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Socially dominant mice in C57BL6 background show increased social motivation

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Abstract

A series of behavioral tests measuring social dominance, social motivation, and non-social motivation are examined in adult male C57BL6 mice. By using the well-known tube dominance test to determine social dominance and rank, we find that, in the absence of competition for resource and mating, group-housed mouse cage-mates display stable and mostly linear and transitive social hierarchies. Mice with top and bottom social ranks are subjected to a three-chamber social interaction test to measure social motivation. The top ranked mice spend more time interacting with a stranger mouse than the bottom ranked mice, suggesting that social dominance may positively influence social motivation. When subjected to a novel environment, mice with different social ranks show similar locomotion and exploring activity in the open field test, suggesting no detectable difference in certain aspects of non-social motivation. These results demonstrate a behavioral correlation between social dominance and social motivation.

Keywords

Behavior in mice; social dominance; social interaction; social rank; social motivation; social cognition

As a major aspect of social hierarchy, social dominance can be observed in different social species across the animal kingdom, from insects to fish, birds, rodents, and human and non-human primates (1, 2). It is known that the establishment of social rank and dominance may help to determine the accessibility of resources and the degree of reproductive success without the cost of significant fighting and society damage (2, 3). When considering the societal organization of social creatures, it has been found that social hierarchy plays a potential role in general health and well-being (2, 4). While recent molecular and imaging studies have identified functional involvement of prefrontal cortex in social hierarchy processing in rodents as well as in humans (5, 6), it is also recognized that prefrontal cortex may regulate other aspects of social and non-social cognition (7). Specifically, how social

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hierarchy and dominance affect social and non-social motivation remains largely unclear. This study, by using a series of behavioral tests, first identifies social rank, and then examines dominant and subordinate mice in social motivation and non-social novelty exploration.

We used the tube dominance test (Fig. 1A), which is a reliable paradigm to measure dominant behavior in rodents (5, 8, 9), to determine social rank. To avoid possible genetic complications, competition on resources and mating, and aggression toward intruders, we used inbred C57BL6 male mice in laboratory cages with free and un-limited access to food and water. Three to five littermates (due to variable litter size in different colonies) were housed in a cage from the time of weaning, and subjected to tube dominance test at about 3 months of age. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University.

Social rank in each individual cage was determined. Among all mice in a total of 8 different cages, no obvious fighting was observed, indicating no significant aggressive attacking. Before social rank determination, each mouse went through three consecutive days of pre-training, during which mouse was exposed to the tube and allowed to enter and pass through the tube (a PVC pipe with a diameter of 3.175 cm, small enough to prevent mouse from turning around inside the tube). On each pre-training day, every mouse went back and forth through the tube three times (six passes in total). During testing, two mice were placed at opposite ends of the tube, where they entered the tube at the same time. The mice were given two minutes to force the other to retreat from the tube (Fig. 1A). If a mouse backed out, it was deemed to be the subordinate, while the one remaining in the tube was the dominant and thus ranked higher in social hierarchy. If after two minutes neither mouse retreated, the match was ruled a tie. Testing was done in a round-robin fashion, with each mouse facing against all of its cage-mates two times (placed at different entry end of the tube for each test). Overall social rank in a specific cage was determined by the total score from all tests (+1 for each dominant result, -1 for each subordinate result, and 0 for tie) with the highest score being the most dominant. The test was repeated on four consecutive days, and we found that, in all eight cages tested, the same mouse was the top ranked mouse on each of the testing days (Fig. 1B). Mice in six cages did not show shifting in social rank throughout the 4 testing days (Fig. 1B1). Mice in the other two cages had some shift in rank after the first test, but no rank shift after the second test (Fig. 1B2 and 1B3). Seven of the eight cages had a linear social structure and a transitive hierarchy as defined in Wang et al (5) (Fig. 1C). The last remaining cage had a non-linear social structure in which complete transitivity was not observed (Fig. 1D). Because the non-linear and non-transitive cage (Fig. 1B3 and 1D) still had a top and bottom ranked mouse, these mice were still used in the other tests. Overall, we found that, even in the absence of competition for resource and mating, group housed mice show stable social hierarchy without obvious involvement of aggressive fighting. These stable hierarchies allowed top- and bottom-ranked mice to be easily identified.

