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Group Social Dynamics in a Seminatural Setup Reflect the Adaptive Value of Aggression in Male Mice

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ABSTRACT

BACKGROUND: Maladaptive aggression in humans is associated with several psychiatric conditions and lacks effective treatment. Nevertheless, tightly regulated aggression is essential for survival throughout the animal kingdom. Studying how social dominance hierarchies regulate aggression and access to resources in an enriched environment (EE) can narrow the translational gap between aggression in animal models and normal and pathological human behavior.

METHODS: The social box is a seminatural setup for automatic and prolonged monitoring of mouse group dynamics. We utilized the social box to decipher tradeoffs between aggression, social avoidance, resource allocation, and dominance in 2 mouse models of increased aggression: 1) a model of early exposure to an EE and 2) a model of oxytocin receptor deficiency (*Oxtr*$^{-/-}$). While environmental enrichment increases aggression as an adaptive response to external stimuli, hyperaggression in *Oxtr*$^{-/-}$ mice is accompanied by marked abnormalities in social behavior.

RESULTS: EE groups exhibited significant social avoidance, and an increased proportion of their encounters developed into aggressive interactions, resulting in lower levels of exploratory activity and overall aggression. The hierarchy in EE groups was more stable than in control groups, and dominance was correlated with access to resources. In *Oxtr*$^{-/-}$ groups, mice engaged in excessive social encounters and aggressive chasing, accompanied by increased overall activity. In *Oxtr*$^{-/-}$ groups, dominance hierarchies existed but were not correlated with access to resources.

CONCLUSIONS: Measuring aggression and social dominance hierarchies in a seminatural setup reveals the adaptive value of aggression in EE and *Oxtr*$^{-/-}$ mice. This approach can enhance translational research on pathological aggression.

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Aggression is an easy and intuitive behavior to detect in humans and other animals, including mice in the laboratory. However, assessing whether aggressive behavior is beneficial or detrimental poses a complex challenge (1). In humans, aggression can be either instrumental or hostile (2); whereas instrumental aggression is a calculated effort to obtain desired objectives, hostile aggression is a spontaneous, impulsive act, the purpose of which is to harm another person (3). Theoretical models further delineate pathological aggression as an extreme and unreasonable act, relying on multiple criteria such as personality and context (4). In contrast to human studies, in ethological research, the term pathological aggression is not applied even to deadly aggressive acts, which are observed as legitimate survival-oriented measures (5). Therefore, maladaptive human aggression currently lacks an appropriate animal model, potentially hampering the translation of research findings to human applications.

Most preclinical studies measure aggression in the resident-intruder (RI) test, quantifying the readiness of a male mouse to defend its cage (the territory) from an unfamiliar intruder (6). The RI test has led to significant advances in describing the central aggression network (7). Nevertheless, from an ethological perspective, short dyadic paradigms that model aggression in a relatively specific context may have limited translational value (8).

As opportunists, mice adapt to diverse habitats, and commensal mice that live near human dwellings, where food resources are patched and concentrated, display more fierce territorial conflicts than feral mice (9). Consistent with that, in laboratory settings, aggression increases with the enrichment of the setup (10). For example, there is an increase in intermale aggression and the frequency of injuries from biting in more vast and crowded arenas (11). Similarly, biting injuries are more common in groups of males following prolonged postweaning housing in an enriched environment (EE) (12). Therefore, the complexity of the setup in the laboratory, the group and arena size, as well as the time of exposure to these enriched factors, all simultaneously affect levels of aggression (10–12).

Although aggression during the competition for scarce resources such as food, mates, and shelter is beneficial, it is also risky and must be tightly controlled. Establishing social dominance hierarchies (SDHs) minimizes aggression by setting

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a priority order of access to desirable resources, thereby avoiding unnecessary conflicts (13). Thus, dominance hierarchy could be set as a reference measure for delineating maladaptive aggression in preclinical studies in laboratory settings (14). SDHs in mice are commonly tested using the tube test (15); however, simultaneously measuring aggression and SDHs in the same group of mice using the RI and the tube tests is not feasible.

We postulated that monitoring aggression and SDHs in groups of mice in a seminatural setup could bridge the translational gap and help elucidate mechanisms that underlie pathological aggression (16,17). A few groups of investigators have developed seminatural setups for continuous monitoring of groups of mice (18,19). Our group developed a seminatural setup called the social box (SB) (20). In the SB paradigm, mice with different color-marked fur are uninterruptedly maintained in a large, complex arena and video recorded over several days. Next, the videos are subjected to automatic tracking of the individual mice, enabling the extraction of multiple behavioral readouts, such as aggressive chasing and escaping (21–23). Consistent within-group imbalance between chase and escape behavior is further mathematically expressed to represent SDHs (24).

Here, we utilized our seminatural setup to test both social and individual behavioral characteristics in 2 mouse models that display increased aggression: 1) aggression due to early and prolonged exposure to EE and 2) aggression due to developmental deficiency of the oxytocin receptor (*Oxtr*) (25,26). Early exposure to EE benefits the individual physiologically and encourages ingroup competition (11). Malfunction of the oxytocinergic system due to *Oxtr* deficiency (*Oxtr*$^{-/-}$) alters social behaviors, including aggression (27–29). We hypothesized that EE-induced aggression would reflect an adaptation contributing to group coherence as measured by social interactions, hierarchy, and exploration in the SB. In contrast, because the hyperaggressive phenotype of *Oxtr*$^{-/-}$ mice is shaped by a malfunction in the oxytocin system rather than by environmental needs, we hypothesized that this manipulation would impair group coherence in the SB.

METHODS AND MATERIALS

All methods and materials are detailed in Supplemental Materials and Methods. The Institutional Animal Care and Use Committee of the Weizmann Institute of Science approved all experimental protocols.

Aggression in the SB Due to Early and Prolonged Exposure to an EE

At the age of weaning (3 weeks), mice were randomly distributed into 2 types of groups: standard condition (SC) mice that were housed in groups of 4 in standard laboratory cages and EE mice that were housed in groups of 16 in a spacious and complex cage with a variety of objects (30) (Figure 1A). Six weeks later, 4 of 16 mice in each group were randomly chosen to constitute the treatment groups (EE), and the fur of each of the 4 mice was colored differently. These 4 mice were returned to the enclosure for an additional week, while the rest of the mice were omitted from the experiment. Then, at the age of 10 weeks, the group was introduced into the SB for uninterrupted tracking for 12 hours each day during the dark phase over 4 consecutive days. As a control, we used 8 groups of 4 male mice housed in a home cage (SC) until they were introduced to the SB at 10 weeks (Figure 1A).

