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Randomly sampled frames from our dataset classified as 'not touching'



Randomly sampled frames from our dataset classified as 'huddling'



Α

Figure S1: Pose estimation, data filtering and huddling classification, Related to Figure 2 and Table 1. (A) Images demonstrating the visibility of the two back keypoints, even when most of the mouse's body is occluded by a conspecific and/or nesting material. (B) An image showing the edge points of the chambers (depicted as pink dots) alongside the real-world cage dimensions, utilized to standardize the coordinate data across all trials. This standardization was necessary due to the varying distances between the cameras and the cages in different recordings. (C) Representative heatmaps illustrating the locations of two sibling mice during the 12-hour light cycle in the ISIA apparatus (log transformed histogram counts). The top row depicts the DLC output coordinates for the two mice, showing one keypoint per mouse. The bottom row depicts the coordinate data that has been filtered and transformed. After transformation, a pixel distance of 1 unit corresponds to 1 mm in the real-world. (D) Randomly sampled frames from the DLC tracking output video file with a likelihood value equal to or below 0.1, demonstrating instances where the mice are visually undetectable, being obscured by conspecifics or nesting material or tucked away inside the tube. (E) A summary likelihood histogram illustrating the distribution of all likelihood values across all DLC output files from the ISIA dataset (32 videos, n = 81,454,640 frames). (F) Histogram illustrating the minimum Euclidean distances for all manually classified frames from our training set, categorized as 'physical contact' and 'no physical contact.' The gray dashed line represents the 5 cm threshold hyperparameter established for physical contact. (G) Two 3x4 image grids generated by randomly sampling frames from our entire dataset, classified as 'not-touching' (left) and 'huddling' (right).

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The immersive social interactions assay (ISIA) apparatus







Ambient temperature (°C)



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Figure S2. The immersive social interactions assay (ISIA), Related to Figure 2. (A) Photographs of the various components of the apparatus. (B) Ambient temperature (mean \pm SE) tracked in a single ISIA chamber (alternating sides across different trials) during the 48-hour 'Room Temperature Condition' experiments (n = 8 recordings). (C) Distribution of mice showing a preference for either of the two chambers (right or left) under the 'Room Temperature Condition.' (D) Ambient temperature (mean \pm SE) recorded in each ISIA chamber throughout the 48-hour 'Optional Thermoneutrality Condition' experiments (n = 8 recordings). (E) Proportion of mice preferring each of the two chambers (the warm and the cold ones) under the 'Optional Thermoneutrality Condition.'



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Males Females Figure S3: Sleep/wake architecture and huddling behavior in male and female sibling mice, Related to Figure 3. (A) Number (left) and duration (right) of NREMs episodes for male mice in solitary habitation and sibling cohabitation across a 24-hour period. n = 9 mice, RM (for number of episodes) and mixed-effects (for episodes duration) two-way ANOVA. NREMs: Number of episodes: p(social status) = 0.0008, p(interaction) = 0.0018; Episodes duration: p(social status) = 0.0088, p(interaction) = 0.0103. REMs: For both number of episodes and episodes duration, p > 0.05 for both 'social status' and interaction. (**B**) Same as (A) but for females. N = 9 mice. RM (for number of episodes) and mixed-effects (for episodes duration) two-way ANOVA. NREMs: Number of episodes: p(social status) = 0.0002, p(interaction) =0.0332; Episodes duration: p(social status) = 0.003, p(interaction) = 0.0151. REMs: Number of episodes: p(social status) = 0.042; Episode duration: p > 0.05 for both 'social status' and interaction. (C) Distribution of NREMs episode durations in male mice during solitary habitation and sibling cohabitation. n = 9 per condition. RM two-way ANOVA, p(social status) = 0.0007, p(interaction) < 0.0001. (**D**) Same as (C), but for females. n = 9 mice per condition. RM two-way ANOVA, p < 0.0001 for both 'social status and interaction. (E) Delta power during NREMs across 24 hours (left, per hour; right, overall). n = 16 female and male mice. Per hour: RM twoway ANOVA, p(social status) = 0.0009. Per 24 hours: Paired t-test, two tailed, p = 0.0007. arb. = arbitrary unit. (F) Average sleep episode duration during ZT 5-6 in mice fitted with wireless Neurologger devices, comparing sleep analysis based on EEG-EMG data and video data. The analysis was conducted for mice in both solitary habitation and sibling cohabitation conditions. n = 6 female and male mice. RM two-way ANOVA. (G) Average sleep episode duration during ZT 5-6, comparing mice fitted with wireless Neurologger devices to those without devices. The analysis is based on video data, and for mice in both solitary habitation and sibling cohabitation conditions. n = 6 female and male mice. RM two-way ANOVA. (H) Images illustrating pose estimation and huddling classification in sibling co-housed mice. (I) Percentage of time spent huddling during NREM and REM sleep in the light in male and female sibling mice. n = 9 female and 9 male mice. Asterisks above the data points in (A-E) depict results obtained using Sidak's multiple comparisons tests. ns, p > 0.05; *, 0.01< p < 0.05; **, 0.001 < p < 0.01; ***, 0.001 < p < 0.01; ****, p < 0.0001.



