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Sex-Specific Mechanism of Social Hierarchy in Mice

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The establishment of social hierarchies is a naturally occurring, evolutionarily conserved phenomenon with a well-established impact on fitness and health. Investigations of complex social group dynamics may offer novel opportunities for translational studies of autism spectrum disorder. Here we describe a robust behavioral paradigm using an automated version of the tube test. Isogenic groups of male and female mice establish linear social hierarchies that remain highly stable for at least 14 days, the longest interval tested. Remarkably, however, their social strategy is sex-specific: females primarily utilize intrinsic attributes, whereas males are strongly influenced by prior social experience. Using both genetic and pharmacological manipulations, we identify testosterone as a critical sex-specific factor for determining which social strategy is used. Males inheriting a null mutation of the sex-determining region Y (*Sry*) gene used a similar social cognitive strategy as females. In contrast, females with transgenic expression of *Sry* utilized a typically male social strategy. Analogously, castration of males and testosterone supplementation of females yielded similar outcomes, with a reversal of their social cognitive strategy. Together, our results demonstrate a sex-specific mechanism underlying social hierarchy, in which both males and females retain the functional capacity to adapt their social strategy. More generally, we expect the automated tube test to provide an important complementary approach for both fundamental and translational studies of social behavior.
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INTRODUCTION

The establishment and maintenance of social norms are among the most fundamental and complex aspects of group dynamics. In particular, the formation of social hierarchies is a naturally occurring and evolutionarily conserved phenomenon, with profound influences on health and disease (Sapolsky, 2005; Barnett, 1955; Zink *et al*, 2008; Nagy *et al*, 2010; Hausfater *et al*, 1982). Aberrant social functioning is a frequent symptom of many neurologic and psychiatric disorders, including autism spectrum disorders (ASDs). However, the biology of social hierarchies remains largely unknown, particularly for mammalian species, owing to the complexity of the outcome measure and the difficulty in standardization of genetic *vs* environmental factors. Accordingly, the development of a robust assay for investigating social hierarchies in a genetically tractable species has the potential to greatly advance our mechanistic understanding.

Lindzey *et al* (1961) reported the development of the Tube Test for assaying social dominance hierarchies in rodents. Over the next 20 years, studies using the Tube Test were predominantly designed to investigate the genetic factors underlying differences in the outcome of matches between distinct inbred mouse strains (Lindzey *et al*, 1961; Messeri *et al*, 1975). Masur & Benedito (1974) performed selective breeding to obtain distinct lines of rats with significantly increased or decreased likelihood of winning tube matches compared with the initial parental strain, thereby confirming the causal relationship between genetic factors and social hierarchies. More recently, an elegant study by Wang *et al* (2011) demonstrated the critical influence of the medial prefrontal cortex on the maintenance of social dominance hierarchies, and provided a strong validation of the Tube Test for investigating group dynamics. Furthermore, and highly relevant for translational mouse modeling studies, the Tube Test has already been utilized in a limited number of studies for phenotyping social behavior in transgenic mouse models of neuropsychiatric disorders (Garfield *et al*, 2011; Spencer *et al*, 2005; Lijam *et al*, 1997; Wallén-Mackenzie et al, 2009).

Studies of ASD have increasingly focused on social behavior, as difficulty in social functioning is the most fundamental symptom of ASD (Lai *et al*, 2014). One of the most well-replicated findings in population-based studies of ASD is the highly skewed male:female (M:F) ratio with a 2–3 times higher prevalence in males (Yeargin-Allsopp *et al*, 2003; Baird *et al*, 2006; Lai *et al*, 2014). Moreover, fundamental differences in social abilities between males and females have also been observed across a wide variety of species (Knight, 2002; Clutton-Brock and Huchard, 2013), including humans (Yamasue *et al*, 2008). Regarding ASD, one of the leading hypotheses of the skewed M:F ratio is the 'Extreme Male Brain' theory proposed by Baron-Cohen *et al* (2005), which posits that the liability for ASD is strongly influenced during fetal development through a pathophysiological dysregulation of testosterone-dependent brain

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masculinization (Knickmeyer and Baron-Cohen, 2006; Baron-Cohen, 2002). Multiple studies have provided increasing support for this hypothesis, including neuropsychological testing and endocrinological studies (Whitehouse *et al*, 2010; Lai *et al*, 2013; Baron-Cohen *et al*, 2014). However, a direct causal relationship has been difficult to demonstrate using human cohort studies. Therefore, we reasoned that the Tube Test might provide a unique opportunity to investigate sex-specific mechanisms of social behavior, in particular, given the ability to utilize mice with a homogenous genetic background and thereby imposing a significant constraint on the sources of emergent differences in social behavior among individuals within each group. Using an automated version of the Tube Test to minimize experimenter variability, we found that male and female mice establish highly linear and stable social hierarchies. Interestingly, however, their social strategy is sex-specific: females primarily utilize intrinsic attributes, whereas males are strongly influenced by prior social experience. Further and highly consistent with the extreme male brain theory, we identify testosterone as a critical sex-specific factor for determining which social strategy is used. Thus, our results demonstrate a sex-specific mechanism underlying social hierarchy, in which both males and females retain the functional capacity to adapt their social strategy, governing social relationships.

