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Adrenocortical and behavioural response to chronic restraint stress in neurokinin-1 receptor knockout mice

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ABSTRACT

Brain substance P and its receptor (neurokinin-1, NK1) have a widespread brain distribution and are involved in an important number of behavioural and physiological responses to emotional stimuli. However, the role of NK1 receptors in the consequences of exposure to chronic stress has not been explored. The present study focused on the role of these receptors in the hypothalamic–pituitary–adrenal (HPA) response to daily repeated restraint stress (evaluated by plasma corticosterone levels), as well as on the effect of this procedure on anxiety-like behaviour, spatial learning and memory in the Morris water maze (MWM), a hippocampus-dependent task. Adult null mutant NK1−/− mice, with a C57BL/6J background, and the corresponding wild-type mice showed similar resting corticosterone levels and, also, did not differ in corticosterone response to a first restraint. Nevertheless, adaptation to the repeated stressor was faster in NK1−/− mice. Chronic restraint modestly increased anxiety-like behaviour in the light–dark test, irrespective of genotype. Throughout the days of the MWM trials, NK1−/− mice showed a similar learning rate to that of wild-type mice, but had lower levels of thigmotaxis and showed a better retention in the probe trial. Chronic restraint stress did not affect these variables in either genotype. These results indicate that deletion of the NK1 receptor does not alter behavioural susceptibility to chronic repeated stress in mice, but accelerates adaptation of the HPA axis. In addition, deletion may result in lower levels of thigmotaxis and improved short-term spatial memory, perhaps reflecting a better learning strategy in the MWM.

1. Introduction

Substance P (SP) was the first peptide characterized as a neurotransmitter/neuromodulator in peripheral nociceptive neurons, which synapse with dorsal spinal cord neurons [1]. It is now well established that SP terminals and cell bodies are present in numerous brain regions [2]. Substance P belongs to the family of neuropeptides called neurokinins, which includes SP, neurokinin A and neurokinin B. Neurokinins act through three cloned receptors NK1, NK2 and NK3, with SP having greater affinity for NK1 [3].

In recent decades, there has been a considerable increase in evidence showing that SP and NK receptors are involved in a wide range of stress-related phenomena, including stress-induced analgesia, anxiety and depression-like behaviour [4]. Although most of these results have been obtained using selective non-peptide NK receptor antagonists, some have also been obtained using mice with functional deletions of the NK1 receptor or the tac1 gene. Administration of SP or NK1 agonists induces anxiogenic effects, whereas blockade of NK1 receptors reduces anxiety [5–7] and has antidepressant properties [8,9]. Moreover, blockade of NK2 and NK3 receptors also appears to have antidepressant properties [9–11]. The above effects are quite consistent, even though there are some reports suggesting that SP can exert protective effects against stress, when administered into the dorsal hippocampus [12], which may suggest regional differences in the effect of SP.

Activation of the hypothalamic–pituitary–adrenal (HPA) axis is the prototypical response to stress in all vertebrates, and is under the control of stimulatory inputs arriving at the paraventricular nucleus of the hypothalamus (PVN). It has been repeatedly found in rodents that plasma levels of peripheral HPA hormones (ACTH and corticosterone) reflect the intensity of a stressful situation [13].

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Therefore, if SP were involved in the control of the emotional response to stress, it would be expected that this peptide had a role in the regulation of the HPA axis. In addition, SP fibres are present in the PVN, the key region in the regulation of the HPA axis, which contains corticotrophin-releasing hormone (CRH) and other putative secretagogues of ACTH that, in response to stressors, are released into the pituitary-portal blood. The role of SP and NK1 receptors, especially on the regulation of the HPA axis, is clearly inconsistent. For example, intracerebroventricular (icv) administration of SP has been found to both inhibit [14–16] and stimulate [17] the HPA axis. Similarly, central administration of non-peptide NK1 antagonists resulted in activation of the HPA axis [18], suggesting a predominantly inhibitory tone, whereas the simultaneous central administration of NK1 and NK2 antagonists reduced, in response to formalin stress, adrenaline, ACTH and PVN c-fos expression [19]. A reduction of c-fos expression induced by exposure to a novel environment has been observed in the PVN and other brain areas, by prior administration of NK1 antagonists [20]. It is important to take into consideration that a wide range of drugs are able to activate the HPA axis, regardless of their specific neurochemical actions [13]. Therefore, in the light of the controversial pharmacological findings, complementary approaches are needed.