Aggression in the SB Due to *Oxtr*$^{-/-}$

Eleven groups of *Oxtr*$^{-/-}$ (treatment) and 8 groups of *Oxtr*$^{+/+}$ (control) weanlings were assembled from heterozygous breeding (*Oxtr*$^{-/+}$ × *Oxtr*$^{-/+}$) of mixed background. Each group consisted of 4 males kept in a standard home cage for 7 weeks. Thereafter, at the age of 10 weeks, the groups were introduced into the SB for 4 days (Figure 1B).

RESULTS

Exploratory and Social Activity Is Decreased Following Adolescent Exposure to an EE but Increased in *Oxtr*$^{-/-}$

Location ethograms depict the time each mouse spent in the different regions of interest of the SB, and dyadic interaction ethograms depict approaches, contacts, and chases between pairs in the group (Figure 2A–C). Dyadic interactions were modeled based on the relative ambulatory trajectories of the mice, the distance d between the mice, and the angle θ of movement of each of the mice respective to each of the others (Figure 2B) (24). All dyadic interactions occur outside the regions of interest large nest and small nest, which are enclosed structures that mice typically choose for rest.

During a chase, no direct aggression is applied. However, in the SB, most chases are fast, tend to end in a shelter, and are considered agonistic interactions. Therefore, we hypothesized that direct aggression before or after a chase would be expected. To measure direct aggression before and after a chase event, we systematically observed 1555 chase episodes in both experiments. We defined the beginning of an episode as the first frame of contact and the end of an episode as the first frame of detachment following a chase. We found that 65% of the episodes were accompanied by direct aggressive displays (push to nest, mount, aggressive grooming, bite to the back, attack, and defensive aggression) either before, during, or after a chase. We found a strong correlation between the number of aggressive events relative to the total chase events per individual (Pearson correlation, $r = 0.76$, $p < .001$) (Figure S1). This suggests that the number of chases could be a reliable proxy for measuring aggression events.

Visual inspection of the locations and dyadic interaction ethograms implies that EE mice exhibited lower exploratory activity and social interactions than SC controls (Figure 2D, E). Comparison between *Oxtr*$^{-/-}$ and *Oxtr*$^{+/+}$ groups (Figure 2F, G) revealed exaggerated exploratory activity and increased dyadic interactions in *Oxtr*$^{-/-}$ groups compared with *Oxtr*$^{+/+}$controls.

Next, we defined a set of 21 a priori chosen, automatically extracted behavioral readouts and quantified the separation of those readouts in EE versus SC and *Oxtr*$^{-/-}$ versus *Oxtr*$^{+/+}$ groups in Euclidean space (22). These behavioral readouts included exploratory phenotypes and social interactions. Repeating this procedure multiple times with random partitioning of data revealed that EE groups and *Oxtr*$^{-/-}$ groups show a statistically significant difference from their corresponding controls across the 21 dimensions of our dataset

