A 16p11.2 deletion mouse model displays quantitatively and qualitatively different behaviours in sociability and social novelty over short- and long-term observation.

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Abstract

Background

Autism spectrum disorders affect more than one percent of the population, impairing social communication and increasing stereotyped behaviours. A micro-deletion of the 16p11.2 BP4-BP5 chromosomic region has been identified in one percent of patients also displaying intellectual disabilities. In mouse models generated to understand the mechanisms of this deletion, learning and memory deficits were pervasive in most genetic backgrounds, while social communication deficits were only detected in some models. Based on previous study (Arbogast et al. 2016 PLoS genetics), we selected the mouse model of 16p11.2 deletion on a hybrid C57BL/6NxC3B genetic background to itemize the social deficits. We examined whether behavioural deficits observed in short observation periods were representative of the phenotype displayed by the same mice over long-term monitoring. We recorded the individual and social behaviours of 16p11.2 Del/+ mice and their wild-type littermates from both sexes in short-term (15 min) and long-term (over two and three consecutive nights) social interactions of familiar mixed-genotype quartets of males and of females, and of same-genotype unfamiliar female pairs.

Results

We observed that Del/+ mice of both sexes increased significantly their activity compared to wild-type littermates only over long-term monitoring. In the social domain, Del/+ mice of both sexes displayed only limited impairments over short-term monitoring, and more visible

deficits over long-term monitoring. When recorded in quartets of familiar individuals, social impairments were stronger in males than in females. In pairs, significant perturbations of the organisation of the social communication and behaviours in Del/+ females appeared mostly over the long-term.

Conclusions

Altogether, this suggests that social and contextual variations affect the phenotype of the 16p11.2 Del/+ mice differently in the activity and the social domains. The social behaviour was also differently affected between the two sexes. These findings confirm the importance of testing models both in short- and long-term conditions to provide a comprehensive view of their phenotype that will be more robust for pre-clinical targeted therapeutic trials.

Keywords

Autism; mouse model; 16p11.2; social behaviour; long-term monitoring; spontaneous behaviour; ultrasonic vocalisations

Background

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterised at the clinical level by atypical social interactions and communication, as well as stereotyped behaviours and restricted interests (1). This condition affects not only the patient but also his/her whole family. There exists a large range of severity between patients, who can also present severe comorbidities such as intellectual disability (ID), epilepsy, sleep disorders or hyper/hypo-sensitivity (2). The prevalence of ASD is more than 1% of the general population with more males than females (3,4). Potential causes can be environmental or genetic. Among these, copy number variations in the 16p11.2 region have been identified as one of the most frequent genetic causes of ASD (5). This region of 600kb between two repeated sequences named BP4 and BP5 includes 28 genes that can be either deleted or duplicated ((6): 550 kb and 26 genes; (7): 500-600 kb containing 27-29 genes). The duplication has been robustly linked with schizophrenia (8,9), while the deletion is associated with 1% of ASD cases accompanied by ID (10).

Patients with a deletion in the 16p11.2 region present diverse phenotypes, such as ASD (15% of cases), speech and language disorders (80-90% of cases; (11,12)), abnormal adaptive behaviours, cognitive behaviours and repetitive behaviours (at least one of these domains affected in 70-90% of cases; (13,14)), sleep disorders (80% of cases; (15)), ID (20% of cases; (14)), hyperactivity or attention disorder (30-40% of cases; (7)), developmental delay (100% of cases; (7,12)), epilepsy (10-20%; (7,12)), facial dysmorphia

(>20% of cases; (7,12)), obesity and macrocephaly (14). Patients may also present atypical brain anatomy, with abnormalities in the cerebellar tonsil (16), auditory and speech pathways, as well as in the cortical and striatal structures (17). Overall, these patients frequently display social interaction and communication impairments, especially in speech development (18). They also show poorer adaptative abilities in their daily life compared to controls (13). These aspects evaluated both during short-term clinical examination and during every-day life observation constitute keys points to examine in pre-clinical models.

The homologous region of the 16p11.2 lies in mouse chromosome 7F3 (6,19,20). Four mouse models were generated, differing in the genetic background used and in the size of the deleted chromosomic region ((6,19–21); see review in **Supplementary Table I** and in (18)). These models were further characterised either in the same genetic background or in different backgrounds. All of the four models displayed a reduced body weight compared to their wild-type littermates. Most of them displayed typical or increased activity in the short-term exploration of an open field, and increased activity over long-term (over one day or more) recordings compared to wild-type mice. Stereotyped behaviours remained subtle. Deficits in novel object recognition were recurrently highlighted in the different models. Sensory abilities were minimally affected, except in one model that appeared to be deaf due certainly to the genetic background (6,22). Over the different models, the variability of the social deficit attracted attention (18). One potential confounding factor is the genetic background of the models. Indeed, for a similar deletion and using identical test procedures, our laboratory did not detect robust social deficits in mutant mice generated on a pure C57BL/6N (B6N) background, while the same deletion crossed to obtain a F1 B6NC3B background (for C3B see the material and methods section) provoked a reduced time of sniffing in mutant mice compared to wild-type littermates (20). This model generated on a mixed background constitutes therefore a choice model to investigate further whether and to what extent the social and communication deficits vary over different observation times, as it is the case for the activity.

In the present study, we focused on social interactions, dissecting the different types of body contacts and their dynamics (23,24) to examine social orientation, seeking and maintenance (25). We analysed the variations in magnitude and nature of the social impairments highlighted in the mouse model deleted for the homologous 16p11.2 region generated over a hybrid F1 B6NC3B genetic background (hereafter Del/+) according to the observation duration in a testing environment. We expect social deficits to become more obvious and of a different nature over the long term (i.e., two or three days of interaction) when the environment gets familiar as compared to short-term observation (i.e., the first 15 min of interaction) when the environmental novelty is predominant and might blur the social deficits.

The long-term recordings aim to grab a phenotype closer to the everyday life of animals, to mimic approaches based on the Social Responsiveness Scale filled by caretakers and the Brief Observation of Social Communication Change (BOSCC) clinical evaluation made on videos of spontaneous play between parents/caretakers and patients (26). This approach complements classical tests focusing on short-term observations of a few minutes and/or providing a simple yes/no answer for social preference (27). In addition, we tested this hypothesis in two contexts of free interactions: interactions between four familiar individuals including one pair of Del/+ mice and one pair of wild-type (WT) mice and social encounters of a pair of unfamiliar individuals of the same-genotype.

Results

Spontaneous behaviours of familiar individuals in quartets

Behavioural profiles of quartets

We monitored the spontaneous behaviours of same-sex groups of four males or four females involving a pair of wild-type mice and a pair of Del/+ individuals, all four housed together from weaning on. These animals were familiar with the experimental room since they underwent other behavioural tests in the Live Mouse Tracker setup (unpublished data). As a short-term observation time, we chose the first 15 min of presence of the animals in the testing environment to grab a period with high activity; this time was under light condition. As a long-term observation period, we also focused on the active periods of the animals (i.e., the nights) over three days and we merged the three nights to increase the robustness of the analyses. We present here the centred and reduced data per cage for each Del/+ individual to control the inter-cage variability.

In male quartets, Del/+ mice significantly reduced their vertical exploration compared to WT mice over short-term monitoring only (**Figure 1A & Figure 2A**). They spent significantly longer time moving alone, with only a trend for the distance travelled (centred and reduced per cage), in long-term monitoring only (**Figure 1B**), which was confirmed with the increased distance travelled in Del/+ males compared to WT mice when considering raw data (**Supplementary Figure S1A & B**). Impairments in social behaviour occurred only over long-term recordings and concerned mostly a reduction of the total time spent in contact and of the contact with only one mouse (group of 2), as well as of the total time spent in side-side contacts compared to WT mice (**Figure 1B**). Del/+ mice also performed less nose-nose, nose-anogenital and side-side (head-to-tail) contacts compared to WT mice (**Figure 2B**). The general contacts, contacts with one and only one animal (i.e., group of 2) and side-side (head-to-tail) had a shorter mean total duration in Del/+ mice compared to WT (**Supplementary Figure S2**).