Several mutant lines with deletion of the NK1 receptor have been generated in mice, which are interesting tools for studying the role of this receptor in stress-induced changes. However, to our knowledge, only a few previous reports have addressed the response to stress in NK1−/− mice. In a first report, maternal separation-induced ultrasound vocalisation in pups was found to be reduced in NK1−/− mice [21]. Other studies focused on corticosterone response to acute stress, with clearly conflicting results [22–24]. For instance, when compared to the corresponding wild-type mice, unaltered corticosterone response to stress was found in NK1−/− mice with a C57BL/6 background [23], whereas a reduced response was found in those with a 129/sv background [22]. However, McCutcheon et al. (2008) [24] compared in the same study NK1−/− mice with either pure C57BL/6 or mixed C57BL/6-129/sv backgrounds and their corresponding wild-type mice. They found differences in corticosterone response to stress between wild-type mice of both strains, but no influence of NK1 receptor deletion.

In addition to the paucity of data about the response to acute stressors in NK1−/− mice, to our knowledge, there is no published information about how genetic deletion of the NK1 receptor can affect endocrine and behavioural responses to chronic stress. It is possible that chronic exposure to stress may reveal effects of NK1 receptors, which are not observed under basal or acute stress conditions. Our hypothesis is that genetic deletion of the NK1 receptor would reduce the negative impact of chronic stress and would favour adaptation of the HPA axis. Hence, in the present work with NK1−/− mice, the effects of daily repeated exposure to restraint stress on the adaptation of the HPA axis were studied, as well as some behavioural aspects that have been found to be sensitive to stress, such as anxiety [25,26] and spatial memory [27,28].

2. Materials and methods

2.1. Animals and general procedures

Adult male NK1−/− mice and wild-type (NK1+/+) littermates were used. Original mice were derived from homologous recombination of C57BL/6J blastocysts implanted with 129/sv stem cells containing targeted disruption of the NK1 receptor gene [29]. Mice used in this study were obtained from the original C-57BL/6×129/sv line back-crossed onto a C57BL/6J background over 10 generations. They were named NK1−/− backcross and NK1+/+ backcross.

The experimental procedures were always carried out in the morning between 8:30 a.m. and 1:00 p.m., when the resting and stress levels of HPA hormones are very stable. All animals were handled for 4 days for approximately 2 min a day, before starting experiments. Animals were kept in groups of 4–5 under standard conditions of temperature (22±1 °C) and a 12 h (8–20 h) dark/light cycle, with ad libitum food and water intake. The experimental protocols were approved by the Ethics Committees of the *Universidad Miguel Hernández de Elche* and of the *Universitat Autònoma de Barcelona*, following the "Principles of laboratory animal care", and were carried out in accordance with the European Community Council Directive (86/609/EEC).

2.2. Experiment 1

The aim of the experiment was to demonstrate possible differences in the response of the HPA axis to acute and repeated stress in NK1−/− mice. After the period of handling, blood samples were taken by the tail nick procedure, under resting conditions, to accustom animals to the procedure. The tail nick procedure consisted of gently wrapping the animals with a cloth, making a 2 mm incision at the end of the tail veins and then massaging the tail while collecting, within 2 min, 100 μl of blood into ice-cold EDTA capillary tubes (Sarsted, Granollers, Spain). This procedure is extensively used in our laboratory [30,31] and results in levels of hormones similar to those obtained after decapitation without anaesthesia [32]. Wild-type and NK1−/− mice were assigned to control or chronic stress groups. In those mice assigned to control groups, blood samples were taken twice daily, with a 2 h interval between taking each sample. Those mice assigned to chronic stress groups were restrained daily for 2 h, with samples being taken just before and after restraint. Restraint procedure consisted in complete immobilization of the animal in open-ended Plexiglas cylindrical restrainers measuring 2.8 cm in diameter and 10 cm in length. The rear top of the apparatus was adapted in relation to the weight of the animal to maintain the same level of restraint, irrespective of animal size. Seven holes (1 cm in diameter) in the walls of the cylinder provided fresh air. The chronic stress protocol lasted for 9 days, sufficient time to verify the adaptation of the HPA axis [33,34]. Blood sampling was carried out on days 1, 4 and 9.

