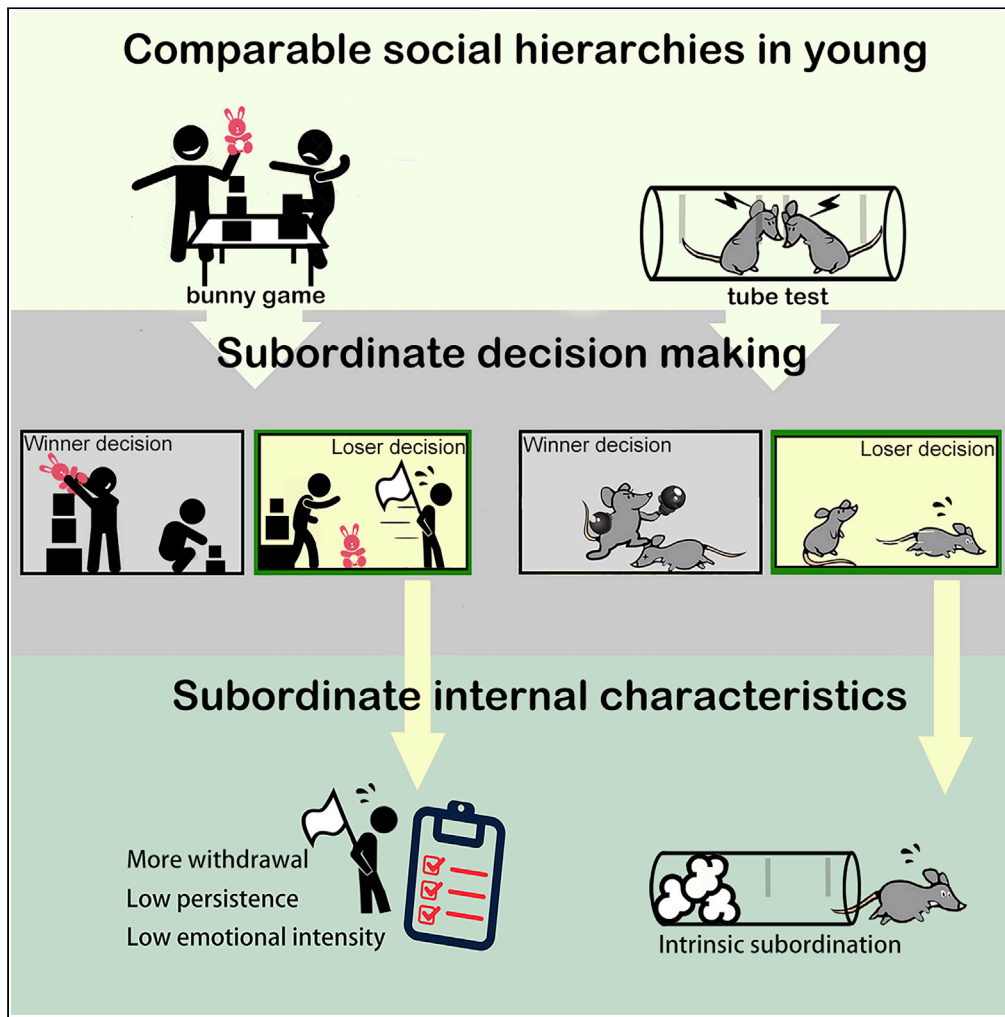


Article

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HIGHLIGHTS

Similar social hierarchy can be established in weanling mice and young children

Social rankings in children are largely influenced by intrinsic characteristics

Conflicts in mouse tube test are not resolved by winner but by loser withdrawal

Subordinate withdrawals are determined by intrinsic status regardless of opponents

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Article

The decisive role of subordination in social hierarchy in weanling mice and young children

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SUMMARY

Social hierarchy plays important roles in maintaining social structures. Despite similarity in concept, frameworks of human hierarchy have seldom been investigated in parallel with other animals. Moreover, the importance of subordination in hierarchical formation has been largely underestimated in previous research. Here we established, compared, and investigated hierarchy in children and weanling mice. Temperament assessments suggested that children who are less persistent, low emotional intensity, and withdrew easily were more likely to be subordinate in competitive scenarios independent of task characteristics and interaction experiences. The tube test further showed that conflicts between mice were not resolved by winner approach but by loser withdrawal, which was mainly determined by intrinsic subordinate status regardless of opponents. Our study presents evolutionary conserved hierarchical relationships in young and a critical role of the intrinsic subordinate characteristics in hierarchical determination. These findings provide a new perspective on social interactions with potential implications for preschool education.

INTRODUCTION

Social hierarchy is a fundamental structure for social interactions in several animal species and has a strong influence on behaviors and physiology of those species. Typically, a hierarchy is established by an agonistic interaction, such as aggression (Wang et al., 2014). Winners of such conflicts eventually dominate their group and have greater access to limited resources, such as food and mates. However, dominance comes at a cost owing to greater stress, physical danger, and metabolic demands (Huntingford and Turner, 1987). In contrast, lower-ranked individuals receive fewer resources but are subjected to less conflict and peril. Either behavioral strategy may benefit the needs of an individual, and, once established, mutual formation of stable dominance hierarchies helps all participants by limiting social conflicts and optimizing group fitness (Wang et al., 2014).

The conceptual framework of social hierarchy has been applied to human studies. Numerous observational studies have shown that dominance hierarchies form early in life and gradually become stable with age (La Freniere and Charlesworth, 1983; Pellegrini et al., 2007; Roseth et al., 2007; Strayer and Trudel, 1984). In the past decades, the stably existing social hierarchies in preschool children have promoted researchers to explore the influences of multiple factors, such as age, sex, intelligence, social skills, parenting style, moral education, and cultural background (Charafeddine et al., 2016; Hawley, 1999, 2002; Hawley and John Geldhof, 2012; Keating and Bai, 1986; McDonald et al., 2013; Neppi and Murray, 1997; Pellegrini et al., 2007; Reifen Targar et al., 2017; Roseth et al., 2011). Children with more aggressive behaviors are usually recognized as higher-ranking status in their group (Hawley, 2007; Roseth et al., 2011; Sluckin and Smith, 1977; Strayer and Strayer, 1976). The adoption and change of resource control strategies of highly dominant children have also been the focus of child development research (Hawley, 1999, 2002; Roseth et al., 2011). Conversely, in recent years, cognitive-oriented studies have indicated that preschool children learn to make judgments of social dominance based on various cues as they gain experience interacting with other children (Brey and Shutts, 2015; Charafeddine et al., 2015; Gülgöz and Gelman, 2017; Lourenco et al., 2016; Over and Carpenter, 2015; Qu et al., 2017). Preschool children even consider social dominance from situation to situation (Charafeddine et al., 2016). This judgment ability suggests that dominance hierarchies in young children are supposed to be unstable or dynamic and would change with time and experience. However, this was not the

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case in past studies based on behavioral observations, raising the question of whether, in addition to direct interactions or observational learning, there is another overlooked fundamental cause impacting the formation of social hierarchies in young children. This question turns us to the influence of innate intrinsic traits in the formation of social hierarchy. An appropriate method that could possibly rule out the complexity of educational or cultural influences and directly address the issue is therefore needed.

Animal models, with a variety of associated basic or translational research assays, have been established to measure social status among these groups. For rodent species, including the mouse and rat, aggression and scent-marking assays have been used to evaluate dominance hierarchy (Wang et al., 2014). Because only adult males perform these dominance behaviors, the social hierarchies of female and young animals have rarely been explored. Compared with methods examining complicated dominance behaviors, the tube test, in which one mouse forces its opponent backward out of a tube, is relatively easy to set up and perform and has been used in recent years largely to study the molecular mechanism(s) and/or neural circuits of social dominance (Larrieu et al., 2017; Lindzey et al., 1961; Saxena et al., 2018; Tada et al., 2016; van den Berg et al., 2015; Wang et al., 2011; Yamaguchi et al., 2017; Zhou et al., 2017). One such study has indicated that social ranks of C57/B6 adult male mice are stable across multiple days, obey a linear (i.e., transitive) relationship and are consistent with the ranks defined by other methods (Wang et al., 2011). Because the only requirement for mice to complete the tube test is the ability to move forward and backward inside the tube, the assay provides a potential opportunity to investigate social ranking in young mice, who do not display dominant behaviors, such as aggression or scent-marking.

While research on social hierarchy in both humans and mice have mostly focused on dominance behaviors (Hawley, 1999, 2002; Islam, 2014; Lindzey et al., 1961; Saxena et al., 2018; Sidanius and Pratto, 2001; Sluckin and Smith, 1977; Strayer and Strayer, 1976; Wang et al., 2011; Yamaguchi et al., 2017; Zhou et al., 2017), the contribution of subordinate behavior or submissive personality to the formation of social hierarchies has largely been underestimated. Moreover, although mice have been used widely as a standard model organism for human biology, in parallel studies between human children and mice have been rare in the past, especially in behavioral research. Because few reports in recent years have found surprising similarities (Esposito et al., 2013), we believe that investigation of social hierarchy in these two species together would provide new insight into the innate intrinsic characteristics in the hierarchical formation from an evolutionary perspective. Since internal characteristics in mice cannot be clearly classified in detail (e.g. different aspects of personality or internal status) and many variables cannot be manipulated in human children (e.g. surgical or pharmacological approach), we expected that the combination and comparison of data from children and mice would be helpful for answering questions that could not be approached by only one of the species.

In this report, we establish and investigate hierarchical relationships in preschool-aged children and weanling mice through parallel comparable task designs. While previous studies based on observation suggested that young children's social hierarchy has initially formed around this time (La Freniere and Charlesworth, 1983; Strayer and Trudel, 1984), these are also the youngest stages in two species with available social tasks for evaluation of hierarchy. Our results showed that, for both species, stable and transitive social rankings develop at a young age. To explore the relationship between intrinsic characteristics and hierarchical formation, we investigated several temperament factors in children and found that while dominant children were as expected to be more likely to approach with stronger emotion expression, subordinate children showed a greater tendency toward withdrawal behavior accompanied by less persistence and less expressiveness of emotions. We next explored the causality between social hierarchy and internal status by applying the mouse tube test and showed that conflicts between two weanling mice were surprisingly not resolved by the dominant winner but by the loser with innate subordinate tendency. Our results therefore not only present a remarkable similarity in social hierarchies of humans and mice but also reveal the determining role of subordinate decision-making that has not been recognized in previous studies for hierarchical formation. These findings potentially provide new insights into the mechanisms underlying the formation of social ranking and contribute to research on social interaction in the fields of biology, psychology, and education.

