7

General discussion

The aim of this thesis was to explore the effects of social housing on chronic stress exposure in male and female rats. We used chronic footshock stress as a model for affective disorders, and investigated if social housing could improve stress coping, and possibly provide an animal model to study the neurobiology of social support.

In the first experiment male and female rats were either individually or socially housed in unisex groups of 4 rats. Rats of the stress groups were exposed to daily footshocks while the control animals were placed in similar non-electrified cages. Of the social groups, 2 rats in each group were exposed to stress while the other 2 served as controls.

In a follow-up experiment the animals were either pair-housed with a rat of the opposite sex, or housed individually. Half of the isolated animals were exposed to chronic stress, and of the paired housed rats, only one of the pair or neither received stress. To provide the socially housed females an escape possibility from the sexual advances of her male partner a plastic tube was added to the cage. A tube was also placed in the cages of individually housed rats so social housing was the only different variable between isolated and socially housed rats.

A wide variety of parameters was used to investigate the impact of chronic stress and the modulating effects of social housing. Locomotor activity in an open field test was used to investigate the impact of stress on behaviour. Weight gain and adrenal weight served as physiological markers and neurobiological effects of chronic stress were examined by measuring Fos, neurogenesis and pCREB expression in response to the adverse environment of the footshock box.

Aim

Behavioural effects

The most commonly found effect of stress in an open field test is a decrease in locomotor activity. We, however consistently found a stress-induced increase in activity, which in both experiments was most pronounced in the female rats. The presence of the tube is most likely the cause for the slight differences between the 2 experiments. Since rats in the second experiment were placed in the tube at the start of the test, this would have an effect, especially on first minute locomotor activity. Despite differences between experiments, exposure to stress increased locomotor activity in both experiments. Both, exposure to footshocks and to stressful components in their home cage, like isolation for females and a stressed female partner for males, resulted in increased activity. This shows that the increases in activity, as found in the current experiments, are a measure of a higher, and not a lower, impact of the stress.

One would expect a "depressed" rat to be less active, similar as found in depressed patients. Most studies indeed found a reduction in activity after stress exposure in rats.[17,26,72] However, like Duncko and coworkers[22] we found a stress-induced increase in activity, which could be prevented by social housing. There are several possible explanations for the discrepancy. Mostly behavioural tests were performed shortly after the stressor, and thus showing an acute behavioural response to stress. We were interested in the chronic effects of stress on behaviour, therefore rats were tested about 16 hours after the previous stress session, before the stress session of that day. Since our rats were housed in reversed light-dark cycle, stress exposure and the open field test occurred during the active period of the rat, in contrast to several other studies.[17,26]

By adding a tube to the open field, we hoped for a more evident behavioural effect, especially in the males since they showed only small changes in locomotor activity.

Placement of the tube in the open field provided an extra behavioural parameter, namely the time an animal spent in the tube. We hypothesised that rats would spent more time in the safe shelter of the familiar tube after chronic stress exposure. Isolated males did show this response, and increased the time spent in the tube. Social housing with a female prevented this response. Surprisingly, the presence of a stressed female partner also increased the time these males spent in the tube. This suggests that a stressed cage mate likely was perceived as stressful for the male, however without an apparent effect on the long-term impact of stress, since adrenal weight was not affected. Females however, showed a different response to stress exposure and housing conditions on the time they spent in the tube. Adrenal weights showed that isolated females were more stressed than socially housed

females, which is in concordance with results from the unisex experiment. However, these isolated females spent less time in the tube than socially housed females, and this effect was further decreased by chronic stress. Socially housed control females spent more time in the tube with repeated exposures to the open field, which was reduced after stress. This indicates that, under control (low stress) conditions, the apparent "normal" response for females is a reduction in exploratory behaviour and spending more time in the tube when an environment like the open field becomes familiar.

These studies show that behaviour provides an interesting and valuable parameter to measure the effects of chronic stress, however the results of behaviour studies are difficult to interpret especially in the females. Since isolation by itself is stressful for females, effects of chronic stress appear to be small because the control group is also stressed. However behavioural differences appear when the individually housed rats are compared with the socially housed females. Another aspect that should be taken into account is the fact that behavioural tests to measure stress effects are validated for males only. Different and even opposite neurobiological and behavioural effects of stress exposure have been observed in males and females,[6,15,31,60,74] it is therefore not odd that females also show a different response in an open field test.