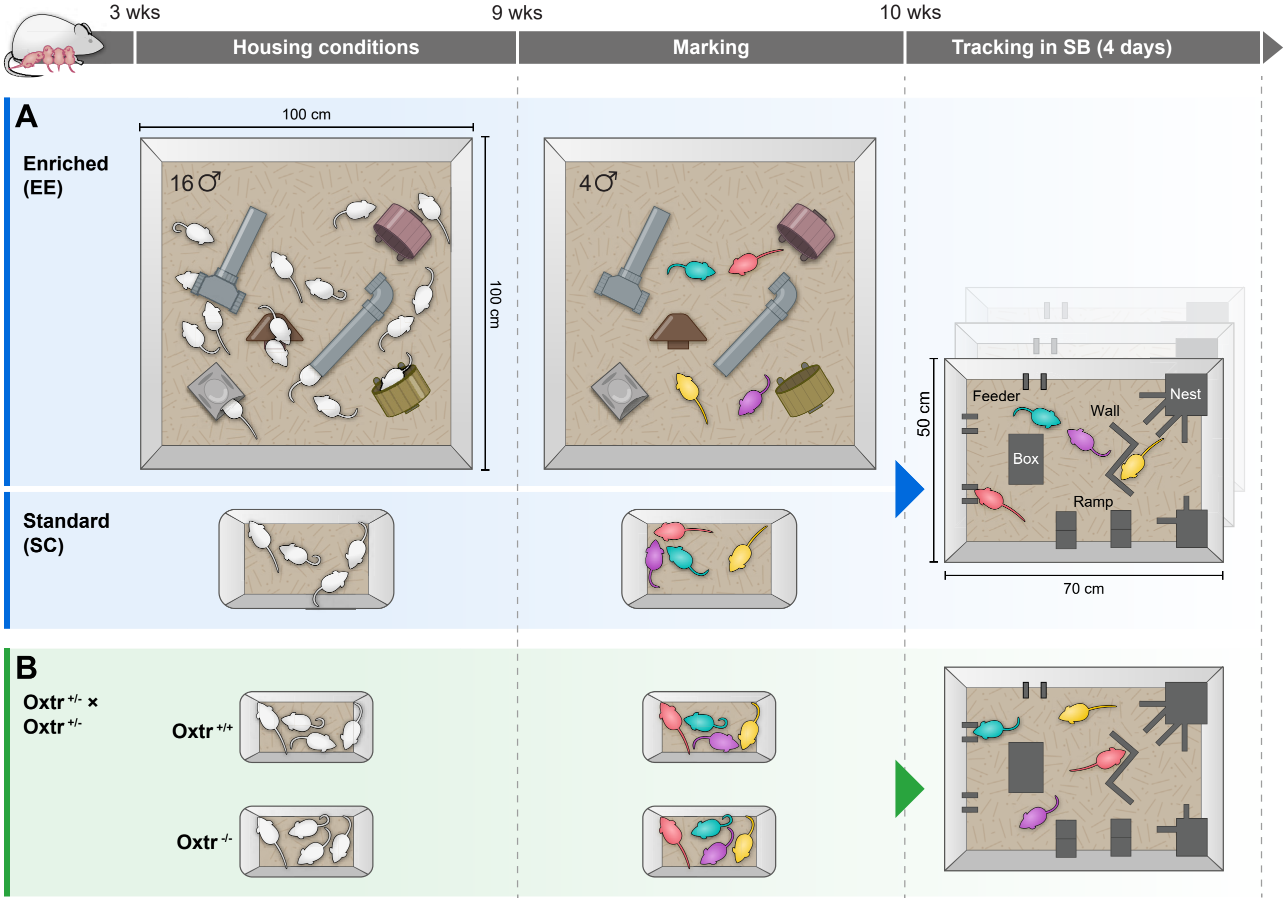

Figure 1. Experimental design. **(A)** EE groups of 16 male mice were kept in the EE cages during adolescence; 4 of them were marked, regrouped and reintroduced to the EE cages for a week and transferred to the SB at the age of 10 weeks. **(B)** *Oxtr*$^{-/-}$ mice were kept in groups of 4 males in a standard home cage until transfer to the SB at the age of 10 weeks. The SB arena was divided into discrete regions of interest: open area, Z wall, water, feeders, large nest, block, and small nest. EE, enriched environment; SB, social box; SC, standard condition.

(Figure S2A; 10^7 permutations, $p = .011$; Figure S2B; 10^7 permutations, $p = 2.11 \times 10^{-5}$). In addition, to illustrate the separation in a lower dimensional space, we plotted the scores of the first 2 principal components that emerged from a principal component analysis for each experiment and observed a considerable degree of separation in EE versus SC (Figure 2H and Figures S3A and S4A) and in *Oxtr*$^{-/-}$ versus *Oxtr*$^{+/+}$ (Figure 2I and Figures S3B and S4B), indicating that the treatment effect (EE or *Oxtr*$^{-/-}$) constitutes a considerable source of variance in the dataset.

To identify the specific behavioral readouts that potentially drove the multivariate phenotypic differences, we fit a logistic regression model that attempts to predict the treatment group (EE vs. SC and *Oxtr*$^{-/-}$ vs. *Oxtr*$^{+/+}$) based on the set of behavioral readouts and estimates the contribution of each behavioral readout. Importantly, to remove potential collinearity between the behavioral features, we first whitened the behavioral data using zero-phase component analysis. This model revealed that the most potent positive predictor variable for EE is the fraction of time spent alone outside the nest ($\beta = 3.2$; 95% CI, 2.3 to 5.7; $p = 2 \times 10^{-4}$) (Figure 2J). In other words, with all other behavioral variables held constant, EE mice tended to spend more time alone outside the nest. Readouts that were found to be significant negative contributors were ones that related to social activity (rate of contacts, being approached or followed) and exploratory behavior (mean distance from walls, total distance traveled, and median speed), (rate of contacts: $\beta = -3.9$; 95% CI, -5.7 to -3.2; $p = 2 \times 10^{-4}$; being approached: $\beta = -2.3$; 95% CI, -3.1 to -1.6; $p = .036$; being followed: $\beta = -2.9$; 95% CI, -5.4 to -2.1; $p = 2 \times 10^{-4}$) (Figure 2J). These results suggest that animals in EE groups show social avoidance and reduced exploratory activity compared with animals in SC groups.

In the *Oxtr*$^{-/-}$ experiment, the prediction of *Oxtr*$^{-/-}$ against *Oxtr*$^{+/+}$ revealed rate of contacts, chasing and escaping, as well as the fraction of time spent outside the nest as significant positive predictor variables, while route predictability (namely, the averaged mutual information of subsequent transitions between regions of interest) as a negative predictor variable (rate of contacts: $\beta = 2.3$; 95% CI, 1.5 to 4.5; $p = .006$; chasing: $\beta = 1.9$; 95% CI, 0.7–3.5; $p = .021$; escaping: $\beta = 2.6$; 95% CI, 1.6 to 5; $p = .031$; fraction of time outside the nest: $\beta = 1.7$; 95% CI, 0.79 to 3.2; $p = .011$; route predictability: $\beta = -2.2$;