RESULTS

Social hierarchy of preschool-aged children

Human studies have shown that dominance hierarchies gradually become stable in preschool years (Strayer and Strayer, 1976; Strayer and Trudel, 1984). To explore the influence of innate characteristics on the

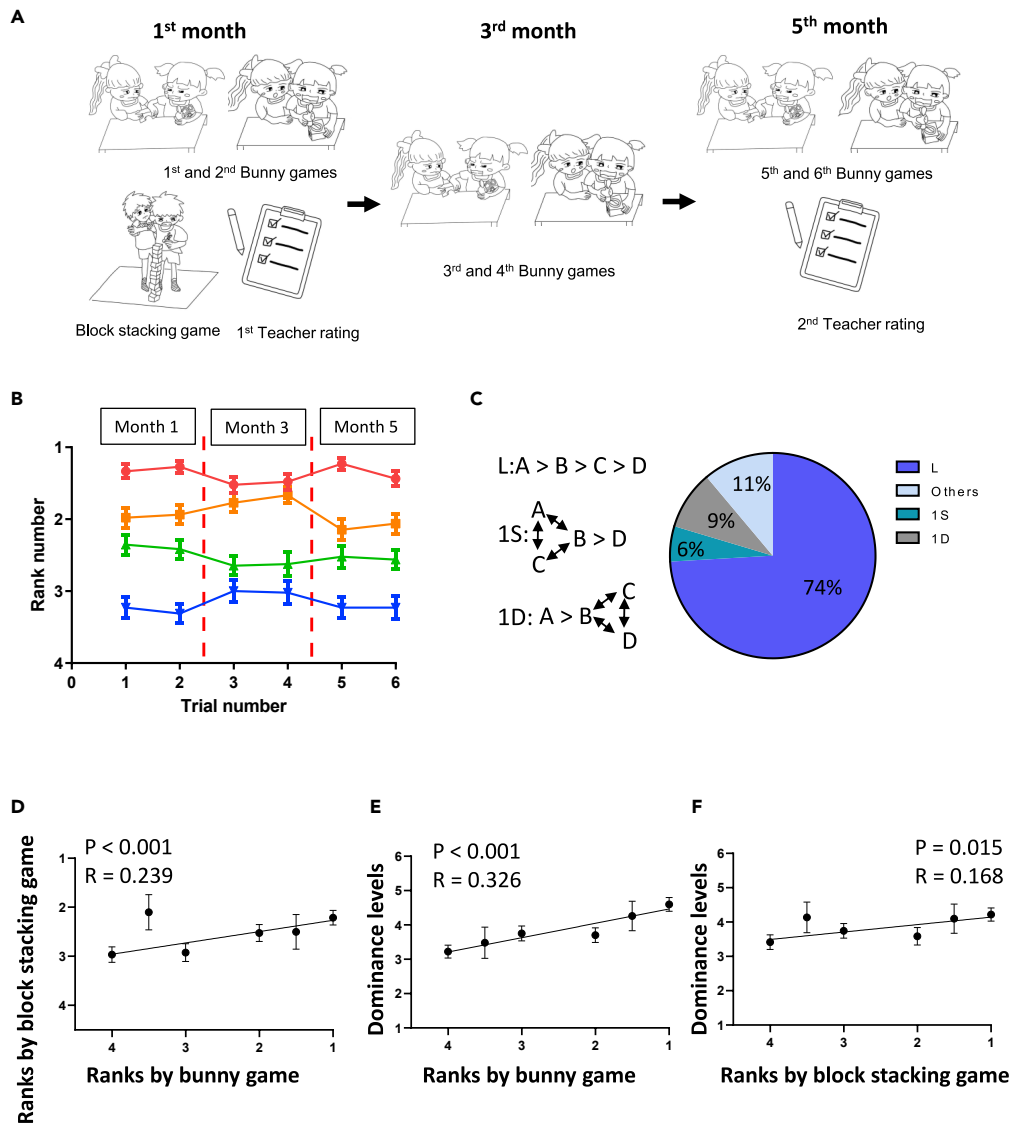


Figure 1. Social hierarchy in preschool-aged children is stable and obeyed a linear relationship

(A) Experimental procedure for evaluating social hierarchy in children. The ranking between two children was tested by the bunny game twice consecutively in the first, third and fifth month. The blocking game was also applied to evaluate hierarchy in the first month. The teacher rating was conducted in both the first and the fifth month.

(B) Summary of social ranks defined by the bunny game for 54 groups of four preschool-aged children over a period of 5 months. Mean ± S.E.M.

(C) Possible social relationships among a group of four children and the percentage of each relationship observed (N = 54 groups).

(D) Correlation between ranks defined by bunny game and ranks by block-stacking game (Pearson Correlation, N = 216).

(E) Correlation between ranks defined by bunny game and dominance levels rated by teacher (Pearson Correlation, N = 216).

(F) Correlation between ranks defined by block-stacking game and dominance levels rated by teacher (Pearson Correlation, N = 216).

formation of social hierarchy, we supposed that preschool young children would be the most appropriate participants because of their least school experience and very limited peer interaction experience. Therefore, we collected data from 216 children aged 3 to 6 years from two preschools and examined their social hierarchies from the beginning of a new semester to the end of the semester. After arranging the children

as groups of four, their social rankings within the groups were examined by the bunny game that they performed twice in one day every other month during a period of 5 months (Figure 1A). The bunny game was new to the children and reflected nonconflicting competitive peer interactions. Our results showed that, as with the social patterns found for animals (Wang et al., 2011), the ranking status of young children was very stable over this 5-month period (Figure 1B). The hierarchical rankings mostly obeyed a linear relationship (i.e., they were transitive, meaning that if A is dominant over B, who is dominant over C, then A is dominant over C) (Figure 1C).

Next, we investigated the consistency of the social rankings under different conditions by comparing the rankings defined by the bunny game with rankings defined by the block-stacking game (Figure 1A). Compared with the bunny game, which requires the children to be more intellectually involved, the block-stacking game mainly demands fast physical movement. Although the task requirements for the two games were different, we found a significantly positive correlation between the rankings defined by these two independent methods (Figure 1D). More importantly, the ranks determined by either game were also consistent with evaluations of the social dominance levels made by the teachers (Figures 1A, 1E, and 1F) (Dodge and Coie, 1987). The significantly positive correlation among these different measures implied a general hierarchical relationship for both the specific conflict situations and daily social interactions.

Correlations between the individual characteristics of the children and their social hierarchy

The consistency among the rankings defined by three completely different methods indicates that certain fundamental root causes, e.g., individual innate traits or personalities, might be important in forming social hierarchies of children. Consequently, since teachers' rating covered various daily social interactions of the young children, exploring the characteristics related to the questionnaire answers given by the teachers would provide insight into the hierarchies formed among those children. To examine the influences of possible individual characteristics, we tested several aspects of temperament using the Temperament Assessment Battery for Children questionnaire, which assesses the intrinsic, neurophysiological basis of an individual (Martin, 1994; Wang, 1995). We found that the rank positions defined by the ratings of the teachers in the beginning of a new semester (with limited social experience with classmates) significantly correlated with almost every aspect of temperament except for activity level (Table S1 and Figure 2A). Moreover, even though the task requirements for two competitive games were quite different, the rankings defined by either game are identically correlated with temperaments in approach/withdraw, emotion intensity and persistence (Table S1, Figures 2B and 2C). Since the preschoolers were not familiar with each other at the beginning of a new semester and thus had limited knowledge about their classmates' abilities or traits, we therefore inferred that the innate internal temperaments of children could have an important influence on the formation of a social hierarchy.

Given that the social rankings can be caused by both the personal characteristics and social experiences in classroom interactions of the children, to determine whether there are personal characteristics that keep influencing the social rankings regardless of the quantity or the quality of social experience, we further investigated the relationship between temperaments and hierarchy defined by the second teacher rating (with accumulated interaction experience with classmates for five months) and the sixth bunny game (after playing six rounds in five months) at the end of the semester. We found that the correlations were extremely similar over the 5-month testing period (Table S1, Figures 2D and 2E), suggesting that the influence of intrinsic traits on rankings were not diminished or changed by learning (including the newly accumulated interactive experiences and preschool education), but in fact pre-existed and persisted over time. Taken together, by simultaneously examining the influence of temperaments on social rankings defined by different methods at the beginning and end of the semester, we found that approach/withdraw, emotion intensity and persistence were the major individual characteristics influencing the formation of social hierarchy.

Social hierarchy in weanling mice

Because the behaviors or performance of children can be influenced by several human factors, to further dissect the causality between intrinsic characteristics and social hierarchy, we also investigated the natural or intrinsic hierarchical relationships in groups of C57BL/6J mice using the tube test. Similar to the bunny game and stock-stacking game in children, tube test also provides a platform to determine mice social ranks unambiguously in a short time without involving physical fighting. However, while there is no any

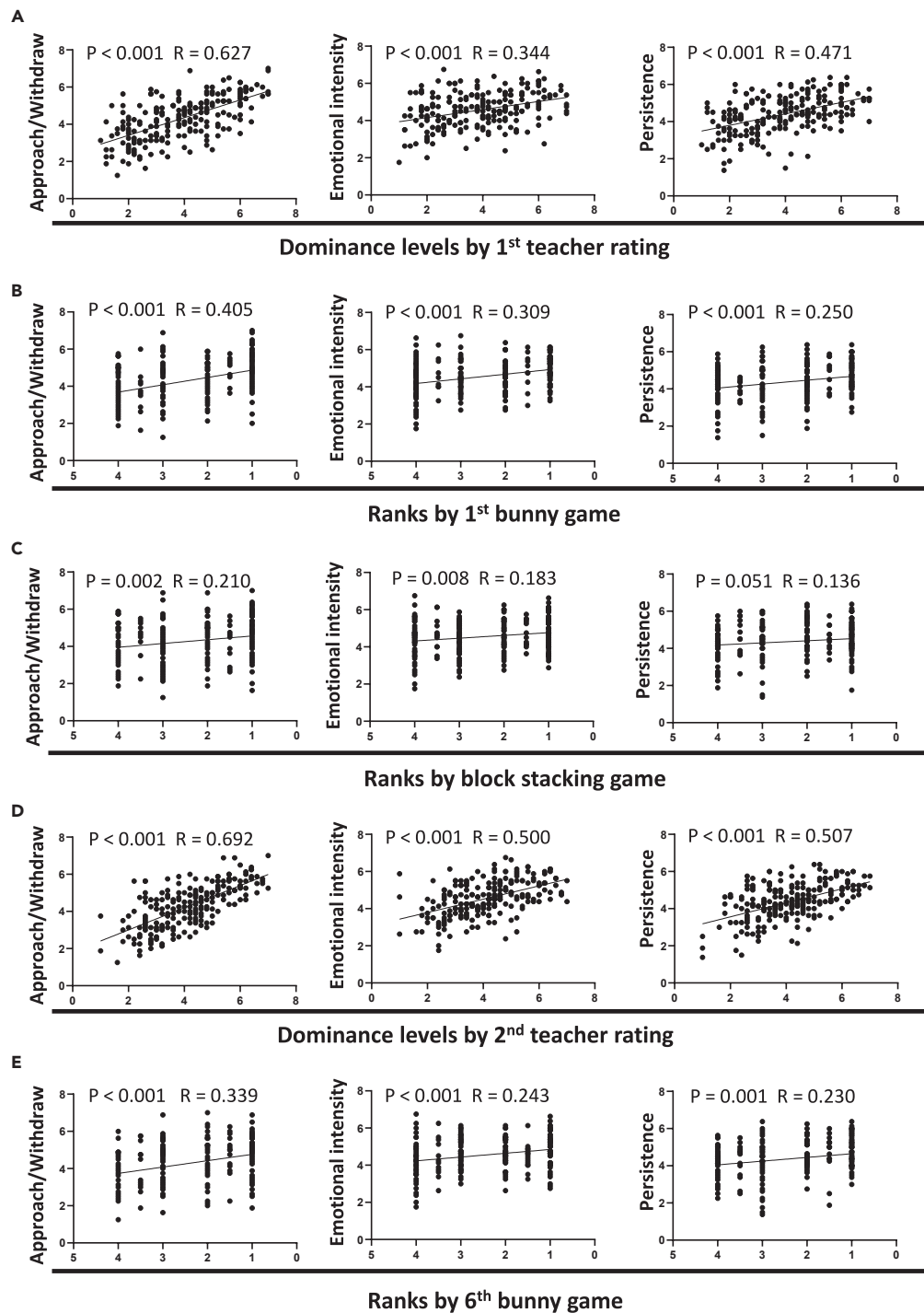


Figure 2. Social ranks defined by teacher rating, bunny game, and block-stacking game in preschool-aged children are positively correlated with temperaments in approach/withdraw, emotion intensity, and persistence Table S1

(A) Correlation between dominance levels defined by first teacher rating and three temperaments.

(B) Correlation between ranks defined by first bunny game and three temperaments.

(C) Correlation between ranks defined by block-stacking game and three temperaments.

(D) Correlation between dominance levels defined by second teacher rating and three temperaments.

(E) Correlation between ranks defined by sixth bunny game and three temperaments (Pearson Correlation, $N = 216$).

See also Table S1.

