat model provides the ability to design experiments to test explicit hypotheses and test biologic plausibility of proposed theories. Due to the homology of many biologic systems of humans and rats, findings in rats can help to inform questions of human health. Such collaboration may ultimately allow us not only to identify biologic mechanism of observed health disparities, but may also allow us to identify critical periods of plasticity and opportunities for intervention during development.

**REFERENCES**


1. Kaufman JS, Cooper RS. Seeking causal explanations in social epidemiology. Am J Epidemiol 1999;150:113-20.
2. Kaplan GA. What's wrong with social epidemiology, and how can we make it better? Epidemiol Rev 2004;26:124-35.
3. Oakes JM, Rossi PH. The measurement of SES in health research: current practice and steps toward a new approach. Soc Sci Med 2003;56:769-84.
4. Kaufman JS, Cooper RS, McGee DL. Socioeconomic status and health in blacks and whites: The problem of residual confounding and the resiliency of race. Epidemiology 1997;8:621-8.
5. Oakes JM. The (mis)estimation of neighborhood effects: causal inference for a practicable social epidemiology. Soc Sci Med 2004;58:1929-52.
6. Oakes JM. Causal inference and the relevance of social epidemiology. Soc Sci Med 2004;58:1969-71.
7. Blane D, Davey Smith G, Bartley M. Social selection: what does it contribute to social class differences in health? Sociology of Health & Illness 1993;15:1-15.
8. West P. Rethinking the health selection explanation for health inequalities. Soc Sci Med 1991;32:373-84.
9. Dohrenwend BP, Levav I, Shrout PE, et al. Socioeconomic status and psychiatric disorders: the causation-selection issue. Science 1992;255:946-52.
10. Rule C. A biologically based theory of human behavior and its implications for psychiatry: Speculations Derived from Recent Studies of Social Behavior of Non-Human Primates. Am J Psychiatry 1964;121:344-52.
11. Blanchard RJ, McKittrick CR, Blanchard DC. Animal models of social stress: effects on behavior and brain neurochemical systems. Physiol Behav 2001;73:261-71.
12. Blanchard DC, Spencer RL, Weiss SM, et al. Visible burrow system as a model of chronic social stress: behavioral and neuroendocrine correlates. Psychoneuroendocrinology 1995;20:117-34.
13. Adams N, Boice R. A longitudinal study of dominance in an outdoor colony of domestic rats. Journal of Comparative Psychology 1983;97:24-33.
14. Barnett S. An analysis of social behaviour in wild rats. Proceedings of the Zoological Society of London 1958;130:107-52.
15. Berdoy M, Smith P, MacDonald DW. Stability of Social Status in Wild Rats: Age and the Role of Settled Dominance. Behaviour 1995;132:193-212.
16. Blanchard DC, Sakai RR, McEwen B, et al. Subordination stress: behavioral, brain, and neuroendocrine correlates. Behav Brain Res 1993;58:113-21.
17. Blanchard RJ, McKittrick CR, Blanchard DC. Animal models of social stress: effects on behavior and brain neurochemical systems. Physiology and Behavior 2001;73:261-71.
18. McKittrick CR, Blanchard DC, Blanchard RJ, et al. Serotonin receptor binding in a colony model of chronic social stress. Biological psychiatry 1995;37:383-93.

19. Tamashiro KLK, Hegeman MA, Nguyen MMN, et al. Dynamic body weight and body composition changes in response to subordination stress. Physiology & Behavior 2007;91:440-8.
20. Blanchard DC, Spencer RL, Weiss SM, et al. Visible burrow system as a model of chronic social stress: Behavioral and neuroendocrine correlates. Psychoneuroendocrinology 1995;20:117-34.
21. Bartolomucci A. Social stress, immune functions and disease in rodents. Frontiers in Neuroendocrinology 2007;28:28-49.
22. Barnum CJ, Blandino P, Deak T. Social status modulates basal IL-1 concentrations in the hypothalamus of pair-housed rats and influences certain features of stress reactivity. Brain, Behavior, and Immunity 2008;22:517-27.
23. Cordero MI, Sandi C. Stress amplifies memory for social hierarchy. Frontiers in Neuroscience 2007;1:175-84.
24. Gentsch C, Lichtsteiner M, Feer H. Competition for sucrose-pellets in triads of male Wistar rats: the individuals' performances are differing but stable. Behavioural Brain Research 1988;27:37-44.
25. Sapolsky RM. The influence of social hierarchy on primate health. Science 2005;308:648-52.
26. Morgan D, Grant KA, Prioleau OA, et al. Predictors of social status in cynomolgus monkeys (Macaca fascicularis) after group formation. American Journal of Primatology 2000;52:115-31.
27. Shively CA, Register TC, Friedman DP, et al. Social stress-associated depression in adult female cynomolgus monkeys (Macaca fascicularis). Biological Psychology 2005;69:67-84.
28. Czoty PW, Gould RW, Nader MA. Relationship between social rank and cortisol and testosterone concentrations in male cynomolgus monkeys (Macaca fascicularis). Journal of Neuroendocrinology 2009;21:68-76.
29. Ottaviani E, Franceschi C. The neuroimmunology of stress from invertebrates to man. Progress in Neurobiology 1996;48:421-40.
30. Sapolsky RM. Social status and health in humans and other animals. Annual Review of Anthropology 2004;33:393-418.
31. McEwen BS, Seeman T. Protective and damaging effects of mediators of stress: elaborating and testing the concepts of allostasis and allostatic load. Annals of the New York Academy of Sciences 1999;896:30-47.
32. Adler NE, Newman K. Socioeconomic Disparities In Health: Pathways And Policies. Health Aff 2002;21:60-76.
33. Kristenson M, Eriksen HR, Sluiter JK, et al. Psychobiological mechanisms of socioeconomic differences in health. Soc Sci Med 2004;58:1511-22.
34. Siegrist J, Marmot M. Health inequalities and the psychosocial environment-two scientific challenges. Soc Sci Med 2004;58:1463-73.
35. Bassuk SS, Berkman LF, Amick BC, III. Socioeconomic status and mortality among the elderly: Findings from four US communities. Am J Epidemiol 2002;155:520-33.

36. Loucks EB, Lynch JW, Pilote L, et al. Life-course socioeconomic position and incidence of coronary heart disease: The Framingham Offspring Study. Am J Epidemiol 2009;169:829-36.
37. Spencer N. Social, economic, and political determinants of child health. Pediatrics 2003;112:704-6.
38. Miller G, Chen E. Unfavorable socioeconomic conditions in early life presage expression of proinflammatory phenotype in adolescence. Psychosom Med 2007;69:402-9.
39. Miller GE, Chen E, Fok AK, et al. Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling. Proceedings of the National Academy of Sciences 2009:-.
40. Farah MJ, Shera DM, Savage JH, et al. Childhood poverty: Specific associations with neurocognitive development. Brain Research 2006;1110:166-74.
41. Hackman DA, Farah MJ. Socioeconomic status and the developing brain. 2009;13:65-73.
42. Evans GW, Schamberg MA. Childhood poverty, chronic stress, and adult working memory. Proceedings of the National Academy of Sciences 2009;106:6545-9.
43. Adler NE, Ostrove JM. Socioeconomic status and health: what we know and what we don't. Annals of the New York Academy of Sciences 1999;896:3-15.
44. Almeida DM, Neupert SD, Banks SR, et al. Do daily stress processes account for socioeconomic health disparities? J Gerontol B Psychol Sci Soc Sci 2005;60:S34-9.
45. Baum A, Garofalo JP, Yali AM. Socioeconomic status and chronic stress: does stress account for SES effects on health? Annals of the New York Academy of Sciences 1999;896:131-44.
46. Brunner E. Socioeconomic determinants of health: Stress and the biology of inequality. BMJ 1997;314:1472-.
47. Lantz PM, House JS, Mero RP, et al. Stress, life events, and socioeconomic disparities in health: Results from the Americans' Changing Lives Study. Journal of Health and Social Behavior 2005;46:274-88.
48. Dohrenwend BS. Social status and stressful life events. J Pers Soc Psychol 1973;28:225-35.
49. Kessler RC. Stress, social status, and psychological distress Journal of Health and Social Behavior 1979;20:259-72.
50. Galsworthy MJ, Paya-Cano JL, Liu L, et al. Assessing reliability, heritability and general cognitive ability in a battery of cognitive tasks for laboratory mice. Behav Genet 2005;35:675-92.
51. Carpenter LL, Carvalho JP, Tyrka AR, et al. Decreased Adrenocorticotropic Hormone and Cortisol Responses to Stress in Healthy Adults Reporting Significant Childhood Maltreatment. Biological psychiatry 2007;62:1080-7.
52. McEwen BS. Protective and damaging effects of stress mediators. N Engl J Med 1998;338:171-9.

53. Boyce WT, Ellis BJ. Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. Dev Psychopathol 2005;17:271-301.
54. Francis DD, Champagne FA, Liu D, et al. Maternal care, gene expression, and the development of individual differences in stress reactivity. Annals of the New York Academy of Sciences 1999;896:66-84.
55. Chase ID, Tovey C, Spangler-Martin D, et al. Individual differences versus social dynamics in the formation of animal dominance hierarchies. Proceedings of the National Academy of Sciences of the United States of America 2002;99:5744-9.

Table 1. Baseline characteristics (mean and standard deviation) of social hierarchy and control animals

**Group-housed animals, mean (SD)**

| | **Subordinate** | **Mid-2** | **Mid-1** | **Dominant** | **ANOVA p-value** |
|---|---|---|---|---|---|
| Weight (g, PND22) | 44.29 (3.42) | 44.43 (3.17) | 44.43 (3.66) | 45.24 (2.82) | 0.78 |
| Maternal behavior (% LG) | 11.5 (1.1) | 12.2 (1.1) | 12.7 (1.2) | 12.3 (1.2) | 0.085 |
| Light dark box latency (s, PND22) | 245.2 (115.5) | 300.0 (0.0) | 270.7 (88.0) | 265.5 (97.6) | 0.24 |

**Pair-housed animals, mean (SD)**

| | **Subordinate** | **Dominant** | **T test p-value** |
|---|---|---|---|
| Weight (g, PND22) | 44.46 (11.42) | 44.40 (11.86) | 0.99 |
| Maternal behavior (% LG) | 11.0 (1.3) | 12.0 (1.2) | 0.20 |
| Light dark box latency (s, PND22) | 227.0 (146.0) | 243.2 (127.0) | 0.86 |