MATERIALS AND METHODS

Experimental Animals

Adult (8–12 weeks) male and female mice (CB6F1/OlaHsd; Harlan Laboratories, The Netherlands) were used in this study. Mice from the CB6F1/OlaHsd genetic background are the F1 offspring derived from the mating of a C57BL/6JOlaHsd male and BALB/cOlaHsd female. *Sry* mutant mice were obtained from The Jackson Laboratory (stock #010905) and backcrossed into C57BL/6JOlaHsd for at least 10 generations. Experiments investigating the influence of the *Sry* gene were performed using mice derived from the mating of a C57BL/6JOlaHsd male (XY–Tg(*Sry*)) and BALB/cOlaHsd wild-type female. Mice were housed with littermates in groups of four per homecage, and maintained on a 12 h light/dark cycle with food and water available *ad libitum*. All experiments were approved by the Dutch Ethical Committee for animal research.

Apparatus

The apparatus is an automated version of the Lindzey Tube (Lindzey, *et al*, 1961) (Benedictus Systems, Rotterdam, the Netherlands, http://www.tubeassistant.nl). It consists of a transparent fibreglass tube connected at each end to a transparent fibreglass box (12 × 8 cm), which can be opened and closed with a manual lid (Figure 1a). An air valve is located at the back wall of each box at 1 cm above the base, opposite to the location of the entrance to the tube. The 50-cm-long tube has a diameter of 2.5 cm, which is just sufficient to permit an adult mouse to pass through readily, but not sufficient to permit two mice to pass one another inside the tube. Transparent fibreglass doors are located at the entrance to each box, leaving a 4-mm space for the mouse's tail when the door closes from behind. An opaque fibreglass door is located midway through the tube. The position of each door and activation of each air valve are fully automated and independently controlled in real-time by 40 Hz infrared photo detectors for automated tracking.

Training Trials

In all experiments, mice receive a 5-day training procedure. On Day 1, mice are given two habituation trials in which they are permitted to freely explore the tube until they proceed from the starting box through the tube into the goal box, up to a maximum of 180 s per trial. On Days 2–5, mice are given six training trials per day, with a maximum of 30 s per trial. At the start of each training trial, all doors are initially closed, with the lid to both boxes open. A single mouse is placed in a randomly assigned starting box, after which both overhead lids are closed. Following a random variable 2–5 s delay, the doors at each end of the tube are positioned open, whereas the opaque center door remains closed. The mouse is now freely able to leave the box. However, when a mouse is located within the starting box for longer than 5 seconds, the air valve is activated. Upon leaving the starting box and entering the tube, the air valve is immediately terminated. The opaque door at the center of the tube is automatically opened after a random variable delay of 1–3 s, initiated when the mouse reaches within 4 cm of the center door. After the center door is open, the mouse is able to proceed forward through the tube towards the goal box. Once the mouse fully enters the goal box, the entrance door is closed and the mouse is returned to their homecage. Following every trial, the apparatus is cleaned with a 70% alcohol solution.

Social Hierarchy Tournament

Tournaments are established in an all-*vs*-all design, for which every possible pairing of mice is tested on each tournament day. Prior to a tournament, mice receive two training trials. With a tournament among eight mice, there are 28 unique paired match combinations, for which each mouse participates in seven matches. Importantly, the ordering of the matches is determined by randomly sampling without replacement in blocks of four matches, to ensure that all matches are between mice with the same number of prior matches. As well, the starting boxes are assigned randomly for each match.

At the start of each match, all doors are initially closed, with the lid to both boxes open. One mouse is placed into each of the boxes, on either end of the tube. Matches initially proceed in an identical manner as the training trials. However, the opaque center door is automatically opened only when both mice are positioned within 4 cm of the door, ensuring similar initial positions. The match ends when one mouse has forced the other to retreat with all four paws into the starting box from which it commenced. The mouse that successfully reaches its goal box is designated as the winner for that trial, whereas the mouse that retreats into its starting box is designated the loser for that trial. Following every trial, the apparatus is cleaned with a 70% alcohol solution.