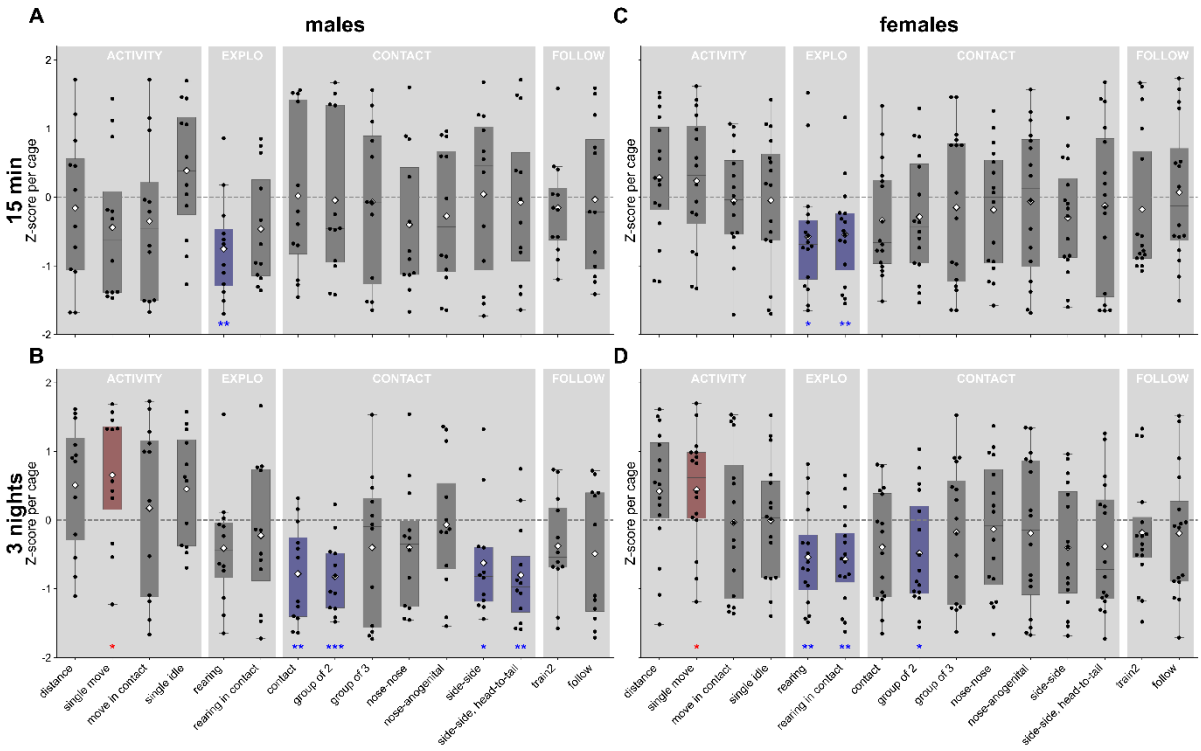

Figure 1: Behavioural profiles for the total duration of events of Del/+ females and males over short and long recording times in quartets. A. Z-score profile of the total duration of each behaviour for each Del/+ mouse compared to the mean behaviour of the four individuals within each quartet for males (n=12) over the first 15 min (A) and over three nights (B), as well as for females (n=16) over the first 15 min (C) and over the three nights (D). One sample T-test: *: p<0.05, **: p<0.01, ***: p<0.001. Red boxes and stars figure behavioural events that are more expressed in Del/+ mice compared to the mean of the whole cage; blue boxes and stars depict behavioural events that are less expressed in Del/+ mice compared to the mean of the whole cage; grey boxes reflect non-significant differences between Del/+ and the other animals of the cage.

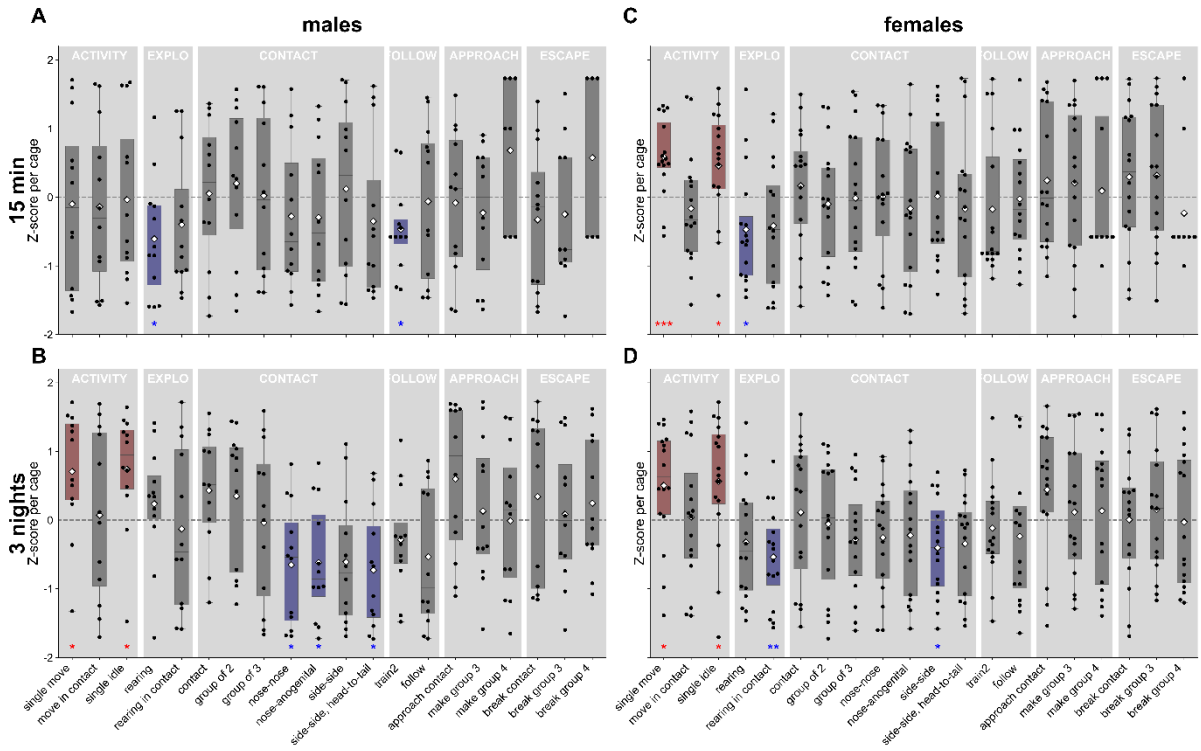

Figure 2: Behavioural profiles for the number of occurrences of events of Del/+ females and males over short and long recording times in quartets. A. Z-score profile of the number of occurrences of each behavioural event for each Del/+ mouse compared to the mean behaviour of the four individuals within each quartet for males (n=12) over the first 15 min (A) and over three nights (B), as well as for females (n=16) over the first 15 min (C) and over the three nights (D). One sample T-test: *: p<0.05, **: p<0.01, ***: p<0.001. Red boxes and stars figure behavioural events that are more expressed in Del/+ mice compared to the mean of the whole cage; blue boxes and stars depict behavioural events that are less expressed in Del/+ mice compared to the mean of the whole cage; grey boxes reflect non-significant differences between Del/+ and the other animals of the cage.

In female quartets, Del/+ mice significantly reduced their vertical exploration (i.e., rearing) compared to WT mice over both short- (**Figure 1C**) and long-term (**Figure 1D**) monitoring. They increased their time spent moving alone compared to WT mice, with only a trend for the distance travelled (centred and reduced per cage), only in long-term monitoring (**Figure 1D**), which was confirmed by the increased distance travelled in Del/+ mice compared to WT mice when considering raw data at the same time scales (see **Supplementary Figure S1C & D**). Interestingly, in both short- (**Figure 2C**) and long-term (**Figure 2D**) monitoring, the number of move and stop events was significantly higher, suggesting more jerking movement/stop episodes in Del/+ mice as compared to WT mice already visible over the first 15 min of recordings. The social behaviour of Del/+ females was minimally affected, and differently so according to the observation time: the time spent in contact with only one other animal (**Figure 1D**) and the number of side-side contacts (**Figure 2D**) were reduced over the long-term, while the mean duration of nose-nose and side-side contacts was reduced only in

short-term recordings (**Supplementary Figure S2**). Altogether, when recorded in familiar quartets, Del/+ females displayed only subtle social abnormalities in both short- and long-term monitoring. In contrast, Del/+ males displayed robust quantitative and qualitative social impairments only over the long term.

Selective interactions with WT mice in mixed-genotypes quartets

Quartet recordings involved a pair of WT and a pair of Del/+ mice. Whether mice of one genotype interacted preferentially with mice of the same genotype remains unknown. We therefore dissected social interactions between individuals and compared them between mice of the same genotype and mice of the other genotype. Over the three nights, both WT and Del/+ males preferred to interact with a WT individual than with a Del/+ one as depicted in the total time spent in contact with another individual (WT: T=5.07, p=0.00036; Del/+: T=-2.84, p=0.016; **Figure 3A**). WT males also performed significantly more approaches leading to a contact towards WT males than towards Del/+ males (**Figure 3B**; U=2.35, p=0.038). In addition, the contacts established with another individual by WT males were also significantly shorter when involving a Del/+ male than a WT male (**Figure 3C**; U=124, p=0.003). Similar preferences occurred only in Del/+ females over the long term (**Figure 3D-F**) but part of them were already visible in the first 15 min of interaction (**Supplementary Figure S3A-C**), while this was not the case in males (**Supplementary Figure S3D-F**). Overall, it appeared that both WT and Del/+ males (and Del/+ females) were able to differentiate the animals according to their genotype and interacted preferentially with WT mice. In females, this was already visible over the short initial period of monitoring.