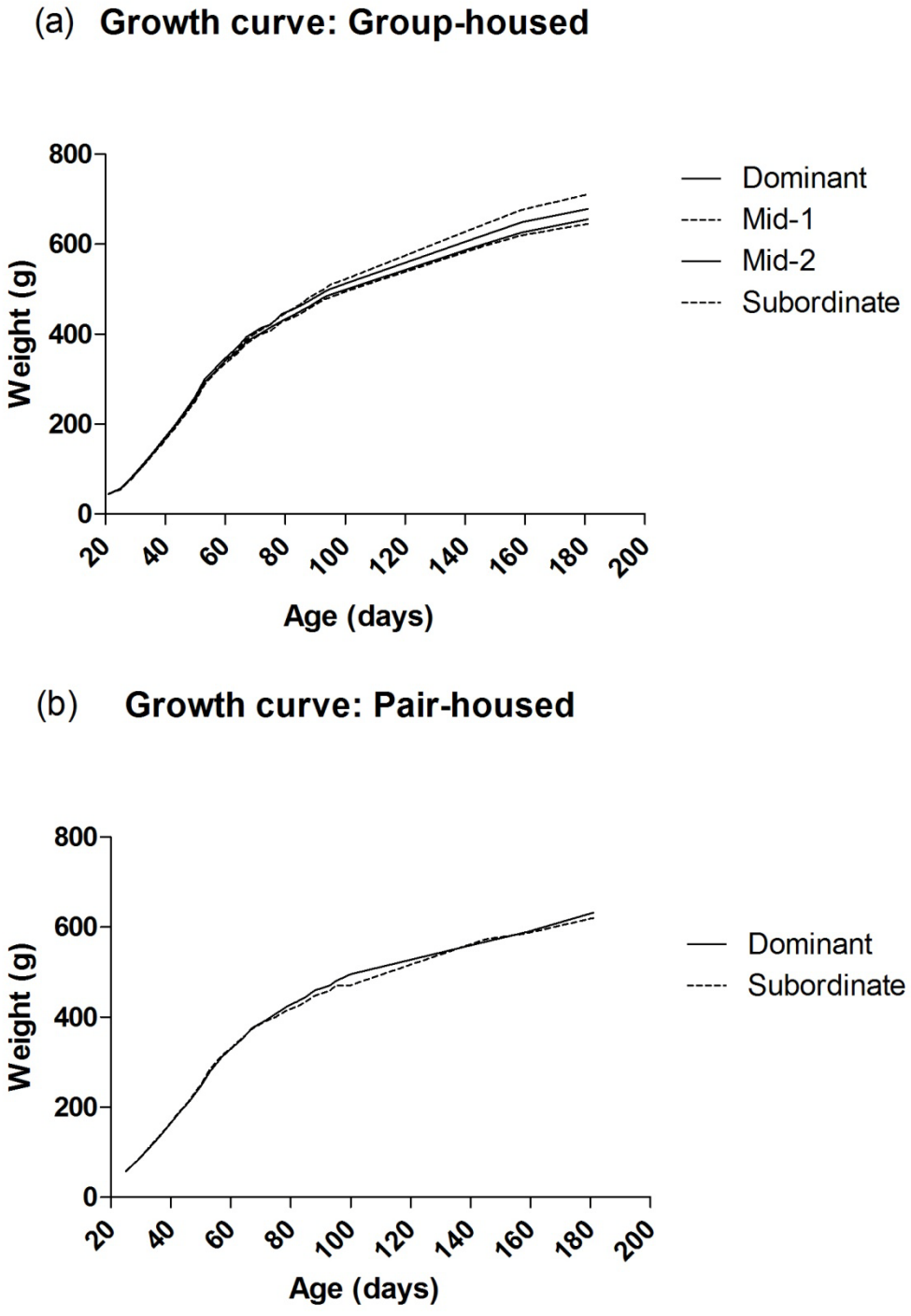



**Figure 1. Growth curves for group-housed and pair-housed animals**
Weight did not differ between rats of different social position at any point during the study for either group-housed (a) or pair-housed (b) animals.

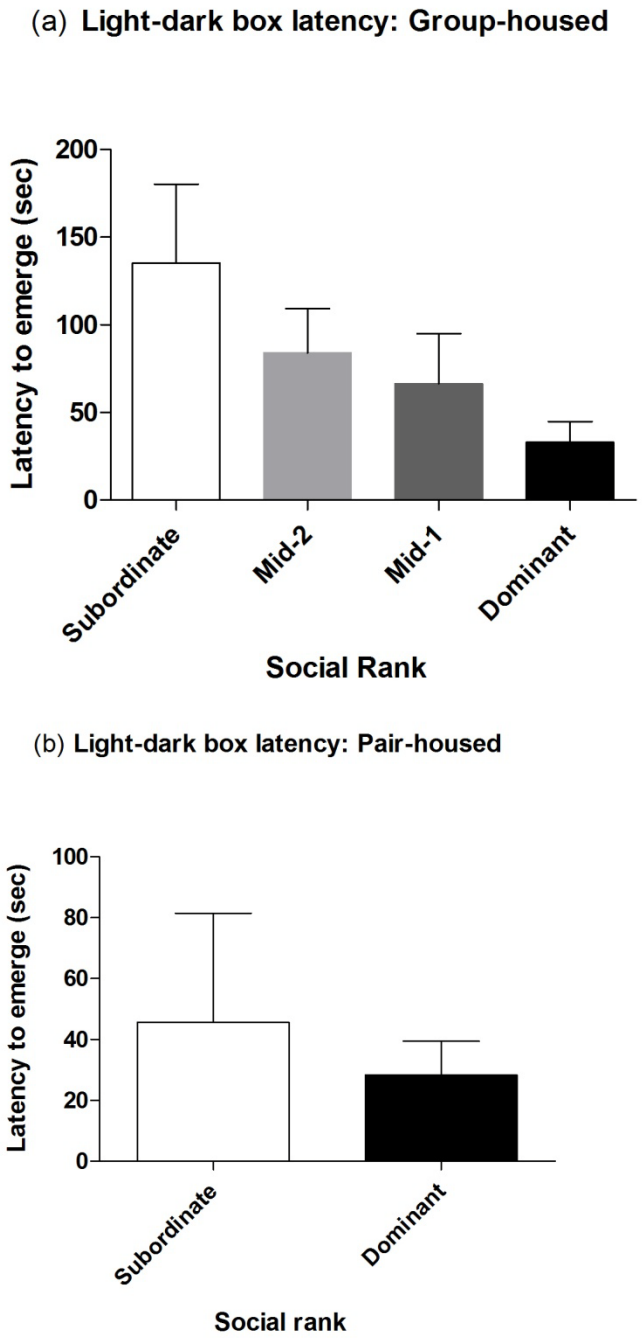
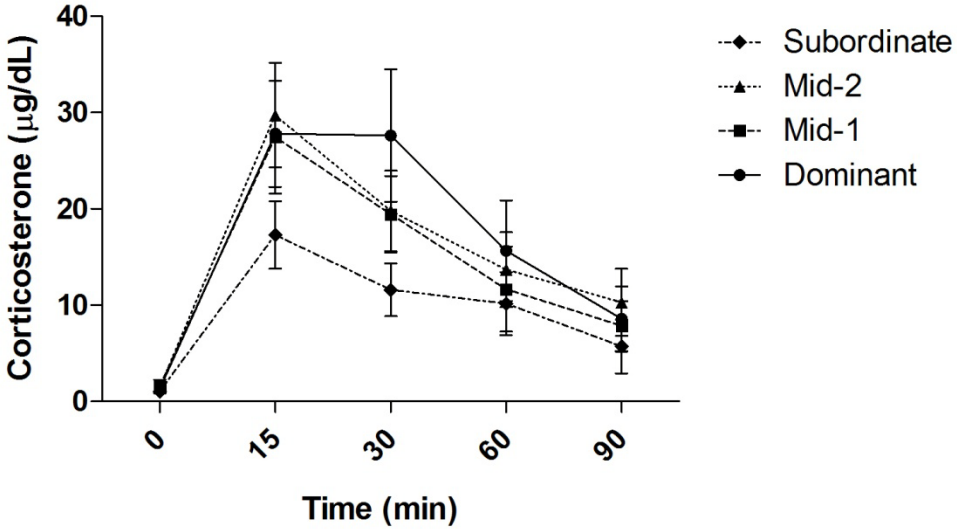
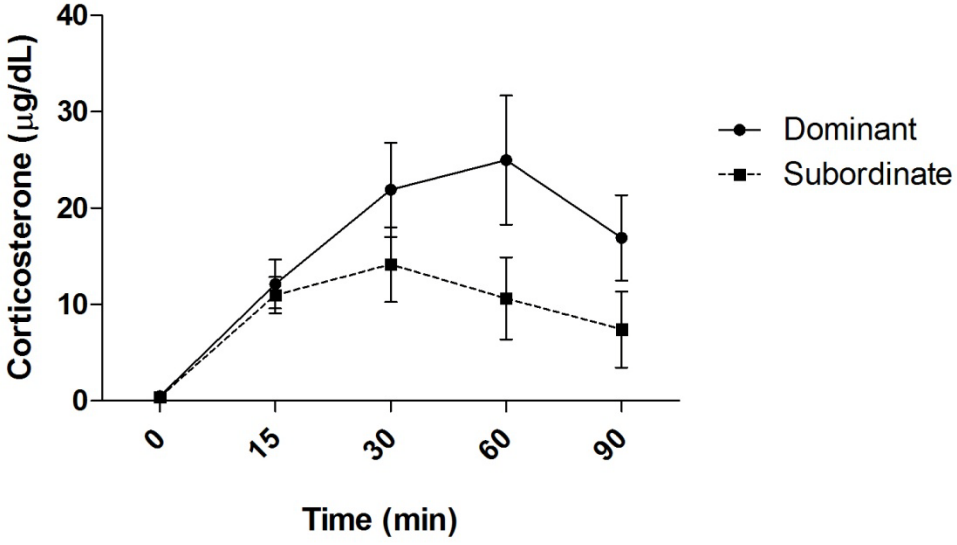



**Figure 2. Behavioral anxiety in adulthood**
A social gradient in behavioral anxiety, as measured by latency to emerge from the dark compartment of the light-dark box, emerged in adulthood among group-housed (a) and pair-housed (b) animals.

(a) **Stress reactivity: Group-housed**




(b) **Stress reactivity: Pair-housed**



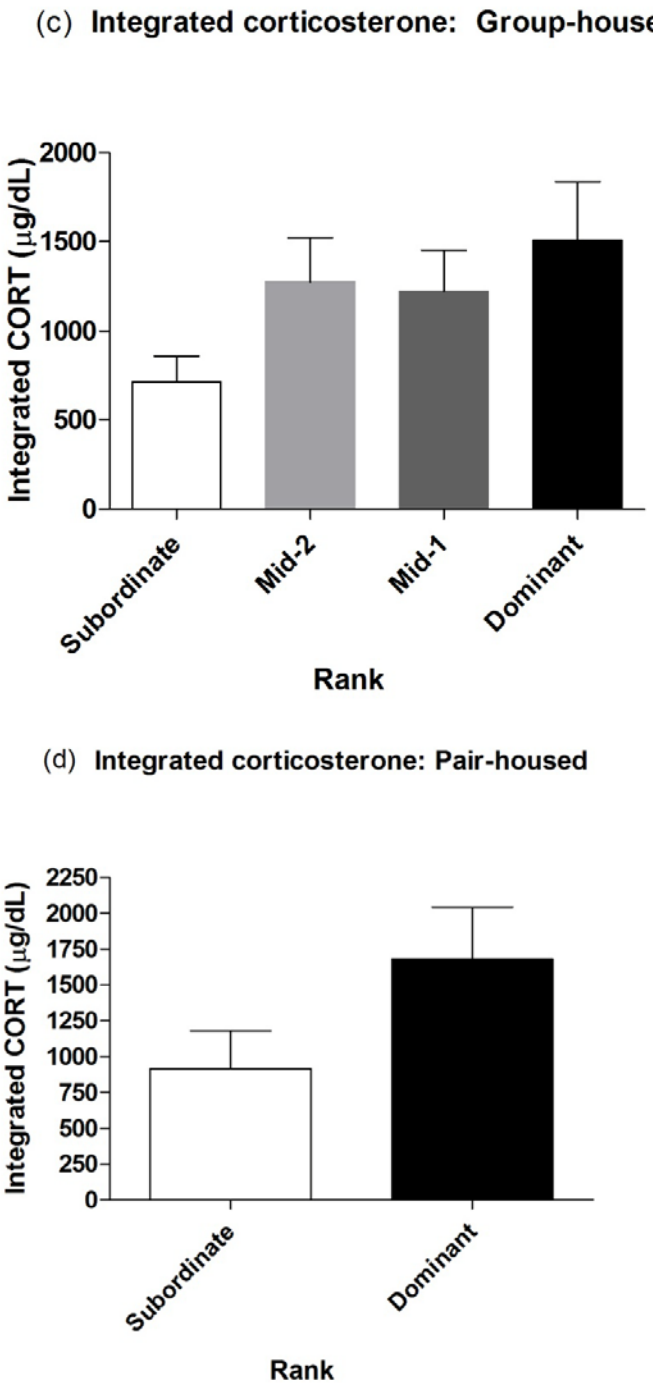



**Figure 3. Stress recovery and integrated corticosterone**
The endocrine response to an acute stress differed by social position. Subordinate rats showed a blunted CORT response to stress in both group-housed (a) and pair-housed animals (b). A social gradient emerged in integrated corticosterone over the stress and recovery time period (c, d).