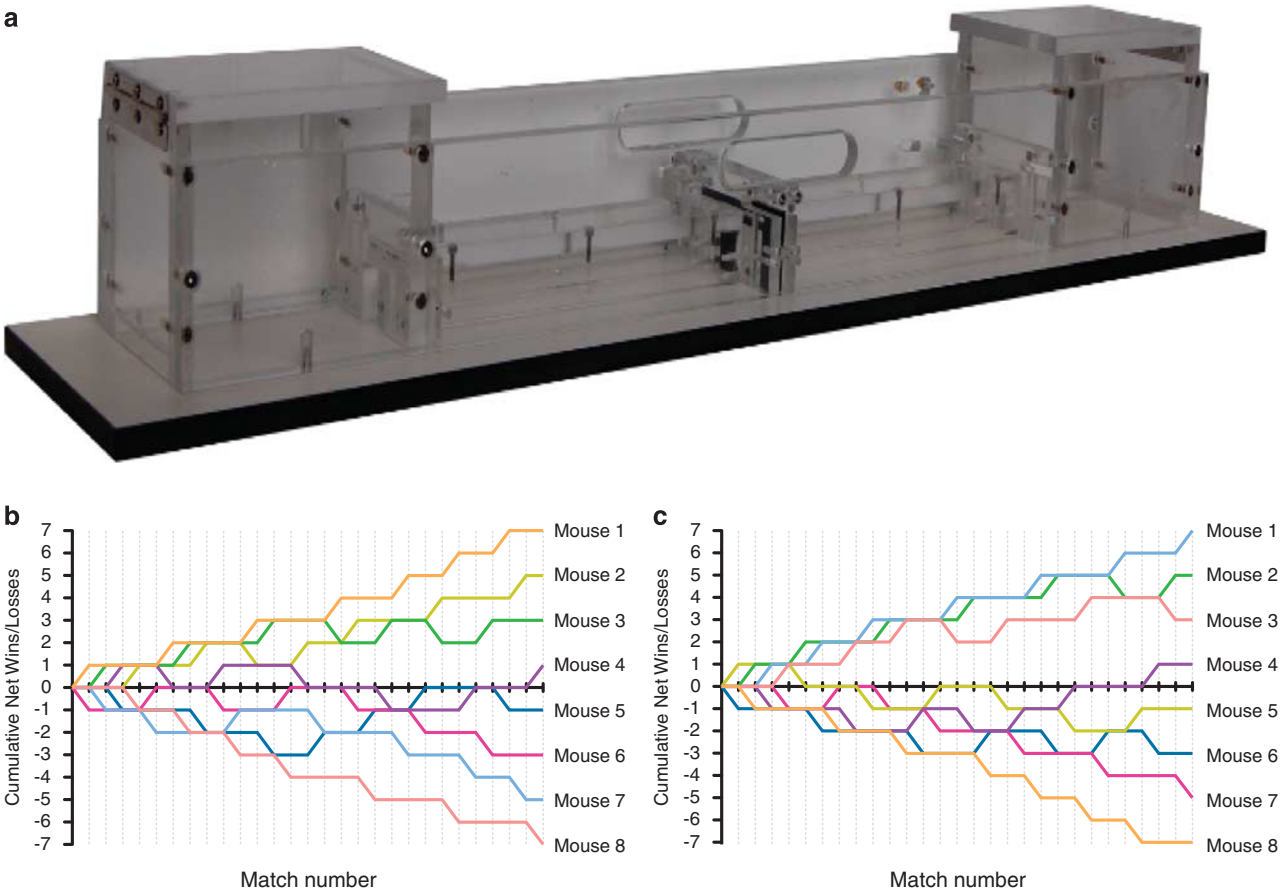

Figure 1 Automated Tube Test. (a) Image of the tube apparatus. (b and c) Representative plot of cumulative wins/losses over the course of a full round-robin tournament for groups of eight male (b) or female (c) mice with seven matches per mouse and 28 matches in total. Match wins are registered as +1, and losses as −1.

'Intrinsic Attributes' Tournament

Transitivity is operationally defined within any group of three mice if, and only if, the following match outcomes occur in succession: mouse A_i wins against mouse B_j ($A_i > B_j$), mouse B_j wins against mouse C_k ($B_j > C_k$), and mouse A_i wins against mouse C_k ($A_i > C_k$), where A_i, B_j, and C_k represent the specific mice in each cage of four mice. In order to quantify the level of transitivity, tournaments were established among groups of 16 mice, housed as four littermates per homecage. Each homecage group was derived from a different litter (age-matched within 7 days), to ensure that mice from different cages had no prior experience with each other.

Matches were performed identically as described above. However, matches were only performed between mice of different cages. This tournament schedule results in 128 possible triads across Days 1–3. Only after the full tournament was completed, matches were evaluated for transitivity. Transitivity assessments were considered valid only in cases for which the successive match outcomes among a triad resulted in $A_i > B_j$ (Day 1) and $B_j > C_k$ (Day 2). In contrast, successive match outcomes of the form $A_i > B_j$ (Day 1) and $C_k > B_j$ (Day 2) were not evaluated, as the Day 3 outcome of the match between A_i *vs* C_k is not informative regarding transitivity. For the overall tournament, the level of transitivity was reported as the percentage of Day 3 match outcomes that were successfully predicted by the matches on Days 1 and 2.

'Prior Experience' Tournament

The 'prior experience' tournament was designed to assess whether prior match outcomes were sufficient to establish a stable social hierarchy. The tournament was performed using an all-*vs*-all design among groups of eight mice, for a total of 28 matches per tournament day, performed on 2 consecutive days. Matches were performed similarly to those described above, except that the outcomes of the matches on Day 1 were determined randomly. Specifically, during every match on Day 1, one of the goal box doors was randomly chosen to be closed coincident with the opening of the center door, leaving the remaining goal box door open. Therefore, for every match on Day 1, there was only one possible randomly determined outcome. In contrast, on Day 2 of the tournament, matches were performed as in the previous experiments, for which both goal box doors remained open throughout the match. The tournament outcome was reported as the % of matches on Day 2 that were similar or different than the equivalent match on Day 1.

