

Dynamic changes in social dominance and mPOA GnRH expression in male mice following social opportunity



Cait M. Williamson^a, Russell D. Romeo^b, James P. Curley^{a,c,*}

^a Department of Psychology, Columbia University, New York, NY 10027, USA

^b Department of Psychology, Barnard College, New York, NY 10027, USA

^c Center for Integrative Animal Behavior, Columbia University, New York 10027, USA

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ABSTRACT

Social competence - the ability of animals to dynamically adjust their social behavior dependent on the current social context - is fundamental to the successful establishment and maintenance of social relationships in group-living species. The social opportunity paradigm, where animals rapidly ascend a social hierarchy following the removal of more dominant individuals, is a well-established approach for studying the neural and neuroendocrine mechanisms underlying socially competent behavior. In the current study, we demonstrate that this paradigm can be successfully adapted for studying socially competent behavior in laboratory mice. Replicating our previous reports, we show that male laboratory mice housed in a semi-natural environment form stable linear social hierarchies. Novel to the current study, we find that subordinate male mice immediately respond to the removal of the alpha male from a hierarchy by initiating a dramatic increase in aggressive behavior towards more subordinate individuals. Consequently, subdominants assume the role of the alpha male. Analysis of brain gene expression in individuals 1 h following social ascent indicates elevated gonadotropin-releasing hormone (GnRH) mRNA levels in the medial preoptic area (mPOA) of the hypothalamus compared to individuals that do not experience a social opportunity. Moreover, hormonal analyses indicate that subordinate individuals have increased circulating plasma testosterone levels compared to subordinate individuals. Our findings demonstrate that male mice are able to dynamically and rapidly adjust both behavior and neuroendocrine function in response to changes in social context. Further, we establish the social opportunity paradigm as an ethologically relevant approach for studying social competence and behavioral plasticity in mammals.

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1. Introduction

Social hierarchies emerge and stabilize over time as individuals engage in competitive or agonistic interactions and relatively subordinate individuals learn to consistently yield to individuals of a relatively higher social status (Chase, 1982). Dominance hierarchies occur frequently in wild species (Muller and Wrangham, 2004; Nakano, 1995; Sapolsky, 1983, 1993) and in laboratory-based studies of cichlids (Grosenick et al., 2007; Oliveira and Almada, 1996), crayfish (Issa et al., 1999), honey bees (Kucharski et al., 2008), and mice (Wang et al., 2011; Williamson et al., 2016). Although findings vary across species, individuals at the top of a social hierarchy may have significantly higher reproductive success, increased neurogenesis, enhanced immune function and better overall health outcomes than those at the bottom of a hierarchy (Archie et al., 2012; Bartolomucci et al., 2001; Kozorovitskiy and Gould, 2004; Maruska and Fernald, 2013; Sapolsky, 1993). It is therefore essential that individuals are capable of recognizing their own social

status relative to others in a hierarchy and of dynamically shifting their behavior when a social system destabilizes (Fernald, 2014).

One approach to studying dynamic changes in social behavior within a social hierarchy is the social opportunity paradigm, where subordinate individuals rapidly ascend a hierarchy following the removal of the most dominant individual. Ascent following social opportunity has been studied in African cichlid fish, with changes in both behavior and physiology occurring in subdominants within minutes of the removal of the alpha male (Maruska et al., 2013; Maruska and Fernald, 2013; Maruska et al., 2011). These physiological changes include alterations within the hypothalamic-pituitary-gonadal (HPG) axis such as increased levels of circulating 11-ketotestosterone (Maruska and Fernald, 2010) and increased brain gonadotropin-releasing hormone 1 (GnRH1) mRNA levels (Maruska and Fernald, 2013) within socially ascending subordinate males.

Previously, we have shown that outbred CD-1 male mice housed in groups of 12 will consistently and rapidly form linear dominance hierarchies in the laboratory (So et al., 2015; Williamson et al., 2016). Housing male mice in large, complex environments for a period of three weeks, we have established that each mouse has a unique rank and behaves appropriately to individuals of relatively higher and lower social status

* Corresponding author at: Department of Psychology, Columbia University, New York, NY 10027, USA.

E-mail address: jc3181@columbia.edu (J.P. Curley).

(Williamson et al., 2016). Similar to cichlid fish (Desjardins et al., 2012), we have also shown that subordinate and subordinate mice are aware of social context, inhibiting their aggressive behavior in the social hierarchy when the alpha male is actively aggressive to other individuals and increasing their aggression when the alpha male is inactive (Curley, 2016b).

The aim of the current study was to first determine whether, following removal of the alpha male mouse from a social hierarchy, subordinate male mice (beta males) would recognize and take advantage of this social opportunity by increasing their aggression to all other individuals in the hierarchy and ascending to alpha male status. The second aim was to determine if such rapid behavioral changes are associated with physiological changes in the HPG axis similar to those observed in cichlid fish. Although subordinate male mice are not fully reproductively suppressed, they do have decreased testes weight (Bronson and Eleftheriou, 1964; McKinney and Desjardins, 1973) and sperm motility (Koyama and Kamimura, 1998) compared to dominant males, suggesting a down-regulation of the HPG axis. In one study investigating groups of three males, subordinate male mice appear to be similar in HPG activation to subordinate males (McKinney and Desjardins, 1973). We hypothesized that compared to subordinate males in stable hierarchies, where no social opportunity occurred, we would observe increased hypothalamic GnRH mRNA levels in subordinate males undergoing social ascent as well as a corresponding increase in plasma testosterone.

2. Methods

2.1. Subjects and housing

Throughout the study, subjects were housed in the animal facility in the Department of Psychology at Columbia University, with constant temperature (21–24 °C) and humidity (30–50%) and a 12/12 light/dark cycle with white light (light cycle) on at 2400 h and red lights (dark cycle) on at 1200 h. For each experiment, all mice were individually and uniquely marked by dyeing their fur with a blue, nontoxic, non-hazardous animal marker (Stoelting Co.). These marks remain for up to 12 weeks and only require one application, thus enabling each animal to be visually identified throughout the study. All procedures were conducted with approval from the Columbia University Institutional Animal Care and Use Committee (IACUC – Protocol Nos: AC-AAAG0054, AC-AAAP5405).

2.2. Experiment #1: behavioral dynamics of a social hierarchy following social opportunity

Twelve male outbred CD1 mice aged 6 weeks were obtained from Charles River Laboratories and housed in groups of 3 for 3 weeks in standard-sized cages containing environmental enrichment (wooden blocks and nestlets). At 9 weeks of age, all twelve mice were weighed and put into a large custom built vivarium (length 150 cm, height 80 cm, width 80 cm; Mid-Atlantic; Supplemental Fig. 1). The vivarium was constructed as described in So et al. (2015) and Williamson et al. (2016). Briefly, each vivarium consists of an upper level consisting of multiple shelves covered in pine bedding and a lower level consisting of a series of nestboxes filled with pine bedding connected by tubes. Mice can access all levels of the vivarium via a system of ramps and tunnels. Standard chow and water were provided ad libitum at the top of the vivarium, encouraging movement and exploration of all the shelves. The one cohort of twelve animals was put into the vivarium just before onset of the dark cycle on Day 1 of the experiment and was observed for 40 days for up to 5 h per day with an average of 3 h of observation per day. All observations were conducted during the first 7 h of the dark cycle. During these observations, trained observers recorded all instances of fighting, chasing, mounting, subordinate posture and induced-flee behaviors recording the identity of the individuals that were dominant and subordinate in the interaction (contests) using all occurrence sampling. Supplemental Table 1 contains an ethogram of these behaviors. On Day 5, the first alpha male was removed from the

system. Upon removal, the alpha male was never returned to the social group. The most dominant male within the social hierarchy continued to be removed every 3–4 days until there were only 2 mice remaining in the system. These removals occurred on Days 8, 12, 15, 19, 22, 26, 29, 33, 36, and 40, and observations were conducted following each alpha removal and on all days in between removals.