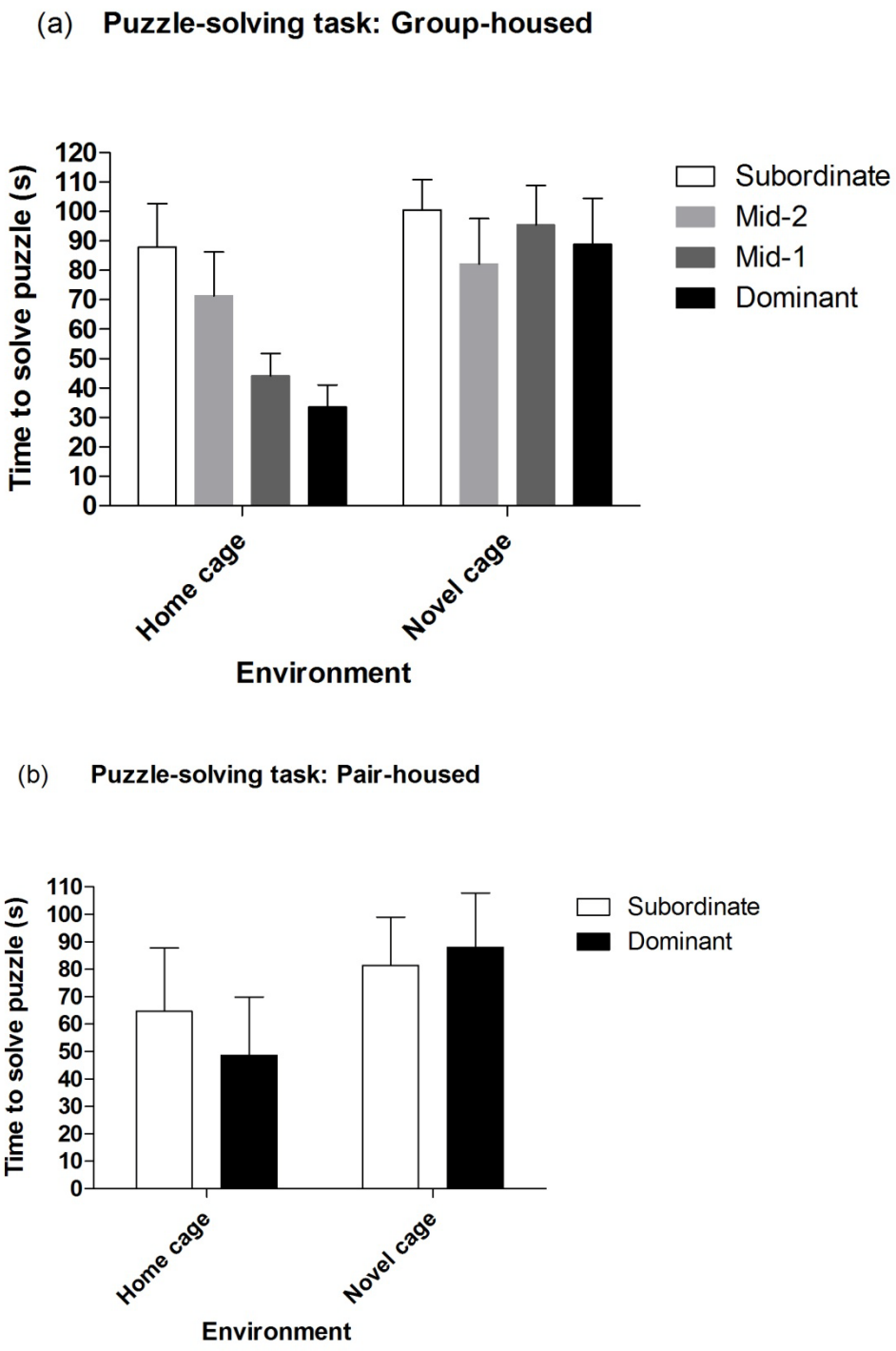



**Figure 4. Puzzle-solving latency in home cage and novel environment**
A social gradient emerged in latency to solve a puzzle in the home cage. There was no difference in a novel environment. Higher ranking rats solved the puzzle more quickly in both group-housed (a) and pair-housed animals (b).

# Chapter 2

## The social environment and inflammatory processes in rats and humans: Interaction between early life experience and adult social status

Katherine B Saxton[1], Neha John-Henderson[2], Matthew W Reid[2], Darlene D Francis[1,3]

[1]School of Public Health, University of California, Berkeley
[2]Department of Psychology, University of California, Berkeley
[3]Hellen Wills Neuroscience Institute, University of California, Berkeley

## INTRODUCTION

Growing evidence links inflammatory processes to a wide range of human diseases, including depression, cardiovascular disease, type 2 diabetes, autoimmune disease, as well as general morbidity and mortality. Inflammatory cytokines including interleukin-6 (IL-6) increase in response to acute psychosocial stress (1) and are elevated among chronically stressed individuals (2). Stress-induced disruptions in neuroendocrine-immune signaling may lead to increased levels of circulating inflammatory mediators, such as interleukin 6, independent of an acute inflammatory response. As elevated IL-6 levels are associated with both risk of disease and stress in humans, IL-6 has been described as a potent psychophysiological health biomarker (3).

Social experiences throughout the lifecourse are associated with inflammatory processes. Socioeconomic status has been inversely associated with inflammatory markers in varied settings and populations (4-6). Providing care for a spouse with dementia predicted a greater rate of increase in plasma IL-6 over time, compared to non-caregivers (2). Social environment in early life has also been associated with inflammation; family home ownership in early childhood predicts better regulation of inflammatory responses in adolescence relative to no home ownership (7). In addition, severe early life adversity, such as childhood maltreatment, predicts increased inflammatory response to acute psychosocial stress later in life (8).

Adversity during critical periods throughout development can influence biologic responses to the social environment and influence risk of disease later in life via developmental programming (9). Epigenetic changes, which affect gene expression but do not change the DNA sequence, may explain the effects of early life stress and adversity on disease processes later in life (10, 11). The variability of IL-6 production in response to differences in experience suggests the involvement of epigenetic processes;

in particular the process of DNA methylation (significantly less is known about histone modifications). For example, differential methylation of a single CpG site in the IL-6 promoter region influences IL-6 gene expression which may play a role in the pathophysiology of rheumatoid arthritis (12). Aged monozygotic twins who have lived apart throughout most of their lives have greater epigenetic differences in global methylation and histone acetylation profiles measured in peripheral blood lymphocytes (13). As concordance rates in monozygotic twins are extremely variable across autoimmune diseases differences in epigenetic processes are likely mediates of observed differences (14). Similar to results in humans, postnatal programming of inflammatory processes has been observed in rats (15). What is much less known in the rat models, however, are the relevant epigenetic mechanisms of action.

Animal studies provide the opportunity to delve deeper into the potential cellular, molecular, and epigenetic mechanisms underlying the associations between stress (particularly early life experience) and inflammatory processes than would be possible in humans alone. Several studies indicate that social stress reliably changes immune system functioning in laboratory (16). More specifically, social stress in mice leads to increased proinflammatory cytokine (IL-6 and TNF-α) production and glucocorticoid resistance (17, 18). Psychological stress causes increased plasma IL-6 in rats (19, 20), which is accompanied by activation of IL-6 neurons, and upregulation of IL-6 gene expression in the brain (21, 22). While these basic models can identify and characterize the biological mechanisms underlying stress-induced inflammatory processes, few studies in laboratory animals have focused on a developmental framework or considered interactions between early life environment and adult social context (i.e. a lifecourse perspective).

Although our questions arise from the field of social epidemiology, human studies face inherent limitations in addressing questions of social experiences over the lifespan. Human studies cannot fully control for all social environments encountered over a lifetime, especially when childhood risks are measured retrospectively. In addition, many risk factors for inflammation are correlated, such as social class, material resources, neighborhood environment, and personal health behaviors. The potential for confounding, both measured and unmeasured, is quite high. When attempting to separate the effects of early life environment and later social status in human studies, researchers face the challenge of including study participants in all combinations of exposure categories. Violation of this assumption (i.e. experimental treatment assignment) can lead to extrapolation of results, in which analyses create statistical estimates which do not reflect people actually studied (23).

Using animal models in combination and in parallel with human studies provides a novel approach to understanding how social experiences become biologically embedded across the lifecourse. Animal models provide an opportunity to explore the questions raised by the epidemiologic work in humans. They allow for full characterization of social exposures and may provide causal inference in support of the correlational evidence in human populations. In the current paper, we present data from parallel studies in college

students and laboratory rats to address the relationship between social status and IL-6, examining the interactions between early life environment and social status in young adulthood. Our study utilizes a younger human sample than those often studied, in concert with a cohort of laboratory rats fully characterized with respect to their relative social rank. We define early adversity using a socioeconomic measure with psychosocial correlates in humans (parental home ownership in Kindergarten) and a psychosocial measure in rats (maternal care). We define adult stress using subjective social status in humans and relative social status in rats. Our innovative approach to interdisciplinary research combines data from parallel studies in humans and an animal model to explore how early life experience interacts with adult social status to influence constitutive expression of IL-6. More generally, we investigate how social experiences that occur over the lifecourse interact to influence markers of inflammation.

**METHODS**

*Humans*

Participants: One hundred and twelve participants (70 females, 42 males) were sampled from the student undergraduate population at UC Berkeley. Participants ranged from 18 to 33 years of age (mean=19). The sample included 19% Caucasian, 47% Hispanic, 3% African American, and 23% Asian students. Only individuals free of acute illness at the time of the study were allowed to participate.

Procedures:

Assessment of childhood SES, social status, and covariates: Participants reported to a designated room on the UC Berkeley campus during academic school hours. They completed a questionnaire upon arrival. The survey collected general demographic information, including age, gender, and race/ethnicity. Participants were also asked to report the highest level of education achieved by their mother and father, and each parent's current income.

As a marker of childhood SES participants were asked if their parents were owners of their home when they were in kindergarten. Homeownership has been associated with improved quality of children's physical and emotional environment, decreased stress, and increased stability (24).

The MacArthur SES ladder was used as a measure of subjective social status. Participants were shown a ladder representing where people stand in the United States. They were asked to place an "x" on the ladder indicating where they place themselves on the 10-rung ladder relative to others in society (25, 26).

The Beck Depression Inventory was used to obtain a marker of mental health. This measure also assesses physical symptoms and lifestyle choices relating to overall health. Participants were also asked to report their self-rated health and perceived stress levels.

IL-6 measurement: After completion of the survey, participants provided baseline samples of oral mucosal transudate (OMT) to reliably assess markers of immune system activity (27). To collect the sample, an Orasure collective device (Epitope, Beaverton, OR) was placed between the lower cheek and gum for two minutes. Immediately following collection, the sample was frozen and stored at -80 C. The samples were thawed and IL-6 concentrations were determined by ELISA using commercially available kits (R&D systems, Minneapolis, MN). All IL-6 samples were run in duplicate. The inter- and intra-assay coefficients of variation were both below 10%. Protein in oral fluids was quantified using the BCA Protein Assay with bovine serum albumin as the standard (Thermo Scientific, Rockford, IL), using HEPES as the diluent. All total protein samples were run in triplicate according to kit instructions. All IL-6 results are reported using analyte to protein ratios, as this measure has been shown to be more reliable than analyte values alone (28) and controls for individual differences in salivary flow rate.

Statistical analysis: All analyses were conducted using Stata v.10 (College Station, TX). We adjusted all IL-6 results for total protein in the sample by taking the ratio of IL-6/total protein. We natural-log transformed the IL-6/total protein ratio for all analyses as the untransformed data were skewed and did not meet the diagnostic criteria for linear regression, according to the IQR and Shapiro-Wilk tests. We then converted the log-transformed IL-6/total protein ratios to z-scores for simpler comparison across species. The z-scores of the log-transformed IL-6/total protein measurement became the dependent variable for all analyses. Linear regression was used to assess the relationship between explanatory variables and IL-6. IL-6 outliers (>3 standard deviations from the mean) were excluded.

*Rats*

Participants: Male rats included in the study were born in our colony from outbred Long Evans rats originally purchased from Charles River Breeding Laboratories (Wilmington, MA). Temperature was kept constant at 20 ± 2 °C and relative humidity was maintained 50 ± 5%. Rats were maintained on a 12-h light–dark cycle (lights on 0700 h to 1900 h) and allowed access to food (Purina Rat Chow, Purina Mills, St. Louis, Missouri) and tap water *ad libitum*. Housing and care of the rats were carried out in accordance with the standards and practices of the UC Berkeley Animal Care and Use Committee.

Procedures:

Maternal behavior and housing conditions: Female rats were bred and permitted to give birth. Day of birth was marked as postnatal day (PND) 0. Maternal observations were performed beginning on PND 1 and continued until PND 5. Each litter was observed for five hours a day at the following times; 0600h-0800h, 1200h-1300h and 1800h-2000h. During each observation session, litters were observed and behaviors recorded every two minutes (each litter was observed 150 times per day) (29-31). Behaviors recorded included: mother on/off the nest and maternal licking behaviors directed at self or at pups. A maternal care distribution curve was generated by calculating the frequency with which pup-directed maternal licking was observed. Maternal licking was expressed as a

percentage of the total number of observations performed for each litter. High and Low licking litters were assessed as those falling above or below the median of all litters. Male pups were weaned at PND 24 and housed in cages of three, with non-littermates matched for weight and maternal care received. Rats were housed in guinea pig cages (20x16x8.5) , which are substantially larger than standard rat cages (10.5x19x8) Food and water were available *ad libitum*, and rats were maintained on a 12 hour light-dark schedule (lights on 0700h-1900h).