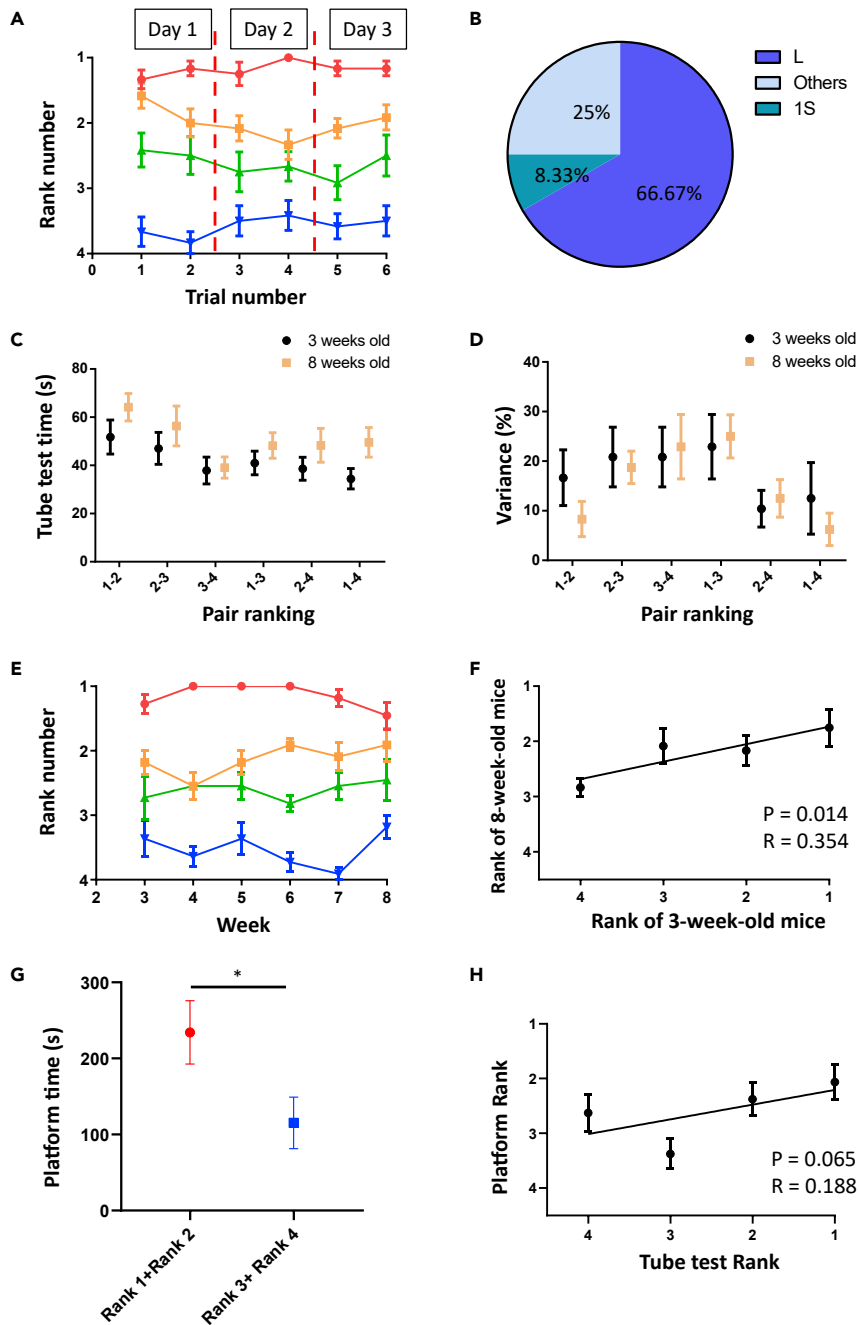


Figure 3. Social hierarchy in weanling mice can be established using the untrained tube test Figure S1

(A) Summary of the social ranks of the weanling mice as determined by the untrained tube test performed two times per day over three days (N = 12 cages).

(B) Percentage of each hierarchy relationship observed in weanling mice (N = 12 cages).

(C) Comparison of assay time across four trials for the 3- and 8-week-old mice (pair 1-2: $p = 0.186$, pair 2-3: $p = 0.370$, pair 3-4: $p = 0.561$, pair 1-3: $p = 0.259$, pair 2-4: $p = 0.267$, pair 1-4: $p = 0.057$, unpaired t test or Mann-Whitney test, N = 12 pairs).

(D) Comparison of assay variation across four trials for the 3- and 8-week-old mice (pair 1-2: $p = 0.369$, pair 2-3: $p > 0.999$, pair 3-4: $p = 0.883$, pair 1-3: $p = 0.626$, pair 2-4: $p > 0.999$, pair 1-4: $p = 0.784$, Mann-Whitney test, N = 12 pairs).

(E) Summary of social ranks tested every week from 3-week-old to 8-week-old (N = 12 cages). (F) Correlation of social ranks for the 3- and 8-week-old mice (Pearson Correlation, N = 12 cages).

Figure 3. Continued

(G) Total time of dominant and subordinate mice on the platform in platform competition assay ($p = 0.011$, Mann-Whitney test, $N = 12$ cages).

(H) Correlation of social ranks defined by tube test and platform competition assay (Pearson Correlation, $N = 12$ cages). Mean \pm S.E.M.

See also [Figure S1](#).

learning procedure as well as immediate advantage/disadvantage for children to compete for bunny or stock-stacking games, the traditional tube test requires training of the mice by applying aversive air pressure to motivate individuals to enter the tube ([Wang et al., 2011](#)). In order to apply an assay comparable to the tasks for young children, we therefore modified the standard tube test to reduce confounds associated with training or stress. We first applied this untrained tube test using adult male mice and found that the mice voluntarily entered the tube and behaved naturally. For the untrained tube test, the majority of social rankings among the mice were stable across 3 days and were mostly transitive ([Figures S1A and S1B](#)). Consistent with a previous study, we also noticed that the assay time was negatively correlated with the rank numbers and rank distances between pairs of mice ([Figure S1C](#)) ([Wang et al., 2011](#)). We compared the results of the untrained tube test to the standard trained tube test and found that the assay time was generally shorter after training ([Figure S1D](#)), but the differences in the percent variance over four consecutive sessions between assays with trained and untrained mice were not significant ([Figure S1E](#)). These results indicated that the tube test can be a reliable experimental platform to study social hierarchy even without the training procedure.

We next used the untrained tube test to examine social hierarchy in young mice. When we tested 2-week-old mouse pups, right before they were weaned, we failed to establish a robust social ranking because the pups had difficulty finishing the assay. For the 3-week-old mice who had just been weaned, most were able to complete the assay successfully, and we observed a stable and mostly transitive social hierarchy ([Figures 3A and 3B](#)). Comparison between adult and weanling mice showed no difference in either the assay time or variance across four consecutive trials ([Figures 3C and 3D](#)). Notably, we found that the ranks of the weanling mice were well maintained as the mice matured to 8 weeks of age, i.e., became adults ([Figure 3E](#)). The correlation of rank positions between 3- and 8-week-old mice were weak but statistical significance ([Figure 3F](#)). Lastly, since young mice do not perform aggression, platform competition assay, in which mice in water compete for a platform to stand, was developed to validate the social ranks by tube test ([Figure 3G](#)). We found dominant mice (first and second rank mice) generally have more time on the platform than subordinate mice (third and fourth mice). The positive correlation between ranks defined by tube test and ranks by platform competition were also close to significant ([Figure 3H](#)), implying a certain degree of consistency between two assays. Taken together, our results suggested that a stable social hierarchy can be established in 3-week-old weanling mice using the untrained tube test and that these social rankings could potentially reflect the hierarchical relationship in adults.

The loser in the untrained tube test determined the social hierarchy of the group

What roles do winners and losers play to determine the outcome of the tube test? Controlling and aggressive actions of a dominant male are thought to limit and influence the response of a subordinate individual ([Benton and Brain, 1979](#); [Darwin, 1871](#); [Long, 1972](#); [Wang et al., 2014](#)); however, our study in children also implied that those of lower rank were more likely to withdraw and be both less expressive and less persistent, which may contribute to the formation of their social hierarchy. Although previous studies using the tube test mainly quantified winners and described the trial as ending when one mouse 'forced' another to retreat out of the tube ([Lindzey et al., 1961](#)), the behaviors of the losers have never been considered carefully. By examining the behaviors and positions of the mice in detail during the tube test with weanling mice ([Figures 4A and 4B](#) and [Video S1](#)), we first found that the behaviors of the two competing individuals during the early phases of the assay were hardly distinguishable. Although rank-1 mice tended to gain slightly more territory, in most cases both winners and losers acquired similar territory in the tube and stood holding their positions ([Figure 4C](#)). Unexpectedly, less than half of the winners physically pushed the losers during the assay ([Figure 4D](#)). Resolution of the social encounters within the tube was therefore not determined by one-sided assertive and dominance behavior of the ultimate winner. Instead, detail examination of 145 loser retreat events in 72 tests showed that the assay was mostly ended by a shift by the ultimate loser from being socially engaged to unilaterally retreating voluntarily ([Figures 4E and 4F](#)). Most importantly, although some winners moved forward as the losers retreated, the assays almost always ended with a certain

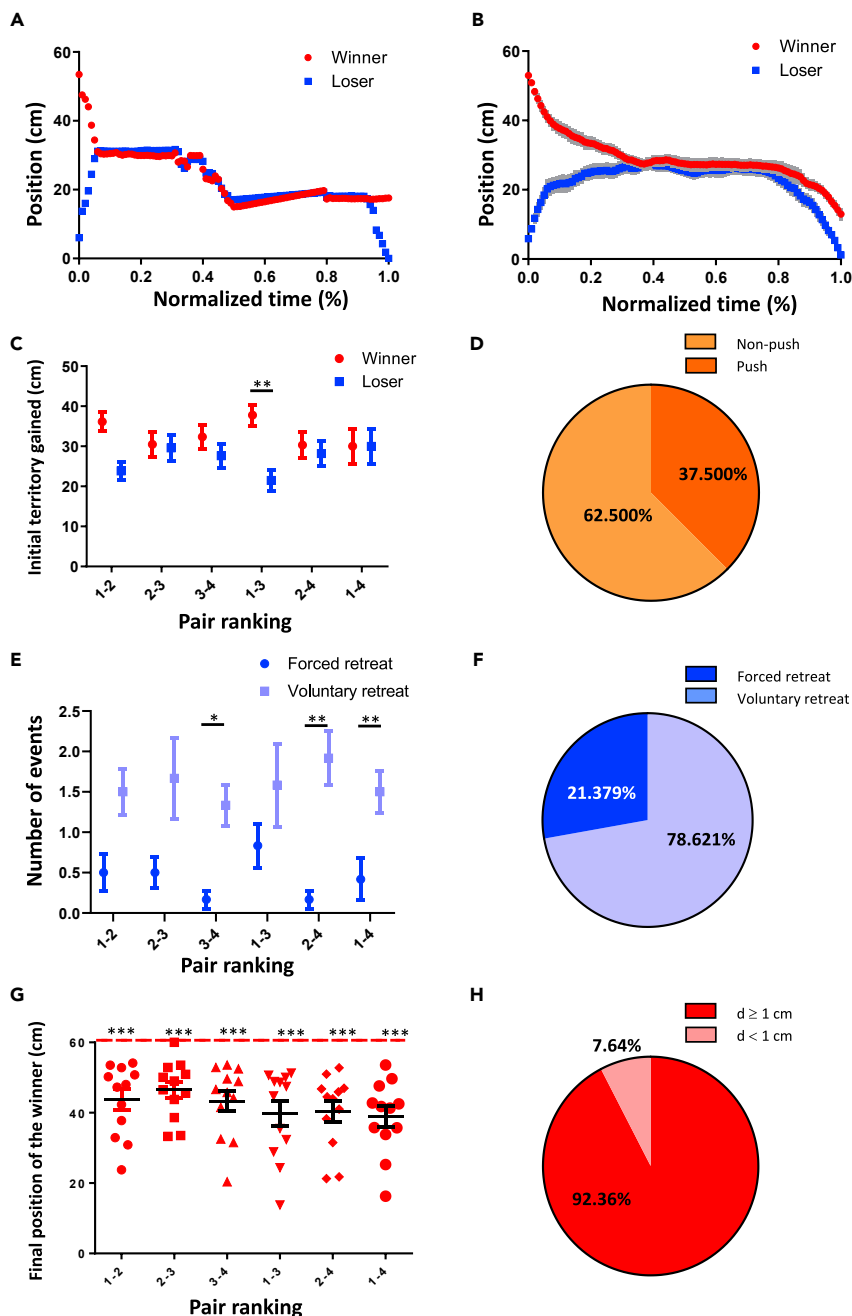


Figure 4. The outcome of the tube test for weanling mice is determined by the withdrawal behavior of the losers [Figure S2](#)

(A) A representative kymograph of mouse positions during the tube test assay (see also [Video S1](#)).