2.3. Experiment #2: behavioral and neuroendocrine changes following exposure to social opportunity vs. social stability

To determine how rapidly individual males socially ascend and the association between ascent and changes in gene expression and circulating hormone levels, we designed a social opportunity manipulation comparing individuals from socially stable groups to those from a group undergoing a social transition. A total of 96 male outbred CD1 mice aged 7 weeks were obtained from Charles River Laboratories and housed in groups of 3 for 2 weeks in standard sized cages. At 9 weeks of age, groups of 12 mice were placed into custom built vivaria (see Supplemental Fig. 1). Social groups ($N = 12$ mice) were paired such that each group was introduced into the vivarium on the same day as one other group, creating 4 sets of paired cohorts. Live behavioral observations occurred as described in Experiment #1 for 2 h a day for each group on Days 1–5 of the experiment. At the end of this initial observation period, a linear dominance hierarchy was verified to have emerged through analysis of the collected behavioral data through calculation of Landau's modified h' (De Vries, 1995). The identity of the alpha male and all other ranks was determined through calculation of Glicko ratings. In the Glicko rating system (Glickman, 1999; Williamson et al., 2016), animals gain or lose points based on the number of wins and losses relative to the difference in ratings between themselves and their opponent (see Williamson et al., 2016 for a more detailed description of the calculations). All social groups formed a linear hierarchy with identifiable individual ranks by Day 5. On Day 6, immediately following the onset of the dark/red light cycle, the alpha male from one of the paired cohorts was removed from the vivarium (social opportunity condition) and placed in a standard cage with food and water. In the other paired cohort, the alpha male was sham-removed. The sham-removal consisted of an experimenter opening the Perspex windows to the vivarium, placing their hand into the vivarium and reaching towards the alpha mouse but not removing him from the vivarium. Thus, in this condition the alpha male was not removed from the social group. This condition controls for behavioral changes that may be occurring in response to a disturbance of the housing system that does not impact the presence of the alpha male. Live behavioral observations occurred for the one-hour period directly following alpha removal or sham-removal. Ascending subordinate males were confirmed as the individual who won most contests post-removal without consistently losing to other males. One hour after the subordinate male in the social opportunity group had won three fights, two mice were removed from each group. From the social opportunity group, the subordinate individual who had risen to dominant status and the most subordinate individual were removed. From the sham-removal group, the subordinate individual who had remained subordinate and the most subordinate individual were taken. This experimental design is detailed in Fig. 1.

Following removal from the social group, mice were immediately euthanized via cervical dislocation, and brains were flash frozen in hexane. Trunk blood was collected into heparinized tubes and plasma was separated and then stored at -80 °C. Following brain and blood collection (subdominant and subordinate), the alpha male who had been removed within the social opportunity condition was returned to his social group. This procedure was repeated at five day intervals for a total of four "removals". However, manipulations were counter-balanced between paired cohorts (i.e. one vivarium had alpha removal for removals 1 and 3 and sham-removals for removals 2 and 4 and the opposite was true of the paired vivarium). Each removal/sham-removal decreased the size of the social group by 2, resulting in $N = 10$ (first

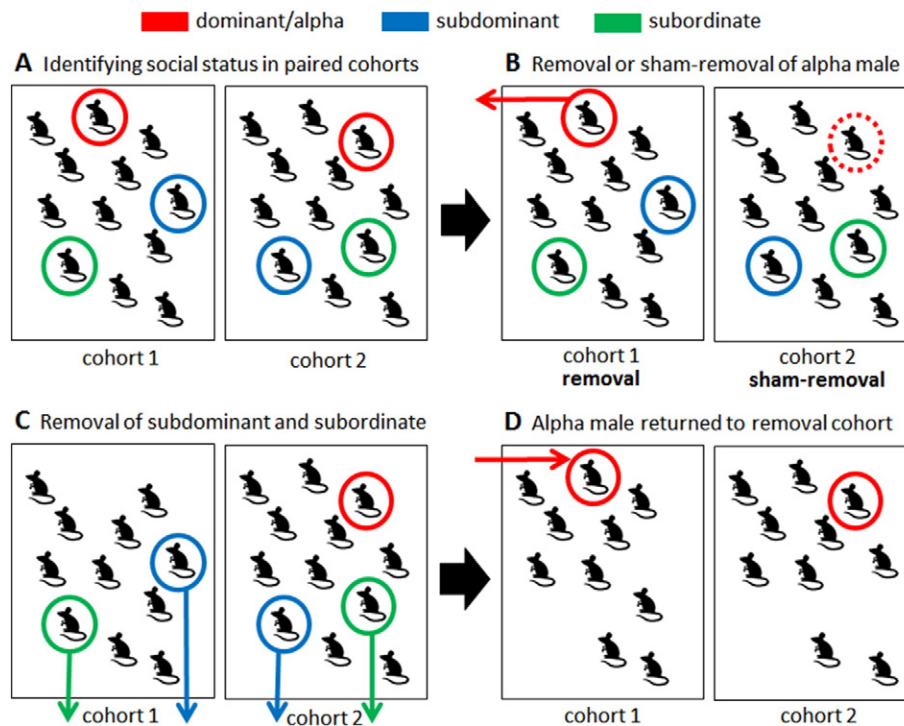


Fig. 1. Schematic of the social opportunity experimental design. (A) Two cohorts of twelve mice are put into separate vivaria and a stable social hierarchy emerges, with clearly defined dominant, subdominant and subordinate individuals. (B) The alpha male is removed from one stable hierarchy and sham-removed from the paired hierarchy. (C) Following removal/sham-removal, behavioral observations are conducted on both cohorts until 1 h after a subdominant rises in the alpha-removed group. At this one-hour time point, the most subdominant and subordinate animal in each hierarchy is removed and brains and trunk-blood collected. (D) One-hour following this removal of the subdominant and subordinate, the alpha male is returned to its social group. This procedure is repeated three more times five days apart for each pair of cohorts.

removal), $N = 8$ (second removal) and $N = 6$ (third removal). This experimental design yielded $N = 16$ mice per group from four groups: subdominant/alpha-removed, subordinate/alpha-removed, subdominant/alpha sham-removed, and subordinate/alpha sham-removed.

2.4. Gene expression

Brains were stored at -80°C until dissection. Samples of the medial preoptic area (mPOA) were collected using a Harris Micro-Punch with reference to coronal plane from the Mouse Brain Atlas (Paxinos and Franklin, 2004). The mPOA was collected as one 1 mm diameter area along the midline from Bregma +0.14 mm to -0.7 mm. RNA was isolated from the mPOA of each individual using the AllPrep RNA Micro Kit (Qiagen) and reverse transcribed to cDNA using the SuperScript III First-Strand Synthesis System for RT-PCR applications (Invitrogen). Quantitative RT-PCR was performed with $1\ \mu\text{l}$ of cDNA using an ABI 7500 Fast Thermal Cycler and the Fast SYBR Green Master Mix reagent (Applied Biosystems). All primer probes (Sigma-Aldrich) were designed to span exon boundaries ensuring amplification of only mRNA. For each gene, C_T values were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH - endogenous control). Relative expression values were obtained by the $\Delta\Delta C_T$ method (Schmittgen and Livak, 2008) with fold-difference being determined relative to subordinates in the sham-removal condition. The following validated quantitative PCR primers were used for mRNA analysis: GAPDH (Forward: TGTGTCCGTCGTGGATCTGA; Reverse: CTGCTTCACCACCTTCTTGA), GnRH1 (Forward: AGCACTGGTCCTATGGGTTG; Reverse: GGTCTGCCATTGATCCAC). Samples that did not yield sufficient RNA for cDNA conversion were eliminated from the analysis.

2.5. Hormone assay

Plasma testosterone concentrations were measured using a commercially available kit (MP Biomedicals, LLC; Orangeburg, NY) and

conducted using the manufacturer's specifications. Samples were run in duplicate and values were averaged. The intra-assay coefficient of variation was 16.2% and lower limit of detectability for the assay was 0.09 ng/ml.

2.6. Statistical analysis

All statistical analyses were undertaken in R version 3.2.3 (R Core Team, 2016).