Next, we subjected the top ranked (n=8) and bottom ranked (n=8) mice to the 3-chamber social interaction test (10), which reflects certain aspects of social motivation (7). Mice were first introduced to the middle chamber (Fig. 2A) of a three-chamber plexiglass cage with

small openings to connect the adjacent chambers and allow free passage from one chamber to another. Following a 5-min acclimation, during which the mouse explored the plexiglass cage, a stranger male C57BL6 mouse was placed in a small wire enclosure in a chamber on one end of the plexiglass container (the social object holding chamber). A novel inanimate object was placed in another wire enclosure in the chamber on the opposite end (the non-social object holding chamber) (Fig. 2A). During the 10-min examination, the mouse being tested was scored for the number of entries to each chamber (Fig. 2B), the amount of time in each chamber (Fig. 2C), and the amount of time spent directly interacting with the stranger mouse or the non-social novel object (i.e. total time spent during putting its nose in between the wire cage bars) (Fig. 2D). Data from the 3-chambered test was analyzed using an unpaired t-test (SPSS statistics software). Top and bottom ranked mice made comparable entries to the chambers holding either the stranger mouse (the social object) (7.25 ± 0.62 versus 6.63 ± 0.84 ; $t(14) = 0.5939$, $p = 0.56$) or the non-social object (7.63 ± 0.86 versus 6.88 ± 0.48 ; $t(14) = 0.7615$, $p = 0.4590$) (Fig. 2B). They also spent comparable time in the social object holding chamber (313.5 ± 25.63 versus 283.625 ± 42.23 , $t(14) = 0.6048$, $p = 0.555$), and the non-social object holding chamber (204.875 ± 14.52 versus 239.5 ± 40.64 ; $t(14) = 0.8023$, $p = 0.4358$) (Fig. 2C). However, the top ranked mice spent significantly more time in the social object holding than the non-social chamber ($t(14) = 3.6876$, $p < 0.05$) (Fig. 2C). The bottom ranked mice did not show preference to the social object holding chamber over the non-social object holding chamber ($t(14) = 0.7925$, $p = 0.464$) (Fig. 2C). We further found that the top ranked mice spent more time in direct interaction with the stranger mouse than did the bottom ranked mice (47.0 ± 4.24 versus 31.9 ± 4.30 ; $t(14) = 2.5005$, $p < 0.05$) (Fig. 2D). In contrast, they showed comparable interaction with the non-social novel object (3.1 ± 0.42 versus 2.75 ± 0.71 ; $t(14) = 0.4243$, $p = 0.6778$) (Fig. 2D). Overall, these data show that mice with higher social rank are more engaged in social interaction, indicating increased social motivation. As past studies have demonstrated that dominant mice may not be necessarily aggressive (11), we expect that the increased social interaction in the top-ranked mice is unlikely due to increased aggression. Interestingly, in some situations, aggressive mice show reduced social interaction (12).

We next determined whether social status would also influence certain aspects of motivation-related behavior in non-social domain. Specifically, we examined novelty exploration behavior by subjecting mice to an open field arena with novel contextual and spatial cues (13). During testing, mice of top or bottom social rank were placed in an E63-12 chamber (Coulbourn Instruments) for thirty minutes. Locomotive movement distance and time were recorded by infrared sensors and the TRUScan 2.01 software (Coulbourn Instruments), and analyzed for each of the 2-min bin during the 30-min test. The top and bottom ranked mice showed similar overall ambulatory movement time ($F_{1,12} = 1.02$, $p = 0.333$, repeated measures ANOVA, SPSS statistics software) (Fig. 3A) and ambulatory travel distance ($F_{1,12} = 0.823$, $p = 0.382$ repeated measures ANOVA, SPSS statistics software) (Fig. 3B). Their exploratory locomotion behavior was also not distinguishable during the first 2 min exposure to the novel open field chamber [80.57 ± 5.29 versus 91.57 ± 5.49 ; $t(14) = 1.4428$, $p = 0.1711$ for movement time; 150.0 ± 19.5 versus 181.0 ± 20.2 ; $t(14) = 1.1041$, $p = 0.2882$ for distance traveled; unpaired t-test (SPSS statistics software)] (Fig. 3A and 3B). These data suggest that social status does not have a detectable influence on certain aspects

of non-social motivation in novelty exploration. This lack of difference in non-social motivation is consistent with the result from the three-chambered test, where top and bottom ranked mice spent comparable time investigating a non-social novel object (Fig. 2D).