(B) Kymograph of mouse positions in an average of 12 pairs.

(C) Initial territory gained by the winners and losers in the tube test (pair 1-2: $p = 0.052$, pair 2-3: $p = 0.8936$, pair 3-4: $p = 0.4362$, pair 1-3: $p = 0.009$, pair 2-4: $p = 0.732$, pair 1-4: $p = 0.996$, paired t test or Wilcoxon signed-rank test, $N = 12$ pairs, four trials for each pair).

(D) Percentage of winners physically pushing losers during the assay ($N = 72$ trials).

(E) Number of forced retreat or voluntary retreat of losers during the assay (pair 1-2: $p = 0.056$, pair 2-3: $p = 0.120$, pair 3-4: $p = 0.009$, pair 1-3: $p = 0.240$, pair 2-4: $p = 0.005$, pair 1-4: $p = 0.039$, Wilcoxon signed-rank test, $N = 12$ pairs).

(F) Percentage of forced retreat and voluntary retreat of losers during the assay ($N = 145$ retreats in 72 trials).

Figure 4. Continued

(G) Final positions of the winners compared with their exit end of the tube at the end of the assay (pair 1-2: $p < 0.001$, pair 2-3: $p < 0.001$, pair 3-4: $p < 0.001$, pair 1-3: $p < 0.001$, pair 2-4: $p < 0.001$, pair 1-4: $p < 0.001$, one-sample t test or one-sample Wilcoxon signed-rank test, $N = 12$ pairs, four trials for each pair).

(H) Percentage of winners found at a distance (D) from the exit of ≥ 1 cm and < 1 cm at the end of the assay ($N = 288$ trials).

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Mean \pm S.E.M.

See also [Figure S2](#).

distance between the winner and its forward exit, which indicated that losers left the tube without direct contact with the winners ([Figure 4G](#)). Among the encounters, only $< 8\%$ concluded with the winner forcibly pushing the loser out, which was defined as a distance between the two animals of < 1 cm once the loser had fully exited the tube ([Figure 4H](#)). Similar phenomenon was also observed in the adult mice ([Figures S2A–S2C](#)) and indicated that the social rank based on the untrained tube test was mainly determined by the loss of motivation and persistence by the subordinate mice and was followed by their withdrawal rather than by an aggressive advance of the dominant mice.

Subordination is driven by internal status

The social rankings in weanling mice were not simply determined by weight or general activity ([Figures S3A and S3B](#)). There were also no detectable differences in anxiety level and exploration activity ([Figures S3C–S3E](#)). How does a mouse decide to switch from persistence to retreat? Since group-housing prior to the test may provide an opportunity for mice to learn and associate the individual social signals of each cagemate with the previous outcomes of their social experiences ([Brennan and Kendrick, 2006](#); [Camats Perna and Engemann, 2017](#); [Hurst et al., 2001](#)), one possibility is that the low-ranking individuals could recognize the learned social signature of their opponent to determine whether they should persist or withdraw in a given encounter ([Figure 5A](#)). To evaluate the role of social memory in promoting social withdrawal, we compared each individual's social outcome against its cagemates with whom they had prior social interactions and against strangers with whom no prior social experience existed ([Figure 5B](#)). We reasoned that if individual recognition was guiding the withdrawal behavior of the losers in the tube test, we would expect that (1) the number of times a mouse wins against an unknown individual would be unbiased (50%) and that (2) on average the assay time between two strangers would be longer than that between two cagemates because there would be no recognizable information that could be used to resolve the conflict. Instead, we found that dominant males mostly dominated against unknown subordinate opponents ([Figure 5C](#)). The assay time across four consecutive trials, surprisingly, was shorter between two strangers than between two cagemates ([Figure 5D](#)). These results, therefore, did not support a role for associative social memories or individual recognition driving the hierarchy in the untrained tube test.

Instead of social memory, most social cues function as instructive signals to induce a receiver's response ([Figure 5E](#)). For example, specialized olfactory cues or pheromones have been suggested to trigger specific dominance behaviors or to promote aggressive motor patterns in mice ([Chamero et al., 2007](#); [Stowers et al., 2002](#)). In the tube test, if subordinate-specific behaviors or social withdrawal are induced by signals released from dominant opponents, mice without sensory perceptions should not retreat and have enhanced persistence in the tube ([Figure 5F](#)). We would then expect that (1) there is an increase in the assay time for mice without sensory inputs and that (2) such mice would be more dominant than mice with intact sensation. However, when we compared mice injected with dichlobenil, which eliminated olfaction ([Yoon et al., 2005](#)), to dimethyl sulfoxide (DMSO) control mice, we did not find longer assay time in anosmic mice ([Figures 5G and S4A](#)). There was also no difference in assay time between mice injected with lidocaine at the whisker pad, which blocked somatosensation, and phosphate buffered saline (PBS)-injected control mice ([Mizuno et al., 2018](#)) ([Figures 5G and S4B](#)). In addition, when the tube test with untrained mice was conducted under infrared light to minimize visual cues ([Jacobs et al., 1999](#)), the assay times were even shorter than when the assay was performed under white-light conditions ([Figures 5G and S4C](#)). This shorter assay time may be due to reduced anxiogenic effect caused by white light. The win rates for mice without both olfactory and tactile sensations and for control mice injected with DMSO and PBS were $\sim 50\%$ under infrared light, suggesting that the outcomes of the competitive interactions between intact mice and mice with sensory deficits were random ([Figure 5H](#)). Therefore, we obtained no evidence suggesting that the subordinate withdrawal of the mice was triggered by instructive social signals.

Our experiments did not support roles for social memory or instructive odors underlying the differences in social persistence, as shown by the tube test. Alternatively, a third possible model suggests that the

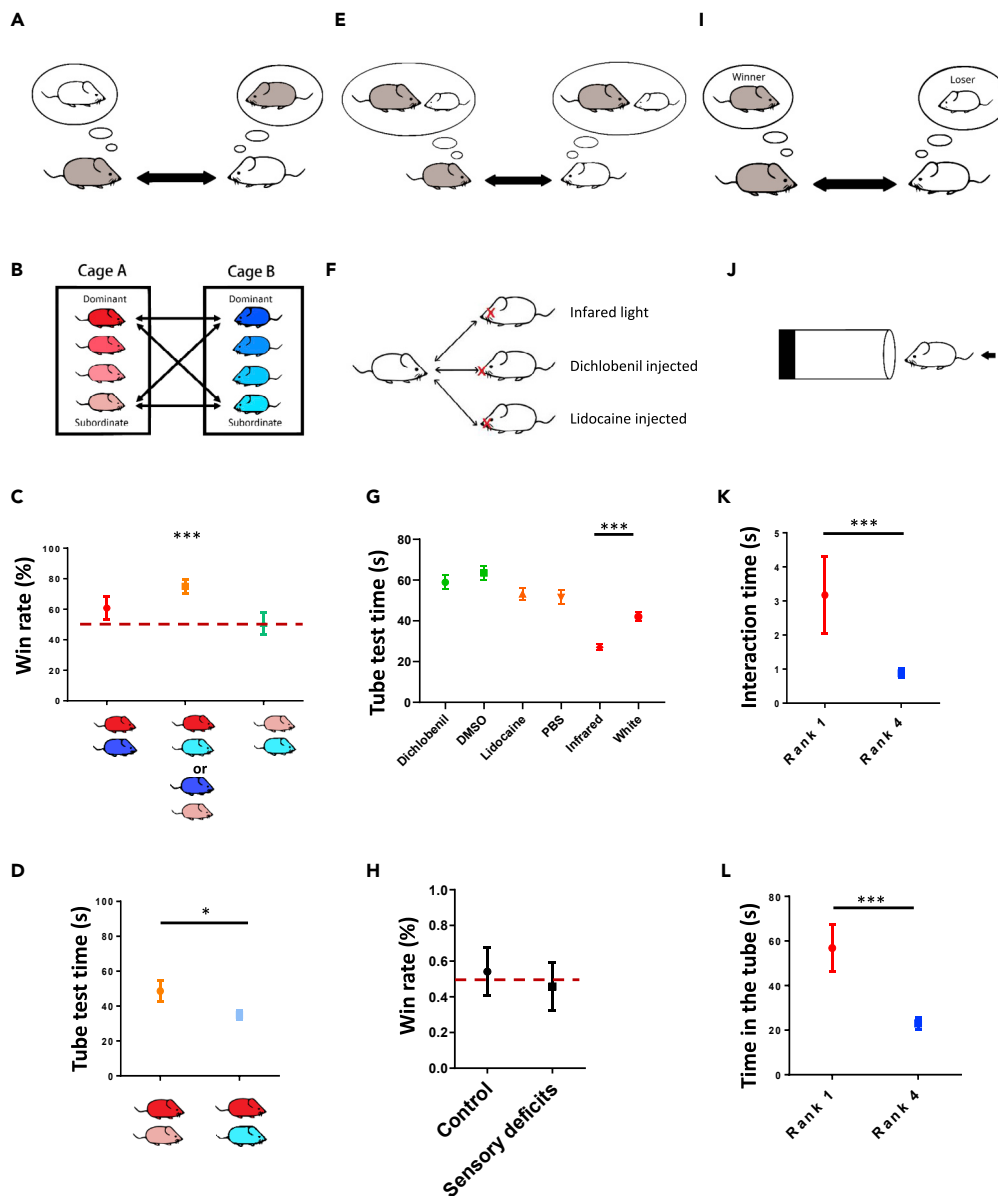


Figure 5. The withdrawal behavior of the subordinate mice is determined by their internal status [Figure S3](#)

(A) Associative memory model. An individual's unique social cues evoke a memory of previous social experiences.
 (B) Stranger assay. The tube test was applied to compare the behavior of two cagemates (individuals housed together) vs. two strangers (individuals housed in different cages).
 (C) The win rate for paired strangers compared with the expected rate of 50% (dashed line) (rank-1 vs. rank-1: $p = 0.188$, $N = 15$ pairs; rank-1 vs. rank-4: $p < 0.001$, $N = 30$ pairs; rank-4 vs. rank-4: $p = 0.991$, $N = 15$ pairs, one-sample Wilcoxon signed-rank test).
 (D) Assay time across four trials for two cagemates and two strangers ($p = 0.0179$, unpaired t test, $N = 12$ pairs of cagemates, $N = 30$ pairs of strangers).
 (E) Specialized social cue(s) model. Dominant winners emit an instructive social signal(s) that promotes retreat by the losers.
 (F) Sensory removal. Social hierarchy was evaluated using mice without normal sensory sensations. Olfactory cues were removed by intraperitoneal injection of dichlobenil. Tactile cues were blocked by injection of lidocaine into the whisker pads of the mice. Visual signals were eliminated by performing the experiments under infrared light.
 (G) Assay time of all paired conditions across four trials for evaluation of the social hierarchy in mice that lacked a sensory input (dichlobenil vs. DMSO mice: $p = 0.57$, lidocaine vs. PBS mice: $p = 0.581$, infrared-light condition vs. white-light condition: $p < 0.001$, Mann-Whitney test, $N = 60$ – 72 pairs) (see also [Figure S4](#)).