Assessment of Social Status in Rats:
Food competition task: Several days prior to weaning all rats were exposed to the novel taste of chocolate. On the first day of habituation training a small amount of melted chocolate was smeared onto the inside of each home cage. The next day mini chocolate chips were placed inside each home cage. Rats were provided with mini chocolate chips daily for five days, until all rats quickly approached and ate the chocolate. To assess within cage social rank we administered a brief food competition task. In this task rats within a single home cage competed for access to a small container of chocolate secured vertically to the cage wall. The chocolate was melted and allowed to cool in a small glass dish before testing, so the rats could not remove pieces of the chocolate, but were required to eat at the dish. All rat behavior was video recorded following chocolate provision and a blinded investigator scored the amount of time each rat spent eating chocolate over the first minute of the task. The food competition task was conducted at 109 days of age.

Water competition task: Rats were deprived of access to water bottles for eight hours prior to testing. Upon return of the water bottles all behaviors were recorded and a blinded investigator scored the amount of time each rat spent drinking over the first two minutes of the task. The water competition task took place on postnatal day 111.
Assignment of Social status: Social status was determined by averaging the proportion of time each rat spent accessing resources (chocolate or water), relative to cage mates. A social status score of 1 for all rats in a cage would represent equal access to resources. Two cages did not form stable hierarchies and were excluded from all analyses.

$$\text{Social status score} = \frac{\text{time eating}}{\text{total time eating/group size}} + \frac{\text{time drinking}}{\text{total time eating/group size}} \; / 2$$

IL-6 measurement: At the termination of the study all animals were sacrificed. Blood was collected from each animal and plasma was extracted, frozen immediately and stored at -80 C for future use. At the time of assay all samples were thawed and IL-6 concentrations were determined by ELISA using commercially available kits (R&D systems, Minneapolis, MN). All samples were run in duplicate. The inter- and intra-assay coefficients of variation were both below 10%. As some samples contained levels of IL-6 below the standard concentrations provided in the assay kit, we extended the

standard curve via serial dilutions until all sample concentrations fell within the values included by the standard curve. Total protein levels in plasma were quantified using the BCA Protein Assay with bovine serum albumin as the standard (Thermo Scientific, Rockford, IL) and HEPES as the diluent. All protein samples were run in triplicate, according to kit instructions. All IL-6 results are reported using analyte to protein ratios.

Statistical analysis: All analyses were conducted using Stata v.10 (College Station, TX). All IL-6 measurements were adjusted for total protein in the sample by taking the ratio of IL-6/total protein. The IL-6/total protein ratio was natural-log transformed for all analyses, because the untransformed data were skewed and did not meet the diagnostic criteria for linear regression, according to the IQR and Shapiro-Wilk tests. The log-transformed IL-6/total protein ratios were converted to z-scores for simpler comparison across species. The z-scores of the log-transformed IL-6/total protein measurement became the dependent variable for all analyses. Linear regression, controlling for clustering by cage, was used to assess the relationship between explanatory variables and IL-6. IL-6 outliers (>3 standard deviations from the mean) were excluded.

**RESULTS**

*Independent effects of early life environment and adult social status in humans and rats:* We used natural-log transformed IL-6 measurements, converted to z-scores, as the dependent variable in all analyses for both rats and humans. In humans, we tested the associations between childhood SES (home ownership), adult subjective social status, and IL-6, adjusting for maternal education, Beck Depression Inventory score, and age.

We conducted analyses separately in males and females, because relationships between our exposures of interest and IL-6 differed markedly by gender. In males, subjective social status (B=-0.16, SE=.061, p=.01), home ownership (B=0.42, SE=0.20, p=0.05), and maternal education (B=-0.46, SE=0.13, p=0.001) each significantly predicted IL-6 levels. Social status and homeownership explained 11% and 5% of the variance, respectively. In females, the effect of subjective social status and home ownership were not significant. Maternal education was the only significant predictor of IL-6 among females (B=-0.43, SE=0.13, p=0.002).

In rats, using linear regression and adjusting for clustering by cage, we tested whether childhood environment (maternal care) and/or adult social status explained IL-6 levels. Social status was inversely associated with IL-6 in plasma, such that rats of higher social status had lower levels of plasma IL-6 (B=-0.56, SE=0.26, p=0.04); social status explained 7.7% of the variance. Early environment (maternal licking and grooming) was not independently associated with IL-6.

*Interactive effects of early life environment and adult social status in humans and rats:* We next examined the interactive effect of early life environment and adult social status on the production of IL-6. The interaction between early life environment and adult social status reached statistical significance in both humans (males only) and rats.

Results show an interaction in male college students; the effect of subjective social status on IL-6 was much larger among people from families who did not own their home during the participant's childhood. The slope of social status versus IL-6 was significantly steeper among individuals who experienced low SES in childhood (Figure 1, Table 2). Maternal education remained significant in the interaction model, with higher maternal education predicting lower IL-6. Age and depression were not associated with IL-6 in the interaction model. Parental home ownership during kindergarten seemed to buffer individuals from later life social status, lessening the effect of social status on IL-6 both at high and low status. Individuals whose parents did not own their home accounted for both the highest and lowest levels of IL-6.

Similarly, we identified an interaction in rats; the effect of social status on IL-6 was stronger among rats that experienced less licking and grooming as pups (Figure 1, Table 2). The slope of social status versus IL-6 is significantly steeper among rats from low licking and grooming litters, compared to those who received higher maternal care. Just as in the human data, the rats that experienced early adversity produced both the highest and lowest levels of IL-6. The low licking and grooming/low adult status rats experienced the highest levels of circulating IL-6, whereas the low licking and grooming/high adult status rats experienced the lowest levels of IL-6.

We then examined the relationship between social status and IL-6 within categories of early life experience (home ownership vs. not, high vs. low licking and grooming). In both humans and rats, the effect of social status on IL-6 was significant among the early life stress group only. In humans, subjective social status predicted IL-6 among people whose families did not own their home (B=-0.32, SE=0.071, p=0.001), but not among those whose families were home owners (B=-0.059, SE=0.087, p=0.5), again adjusting for maternal education, age, and depression. In the no home ownership group, subjective social status explained 36% of the variance in IL-6. In rats, social status predicted IL-6 production among pups from low licking and grooming litters (B=-1.10 SE=0.37, p=0.01), but not high licking and grooming litters (B=-0.086, SE=0.26, p=0.8). Among rats who received little maternal care as pups, social status explained 21% of the variance in IL-6 production.

The interaction effects in humans and rats were strikingly similar. In both species, high childhood SES seemed to buffer individuals from later life risks. However, this interaction occurred at both extremes of social status, insulating individuals both from the negative effects of low social status and the beneficial effects of high social status. As a result, the highest and lowest IL-6 levels were found among the early life stress group in each species.

**DISCUSSION**

Results of parallel studies in humans and rats demonstrate an interaction between early life experience and adult social status in relation to IL-6 production, in which childhood

stress increased sensitivity to adult social status. We found parallel results in college students (males only) and a laboratory rat model of social hierarchy. Early life conditions seem to influence the plasticity of the inflammatory response to later social experiences. Rats and humans who experienced low maternal care (rats) or low childhood SES (humans) represented both the highest and lowest levels of IL-6 in adulthood, depending on their social status as young adults. Therefore, stress in childhood may not have a monotonically negative effect on later life health, but may alter responsiveness to later exposures.

Our interactive view of social experiences over the lifecourse bears similarities to Boyce and Ellis' theory of biological sensitivity to context, in which they propose that early life can prime biological reactivity to the environment. The ultimate outcome for an individual depends on both biological sensitivity and the quality of that later environment, with the highly reactive individuals experiencing both the best and worst outcomes (32, 33). Boyce and Ellis describe this sensitivity to context in relation to stress reactivity, but our study expands their definition to include family home ownership in humans and maternal care in rats as modifying factors. Thus, our results support home ownership and maternal care as plasticity factors (34).

These markers of early life adversity could reflect prenatal environment as well as postnatal exposures. The increased plasticity that appears to be induced by stress early in life could instead be selected for during gestation, in anticipation of an unpredictable or stressful environment after birth. Our study cannot differentiate between possible prenatal and postnatal exposures in humans; however, mothers in our rat model experienced identical environments during their pregnancies.

The predominant view of early life stress is that it increases vulnerability to later stressors by creating a biologic vulnerability. For instance, a recent review suggests that the effects of stress on health in adulthood appear more consistent among populations with underlying vulnerability, perhaps induced in response to early life environment (10). Miller and Chen found such an interactive effect when examining the interaction between harsh parenting experiences and life stress among adolescent girls. The combination of the two exposures predicted the emergence of a proinflammatory phenotype (35). An animal model of colitis also demonstrated an interactive effect between juvenile and adult environment in predicting inflammation. Neuroendocrine changes and exacerbated colitis symptoms were observed among mice exposed to maternal separation in early life and chronic psychosocial stress in adulthood. Adult stress most strongly affected the mice who had also been stressed as juveniles (36). These studies reinforce the paradigm that adult experiences are most potent among those already vulnerable due to early life circumstances. Although both of these examples point to the interactions of stressful experiences over the lifecourse, neither study directly examines the potential beneficial effects of a positive environment for the more biologically sensitive group, as our study does.

Our study used family home ownership and maternal licking and grooming to capture early life environment. Both home ownership and maternal care in the rat have been studied in relation to programming of hypothalamic-pituitary-adrenal (HPA) axis activity, and both early life exposures have been associated with increased activity (30, 37). In addition, living in a home owned by one's parents seems to be associated with decreased threat interpretations and decreased chaos, which mediate the relationship between childhood SES and cortisol trajectories (37). Therefore, early life SES in humans and maternal care in rats may be increasing responsiveness of the HPA axis, which in turn increases plasticity to later social experiences, as suggested in the biological sensitivity to context model.

Epigenetic mechanisms may underlie the interactions observed in our studies. Even among monozygotic twins, differences in environment throughout life correlate with diverging epigenetic profiles, suggesting that epigenetic profiles remain malleable beyond prenatal and early postnatal windows (13). Transcription of the IL-6 gene is tightly controlled through dynamic epigenetic mechanisms. Expression of IL-6 mRNA can be induced by tissue-specific signals, is regulated by the transcription factor NF-kB (38), which itself is differentially recruited depending on epigenetic profiles (39). However, the IL-6 gene can be repressed by DNA methylation (40). Although these studies suggest possible mechanisms leading to the results we observe, they do not evaluate the effects of social stress throughout life on the epigenetic regulation of inflammation. Further research may elucidate the epigenetic mechanisms underlying the effects of social stress on inflammatory processes.

Although not focused on inflammation, research in both laboratory rats and humans identifies epigenetic changes in the HPA axis resulting from stress early in life. The effects of early life stress on epigenetic profiles in laboratory rats (41) and humans (42) suggest that increased methylation of the glucocorticoid receptor promoter in the hippocampus results from low maternal care (rats) or child abuse (humans). Although environmental interventions in the peripubertal period functionally reversed the increased HPA axis reactivity resulting from early life stress, the intervention led to compensatory changes, rather than direct reversal of the cellular mechanisms (43). Therefore, further examination of the effects of peripubuteral and adult exposures at the cellular level is warranted.

Our study's limitations include the relatively small sample size and that the human sample includes only college students, possibly limiting external validity. Both studies measured IL-6 at only one time point, so we were unable to examine changes in inflammatory processes over time. However, the parallel results in rats and humans increase our confidence in the observed effect. In humans, the SES-inflammation relationship could be confounded by unmeasured health behaviors, and several studies failed to find associations between SES and IL-6 after adjusting for behavioral risk factors (44, 45). In the rat model of social hierarchy, the results could not be caused by

material differences or health behaviors, because the rats experienced identical environmental conditions.

In addition, rats provide the ability to fully measure and control for early life. We are able to directly observe naturally occurring variation in maternal licking and grooming in the rat. By matching rats on maternal behavior, we ensure that it does not determine social status, but we are able to examine the effects of adult social status within levels of early life care. Living in a house that one's parents own has been associated with improved physical and psychosocial environment. However, home ownership does not perfectly capture childhood SES. Home ownership does not describe parenting style or childhood maltreatment, both of which have been identified as powerful predictors of later life health.