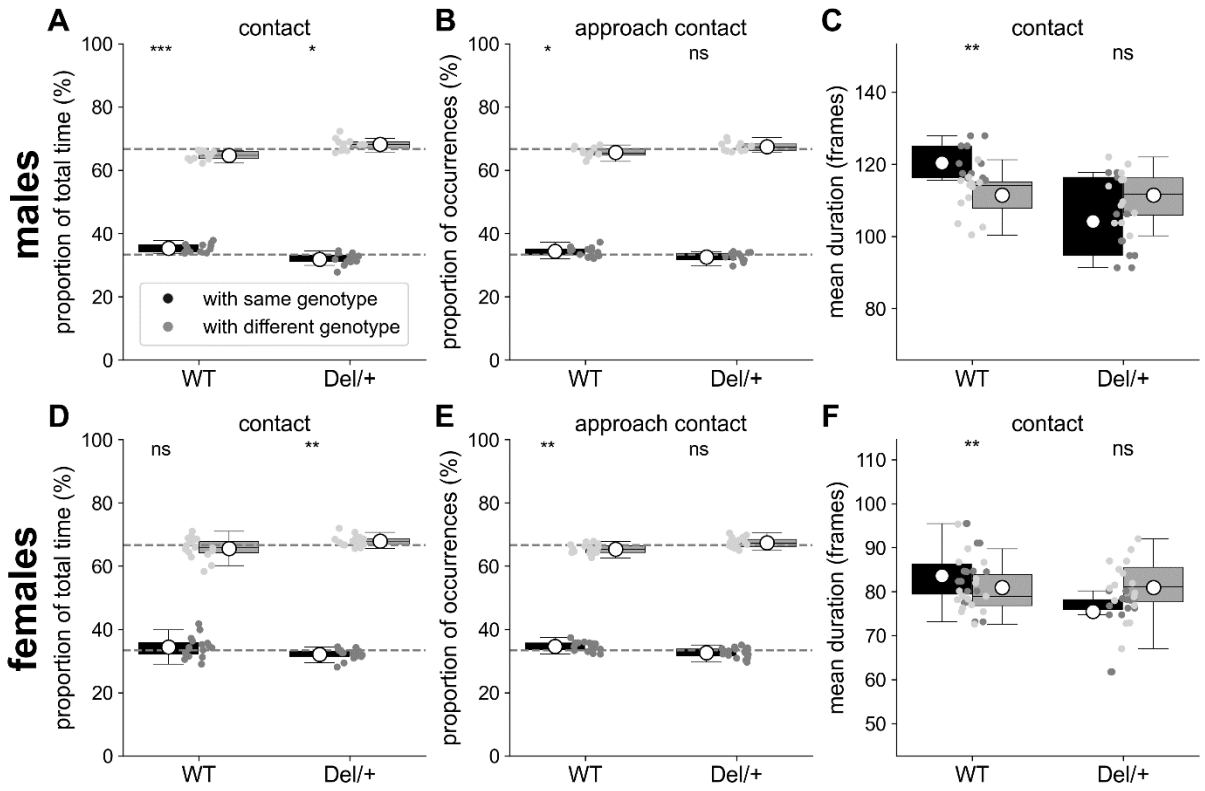

Figure 3: Selective interactions between genotypes for quartets of males and females recorded over three nights. A. Proportion of the total time spent in contact with individuals of the same or of the different genotype for WT (n=12) and Del/+ (n=12) males. B. Proportion of the number of approaches leading to a contact with individuals of the same or of the different genotype for WT (n=12) and Del/+ (n=12) males. C. Mean duration (in frames) of the contacts established with individuals of the same or of the different genotype for WT (n=12) and Del/+ (n=12) males. D. Proportion of the total time spent in contact with individuals of the same or of the different genotype for WT (n=16) and Del/+ (n=16) females. E. Proportion of the number of approaches leading to a contact with individuals of the same or of the different genotype for WT (n=16) and Del/+ (n=16) females. F. Mean duration (in frames) of the contacts established with individuals of the same or of the different genotype for WT (n=16) and Del/+ (n=16) females. A, B, D & E: One sample T-tests compared to expected proportions; dashed horizontal lines represent the expected proportions: 1/3 with individuals of the same genotype and 2/3 with individuals of the different genotype. C & F. Non-parametric Mann-Whitney U-tests. ns: not significant, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

Social encounters of unfamiliar females in pairs

After examining the behaviours of a pair of WT with a pair of Del/+ mice in both sexes in the quartet interactions, we aimed at exploring further social communication deficits and the organisation of behaviours. For that purpose, we examined a simpler type of interaction: the dyadic interaction within pairs of same-sex unfamiliar individuals of the same genotype. In this context, we restricted our analyses to females since males were too aggressive toward an unfamiliar conspecific when left over for more than one hour (personal observation).

Activity in unfamiliar female pairs

We recorded the spontaneous behaviour of WT and Del/+ females during the encounter of a same-genotype unfamiliar individual over short- (the first 15 min) and long- (2 nights) term sessions. As for the quartet recordings, for the long-term sessions, we analysed data from the dark phases (nights) only to focus on the active periods of the animals. We present here the data per time unit to display in parallel the short- and the long-term results. The genotype-related variations in activity levels differed according to the duration of the experiment. Indeed, over the first 15 min, Del/+ mice travelled similar distance per hour as WT mice (Linear Mixed Model (LMM): β=7.378, SE=3.894, p=0.058, with β as the coefficient estimate of the fixed factor 'genotype', i.e., the slope of the line between WT and Del/+ values, SE as the standard error of this coefficient estimate and p as the probability of the current data to occur assuming the difference between genotypes is null: the lower it is, the most likely the genotype has an effect; **Figure 4A**). In contrast, when tested over long term (2 nights), Del/+ females travelled significantly longer distances per hour (LMM: β=-828.470, SE=251.478, p=0.001) compared with their WT littermates (**Figure 4A;** see **Supplementary Figure S1C & D** for raw distance travelled and **Supplementary Table II** for complementary statistics), confirming our findings during quartet recordings (see above). This suggests that the environmental exploration due to novelty masks the increased activity induced by the deletion in a social context.

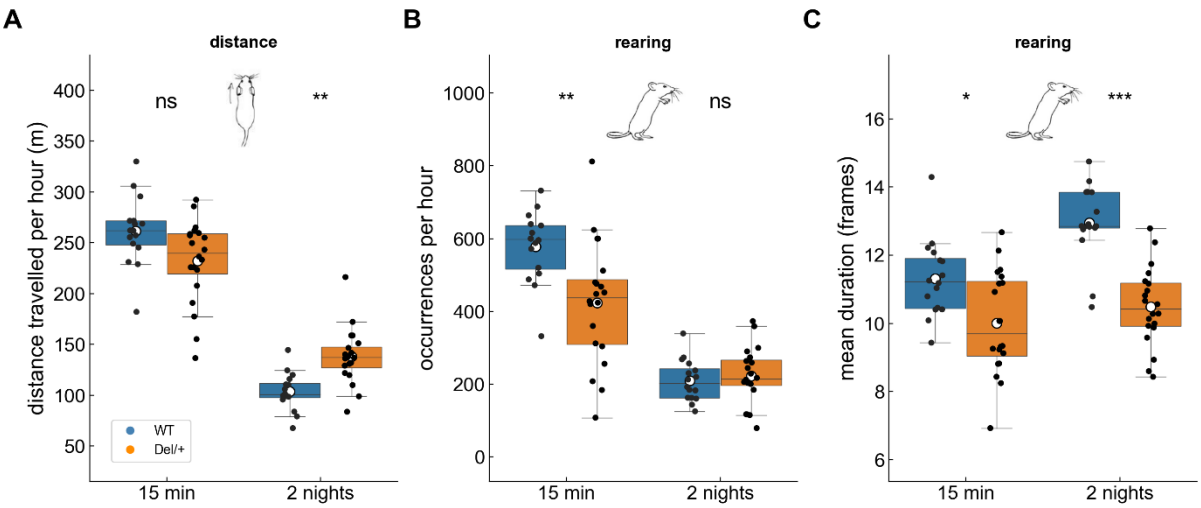

Figure 4: Activity and exploration in pairs of unfamiliar females over the first 15 min (short term) and over 2 nights (long term). A. Distance travelled per hour estimated over the first 15 min and over the two nights. B. Number of rearing events per hour over the first 15 min and over the two nights. C. Mean duration of the rearing events over the first 15 min and over the two nights. Linear mixed model with genotype as fixed factor and pair as random factor (n(WT)=16, n(Del/+)=20); p: probability of the occurrence of the data assuming there is no effect of genotype: ns: p>0.05, *: p<0.05, **: p<0.01, ***: p<0.001.

Exploration in unfamiliar female pairs

When considering behaviours reflecting exploration, we observed that, in short-term conditions, Del/+ females displayed significantly fewer rearing events per hour (LMM: β=38.55, SE=12.229, p=0.002; **Figure 4B**) and stayed reared for significantly shorter time (data not shown) compared to WT littermates. Del/+ mice performed rearing of significantly shorter mean duration compared to their WT littermates (LMM: β=1.308, SE=0.543, p=0.016; **Figure 4C**). In contrast, over the two nights of recording, Del/+ females in pairs were not affected in their rearing behaviour (except for the mean duration of rearing events: β=2.464, SE=0.504, p<0.001; **Figure 4C**). These results suggest that Del/+ mice explored differently the novel environment compared to WT mice.

Social contacts between unfamiliar females in pairs

We next focused on the time spent in contact and on the different types of contacts. Over the first 15 min of interaction, Del/+ unfamiliar female pairs spent a significantly smaller proportion of time in contact with their conspecific (Mann-Whitney U-test (MW) U=66, p=0.021; **Figure 5A**). This decrease appeared to be related to a non-significant decrease in the number of contacts per hour (MW: U=60, p=0.083; **Figure 5B**), while the organisation of these contacts was similar compared to WT female pairs (MW: U=55 p=0.203; **Figure 5C**). In contrast, over the two nights of recording, the proportion of time spent in contact did not differ anymore between genotypes (MW: U=55, p=0.203; **Figure 5A**), while their temporal structure did. Indeed, Del/+ female pairs established significantly more contacts per hour (MW: U=15, p=0.027; **Figure 5B**) of shorter mean duration (MW: U=70, p=0.006; **Figure 5C**) compared to their WT littermates. This perturbed temporal structure of global contact might be related to the increased activity of the Del/+ mice compared to WT mice. Nevertheless, this increased activity compared to WT mice affected mostly other contacts than the ones specified (nose-nose, side-side, and side-side head-to-tail), that did not differ between genotypes in short- or long-term observation (except nose-nose contacts over short-term, which were reduced in the number of occurrences; **Supplementary Figure S4**). Altogether, the structural impairments of contacts displayed by Del/+ mice over the long-term suggest a profound social deficit that could impair the everyday life of the animals, and not just complicate initial encounters with unfamiliar individuals as observed in the short term. Indeed, the structural impairments of contacts visible in the long-term seem to affect differently the various types of contacts and therefore impair social maintenance.