Fos-reactivity

Behavioural and physiological data from both unisex and mixed-gender groups show that isolation was perceived by females as stressful. However it appears that not a stress-induced increase in Fos-expression, but the lack of such a response, is an indication of the impact of chronic stress in brains of these females. While the PVN showed a normal stress-induced increase in activity, brain regions known to modulate the response to affective stimuli, like amygdala, VTA and raphe nuclei,[1,19,42] failed to show an response to stress in isolated females, whereas socially housed females responded with increased Fos levels, like the males. Especially the reactivity of the serotonergic systems seems to be related to the impact of chronic stress. Isolated females were affected the most by chronic stress exposure and demonstrated a lack of response of the DRN, whereas the females that were the least stressed, namely the rats housed in unisex groups, showed an stress-induced increase in DRN Fos-activity. The DRN of stressed females housed with a male partner also failed to show a stress-induced increase in Fos-ir. However, the MRN of these rats did show a response, in contrast to that of their isolated counterparts, indicating a slight positive effect of social housing with a male but not to the same extend as social housing in unisex groups. Serotonergic neurons in the DRN are activated by inescapable, but not escapable stress,[36,49] and in response to psychological stress serotonin is released from the DRN.[30] It is therefore tempting to relate the absence of Fos-reactivity

in the DRN of isolated females to dysfunctions found in the serotonergic system of depressed patients.[32,45,47,61] Our data could provide an explanation for the higher effectiveness of SSRI's for treatment of depression in women.[40,48] Since in our experiment only the stressed isolated females and not males showed a dysfunction in the serotonergic system, it is tempting to speculate that especially in women chronic stress and/or stressful life events result in disturbances in the serotonergic system. So especially antidepressant drugs that increase the availability of serotonin in the brain would ameliorate symptoms in women.

Control males housed in unisex groups showed a similar level of Fos expression as isolated and socially housed stressed males in several brain regions. This indicates that socially housed control males were stressed by the presence of (stressed) male cage mates, in contrast to the females. On the other hand, males housed with a stressed female partner did not show this stress-like increase in Fos expression. This demonstrates that the elevated Fos expression in non-stressed socially housed rats is not solely due to the of "fear smelling" cage mates and increased social interactions, but likely a result of differences in the nature of the social interactions occurring between male-male and male-female rats.

The variation in basal and stress-induced Fos expression in some brain regions is quite high, and could be due to the fact that we have performed the experiments during the active period of the rats. Most studies using Fos expression to investigate the reactivity to stress were performed during the light/resting period of the rats, when brain activity is reduced compared to the active/dark period.[14] During the resting period Fos levels are most likely less variable between rats, and the reduced baseline/control levels could result in a more distinct and more homogenous stress-induced Fos response. In addition, the response to a stressor could be more 'intense' during the resting period because when the nervous system is not primed for stress coping.

In females high variability in Fos expression could also be caused by the estrus cycle. Sex steroids are known to modulate the stress response[11] and Fos mRNA expression after acute restraint stress is affected by stage of the oestrus cycle.[27] However in our chronic stress protocol females were stressed during all stages of the estrus cycle, which would most likely overrule sex hormone related differences in stress sensitivity. Concordant with previous findings, a synchronisation of the estrus cycle among females was not observed.[58] Females in every group were normal cycling females, it is therefore unlikely that the estrus cycle can explain the differences between the groups.

The absence of a stress-induced Fos expression in most brain regions of the isolated rats described in chapter 6 (pair-housed rats vs. isolated) suggests that these rats had habituated to the footshock,[62] in contrast to rats described in chapters 2

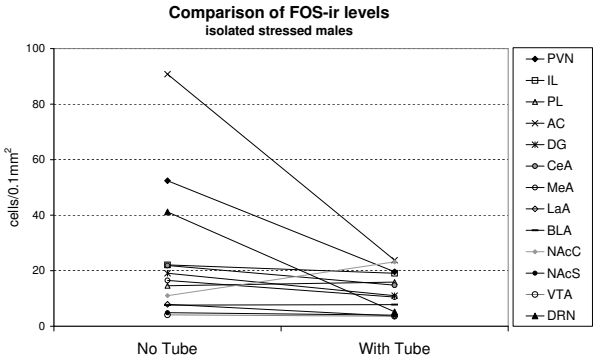

Figure 1. Comparison of Fos expression in several brain regions between isolated males housed with and without a tube.

and 3. However rats were subjected to the same stress procedure and it seems unlikely that one group of rats would habituate while the other would not. Since the paired-housed rats showed a normal stress-induced Fos response, the equipment functioned normally and all rats received footshocks. The isolated rats did show adrenal hypertrophy and also behavioural data showed that they suffered from chronic stress, it is therefore unlikely that these isolated rats were habituated to the stressor.