Viewing early life SES as a plasticity factor, rather than simply as a risk factor, suggests a more hopeful perspective. If low childhood SES increases responsiveness to later life experiences, then interventions targeted in low-SES communities may have significant impacts. Our rat model of social hierarchy provides the opportunity to test interventions in a controlled setting. Continued parallel studies between laboratory models and human populations may help explain relationships between exposures at different time points and may suggest opportunities to intervene and prevent negative health outcomes.

**REFERENCES**


1. Steptoe A, Hamer M, Chida Y. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. Brain Behav Immun 2007;21:901-12.
2. Kiecolt-Glaser JK, Preacher KJ, MacCallum RC, et al. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. Proc Natl Acad Sci U S A 2003;100:9090-5.
3. Hänsel A, Hong S, Cámara RJA, et al. Inflammation as a psychophysiological biomarker in chronic psychosocial stress. Neuroscience & Biobehavioral Reviews 2010;35:115-21.
4. Gimeno D, Brunner EJ, Lowe GD, et al. Adult socioeconomic position, C-reactive protein and interleukin-6 in the Whitehall II prospective study. Eur J Epidemiol 2007;22:675-83.
5. Loucks EB, Pilote L, Lynch JW, et al. Life course socioeconomic position is associated with inflammatory markers: the Framingham Offspring Study. Soc Sci Med 2010;71:187-95.
6. Friedman EM, Herd P. Income, education, and inflammation: differential associations in a national probability sample (The MIDUS study). Psychosom Med 2010;72:290-300.
7. Miller G, Chen E. Unfavorable Socioeconomic Conditions in Early Life Presage Expression of Proinflammatory Phenotype in Adolescence. Psychosom Med 2007;69:402-9.
8. Carpenter LL, Gawuga CE, Tyrka AR, et al. Association between Plasma IL-6 Response to Acute Stress and Early-Life Adversity in Healthy Adults. Neuropsychopharmacology 2010.
9. Shonkoff JP, Boyce WT, McEwen BS. Neuroscience, molecular biology, and the childhood roots of health disparities: building a new framework for health promotion and disease prevention. JAMA 2009;301:2252-9.
10. Champagne FA. Early adversity and developmental outcomes: Interaction between genetics, epigenetics, and social experiences across the lifespan. Perspectives on Psychological Science 2010;5:564-74.
11. McGowan PO, Szyf M. The epigenetics of social adversity in early life: implications for mental health outcomes. Neurobiol Dis 2010;39:66-72.
12. Nile CJ, Read RC, Akil M, et al. Methylation status of a single CpG site in the IL6 promoter is related to IL6 messenger RNA levels and rheumatoid arthritis. Arthritis Rheum 2008;58:2686-93.
13. Fraga MF, Ballestar E, Paz MF, et al. Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci U S A 2005;102:10604-9.
14. Ballestar E. Epigenetics lessons from twins: prospects for autoimmune disease. Clin Rev Allergy Immunol 2010;39:30-41.
15. Spencer SJ, Galic MA, Pittman QJ. Neonatal programming of innate immune function. Am J Physiol Endocrinol Metab 2010.

16. Bartolomucci A. Social stress, immune functions and disease in rodents. Frontiers in Neuroendocrinology 2007;28:28-49.
17. Kinsey SG, Bailey MT, Sheridan JF, et al. The inflammatory response to social defeat is increased in older mice. Physiology & Behavior 2008;93:628-36.
18. Powell ND, Bailey MT, Mays JW, et al. Repeated social defeat activates dendritic cells and enhances Toll-like receptor dependent cytokine secretion. Brain, Behavior, and Immunity 2009;23:225-31.
19. Takaki A, Huang QH, Somogyvari-Vigh A, et al. Immobilization stress may increase plasma interleukin-6 via central and peripheral catecholamines. Neuroimmunomodulation 1994;1:335-42.
20. LeMay LG, Vander AJ, Kluger MJ. The effects of psychological stress on plasma interleukin-6 activity in rats. Physiol Behav 1990;47:957-61.
21. Shizuya K, Komori T, Fujiwara R, et al. The influence of restraint stress on the expression of mRNAs for IL-6 and the IL-6 receptor in the hypothalamus and midbrain of the rat. Life Sci 1997;61:PL 135-40.
22. Jankord R, Zhang R, Flak JN, et al. Stress activation of IL-6 neurons in the hypothalamus. Am J Physiol Regul Integr Comp Physiol 2010;299:R343-51.
23. Oakes JM. The (mis)estimation of neighborhood effects: causal inference for a practicable social epidemiology. Soc Sci Med 2004;58:1929-52.
24. Haurin DR, Parcel TL, Haurin RJ. Does homeownership affect child outcomes? Real Estate Economics 2002;30:635-66.
25. Adler NE, Epel ES, Castellazzo G, et al. Relationship of subjective and objective social status with psychological and physiological functioning: preliminary data in healthy white women. Health Psychol 2000;19:586-92.
26. Operario D, Adler NE, Williams DR. Subjective social status: Reliability and predictive utility for global health. Psychology & Health 2004;19:237-46.
27. Nishanian P, Aziz N, Chung J, et al. Oral fluids as an alternative to serum for measurement of markers of immune activation. Clin Diagn Lab Immunol 1998;5:507-12.
28. Dickerson SS, Kemeny ME, Aziz N, et al. Immunological Effects of Induced Shame and Guilt. Psychosom Med 2004;66:124-31.
29. Champagne FA, Francis DD, Mar A, et al. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav 2003;79:359-71.
30. Francis DD, Champagne FA, Liu D, et al. Maternal care, gene expression, and the development of individual differences in stress reactivity. Annals of the New York Academy of Sciences 1999;896:66-84.
31. Liu D, Diorio J, Tannenbaum B, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 1997;277:1659-62.
32. Ellis BJ, Essex MJ, Boyce WT. Biological sensitivity to context: II. Empirical explorations of an evolutionary-developmental theory. Dev Psychopathol 2005;17:303-28.

33. Boyce WT, Ellis BJ. Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. Dev Psychopathol 2005;17:271-301.
34. Belsky J, Pluess M. Beyond diathesis stress: differential susceptibility to environmental influences. Psychol Bull 2009;135:885-908.
35. Miller GE, Chen E. Harsh family climate in early life presages the emergence of a proinflammatory phenotype in adolescence. Psychol Sci 2010;21:848-56.
36. Veenema AH, Reber SO, Selch S, et al. Early life stress enhances the vulnerability to chronic psychosocial stress and experimental colitis in adult mice. Endocrinology 2008;149:2727-36.
37. Chen E, Cohen S, Miller GE. How low socioeconomic status affects 2-year hormonal trajectories in children. Psychol Sci 2010;21:31-7.
38. Libermann TA, Baltimore D. Activation of interleukin-6 gene expression through the NF-kappa B transcription factor. Mol Cell Biol 1990;10:2327-34.
39. Grolleau-Julius A, Ray D, Yung RL. The role of epigenetics in aging and autoimmunity. Clin Rev Allergy Immunol 2010;39:42-50.
40. Armenante F, Merola M, Furia A, et al. Repression of the IL-6 gene is associated with hypermethylation. Biochem Biophys Res Commun 1999;258:644-7.
41. Weaver IC, Cervoni N, Champagne FA, et al. Epigenetic programming by maternal behavior. Nat Neurosci 2004;7:847-54.
42. McGowan PO, Sasaki A, D'Alessio AC, et al. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci 2009;12:342-8.
43. Francis DD, Diorio J, Plotsky PM, et al. Environmental enrichment reverses the effects of maternal separation on stress reactivity. J Neurosci 2002;22:7840-3.
44. Ramsay S, Lowe GD, Whincup PH, et al. Relationships of inflammatory and haemostatic markers with social class: results from a population-based study of older men. Atherosclerosis 2008;197:654-61.
45. Petersen KL, Marsland AL, Flory J, et al. Community Socioeconomic Status is Associated With Circulating Interleukin-6 and C-Reactive Protein. Psychosom Med 2008;70:646-52.

Table 1. Descriptive characteristics of both study populations

| **Humans** | Males | Females |
|---|---|---|
| Age (mean (SD)) | 20.1 (2.9) | 19.3 (2.0) |
| Social status (mean (SD)) | 5.8 (2.1) | 5.4 (1.9) |
| Home ownership (%) | 57% | 49% |
| IL-6/total protein (pg/μg) (mean (SD)) | 0.0012 (0.0013) | 0.0015 (0.0025) |
| Maternal education (mean(SD)) | 2.2 (0.88) | 2.1 (0.99) |
| Beck Depression Index (mean(SD)) | 6.4 (5.8) | 8.8 (6.2) |
| | | |
| **Rats** | | |
| Age | 163 days | |
| Social status | 1.02 (0.38) | |
| Maternal care (mean (SD)) | 6.8% (2.0) | |
| IL-6/total protein (pg/μg) (mean (SD)) | 0.017 (0.0076) | |

Table 2. Social status and early life environment predict IL-6 production in male humans and rats

| **Humans (males)** | B | SE | t | p |
|---|---|---|---|---|
| Social status | -0.27 | 0.074 | -3.64 | 0.001 |
| Home ownership | -0.78 | 0.61 | -1.28 | 0.2 |
| Interaction (social status*home ownership) | 0.20 | 0.10 | 2.02 | 0.05 |
| Maternal education | -0.42 | 0.12 | -3.41 | 0.002 |
| Depression score | -0.025 | .019 | -1.33 | 0.2 |
| Age | -0.051 | 0.038 | -1.34 | 0.2 |
| Constant | 3.46 | 0.78 | 4.46 | <0.001 |
| $R^2$=.56<br>F(6,35)=9.14, p<0.001 | | | | |

| **Humans (females)** | B | SE | t | p |
|---|---|---|---|---|
| Social status | -0.10 | 0.088 | -1.17 | 0.2 |
| Home ownership | -0.097 | 0.72 | -0.13 | 0.9 |
| Interaction (social status*home ownership) | 0.042 | 0.12 | 0.36 | 0.7 |
| Maternal education | -0.44 | 0.13 | -3.27 | 0.002 |
| Depression score | -0.078 | 0.020 | -0.39 | 0.7 |
| Age | -0.053 | 0.040 | -1.32 | 0.2 |
| Constant | 2.45 | 0.85 | 2.88 | 0.005 |
| $R^2$=.23<br>F(6,62)=4.52, p=0.007 | | | | |

| **Rats** | B | SE | t | p |
|---|---|---|---|---|
| Social status | -1.04 | 0.36 | -2.91 | 0.008 |
| High maternal care | -0.95 | 0.48 | -1.99 | 0.06 |
| Interaction (social status*high maternal care) | 0.95 | 0.44 | 2.16 | 0.04 |
| Constant | 1.09 | 0.41 | 2.67 | 0.01 |
| $R^2$=0.15<br>F(3,21)=3.77, p=0.03 | | | | |