Figure 5. Continued

(H) Win rate for control mice (DMSO + PBS) and mice without olfaction and somatosensation (dichlobenil + lidocaine) compared with the expected rate of 50% (dashed line) under infrared light (Intact: $p = 0.877$, Sensory deficits: $p = 0.877$, one-sample Wilcoxon signed-rank test, $N = 10$ pairs).

(I) Internal status model. Losers are less persistent and more likely to withdraw when obstructed.

(J) Blocker assay. Further forward motion of winners or losers was blocked by an immovable object placed midway in the tube.

(K) Assay time across four trials that higher-ranked mice and lower-ranked mice interacted with the blocking material ($p < 0.001$, Mann-Whitney test, $N = 10$ pairs).

(L) Assay time across four trials that higher-ranked mice and lower-ranked mice spent in the tube containing the blocking material ($p < 0.001$, Mann-Whitney test, $N = 10$ pairs). * = $p < 0.05$, *** = $p < 0.001$. Mean \pm S.E.M.

See also [Figure S3](#).

behavioral switch from persistence to withdrawal can be simply determined by an animal's intrinsic status rather than via social signals ([Figure 5I](#)). If this was true, we would expect that losers would retreat more readily from everything regardless of the identity of their opponents. For the blocker assay, the material in the tube prevented further forward movement to the exit of the tube ([Figure 5J](#)). Notably, the higher-ranked animals spent significantly more time interacting with the blocking material ([Figure 5K](#)). The losers withdrew and retreated out of the tube much faster than the winners ([Figure 5L](#)), which indicated that the losers inherently were less persistent and preferred to withdraw regardless of whether the obstruction was a mouse or a blockage of the tube. In summary, while our experiments cannot support the first and second models, the blocker assay evidently indicated that the withdrawal decision of subordinate animals can be solely made by intrinsic status without involvement of the opponents' identity.

DISCUSSION

By investigating social hierarchy in both children and weanling mice together, our study aimed to uncover the importance of individuals' intrinsic characteristics in hierarchical formation. Through simply behavioral observation and manipulation, we provide evidences related to three aspects of social hierarchy in the young. First, a stable social hierarchy can be established similarly in young children and mice. Second, social ranking in children is largely influenced by intrinsic characteristics independent of the social experience, not only the active approach in dominants but also withdrawal or retreating in subordinates. Third, in the tube test, individuals with subordinate tendency play the determining role in the formation of a social hierarchy. We carefully ruled out the effects of past interaction experiences and other confounding dominance cues from competitors. Our study not only shows a remarkable similarity in social hierarchy between children and weanling mice but also reveals the critical role of subordinate characteristics in hierarchical formation. We believe that this simple but novel concept has not been well recognized in previous studies.

The concept of dominance hierarchy in preschool-aged children has been suggested either by observing a small sample size or questionnaire rating by teachers in previous studies ([Dodge and Coie, 1987](#); [Hawley, 1999, 2002](#); [Pellegrini et al., 2007](#); [Roseth et al., 2007](#); [Sluckin and Smith, 1977](#); [Strayer and Strayer, 1976](#); [Vaughn and Waters, 1981](#)). To our knowledge, this is the first study to adopt multiple methods to evaluate young children's dominance rank in such a large sample of over two hundred preschool children in a five-month longitudinal design. Through the carefully designed tasks that depend on substantially different cues, types of interactions, behavioral requirements, and outcome measures, the consistent results we got therefore strongly support the possibility that intrinsic traits fundamentally influence the formation of social hierarchy. More importantly, we present our findings in parallel with mouse hierarchy, reflecting the similarity between these two species. Given that dominance hierarchy has been studied extensively in children, it is surprising that the hierarchy in young mice has not been reported previously. Most studies concerning social hierarchy in mice have focused on dominance behaviors, e.g., aggression and scent-marking, and were, therefore, performed using adult males. One study recently applied the tube test to investigate the influence of social isolation on ranking in the juvenile rat ([Tada et al., 2016](#)), but it did not provide general information concerning the hierarchical relationships in a group. Our study shows a detectable ranking among weanling mice, providing a fundamental basis for exploring social interactions in young animals and having potential implications for human children.

While previous studies have mostly focused on the dynamics of peer dominant relationship or on the changes in children's controlling strategies with interactive experiences or education ([Hawley, 1999](#),

2002; La Freniere and Charlesworth, 1983; Roseth et al., 2011), our findings suggest stable and persisting influences of innate intrinsic traits in the formation of social hierarchy. Although the relationship between temperament and social dominance may have been examined in previous studies of adults, it was defined either as one dimension of personality or just one aspect of emotion (Burgoon et al., 1998; Hall et al., 2005; Keltner et al., 2003; Mehrabian, 1996; Russell and Mehrabian, 1977). The influence of temperament on social dominance of young children has rarely been explored. Even if there was, the focus was just on one single aspect of temperament (persistence) (Hawley and Little, 1999). Our work therefore provides a more comprehensive examination of the relationship between social rankings and multiple temperaments and suggests that certain fundamental individual traits are especially crucial in determining social hierarchies. Children who can adopt a more accepting attitude toward a novel social situation (approach/withdraw), tend to express emotions more strongly (emotional intensity), and to continue with difficult learning tasks (persistence) would gain a higher rank in their social group. In contrast, regardless of the task characteristics and past social experience, children who withdraw more rapidly from a new situation, are less daring in expressing emotions and exhibit less persistence are more likely become subordinates. Our findings suggest that social hierarchies are strongly determined by certain intrinsic traits of the individual during the very beginning of social interactions. The formation of social hierarchy might be not caused solely by someone dominating or controlling others but also by someone with submissive characteristics allowing the other to successfully become the winner. This phenomenon prompted us to reconsider the possible even more important role of subordinates in the formation of social hierarchies.

Our study in mice further confirms this concept concerning the determining role of the loser in conflict situations. The untrained tube test shared some important features with the bunny game or stock-stacking game for us to investigate these two species comparably. First, there is no any form of physical fighting. Second, the conflicts are resolved in a short time with no ambiguous condition between winner and loser. Third, active motivation is important since there is no any punishment or disadvantage for losers. Using this untrained tube test, we found that hierarchy surprisingly was not determined by displays of antagonism from winners or by a lack of such dominance displays by losers. Instead, the outcomes were determined by the behavior of the losers as they disengaged from the conflict. Since winners needed only to hold their competitive intention, it was the losers who changed their action to resolve a conflict. A hierarchy basically cannot be formed without a loser or subordinate decision. From an evolutionary perspective, the decision to withdraw or be subordinate is an important survival strategy, because losing a conflict without avoiding the winner might be lethal for the loser. Consequently, an intrinsic, neurophysiological basis for the hierarchical position of an individual helps the social hierarchy of a group to form easily and remain stable without the need for fighting or other interactions. It is, therefore, important to uncover the mechanism(s) underlying the decision(s) of losers to understand the fundamental structure of a social hierarchy. Glucocorticoid hormones, for example, have been reported to play a crucial role in social rank attainment in rodents (Papilloud et al., 2020; Timmer and Sandi, 2010). While the effect of glucocorticoid on aggression have been investigated extensively (Mikics et al., 2004, 2007), it would be interesting to explore the influence of the hormones on subordinate behaviors. The untrained tube test, thus, presents as a novel platform to evaluate tendency toward persistence or withdrawal, and may help to explore subordinate decisions in the future.

We believe that both dominance and subordinate individuals play important roles in hierarchy determination. Unfortunately, most assays for social behaviors in mice have focused only on dominance displays, whereas the behavior of subordinate animals was simply not assessed (Wang et al., 2014). Our study provides evidence showing that, in certain circumstance, subordinate individuals can play the major role to resolve the conflict. Like studies involving nonhumans, human studies concerning social hierarchy have focused on primarily dominance behaviors, whether the target behavior was aggression or resource control (Sluckin and Smith, 1977; Strayer and Strayer, 1976). In a preschool classroom, aggressive and coercive-controlling behaviors are not preferred and thus attract the attention of and intervention by the teacher. However, our study shows that young children with retreating, less-emotionally expressive, and less persistent temperaments are most likely to become subordinate. Although this may have evolutionary roots or significance, this silent formation of a social hierarchy with an absence of noticeable conflicts may mean that some children lose their opportunities to play toys, use classroom resources, or partake in important learning experiences without being noticed by the teacher. The findings of this study, therefore, provide an important reminder to educators that more attention should be paid to subordinates in the classroom rather than to only the dominant students.

Limitations of the study

In this study, we applied surgical and pharmacological manipulations in mice to study the influence of internal status on conflict solving, which cannot be conducted in human children. We also study children temperaments to figure out crucial internal characteristics on the formation of social hierarchy, which cannot be classified clearly in mice. Our study therefore made a breakthrough to use the two species to answer different questions and reveal information that could not be approached solely by one of them. However, compared to the study of a single species, cross-species behavioral research is more difficult in data comparison and interpretation. Although each of our research tasks has been carefully designed and the consistent results solidified our discovery, this research still has many unresolved limitations.

First, from behavioral display to outcome measurements, there are still substantial differences among bunny game, block-stacking and tube tests. Although consistent results based on these different tasks solidified our discovery, interpretation of the data need to be very careful. For example, while data from tube test provided proof of concept that social conflict can be mainly determined by subordinate decision, the same conclusion cannot be inferred from our children study. Second, in spite of that our study emphasized the determining role of the subordination on resolving the conflict, we had no intention to decline the contribution of dominate individuals involved in the subordinate decision. The development of new and comparable tasks in both species will be therefore important to answer whether conflict can be resolved solely by subordinate decision without any influence from dominant others. Third, although our data examining three different potential models suggested that the withdrawal of subordinate mice are mainly caused by their intrinsic status, i.e., being less persistent, we, however, cannot completely exclude the influence of opponents or associated memory involved in subordinate retreat in the tube test. Further studies will be needed to clarify the involvements of the other two models in tube test assay. Given that social stress is known to affect social hierarchy and enhance social memory (Cordero and Sandi, 2007), the roles of stress hormones should be focused in particular. Finally, we found that the formations of social hierarchy in children and mice are influenced by temperament or intrinsic status, the underlying neurocognitive or physiological mechanisms, however, were not investigated in this study and remain to be further explored. Future research in the examination of neural activity during conflicts should put more focus on the decision-making of subordinate behaviors. And once again, we believe that comparative studies between human and model organisms would provide us more information about the neural basis as well as evolutionary mechanisms behind hierarchical formation.

Resource availability

Lead contact

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Materials availability

This study did not generate any new unique reagent.

Data and code availability

This study did not use any custom code, software, or algorithm.

METHODS

All methods can be found in the accompanying [Transparent Methods supplemental file](#).

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.isci.2021.102073>.

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AUTHOR CONTRIBUTIONS

YJ Chou, YH Lu, YS Su, and TH Kuo designed the experiments; YJ Chou, YH Lu, YK Ma, and YS Su performed the experiments; YJ Chou, YH Lu, YS Su, and TH Kuo analyzed the data, and YJ Chou and TH Kuo wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing financial interests.