Representative frames from four experiments illustrating male-male dyads huddling, even when provided with two separate shelters



Figure S4. Huddling behavior and changes in sexual and agonistic behaviors across days of cohabitation in mouse dyads, Related to Figure 5. (A) Number (left) and duration (right) of 'Mounting,' 'Attacking,' 'Chasing,' 'Aggressive grooming,' and 'Fleeing' bouts in male-male dyads during ZT 23-0, immediately following the introduction of the male intruder into the home-cage of the male resident on day 1 and four days later. n = 8 dyads. RM two-way ANOVA's followed by Sidak's multiple comparisons tests. Number of bouts: p(time) = 0.0027, p(behavior) = 0.0185, p(behavior x time) = 0.0216. Bout duration: p(time) = 0.0039, p(behavior) = 0.0019, p(behavior x time) = 0.0024. (B) Number (left) and duration (right) of 'Seeking,' 'Mounting without lordosis,' and 'Mounting with lordosis' bouts in male-female dyads during ZT 23-0, immediately following the introduction of the female intruder into the home-cage of the male resident on day 1 and four days later. n = 8 dyads. RM two-way ANOVA's followed by Sidak's multiple comparisons tests. Number (left) and duration (right) of 'Seeking,' 'Mounting without lordosis,' and 'Mounting with lordosis' bouts in male-female dyads during ZT 23-0, immediately following the introduction of the female intruder into the home-cage of the male resident on day 1 and four days later. n = 8 dyads. RM two-way ANOVA's followed by Sidak's multiple comparisons tests. p < 0.0001 for 'time,' 'behavior,' and 'behavior x time.' For both number and duration of sexual behaviors. ***, 0.001 < p < 0.01; ****, p < 0.0001. (C) Images from four different trials depicting huddling behavior in male-male dyads, both with and without shelters.





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Male-female dyads: REM sleep during the light phase



- Male Resident while with his siblings
- Male Resident while with a female Intruder
- Female Intruder while with her siblings
- Female Intruder while with a male Resident

Figure S5: Sleep/wake architecture in non-familial mice dyads, Related to Figure 5. (A-F) Sleep/wake architecture across the light and dark cycles in Resident and Intruder mice compared to the architecture of the same mice while cohabiting with their siblings. (A) Wake architecture during the light phase in Resident mice (left) and Intruder mice (right) compared to their wake architecture while cohabiting with their siblings. Top, number of episodes; Bottom, episode duration. n = 9 mice per group, RM two-way ANOVA's. Number of episodes: Residents, p(social status) = 0.002; Intruders, p(social status) = 0.031. Episode duration: Residents, p(social status) = 0.0033; Intruders, p > 0.05 for both 'social status' and interaction. (**B**) Same as (A), but for NREMs. Number of episodes: Residents, p(social status) = 0.002; Intruders, p(social status) = 0.0305. Episode duration: Residents, p(social status) = 0.035; Intruders, p > 0.05 for both 'social status' and interaction. (C) Same as (A), but for REMs. Number of episodes: Residents, p(social status) = 0.0157; Intruders, p > 0.05 for both 'social status' and interaction. Episode duration: Residents and Intruders, p > 0.05 for both 'social status' and interaction. (D) Wake architecture during the dark phase in Resident mice (left) and Intruder mice (right) compared to their wake architecture while cohabiting with their siblings. Top, number of episodes; Bottom, episode duration. n = 9 mice per group, RM two-way ANOVA's. Number of episodes: Residents, p > 0.05 for both 'social status' and interaction; Intruders, p(social status) = 0.0001, p(interaction) = 0.0042. Episode duration: Residents, p > 0.05 for both 'social status' and interaction; Intruders, p(social status) = 0.0189. (E) Same as (D), but for NREMs. Number of episodes: Residents, p > 0.05 for both 'social status' and interaction; Intruders, p(social status) = 0.0002, p(interaction) = 0.0225. Episode duration: Residents and Intruders, p > 0.05 for both 'social status' and interaction. (F) Same as (D), but for REMs. Number of episodes: Residents, p(social status) = 0.0287, p(sex of intruder) = 0.0359; Intruders, p(social status) = 0.0076. Episode duration: Residents and Intruders, p > 0.05 for both 'social status' and interaction. (G) Percentage of time spent in REM sleep during the light phase in male and female mice while in a dyad compared to when in the presence of their siblings. n = 9 mice per group, RM two-way ANOVA's. p(social status) = 0.0126, p(sex) = 0.0549 p(social status x sex) = 0.0666. Asterisks over data points were obtained using Sidak's multiple comparisons tests. Asterisks displayed in the center of a panel without an underlying line signify a significant main effect (no significant differences were observed in multiple comparison tests). ns, p > 0.05; *, 0.01< p < 0.05; **, 0.001 < p < 0.01; ***, 0.001 < p < 0.01; ****, p < 0.0001.