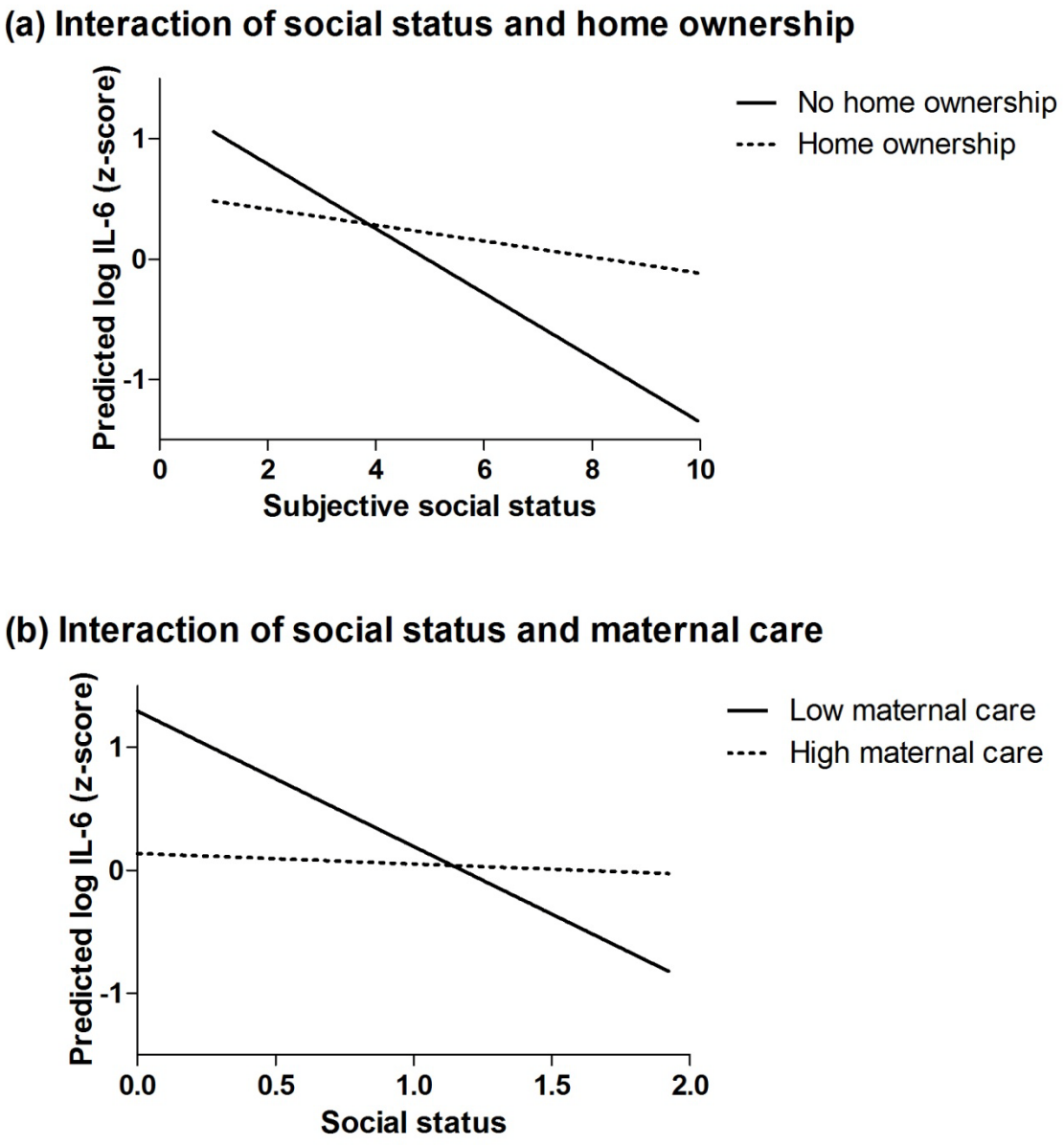



Figure 1. Interaction between early life adversity and adult social status in humans (a) and rats (b) predicts plasma IL-6 concentration.

# Chapter 3

## Emergence and stabilization of social hierarchy: Importance of developmental context and relevance to human studies

### INTRODUCTION

Social dominance in rats relates to a number of behaviors and biological markers that may confer an evolutionary advantage. For instance, dominance predicts reproductive behavior and success (1), metabolic and endocrine (2), behavioral (3), and neurobiological (4) outcomes. However, multiple housing conditions, metrics of dominance, and age of rats studied make the results of social hierarchy investigations difficult to reconcile. My goal in studying social hierarchy in the laboratory rat is to gain insight into such relationships in humans. Therefore, all of these seemingly minor differences in study design are crucial to consider. In this paper, I compare existing models of social hierarchy in rodents and describe a new model of social status intended to parallel human relationships.

Researchers have used several methods for determining social dominance in rodents, including agonistic behaviors, resident-intruder paradigms, and competition for resources. Methods of determining dominance often depend on housing conditions. For example, rats housed in semi-natural environments display fighting and overt aggression towards other residents, whereas rats housed in laboratory cages often do not (5). Therefore, semi-natural colonies, with larger populations, mixed-sex groups, and more varied physical environment than standard lab housing, generally measure dominance using a metric of aggressive interactions won versus lost (5) or wounding (6).

Because rats housed in standard laboratory cages continue play fighting well into adulthood, and often do not exhibit clear aggression towards cagemates, studies using laboratory housing rarely measure dominance using aggression metrics (Blanchard 2001). Of course, there are exceptions (7). Instead of aggressive behaviors between cagemates, laboratory studies of social hierarchy often employ resident-intruder models or competition for resources to measure dominance relationships.

The resident-intruder paradigm and social defeat models include either a single confrontation or repeated interactions separated by periods of "recovery" in the home cage (8, 9). These models introduce unfamiliar rats in adulthood and lead to higher levels of fighting, compared to models that house animals together. Such studies define social status based on intermittent exposure to the intense stress of fighting and include primarily adult male rats. Therefore, they may provide insight into the biological effects

of agonistic experiences, but do not reflect the impact of social relationships among consistent social groups.

Another group of social hierarchy models uses access to resources to measure hierarchy among a group of rats. Food and water competition tasks have been used to measure the social status of rats both in the homecage and with novel social partners. Competition for chocolate cereal and for water (following six hours of water deprivation) produced stable hierarchies in both conditions (10). Another model used competition for sucrose pellets to measure social hierarchy. The competitions were repeated daily among triads of male rats, who were housed together. This model identified stable hierarchies within the homecage for 75% of groups (11). Another study of social competition for resources used pairs of male rats matched on weight and food consumption. Competition for graham cracker crumbs was repeated daily for six weeks. Researchers determined dominance based on time eating, with a stable dominant-subordinate relationship defined by one rat eating a minimum of 70% of the time over a three day period. Eight of eleven pairs (73%) formed stable hierarchies (3). However, in all of these studies, rats were not observed until adulthood, preventing consideration of developmental context.

Because social rank relates to multiple outcomes in so many experimental settings, understanding the stability and temporal dynamics of hierarchy formation would help to inform further research. However, many studies of social dominance measure rank at a single time point and introduce individuals only in adulthood, ignoring developmental differences. Even in well-established paradigms (i.e., the visible burrow system), researchers do not control for developmental differences or experiences of animals before they begin the study, as adults, and interaction between animals is limited (12).

Very few studies have examined dominance relationships longitudinally. Those that do so find that stable dominance relationships are formed in most groups of male rats. However, social groups may not be formed until adulthood, so hierarchy development does not begin until after adolescence, even in studies that follow rats from birth (13). For example, a study of postweaning social experience and aggression used rats purchased at 28 days of age and began the experimental housing conditions at 46 days of age, or late adolescence (14). Although rats were under control of the investigators throughout adolescence, this time period was not included in the study design. Another study, focused on changes in aggression and dominance "across the lifespan," used rats housed with littermates until adulthood, forming experimental groups at 100 days of age (13). Thus, even studies that examine social status and dominance longitudinally generally begin with adult animals.

Some studies suggest that rodents housed together from weaning are less aggressive than those only introduced as adults (14). While this observation may be accurate, it does not validate the exclusive use of adult animals, without regard to their developmental experience. Also, although studies described above provide insight into the consequences of winning or losing agonistic encounters or losing competitive challenges

outside of the homecage in adulthood, they may not reflect the effects of constant, stable, lifelong social status, such as that often experienced in contemporary human societies. In addition, studies that begin in adulthood could suffer from confounding effects of differences in early life experience between group members. I examine the development of social hierarchy among group-housed laboratory rats, using competition for resources to measure social status, and controlling for developmental experience.

**METHODS**

Animals: Rats included in the study were born in our colony from Long Evans rats originally purchased from Charles River. For all animals, temperature was kept constant at 20 ± 2 °C and relative humidity was maintained 50 ± 5%. Rats were maintained on a 12-h light–dark cycle (lights on 0700 h to 1900 h) and allowed access to food (Purina Rat Chow, Purina Mills, St. Louis, Missouri) and tap water *ad libitum*. Housing and care of the rats were carried out in accordance with the standards and practices of the UC Berkeley Animal Care and Use Committee.

Maternal behavior: Female rats were bred and permitted to give birth. Day of birth was marked as postnatal day (PND) 0. Maternal observations were performed beginning on PND 1 and continued until PND 5. Each litter was observed for five hours a day at the following times; 0600h-0800h, 1200h-1300h and 1800h-2000h. During each observation session, litters were observed and behaviors recorded every two minutes (each litter was observed 150 times per day) (15-17). Behaviors recorded included: mother on/off the nest and maternal licking behaviors directed at self or at pups. A maternal care distribution curve was generated by calculating the frequency with which pup-directed maternal licking was observed. Maternal licking was expressed as a percentage of the total number of observations performed for each litter. High and Low licking litters were assessed as those falling above or below the median of all litters.
Housing: Male pups were weaned at PND 24 and housed in cages of three, with non-littermates matched for weight and maternal care received. Rats were housed in guinea pig (20x16x8.5) cages, which are substantially larger than standard rat cages (10.5x19x8) Food and water were available *ad libitum*, and rats were maintained on a 12 hour light-dark schedule (lights on 0700h-1900h).

*Assessment of Social Status in Rats:*

Food competition task: Before weaning, animals were habituated to chocolate. On the first day of training, a small amount of melted chocolate was smeared onto the inside of each home cage. The next day, mini chocolate chips were dropped into each cage. Rats were provided with mini chocolate chips daily for five days, until all rats quickly approached and ate the chocolate. During the food competition task, rats competed for access to a small container of chocolate secured vertically to the cage wall. The chocolate was melted and allowed to cool in a small glass dish before testing, so the rats could not remove pieces of the chocolate, but were required to eat at the dish. Rats' behavior was video recorded following chocolate provision, and a blinded investigator scored the amount of time each rat spent eating the chocolate over the first two minutes.

Water competition task: Rats were deprived of water for eight hours prior to testing, by removing water bottles from the cages. Upon return of the water bottles, rats' behavior was video recorded, and a blinded investigator scored the amount of time each rat spent drinking over the first two minutes.

Assignment of Social status: Chocolate and water tasks were performed within 3 days of each other, at postnatal day 25, 35, 45, 61, 75, 100, 110. Social status at each time point was determined by averaging the proportion of time each rat spent accessing resources (chocolate or water), relative to cagemates. A social status score of 1 for all rats in a cage would represent equal access to resources. Ranks were created based on the social status score, such that for each cage, the rat with the highest social status score was ranked dominant, etc.

$$\textit{Social status score} = \frac{\textit{time eating}}{\textit{total time eating/group size}} + \frac{\textit{time drinking}}{\textit{total time eating/group size}} \; / 2$$

Statistical analysis: Analyses were conducted using Stata v.10 (College Station, TX) and GraphPad Prism 5 (La Jolla, CA). To examine the age at first emergence of a hierarchy and the age of rank stabilization, survival analysis was used, with age as the time variable.

**RESULTS**

Full characterization of maternal care received allowed us to match group-housed rats on early life experience (all rats in a cage received equivalent maternal care). Through this matching procedure, I ensured that maternal care and social status were not correlated within housing groups and that maternal care could not determine social status. I confirmed that maternal care did not correlate with maternal care either in adolescence ($r=.11$, $p=.37$) or in adulthood ($r=-.027$, $p=.83$).

Repeated measures of social status over development allows for the examination of the age at which a social hierarchy can first be detected, the age at which the hierarchy stabilizes within a cage, and changes in social status over time. I conducted competition tasks in all cages beginning in the juvenile stage and continuing through adolescence into adulthood (postnatal day (PND) 25, 35, 45, 61, 75, 100, and 110).

Rats were weaned and housed in groups of three at PND 24. To determine the timing of hierarchy emergence, I identified the age at which each cage first formed an identifiable hierarchy in the food and water competition tasks. 25% of cages do not form a measurable hierarchy on the first or second test. In these cages, the rats do not compete; instead, at least two of the rats ignore the chocolate or water. Half of cages in the cohort formed a distinct hierarchy on first competition task (see Figure 1). 75% of cages show a distinct hierarchy at PND 35, with the remainder showing a hierarchy at PND 45. Level

of maternal care did not predict the age at which hierarchy first emerged ($X^2$=.15, df=2, p=.93).

To determine when the hierarchy stabilized, I examined the age at which the rankings of animals within each cage matched their final adult ranking (measured at PND110). Survival analysis indicated that the median time to hierarchy stabilization in the cohort was 45 days (see Figure 2). 75% of cages showed stable hierarchies at PND 100. Level of maternal care did not predict the age at which hierarchy stabilized ($X^2$=1.038, df=2, p=.60). These results indicate that social status begins to stabilize during late adolescence, and in some groups that process continues into adulthood.