Intriguingly, we found that the top ranked mice spent less time in the center area of the open field arena than the bottom ranked mice ($F_{1,12}=4.894$, $p<0.05$, repeated measures ANOVA) (Fig. 3C). The top ranked mice had slightly less entries to the center region than the bottom ranked mice without statistical significance ($F_{1,12}=4.666$, $p=0.052$, repeated measures ANOVA) (Fig. 3D). Although it has been speculated that lower activity in the center region may reflect anxiety- and stress-related behavioral outcome (14), it remains unclear whether it also reflects less risk-taking. Regarding whether social status impinges on anxiety and stress, there are seemingly controversial opinions. Previous studies have shown that in some species, such as savannah baboons, animals at the top of the social hierarchy have high basal levels of circulating glucocorticoids (15, 16). However, other studies indicate that dominant animals have lower basal glucocorticoid levels (15, 17). Interestingly, Blanchard et al. (17) found that subordinate rodents have higher basal glucocorticoid levels, but the dominant ones have higher acute glucocorticoid levels in response to a stress stimulus. Considering that a certain level of stress is essential for the peak performance of many fundamental brain functions and not necessarily pathological (18), variation and changes in hormone levels may not directly reflect the physiological or pathological condition of the brain. Further, depending on the resource and mating availability, social status may not always have the same impact on motivation, anxiety, and risk-taking.

In summary, we used the tube dominance test as a reasonably reliable paradigm to determine social rank (5, 8, 9) in C57BL6 littermates, which were housed together since their birth. The mice with stable social dominance status show increased motivation in certain aspect of social but not non-social novelty exploration. Human and rodent studies have implicated that increased activity in the medial prefrontal cortex (mPFC) predicts a higher social rank (5). Additionally, the mPFC has widespread projections to numerous regions including the dorsal raphe nucleus, hippocampus, amygdala, and ventral tegmental area (19, 20). It is interesting to note that, through these pathways, the mPFC can also exert control over motivation, fear/anxiety response, and stress responsiveness (21–23). Future investigations may aim to determine whether and how distinct neuronal population in mPFC and their projection to other brain regions cross talk and co-regulate social status and social motivation.

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Fig. 1.

Performance in the tube dominance test indicates stable social hierarchy in group housed male C57BL6 mice. (A) Tube test as a behavioral paradigm to determine social dominance and rank. During testing, two mice are placed at the opposite ends of the tube, and encounter inside the tube. The mouse which forces the other to retreat and exit the tube is considered to be socially dominant and is ranked higher than the retreating mouse. (B) Social ranks in the eight social groups tested on 4 consecutive days. (B1) Pattern of rank in six of the tested social groups. (B2) Pattern of rank in one of the tested social groups. (B3) Pattern of rank in another social group. (C and D) Social rank pattern in descending order (from top to bottom: 1 represents the most dominant and 5 represents the least dominant). (C) Linear social structure seen in seven of the eight cages. (D) Non-linear social structure seen in one cage.

Fig. 2.

Socially top ranked mice show more direct interaction with stranger mouse. (A) A 3-chamber social interaction set up, of which one chamber holds an enclosed stranger mouse as the novel social object and another chamber holds an enclosed novel non-social object. (B) The top ranked mice (n=8) and bottom ranked mice (n=8) show comparable entries to the social and non-social object chambers. (C) The top ranked, but not the bottom ranked, mice spend more time in the social object chamber than the non-social object chamber. (D) The top ranked mice spend more time than the bottom ranked mice in direct interaction with the social object (i.e. the stranger mouse). Data are presented as mean $\pm$ SE *: $p < 0.05$; ns: not significant; determined by t-test.

Fig. 3.

Non-social novelty exploration activity. The top ($n=8$) and bottom ranked mice ($n=8$) were placed into a novel open field test chamber. Their activity was recorded and analyzed for each of the 2-min duration during the 30-min test. (A) The top and bottom ranked mice show similar movement time in the novel open field arena. (B) The top and bottom ranked mice show similar movement distance in the novel open field arena. (C) The top ranked mice spend less time in the center area of the novel open field arena. (D) The top and bottom ranked mice show comparable number of entries to the center area of the novel open field arena. Data are presented as mean $\pm$ SE.