2.6.1. Behavior analysis

The linearity of each hierarchy was calculated using Landau's modified h' . Briefly, the total number of wins by each individual against all other individuals is entered into a sociomatrix. Landau's method then assesses the degree to which each individual consistently dominates others in contests and whether individuals can be linearly ordered based upon their wins and losses. It ranges from 0 (no linearity) to 1 (completely linear). The significance of h' is determined by performing 10,000 two-step Monte Carlo randomizations of the sociomatrix and comparing the observed h' against a simulated distribution of h' (De Vries, 1995; Williamson et al., 2016). Temporal changes in individual dominance ratings were calculated using Glicko ratings (Glickman, 1999; So et al., 2015). Glicko ratings are a pairwise-contest model rating system where rating points are recalculated following each successive win or loss. All individuals start with a rating of 2200. Ratings are gained after wins and lost after losses with the magnitude of points gained or lost dependent upon the difference in rating scores between the two individuals in each contest (Glickman, 1999; Williamson et al., 2016). Landau's modified h' was calculated using the R package *compete* v0.1 (Curley, 2016a). Glicko ratings were calculated using the *PlayerRatings* package v1.0 in R (Stephenson and Sonas, 2012).

Differences in proportions of individuals engaging in behavior were assessed using a Binomial test or Chi-squared test as appropriate. Differences between two groups in the frequency or latency of behaviors

were assessed using paired or unpaired Wilcoxon Signed Rank Tests as appropriate. Individual David's Scores were calculated based on wins and losses in the hour after alpha removal or sham-removal to confirm individual dominance scores during these periods (De Vries, 1995). To compare changes in total wins between the day prior to alpha removal/sham-removal and the day of alpha-removal/sham-removal, we used a zero inflated negative binomial generalized mixed effect model with counts of wins as the outcome variable, alpha removal status (removal or sham-removal) and day (day prior to removal or day of removal) as fixed effects and cohort and removal number as random effects using the R packages glmmADMB (Skaug et al., 2015) and lmerTest (Kuznetsova et al., 2015).

2.6.2. GnRH expression and testosterone analysis

To examine the effect of alpha removal and social status (subdominant or subordinate) on GnRH mRNA levels and circulating plasma testosterone, we used general linear models using the R package lme4 (Bates et al., 2015). GnRH and testosterone data were log-transformed to ensure that assumptions of normal distribution were satisfied. To determine the effect of number of wins, number of losses, and total fights occurring within each social group in the hour post-alpha removal or sham-removal on GnRH mRNA levels and circulating plasma testosterone we performed Spearman rank correlations between number of wins, losses, total fights and GnRH or testosterone levels. These correlations were run separately for each condition and male social status giving four groups (subdominant/alpha-removed; subdominant/sham-removal; subordinate/alpha-removed; subordinate/sham-removal).

2.6.3. Effect size calculations

For all Wilcoxon Rank Sum Tests and Wilcoxon Signed Rank Tests, effect sizes were calculated with the formula $r = \frac{z}{\sqrt{N}}$. An r value below 0.3 indicates a low effect, between 0.3 and 0.5 indicates a moderate effect, and between 0.5 and 0.7 indicates a large effect. Cohen's d was calculated for all Chi-squared tests. A Cohen's d value between 0.2 and 0.5 indicates a small effect size, 0.5 to 0.8 indicates a medium effect size, and values above 0.8 indicate a large effect size.

3. Results

3.1. Experiment #1: subdominant males socially ascend and assume alpha male status following social opportunity

Following each of the 10 removals of the most dominant alpha male from the social group, the next most socially dominant male ascended

to become the new alpha male within one day (Binomial test, $N = 10$, $p = 0.002$). Glicko dominance ratings indicated that the socially ascending male rapidly increases their dominance rating relative to all other males following each alpha male removal (Fig. 2A). All rising subdominant males showed a significant increase in their daily relative share of aggressive behaviors in the hierarchy on the day of alpha removal compared to the day prior to alpha removal (Wilcoxon Paired Test: $V = 0$, $p = 0.002$, $r = 0.63$, Fig. 2B). There was no difference in relative share of aggression by each male between the day of ascending to alpha rank and the day after (Wilcoxon Paired Test: $V = 17$, $p = 0.32$, $r = 0.24$) or between the next two days (Wilcoxon Paired Test: $V = 22$, $p = 0.62$, $r = 0.09$), indicating that socially ascended males maintain their new alpha male status over several days. Additionally, the day before the alpha male was removed, subdominant males lost a median of 12.9% of all fights in the hierarchy (nearly all to alpha males), whereas after alpha removal this value significantly dropped to a median of 0% fights lost (Wilcoxon Paired Test: $V = 55$, $p = 0.002$, $r = 0.62$) and stayed at this value for the next three days. Thus, the most dominant subdominant male socially ascends to become the new and stable unequivocal alpha male following removal of the previous alpha male.

3.2. Experiment #2: social ascent dynamics and neuroendocrine impact

Prior to the first alpha or sham-removal, all eight social groups of twelve males had formed a stable social hierarchy with a clear alpha male (all h' values >0.43 – mean $h' = 0.54$; all $p < 0.05$ – mean $p = 0.018$). All alpha males maintained their social rank for the duration of the experiment.

3.2.1. Subdominant males rapidly socially ascend following social opportunity

After each of the 16 removals of alpha males, one subdominant male clearly rapidly ascended within 1 h. Rising subdominants had on average 10 times as many wins as the individual with the second most wins in this time period and 15/16 rising males never lost any fight (the remaining rising male only lost one fight, Supplemental Figs. 2 & 3).

The identity of the rising subdominant could be predicted from analyzing the behavior in the five days prior to each alpha removal. A significant proportion of males that rose (13/16) were those with the second highest Glicko ranking (i.e. second to alpha male) prior to removal (Binomial test, $p = 0.02$). In 3/16 instances, an individual that ascended was another subdominant male with a slightly lower Glicko rating than the highest subdominant. Notably, two of these instances were during the fourth removal (i.e. following several manipulations of the social group).

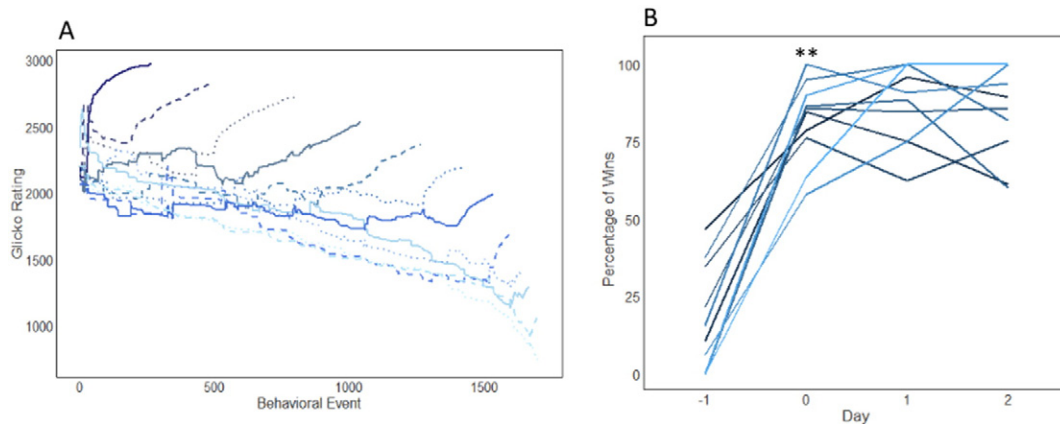


Fig. 2. Behavioral changes following social opportunity. (A) Glicko dominance rating as recalculated after each observed agonistic interaction. Separate lines represent different individuals. (B) The percentage of all contests occurring in a social hierarchy that are won by the subdominant male on the day of social ascent (0), the following two days (1, 2) and the day before social ascent when a more dominant alpha male was present (–1). Separate lines represent different individuals. Asterisks denote a significant difference in percentage of wins ($p < 0.01$) from the day prior to removal of the dominant to the day of dominant removal.