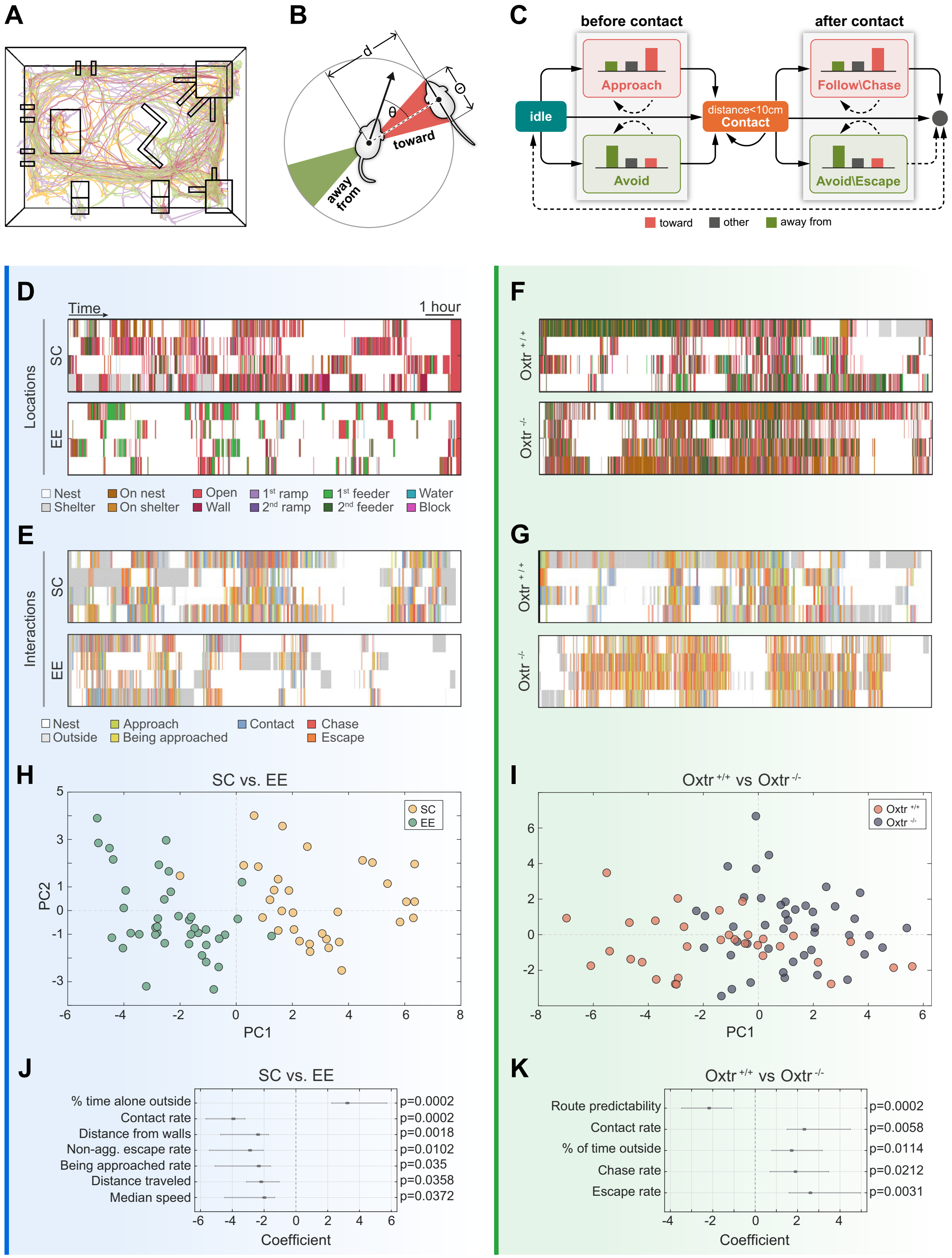

Figure 2. Exploratory and social activity decreases following adolescent exposure to EE, but increases in *Oxtr*$^{-/-}$ mice. **(A)** A representative group depicting a 15-minute segment of each mouse's tracked paths (ambulatory trajectories). **(B, C)** Computational model of dyadic interactions. The algorithm used the distance (*d*) and the relative direction of the mouse respective to its conspecifics (θ) to determine whether a mouse was moving toward another mouse, away from it, or neither. A contact is defined as a dyadic distance of <10 cm between the 2 centroids of the mice. A Hidden Markov Model detects the events leading

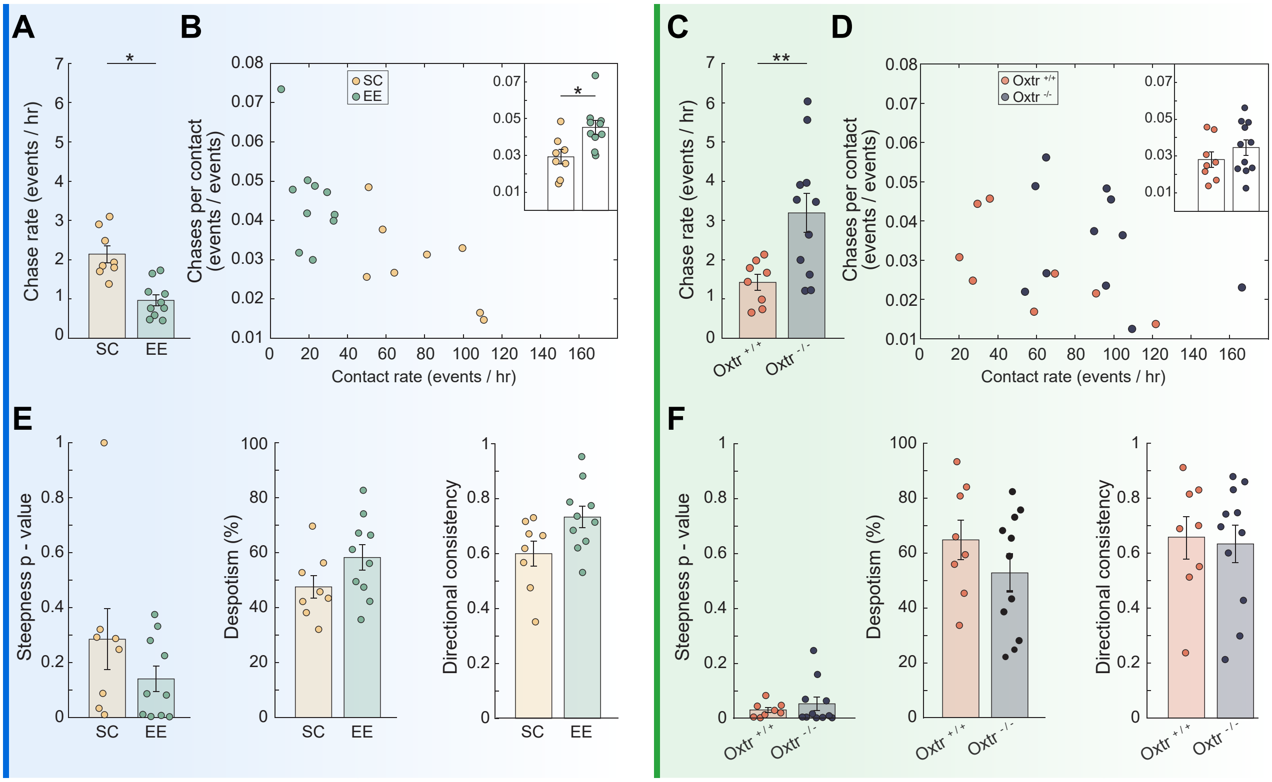

Figure 3. Divergent expression of hyperaggression, but enduring social dominance hierarchies in EE and *Oxtr*$^{-/-}$ groups. **(A–D) (A)** EE groups chase significantly less than SC groups. **(B)** While the rate of social contact is lower in EE than in SC groups, the probability of a contact ending in aggression (a chase) is higher. The inset depicts a quantitative analysis of the chases per contact (by definition, each chasing event is preceded by a contact). **(C)** *Oxtr*$^{-/-}$ groups show an elevated rate of chases compared with *Oxtr*$^{+/+}$ groups. **(D)** While the rate of contacts in *Oxtr*$^{-/-}$ groups is higher than that of *Oxtr*$^{+/+}$ groups, the probability of each contact ending in aggression (a chase) is similar. The inset depicts a quantitative analysis of the chases per contact. **(E)** No differences between EE and SC groups were found in the steepness of hierarchy, despotism, or directional consistency. **(F)** No differences were found between *Oxtr*$^{-/-}$ and *Oxtr*$^{+/+}$ groups in the steepness of hierarchy, despotism, or directional consistency. Values are mean ± SEM; * and ** differ from the corresponding control value ($p < .05$ and $p < .01$, respectively). EE, enriched environment; SC, standard condition.