The isolated rats of the first and third experiment (Chapter 2 and 3 vs. 6) however also show different levels of Fos expression in several brain areas. Especially the isolated stressed rats have lower Fos levels and only few regions show a stress response in the third experiment (chapter 6)(Figure 1). While this could be due to differences in pre- and perinatal influences, another explanation could be the presence of a tube in the home cage, which was present in the third but not the first experiment. Rats show thigmotaxic behaviour, i.e. the avoidance of open spaces and tendency to hide in sheltered areas. The opportunity to hide in a tube when returned from the stress exposure, opposite to an "exposed" home cage, might reduce the reactivity of the stress response, especially of Fos-ir. The expression of Fos mRNA is maximal after half an hour after the stimulus,[75] (see also figure 2A), which is also the time the animal spent in the stress box during the last exposure, allowing the mRNA response to be maximal in our experiments. However, when a tube is present in the home cage, the stress response is minimal compared to rats without a home tube. It is tempting to speculate that a sheltered home tube signals "safe" and suppresses the translation of the Fos mRNA to protein (figure 2B).

This hypothesis is supported by preliminary data collected by M. Gerrits from our lab (unpublished results), who looked at stress responses of Fos-ir and Fos mRNA in ovariectomized (OVX) females with and without estradiol replacement. These rats were subjected to the same stress protocol and were also housed with a tube. She found an increase of mRNA levels in the PVN in both groups, however

Fos-ir was only increased in the OVX group, thus showing dissociation between Fos mRNA and protein expression (figure 3).

The translation of mRNA to protein is subjected to modulation, so transcription of mRNA does not automatically lead to translation of the mRNA to protein. Regulation of mRNA translation has been studied in mRNA that is transported to the dendrites. It has been suggested that binding of mRNA to a transport apparatus could serve as a mechanism to suppress synthesis of the protein until required,[3] also a non-translatable small RNA has been shown to act as a repressor of mRNA translation in dendrites.[70] Another possible mechanism could be translation repression by small antisense sequences during different levels of translation.[13,33] These mechanisms provide a reversible posttranscriptional suppression of mRNA translation, which could allow fast responses to environmental changes. While isolated rats got the change to hide quietly in the shelter of the tube, socially housed rats did not get this opportunity because of exposure to social interactions with the cage mate after reintroduction in the home cage, preventing the suggested inhibition of Fos mRNA translation into Fos protein. Also this proposed suppression is region specific, since Fos levels did not differ in all studied regions. Interestingly, even though the stress-reactivity of Fos is dampened by the presence of a tube in the cage of isolated rats, the impact of the chronic stress was not, since they still suffered from adrenal hypertrophy and behavioural changes in the open field test. In the described studies Fos expression was used a marker for neuronal activation.[59] The function of the Fos protein is however far from understood. The current study suggests that Fos expression is not essential for the impact of chronic stress exposure, since

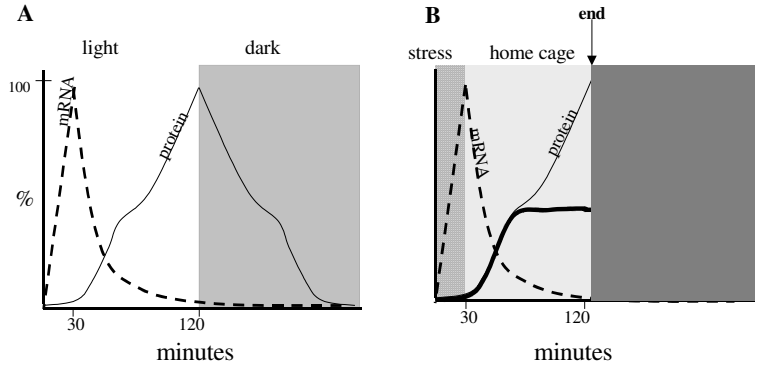

Figure 2. Graphs adjusted from Zangenehpour.[75] (**A**). Exposure to light quickly induces the expression Fos-mRNA, which is maximal 30 minutes after initiation of the stimulus. The level of the protein Fos is maximal 2 hours after the start of the stimulus. (**B**) In our experiment rats were exposed to the stress box for 30 minutes, allowing the Fos-mRNA response to be maximal. After the stress exposure they were put back into the home cage with or without tube. The opportunity of taking shelter in the tube could possibly inhibit translation of Fos mRNA, resulting in a lower level of Fos protein (bold line).