We compared the frequency of aggressive behavior exhibited by rising subdominant males compared to subdominant males of the equivalent rank when the alpha male was sham-removed. A significantly higher proportion of subdominant males showed aggression within 1 h following the alpha male being removed versus sham-removed (alpha removed = 16/16 males, alpha sham-removed = 8/16 males; Chi-squared test $X^2 = 8.17$, $df = 1$, $p = 0.004$, $d = 1.17$). When the alpha male was sham-removed, no beta male won more than ten contests. Conversely, one beta male in the alpha removed group won 48 contests in 1 h. A significantly higher proportion of subdominant males from the alpha male removed group achieved each number of wins compared to subdominant males from the sham-removed group (Chi-squared tests, all $p < 0.05$; Fig. 3A).

The social ascent of rising subdominants was rapid. The latency to each successive win was significantly shorter when the alpha male was removed compared to when the alpha male was sham-removed (Fig. 3B, Wilcoxon Rank Sum Test all $p < 0.001$, all r between 0.53 and 0.62). Most strikingly, the average latency to winning a fight was under 3 min after the alpha male was removed (median [IQR] = 165 s [78 s, 300 s]) but was over 38 min for subdominant males following sham-removal (2306 s [338 s, 3600 s]). Even when considering only those males that were aggressive during the observation (alpha removed $N = 16$, alpha sham-removed $N = 8$), subdominant males were significantly faster to record their first win when the alpha male was removed (Wilcoxon Rank Sum Test, $W = 33.5$, $p = 0.03$, $r = 0.38$).

Further, as shown in Fig. 3C, in the one-hour period directly following alpha male removal, subdominant males displayed significantly increased aggression; compared to behavior during the same 1 h on the previous day (alpha male present) and compared to behavior of subdominants following sham-removal. Using a negative binomial mixed effect model with frequency of aggressive behavior as the outcome variable and cohort and removal number as random effects, there was a

significant interaction between alpha removed/sham-removed and day (NB-GLMM: $\beta = 1.69 \pm 0.30$, $N = 64$, $P < 0.001$). Subdominant males were significantly more aggressive when alpha males had been removed compared to sham-removed (Wilcoxon Signed Rank Test, $W = 4.5$, $p < 0.001$, $r = 0.62$) and compared to the day prior to removal (Wilcoxon Paired Signed Rank Test, $V = 0$, $P < 0.001$, $r = 0.62$). There was no significant difference in the frequency of aggression of subdominant males when the alpha male was sham-removed; compared to behavior the day prior to sham-removal ($p = 0.89$) or compared to subdominant males from either group the day prior to removals ($p = 0.66$).

We also assessed how subordinate mice changed their behavior in response to the alpha-removal or sham-removal. In 5/16 alpha-removals, the most subordinate animal had one win post-removal and in 1/16 removals the subordinate animal had two wins. Proportionally this is a significantly higher number of subordinate animals showing any aggression during removals than was observed during sham-removals (0/16, Chi-squared test: $X^2 = 5.13$, $df = 1$, $p = 0.024$, $d = 0.87$). It is also a significantly smaller proportion of animals showing any aggression than the proportion of subdominant animals that exhibited aggression (Chi-squared test: $X^2 = 11.78$, $df = 1$, $p < 0.001$, $d = 1.53$).

3.2.2. GnRH mRNA gene expression and plasma testosterone levels following social opportunity

One hour following the subdominant male's rise to dominant status both subdominant males and subordinate males showed elevated GnRH mRNA levels in the mPOA, as compared to subdominant and subordinate males in the sham-removed group (GLM: $F_{2,38} = 3.02$, $p = 0.04$, Fig. 4A).

Social status was significantly associated with plasma testosterone levels with subordinate male mice having lower testosterone than

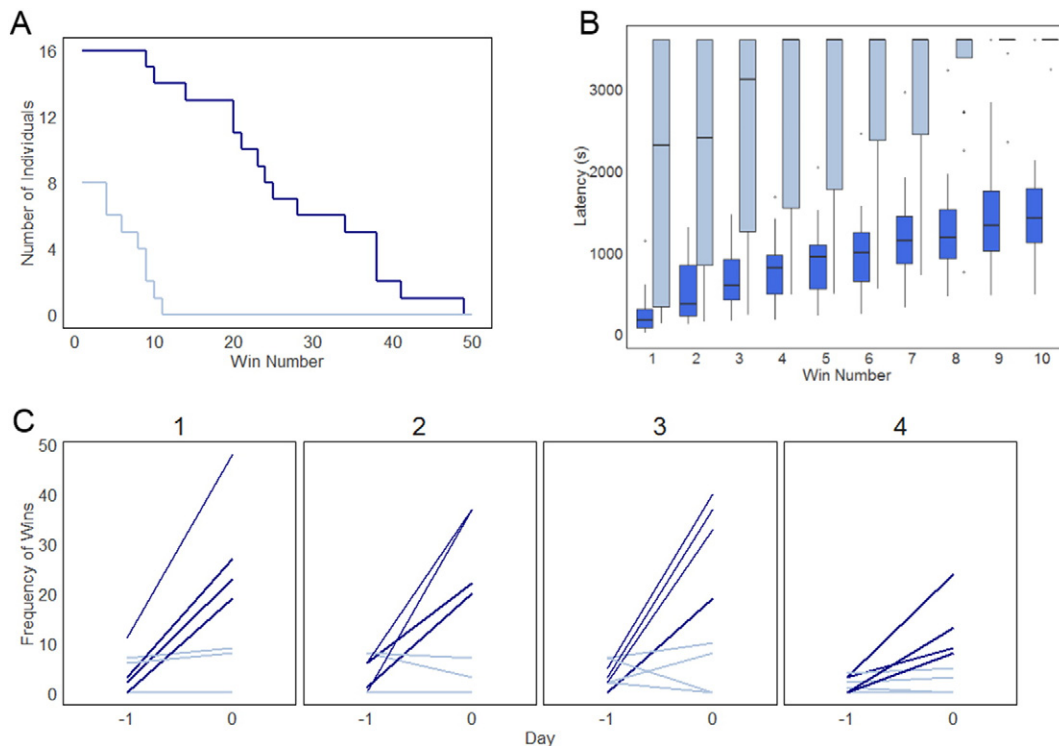


Fig. 3. Behavioral changes in subdominant males following removal of the alpha male. (A) Total number of subdominant beta males winning each number of social contests after alpha removal (dark blue) or sham removal (light blue). (B) Latency of subdominant males to win successive contests within 1 h after the alpha male was removed (dark blue) or sham-removed (light blue). (C) Frequency of all wins won by subdominant males during one-hour time-matched observations on the day prior (−1) or day of (0) the alpha male being removed (dark blue) or sham-removed (light blue) at each removal, separated by removal number. Removal 1 occurred when there were 12 mice in the group, removal 2 occurred when there were 10 mice in the group, removal 3 when there were 8 mice in the group, and removal 4 when there were 6 mice in the group. $N = 16$ males per condition.

subdominant mice (GLM, $F_{2,58} = 2.46$, $p = 0.03$, Fig. 4B). There was no significant interaction between alpha removal and social status in the GLM. However, it is notable that in the alpha male removed group, we did find a significant effect of status on plasma testosterone levels (GLM: $F_{1,30} = 4.59$, $p = 0.04$), which was not observed in the sham-removal group (GLM: $F_{1,28} = 1.221$, $p = 0.28$).

There was no relationship between the frequency of wins or losses by each individual and their mPOA GnRH mRNA levels or circulating plasma testosterone levels in any of the four groups (Supplemental Tables 2 and 3). There was also no relationship between the frequency of all contests that occurred between all animals in the group and GnRH mRNA or circulating plasma testosterone levels in any of the four groups (Supplemental Tables 2 and 3).