Testosterone Manipulation

Male CB6F1/OlaHsd mice were surgically castrated at 8 weeks of age, as previously described (Nagy *et al*, 2003). Female CB6F1/OlaHsd mice received subcutaneous implants of testosterone pellets (A-151: 5 mg, Innovative Research of America) at 8 weeks of age. Social behavior was tested 2 weeks following castration or pellet implantation. Serum was obtained on the day following the final behavioral test, and stored at $-20\,^{\circ}C$. Testosterone levels were measured following the protocol of the Testosterone EIA-5179 ELISA assay (DRG Diagnostics): castrated males (0.56 ± 0.03 ng/ml), females with testosterone pellet implants (6.58 ± 0.61 ng/ml). Calibration was established using the provided standards and confirmed with intact control males and females.

Statistical Analysis

Tournament outcomes were analyzed using the binomial distribution as the outcome of the matches fall into one of two mutually exclusive categories: winning or losing. The binomial test was performed for the observed outcome against the null hypothesis that for any given match both mice were equally likely to win ($P = 0.50$). Comparisons between continuous variables were performed using ANOVA or Pearson's *r*. Proportions of transitive relationships were compared using the Pearson χ^2. Hypothesis testing was based on a two-tailed *P*-value. Statistical significance was considered at $\alpha = 0.05$. Data were analyzed using SPSS version 18.0 (Chicago, IL).

RESULTS

We investigated social hierarchies in mice using a modified version of the tube test (Lindzey *et al*, 1961; Wang *et al*, 2011) (Figure 1a). Initially, mice were habituated to the apparatus and trained individually to cross through the tube (Supplementary Fig. S1A-B; Supplementary Movie S1). Following training, we used a round-robin tournament design among groups of eight mice in order to establish the social hierarchy. For each tournament there were 28 matches, corresponding to every unique pairing within a group of eight mice. Tournament matches began with one mouse being placed in each starting box, and concluded when both mice were located in a single box (Supplementary Movie S2). The mouse advancing forward through the entire tube was designated as the winner of the match.

We utilized mice on a CB6F1 genetic background, derived from the mating of a C57BL/6JOlaHsd male and BALB/cOlaHsd female. This breeding scheme yields isogenic mice, while substantially reducing the frequency of homozygosity of pure inbred lines and controlling for parent-of-origin allelic expression (Gregg *et al*, 2010; Garfield *et al*, 2011). Our initial experiment was designed to characterize the social hierarchy distribution and stability of tournament outcomes (Figure 1b and c). Remarkably, despite an isogenic genetic background, both male and female mice formed highly linear social hierarchies (Figure 2a and b). Importantly, however, no biases were observed in the outcome of matches related to training performance, starting box, homecage, or weight (Supplementary Fig. S1C-F).

In order to examine the stability of the social hierarchy over time, we conducted tournaments on 5 consecutive days. Overall, tournament rankings were highly stable in both males and females (Figure 2c–h). At the group level, the distribution of changes in rank across each successive tournament day confirmed a significant overrepresentation of stable rankings (Figure 2c and d). In particular, the outcome of any given match in the first tournament was highly predictive of the outcome for the same match in subsequent tournaments (Figure 2e and f). Furthermore, even following a 14-day rest interval, match outcomes remained highly stable, confirming the long-term stability of the observed social hierarchies (Figure 2g and h).

Identification of Sex-Specific Social Strategies

Two distinct models have been proposed to account for the widespread observation of linear hierarchies across the animal kingdom (Beacham, 2003; Chase *et al*, 2002; Hsu *et al*, 2006). The first hypothesizes that stable linear hierarchies can arise based upon stable differences in the intrinsic attributes of individuals within a social group (Wilson, 1975). In contrast, the second model predicts that social hierarchies are organized based upon the outcomes of prior competitive encounters between individuals in the group (Rutte *et al*, 2006; Seebacher and Wilson, 2007). However, distinguishing between these models has remained difficult, in part, owing to the genetic heterogeneity within the groups examined in previous studies (Fowler *et al*, 2009; Turkheimer, 2000). Therefore, given our findings that isogenic mice establish linear and stable hierarchies, we developed novel tournament designs to independently assess these models.

In the first set of experiments, we sought to directly examine the influence of intrinsic attributes on the social hierarchy, independent of prior experience. In particular, we took advantage of the well-described transitivity relationship (if $A > B$ and $B > C$, then $A > C$) that occurs in linear hierarchies (Kendal, 1962; Appleby, 1983). We reasoned that for groups of animals in which stable intrinsic attributes are the primary mechanism responsible for determining the social hierarchy, then the outcome of the final match between A and C should be predictable when $A > B$ and $B > C$, even when A and C have no prior experience together.

Figure 2 Stable linear social hierarchies in male and female isogenic mice. (a and b) Histogram of observed tournament rankings (black) compared with the expected binomial proportions (red) demonstrates highly linear hierarchies in male (a; $P < 0.001$) and female (b; $P < 0.001$) mice. (c and d) Distribution of observed tournament rank stability (black) compared with the expected binomial proportions (red) indicates a significant increase in perfectly stable rankings for males (c; $P < 0.001$) and females (d; $P < 0.01$). (e and f) Observed (black) and expected (red) distribution of match outcomes compared with the initial tournament, across five successive tournaments. Both males (e; $P < 0.001$) and females (f; $P < 0.001$) show a substantial shift toward highly stable hierarchies. (g and h) Stability of match outcomes is maintained even after a 14-day period in both males (g) and females (h). Males: $n = 16$ mice; females, $n = 16$ mice. ***$P < 0.001$.