2.3. Experiment 2

The aim of the experiment was to demonstrate possible differences in the behavioural consequences of exposure to repeated restraint stress in NK1−/− mice. Wild-type and NK1−/− mice were assigned to control or chronic stress groups. Chronic stress consisted of daily exposure to 2 h restraint stress (previously described) for 14 days. A longer period of chronic stress than in the previous experiment was chosen to maximize possible effects. Two days after the last restraint session, control and chronically stressed mice were tested in the dark–light test, and 2 days later the Morris water maze protocol was started.

2.4. Corticosterone radioimmunoassay

Plasma corticosterone levels were determined by double-antibody radioimmunoassays (RIA) as previously reported [35]. In brief, the corticosterone RIA used 125I-corticosterone–carboximethyloxime–tyrosine-methyl ester (ICN-Biolink 2000, Barcelona, Spain) as the tracer, synthetic corticosterone (Sigma, Barcelona, Spain) as the standard, and an antibody raised in rabbits against corticosterone–carboximethyloxime-BSA kindly provided by Dr. G. Makara (Institute of Experimental Medicine, Budapest, Hungary). We followed the RIA protocol recommended by Dr. G. Makara (plasma corticosteroid-binding globulin was inactivated by low pH). All samples to be statistically compared were run in the same assay to avoid inter-assay variability. The intra-assay coefficient of variation was 6% and the sensitivity 0.1 μg/dl.

2.5. Dark–light test

The dark–light test was performed 48 h after the last stress session. The dark–light box consisted of a small dark chamber (27×18×27 cm high) connected by a 7×7 cm opening to a larger white chamber (27×27×27 cm) without a top cover. The light intensity in the white compartment was 500 lx compared with 10 lx in the dark compartment. The mouse was placed in the dark compartment facing away from the light side. Behaviour of the mice was recorded for 10 min on video and analysed by a trained observer blind to the experimental conditions. The number of transitions from the dark to the light compartments, and the total time and activity in the light compartment were measured [36]. Entry into either side of the dark–light box was defined as the placement of all four paws into that side. The stretch-attend and flat-back postures were also measured as possible indices of anxiety [37].

2.6. Morris water maze

Mice were tested in the water maze for their spatial learning capabilities. A pool (white, diameter 120 cm) was filled with warm water (26±1 °C), made opaque by the addition of non-toxic paint. A platform (10 cm in diameter) was situated 5 mm below the surface of the water (invisible condition) or 8 mm above the water level (dark coloured rim; visible condition). The pool was divided into four quadrants with the platform in the middle of one of the quadrants. The protocol established by Grootendorst et al. (2001) [38] was followed with some modifications. For each trial, the mouse was placed in the water at one of four locations, equally spaced along the sidewall of the pool. A maximum of 60 s was allowed, during which the mouse had to find the platform and climb onto it. It remained there for 20 s (day 1) or 10 s (remaining trials and days). If the animal did not find the platform, it was guided there with a grid and was allowed to stay for 20 s on the platform. Four animals were run sequentially for the same trial during one session. After each trial, mice were placed under a red-light warming lamp to dry. One day before spatial training in the water maze started, the pool was filled with 2 cm of warm water and a large flat object was added to aid climbing. This was the animal's first contact with water and each mouse was allowed to move around for 120 s (water adaptation trial). Water maze training on day 1 started with a 120 s free swim in absence of the platform. It was expected that this would motivate the animal to search for escape from the novel aversive environment and to consider the platform as a safe place when first encountered. It also allowed estimation of the ability of the mice to swim. A training trial with a visible platform followed 60 min later. Another trial, now with the invisible platform (trial 2) followed 60 min later. On days 2 and 3 the platform remained submerged and the interval between trials was about 5 min, except when otherwise stated. On day 2, seven training trials (trials 3 to 9) were run with a 120 min interval between trials 6 and 7. On day 3 four trials (trials 10 to 13) were run. For all training trials we assessed the latency (s) and travelled distance (cm) to find and climb on the platform, the swim speed (cm/s) and the time spent in the peripheral zone (thigmotaxis) as a possible measure of anxiety. Thigmotaxis was considered when the animal swam by the periphery of the pool, through a 13 cm corridor near the wall.