4. Discussion

In the current study, we show that removing an alpha male mouse from a social hierarchy leads to a rapid increase in aggression and a subsequent ascent to alpha status by the most subdominant male. This is a robust effect that occurred following every single removal of alpha males regardless of whether the group consisted of as many as 12 individuals or as few as 3 individuals in the social group. Subordinate males expressed aggression during social opportunity, but these males were quickly defeated by the socially ascending subdominant males. Though subordinate individuals clearly respond to the dynamic change in social context, these individuals are unable to take advantage of the opportunity. These findings support and extend previous findings of an attentional hierarchy - where we observed that the aggressive behavior of subdominant and subordinate males is suppressed when alpha male mice are actively aggressive within a social hierarchy (Curley, 2016b).

The social ascent by subdominant male mice observed in the current study is consistent with what has been observed to occur during a social opportunity in African cichlid fish (Maruska et al., 2013). In this species, individual fish respond behaviorally and physiologically within seconds to minutes to the change in social context. Likewise, we observed that rising subdominant male mice respond to the removal of alpha males rapidly with the first fight occurring in less than 3 min. This is remarkable given that we removed the alpha male at the change of light cycle (white light to dark light) – a time when the subdominant male was not always active or even awake. Regardless, removal of the alpha male always led to individuals attempting to take advantage of the social opportunity which in turn aroused the subdominant male even if he was not originally aware of the opportunity. Although it has long been established that individuals across all species are able to re-establish social hierarchies over time following the death, removal or other disturbance of dominant individuals (Chase and Seitz, 2011; Franz et al., 2015; Rosvold et al., 1954), we and others have argued that this ability to respond rapidly and dynamically to changes in social context is a fundamental feature of group-living social cognition and social competence (Desjardins et al., 2012; Fernald, 2014; Oliveira, 2009; Williamson et al., 2016). Individuals that are unable to respond flexibly to social challenges such as these are likely to be at a great social, reproductive and health fitness disadvantage (Hofmann et al., 2014; Taborsky and Oliveira, 2012). Our data are consistent with experimental findings in cichlid fish (Burmeister et al., 2005; Carpenter et al., 2014; Maruska et al., 2013; Maruska and Fernald, 2010, 2011; Maruska et al., 2011) and rhesus monkeys (Rosvold et al., 1954), as well as naturalistic observations of hierarchy maintenance in primates (Chase and Seitz, 2011; Franz et al., 2015), suggesting that rapid social ascent following social opportunity may be a universal feature of linear social hierarchies.

Increased aggression exhibited by the subdominant and recognition of the absence of the alpha male by the group as a whole lead to physiological as well as behavioral shifts. We find that both the ascending subdominant and the most subordinate male in the group express higher levels of GnRH mRNA in the mPOA of the hypothalamus 1 h following the removal of the alpha male compared to individuals in the sham-removal condition. Similar rapid increases in mRNA expression are observed in male mice exposed to soiled bedding from an unfamiliar male (Gore et al., 2000) and in doves following a one-hour courtship period (Mantei et al., 2008). Increases in hypothalamic GnRH mRNA of subdominant males during such a social opportunity are observed in African cichlid fish where up-regulation of GnRH and the HPG axis occurs during social ascent (Maruska and Fernald, 2013; Maruska et al., 2011). Importantly, in cichlid fish subordinate males are truly reproductively suppressed, and the transition from being reproductively inactive to becoming reproductively active upon social ascent requires large changes in reproductive physiology that are regulated by the HPG axis (Maruska and Fernald, 2011). In mice, there is some evidence that more subordinate individuals do have a down-regulated HPG axis, as subordinates have been found to have lower seminal vesicle weight and decreased

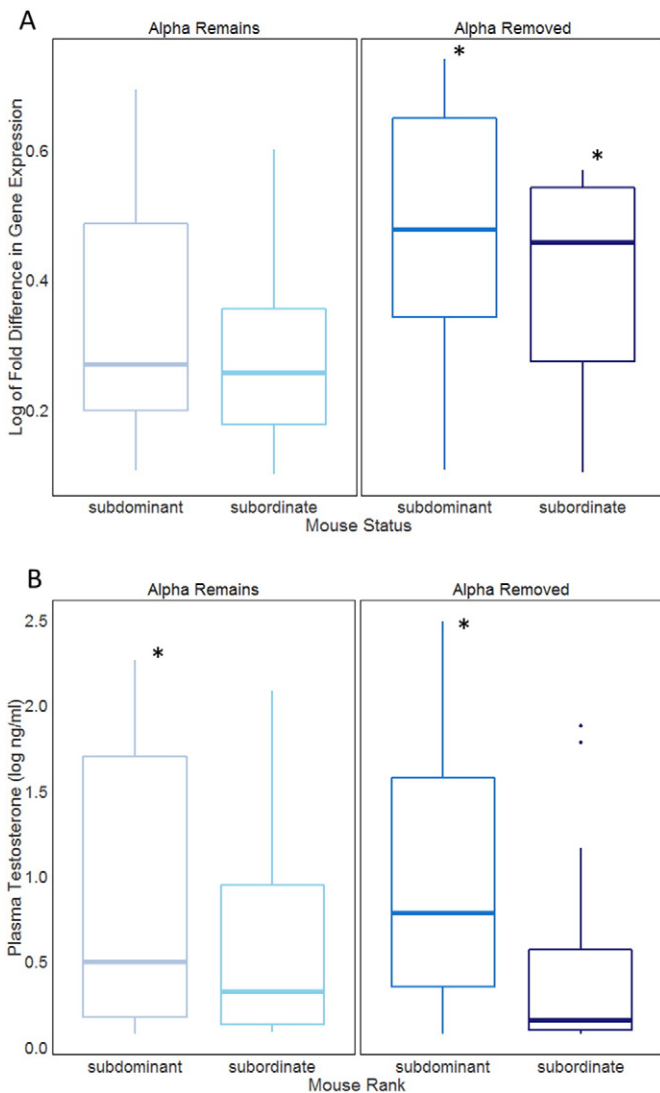


Fig. 4. HPG measures correlated to social status and impacted by social opportunity. (A) Log-transformed fold difference in mPOA GnRH mRNA levels. Asterisks denote a significant difference between GnRH levels in individuals in the group undergoing social ascent and the stable group ($p < 0.05$). (B) Plasma testosterone levels in subdominant and subordinate males following alpha removal (dark blue) or sham-removal (light blue). Boxplots show median, IQR and 95% confidence interval of data. Asterisks denote a significant difference between plasma testosterone levels in subdominant individuals and subordinate individuals, regardless of alpha removal ($p < 0.05$).

testes weight (Bronson and Eleftheriou, 1964; Mckinney and Desjardins, 1973) as well as decreased sperm motility (Koyama and Kamimura, 1998). Therefore, although subordinate male mice are not necessarily entirely reproductively suppressed, it is not completely unexpected that ascent to dominant status would involve changes along the HPG axis. The mechanism for this GnRH plasticity is still poorly understood, however, it is possible that changes in social experience could trigger dynamic changes in GnRH expression through direct neural input from different sensory modalities rather than via steroid hormone effects (Stevenson et al., 2012).

Increased hypothalamic GnRH mRNA levels in subordinate individuals were not expected, as these animals are not consistently engaging in increased aggression and do not socially ascend during the social opportunity. One potential explanation is that subordinates sense that the social context has altered, and observing changes in social interactions between other individuals leads to the increased GnRH. Several species including cichlid fish and corvids are able to infer social ranks through observation (Bond et al., 2003; Grosenick et al., 2007) and are able to adjust their own behavior by closely monitoring the behavior of other more dominant individuals and recognizing when these animals are absent (Desjardins et al., 2012; Freniere and Charlesworth, 1983). Indeed, we have previously described how subordinate and subordinate male mice attend to alpha males and change their own behavior when alpha males are less active (Curley, 2016b). Further, watching fights leads to increased androgen levels in observers across species including fish and humans (Bernhardt et al., 1998; Oliveira et al., 2001), suggesting that simply observing the changes in social interactions may be sufficient to change GnRH gene expression in all group members. Alternatively, increased engagement in aggressive interactions, even if the vast majority of those interactions resulted in losses, may underlie the increased hypothalamic GnRH expression in subordinate individuals. Though losing fights has not been previously associated with an increase in GnRH, losers and winners in social contests between male mice exhibit similar decreases in c-fos activation of RFamide-related peptide (RFRP) cells (Jennings et al., 2016). Given that RFRP (gonadotropin-inhibitory hormone in birds), is a negative regulator of the reproductive axis (Kriegsfeld et al., 2015), this decrease in RFRP activation due to any type of aggressive encounter (win or loss) could lead to increases in GnRH both following winning and subsequent social ascent in subdominants and following losing experienced as others socially ascend. Although there may be multiple mechanisms through which changes in GnRH mRNA levels may be modulated, we did not observe any relationship between total wins or losses or the number of total contests that occurred in each hour following removal or sham-removal and mPOA GnRH mRNA levels. This suggests that the relationship between behavior and gene expression is not a simple linear association. Nevertheless, it is evident that all individuals in each social hierarchy, including those undergoing transition from subordinate to alpha status as well as subordinate males, are exhibiting a behavioral and neuroendocrine response to the increased social instability induced by removal of the alpha male.