95% CI, −3.5 to −1.1; $p = 2 \times 10^{-4}$) (Figure 2K). Together, these results suggest that, compared with the *Oxtr*$^{+/+}$ control groups, the *Oxtr*$^{-/-}$ groups showed increased exploratory behavior and social activity, especially reciprocal aggressive chasing (Figure 2K). These findings suggest distinct patterns of group social dynamics in these 2 models that display elevated aggression that likely represent opposite behavioral effects—an overall reduction in exploration and social engagement in groups exposed to EE during adolescence and an increase in exploration and social engagement in *Oxtr*$^{-/-}$ groups.

Divergent Expression of Hyperaggression in EE and *Oxtr*$^{-/-}$ Groups

Next, we directly compared the number of social encounters and aggressive chases in EE versus SC and *Oxtr*$^{-/-}$ versus *Oxtr*$^{+/+}$ groups. While the absolute rate of aggressive chasing in EE groups was drastically lower than in SC control groups (permutation test, 10^7 permutations, $p = .0002$) (Figure 3A), the EE mice engaged in significantly fewer contacts than SC control mice (permutation test, 10^7 permutations, $p = .00002$) (Figure 3B). Overall, the fraction of contacts that developed into an aggressive chase was significantly higher in EE groups than in the SC group (permutation test, 10^7 permutations, $p = .008$) (Figure 3B inset). In contrast, the *Oxtr*$^{-/-}$ groups' absolute rate of aggressive chasing was drastically higher than that of the *Oxtr*$^{+/+}$ control groups (permutation test, 10^7 permutations, $p = .009$) (Figure 3C), and they also engaged in significantly more contacts than *Oxtr*$^{+/+}$ control groups (permutation test, 10^7 permutations, $p = .037$) (Figure 3D). However, the fraction of contacts that developed into chasing was not

up to contact (Supplement). **(D)** Representative second-day EE and SC location ethograms. **(E)** Representative second-day EE and SC dyadic interaction ethograms. **(F)** Representative second-day *Oxtr*$^{-/-}$ and *Oxtr*$^{+/+}$ location ethograms. **(G)** Representative second-day *Oxtr*$^{-/-}$ and *Oxtr*$^{+/+}$ dyadic interaction ethograms. **(H, I)** A scatterplot of the PC analysis' PC1 and PC2 scores represents the behavioral readouts of EE vs. SC **(H)** and *Oxtr*$^{-/-}$ vs. *Oxtr*$^{+/+}$ **(I)** groups. Each dot represents a single mouse. **(J, K)** Coefficients and 95% CIs of logistic regression models that predict EE vs. SC **(J)** and *Oxtr*$^{-/-}$ vs. *Oxtr*$^{+/+}$ **(K)**. Positive or negative values correspond to behavioral readouts that, with the other behavioral readouts held constant, are more or less frequent in treatment groups (EE or *Oxtr*$^{-/-}$) than in control groups. agg., aggressive; EE, enriched environment; PC, principal component; SC, standard condition.

significantly different (permutation test, 10^7 permutations, p = .29) (Figure 3D inset). Therefore, EE groups showed reduced social interactions and aggression compared with SC groups, with an increased likelihood of each interaction being aggressive. Such a strategy potentially minimizes the risks of injury. In contrast, compared with $Oxtr^{+/+}$ groups, the $Oxtr^{-/-}$ groups showed increased social interactions and aggression, thus putting individuals at a higher risk.

Next, we tested whether the increase in aggressive chases per contact in EE compared with SC and the increase in total aggressive chases in $Oxtr^{-/-}$ compared with $Oxtr^{+/+}$ was accompanied by a shift toward more direct aggression. Toward that aim, we divided the 1555 observations of chase episodes into 3 categories: 1) no direct aggression during a chase episode, 2) mild aggression (push to nest, mount, aggressive grooming), and 3) intense aggression (bite to back, attacks, defensive aggression). We found that the relative proportions of each category did not differ between the EE and SC groups (Figure S5). However, $Oxtr^{-/-}$ groups displayed more mild aggression and fewer chases with no direct aggression than $Oxtr^{+/+}$ groups (2-proportion z tests $p < .01$ for both no direct aggression and mild aggression).

SDHs Endure in Both EE and $Oxtr^{-/-}$ Groups

Next, we asked how social avoidance and increased aggression per contact in EE groups and increased social interactions and total levels of aggression in $Oxtr^{-/-}$ groups affect the SDHs. Using the David's score (DS) method (31) and calculating the imbalance of weaning of aggressive chases in the SB, we previously demonstrated that groups of 4 male mice in the SB tended to form prominent and stable hierarchies (24). Here, based on the DS, we calculated a set of metrics that reflect different characteristics of the hierarchy in each group, namely, the steepness, despotism (the fraction of total encounters dominated by the top-ranking subject), and directional consistency (the fraction of encounters dominated by the higher-ranked subject within each dyad) (Figure 3E, F). Using a randomization procedure, we calculated p values for the steepness of EE versus SC and $Oxtr^{-/-}$ versus $Oxt^{+/+}$ groups and found no significant differences (permutation test, 10^7 permutations, p = .22 and p = .51, respectively). There were also no significant differences in despotism (p = .11 and p = .024, respectively). Directional consistency was slightly higher in EE than SC groups (p = .04) and did not differ between $Oxtr^{-/-}$ and $Oxtr^{+/+}$ groups (p = .82).