To examine the differences in the slope of the hierarchy over development, I examined the social status score by rank for each time point (Figures 3-9). The results of the first two competition tasks appear to indicate the strongest hierarchies. However, this appearance results from the lack of competition by a minority of rats. Because some of the rats did not compete for chocolate or water at PND 25 or 35, perhaps because of the novelty, social status score captures something different than when all the rats compete for resources at the later time points. This issue shows up in the data as high variance in social status score by rank; the standard deviation of social status score decreases as the rats age. Understanding the details of these behavioral tasks prevents misinterpretation of the data.

Using social status measured in adolescence (PND 45) as a proxy for adulthood social status would lead to misclassification for 42% of rats (see Table 1). The majority of rats maintain the same social status from adolescence to adulthood, as 58% of rats have the same rank at PND 45 and PND 110. However, 30% of rats change their rank by one step in either direction (i.e. moving from the subordinate rank to the middle rank, or from the middle rank to the dominant rank), and 12% of rats change from subordinate to dominant or vice versa. Misclassification of rats switching from dominant to subordinate or vice versa would be more misleading than those of a single rank in either direction. These results increase our understanding of the temporal dynamics of hierarchy formation between rats housed together from weaning with rats of similar early life experience.

## DISCUSSION

This study examined the formation of social hierarchies within cages of group-housed male laboratory rats. Rats were housed together at weaning and competed for access to chocolate and water within their homecage throughout the juvenile, adolescent, and adult periods. Late adolescence (PND 45) emerged as a crucial time for hierarchy development. All cages showed a distinct hierarchy at PND 45, and half of the groups maintained stable hierarchies from PND 45 into adulthood. I fully characterized early life environment by observing and scoring maternal care received during the first five days of life, so social status results were not influenced by differences in early life experience.

This study differs in several ways from previous studies of social hierarchy in laboratory rats. Maternal care has never been considered in this context, as early life environment is often ignored in studies of social dominance. However, it is important to know the life histories of animals before groups are formed, so that differences in exposures before hierarchies are formed do not influence study outcomes. In addition, repeated measurement of competition at important developmental time points allowed us to determine the timing of hierarchy emergence and stabilization.

Researchers have debated the importance of repeated behavioral interactions versus individual characteristics in predicting social status. Several studies suggest that behavioral processes lead to development of hierarchical social structures – that repeated interactions between group members determine social status, rather than endogenous individual characteristics (18, 19). Other studies argue that both behavioral interactions and individual attributes influence social status (20). The results of this study suggest that in many groups, repeated competitions are required for hierarchy stabilization; most groups do not form stable hierarchies when first presented with a competition task.

Consideration of the timing of social groupings and competition testing is essential. These results show that all groups formed hierarchies by late adolescence and that half of the groups formed stable hierarchies by that time. Adolescence is a time of rapid neurological change, physiological growth, and may represent a window of heightened vulnerability. For instance, late adolescent rats show more susceptibility to physiological, HPA axis, and neurobiological effects of chronic stress, compared to early adolescent and adult rats (21). However, many studies of such outcomes include rats who are still in the adolescent window, without explicitly measuring, controlling, or considering the important changes occurring in that developmental period (22). When studying the development of social status and dominance relationships, it is crucial to consider such developmental time periods and their effects.

If one goal of animal research is to gain insight into biological mechanisms in humans, then ensuring that the exposure also parallels human experience as much as possible is important. Therefore, in models of social hierarchy using laboratory rodents, development and parallels to human experience should be considered. For instance, housing rats in experimental groups at weaning may more closely parallel human social experience than purchasing rats as adults, without consideration of their earlier experiences. However, humans do not live under laboratory conditions – small same-sex groups without exposure to varied individuals or environments. Although the ideal model has not yet been developed, our model of social hierarchy presents distinct advantages over those historically used.

**REFERENCES**


1. Dewsbury DA. Dominance rank, copulatory behavior, and differential reproduction. Q Rev Biol 1982;57:135-59.
2. Tamashiro KL, Nguyen MM, Fujikawa T, et al. Metabolic and endocrine consequences of social stress in a visible burrow system. Physiol Behav 2004;80:683-93.
3. Hoshaw BA, Evans JC, Mueller B, et al. Social competition in rats: cell proliferation and behavior. Behav Brain Res 2006;175:343-51.
4. Kozorovitskiy Y, Gould E. Dominance hierarchy influences adult neurogenesis in the dentate gyrus. J Neurosci 2004;24:6755-9.
5. Adams N, Boice R. Development of dominance in domestic rats in laboratory and seminatural environments. Behavioral Processes 1988;19:127-42.
6. Blanchard DC, Spencer RL, Weiss SM, et al. Visible burrow system as a model of chronic social stress: Behavioral and neuroendocrine correlates. Psychoneuroendocrinology 1995;20:117-34.
7. Willner P, D'Aquila PS, Coventry T, et al. Loss of social status: preliminary evaluation of a novel animal model of depression. Journal of Psychopharmacology 1995;9:207-13.
8. Blanchard RJ, McKittrick CR, Blanchard DC. Animal models of social stress: effects on behavior and brain neurochemical systems. Physiology and Behavior 2001;73:261-71.
9. Bartolomucci A. Social stress, immune functions and disease in rodents. Frontiers in Neuroendocrinology 2007;28:28-49.
10. Cordero MI, Sandi C. Stress amplifies memory for social hierarchy Frontiers in Neuroscience 2007;1:175-84.
11. Gentsch C, Lichtsteiner M, Feer H. Competition for sucrose-pellets in triads of male Wistar rats: the individuals' performances are differing but stable. Behavioural Brain Research 1988;27:37-44.
12. McKittrick CR, Blanchard DC, Blanchard RJ, et al. Serotonin receptor binding in a colony model of chronic social stress. Biological psychiatry 1995;37:383-93.
13. Blanchard RJ, Flannelly KJ, Blanchard DC. Life-span studies of dominance and aggression in established colonies of laboratory rats. Physiol Behav 1988;43:1-7.
14. Lore RK, Stipo-Flaherty A. Postweaning social experience and adult aggression in rats. Physiol Behav 1984;33:571-4.
15. Champagne FA, Francis DD, Mar A, et al. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. Physiol Behav 2003;79:359-71.
16. Francis DD, Champagne FA, Liu D, et al. Maternal care, gene expression, and the development of individual differences in stress reactivity. Annals of the New York Academy of Sciences 1999;896:66-84.
17. Liu D, Diorio J, Tannenbaum B, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 1997;277:1659-62.

18. Chase ID. Dynamics of Hierarchy Formation: The Sequential Development of Dominance Relationships. Behaviour 1982;80:218-40.
19. Chase ID. Social process and hierarchy formation in small groups: a comparative perspective. American Sociological Review 1980;45:905-24.
20. Chase ID, Tovey C, Spangler-Martin D, et al. Individual differences versus social dynamics in the formation of animal dominance hierarchies. Proceedings of the National Academy of Sciences of the United States of America 2002;99:5744-9.
21. Jankord R, Solomon MB, Albertz J, et al. Stress Vulnerability during Adolescent Development in Rats. Endocrinology 2010.
22. McCutcheon JE, Marinelli M. Age matters. Eur J Neurosci 2009;29:997-1014.

Table 1. Stability of social rank from adolescence to adulthood.

| | | Rank in adolescence (PND 45) | | |
|---|---|---|---|---|
| | | Subordinate | Mid | Dominant |
| Rank in adulthood (PND 110) | Subordinate | 12 | 7 | 5 |
| | Mid | 7 | 13 | 4 |
| | Dominant | 3 | 3 | 15 |

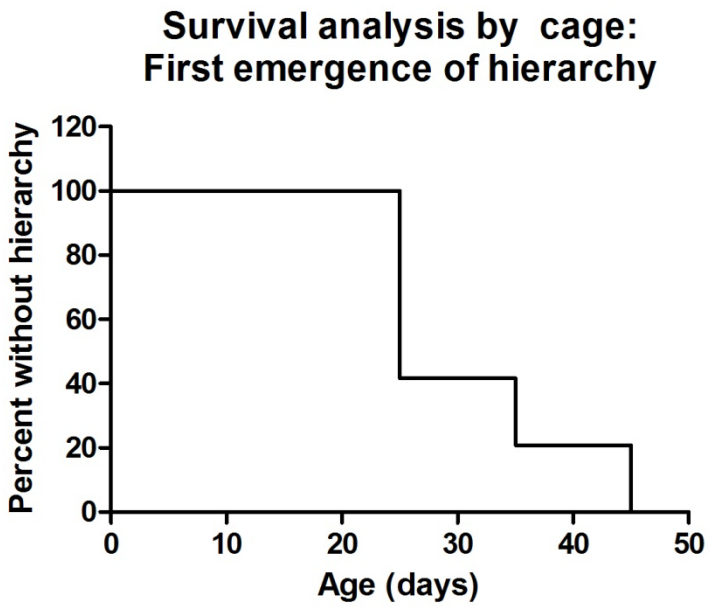



Figure 1. Hierarchy emerges by postnatal day 45 for all cages.

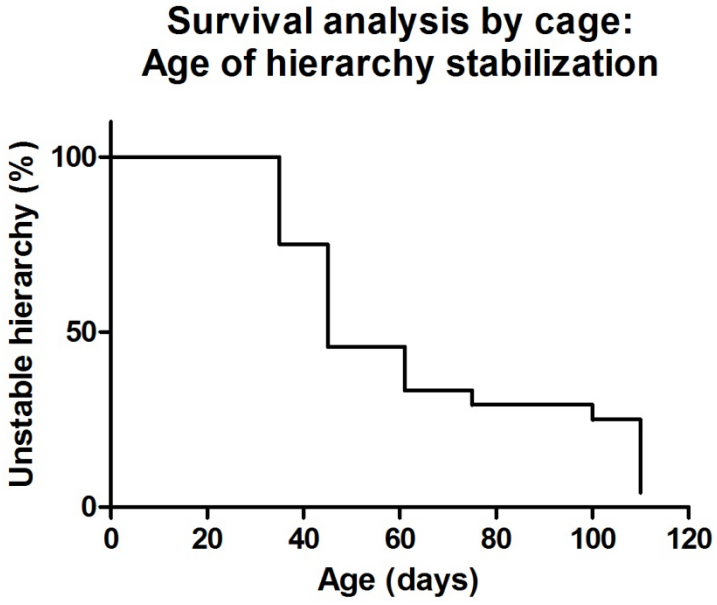



Figure 2. Hierarchy stabilizes between postnatal day 35 and 110.

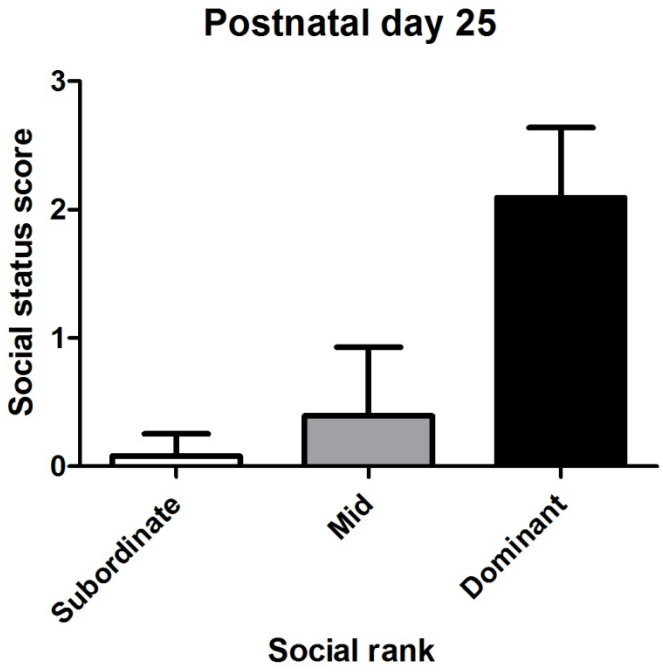



Figure 3. Social status score (proportion of time accessing resources) by rank the first day following weaning, PND 25.