Five minutes after the last trial, a probe trial was carried out without the platform to evaluate short-term spatial memory. In the absence of the platform, if the animals remembered the usual location of the platform they would swim around the place where it used to be located [39]. Memory was then evaluated by measuring the distance travelled and the percentage of time spent in each quadrant: target (TQ), opposite (OQ), adjacent left (ALQ) and adjacent right (ARQ). Thigmotaxis and other parameters usually measured during regular trials were also recorded in the probe trial.

2.7. Statistical analysis

Experiment 1: to analyse the effects of repeated restraint stress on plasma corticosterone, generalized linear models (GzLM) were used [40], with repeated measurements (generalized estimating equations, GEE) [41], with stress and genotype as the between-subjects factors and day and sampling time as the within-subjects factors. Further comparisons were undertaken when appropriate.

Experiment 2: to analyse the influence of daily repeated restraint on behaviour in the dark–light test, a GzLM was used with stress and genotype as the between-subjects factors. To analyse the learning processes across days in the MWM, a GEE analysis was undertaken with the same between-subjects factors as above (stress and genotype), and days as the within-subjects factor. To analyse spatial memory in the probe trial a GEE was used, with stress and genotype as between-subjects factors and quadrant as the within-subjects factor. Data of thigmotaxis in the MWM were analysed by a GzLM, with stress and genotype as the between-subjects factors. If a statistically significant interaction was found, additional comparisons were made.

As a method of estimation, the maximum likelihood (ML) was used in all cases. The generalized linear model is a more flexible statistical tool than the standard general lineal model (GLM), because several types of data distribution can be chosen. Normality distribution was chosen with identity as a link function, because it was a better fit for the data. The significance of the effects was determined by the Wald chi-square statistic.

3. Results

3.1. Experiment 1

Plasma corticosterone levels in control and repeatedly restrained mice are shown in Fig. 1. The GEE analysis revealed significant effects for stress (Wald $\chi^2(1)=41.21$, $p<0.001$), day (Wald $\chi^2(2)=20.84$, $p<0.001$), sampling time (Wald $\chi^2(1)=88.10$, $p<0.001$) and the interactions stress×day (Wald $\chi^2(2)=17.15$, $p<0.001$), stress×sampling time (Wald $\chi^2(1)=110.94$, $p<0.001$), genotype×day×sampling time (Wald $\chi^2(2)=11.44$, $p<0.005$) and stress×day×sampling time (Wald $\chi^2(2)=23.46$, $p<0.001$). Appropriate further comparisons revealed that no differences emerged regarding resting corticosterone levels, whereas an interaction genotype×day was found in response to stress: in wild-type mice the reduction of corticosterone response after repeated restraint did not reach statistical significance on day 4, but on day 9 the reduction was significant as compared to both day 1 ($p<0.001$) and day 4 ($p<0.05$); in contrast, in NK1−/− mice the reduction was already significant on day 4 and was maintained on day 9 ($p<0.001$ vs. day 1 in the two cases), with no additional reduction from day 4 to day 9.

3.2. Experiment 2

Statistical analysis of the number of entries in the white box (Fig. 2a) showed a significant effect of stress (Wald $\chi^2(1)=4.46$; $p<0.05$), but there was no significant effect of genotype or of the interaction stress×genotype. Animals exposed to restraint, regardless of genotype, showed a reduced number of entries into the white area. GzLM analysis of the time spent in the white box (Fig. 2b) did not reveal statistically significant effects of either stress or genotype. Activity and stretch-attend behaviour were also measured in the illuminated area. No differences between groups were observed regarding activity (not shown) and strecht-attend behaviour was very low in all animals and, therefore, was not quantified.