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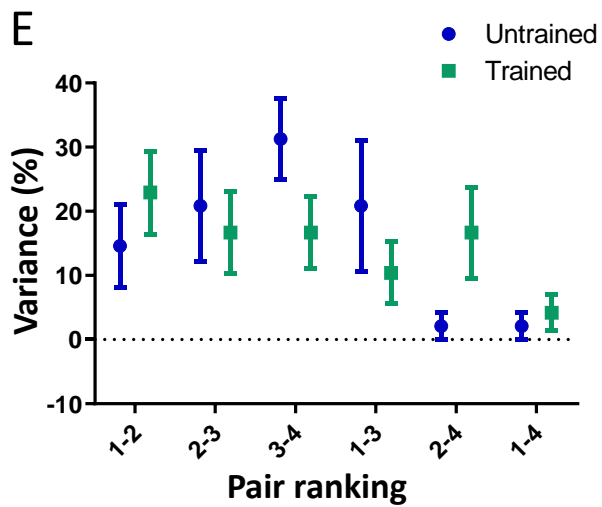
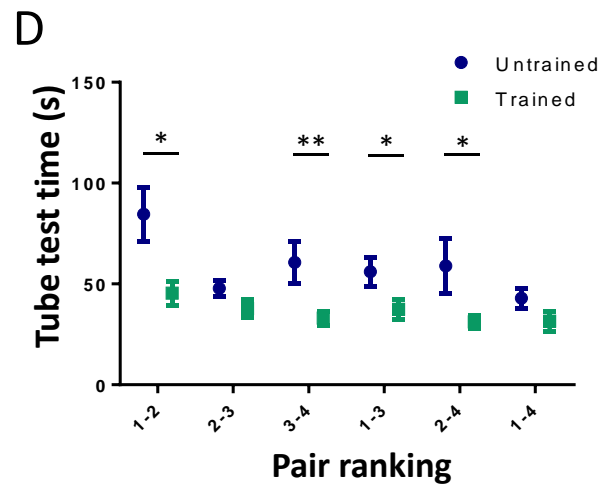
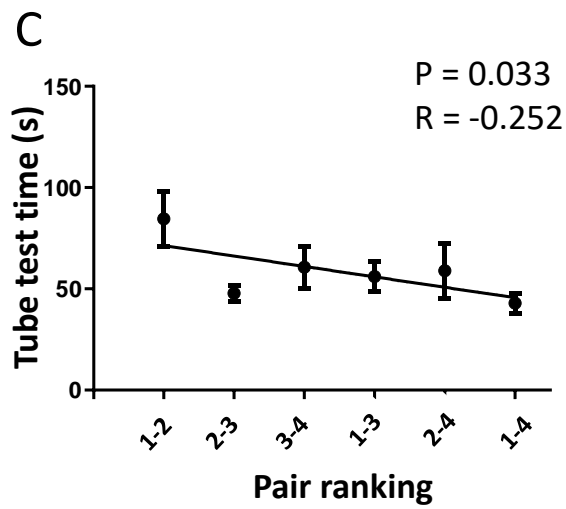
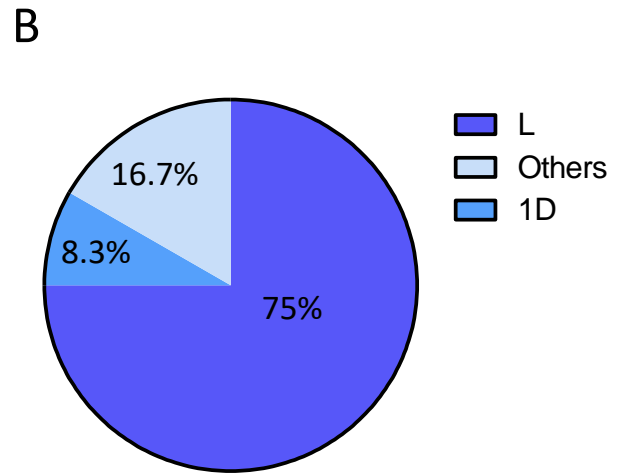
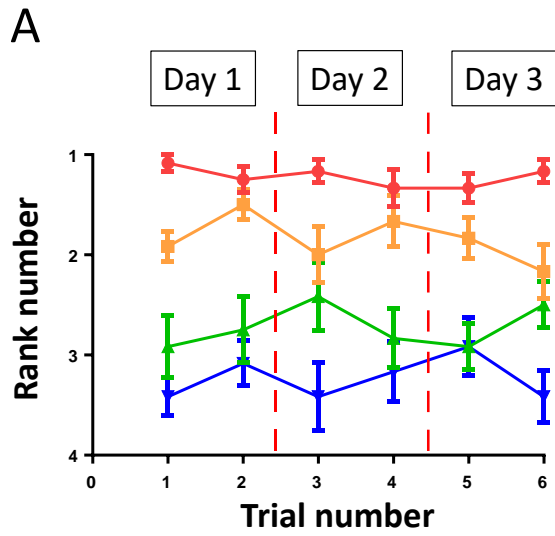
Supplemental Information

**The decisive role of subordination
in social hierarchy in weanling
mice and young children**

Yu-Ju Chou, Yi-Han Lu, Yu-Kai Ma, Yu-Shan Su, and Tsung-Han Kuo

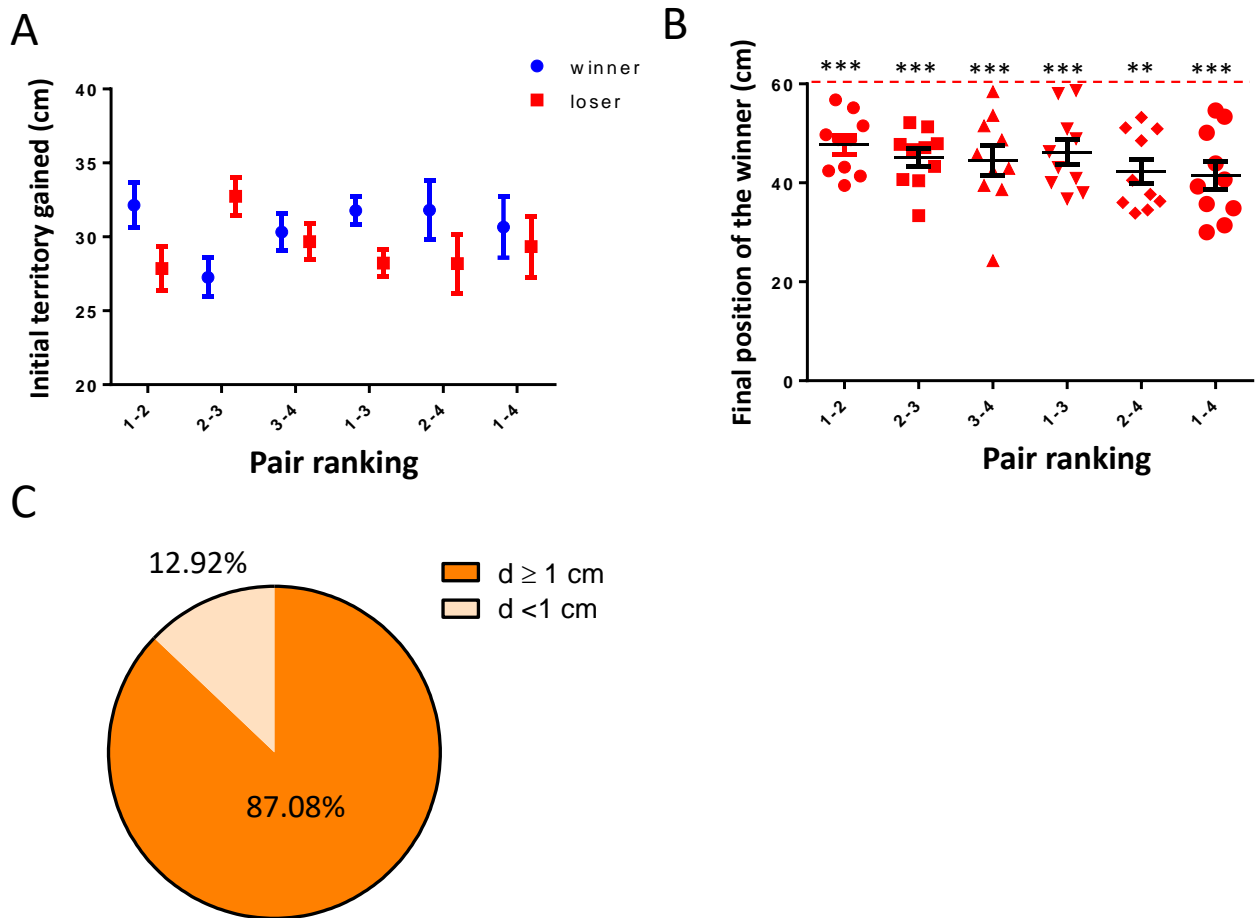
1 Supplemental Figures

2 Figure S1



4 **Figure S1 Social hierarchy in adult mice can be established using the untrained tube test, related**
5 **to Figure 3.** (A) Summary of the social ranks of the adult mice as defined by the untrained tube test
6 performed two times per day over three days. (N = 12 cages). (B) Percentage of each type of hierarchy
7 relationship observed in 8-week-old adult mice. (N = 12 cages). (C) Assay time across four trials
8 versus different ranked pairings (Pearson correlation, N = 12 cages). (D) Comparison of assay time
9 across four trials for trained and untrained mice (pair1-2: $p = 0.015$, pair2-3: $p = 0.099$, pair3-4: $p =$
10 0.004 , pair1-3: $p = 0.047$, pair2-4: $p = 0.047$, pair1-4: $p = 0.091$, unpaired t -test or Mann-Whitney test,
11 N = 12 pairs). (E) Comparison of assay variance across four trials for trained and untrained
12 mice (pair1-2: $p = 0.516$, pair2-3: $p > 0.999$, pair3-4: $p = 0.125$, pair1-3: $p = 0.555$, pair2-4: $p = 0.156$,
13 pair1-4: $p > 0.999$, Mann-Whitney test, N = 12 pairs). * = $p < 0.05$, ** = $p < 0.01$. Mean \pm S.E.M.
14

15 **Figure S2**



16

17

18 **Figure S2 The outcome of the tube test is determined by the withdrawal behavior of the losing**

19 **adult mice, related to Figure 4. (A) Initial territory gained by the winners and losers of the tube**

20 **test (pair 1-2: $p = 0.193$, pair 2-3: $p = 0.067$, pair 3-4: $p = 0.804$, pair 1-3: $p = 0.087$, pair 2-4: $p = 0.390$,**

21 **pair 1-4: $p = 0.756$, paired t -test, $N = 10$ pairs, four trials for each pair). (B) Final positions of the**

22 **winners compared with their exit end of the tube at the end of the assay (pair 1-2: $p < 0.001$, pair 2-**

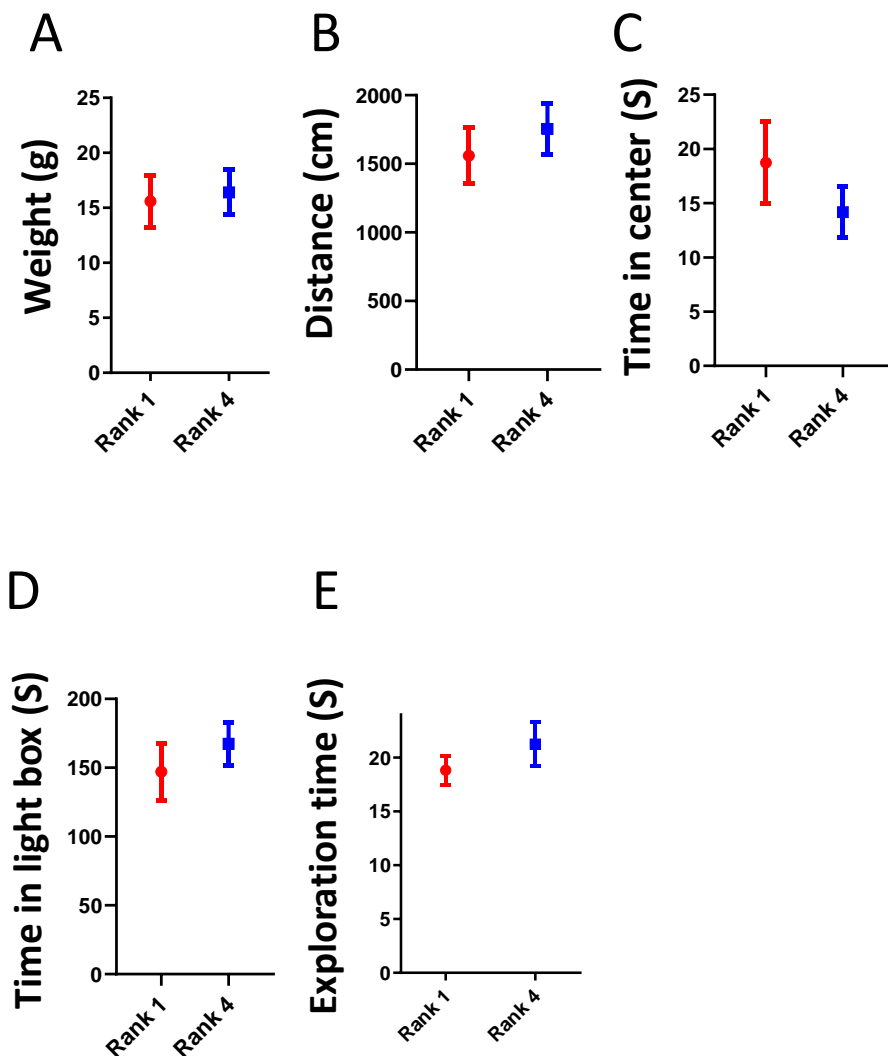
23 **3: $p < 0.001$, pair 3-4: $p < 0.001$, pair 1-3: $p < 0.001$, pair 2-4: $p = 0.002$, pair 1-4: $p < 0.001$, a one-**