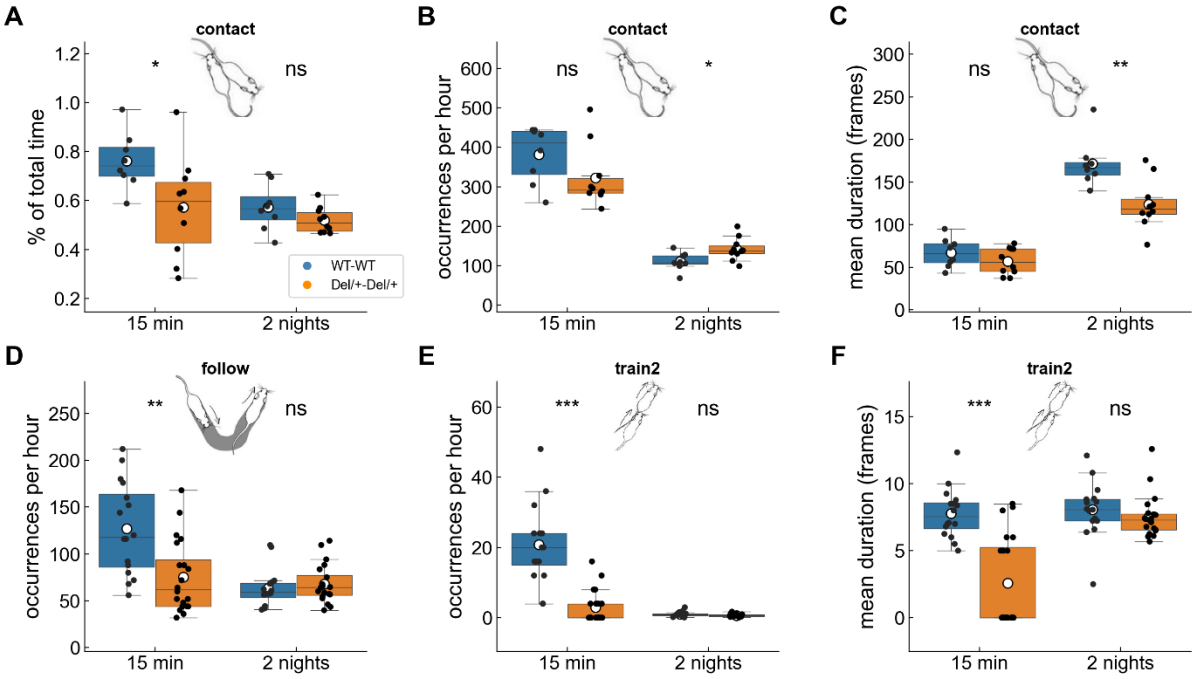

Figure 5: Social behaviours in pairs of unfamiliar females over the first 15 min (short term) and over 2 nights (long term). A. Proportion of time spent in contact estimated over the first 15 min and over the two nights. B. Number of contact events per hour estimated over the first 15 min and over the two nights. C. Mean duration of the contact events over the first 15 min and over the two nights. D. Number of follow events per hour estimated over the first 15 min and over the two nights. E. Number of train2 events (i.e., following another animal in ano-genital contact) estimated over the first 15 min and over the two nights. F. Mean duration of the train2 events estimated over the first 15 min and over the two nights A, B & C: Non-parametric Mann-Whitney U-test, n(WT-WT)=8, n(Del/+ Del/+)=10. D, E & F: Linear mixed model with genotype as fixed factor and pair as random factor (n(WT)=16, n(Del/+)=20); ns: not significant genotype effect, *: p<0.05, **: p<0.01, ***: p<0.001.

Follow behaviours between unfamiliar females in pairs

Follow events are rare but well recognisable behaviours that occur mostly when animals are aroused during intense social interactions. These behaviours are therefore more likely to occur in the initial exploration of an unfamiliar conspecific, but they can still be observed between familiar animals housed together (24). In our conditions, the number of follow behaviours (with and without ano-genital contacts, i.e., train2 and follow behaviours, respectively) were significantly reduced in pairs of Del/+ females compared to pairs of WT females only during the first 15 min of interaction (LMM for follow: β=51.60, SE=18.059, p=0.004; Train2: β=17.750, SE=2.928, p<0.001), and not over the two nights of recordings (follow: β=-4.530, SE=9.900, p=0.647; train2: β=0.320, SE=0.239, p=0.181; **Figure 5D-E)**. The same was true for the proportion of time spent in follow behaviour (data not shown). In addition, each follow event was also significantly shorter in Del/+ female pairs compared to WT female pairs over short term (LMM train2: β=5.186, SE=1.034, p<0.001; **Figure 5F**). Therefore, the Del/+ mice displayed a quantitative reduction of follow behaviour during the

initial exploration of unfamiliar individuals suggesting that Del/+ mice were less interested by the encounter of unfamiliar conspecific. The blurring of the genotype-effect on the quantity of these follow events over the long-term might be related to a bottom effect due to the scarcity of these events once females were getting used to each other. Nevertheless, over the long term, Del/+ females performed follow behaviours at a lower speed and travelled less distances during these behaviours compared to WT females (see **Supplementary Figure S5** for train2; such an effect was not significant in follow behaviours without ano-genital contacts (data not shown)). These qualitative variations suggest that Del/+ mice displayed decreased social motivation and arousal compared to WT mice also over long-term experiments.

Social communication between unfamiliar females in pairs

Ultrasonic vocalisations (USVs) are communicative signals emitted by pups during development and by juvenile and adult mice during social or sexual encounters (28). At the juvenile or adult stages, USVs reflect the arousal status of the animal and the emotional perception of the interactions (24,29). During these encounters between unfamiliar adult females, Del/+ pairs emitted significantly less USVs per hour compared to WT pairs in the first 15 min (W=63.0, p=0.045), while this reduction became not significant over the long term (W=60.0, p=0.083; **Figure 6A**). This parallels the reduction of social contacts (see above) and suggests a reduced arousal triggered by social initiation in Del/+ pairs compared to WT pairs.

In pairs of both genotypes, USVs were organised in sequences, i.e., consecutive USVs separated by less than 750 ms (24). Both in short- and long-term recordings, USVs were organised in significantly shorter sequences in Del/+ pairs compared to WT pairs (LMM: short term: n(WT)=191, n(Del/+)=136, β=6.892, SE=3.251, p=0.034; long term: n(WT)=2923, n(Del/+)=2578, β=4.378, SE=1.242, p=0.002; **Figure 6B**). This also reflects the reduced arousal of Del/+ pairs compared to WT pairs, both in the initial social exploration and in the maintenance of these interactions.

When testing the effect of the deletion on variables reflecting the acoustic structure (**Figure 6C**), we observed broader impairments of the acoustic structure over long-term recordings than over short-term recordings. Indeed, USVs recorded over the two nights from Del/+ pairs (n=15885) were acoustically simpler, with shorter duration (**Figure 6D**), a smaller frequency range covered (**Figure 6E**), less frequency modulations (**Figure 6F**) compared to those recorded in WT pairs (n=29079); frequency characteristics (**Figure 6G**) and frequency jumps (data not shown) did not differ between genotypes. In contrast, over the short-term, USVs recorded from Del/+ pairs (n=1091) displayed reduced acoustic variations by being flatter and higher pitched compared to those recorded from WT pairs (n=2875), with a

smaller frequency range covered (**Figure 6E**) and higher mean peak frequency (**Figure 6G**); no significant differences emerged in the duration (**Figure 6D**), frequency modulations (**Figure 6F**) and number of frequency jumps (data not shown).