Temporal Dynamic Analysis of SDH

Although $Oxtr^{-/-}$ mice display elevated social interactions, exploration, and aggression, they maintain SDH. This points to the importance of the hierarchical structure in the social dynamics of the group. SDH can be maintained with minimal aggression or continuous effort. Therefore, we asked how efficient the maintenance of a dominance hierarchy is in EE versus SC groups and in $Oxtr^{-/-}$ versus $Oxtr^{+/+}$groups. DS for each group member is based on the total number of chases (wins) and escapes (losses) over the 4 days in the SB. We hypothesized that a temporal model of wins/losses over the 4 days of the experiment could help decipher intricate differences in hierarchy dynamics. We expected improved maintenance of dominance in EE and therefore fewer temporal changes in EE than in SC groups. In contrast, we expected more temporal changes in $Oxtr^{-/-}$ than in $Oxtr^{+/+}$ groups. Toward that aim, we analyzed the dyadic aggressive chases in the group using the Elo rating model. The Elo rating is a standard method for modeling SDHs where the winner and loser scores are symmetrically adjusted according to the outcome and proportionally to the difference between the current ratings. As such, an unexpected outcome, such as a subordinate's win against a powerful competitor, results in a more significant adjustment and vice versa (32). Visual qualitative inspection of the Elo rating trajectories of SC groups suggested unstable dynamics with frequent changes (Figure 4A). EE groups showed a divergence between one of the mice and the rest, with minimal changes occurring from the second experimental day onward (Figure 4B). By integrating the fluctuations of the Elo rating values throughout the experiment, we quantitatively estimated the inconsistency of each encounter outcome (i.e., the temporal instability of the hierarchy). As predicted, significantly fewer changes occurred in EE groups than in SC groups ($t_{16} = -3.5$, p = .003) (Figure 4C). Qualitative (Figure 4D, E) and quantitative (Figure 4F) inspection of the $Oxtr^{-/-}$ versus $Oxtr^{+/+}$ groups did not reveal apparent differences in $Oxtr^{-/-}$ versus $Oxtr^{+/+}$ (t_{17} = 0.76, p = .46).

Differential Access to Resources Is Strengthened in EE Compared With SC Groups and Weakened in $Oxtr^{-/-}$ Compared With $Oxtr^{+/+}$ Groups

SDHs prioritize access to food, water, territory, and mates, thereby minimizing energy expenditure and the risk of injury through an aggressive encounter. Differential access to resources in the SB can be quantified as the time spent occupying the territory outside the shelter, being close to the feeders or water, and the patterns of ambulation trajectories outside the nest. To study the link between access to resources and dominance as measured using within-group chasing (DS), we correlated (Pearson correlation) the DS with the within-group normalized values of the percentage of the time spent outside, the fraction of time near food or water, and route predictability.

In both SC and EE groups, the DS had a positive linear relationship with fraction of time spent near food and water (SC: r = 0.27, p = .129; EE: r = 0.63, $p = 1.29 \times 10^{-5}$) (Figure 5A). A permutation test revealed a trend toward a stronger correlation in EE (p = .1, 10^6 permutations). In both SC and EE groups, the DS had a positive linear relationship with the fraction of time outside (SC: r = 0.39, p = .026; EE: r = 0.72, $p = 1.3 \times 10^{-7}$) (Figure 5B). There was no significant difference in the correlations between SC and EE (permutation test, p = .45, 10^5 permutations). Route predictability was negatively correlated with the DS in EE groups, but not in SC groups (SC: $r = -0.19$, p = .3; EE: $r = -0.43$, p = .006) (Figure 5C). Nevertheless, there was no significant difference in the correlations between SC and EE (permutation test, p = .35, 10^5 permutations).

In both $Oxtr^{+/+}$ and $Oxtr^{-/-}$ groups, the DS had a positive linear relationship with the fraction of time spent near the food and water ($Oxtr^{+/+}$: r = 0.55, p = .0009; $Oxtr^{-/-}$: r = 0.34, p = .02) (Figure 5D). The $Oxtr^{-/-}$ correlation with DS was significantly weaker than the $Oxtr^{+/+}$ correlation with DS