The 'intrinsic attributes' tournament structure is shown in Figure 3a. Specifically, tournaments were conducted among groups of 16 mice, housed in four groups of 4 mice per cage. Importantly, although the entire group of mice was born within a 1-week period, each homecage was derived from a different litter to ensure that the mice in any

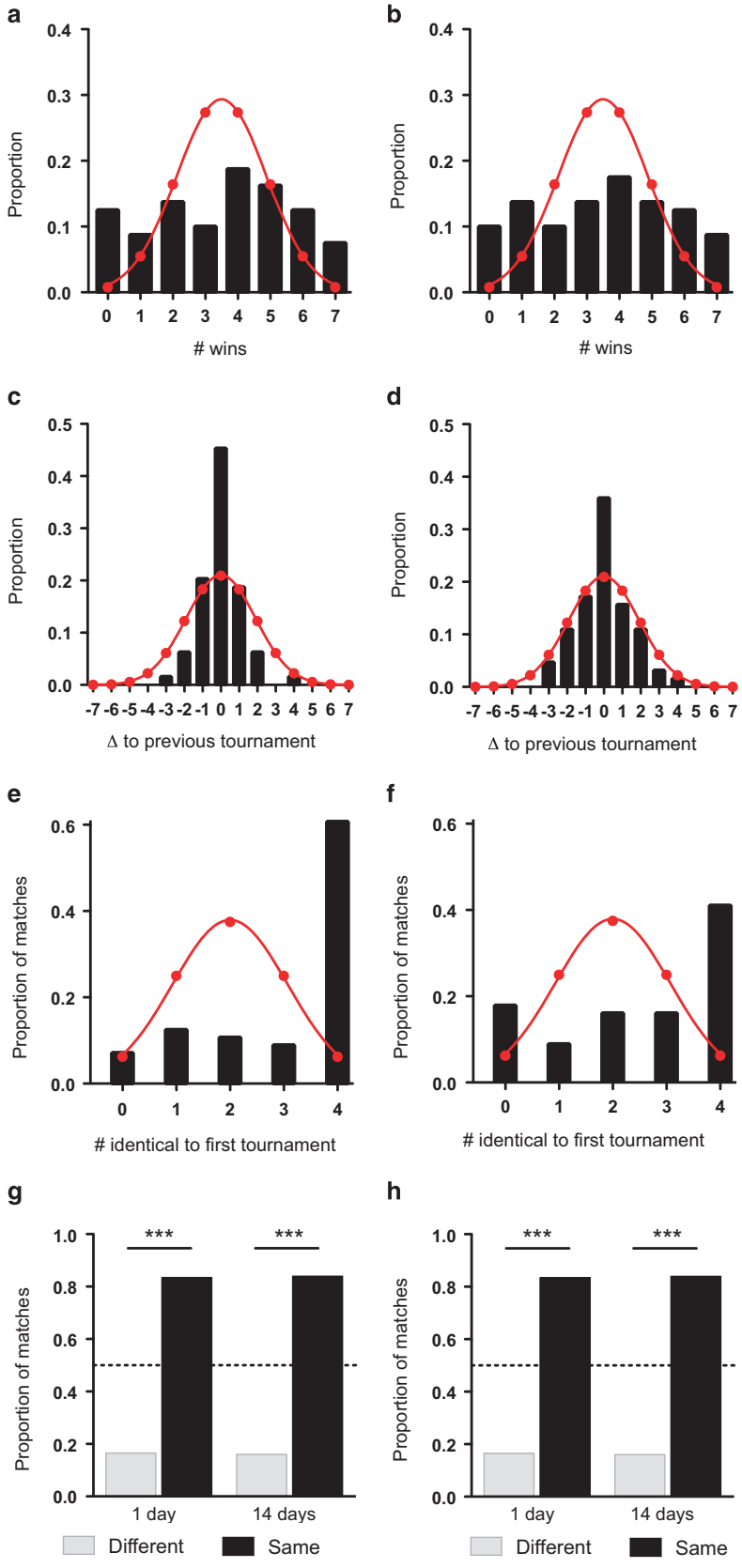

Figure 2 For caption see page 1367.