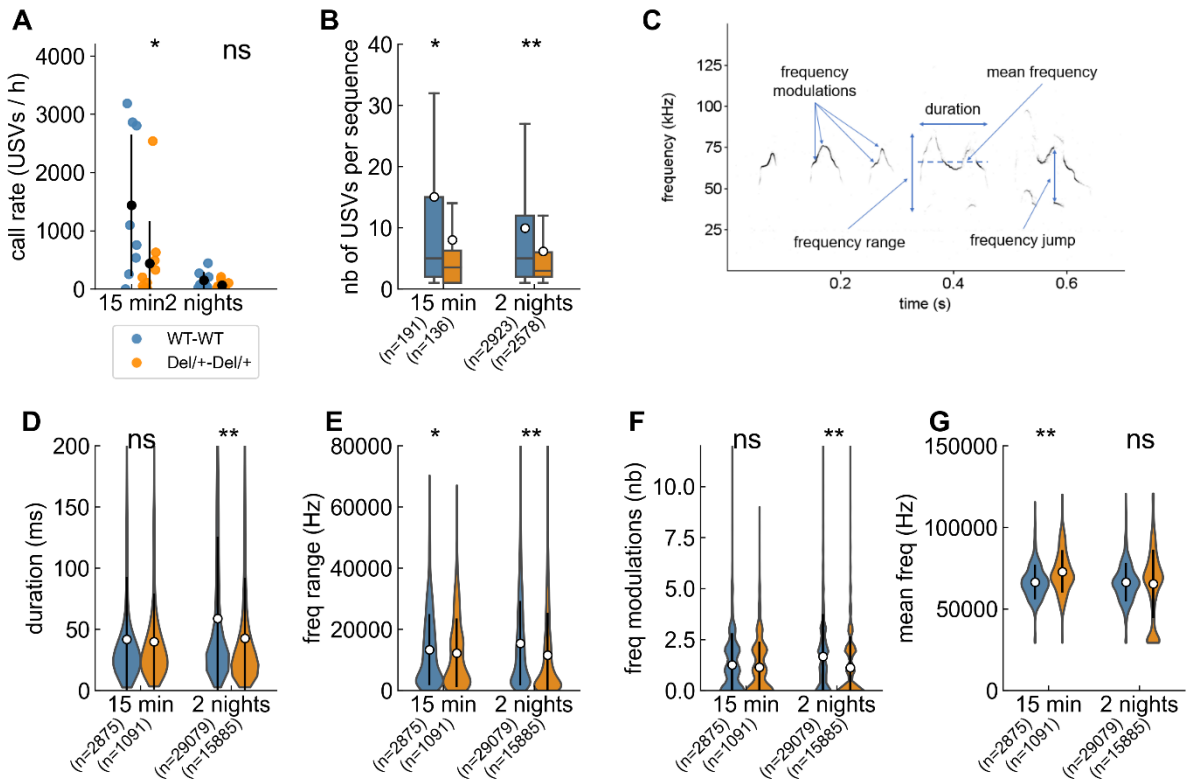

Figure 6: Characteristics of ultrasonic vocalisations (USVs) emitted during social encounters between two unfamiliar females of the same genotype. A. Number of USVs emitted per hour estimated over the first 15 min and over 2 nights; non-parametric Mann-Whitney U-test: n(WT-WT)=8, n(Del/+ Del/+)=10. B. Number of USVs per sequence (i.e., successive USVs separated by less than 750 ms) estimated over the first 15 min and over 2 nights; sample sizes represent the number of sequences analysed and the white dots represents the mean values. C. Spectrogram (300 kHz sampling frequency, 16-bits format, FFT length: 1024 points, 75% overlap, Hamming window, 0.853 ms time resolution) of five USVs depicting the main acoustic features measured by LMT USV Toolbox. D. Duration of USVs. E. Range of frequencies covered by USVs. F. Number of frequency modulations (i.e., inflexion points in the peak frequency) per USV. G. Mean peak frequency computed over each USV. D, E, F & G: sample sizes represent the number of USVs analysed; the white dot and black error bars represent the mean values and standard deviations; linear mixed model with genotype as fixed factor and pair as random factor. ns: not significant, *: p<0.05, **: p<0.01, ***: p<0.001.

The call rates specific to behavioural events did not differ significantly between WT and Del/+ pairs, with the lowest call rate in single idle and the highest in the different types of contacts, in both short- and long-term experiments (**Supplementary Figure S6**). Over the short term, the proportion of USVs given with the different behaviours did not differ significantly between WT and Del/+ pairs, except for train2 events (**Supplementary Figure S7A**). In contrast, over the long term, Del/+ pairs emitted significantly more USVs in the single idle context and significantly less USVs in nose-anogenital contact, and in side-side head-to-tail contacts

compared to WT pairs (**Supplementary Figure S7B**). This reflects the reduced expression of these behaviours in Del/+ mice compared to WT mice (see above). In addition, over the short term, around 35% of the side-side events and 35% of the train2 events were accompanied by USVs in WT pairs, while only 10% and 1% of these respective events were accompanied by USVs in Del/+ pairs (**Supplementary Figure S7C**). The genotype-related differences extended to other behaviours over the long term (**Supplementary Figure S7D**). Altogether, this suggests that Del/+ mice were less aroused by intense social contacts over the short-term; this reduced arousal broadened to other types of interactions over the long-term.

Transitions between behavioural events in unfamiliar pairs

As the structure of behavioural events such as contacts was perturbed in Del/+ mice (see above), we investigated whether the basic organisation of the behaviour was affected by examining the temporal succession of simple behavioural events, that should contribute to a better understanding of the functions of the different behaviours (30,31). For this analysis, we needed to define exclusive behavioural events (i.e., events that could not occur at the same time; see methods for definitions). We focused on simple behavioural blocks to explore the bases of the behaviour: the animal is alone and moving or idling, and the different types of contacts; more complex social events such as train2 were not examined in the present analysis. The exclusive behavioural events were computed by combining the existing non-exclusive events. We excluded any overlap between events and each animal of the pair was engaged in one and only one event at each time frame. Recomputing the behavioural profiles with these exclusive events allowed to precise social contact deficits: Del/+ pairs displayed a quantitative reduction of nose-nose and nose-anogenital contacts compared to WT pairs in the short-term recordings, while there were structural variations (mean duration) of events involving side-side contacts between genotypes over the long-term recordings (**Supplementary Figure S8**). To analyse the temporal succession of events, we compared the transitions from one behavioural event to another between pairs of WT females and pairs of Del/+ females over the two different time scales (see example in **Figure 7A**).

Over the first 15 min of experiments, most of the transitions between exclusive events are similar in pairs of WT females and in pairs of Del/+ females. It only appeared that moving alone (move) was significantly more followed by being sniffed in the ano-genital region (N-G pass) or performing other contacts (other cct) in WT females than in Del/+ females, while other contacts (other cct) were ending a social sequence (i.e., followed by a single idle) more frequently in Del/+ females than in WT females (**Figure 7B**). This suggests that Del/+ mice did not initiate their contacts by anogenital sniffing as frequently as WT mice and that contact

sequences of Del/+ terminated in an unspecific way more frequently than in WT mice in the initial exploration of an unfamiliar mouse.

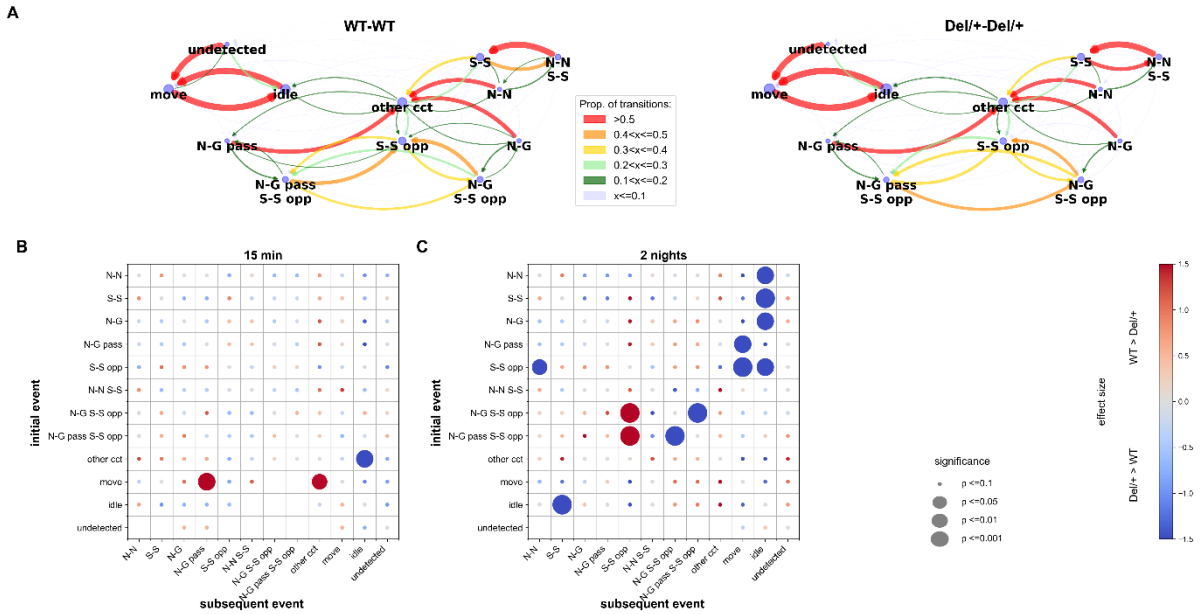

Figure 7: Transitions between exclusive behavioural events in unfamiliar female pairs. A. Examples of the transitions between behavioural events in pairs of WT (left panel) and in pairs of Del/+ (right panel) females over the long-term recording. The proportion of each possible transition is represented by the colour and weight of the arrows oriented from initial to subsequent events. The size of the blue nodes represents the proportion of each event. B. Overview of the comparisons between WT-WT and Del/+ Del/+ pairs of the transitions for the events in the y-axis towards the events of the x-axis for the first 15 min (B) and over the two nights (C) of recordings. B & C: n(WT-WT)=16, n(Del/+ Del/+)=20; Mann-Whitney U-tests; significance levels are represented by the diameter of the circles, and the effect size is represented by the colour of the points. N-N: nose-nose contact, N-G: nose-anogenital contact, N-G pass: passive nose-anogenital contact, S-S: side-side contact, S-S opp: side-side contact head-to-tail, N-N S-S: nose-nose contact during side-side contact, N-G S-S opp: nose-anogenital contact during side-side contact head-to-tail, N-G pass S-S opp: passive nose-anogenital contact during side-side contact head-to-tail, other cct: other types of contacts than the ones described above, idle: single idle, move: single move, undetected: the animal is not detected.

Over the two nights of recording, more significant differences emerged between WT and Del/+ mice. Del/+ mice appeared to show more transitions back and forth between ‘side-side head-to-tail & ano-genital sniffing’ (being sniffed or sniffing; N-G S-S opp or N-G pass S-S opp) and less transitions between ‘side-side head-to-tail & anogenital sniffing’ (N-G S-S opp) and pure ‘side-side head-to-tail’ (S-S opp) compared to WT mice, as if Del/+ mice performed more continuous ano-genital sniffing during side-side head-to-tail behaviours. Social sequences appeared to end in an atypical way in Del/+ mice since nose-nose (N-N), nose-anogenital (N-G), passive ano-genital (N-G pass), side-side (S-S), and side-side head-to-tail (S-S opp) ended a social sequence (i.e., were followed by idle or move) more frequently in Del/+ mice compared to WT mice (**Figure 7C**). In addition, Del/+ mice also displayed one

atypical start of a social sequence: they used side-side contacts (S-S) as a social sequence start (i.e., following an idle event) more frequently compared to WT mice. Altogether, this suggests that the perturbations of the behavioural organisation in Del/+ mice concerned the initiation and termination of social contact sequences but did not affect the most frequent transitions between behavioural events. These perturbations were more important over the long-term than in the initial encounter.