The GEE analysis of the spatial learning behaviour in the MWM (Fig. 3) revealed a significant effect of training day (Wald $\chi^2(2)=105.01$; $p<0.005$), but no significant effects were found for genotype or stress, or for the interactions stress×genotype and training

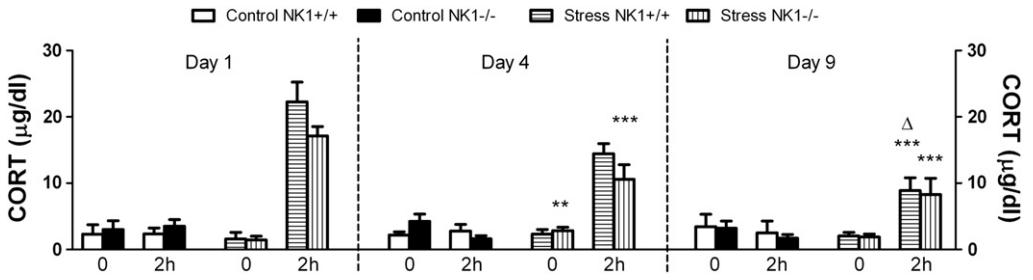

Fig. 1. Plasma corticosterone levels for stress-naïve (control) or chronic restraint NK1+/+ and NK1−/− mice. Means and SEM (n = 7–8/group) are represented. Samples were taken before the daily stressor (0) and immediately after stress (2 h) on days 1, 4 and 9 (or corresponding times for controls). **p<0.01; ***p<0.001 vs. the same genotype and sampling time on day 1; Δ p<0.05 vs. the same genotype and sampling time on day 4.

day × genotype. These results showed that mice of all groups learned to find the platform, with a similar pattern during the training days. A marginal effect was also found in the interaction stress × training day (Wald $\chi^2(2) = 5.74$; $p = 0.056$), suggesting that animals of both genotypes exposed to restraint learnt to find the platform more avidly during the course of the training days than did the control mice.

The analysis of the time spent searching in each quadrant during the probe trial in the MWM (Fig. 4) showed a significant effect of quadrant (Wald $\chi^2(3) = 180.13$; $p<0.001$). There were no significant effects for genotype or stress, and neither stress × genotype nor stress × quadrant was found to be significant interactions. However, the interaction quadrant × genotype was significant (Wald $\chi^2(3) = 13.92$; $p<0.005$). Decomposition of this interaction showed that knockout mice spent more time in the TQ and less time in the OQ than wild-type mice, regardless of stress; marginally significant differences were also found between genotypes in the ALQ quadrant, with NK1−/− spending less time in the ALQ quadrant than wild-type mice.

The GzLM analysis of thigmotaxis in the MWM (Fig. 5) showed a significant effect of genotype (Wald $\chi^2(1) = 4.41$; $p<0.05$), but no significant effects were found for stress or the interaction stress × genotype. These results suggest a higher preference for swimming in the peripheral area of the swimming pool during the probe trial in wild-type than in NK1−/− mice.