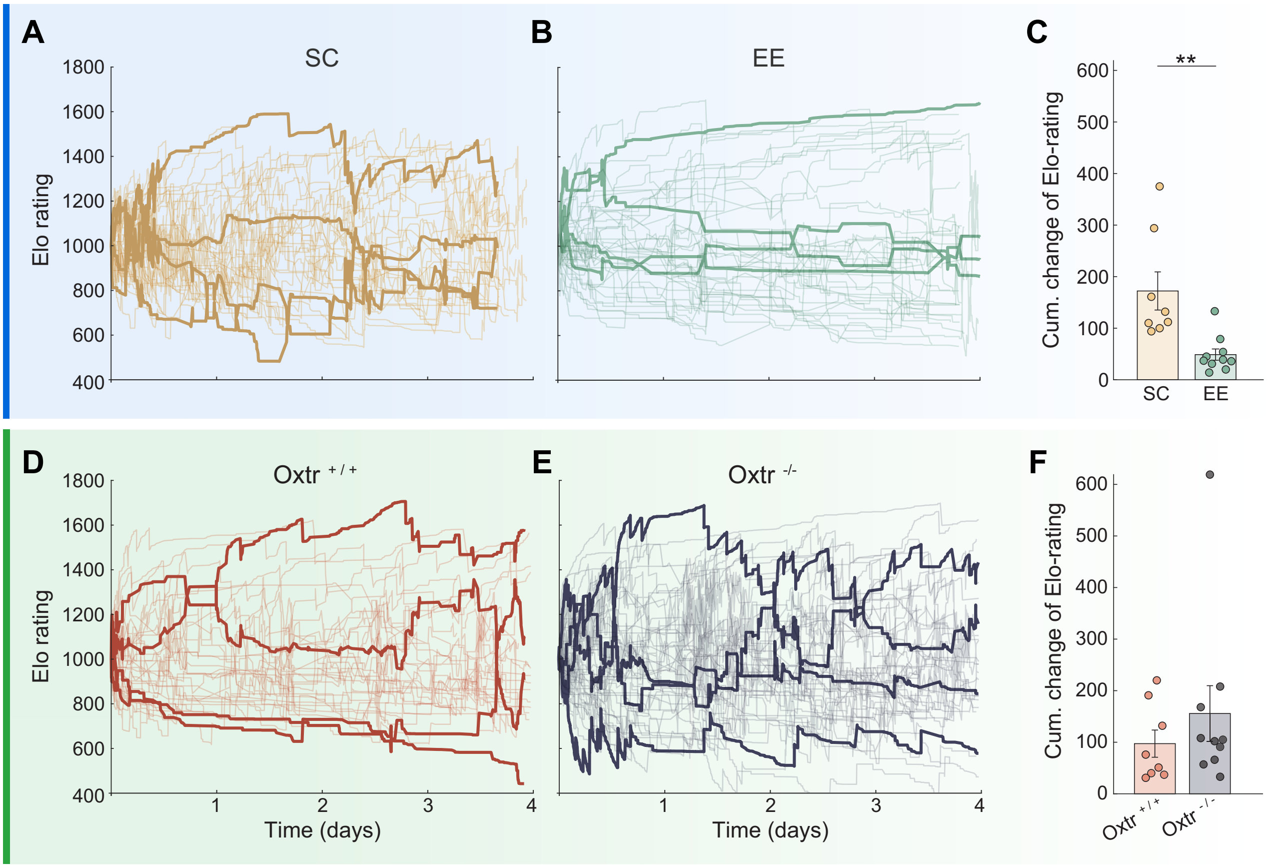

Figure 4. Temporal dynamic analysis of social dominance hierarchy. **(A, B)** Elo rating scores of all SC **(A)** and EE **(B)** mice. In bold, a single representative group of 4 mice (each line represents a single mouse). **(C)** Summation of the perturbations of the Elo rating values throughout the experiment in SC vs. EE groups. Fewer perturbations occurred in EE groups than in SC groups. **(D, E)** Elo rating scores of all *Oxtr*$^{+/+}$ **(D)** and *Oxtr*$^{-/-}$ **(E)** mice. In bold, a single representative group of 4 mice. **(F)** Summation of the perturbations of the Elo rating values throughout the experiment in *Oxtr*$^{+/+}$ vs. *Oxtr*$^{-/-}$ groups. Values are mean ± SEM; ** differs from the corresponding control value ($p < .01$). Cum., cumulative; EE, enriched environment; SC, standard condition.

(permutation test, p = .03, 10^6 permutations). In *Oxtr*$^{+/+}$, but not *Oxtr*$^{-/-}$, groups, the DS had a positive linear relationship with the fraction of time spent outside (*Oxtr*$^{+/+}$: r = 0.61, p = .0002; *Oxtr*$^{-/-}$: r = 0.11, p = .46) (Figure 5E). A permutation test revealed a trend toward a difference between *Oxtr*$^{+/+}$ but not *Oxtr*$^{-/-}$ (permutation test, p = .08, 10^6 permutations). In *Oxtr*$^{+/+}$, but not *Oxtr*$^{-/-}$, groups, the DS had a negative linear relationship with route predictability (*Oxtr*$^{+/+}$: r = −0.56, p = .0008; *Oxtr*$^{-/-}$: r = −0.05, p = .75) (Figure 5F), and the difference between the treatments was significant (permutation test, p = .03, 10^5 permutations). This analysis indicates that differential access to resources in EE (compared with SC) was maintained, but in *Oxtr*$^{-/-}$ (compared with *Oxtr*$^{+/+}$), it was altered.

DISCUSSION

Aggression must be tightly regulated to be displayed in the correct time and place (5,33). Disinhibition of aggression is a hallmark of many mental disorders, and the lack of appropriate behavioral mouse models hampers the effort to find an effective treatment for this symptom (4). Here, we presented an ethologically relevant approach in mice that quantifies group behavior in a seminatural setup and studies the reciprocal relationships between aggression, social avoidance, hierarchy, and access to resources. Such an approach enabled the study of the effect of EE and *Oxtr*$^{-/-}$ on adaptive aggression in a way that was not feasible before.

In the EE experiment, we exposed mice to a physically and socially EE during adolescence. The EE paradigm can be considered a model for commensal habitats such as barns. Such habitats, which are typically rich in food and shelter, are characterized by intense competition between conspecifics (9). Exposure of mice to an EE in laboratory conditions has previously been shown to enhance cognitive and physiological well-being and also aggression and competition (11,30,34,35). Multivariate analysis of the behavioral data acquired in our SB paradigm revealed marked differences between EE and SC groups. Further investigation of specific readouts revealed that EE groups combined a social avoidance strategy with an increased probability of aggressive encounters once an encounter occurred (Figures 2 and 3). Such a strategy can strengthen the hierarchy and the differential access to resources while minimizing the risks of injuries (Figure 2 and Figure S2). Social avoidance following intense aggression is a