Discussion

In the present study, Del/+ mice displayed differential impairments according to sex and time scale (**Figure 8**). The hyperactive phenotype appeared only during long-term monitoring, while the reduced environmental exploration was visible mostly during short-term monitoring in both sexes. In the social domain, among familiar quartets, Del/+ males displayed reduced social interactions only over long-term monitoring, while these deficits were subtler in Del/+ females. Interestingly, the behavioural variations were perceived by the animals themselves, as both WT and Del/+ mice displayed a social preference toward WT animals as opposed to Del/+ mice. In encounters between unfamiliar females, Del/+ mice displayed quantitative reduction of social contacts and ultrasonic vocalisations over short-term, and qualitative variations in ultrasonic vocalisations and in the organisation of their social interactions over long-term compared to wild-type mice.

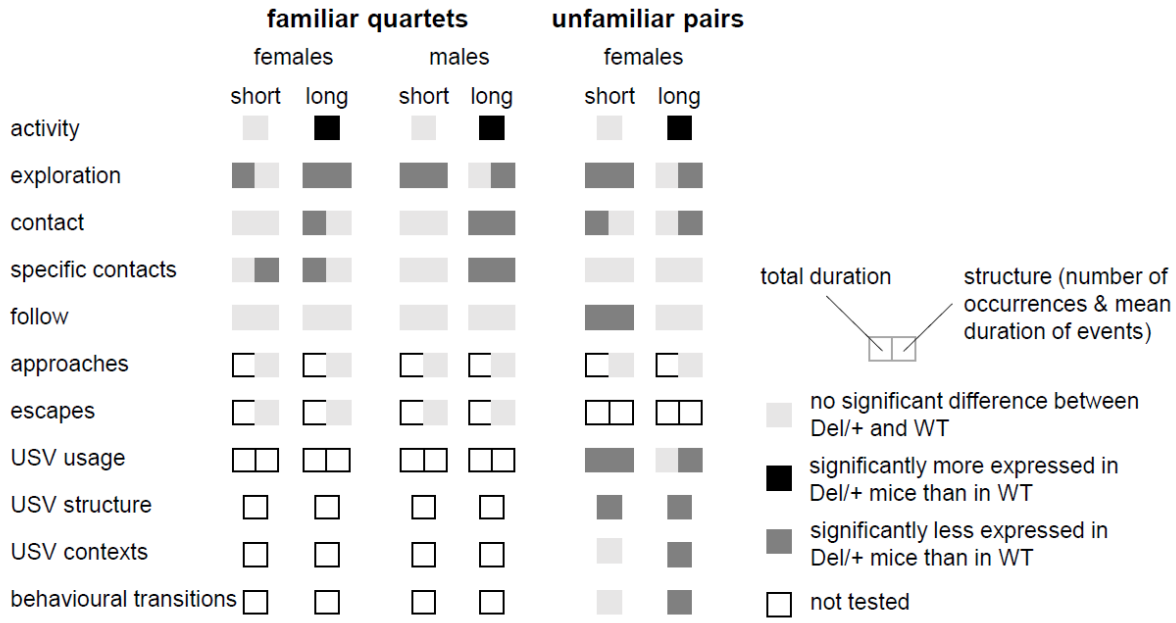

Figure 8: Summary of behavioural variations between Del/+ and wild-type mice. Variations were explored in both sexes and in both contexts (familiar quartets and unfamiliar pairs) over short- and long-term monitoring. Variations between genotypes are depicted in colours. The left part of the coloured rectangles represents variations in total duration (quantitative variations) and the right part

represents variations in the structure of the events (qualitative variations in number of events and in mean duration).

Disentangling activity and social phenotypes

As activity and exploration might be traits which can affect social behaviours, we tested them simultaneously with social behaviours. Our comparison of genotype-related differences in activity in both sexes between the different time scales confirmed previous findings on the same model in different protocols. Indeed, in the study of Arbogast using the same model, the hyperactivity displayed by Del/+ mice was visible only over the dark phase in the circadian activity test, while it was not observed in the 30 min exploration of an open field (20). In contrast to our previous study (20) in which we observed increased rearing over long-term in isolated Del/+ individuals, we observed a different pattern for vertical exploration, with a decreased number of rearing events in Del/+ mice over the short- and the long-term, in quartets of both sexes and in pairs of females. Reduced muscle strength can be ruled out (20). This discrepancy might be related to differences in the test cage: in the present study, mice were tested in a social context with bedding and nesting material, while in (20) mice were isolated without bedding and nesting material. Reduced unsupported rearing is expected in more anxious animals (32). However, as the proportion between supported and unsupported rearing did not vary significantly between genotypes (data not shown), other causes than increased stress and anxiety in Del/+ mice of the present study remain to be investigated. The increased activity displayed by Del/+ mice might explain the shorter mean duration of rearing events over long-term monitoring. Interestingly, impairments in activity and exploration did not occur simultaneously with social deficits in our testing conditions. This precludes the explanation of social deficits by hyperactivity, as in some other mouse models of autism (e.g., *Shank2/ProSAP1*$^{-/-}$ *mice*: (33,34)).

Decoding social defects

We took advantage of the different test contexts to better understand the extent to which the deletion interferes with the social life in mice. Indeed, the behaviour of mice is evolving over time (visible burrow system, (35)) and this evolution should be considered in mouse phenotyping studies. In the present case, in unfamiliar female pairs, we observed simple quantitative reduction of time spent in contact and follow behaviours over the short-term in Del/+ mice compared to WT mice. In contrast, over the long-term, when the animals get more familiar with each other, these quantitative impairments turned to more qualitative impairments such as atypical ways of starting and ending contact sequences. Observations in the short-term phase parallel classical tests for social interactions, with a decreased time spent sniffing the conspecific (males; (20)) and reflect the atypical way of initiating social

encounters, in which ano-genital sniffing and following appeared to play a crucial role. In contrast, observations of structural abnormalities in the social behaviour over the long-term reflect the difficulties in maintaining social interactions (25), which has been, up to now, under-studied given the short duration of social experiments. Such structural defects might be more complicated to improve through behavioural intervention (as in (36)) compared to motivational defects and require further study for a better understanding of the neuronal circuits involved. Future studies will unravel the time course of social interactions to identify the time point at which the initial social contacts turn to social maintenance.

Sex differences in the social phenotype

We were not able to run the social encounters between unfamiliar males. Indeed, sexually mature males are highly aggressive and could not be left for two days and two nights together without severe fighting outcomes despite the large surface of the test cage (personal observation). We nevertheless observed robust social impairments in males over the long-term when tested with familiar cage mates. The reduced time, number and mean duration of some specific contacts were even stronger in males than in females, while the activity level was increased in Del/+ mice of both sexes to a similar extent. Interestingly, the fact that social deficits were more visible over the short-term in females (even more so when encountering unfamiliar individuals) than in males might reflect the fact that Del/+ females might be more vulnerable to the stress related to the new physical environment compared to Del/+ males as it has been found in another model (37). These findings might be reminiscent of observations in patients. Indeed, in patients carrying a 16p11.2 deletion, the sex ratio was almost balanced, with 1.3 males for 1 female for autism and 1.6 male for 1 female for ID/DD. However, females carrying a 16p11.2 deletion displayed comorbid features more frequently than males (38). There was an increased tendency of female patients to display anxiety-like disorders (discussed in (37), which might also affect the diagnostic of patients (39,40)).

Effect of familiarity with the environment

As suggested by previous studies (6), the Del/+ mice might have difficulties in habituating to new environments. The social deficits in Del/+ male mice did not occur over the short-term recording in our conditions of familiar cage mates. In this case, the arousal triggered by the environmental change might mask social impairments over the short-term experiment. Robust social deficits were observed over the long term; at this time scale, we can suppose that wild-type mice got habituated while Del/+ mice did not. In contrast, when interacting with an unfamiliar conspecific, Del/+ females displayed quantitative reduction of social contacts over the short-term already. This might suggest that initial social encounters might be even more stressful for Del/+ female mice. This increased behavioural reaction might be triggered

by the fact that these mice were tested in unfamiliar pairs in the testing environment that they already visited for the recordings in quartets. Such a re-exposure to the unfamiliar testing environment might boost behavioural deficits, as in the *Shank3*$^{\Delta C/\Delta C}$ mice (41). To explore this aversion towards unfamiliarity, further studies should incorporate social cognitive challenges in long-term monitoring of mixed-genotype groups within a complex environment to better fit the natural needs of mice (42) and to provide cognitive tasks to unravel social phenotypes (43,44).

Atypical ultrasonic vocalisations

Speech and language abnormalities were frequently described in patients carrying a 16p11.2 deletion. Indeed, 94% of patients were diagnosed with speech problems or delays (delay, poor articulation, lower verbal intelligence, difficulty in reading skills; (12)). Childhood apraxia of speech was detected in the majority of children and half of adults, as well as articulation or phonological errors, dysarthria, minimal verbal output and typical speech in some cases (11).

Mouse ultrasonic vocalisations cannot be considered as direct proxies for speech abnormalities since they are mostly innate. In 16p11.2 Del/+ models, vocal production impairments were minimal. Indeed, previous studies highlighted that Del/+ mice were able to utter all types of ultrasonic vocalisations in adults (Portmann's model: (22)) and in pups (Horev's model: (45)). In our study, we only observed slight variations in the acoustic structure of the USVs, which might reflect a simplification of the calls (shorter, less frequency modulated). The reduction of usage that we observed (less USVs, in shorter sequences) might reflect more closely the reduced arousal during social interactions in Del/+ mice compared to wild-type mice. This corroborates and refines behavioural findings and represents a proxy for social arousal (24).