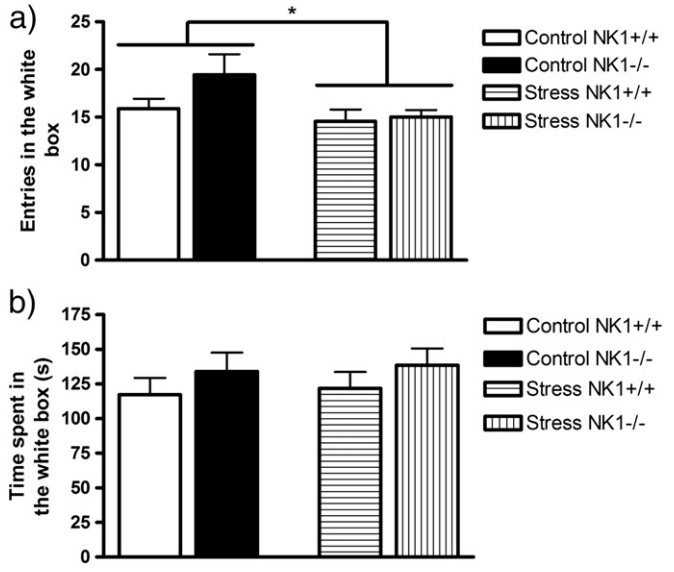

Fig. 2. Behaviour of NK1+/+ and NK1−/− mice in the dark–light test after chronic restraint stress. Each bar shows mean ± SEM (n = 8–9/group) of the number of entries (a) and the time spent (b) in the white box. The GzLM analysis revealed no effect of genotype or the interaction treatment by genotype, but a significant effect of chronic stress (*p<0.05) in the number of entries. Any significant effect was found in the time spent in the white box.

4. Discussion

The present results demonstrate that NK1−/− mice showed unaltered corticosterone response to acute restraint stress and faster adaptation to daily repeated exposure to the stressor than wild-type mice. In addition, repeated exposure to stress slightly increased anxiety-like behaviour in both genotypes, but did not affect spatial learning in the MWM. In the latter test, NK1−/− mice appeared to be more efficient than wild-type mice in that they showed lower levels of thigmotaxis and improved short-term memory.

In the first experiment, resting activity of the HPA axis and its response to acute and chronic restraint stress were studied. Mutant mice showed plasma corticosterone levels similar to those of wild-type mice, suggesting no changes in resting HPA activity. The response to acute restraint was also similar in mutant and wild-type mice. When mice were exposed daily to restraint, both genotypes showed reduced corticosterone response after repeated exposure, although there were some differences between the two genotypes. In wild-type mice, reduced corticosterone response to restraint was only observed on day 9 as compared with day 1, whereas in NK1−/− mice reduction was already observed on day 4 and this level was maintained until day 9. Reduced corticosterone response to daily repeated restraint reflects partial adaptation of the HPA axis to this stressor, further confirming previous results in rats [42–45]. As the time-course of adaptation differed between the two genotypes, NK1 receptor activation may play some role in maintaining the response of the HPA axis, despite repeated experience with the same situation.

On the basis of previously available data, the role of NK1 receptors in HPA responsiveness to stressors is unclear. In NK1−/− mice, unaltered [23] or reduced [22] corticosterone response to stress has been reported, which could be at least in part explained by the genetic background (C57BL/6 in the former case, 129/sv in the latter) [22]. In this regard, McCutcheon et al. (2008) [24] compared in the same study NK1−/− mice with either pure C57BL/6 or mixed C57BL/6-129/sv backgrounds and their corresponding wild-type mice. Although they found differences in corticosterone response to stress

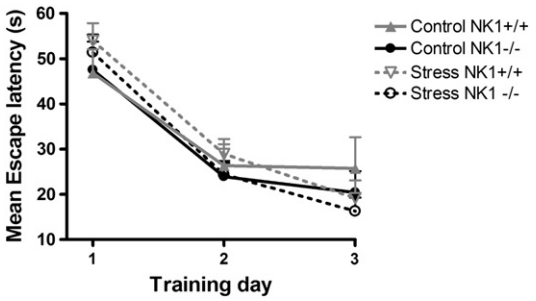

Fig. 3. Spatial learning behaviour of NK1+/+ and NK1−/− mice in the MWM after chronic restraint stress. Means ± SEM (n = 8–10/group) of average escape latencies on each training day are represented. The GEE analysis revealed a significant effect of day ($p<0.005$), but not of other factors or interactions.