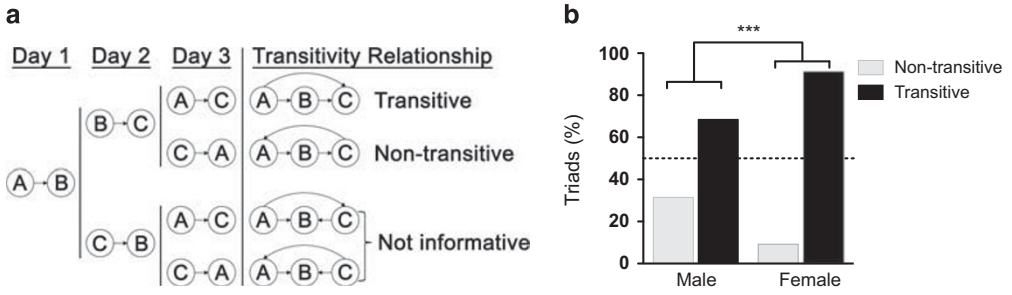

Figure 3 Intrinsic attributes are sufficient for stability in females, but not in males. (a) Diagram of 'intrinsic attributes' tournament. (b) Males demonstrate a significantly lower proportion of transitive relationships compared with females (Pearson χ^2: Sex × transitivity interaction, $F = 17.393$, $P < 0.001$). Males: $n = 32$ mice; females, $n = 32$ mice.

given cage had never previously encountered mice from any other cage. Accordingly, matches were conducted exclusively between mice from different cages, none of whom had ever previously been in direct contact. Remarkably, the outcome of the Day 3 matches were successfully predicted in over 91% of female triads (Figure 3b), consistent with a social strategy by which females utilize intrinsic attributes to maintain stable hierarchies. In contrast, the transitivity relationships for males yielded significantly fewer successful predictions (69%, Figure 3b), consistent with a model in which intrinsic attributes alone are unlikely to explain the highly stable observed hierarchies in males. Therefore, these data suggest a sex-specific model of social hierarchy by which female mice are capable of using intrinsic attributes in the absence of prior experience, whereas males appear to require additional information obtained from prior competitive encounters.

In order to further evaluate this sex-specific model, we again modified the tournament design in an effort to more directly examine the influence of prior experience (Figure 4). In the 'prior experience' tournament, mice were randomly assigned to occupy each position on a linear ranking during the initial tournament, and subsequently evaluated for whether this randomly assigned hierarchy would remain stable. We reasoned that a high stability of randomly determined match outcomes would provide strong support for the 'prior experience' model. In contrast, low stability of a randomly established hierarchy would suggest that prior experience is insufficient, giving support to the 'intrinsic attributes' model.

The 'prior experience' tournament was conducted over 2 days, following the standard initial training period. On the first tournament day, match outcomes were fixed in accordance with the *a priori* random assignments, whereas the second tournament was unbiased to examine whether the initial match outcomes remained stable. Match outcomes during the first tournament were fixed by closing one of the goal box doors after both mice had entered the tube, leaving only a single possible exit. Therefore matches were recorded as either forced or natural, depending upon whether the mice first attempted to leave through the closed door or directly exited through the open side, respectively (Figure 4a and Supplementary Movies S2-S4). Importantly, forced and natural matches occurred with the expected binomial proportion in males and females (Supplementary Fig. S3A and B). Remarkably, the *a priori* random assignment of a linear hierarchy to male mice was highly stable for both forced and natural matches (Figure 4b). In contrast, whereas natural outcomes remained stable in female mice, the outcome of forced matches was at chance levels (Figure 4c). Together, these data identify a sex-specific mechanism underlying social hierarchies in mice, whereby males are strongly influenced by prior experience, whereas females are reliant upon intrinsic attributes.

Testosterone Dynamically Regulates Sex-Specific Social Behavior

Given the uniform genetic background of the mice used in our experiments, we reasoned that the major etiological factor underlying the sex-specific differences in social hierarchies might be located on a sex chromosome, as the autosomes are isogenic between males and females (Goy and McEwen, 1980). Therefore, in an attempt to isolate the sex chromosome complement from gonadal sex, we took advantage of a mouse model in which the sex chromosome complement (XX *vs* XY) and gonadal phenotype (ovaries *vs* testes) segregate independently through mutations in the sex-determining region Y (*Sry*) gene (Arnold, 2009). Using this strategy, we derived CB6F1 mice with either an XY genotype and female gonadal phenotype (XY_{Female}—deletion of *Sry* gene), or XX genotype and male gonadal phenotype (XX_{Male}—autosomal *Sry* transgene). Remarkably, XY_{Female} mice performed similarly to intact XX females with a high stability of natural matches, but chance-level outcomes in forced matches (Figure 5a). In contrast, the performance of XX_{Male} mice resembled that of intact XY males, with high stability in both natural and forced matches (Figure 5b). Therefore, these results strongly implicate gonadal sex as the critical determinant underlying the sex-specific mechanism of social hierarchies in mice.

Sry functions as the testis-determining factor during early development, yet has also been suggested to function within the adult male brain (Dewing *et al*, 2006). Therefore, in order to distinguish between these possibilities, we inversely manipulated testosterone levels in adult male and female mice, by castration and testosterone supplementation, respectively. Remarkably, adult castrated males performed similarly to intact and sham-operated females, with high stability of natural matches but chance-level outcomes resulting from forced matches (Figure 5c, Supplementary Fig. S3D). Conversely, adult females that were given subcutaneous testosterone pellets had similar outcomes as intact and sham-operated males, with stability of both natural and forced matches (Figure 5d, Supplementary Fig.