Perspectives

The characterisation of the present model highlighted robust social deficits, that also seemed to parallel sex-related variations in patients. The same framework could be used to examine the contribution to the social phenotype of each gene within the deleted region, as it has been done to identify the contribution of *Kctd13* gene to the cognitive impairment phenotype (46,47). To ascertain the robustness of these findings, a cross-species comparison should be conducted in the rat model, as recommended in recent guidelines to increase the value and robustness of preclinical models (48). Rescue strategies could then be attempted, with for instance R-baclofen, a GABAb agonist, or Fasudil, an inhibitor of the Rho-associated protein kinase, both restoring the cognitive deficits in the mouse model (46,49). Currently, the effects

of such treatment on the social phenotype is not documented and it would be of interest to evaluate its therapeutic value.

Material and methods

Animals

Mice were generated according to the breeding scheme used in (20). In brief, C57BL/6N.16p11.2 Del/+ females were bred with sighted C3H/HeH (C3B) males (50) (16p11.2+/+) to obtain F1 C57BL/6N x C3B.16p11.2 Del/+ (hereafter Del/+) and F1 C57BL/6N x C3B.16p11.2 +/+ (hereafter WT) mice. The cohort included 24 males (12 WT and 12 Del/+) and 32 females (16 WT and 16 Del/+). Animals were grouped in cages of four animals at weaning (quartets: 2 WT and 2 Del/+), therefore leading to 6 cages of males and 8 cages of females. In addition, for paired social encounters, we added two pairs of Del/+ females of the same age and housed in similar conditions. All mice were housed under 21-23°C with 12h/12h light/dark cycle (lights on at 7:00 AM). Hemp squares, food and water were available ad libitum. All mice were weighted at 11 weeks.

Individual identification

Mice were identified through finger cuts realised between 2 and 7 post-natal days. Genotyping was conducted on these finger biopsies according to the protocol described in (20). In brief, DNA was extracted in NaCl. PCR reaction used the primers Del70 F (CCTGTGTGTATTCTCAGCCTCAGGATG) and primer Del71 R (GGACACACAGGAGAGCTATCCAGGTC) with the following cycles: one cycle of 4 min at 95°C, 35 cycles of 30°C at 94°C + 30 s at 62°C + 1 min at 72°C, one cycle of 7 min at 72°C.

At least two weeks before starting the recordings, we inserted a Radio Frequency IDentification (RFID) tag (APT12 PIT tags; Biomark, Inc., Boise, The United States of America) under the skin of each individual under gas anaesthesia (Isoflurane) with local analgesia (Lidor 20 mg/ml, with 40 ul/10 g mouse). RFID tags were located in the lower part of the left flank. Mice were allowed to recover for one week. They were manipulated three days before starting the behavioural experiments to get them used to the experimenters and to being held within a cup. Mice were habituated to the experimental room and the setup since they underwent the novel object recognition test (data not presented) in the same room and setup at least one week before the quartet recordings. They underwent the dyadic encounters at least one week after the quartet recordings, and were therefore also familiar with the experimental room.

Behavioural monitoring in quartets

We monitored the individual and social behaviours of each quartet of mice over three days and nights in the Live Mouse Tracker system (LMT, plugin 931; (34)). This system tracks individually mice living in a group over several days and nights and extracts automatically the number, total duration and mean duration of more than thirty behavioural events describing the posture of the mouse, the types of social contacts, the dynamic social approach and escapes and complex social groupings (see (34)). In this system, the four mice (10-14 weeks of age) of each housing cage were left undisturbed for 71 hours in a large transparent Plexiglas cage (50 x 50 x 40 cm), with fresh bedding, a house (width: 100 mm, depth: 75 mm, height: 40 mm) in red Plexiglas, 6 dental cotton rolls as well as food and water ad libitum. Light/dark cycle and temperature conditions were similar to those of the housing room (12/12h light/dark, lights on at 07:00 AM, 75-90 lux when the lights were on). Each recording session started between 03:00 and 04:00 PM. At the end of the session, mice were placed back in their home cage and the LMT setup was cleaned with soap water and dried with paper towels. Altogether, we recorded the six cages of males and the eight cages of females, keeping the animals with their familiar cage mates. For each individual, we extracted the total distance travelled. We also automatically recorded the following behavioural events (based on the original publication of LMT (34); the type of quantification extracted is indicated in brackets):

Single move: The focal animal is moving (speed > 5 m/s) without being in contact with any other animal (total duration, number of events, mean duration of events).

Move in contact: The focal animal is moving (speed > 5 m/s) while being in contact with another animal (total duration, number of events, mean duration of events).

Jumps: The focal animal is jumping against the wall (total duration, number of events, mean duration of events).

Single idle: The focal animal is resting (not moving) without being in contact with any other animal (total duration, number of events, mean duration of events).

Rearing: The focal animal is straightened on its hindlegs (either unsupported or against the wall). Rearing is considered when the body slope is higher than a threshold (total duration, number of events, mean duration of events).

Rearing in contact: The focal animal is straightened on its hindlegs (either unsupported or against the wall) while being in contact with another individual. Rearing is considered when the body slope is higher than a threshold (total duration, number of events, mean duration of events).

Contact: The focal animal is touching another individual (total duration, number of events, mean duration of events).

Group of 2: The focal animal is touching one and only one other individual (total duration, number of events, mean duration of events).

Group of 3: The focal animal is touching two and only two other individuals (total duration, number of events, mean duration of events).

Nose-nose: The focal animal is sniffing the nose of another animal (i.e., the nose is at a whisker distance from the nose of the other animal) (total duration, number of events, mean duration of events).

Nose-anogenital: The focal animal is sniffing the ano-genital region of another animal (i.e., the nose is at a whisker distance from the tail basis of the other animal) (total duration, number of events, mean duration of events).

Side-side: The flank of the focal animal is in contact with the flank of another animal; both animals head in the same direction (total duration, number of events, mean duration of events).

Side-side head-to-tail: The flank of the focal animal is in contact with the flank of another animal; both animals head in opposite directions (total duration, number of events, mean duration of events).

Train2: The focal animal is moving (speed > 5 m/s) while sniffing the ano-genital region of another animal also moving (total duration, number of events, mean duration of events).

Follow: The focal animal is walking in the path of another individual: the two animals are moving at a speed >5 cm/s, the angles between the two animals are less than 45° apart, and the mass centre of the follower (the focal animal) is within a follow zone of one mean body length of width and two mean body lengths of length (total duration, number of events, mean duration of events).

Approach contact: The focal animal gets closer to another one, with the approaching animal walking at a higher speed than the approached animal; the approach ends by a contact between the two animals (total duration, number of events, mean duration of events).

Make group3: The focal animal is joining a group of two animals to form a group of three animals in contact (number of events).

Make group4: The focal animal is joining a group of three animals to form a group of four animals in contact (number of events).

Break contact: The focal animal is getting away (higher speed) from the animal it has been in contact with; the speed of the focal animal is higher than the speed of the other animal (number of events).

Break group3: The focal animal is leaving a group of three animals to leave a group of two animals in contact; the focal animal has the highest speed among the three animals in contact (number of events).

Break group4: The focal animal is leaving a group of four animals, that remain as a group of three animals in contact; the focal animal has the highest speed among the four animals in contact (number of events).

For social events, we computed the variables either in general or separately according to the identity of the interacting individual. These behaviours are not exclusive: one animal can be involved in several of them simultaneously.

Social encounter between unfamiliar individuals in pairs

We evaluated the social interactions and communication between unfamiliar individuals in pairs. For these recordings of social behaviour and ultrasonic communication, we focused on pairs of individuals since we currently cannot identify the emitter of USVs when animals were interacting closely. Therefore, we recorded undisturbed dyadic interactions between two unfamiliar individuals (from two different housing cages) of the same age (14-20 weeks of age) and genotype for 47h (two days and nights, starting between 03:00 and 04:00 PM). For that purpose, we coupled the LMT system (plugin 931) with one CM16/CMPA microphone (Avisoft Bioacoustics, Glienecke, Germany) connected to the Avisoft Ultrasound Gate 416 (300 kHz sampling rate, 16-bit format; trigger: level of this channel; pre-trigger: 1 s; hold time: 1 s; duration > 0.005 s; trigger event: 2 % energy in 25-125 kHz with entropy < 50%; Avisoft Bioacoustics, Glienecke, Germany). LMT and Avisoft systems were synchronised based on the protocol described in (24). Altogether, we recorded eight pairs of WT females and ten pairs of Del/+ females. We focused on females since males were too aggressive toward each other when they were taken out of their housing group to conduct robust (and safe) social monitoring. We recorded the same behaviours as in quartets recordings, except those involving more than two animals. USVs were analysed using LMT – USV Toolbox (24).

Transitions between exclusive behavioural events

To investigate the transitions between two events in paired encounters, we needed to compute exclusive events, i.e., events that do not overlap in time for each individual. For that purpose, we split the existing overlapping events in more simple events that were not

overlapping in time to obtain new exclusive events (script ComputeTransitionsBetweenEvents.py). We obtained the following exclusive events:

Move: The focal animal is moving (speed > 5 m/s) without being in contact with any other animal.

Idle: The focal animal is resting (not moving) without being in contact with any other animal.

Nose-nose: The focal animal is sniffing the nose of another animal (i.e., the nose is at a whisker distance from the nose of the other animal).

Nose-anogenital: The focal animal is sniffing the ano-genital region of another animal (i.e., the nose is at a whisker distance from the tail basis of the other animal).

Passive nose-anogenital: The focal animal is being sniffed in the ano-genital region by another animal (i.e., the nose is at a whisker distance from the tail basis of the focal animal).

Side-side: The flank of the focal animal is in contact with the flank of another animal; both animals head in the same direction.

Side-side head-to-tail: The flank of the focal animal is in contact with the flank of another animal; both animals head in opposite directions.

Nose-nose & Side-side: The focal animal is sniffing the nose of the other animal during a side-side contact with this same animal.