24 **sample t -test or a one-sample Wilcoxon signed-rank test, $N = 10$ pairs, four trials for each pair). (C)**

25 **Percentages of winners at a distance from the exit of ≥ 1 cm at the end of the assay ($N = 240$ trials). ****

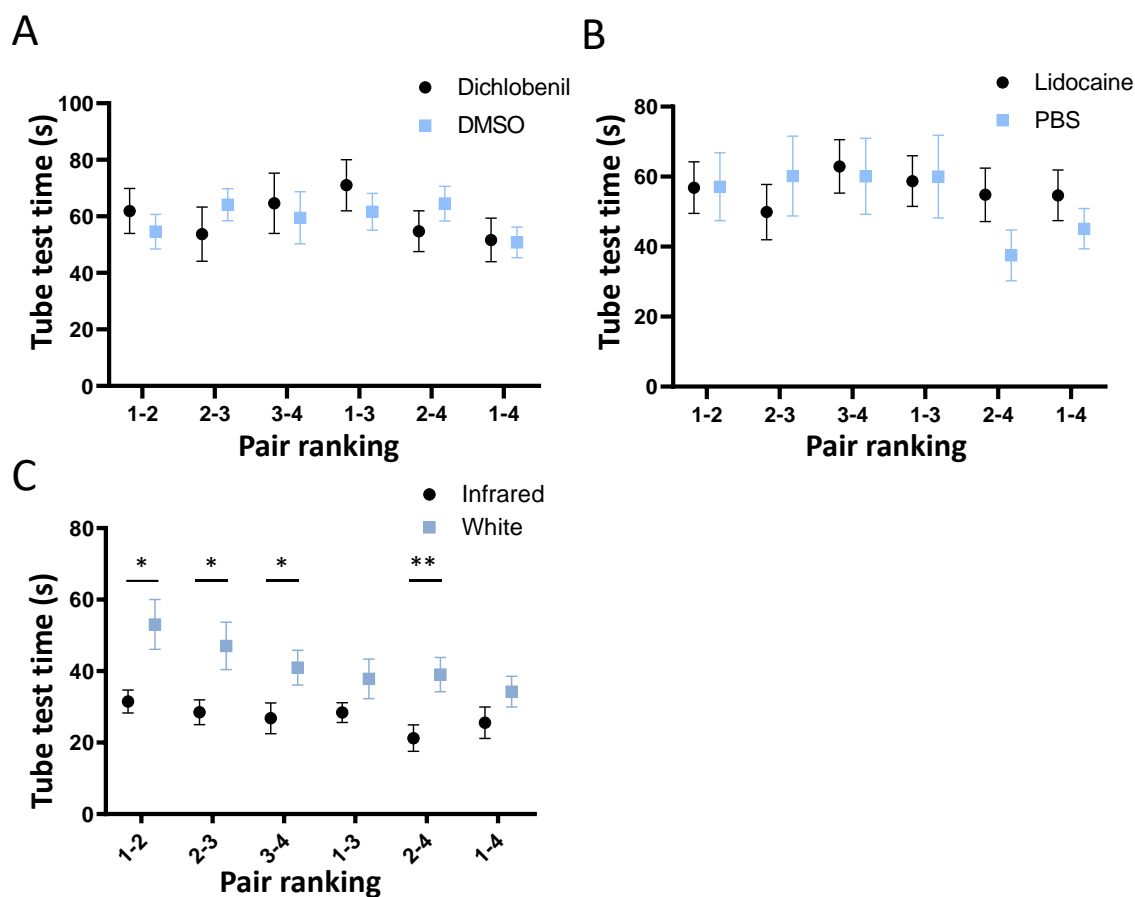
26 **= $p < 0.01$, *** = $p < 0.001$. Mean \pm S.E.M.**

27



29

30 **Figure S3 Weight or activity has no influence on social rankings, related to Figure 5.** (A) Weight
 31 of higher-ranked and lower-ranked mice ($p = 0.091$, paired t -test, $N = 12$ pairs). (B) Total moving
 32 distance of higher-ranked and lower-ranked mice in open field test ($p = 0.332$, paired t -test, $N = 12$
 33 pairs). (C) Total time of higher-ranked and lower-ranked mice in the center area of open field box (p
 34 $= 0.233$, Wilcoxon signed-rank test, $N = 12$ pairs). (D) Total time of higher-ranked and lower-ranked
 35 mice in the light box in light-dark box assay ($p = 0.336$, paired t -test, $N = 12$ pairs). (E) Total time
 36 of higher-ranked and lower-ranked mice in the exploration of novel objects ($p = 0.342$, paired t -test,
 37 $N = 12$ pairs). Mean \pm S.E.M.



39

40 **Figure S4 Sensory inputs have minimal influences on the assay time for the six pair ranking**41 **among cagemates of four weanling mice, related to Figure 5. (A) Assay time across four trials for**42 **different ranked pairs of weanling mice with ablated olfactory epithelium (pair 1-2: $p = 0.476$, pair 2-**43 **3: $p = 0.426$, pair 3-4: $p = 0.719$, pair 1-3: $p = 0.411$, pair 2-4: $p = 0.311$, pair 1-4: $p = 0.770$, unpaired**44 **t -test or Mann-Whitney test, $N = 10$ pairs). (B) Assay time across four trials for different ranked pairs**45 **of weanling mice with blocked somatosensation (pair 1-2: $p = 0.203$, pair 2-3: $p = 0.460$, pair 3-4: $p =$** 46 **0.846 , pair 1-3: $p = 0.928$, pair 2-4: $p = 0.122$, pair 1-4: $p = 0.317$, unpaired t -test or Mann-Whitney**47 **test, $N = 10$ pairs). (C) Assay time across four trials for different ranked pairs of weanling mice under**48 **infrared light (pair 1-2: $p = 0.013$, pair 2-3: $p = 0.016$, pair 3-4: $p = 0.041$, pair 1-3: $p = 0.154$, pair 2-**49 **4: $p = 0.009$, pair 1-4: $p = 0.173$, unpaired t -test or Mann-Whitney test, $N = 10-12$ pairs). * = $p < 0.05$,**50 **** = $p < 0.01$. Mean \pm S.E.M.**

51 **Supplemental Table 1. Pearson correlations among social ranks and temperament assessments**
 52 **in preschool-aged children (N=216), related to Figure 2.**

	activity level	adaptability	approach/ withdraw	emotional intensity	distractibility	persistence
1 st Teacher rating	0.10	0.61***	0.63***	0.34***	-0.22**	0.47***
2 nd Teacher rating	0.13	0.56**	0.69**	0.50**	-0.18*	0.51**
1 st Bunny game	0.18**	0.26**	0.41**	0.31**	0.02	0.25**
6 th Bunny game	0.09	0.22**	0.34**	0.24**	-0.10	0.23**
Block stacking game	0.14*	0.09	0.21**	0.18**	0.04	0.14 [#]

53 [#] $p = 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

54 **Supplemental Table 2. Experimental designs for children and mice, related to all Figures.**

Figure /Table	Experiment	Sample size	Number of children or mice (Cohorts)	Number of matchups	Number of Trials per match-up	Age
Children						
1B-C	Transitivity, Stability	54 groups	216	324	2 trials for 1 st , 3 rd , 5 th months	4-6 years
1D-F	Correlation among social ranks by different methods	54 groups	216	324 (block) 324 (bunny)	1 trial for each method	4-6 years
2A-E, Table S1	Correlation among social ranks and temperament assessments	54 groups	216	324 (block) 324 (bunny)	1 trial for each method	4-6 years
Mice						
3A-B	Transitivity, Stability in weanling mice	12 cages	48 (Cohort A)	72	2 trials per day for 3 days	3 weeks
3C-D	Assay time and variation in weanling and adult mice	12 pairs (adult mice) 12 pairs (weanling mice)	96 (Cohort A)	144	4	3 and 8 weeks
3E-F	Long-term stability and correlation between 3- and 8-week-old mice	12 cages	48 (Cohort A)	72	2 trials every week for 6 weeks	3-8 weeks
4B, 4D-F	Kymograph, winner push, loser retreat in weanling mice	12 pairs	48 (Cohort B)	72	1	3 weeks
4C, 4G-H	Initial territory gained, winners' final position in weanling mice	12 pairs	48 (Cohort B)	72	4	3 weeks
S1A-C	Initial territory gained, winners' final position in adult mice	10 pairs	40 (Cohort C)	60	4	8 weeks
S2A-B	Weight and Global activity	12 pairs	48 (Cohort B)	72	4	3 weeks
5C	Win rate for Stranger Assay	15 pairs (same ranks)	30 (Cohort B)	60	4	3 weeks

		30 pairs (different ranks)				
5D	Assay time for Stranger Assay	30 pairs	60 (Cohort B)	30	4	3 weeks
5G	Assay time for all pair rankings in mice with sensory defects	dichlobenil-60 matchups DMSO-60 matchups lidocaine-60 matchups PBS-60 matchups infrared-60 white-72 matchups	dichlobenil-60 (Cohort D) DMSO-60 (Cohort D) lidocaine-60 (Cohort D) PBS-60 (Cohort D) infrared-60 (Cohort A) white-72 (Cohort A)	dichlobenil-60 DMSO-60 lidocaine-60 PBS-60 infrared-60 white-72	4	3-4 weeks
S3A-C	Assay time for each pair ranking in mice with sensory defects	dichlobenil-10 cages DMSO-10 cages lidocaine-10 cages PBS-10 cages infrared-10 cages white-12 cages	dichlobenil-60 (Cohort D) DMSO-60 (Cohort D) lidocaine-60 (Cohort D) PBS-60 (Cohort D) infrared-60 (Cohort A) white-72 (Cohort A)	dichlobenil-60 DMSO-60 lidocaine-60 PBS-60 infrared-60 white-72	4	3-4 weeks
5H	Percentage of win rate between intact and sensory defects	10 pairs	20 (Cohort E)	10	4	3 weeks

56 **Transparent Methods**

57 **Participants.** The study participants were recruited from two preschools in Hsinchu City, Taiwan,
58 from the beginning of a new semester to the end of the semester, across five months. A total of 216
59 children participated after their parents provided informed consent. The children included 112 boys
60 and 104 girls between the ages of 3 years and 3 months and 6 years and 3 months (average age, 57.50
61 months). Information about sample size, number of children, number of trials, number of matchups
62 and age for each experiment is listed in Table S2.

63

64 **Social dominance games.** All children participated in competitive games of social dominance, i.e.,
65 the bunny and block-stacking games at preschool before lunch time (9am-11am). The participating
66 children were divided into groups of four. Paired encounters were staged with a round robin design
67 (comparable to the design in mouse), such that each child would encounter every other child of the
68 group, leading to a total of six combinations of pairs. During the first month of the study, the same
69 groups of children played the bunny game twice and the block-stacking game once. The same
70 groupings of four children then played the bunny game twice consecutively every other month. Thus,
71 that the bunny game was played a total of six times over the course of 5 months.