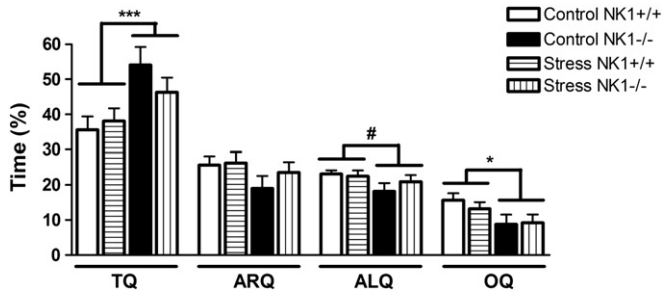

Fig. 4. Time spent searching in each quadrant of the MWM during the probe trial in NK1+/+ and NK1−/− mice after chronic restraint. Means ± SEM (n = 8–10/group) are represented. Quadrants are as follows: TQ, target quadrant; ARQ, adjacent right; ALQ, adjacent left; OQ, opposite. The GEE analysis revealed a significant interaction quadrant by genotype ($p<0.005$). The decomposition of this interaction showed that NK1−/− spent more time in the TQ than NK1+/+ (***$p=0.001$) and less in the OQ (*$p=0.012$); a marginally significant effect of genotype was found in the ALQ (#$p=0.055$).

between wild-type mice of both strains, no influence of NK1 receptor deletion was observed. Our present results confirm that NK1 deletion in a C57BL/6 background does not alter corticosterone response to acute stress. It is thus possible that the effects of NK1 deletion on corticosterone response to stress are restricted to mice with a pure 129/sv background.

In the second experiment, the effect of 14 days of chronic restraint on anxiety-like behaviour and learning in the MWM was studied in mutant and wild-type mice. Anxiety-like behaviour was assessed in the dark–light test. Two days after the termination of the chronic stress protocol, chronically restrained mice showed a significantly reduced number of entries from the dark to the illuminated area, but no change in the time spent in the latter area. In the same test, few signs of other anxiety-related behaviours, such as stretch-attend and flat-back postures, were observed. The overall results suggest a modest anxiogenic effect of chronic restraint stress that was genotype-independent.

Our results show a lack of effect of the deletion of NK1 receptor on anxiety-like behaviour, in both stress-naïve and chronically stressed mice. Previous research has shown a reduction in anxiety-like behaviour, as evaluated in the elevated plus-maze (EPM) ([22]; 129/sv background) or an unaltered level of such behaviour ([46,47] mixed background) in NK1−/− mice. Indeed, when the latter mice were crossed to introduce the MF1 genetic background, NK1−/− mice showed hyperactivity in novel environments and some signs of reduced anxiety in the light–dark test [48,49], thereby suggesting a contribution of genetic background. Interestingly, diminished anxiety was found after deletion of the tac1 gene, which encodes the neuropeptides substance P and neurokinin A, in a C57BL/6 background [50]. This suggests that lack of both SP and NKA exerts stronger effects on anxiety than a lack of NK1 receptors.

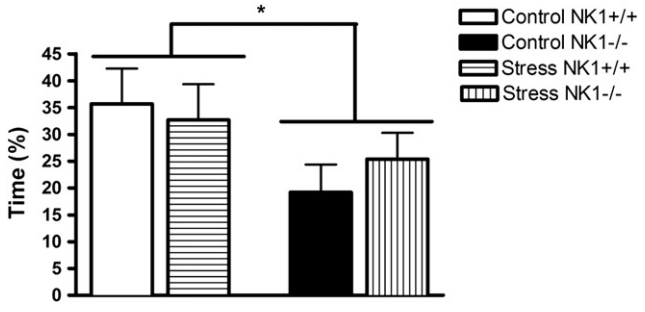

Fig. 5. Thigmotaxis behaviour in the MWM during the probe trial in NK1+/+ and NK1−/− mice after chronic restraint. Means ± SEM (n = 8–10/group) of the time spent in the peripheral zone in the MWM are represented. The GzLM analysis revealed a significant effect of genotype (*$p<0.05$), with NK1−/− mice spending less time in the peripheral zone than NK1+/+ mice, regardless of the stress condition.

