

Does social isolation rearing induce prefrontal cortex volume loss – a MR volumetry study in rats at 7T.

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Introduction

Rearing rats in social isolation from weaning induces robust behavioral and neurobiological alterations resembling several core symptoms of schizophrenia, such as neophobia¹, impaired memory² and sensorimotor gating³. The latter can be measured by prepulse inhibition (PPI) of startle, and is regulated by the medial prefrontal cortex (mPFC)⁴ which has been shown to be reduced by 5-11% in schizophrenic patients⁵⁻⁸. However, few studies have examined whether social isolation rearing in rats induces subtle brain structural alterations similar to those reported in schizophrenic patients^{9,10}. The feasibility of using MR volumetry to measure limbic brain areas in rodents at 7T has been demonstrated^{11,12}. Therefore, this study investigated whether social isolation (i) causes structural changes of limbic brain areas comparable to those seen in schizophrenia, (ii) whether such subtle changes can be detected using MR volumetry, and (iii) if volume alterations correlate with altered PPI behavior in the social isolation animal model.

Material and Methods

Male Lister Hooded rats (n=16, Charles River, UK) were either reared in social isolation from weaning at post-natal day 23 (ISO, n=8), with auditory and visual but no physical contact with littermates, or housed in social groups of four (control rats, CON, n=8). On PND 61 the effect of social isolation was recorded on pre-pulse inhibition of startle (PPI) using computerised startle boxes (San Diego Instruments, USA). After 5 mins acclimatisation rats were exposed to 40 auditory startles composed of either a pulse alone (120dB for 40ms) or preceded (100ms) by a prepulse (72, 76, 80 or 84 dB for 20ms) presented in a pseudorandom order, using a variable intertrial interval, over 15 mins. PPI was defined as percentage reduction in startle produced by each prepulse intensity analysed by two-way ANOVA. After seven weeks isolation-reared (ISO, n=8, 389±24 g) and group-reared (CON, n=8, 391±24 g) adult rats were examined by MRI after terminal anaesthesia following a pharmacological MRS study not reported herein (7T Bruker Biospec, Germany; 40 coronal slices, TR=4096.8 ms, TE=43.8 ms, RARE factor 4, one average (n=13; 6 min 33 s, same scan repeated six times), 6 averages (n=3; 39 min 20 s), resolution: 0.068x0.068x0.5 mm³). Both medial prefrontal cortices (mPFC; from approx. 3.7 to 1.2 mm from Bregma) and anterior cingulate cortices (ACC; from approx. 1.6 to -1.4 mm from Bregma) were manually outlined according to the Paxinos and Watson¹³ rat brain atlas using the manufacturer's software^{14,15} (ROItool, Bruker). Volumes were calculated by multiplying the area with interslice distance. Volumetric data (right and left mPFC and ACC) was analysed by MANOVA deploying a group comparison design. Significance was considered if $p < 0.05$.

Results

There was an overall significant group effect ($F(1,14)=4.540$, $p=0.021$; Wilks' Lambda = 0.377) with significantly reduced right mPFC volume ($p<0.05$) and a trend of reduced left mPFC volume ($p=0.059$) in isolation- compared to group-reared rats, but no significant differences in right ($p=0.680$) or left ACC ($p=0.132$). Body weight was equivalent in the two groups (univariate ANOVA; $F(1,14)=0.35$, $p=0.855$). There was a main effect of rearing condition ($F(1,3)=4.548$, $p=0.037$) and prepulse intensity ($F(3,3)=14.83$, $p<0.0001$) but no significant interaction. No correlation between mPFC volumes and PPI was found. Evaluation of intrarater variability is pending, but our previous experiments¹² found good intrarater variability of rodent brain MR volumetry.

Conclusion

The finding of reduced mPFC volume in isolation-reared rats (5.4% right, 5.2% left, 5.3% total mPFC) is in line with a recent report using histological stereology⁹ and MRI studies in schizophrenic patients where mPFC reductions range from 5-11%⁵⁻⁸. A previous study⁹ demonstrated that the reduction in mPFC volume was not due to an overall decrease in brain volume. Interestingly, as in the previous report⁹, there was no correlation between mPFC volume and PPI response, although this may be due to the small sample size. So far, our findings confirm regionally distinct volume loss due to social isolation rearing. Moreover, this study demonstrates that such subtle volumetric changes can be detected using MRI at 7T. Currently, the hippocampus, cerebellum and total brain volume are under investigation to gain further insight into the neuroanatomical basis and circuitry¹⁶ affected in isolation-reared rats, and potentially schizophrenia.

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