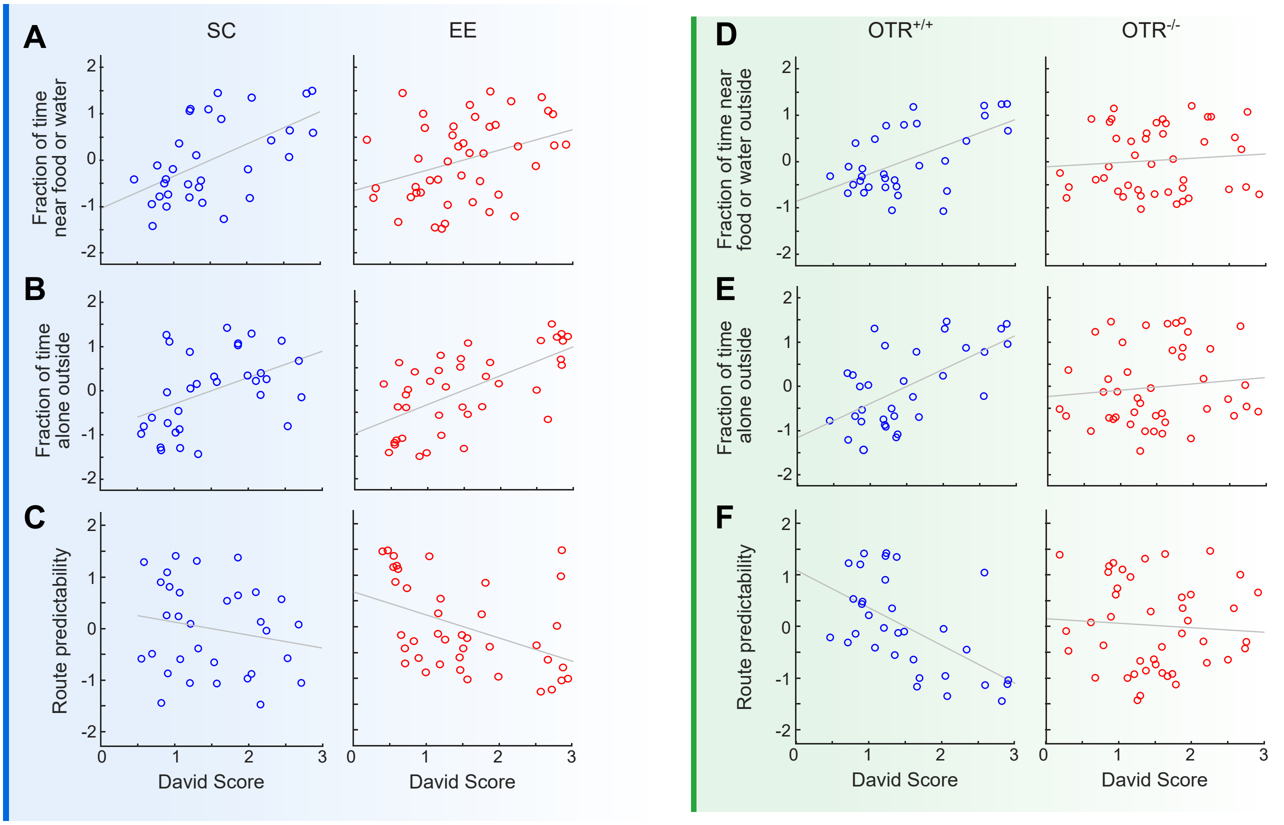

Figure 5. Organized priority order of access to resources in SC, EE, and *Oxtr*$^{+/+}$ groups, but not in *Oxtr*$^{-/-}$ groups. **(A–C)** Scatterplots of time spent near food or water **(A)**, time spent outside **(B)**, and route predictability **(C)** in SC and EE groups. **(D–F)** Scatterplots of time spent near food or water **(D)**, time spent outside **(E)**, and route predictability **(F)** in *Oxtr*$^{+/+}$ and *Oxtr*$^{-/-}$ groups. EE, enriched environment; SC, standard condition.

well-documented phenomenon. For example, sequential exposures to intense aggression in the chronic social defeat paradigm can result in defeated mice avoiding unfamiliar males (36). Temporal analysis of winning/losing consistency in dyadic chases (Figure 4) confirmed that EE groups maintained hierarchy relationships more efficiently than SC groups and that differential access to resources was strengthened in EE compared with SC groups (Figure 5A–C). These findings suggest that exposure to EE challenges during adolescence can reshape the group dynamics in adulthood to maintain differential access to resources with minimal but efficient levels of aggression.

In the second experiment, we modeled dysregulated aggression by introducing groups of mice to the SB that, since early development, lack the *Oxtr* (Figure 1). This receptor is widely expressed throughout the central nervous system, mediates a substantial part of the effects of oxytocin, and plays a pivotal role in many social behaviors (37–39). It is known that *Oxtr*$^{-/-}$ mice display excessive aggression in the RI paradigm (40), and it has also been suggested that the oxytocinergic system is involved in territorial behavior (27). Dimensionality reduction analysis of the multivariate behavioral data revealed that *Oxtr*$^{-/-}$ mice behaved differently in the SB than mice of the *Oxtr*$^{+/+}$ control groups. A logistic regression model to predict the treatment based on the behavioral readouts suggested that *Oxtr*$^{-/-}$ mice spent more time outside the nest, were more explorative, and were more aggressive to each other than mice in *Oxtr*$^{+/+}$ groups (Figures 2 and 3). Interestingly, *Oxtr*$^{-/-}$ groups were still able to maintain a hierarchy, suggesting that hierarchy is essential for group dynamics and survival even in genetic knockout mouse lines that display severe behavioral dysfunction in classic tests (Figure 3). Nevertheless, differential access to resources in *Oxtr*$^{-/-}$ mice was weakened compared with *Oxtr*$^{+/+}$ mice (Figure 5D–F). Overall, *Oxtr*$^{-/-}$ mice displayed heightened aggression in a group context in the SB, and their hierarchy was not reflected by differential access to territory and food. These findings suggest that the increased aggression of *Oxtr*$^{-/-}$ mice is not constructive in terms of group coherence but is rather a manifestation of irritation and anxiety that characterize this genetic manipulation. Notably, the food supply was ad libitum, and the group members faced no outside danger like predation. It can be hypothesized that introducing food restriction and/or danger in *Oxtr*$^{-/-}$ in the SB might induce differential access to resources or otherwise deteriorate the hierarchy.

Because aggression, SDH, and competition over resources are common throughout the animal kingdom, it is reasonable to assume that the neuronal mechanisms that underlie them are evolutionarily conserved to some extent between mammals. Therefore, revealing the mechanisms that underlie ethologically relevant dysregulated aggression in animal models is crucial for translational research. In mice, the RI test measures reactive aggression toward immediate unexpected threats. Escalated aggression toward an intruder in the RI test, such as excessive attacks, targeting vulnerable body parts, or ignoring submissive signals, has been suggested as a model of pathological human aggression (1). However, intense aggression and even conspecific killing are not rare among territorial species, such as mice in their natural habitats (5); therefore, escalated aggression is not necessarily pathological. The aggression self-administration test is an appetitive model for proactive or instrumental aggression in mice, and exaggerated appetitive aggression is a hallmark of aggression seen in psychiatric disorders (41). Nevertheless, instrumental aggression is not rare in mammals, and its adaptive value depends on the context.

Therefore, the types and intensity of aggressive displays depend on the context and may differ between classical tests such as the RI or aggression self-administration test and the SB. Moreover, different challenges in the SB can also affect the types of aggressive displays and their intensity. A systemic comparative study of such different methodologies could help assess the complementary advantage of each paradigm. However, the unique advantage of a seminatural approach over classical tests is the ability to simultaneously measure aggression, SDH and resource allocation, and reveal the intricate thresholds that separate adaptive from maladaptive aggression.

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ARTICLE INFORMATION

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