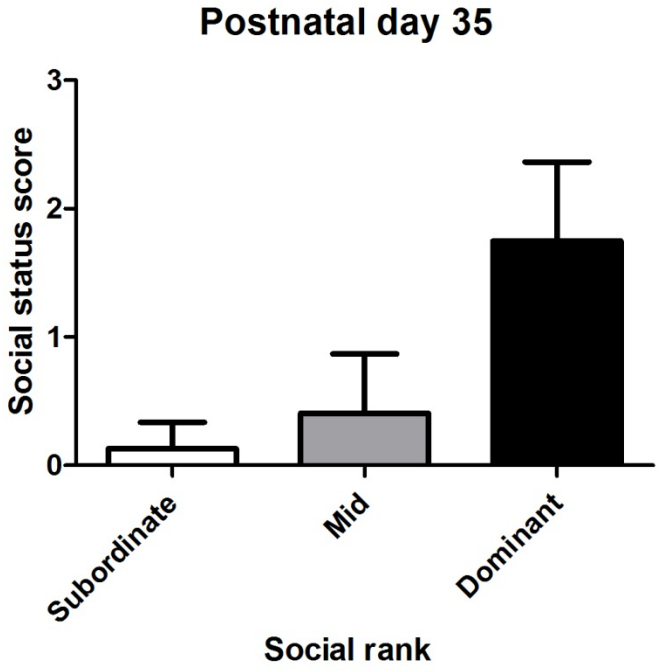



Figure 4. Social status score (proportion of time accessing resources) by rank in early adolescence, PND 35.

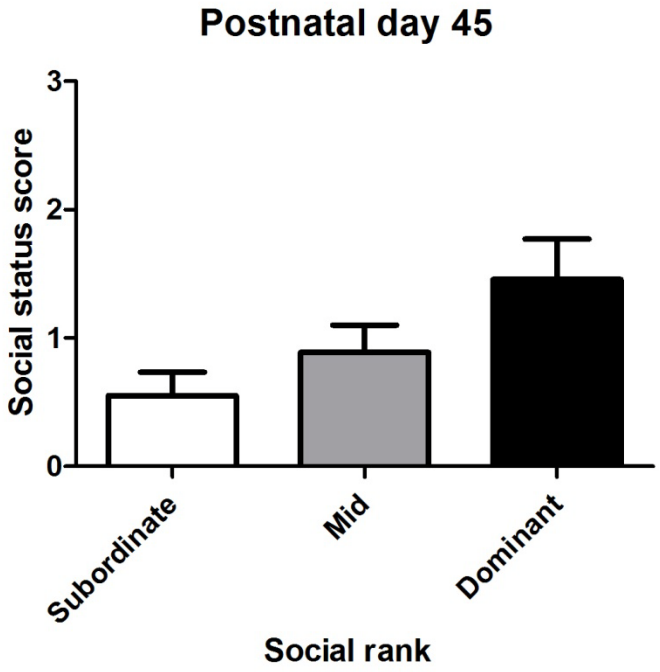



Figure 5. Social status score (proportion of time accessing resources) by rank in late adolescence, PND 45.

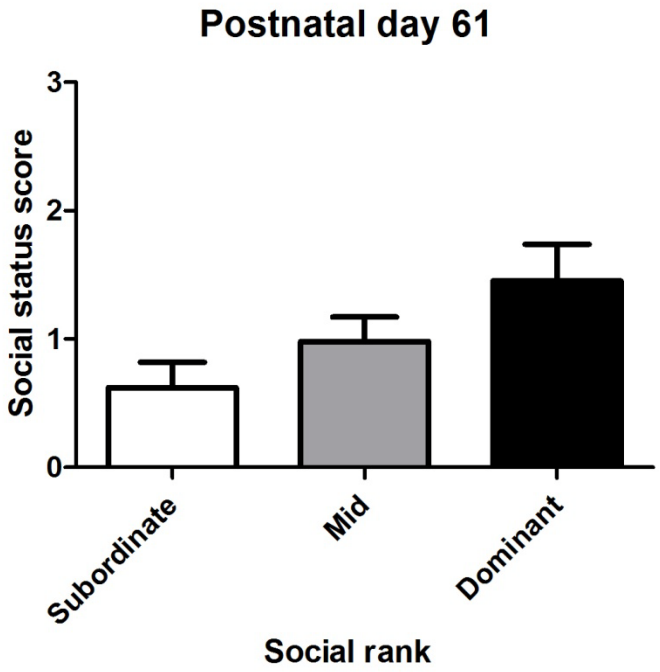



Figure 6. Social status score (proportion of time accessing resources) by rank in late adolescence/early adulthood, PND 61.

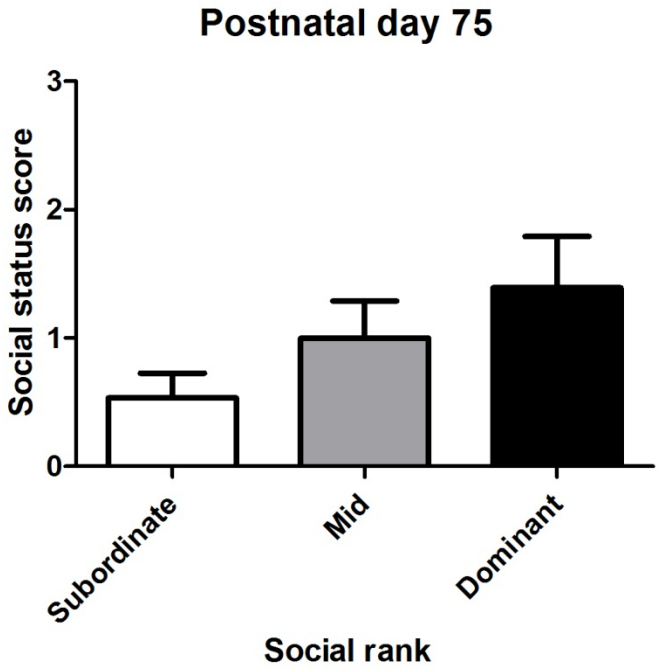



Figure 7. Social status score (proportion of time accessing resources) by rank in early adulthood, PND 75.

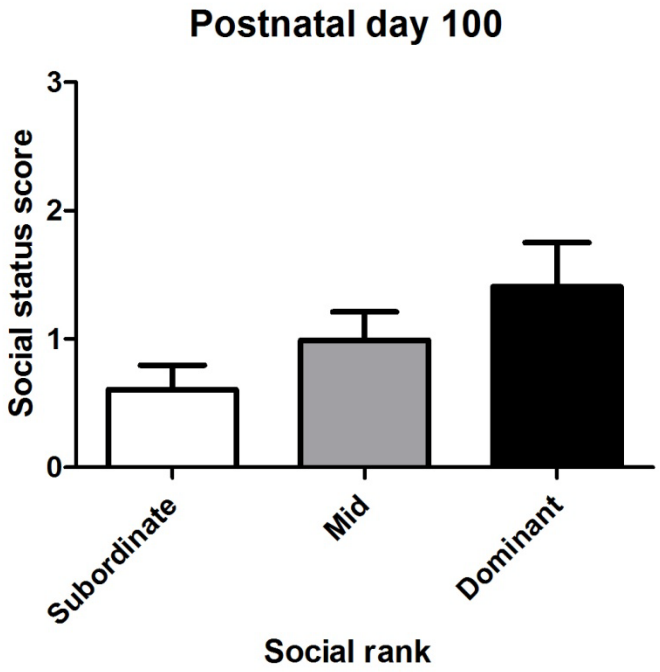



Figure 8. Social status score (proportion of time accessing resources) by rank in adulthood, PND 100.

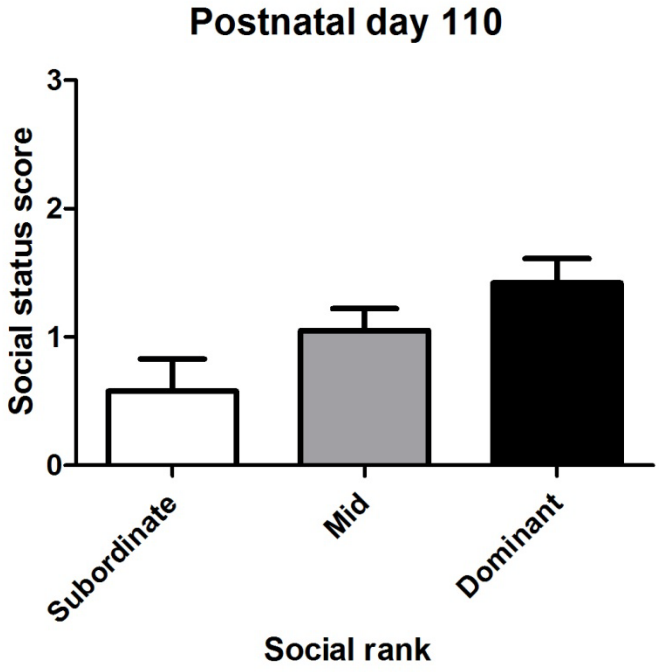



Figure 9. Social status score (proportion of time accessing resources) by rank in adulthood, PND 110.

## CONCLUSIONS

Social health disparities have garnered increasing scientific, media and political interest over the past several decades. Epidemiologic studies on the health effects of relative social position face complex challenges including; the clustering of risk factors, the inability to adequately control confounders, questions of temporality and difficulty in measuring complicated social environments. Much debate exists concerning the ability of epidemiologic studies to differentiate between the social selection (i.e. downward social mobility due to poor health) and social causation (poor health due to adversity) hypotheses, and to accurately measure temporality between social exposures and health. In addition, epidemiologists have debated the importance of absolute levels of resources versus relative social position. Many of these challenges can be addressed using animal models, but the application of animal studies to public health questions has not yet been fully explored.

Lack of progress in this area may speak to the current research and health models used across disciplines. Fields that are able to experimentally control environment and temporality often fail to fully conceptualize and measure social context. To gain traction into the etiology and determinants of the powerful social gradient requires the application of multiple approaches across disciplines. This dissertation provides one approach to tackling these challenges, namely better integration of human studies and animal models. Such an approach can greatly contribute to moving the field forward.

Specifically, this dissertation describes a novel approach to interdisciplinary work: the use of a laboratory animal model to explicitly address questions and test hypotheses from social epidemiology. A better understanding of the biological effects of social context and social experience in an animal model will shed light on the possible mechanistic connections in humans.

Chapter 1 describes a social gradient in multiple domains among group-housed laboratory rats. This animal model of social hierarchy was developed with the goal of directly modeling public health questions. Therefore, the study included careful and extensive characterizing of early life environment and social status. Competition for resources (chocolate and water) was used to determine social status. Because groups were matched on maternal care received, this study separated the effect of early life experience and later social status. Results demonstrate a social gradient in endocrine response to acute stress, exploratory behaviors, and cognitive performance. Subordinate rats showed a blunted corticosterone response to acute stress, lowest levels of exploratory behavior, and poorest cognitive performance in the homecage. Stress of social subordination likely causes the observed differences. This model replicates relationships observed in human populations and presents the opportunity for exploring the ways in which social experiences become biologically embedded to affect lifetime health and disease processes. However, further research is necessary to fully determine causation and to identify factors that predict social status.

Chapter 2 describes an innovative approach to interdisciplinary research, combining data from parallel studies in humans and laboratory rats. This study explores the effects of early life experience and adult social status in predicting inflammatory profiles, investigating how social experiences that occur over the lifecourse interact to influence health. Results of parallel studies in humans and rats demonstrate an interaction between early life experience and adult social status in relation to IL-6 production, such that childhood stress increased sensitivity to adult social status. We found parallel results in college students (males only) and laboratory rats. Early life conditions seem to influence the plasticity of the inflammatory response to later social experiences. Rats and humans who experienced early life adversity (low maternal care in rats and low childhood SES in humans) represented both the highest and lowest levels of IL-6 in adulthood, depending on their social status. Therefore, stress in childhood may not have a monotonically negative effect on later life health, but may alter responsiveness to later exposures. Further research should examine whether interventions aimed at improving the social environment of children experiencing early life stress could prevent or ameliorate negative health effects in vulnerable populations.

Chapter 3 examines the temporal dynamics of hierarchy formation in the laboratory rat model of social hierarchy, and it identifies adolescence as a critical period. Hierarchies emerge in early adolescence and begin to stabilize in late adolescence. Consideration of developmental time periods is critical when studying social experiences, as social status appears to emerge during a time of rapid neurological change. If future research examines the effect of environmental or social interventions on hierarchy status or health outcomes, developmental context and timing will be crucial.

To this point, the utility of animal models in public health research has not been fully recognized. However, these approaches can offer insight into biologic mechanisms under controlled conditions, allowing for the examination of temporal relationships, determination of causality, and experimentation which are not so straightforward in traditional epidemiologic investigations. The work described in this dissertation demonstrates the utility of applying carefully designed animal models, combined with human studies, in addressing questions from public health and social epidemiology.