In the literature, controversial effects of chronic exposure to restraint stress on anxiety-like behaviour have been reported, using EPM measurements and other tests [25,51]. Although some of these controversies may be related to the particular stress protocol and the use of animals differing in susceptibility to stress, it is still difficult to precisely determine the origin of such conflicting findings. The varied susceptibility of different animals has been directly tested in various studies. For example, long-lasting exposure to chronic stress enhanced anxiety and depression-like behaviour in BALB/cByJ, but not in C57BL/6ByJ mice [52]. In another study, C57BL/6 mice were again more resistant than BALB/cJ and DBA/2J to chronic unpredictable stress-induced increases in anxiety-like behaviour in the light–dark test [53]. Yet, there is also some evidence in rats and mice that chronic unpredictable stress can reduce rather than enhance anxiety [54,55]. Therefore, special attention should be paid to the factors determining susceptibility versus resilience to stressors.

A few days after finishing the chronic stress protocol, mice started training in the MWM to evaluate spatial memory, a hippocampus dependent task. No effects of chronic restraint stress were observed, whereas genotype differences in some parameters were noted. For example, during the probe trial (platform removed) mutant mice showed improved short-term (5 min) retention of the task in comparison to wild-type mice, as reflected by the greater time spent in the quadrant where the platform was located during learning. Both genotypes appeared to learn, in a similar way, how to find the hidden platform, as judged by the latency in finding the platform throughout the days of the experimental protocol. However, NK1−/− mice showed reduced thigmotaxis, suggesting an improved learning strategy. As no evidence for altered anxiety was observed in mutant mice in the dark–light test, it is unlikely that reduced thigmotaxis was related to a reduced anxiety. In fact, the anxiolytic diazepam did not alter the enhanced thigmotaxis observed in mice over-expressing the alpha-2C receptors [56], suggesting an altered strategy not linked to changes in anxiety. In addition to changes in thigmotaxis, the improved retention performance observed in NK1R−/− mice is compatible with their increased neurogenesis in the dentate gyrus and their higher levels of hippocampal BNDF [57]. Both neurogenesis and BDNF expression have been repeatedly found to be positively associated to hippocampus-dependent tasks [58,59], even though in a previous study improved performance in the MWM or in trace fear conditioning (two tasks assumed to be hippocampus-dependent) was not observed [57].

Under the conditions present in our study, chronic restraint stress did not appear to impair spatial learning in any genotype; thereby ruling out that the lack of NK1 receptors could sensitize the mice to the effects of stress. The lack of an effect by chronic restraint stress merits further comment. In a recent review on the topic [60], there is an excellent discussion of the factors putatively involved in the discrepancies between experiments. In addition to the use of different spatial learning tasks and outcomes, the length and type of chronic stress protocols appear to be important. For example, in rats exposed to chronic restraint stress, more than 2 weeks of exposure appears to be needed to induce some deficits [27,61], which may be compatible with our results. Another critical factor is the particular strain used, as marked strain differences have been repeatedly reported regarding the impact of stress in both rats and mice. More precisely, C57BL/6 mice have been found to be resistant to the consequences of exposure to chronic unpredictable stress in terms of impaired passive avoidance [62]. Pothion et al. (2004) [63] reported no effect of chronic unpredictable stress on spatial learning in the MWM in 3 different mice strains (CBA/H, DBA/2J and C57BL/6), whereas impaired long-term memory was observed only in the former. It is possible that radial maze tasks are more sensitive than the MWM, because chronic acoustic stress impaired radial maze learning in the C57BL/6 strain, but improved it in the DBA/2 [64].

In conclusion, the present results indicate that mice exposed to daily restraint stress showed evidence of the adaptation of the HPA axis, a modest increase in anxiety and no altered learning or memory in the MWM. Null mutation of the NK1 receptor resulted in unaltered corticosterone response to acute restraint stress, but faster adaptation to repeated restraint. Genotype differences were not found in the behavioural consequences of stress, although NK1−/− mice showed some evidence of improved short-term spatial memory. The previously reported enhancement of dentate gyrus neurogenesis in NK1−/− mice and their depression-resistant phenotype deserve further attention, regarding the putative role of NK1 receptors in depression [57]. The present results favour the hypothesis that the deletion of NK1 receptors does not have negative consequences when animals are exposed to chronic stress, thereby suggesting that there will be no negative consequences when NK1 antagonists are used in therapy against stress-related pathologies.

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