We observed elevated circulating plasma testosterone levels in subdominant individuals compared to subordinate males. In animals undergoing social opportunity, although both individuals exhibited increased levels of GnRH mRNA, subdominant males had significantly higher testosterone than the subordinates. This dissociation between increased GnRH and testosterone levels may be related to an inability of subordinate individuals to respond to GnRH as occurs in group-living subordinate sugar gliders (Bradley and Stoddart, 1997). In this species, exogenous administration of GnRH to dominant and subordinate individuals leads to an increase in plasma testosterone in dominant but not subordinate individuals. Similarly, male wild dark-eyed juncos alter their behavioral and physiological responses to GnRH administration dependent upon the particular social context (McGlothlin et al., 2007). A related phenomenon also occurs in naked mole rats – nonbreeding females do not show an LH surge of the same

magnitude as breeding females in response to exogenous GnRH administration (Faulkes et al., 1990). Thus, it is possible that subordinate individuals in our social hierarchies are able to increase GnRH in the mPOA but are unable to successfully respond to that GnRH increase with increased testosterone levels and ultimately higher HPG activation.

In the current study, socially subdominant males in the sham-removal group had equivalent levels of testosterone to subordinate males in the alpha-removal group. There is an extensive literature on the relationship between circulating testosterone and aggression and social dominance with higher circulating testosterone levels being commonly observed in the dominants of many species (Gesquiere et al., 2011; Higham et al., 2012; Mendonça-Furtado et al., 2014; Sapolsky, 2005). In mice, more dominant males have been reported to have higher circulating testosterone levels than subordinates but these findings are inconsistent (Bronson, 1973; Ely and Henry, 1978; Haemisch et al., 1994; Hiadlovská et al., 2015; Oyegbile and Marler, 2005; Selmanson et al., 1977; Zielinski and Vandenbergh, 1993). One possible explanation for this inconsistency is variation in social context. Indeed, it has been proposed that testosterone will be more highly correlated with dominance status and agonistic behavior during times of social instability (Wingfield et al., 1990) when it is essential for individuals to attempt to rise in social status (Liening et al., 2012). Evidence in support of this “challenge hypothesis” has been seen in fish (Almeida et al., 2014), lizards (Greenberg and Crews, 1990), and chimpanzees (Cavigelli and Pereira, 2000). It is likely that given the repeated removals of beta subdominant males from our social system every five days that these subdominant males may have been consistently exerting their dominance to maintain their newly established social position and as such exhibited higher circulating plasma testosterone compared to more subordinate individuals. While these subdominant individuals do exhibit higher levels of testosterone, they do not have the elevated GnRH mRNA levels that the socially ascending subdominants do immediately after alpha removal. This finding could be due to consistently increased testosterone levels over the previous 5-day period leading to an overall down-regulation of GnRH in the hypothalamus (Lee et al., 2008) in these males that is overridden in the subdominants in the alpha-removal group.

5. Conclusion

We have demonstrated that following the removal of the alpha male from a stable social hierarchy, the subdominant male responds within minutes to this social opportunity by increasing their aggression against all other individuals. If the alpha is permanently removed, this leads to the subdominant assuming the alpha male role. Other males also respond behaviorally to the social opportunity but are not as capable at ascending the social hierarchy. Associated with these behavioral changes are rapid increases in mPOA GnRH gene expression which may lead to further changes in the HPG axis regulation of behavior. Further, recently socially risen subdominant males possess higher circulating plasma testosterone which is likely associated with their increased aggression following social ascent. Such dramatic and rapid behavioral and physiological modifications in response to dynamic alterations in social contexts are consistent with individuals engaging in socially competent behaviors similar to those that occur in other animals that similarly live in dynamically transitioning social hierarchies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.yhbeh.2016.11.001.