72

73 **Bunny game.** The children were told that the bunny wanted to go home, but first three wooden blocks,
74 colored red, blue, and yellow, must be placed in the correct positions as specified by the test picture,
75 and only then could the rabbit be put into the hole that represents its home. Each time the tester
76 presented a picture card showing the required placement of the blocks, she first instructed the children
77 in a competing pair to place their hands on their thighs. When the tester said, “Start,” the two children
78 would compete to place the three wooden blocks. The children could only place their bunny doll in its
79 hole when the blocks were in the correct positions as depicted in the picture card. The first child to
80 place his or her bunny doll into its hole was declared the winner.

81

82 **Block-stacking game.** This game involved stacking 12 large wooden blocks into a tower and then
83 placing a bunny doll on top of the tower. As with the bunny game, each competing pair was provided
84 with 12 blocks and a bunny doll. The children were asked by the tester to place their hands on their
85 thighs until she said, “Start,” at which time the children started stacking the blocks. After the 12 blocks
86 were stacked, the bunny doll was placed on top of the blocks, and the winner was the child who first
87 placed their bunny doll on top of their tower without collapsing it.

88

89 **Teacher questionnaires.** The teachers answered the questions in the Young Children's Social
90 Dominance Rating Scale and Temperament Assessment of Young Children questionnaires in the first
91 month. The Young Children's Social Dominance Rating Scale was evaluated again at the fifth month.
92 The teachers who worked with the children every day carried out the evaluations with respect to the
93 children's daily behaviors in the classroom. A total of eight teachers carried out the evaluations, and
94 the chief teacher of each preschool helped to check for differences in ratings across the teachers and
95 made the final decision.

96

97 **Social dominance rating scale.** Dodge's teacher rating scale was adopted for assessing the social
98 dominance levels of the young children, which was based on the Teacher Checklist of Dodge and Coie
99 (Dodge and Coie, 1987). There were five items related to social dominance (Cronbach alphas = .89):
100 “this child is a leader”, “this child gets what he or she wants”, “this child is competitive”, “this child
101 suggests to other children how things should be done”, and “this child is frequently the center of the
102 group”. The teachers respond to each statement with a seven-point Likert scale, with “1” indicating
103 “never” and “7” indicating “always”. The five scores were averaged and represented a child's social
104 dominance score.

105

106 **Temperament assessment of young children.** The Temperament Assessment of Young Children
107 questionnaire is used to guide educators in the assessment of the temperament of their students. The
108 Chinese version was developed by Wang with the reliability and validity verified in Taiwan with a
109 large sample of preschool children (Wang, 1995). This scale defines temperament as a behavioral
110 response tendency with genetic and physiological bases. The reliability of each temperament
111 dimension range from 0.73~0.89. The internal consistency coefficient for the full scale is 0.86. This
112 scale consists of 48 questions covering six temperaments: activity level, adaptability,
113 approach/withdraw, emotional intensity, distractibility, and persistence. Each question is scored on a
114 seven-point Likert scale, with “1” indicating “never” and “7” indicating “always”. The average score
115 of all questions for each dimension constitutes the score for each temperament item. The higher the
116 score, the stronger is the performances of the child in that particular temperament dimension.
117

118 **Mice.** C57BL/6J adult male mice between the ages of 8 to 10 weeks and weanling mice between the ages
119 of 3 to 4 weeks were purchased from the National Laboratory Animal Center in Taiwan. Mice were housed
120 in a controlled animal room with a 12-h light/dark cycle (0700–1900 hr). All tests were conducted during
121 the light period. To eliminate olfaction, 50 mg/mL 2,6-dichlorobenzonitrile (dichlobenil, Sigma D57558)
122 in DMSO was injected intraperitoneally into the mice on days 1, 3, and 5 before experimentation at a dose
123 of 100 µg/g body weight (Yoon et al., 2005). To block somatosensation of the mice, 10 mg/mL lidocaine
124 hydrochloride (lidocaine, Sigma L5647) in PBS was bilaterally injected into their whisker pads 10 min
125 before experimentation at a dose of 25 µg/g body weight (Mizuno et al., 2018). All animal procedures were
126 in compliance with institutional guidelines established and approved by the Institutional Animal Care and
127 Use Committee of National Tsing Hua University. Information about sample size, number of animals,
128 number of trials, number of matchups, age for each experiment and cohort information is listed in Table S2.
129

130 **Standard tube test behavioral assay for the mice.** The standard tube test for the adult mice was modified
131 from that of Wang et al. and utilized a clear Plexiglas tube with a length of 60 cm and diameter of 3.75 cm
132 (Wang et al., 2011). For training, each mouse was released at the end of a tube and allowed to run through
133 the tube, with an initial push from a plastic stick if necessary. Each animal had eight training trials on three
134 successive days. On the fourth day, a tube test trial was carried out involving two mice that were
135 simultaneously released at opposite ends of the tube and then ran toward the middle. When a mouse
136 retreated and set all four paws outside the tube, the test trial was over and that mouse was considered the
137 loser. The interior of the tube was cleaned after every pairwise with 70% ethanol. A round robin design
138 was applied to the four mice housed in each cage to allow the six possible pairs to compete. Mice were
139 tested against one another in this manner in consecutive trials, with each mouse starting at an alternative
140 end of the tube for each trial. The assay time (from mice released to loser leaving the tube) was recorded
141 for each trial.

142

143 **Tube test behavioral assay with untrained mice.** The tube test with untrained mice was performed in
144 the same manner as the standard tube test (Wang et al., 2011), but the mice were not trained first. Mice
145 were habituated to the procedure room for 1 h on two consecutive days and were tested on the third day
146 without having any previous exposure to the tube. The standard tube (3.75 cm diameter, 60 cm length)
147 was used for adult mice, and a narrower tube (2.5 cm diameter, 60 cm length) was used for weanling mice.
148 In the tube test with untrained mice, we also recorded the assay digitally and marked the position of both
149 mice at first contact as the initial territory gained and the final position of the winner when the assay ended
150 (loser left the tube).

151

152 **Platform competition assay.** A 2 cm-diameter platform was set in a 50 cm (L) ×25 cm (W) ×25 cm (H)
153 water tank with 15 cm deep water. In each trial, four mice were simultaneously placed into the water and

154 recorded for 5 minutes. Each group was tested in three trials with 35 minutes interval under an infrared
155 lamp in their homecage. Videos were analyzed to obtain total time for each mouse standing on the platform.
156 Time that mouse leaned on the platform or climbed on the mouse on the platform would not be calculated.
157 The rank among four mice were based on the time on the platform.

158

159 **Blocker assay.** For the blocker assay, we placed sufficient tissue paper in the middle of the tube to block
160 further forward movement by the mice. A mouse was released into the tube and allowed to freely interact
161 with the tissue block. The trial was over when the mouse retreated and set all four paws outside the tube.
162 Each mouse was tested in four consecutive trials and was placed at an alternating end for each trial. The
163 tube was cleaned with 70% ethanol before introduction of a new mouse.

164

165 **Open field test.** The open field test was modified from a previous study to evaluate locomotion and
166 anxiety level (Seibenhener and Wooten, 2015). A mouse was placed into a clean 50 cm x 50 cm open field
167 apparatus for 6 minutes. Animal movement was filmed from overhead, and the open field apparatus was
168 cleaned with 70% ethanol between each trial. Videos were evaluated using SMART VIDEO TRACKING
169 Software (Panlab) to determine the total moving distance to present the activity and total time in the box
170 center (25 cm x 25 cm) to present the anxiety level.

171

172 **Light-Dark box assay.** The Light-Dark Box Assay was modified from a previous study to evaluate mouse
173 anxiety level (Takao and Miyakawa, 2006). The light-dark box was made of opaque Plexiglas (50 cm x
174 25 cm white open chamber for the light box, 50 cm x 25 cm x 30 cm black closed chamber for the dark
175 box). The chambers were connected by a 5.5 cm x 4.5 cm door in the middle of the wall separating the
176 two chambers. Animals were placed in the middle of the light chamber for 6 minutes. The total time in
177 the light box was evaluated to present the anxiety level. The apparatus was cleaned with 70% ethanol
178 between each trial.

179

180 **Novelty investigation test.** The novelty investigation test was modified from the novel object recognition
181 test (Leger et al., 2013). A mouse was introduced into a box with two identical objects in the center of an
182 empty open cage [28 cm x 16.5 cm x 13 cm] for free exploration for 10 minutes. The investigation time
183 of both objects was recorded for exploration activity. The apparatus was cleaned with 70% ethanol
184 between each trial.

185

186 **Determination of stability and hierarchy relationships.** To assess the stability of the rankings of the
187 children over time, each pairwise test for the bunny game among the four children within a group was
188 conducted twice during one day in the first, third, and fifth months. For the groups of four mice, each
189 pairwise tube test was performed twice per day for three consecutive days. The final rankings were based
190 on the total winning times across all trials among all comparisons (18 trials for each child or mouse). For
191 the weanling mice, two trials were also conducted every week from the ages of 3 to 8 weeks to examine
192 their long-term social rankings. For the hierarchical relationships, winners and losers were determined
193 from the 6 trials between two children or mice. When the number of wins between two individuals was
194 equal, i.e., both with three wins and three loses, the ranking was based on the total winning numbers
195 against 3 opponents (18 trials). If the individuals in a group were ranked such that $A > B$ and $B > C$ so that
196 $A > C$, the structure was labeled as linear (L). If one individual was always the winner and no linear
197 relationship among the others existed in the group, the structure was labeled as “1 dominant” (1D). If one
198 individual was always the loser and no linear relationship among the others existed in the group, the
199 structure was labeled as “1 subordinate” (1S). All other outcomes were denoted as “other.”

200

201 **Tube test parameters.** For each mouse, the assay time for tube test and blocker assay was the average of
202 four consecutive trials. The win rate for each mouse was defined as the percentage of the number of
203 winning trials for each of the four trials between two mice and was reported as 0%, 25%, 50%, 75%, or

204 100%. The assay variation for each comparison was defined by the variability among the four trials
205 between two mice as 0, 25, or 50%. To generate a kymograph of the mouse position in tube test, the trial
206 was digitally recorded and analyzed using SMART VIDEO TRACKING software (Panlab). The assay
207 time for each trial was normalized or transformed to 100 intervals. The value for the initial territory gained
208 for each mouse was the distance from the entrance to the position that an animal first made contact with
209 its opponent in the average of four trials. The winner push was defined by winner's body moving forward
210 during physical contact between two animals. The voluntary retreat of losers was defined by separation
211 between two animals without winner push. The final position of the winner was the average of positions
212 across four trials when the loser had all four paws outside the tube.

213

214 **Statistics.** SPSS or GraphPad Prism 6.0 software was used for all statistical analyses. The Shapiro-
215 Wilkerson normality test was used to analyze the distribution of the data. For normally distributed data,
216 the paired *t*-test (two-tailed) was applied to examine the difference between winners and losers or between
217 rank-1 and rank-4 children or mice. The unpaired *t*-test (two-tailed) was used to evaluate assay time. For
218 data not normally distributed, the Wilcoxon signed-rank test (two-tailed) was applied to examine the
219 difference between winners and losers, between forced and voluntary retreat, or between rank-1 and rank-
220 4 mice. The Mann-Whitney test (two-tailed) was used to evaluate the assay time or assay variation in tube
221 test and platform time in platform competition assay. Comparison of the final position of the winning
222 mouse to forward exit and of the win rate to 50% were tested by the one-sample *t*-test (two-tailed) or one-
223 sample Wilcoxon signed-rank test (two-tailed). Pearson correlation was applied to all correlation analyses.
224 All data are represented as the mean \pm standard error of the mean (S.E.M.).

225

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