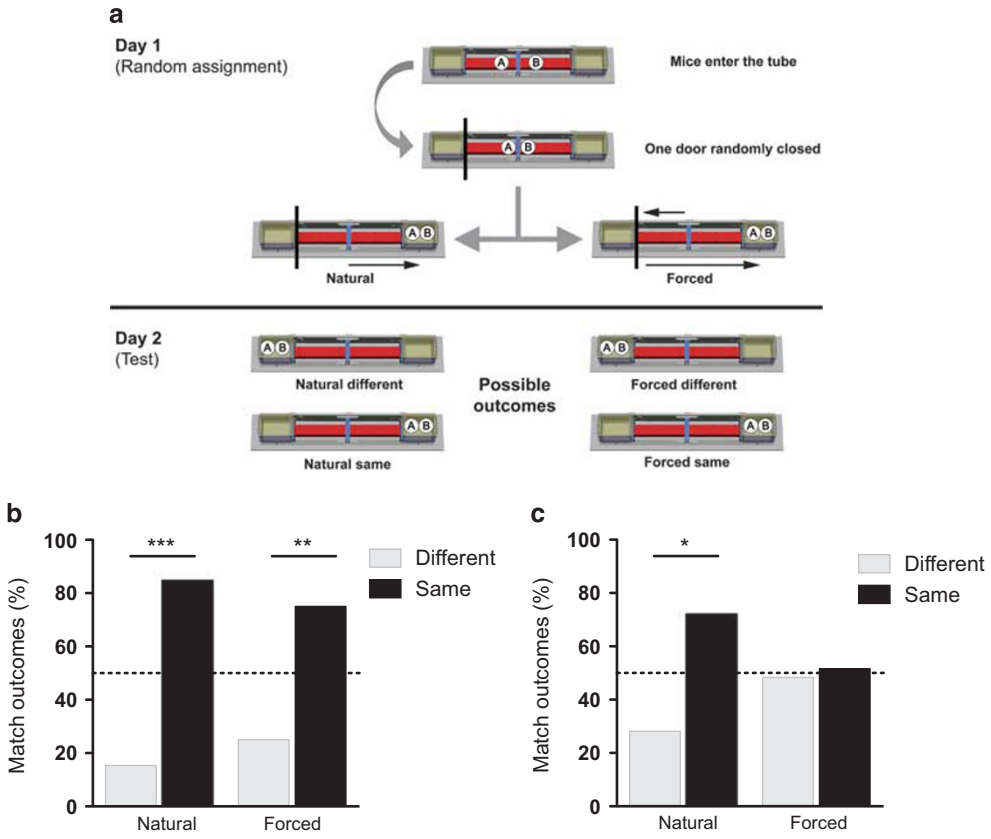

Figure 4 Prior experience is sufficient to establish stable linear hierarchies in males, but not in females. (a) Diagram of 'prior experience' tournament. (b and c) Stability of randomly assigned linear hierarchies. Males (b) show a high stability of both forced and natural matches. In contrast, whereas females (c) maintain a high stability for natural outcomes, the forced matches have inconsistent outcomes. Males: $n = 24$ mice; females, $n = 16$ mice. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

S3C). Importantly, and consistent with previous findings (Wang *et al*, 2011; Klomberg *et al*, 2002), we never observed fighting or overt physical aggression during tournament matches with intact or testosterone-manipulated mice. Furthermore, stable hierarchies were readily established among eight castrated males (Supplementary Fig. S2A), or within a group of four castrated and four normal males (Supplementary Fig. S2B). Moreover, the outcome of matches between normal and castrated males was not significantly different than chance (Supplementary Fig. S2C), thereby excluding testosterone-mediated aggression as a meaningful determinant of match outcomes. Taken together, our findings demonstrate that social cognitive strategies used by mice are dynamically and bidirectionally regulated throughout the lifespan by the sexually dimorphic production of testosterone.

DISCUSSION

The establishment of social hierarchies is a naturally occurring, evolutionarily conserved phenomenon with significant implications on fitness and health (Clutton-Brock, 1988; Ellis, 1995; Sapolsky, 2005). However, causal models for investigating the neurobiology underlying social hierarchies have remained limited, owing to the requirement for a genetically tractable model system, with a robust behavioral paradigm. From a translational perspective, the study of more complex social behaviors in mice offers a potential for unique insight into the evolutionary antecedents of human social functioning. Here, we demonstrate that isogenic mice are fully capable of establishing stable linear hierarchies, and reveal a novel sex-specific mechanism of social hierarchy mediated by testosterone. More generally, the automated tube test provides a robust paradigm for studying fundamental mechanisms of group social dynamics in mice, including transgenic models of ASD (Silverman *et al*, 2010). As an example of the potential advantages of the automated tube test, de Esch *et al* (2014) have demonstrated a highly robust and mGluR5-dependent phenotype in the *Fmr1*$^{-/y}$ mouse model for Fragile X Syndrome.