References

- Almeida, O., Gonçalves-de-Freitas, E., Lopes, J.S., Oliveira, R.F., 2014. Social instability promotes hormone-behavior associated patterns in a cichlid fish. *Horm. Behav.* 66 (2): 369–382. <http://dx.doi.org/10.1016/j.yhbeh.2014.05.007>.
- Archie, E.A., Altmann, J., Alberts, S.C., 2012. Social status predicts wound healing in wild baboons. *Proc. Natl. Acad. Sci. U. S. A.* 109 (23):9017–9022. <http://dx.doi.org/10.1073/pnas.1206391109>.
- Bartolomucci, A., Palanza, P., Gaspani, L., Limiroli, E., Panerai, A.E., Ceresini, G., ... Parmigiani, S., 2001. Social status in mice: behavioral, endocrine and immune changes are context dependent. *Physiol. Behav.* 73 (3):401–410. [http://dx.doi.org/10.1016/S0031-9384\(01\)00453-X](http://dx.doi.org/10.1016/S0031-9384(01)00453-X).
- Bates, D., Maechler, R., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., ... Grothendieck, G., 2015. lme4: linear mixed-effects models using “Eigen” and S4 (version 1.1–10). Retrieved from: <https://cran.r-project.org/web/packages/lme4/index.html>.
- Bernhardt, P.C., Dabbs Jr., J.M., Fielden, J.A., Lutter, C.D., 1998. Testosterone changes during vicarious experiences of winning and losing among fans at sporting events. *Physiol. Behav.* 65 (1):59–62. [http://dx.doi.org/10.1016/S0031-9384\(98\)00147-4](http://dx.doi.org/10.1016/S0031-9384(98)00147-4).
- Bond, A.B., Kamil, A.C., Balda, R.P., 2003. Social complexity and transitive inference in corvids. *Anim. Behav.* 65 (3):479–487. <http://dx.doi.org/10.1006/anie.2003.2101>.
- Bradley, A.J., Stoddart, D.M., 1997. Plasma androgen, social position, and response to GnRH in the marsupial sugar glider *Petaurus breviceps* (Marsupialia: Petauridae). *J. Zool.* 241 (3):579–587. <http://dx.doi.org/10.1111/j.1469-7998.1997.tb04849.x>.
- Bronson, F.H., 1973. Establishment of social rank among grouped male mice: relative effects on circulating FSH, LH, and corticosterone. *Physiol. Behav.* 10 (5):947–951. [http://dx.doi.org/10.1016/0031-9384\(73\)90065-6](http://dx.doi.org/10.1016/0031-9384(73)90065-6).
- Bronson, F.H., Eleftheriou, B.E., 1964. Chronic physiological effects of fighting in mice. *Gen. Comp. Endocrinol.* 4 (1):9–14. [http://dx.doi.org/10.1016/0016-6480\(64\)90033-4](http://dx.doi.org/10.1016/0016-6480(64)90033-4).
- Burmeister, S.S., Jarvis, E.D., Fernald, R.D., 2005. Rapid behavioral and genomic responses to social opportunity. *PLoS Biol.* 3 (11), e363. <http://dx.doi.org/10.1371/journal.pbio.0030363>.
- Carpenter, R.E., Maruska, K.P., Becker, L., Fernald, R.D., 2014. Social opportunity rapidly regulates expression of CRF and CRF receptors in the brain during social ascent of a teleost fish, *Astatotilapia burtoni*. *PLoS One* 9 (5), e96632. <http://dx.doi.org/10.1371/journal.pone.0096632>.
- Cavigelli, S.A., Pereira, M.E., 2000. Mating season aggression and fecal testosterone levels in male ring-tailed lemurs (*Lemur catta*). *Horm. Behav.* 37 (3):246–255. <http://dx.doi.org/10.1006/hbeh.2000.1585>.
- Chase, I.D., 1982. Dynamics of hierarchy formation: the sequential development of dominance relationships. *Behaviour* 80 (3), 218–239.
- Chase, I.D., Seitz, K., 2011. Self-structuring properties of dominance hierarchies: a new perspective. *Adv. Genet.* 75, 51.
- Core Team, R., 2016. R: A Language and Environment for Statistical Computing (Version 3.2.3). R Foundation for Statistical Computing, Vienna, Austria (Retrieved from <https://www.R-project.org/>).
- Curley, J.P., 2016a. compete: organizing and analyzing social dominance hierarchy data. (Version 0.1). Retrieved from: <https://cran.r-project.org/web/packages/compete/index.html>.
- Curley, J.P., 2016b. Temporal pairwise-correlation analysis provides empirical support for attention hierarchies in mice. *Biol. Lett.* 12 (5):20160192. <http://dx.doi.org/10.1098/rsbl.2016.0192>.
- De Vries, H., 1995. An improved test of linearity in dominance hierarchies containing unknown or tied relationships. *Anim. Behav.* 50 (5), 1375–1389.
- Desjardins, J.K., Hofmann, H.A., Fernald, R.D., 2012. Social context influences aggressive and courtship behavior in a cichlid fish. *PLoS One* 7 (7), e32781. <http://dx.doi.org/10.1371/journal.pone.0032781>.
- Ely, D.L., Henry, J.P., 1978. Neuroendocrine response patterns in dominant and subordinate mice. *Horm. Behav.* 10 (2), 156–169.
- Faulkes, C.G., Abbott, D.H., Jarvis, J.U.M., Sherriff, F.E., 1990. LH responses of female naked mole-rats, *Heterocephalus glaber*, to single and multiple doses of exogenous GnRH. *J. Reprod. Fertil.* 89 (1):317–323. <http://dx.doi.org/10.1530/jrf.0.0890317>.
- Fernald, R.D., 2014. Cognitive skills needed for social hierarchies. *Cold Spring Harb. Symp. Quant. Biol.* 79:229–236. <http://dx.doi.org/10.1101/sqb.2014.79.024752>.
- Franz, M., McLean, E., Tung, J., Altmann, J., Alberts, S.C., 2015. Self-organizing dominance hierarchies in a wild primate population. *Proc. R. Soc. B* 282 (1814):20151512. <http://dx.doi.org/10.1098/rspb.2015.1512>.
- Freniere, P.L., Charlesworth, W.R., 1983. Dominance, attention, and affiliation in a preschool group: a nine-month longitudinal study. *Ethol. Sociobiol.* 4 (2):55–67. [http://dx.doi.org/10.1016/0162-3095\(83\)90030-4](http://dx.doi.org/10.1016/0162-3095(83)90030-4).
- Gesquiere, L.R., Learn, N.H., Simao, M.C.M., Onyango, P.O., Alberts, S.C., Altmann, J., 2011. Life at the top: rank and stress in wild male baboons. *Science* 333 (6040):357–360. <http://dx.doi.org/10.1126/science.1207120>.
- Glickman, M.E., 1999. Parameter estimation in large dynamic paired comparison experiments. *J. R. Stat. Soc. Ser. C: Appl. Stat.* 48 (3), 377–394.
- Gore, A.C., Wersinger, S.R., Rissman, E.F., 2000. Effects of female pheromones on gonadotropin-releasing hormone gene expression and luteinizing hormone release in male wild-type and oestrogen receptor- α knockout mice. *J. Neuroendocrinol.* 12 (12): 1200–1204. <http://dx.doi.org/10.1046/j.1365-2826.2000.00578.x>.
- Greenberg, N., Crews, D., 1990. Endocrine and behavioral responses to aggression and social dominance in the green anole lizard, *Anolis carolinensis*. *Gen. Comp. Endocrinol.* 77 (2):246–255. [http://dx.doi.org/10.1016/0016-6480\(90\)90309-A](http://dx.doi.org/10.1016/0016-6480(90)90309-A).
- Grosenick, L., Clement, T.S., Fernald, R.D., 2007. Fish can infer social rank by observation alone. *Nature* 445 (7126), 429–432.
- Haemisch, A., Voss, T., Gärtner, K., 1994. Effects of environmental enrichment on aggressive behavior, dominance hierarchies, and endocrine states in male DBA/2J mice. *Physiol. Behav.* 56 (5), 1041–1048.
- Hiadlovská, Z., Mikula, O., Macholán, M., Hamplová, P., Vošlajerová Bímová, B., Daniszová, K., 2015. Shaking the myth: body mass, aggression, steroid hormones, and social dominance in wild house mouse. *Gen. Comp. Endocrinol.* <http://dx.doi.org/10.1016/j.ygcen.2015.09.033>.
- Higham, J.P., Heistermann, M., Maestriperi, D., 2012. The endocrinology of male rhesus macaque social and reproductive status: a test of the challenge and social stress hypotheses. *Behav. Ecol. Sociobiol.* 67 (1):19–30. <http://dx.doi.org/10.1007/s00265-012-1420-6>.
- Hofmann, H.A., Beery, A.K., Blumstein, D.T., Couzin, I.D., Earley, R.L., Hayes, L.D., ... Rubenstein, D.R., 2014. An evolutionary framework for studying mechanisms of social behavior. *Trends Ecol. Evol.* 29 (10):581–589. <http://dx.doi.org/10.1016/j.tree.2014.07.008>.
- Issa, F.A., Adamson, D.J., Edwards, D.H., 1999. Dominance hierarchy formation in juvenile crayfish *Procambarus clarkii*. *J. Exp. Biol.* 202 (24), 3497–3506.
- Jennings, K.J., Chang, J., Cho, H., Piekarski, D.J., Russo, K.A., Kriegsfeld, L.J., 2016. Aggressive interactions are associated with reductions in RFamide-related peptide, but not kisspeptin, neuronal activation in mice. *Horm. Behav.* 78:127–134. <http://dx.doi.org/10.1016/j.yhbeh.2015.10.021>.
- Koyama, S., Kamimura, S., 1998. Lowered sperm motility in subordinate social status of mice. *Physiol. Behav.* 65 (4–5):665–669. [http://dx.doi.org/10.1016/S0031-9384\(98\)00205-4](http://dx.doi.org/10.1016/S0031-9384(98)00205-4).
- Kozorovitskiy, Y., Gould, E., 2004. Dominance hierarchy influences adult neurogenesis in the dentate gyrus. *J. Neurosci.* 24 (30):6755–6759. <http://dx.doi.org/10.1523/JNEUROSCI.0345-04.2004>.
- Kriegsfeld, L.J., Ubuka, T., Bentley, G.E., Tsutsui, K., 2015. Seasonal control of gonadotropin-inhibitory hormone (GnIH) in birds and mammals. *Front. Neuroendocrinol.* 37: 65–75. <http://dx.doi.org/10.1016/j.yfrne.2014.12.001>.
- Kucharski, R., Maleszka, J., Foret, S., Maleszka, R., 2008. Nutritional control of reproductive status in honeybees via DNA methylation. *Science* 319 (5871):1827–1830. <http://dx.doi.org/10.1126/science.1153069>.
- Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2015. lmerTest: tests in linear mixed effects models (version 2.0–29). Retrieved from: <https://cran.r-project.org/web/packages/lmerTest/index.html>.
- Lee, V.H.Y., Lee, L.T.O., Chow, B.K.C., 2008. Gonadotropin-releasing hormone: regulation of the GnRH gene. *FEBS J.* 275 (22):5458–5478. <http://dx.doi.org/10.1111/j.1742-4658.2008.06676.x>.
- Liening, S.H., Mehta, P.H., Josephs, R.A., 2012. Competition A2 – Ramachandran, V.S. Encyclopedia of Human Behavior, second ed. Academic Press, San Diego:pp. 556–562 (Retrieved from <http://www.sciencedirect.com/science/article/pii/B978012375006001002>).
- Mantei, K.E., Ramakrishnan, S., Sharp, P.J., Buntin, J.D., 2008. Courtship interactions stimulate rapid changes in GnRH synthesis in male ring doves. *Horm. Behav.* 54 (5): 669–675. <http://dx.doi.org/10.1016/j.yhbeh.2008.07.005>.
- Maruska, K.P., Fernald, R.D., 2010. Behavioral and physiological plasticity: rapid changes during social ascent in an African cichlid fish. *Horm. Behav.* 58 (2):230–240. <http://dx.doi.org/10.1016/j.yhbeh.2010.03.011>.
- Maruska, K.P., Fernald, R.D., 2011. Social regulation of gene expression in the hypothalamic-pituitary-gonadal axis. *Physiology* 26 (6):412–423. <http://dx.doi.org/10.1152/physiol.00032.2011>.
- Maruska, K.P., Fernald, R.D., 2013. Social regulation of male reproductive plasticity in an African cichlid fish. *Integr. Comp. Biol.* <http://dx.doi.org/10.1093/icb/ict017>.
- Maruska, K.P., Levavi-Sivan, B., Biran, J., Fernald, R.D., 2011. Plasticity of the reproductive axis caused by social status change in an African cichlid fish: I. Pituitary gonadotropins. *Endocrinology* 152 (1):281–290. <http://dx.doi.org/10.1210/en.2010-0875>.
- Maruska, K.P., Zhang, A., Neboori, A., Fernald, R.D., 2013. Social opportunity causes rapid transcriptional changes in the social behaviour network of the brain in an African cichlid fish. *J. Neuroendocrinol.* 25 (2):145–157. <http://dx.doi.org/10.1111/j.1365-2826.2012.02382.x>.
- McCloughlin, J.W., Jawor, J.M., Ketterson, E.D., Adkins-Regan, A.E.E., Whitlock, E.M.C., 2007. Natural variation in a testosterone-mediated trade-off between mating effort and parental effort. *Am. Nat.* 170 (6):864–875. <http://dx.doi.org/10.1086/522838>.
- Mckinney, T.D., Desjardins, C., 1973. Internale stimuli and testicular function in adult and immature house mice. *Biol. Reprod.* 9 (4), 370–378.
- Mendonça-Furtado, O., Edaes, M., Palme, R., Rodrigues, A., Siqueira, J., Izar, P., 2014. Does hierarchy stability influence testosterone and cortisol levels of bearded capuchin monkeys (*Sapajus libidinosus*) adult males? A comparison between two wild groups. *Behav. Process.* 109, Part A:79–88. <http://dx.doi.org/10.1016/j.beproc.2014.09.010>.
- Muller, M.N., Wrangham, R.W., 2004. Dominance, aggression and testosterone in wild chimpanzees: a test of the “challenge hypothesis.”. *Anim. Behav.* 67 (1):113–123. <http://dx.doi.org/10.1016/j.anbehav.2003.03.013>.
- Nakano, S., 1995. Individual differences in resource use, growth and emigration under the influence of a dominance hierarchy in fluvial red-spotted masu salmon in a natural habitat. *J. Anim. Ecol.* 64 (1):75–84. <http://dx.doi.org/10.2307/5828>.
- Oliveira, R.F., 2009. Social behavior in context: hormonal modulation of behavioral plasticity and social competence. *Integr. Comp. Biol.* <http://dx.doi.org/10.1093/icb/0055>.
- Oliveira, R.F., Almada, V.C., 1996. On the (in)stability of dominance hierarchies in the cichlid fish *Oreochromis mossambicus*. *Aggress. Behav.* 22 (1):37–45. [http://dx.doi.org/10.1002/\(SICI\)1098-2337\(1996\)22:1<37::AID-AB4-3.0.CO;2-R](http://dx.doi.org/10.1002/(SICI)1098-2337(1996)22:1<37::AID-AB4-3.0.CO;2-R).