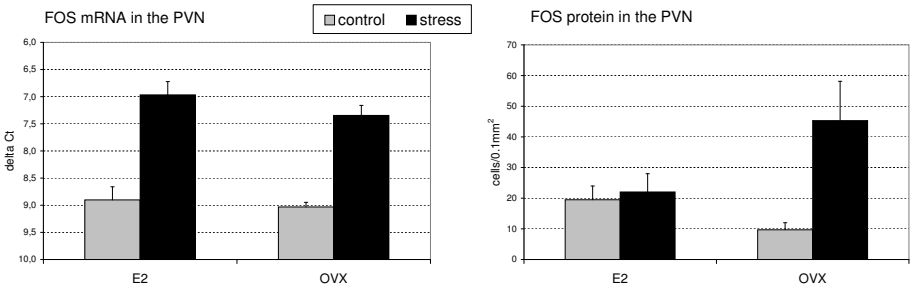

Figure 3. Fos-mRNA and Fos protein expression in the PVN of ovariectomized females, with and without estradiol replacement. Both groups were housed with a tube in the home cage. While Fos mRNA levels were increased after chronic stress in both groups, Fos protein was increased only in females without estradiol replacement, showing dissociation of Fos mRNA and protein expression (preliminary results, courtesy of M. Gerrits).

behaviour, adrenal weight and pCREB expression in the hippocampus showed changes that were related to chronic stress exposure.

Simple environmental enrichment can have an effect even on the response to an acute stressor. Belz et al. showed that placing a kong toys and nestlets in the cage of singly housed rats reduces baseline levels of stress hormones and the stress response to the acute stressor of a ip. saline injection in females.[2] It is reasonable to assume that if the presence of simple objects the rat can chew on, suppresses activity of the HPA-axis, a tube has an even more profound effect on HPA-axis reactivity, since a tube can also be used as a hiding place, besides providing a chewing object. These data show that even simple cage enrichment has profound effects on how a rats responds to stimuli, which are likely not limited to the stress response.

Confounding factors

Positive effects of social housing of unisex groups of male rats have been found.[56,69] An explanation for the absence of such a positive effect, and even an aggravation of stress symptoms, found in the current study, could be due to the combination of control and stressed males in the same cage. It is possible that stress exposure resulted in aggressive behaviour (although not observed) increasing the stress also for control males. It would have been useful to have added an extra control group of 4 control males housed in the same cage. In these undisturbed groups of males, social housing could have been perceived as positive compared to isolation. It is unlikely that aggression due to the formation of a social hierarchy in male groups was the cause of the increased impact of chronic stress. In all male colonies housed in a visible burrow system, no aggression was observed and no hierarchy was formed in contrast to colonies where females were present [4,5] (R.R. Sakai, University of Cincinnati, personal communication).

Variation in stress responses between experiments could be caused by differences

in pre- and postnatal factors, which are known to have long lasting effects on the adult nervous system. Prenatal stress increases the sensitivity to stressors during adulthood.[18,71] Also maternal behaviour has major effects on the offspring, which are still apparent during adulthood. Offspring raised by high licking mothers were less fearful than pups from low licking mothers.[28] Pups from high licking mothers also showed an increased neuronal survival in the hippocampus compared to low licking mothers, which was still evident after 3 months. [8,9,28] Interestingly, some of the effects induce by lower maternal care could be reversed by environmental enrichment.[9] These long-lasting effects of pre- and postnatal treatment could be a confounding factor when attempting to duplicate effects induced by chronic stress when rats are obtained commercially. Unpublished results from our lab showed that female rats obtained at the same time, but from different pavilions, were differently affected by chronic stress exposure. The factors that cause these differences are impossible to trace, and could vary from different treatment by the keepers of the mothers and/or pups to genetic variation between breeding pavilions. Therefore exact duplication of one variable between different experiments could be difficult, however consistency in the overall picture can be achieved by measuring several parameters, even when specific results cannot be exactly duplicated.