Nose-anogenital & side-side head-to-tail: The focal animal is sniffing the ano-genital region of the other animal during a side-side head-to-tail contact with this same animal.

Passive nose-anogenital & side-side head-to-tail: The focal animal is being sniffed in the ano-genital region by the other animal during a side-side head-to-tail contact with this same animal.

Other contact: The focal animal is in contact with another animal and this type of contact is not one of the above described ones (i.e., nose-nose, nose-anogenital, side-side, side-side head-to-tail, nose-nose & side-side, or nose-anogenital & side-side head-to-tail).

Undetected: The focal animal is not detected (tracking issues). This event was needed to have each animal engaged in one event at each time frame.

We computed the proportion of transitions ‘A to B’ from one event (event A) to another (event B) by dividing the number of transitions ‘A to B’ by the total number of occurrences of event A. This was conducted for each individual separately, as each individual was involved in one and only one event at each moment.

Statistical analyses

We did not exclude any outlier. For the behavioural profiles computed in quartets, we centred and reduced data of the Del/+ mice per cage (i.e., per quartet) and compared these z-score values for each Del/+ individual to 0 using Student's one-sample T tests (*ttest_1samp()* function from the *SciPy 1.8.0* package of Python 3.8). Given the small sample sizes of our data for social behaviours at the pair level (e.g., contact, nose-nose contact, side-side contact, side-side head-to-tail, total number of USVs) in encounters between unfamiliar individuals, we used non-parametric Mann-Whitney U tests from the *SciPy 1.8.0* package of Python 3.8. For behaviours in quartet monitoring and at the individual level during paired encounters (e.g., activity, exploration, asymmetric social events), and acoustic features of USVs and USV sequences, we used linear mixed models (*mixedlm()* function from the *statsmodels 0.13.2* package in Python 3.8), with genotype as fixed factor and cage as random factor. Proportion of transitions between exclusive behavioural events were compared at the individual level between genotypes using Mann-Whitney U-tests from the *SciPy 1.8.0* package of Python (3.8). In this case, P-values were corrected by the number of tests conducted (12*11) and effect size was estimated using the Cohen's D indicator. All scripts are available (github link available after publication).

List of abbreviations

RFID: Radio Frequency IDentification

LMT: Live Mouse Tracker

USV: UltraSonic Vocalisation

LMM: Linear Mixed Model

MW: Mann-Whitney U-test

Description of additional files

Additional file 1 (additional_file_1_table_review_animal_models_16p11.xlsx): **Synthetical view of the phenotypes displayed by the different mouse models of the 16p11.2 deletion syndrome**.

Additional file 2 (additional_file_02_Suppl_Figure_1.pdf): **Distance travelled by mice of both genotypes in both conditions.** A. Distance travelled over the first 15 min of recordings of spontaneous behaviours of mixed-genotype quartets of familiar males (12 WT and 12 Del/+ distributed in 6 cages) or females (16 WT and 16 Del/+ distributed in 8 cages). B. Distance travelled over the three nights of recordings of spontaneous behaviours of mixed-

genotype quartets of familiar males (12 WT and 12 Del/+ distributed in 6 cages) or females (16 WT and 16 Del/+ distributed in 8 cages) (Linear Mixed Model, with genotype as fixed factor and cage as a random factor). C. Distance travelled over the first 15 min of recordings of social encounters between two unfamiliar females of the same genotype. D. Distance travelled over the two nights of recordings of social encounters between two unfamiliar females (16 WT distributed in 8 pairs, 20 Del/+ distributed in 10 pairs; Linear Mixed Model, with genotype as fixed factor and pair as a random factor). ns: no significant difference, *: $p<0.05$, **: $p<0.01$.

Additional file 3 (additional_file_03_Suppl_Figure_2.pdf): **Behavioural profiles for the mean duration of events of Del/+ females and males recorded in quartets over short and long recording times**. Z-score profile of the total duration of each behaviour for each Del/+ mouse compared to the mean behaviour of the four individuals within each quartet for males (n=12) over the first 15 min (A) and over three nights (B), as well as for females (n=16) over the first 15 min (C) and over the three nights (D). One sample T-test: *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. Red boxes and stars figure behavioural events that are more expressed in Del/+ mice compared to the mean of the whole cage; blue boxes and stars depict behavioural events that are less expressed in Del/+ mice compared to the mean of the whole cage; grey boxes reflect non-significant differences between Del/+ and the other animals of the cage.

Additional file 4 (additional_file_04_Suppl_Figure_3.pdf): **Selective interactions between genotypes for quartets of females and males recorded over short-term (15 min) periods.** In female quartets recorded over short-term (i.e., 15 min) period: A. proportion of the total time spent in contact, B. proportion of the number of approaches leading to a contact and C. mean duration (in frames) of the contacts established with individuals of the same or of the different genotype for WT (n=16) and Del/+ (n=16) females. In male quartets recorded over short-term (i.e., 15 min) period: D. proportion of the total time spent in contact, E. proportion of the number of approaches leading to a contact and F. mean duration (in frames) of the contacts established with individuals of the same or of the different genotype for WT (n=12) and Del/+ (n=12) males. A, B, D & E: One sample T-tests compared to expected proportions; dashed horizontal lines represent the expected proportions: 1/3 with individuals of the same genotype and 2/3 with individuals of the different genotype. C & F: Non-parametric Mann-Whitney U-tests. ns: not significant, **: $p<0.01$.

Additional file 5 (additional_file_5_supplementary_table_II_statistics_LMM.xlsx): **Summary of statistics in linear mixed models used in the encounters of unfamiliar pairs of females**. Linear mixed models involve 'genotype' as a fixed factor and 'pair' as a random

factor. Statistical results are presented for short- and long-term analyses. β is the coefficient estimate for the fixed factor 'genotype'; it corresponds to the slope of the line between the WT and the Del/+ values. SE(β) is the standard error of this slope. z is the associated Wald's z-score: z=β/SE(β). The lower bound and the upper bound are the limit of the 95% confidence interval. The p-value is the probability of the current data to exist if the genotype effect is null; the lower the p-value, the most likely the genotype has an effect. We also give the coefficient estimate and the standard error of this coefficient estimate for the random factor 'pair', as well as the log likelihood of the model.

Additional file 6 (additional_file_06_Suppl_Figure_4.pdf): **Specific symmetric social contacts recorded in social encounter in same-genotype pairs of unfamiliar females**. Proportion of the total time spent in nose-nose contacts (A), number of occurrences of nose-nose contacts (B) and mean duration of nose-nose contacts (C) over short- and long-term recordings. Proportion of the total time spent in side-side contacts (D), number of occurrences of side-side contacts (E) and mean duration of side-side contacts (F) over short- and long-term recordings. Proportion of the total time spent in side-side head-to-tail contacts (G), number of occurrences of side-side head-to-tail contacts (H) and mean duration of side-side head-to-tail contacts (I) over short- and long-term recordings. 8 pairs of WT-WT and 10 pairs of Del/+ Del/+; Mann-Whitney U-test; ns: no significant difference, **: $p<0.01$.

Additional file 7 (additional_file_08_Suppl_Figure_5.pdf): **Characterisation of the follow behaviour with ano-genital sniffing (Train2) in the social encounters between same-genotype pairs of unfamiliar females**. A. Mean speed of the animal during the Train2 events over short- and long-term recordings. B. Mean distance travelled by the animal during the Train2 events over short- and long-term recordings. Linear Mixed Models, with genotype as a fixed factor and pair as a random factor; ns: no significant difference, *: $p<0.05$.

Additional file 8 (additional_file_09_Suppl_Figure_6.pdf): **Rate of ultrasonic vocalisations emission during each behavioural event** during the social encounter between same-genotype pairs of unfamiliar females over short- (A) and long-term (B) recordings. Linear Mixed Model with genotype as fixed factor and pair as random factor; ns: no significant difference.

Additional file 9 (additional_file_10_Suppl_Figure_7.pdf): **Comparisons of the contexts of emission of ultrasonic vocalisations between WT-WT and Del/+-Del/+ pairs of unfamiliar females**. Proportion of the total number of USVs recorded occurring synchronously with the different behavioural events over short-term (A) and long-term (B). Proportion of events occurring synchronously with USVs over short-term (C) and long-term

(D). Mann-Whitney U-tests between 8 WT-WT pairs and 10 Del/+-Del/+ pairs; ns: no significant difference, *: p<0.05, **: p<0.01, ***: p<0.001.

Additional file 10 (additional_file_11_Suppl_figure_8): **Behavioural profiles built with exclusive events in pairs of same-genotype unfamiliar females over short- and long-term recordings**. Each row represents one exclusive behaviour, and for each exclusive behaviour the first column represents the proportion of total time spent in this behaviour, the second column represents the number of occurrences per hour and the third column represents the mean duration of this behaviour. 16 WT distributed in 8 pairs and 20 Del/+ distributed in 10 pairs; Linear Mixed Model or Mann-Whitney U-tests conducted; ns: no significant difference, *: p<0.05, **: p<0.01, ***: p<0.001.

Declarations

Ethics approval

In compliance with ethical rules and regulatory requirements on use and welfare of laboratory animals, this protocol and all procedures have been reviewed and approved by our ethical committee (Com'Eth, CE17) registered at the French Ministry of Research under the reference: 2018062715092398 (DAP15692).

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the Zenodo repository, [PERSISTENT WEB LINK TO DATASETS here available upon publication]. Ultrasonic vocalisations data will also be available on the MouseTube database (51).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AR, YH and EE designed the study and wrote the manuscript. AR and EE conducted the behavioural experiments and analysed the data. FdC provided the pipeline to analyse ultrasonic vocalisations. AR, CC, VB and VN genotyped the animals.

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