- Oliveira, R.F., Lopes, M., Carneiro, L.A., Canário, A.V.M., 2001. Watching fights raises fish hormone levels. *Nature* 409 (6819):475. <http://dx.doi.org/10.1038/35054128>.
- Oyegbile, T.O., Marler, C.A., 2005. Winning fights elevates testosterone levels in California mice and enhances future ability to win fights. *Horm. Behav.* 48 (3), 259–267.
- Paxinos, G., Franklin, K.B.J., 2004. *The Mouse Brain in Stereotaxic Coordinates*. Gulf Professional Publishing.
- Rosvold, H.E., Mirsky, A.F., Pribram, K.H., 1954. Influence of amygdectomy on social behavior in monkeys. *J. Comp. Physiol. Psychol.* 47 (3), 173–178.
- Sapolsky, R.M., 1983. Endocrine aspects of social instability in the olive baboon (*Papio anubis*). *Am. J. Primatol.* 5 (4):365–379. <http://dx.doi.org/10.1002/ajp.1350050406>.
- Sapolsky, R.M., 1993. The physiology of dominance in stable versus unstable social hierarchies. In: Mason, W.A., Mendoza, S.P. (Eds.), *Primate Social Conflict*. State University of New York Press, Albany, NY, US, pp. 171–204.
- Sapolsky, R.M., 2005. The influence of social hierarchy on primate health. *Science* 308 (5722):648–652. <http://dx.doi.org/10.1126/science.1106477>.
- Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative C(T) method. *Nat. Protoc.* 3 (6), 1101–1108.
- Selmanoff, M.K., Goldman, B.D., Ginsburg, B.E., 1977. Serum testosterone, agonistic behavior, and dominance in inbred strains of mice. *Horm. Behav.* 8 (1):107–119. [http://dx.doi.org/10.1016/0018-506X\(77\)90026-5](http://dx.doi.org/10.1016/0018-506X(77)90026-5).
- Skaug, H., Fournier, D., Bolker, B., Magnusson, A., Nielsen, A., 2015. *Generalized Linear Mixed Models Using "AD Model Builder" (Version 0.8.3.2)*.
- So, N., Franks, B., Lim, S., Curley, J.P., 2015. A social network approach reveals associations between mouse social dominance and brain gene expression. *PLoS One* 10 (7), e0134509. <http://dx.doi.org/10.1371/journal.pone.0134509>.
- Stephenson, A., Sonas, J., 2012. PlayerRatings: dynamic updating methods for player ratings estimation (version 1.0-0). Retrieved from. <http://CRAN.R-project.org/package=PlayerRatings>.
- Stevenson, T., Hahn, T., MacDougall-Shackleton, S., Ball, G., 2012. Gonadotropin-releasing hormone plasticity: a comparative perspective. *Front. Neuroendocrinol.* 33 (3): 287–300. <http://dx.doi.org/10.1016/j.yfrne.2012.09.001>.
- Taborsky, B., Oliveira, R.F., 2012. Social competence: an evolutionary approach. *Trends Ecol. Evol.* 27 (12):679–688. <http://dx.doi.org/10.1016/j.tree.2012.09.003>.
- Wang, F., Zhu, J., Zhu, H., Zhang, Q., Lin, Z., Hu, H., 2011. Bidirectional control of social hierarchy by synaptic efficacy in medial prefrontal cortex. *Science* 334 (6056):693–697. <http://dx.doi.org/10.1126/science.1209951>.
- Williamson, C.M., Lee, W., Curley, J.P., 2016. Temporal dynamics of social hierarchy formation and maintenance in male mice. *Anim. Behav.* 115, 259–272.
- Wingfield, J.C., Hegner, R.E., Dufty, A.M., Ball, G.F., 1990. The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am. Nat.* 136 (6), 829–846.
- Zielinski, W.J., Vandenbergh, J.G., 1993. Testosterone and competitive ability in male house mice, *Mus musculus*: laboratory and field studies. *Anim. Behav.* 45, 873–891.