Sex-specific effects

Different behavioural responses to stress exposure have been found previously, especially with regard to learning and memory. Spatial memory is negatively affected by chronic stress exposure in male rats, but is improved in females.[7,15,41] Opposite effects of stress on classical eyeblink conditioning have also been found, which was improved in males, but impaired in females.[74] Several of the investigated parameters in the current experiments also showed gender-specific responses to both stress and housing conditions. While locomotor activity in the open field tests showed a stress-induced increase in both males and females, the response to stress and the influence of social housing on the time the animals spent in the tube showed a gender-specific response.

The parameter time spent in the tube during an open field test could provide a sensitive parameter to test the effects of antidepressants in both males and females, however with responses in the opposite direction. Chronically stressed individually housed males showed an increase in the time spent in the tube and pair-housing with a female appeared to have "antidepressant" effects and prevented this increase. The expected antidepressant response for males would therefore be a decrease in the time the animals spent in the tube. The most stressed females, namely the isolated stressed animals, spent the least time in the tube. Socially housed control females however showed an increase in the time in tube with repeated OF exposures, which

was inhibited by stress exposure. This indicates that an increase in time spent in the tube of female rats corresponds to reduced stress levels. So effective antidepressant treatment in females should result in an increase in time spent in the tube, which is opposite to the expected response in males.

We have founds sex-specific neurobiological changes after chronic stress exposure mainly in markers related to neuronal plasticity in the DG of the hippocampus, namely BrdU labelling (neurogenesis) and pCREB expression. Chronic stress-exposure induced a decrease in neurogenesis in male rats, which was prevented by social housing in unisex groups. This observed decrease is most likely caused by a decrease in cell proliferation and not survival, since others have found that acute as well as chronic stress decreases cell proliferation in males.[21,34,35] In isolated but not socially housed females we have found an increase in number of surviving neurones after stress, without a change in cell proliferation. The latter has also been found by Falconer et al.[25] Chronic stress exposure has been found to reduce pCREB labelling in several brain regions,[66] while antidepressant treatment results in increased pCREB expression. This implies that antidepressant exert their function by maintaining or increasing neuronal plasticity.[46] We have found a gender-specific effect of pCREB expression to chronic stress exposure and housing conditions. Socially housed males with a female partner showed a stress-induced increase in pCREB expression in the DG, suggesting an antidepressant effect of social housing.[64] No stress effect was observed in isolated males, while isolated females showed a strong decrease in pCREB expression after stress, which could not be prevented by social housing with a male partner, although this expression was slightly higher than in isolated counterparts.

The cAMP-CREB cascade is involved in cell proliferation in the subgranular cell layer of the DG. Activation of the cAMP-CREB cascade has been found to increase the number of newly born neurons in the DG.[51] In our experiments isolated females showed an increase in neurogenesis in the DG, while pCREB labelling in the DG showed a decrease in isolated females (although from a different experiment). Bromodeoxyuridine (BrdU), a thymidine analog which is incorporated in the DNA during cell division, was used to measure neurogenesis, and was given to the rats on days 3 to 7 of the three week stress protocol. Phospho-CREB however was measured on the last day of the experiment after 3 weeks of stress, and might therefore not relate to the number of BrdU-positive cells.

It is possible that males still show normal adaptational responses, like a normal stress-induced increase in Fos-ir, decrease in neurogenesis, increase in pCREB expression in response to chronic stress, similar to a response to an acute stressor. Whereas females showed possibly pathological changes after chronic stress exposure, like the absence of stress-induced Fos-reactivity in several regions, increase in

survival of newly born neurons, and a decreased pCREB expression.

Reductions in neuronal plasticity are thought to play an important role in affective disorders.[20,46] If neuronal plasticity in the female brain is more sensitive to stress, as implied by pCREB expression in the DG in our experiments, this could also provide an explanation for the higher prevalence of affective disorders in women.

Our data seem to support the recently suggested tend-befriend stress response of females, in contrast to the fight-flight response of males.[63] This hypothesis states that in times of stress females seek support and give support to each other, instead of a fight or flight response during stressful situations. In our experiments female rats housed in unisex groups showed an improved stress-coping, while stress symptoms increased in unisex groups of male rats. Males however were sensitive to "support" provided by (preferably unstressed) females, as shown in the pair-housed rats, whereas the support provided by the males was less successful for females. These data indicate that female rats are more apt at giving support than male rats, interestingly this seems to correspond with the human situation.[38]

Can the placebo effect of antidepressants be explained by social support?