We identified sex-specific cognitive strategies by which males rely upon prior experience, and females utilize stable intrinsic traits, to maintain the stability of social hierarchies. This finding is highly consistent with the differing social functions for males and females of many animal species, particularly regarding mate selection and the care of offspring (Kimchi *et al*, 2007). Moreover, we identified testosterone as a critical factor underlying the specification of social cognitive strategies using a combination of genetic (*Sry*) and pharmacological manipulations. Notably, these findings are highly consistent with the 'extreme male brain' theory of social functioning and ASD, regarding the critical influence of early fetal testosterone (Baron-Cohen, 2002; Baron-Cohen *et al*, 2014). Our experiments demonstrated that Sry-mediated gonadal differentiation during fetal development was a critical factor in determining the

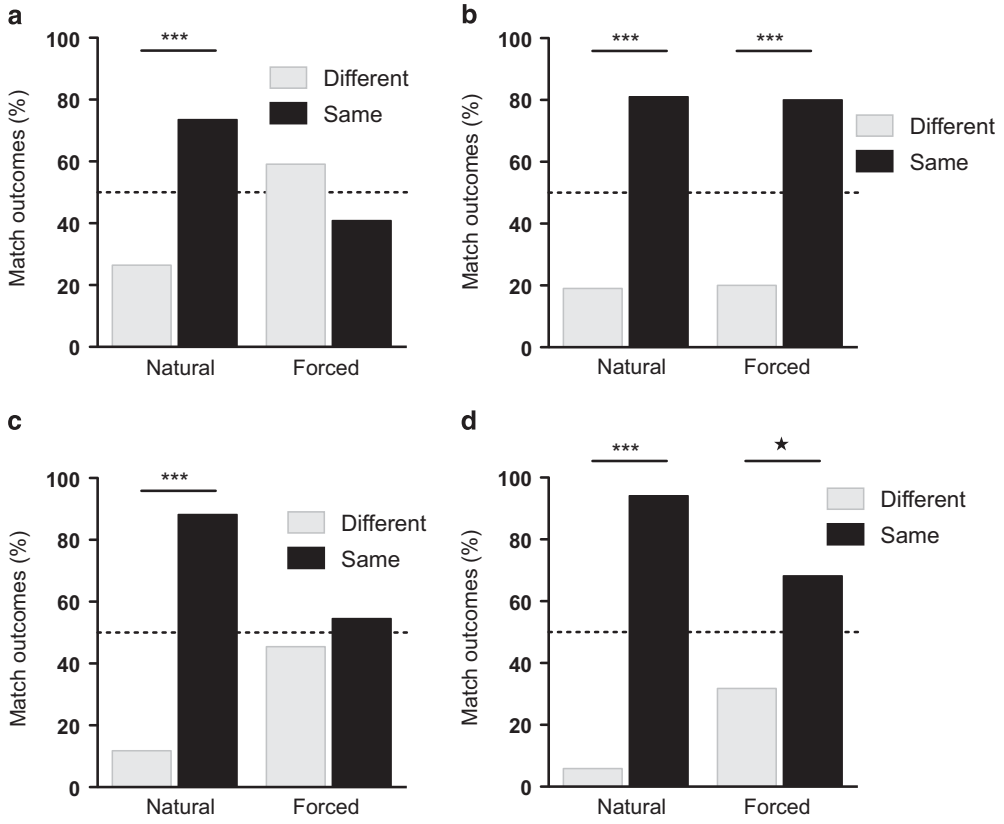

Figure 5 Testosterone is a critical sex-specific factor. (a and b) *Sry* gene regulates the influence of prior experience. XY_{Female} mice (a) perform at chance levels during forced matches, while maintaining the high stability of natural outcomes, similar to intact females. In contrast, XX_{Male} mice (b) exhibit high stability of both natural and forced outcomes, similar to intact males. Males: $n = 16$ mice; females: $n = 16$ mice. (c and d) Testosterone levels dynamically regulate the influence of prior experience. Castrated males (c) show random outcomes following forced matches, while maintaining the stability of natural outcomes. In contrast, females implanted with testosterone pellets (d) resemble intact males through a high stability of both natural and forced outcomes. Males: $n = 8$ mice; females, $n = 16$ mice. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, $^{*}P = 0.052$.

cognitive strategy utilized to maintain social hierarchies in adulthood: XX_{males} behaved like females, whereas $XY_{females}$ behaved like males. Although previous studies have reported sexually dimorphic expression of Sry in human midbrain dopaminergic neurons (Dewing *et al*, 2006; Czech *et al*, 2012), it is important to note that this mechanism cannot account for our finding that administration of testosterone to wild-type females, who lack the *Sry*-containing Y chromosome, was sufficient to induce male-type social cognition. Importantly, however, our findings do not exclude the influence of other gonadal factors, including estradiol, which is produced in the brain by aromatization of testosterone. Furthermore and similar to other investigators, we observed that testosterone manipulations in adulthood were also effective in dynamically regulating the social cognitive strategy in males and females (van Honk *et al*, 2001, van Honk, Schutter, 2007; Bos *et al*, 2010). Future studies will be required to understand the mechanistic distinction and translational relevance of early life *vs* adult manipulations on social functioning.

In conclusion, we have demonstrated a sex-specific and testosterone-dependent mechanism underlying the establishment of social hierarchies in mice, using an automated version of the tube test. Moreover, our findings are highly consistent with the extreme male brain theory of ASD, and therefore could be of significant translational relevance.

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