The effective response to a treatment (both physical and pharmacological) can be related to the treatment itself or to a placebo response. The placebo effect of antidepressants is quite extensive. It is speculated that about 60% of the patients who respond to treatment also would respond to treatment with a placebo.[39] Although this does not shed a positive light on the efficacy of antidepressants, it is an interesting phenomenon. The placebo response is not limited to antidepressant treatment, also pain and Parkinson's disease show a positive reaction to placebo treatment.[29] This placebo effect could be induced by the expectancy of benefit, confidence in the healing power of the physician[65] or increased attention the patient receives when treated with a drug, and subsequently an increased amount of perceived social support. In humans "formalised" social support, like psychotherapy, is able to normalise brain function similarly as successful antidepressant treatment.[10] Successful treatment with a placebo was indistinguishable from successful antidepressant treatment when looking at symptom improvement, however they had different effects on brain activity.[44,50] These data indicate that although clinical improvement is similar the neurobiology of placebo and antidepressants is not. Since the positive effects of social support might mediate part of the placebo response, the neurobiological changes induced by placebo could be similar as those of social support. Unravelling the biological mechanisms of the response to placebo and social support could provide a better understanding of the neurobiology of symptom improvement and possibly provide new insights in the depressed brain.

Future prospects

A likely candidate underlying the beneficial effects of social housing/social support is oxytocin. Oxytocin is well known for its role in parturition, lactation and maternal behaviour, [24] but it also plays an important role in affiliative behaviours and social recognition in rodents.[12,37] Oxytocin is also involved in the stress response. Stress responses are attenuated in rodents after i.c.v. administration of oxytocin and increased after treatment with an oxytocin antagonist.[52,53,73] Links between oxytocin and psychopathology have been found,[43,68] and positive associations between plasma oxytocin levels induced by massage and positive emotions in humans have been observed,[67] providing a link between mood and oxytocin. However, central oxytocin and peripheral oxytocin release are not correlated,[23] which makes it difficult to study the brain oxytocin system in humans. It would be interesting to investigate if central administration of an oxytocin antagonist or anti-sense nucleotides blocks the stress-reducing effects of social housing in females, since they showed the most pronounced positive effects of social housing. When the hypothesis is correct we expect that administration of oxytocin or an oxytocin agonist would improve stress-coping in isolated females.

Specifically the serotonergic system of female rats appears to be sensitive to chronic stress exposure, shown by a non-response of Fos-ir of the raphe nuclei to stress, which implies a dysfunctional serotonergic system. Microdialysis provides a method to study the serotonin release in the raphe nuclei and in its projection areas, like the prefrontal cortex.[54,55] If our hypothesis of a disturbed serotonergic system is valid one would expect that chronically stressed females fail to show an increase in 5TH release in response to stress, whereas socially housed females in unisex groups show a normal stress-induced increase. However, performing microdialysis in socially housed rats likely will give some practical complications.

Especially plasticity related stress-induced changes show gender specific effects,[25,31,60] and are modulated by social housing. Exploration of pathways involved in synaptic plasticity, like the ERK MAP-kinase pathway, cAMP-CREB cascade, (anti)apoptotic factors and neurotrophic factors, and their receptors,[20,46] during stress exposure and its modulation by social housing could provide new insights in the neurobiological mechanisms of social support. It would also be fascinating to see if manipulation of the cAMP-CREB pathway would influence the effects of social housing, as it influences the effects of antidepressant treatment.[16,57]

Concluding remarks

Male and female rats react different, sometimes even opposite, to chronic stress exposure and housing conditions. Also at first glance different parameters appear to

contradict one another, especially in females. However one should take into account that until recently most research was performed in male subjects only. So available data on behavioural and neurobiological responses to stimuli, like stress, reflect a masculine reaction, which probably cannot always be extrapolated to females. This can make it difficult to explain results found in females, since comparable studies in the literature are performed in males.

We found that isolation by itself was stressful for females, whereas males did not appear to be affected by being housed individually. Social housing in unisex groups reduced the impact of chronic stress in females but not in males. Males however were positively affected by the presence of a female cage-mate during exposure to chronic stress, whereas females did not appear to benefit from the presence of a male partner. Although for females the presence of a male partner was favourable over being housed alone during stress exposure.

The social environment can modulate the impact of chronic stress in rats and does so in a gender-specific fashion. Especially 'support' provided by females was the effective in ameliorating stress effects. As chronic stress exposure appears to provide a valuable animal model for affective disorders, social housing at the time of stress exposure could represent an interesting model for social support.

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