Multi-parametric Magnetic Resonance to Investigate Aggression: A Study at 11.7T on the BALB/cJ Mouse Model

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Target audience – Radiologists, Psychiatrists, Psychologists and Neuroscientists.

Purpose – Juvenile aggression and antisocial behavior in childhood and adolescence particularly conduct disorder (CD), represents an increasing socioeconomic burden due to the persistent and repeated nature of offences. The age of onset varies but cases with childhood onset are at increased risk of developing antisocial personality disorder in adulthood and when coupled to callous unemotional traits typically present with more severe treatment aggression and antisocial behavior. Aggressive behaviour is a key symptom domain of CD. Therefore, animal models of aggression could provide more knowledge about the neural substrates underlying CD and could eventually provide new insights in possible treatments to treat or prevent this disorder. The BALB/cJ mouse has been reported to exhibit aggressive behavior when compared to its genetically related substrain, BALB/cByJ. In the current study, we first extensively phenotyped the BALB/cJ mice in terms of aggression, anxiety, impulsivity, compulsivity and attention. Following this, a multiparametric magnetic resonance experiment including diffusion tensor imaging (DTI), arterial spin labelling (ASL), and magnetic resonance spectroscopy (MRS) was performed on the same animals. Three brain regions involved in cognitive processing, i.e. anterior cingulate cortex (ACC), orbitofrontal cortex (OFC) and dorsomedial striatum (DMS) were selected for further study.

Methods – A total of 64 mice (32 BALB/cJ, 32 BALB/cByJ) were included in the study. A cognitive profile of the mice was created by testing aggression in the resident-intruder test, attention and impulsivity in the touchscreen operant boxes and locomotion and anxiety in the open field and elevated plus maze. Interactions took place for 5 consecutive days. In each interaction, C57BL/6J intruders of lower weight were randomly assigned to a different resident. Aggressive behavior was analyzed on the last interaction day using The Observer XT (Noldus). Open field and elevated plus maze experiments were performed for 5 min. Locomotion activity and place preference was quantified using Ethovision (Noldus). Since CD is highly comorbid with ADHD [1], the BALB/cJ mice were tested in the five-choice serial reaction time task in touchscreen operant chambers (Campden Instruments) to assess impulsivity, compulsivity and attention. MR experiments were performed using an 11.7T scanner (Biospec, Bruker). To visualize the brain anatomy a gradient echo sequence in three orthogonal orientations was used. For DTI experiments, 20 axial slices covering the whole brain were acquired with a spin-echo echo-planar imaging (EPI) sequence (b=0 and 1000 s/mm^2) along 30 non-collinear directions; resolution was 156×156×500µm^3 and total acquisition time (TA) was 18 min. Pulsed-ASL data were acquired by a flow sensitive alternating inversion recovery (FAIR) EPI sequence with the resolution as 195×260×1000µm^3 and TA=12min. Brain metabolite concentrations were quantified by single-voxel ^{1}H-MRS using PRESS sequence (TR/TE=2500/11.6ms, TA=17min) with a voxel size of 3.75µL (for DMS) and 2.74µL (for ACC and OFC) and LCModel software. Metabolite concentrations are reported relative to the total Creatine (tCr) as it was found to be unchanged in our models when using water concentrations as references. Imaging data were analyzed using ParaVision software (5.2, Bruker) and MATLAB scripts. Statistical analysis were performed using GraphPad Prism software p-values less that 0.05 were considered significant.

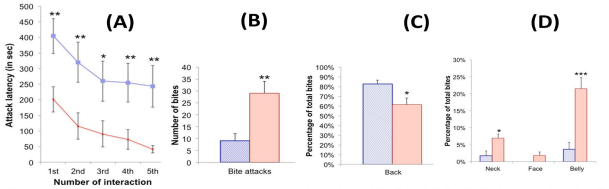

Fig 1. As proofs of aggressiveness, compared to the BALB/cByJ (blue), BALB/cJ mice (red) had significantly (A) lower attack latencies (B) more attack bites on the last day of the interactions (C,D) lower percentage of bites directed to the back and higher to the vulnerable body parts.

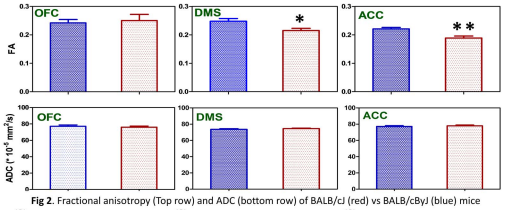

Fig 2. Fractional anisotropy (Top row) and ADC (bottom row) of BALB/cJ (red) vs BALB/cByJ (blue) mice

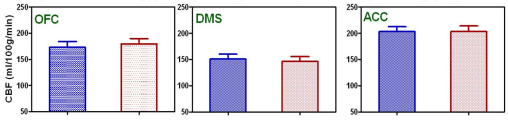

Fig 3. Cerebral blood flow (CBF) in the OFC, DMS and ACC of BALB/cJ (red) vs BALB/cByJ (blue) mice

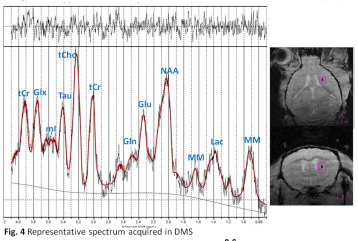

Fig. 4 Representative spectrum acquired in DMS

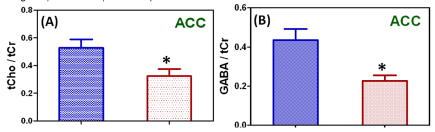

Fig 5. Significant decrease of tCho (A) and GABA (B) in the ACC of BALB/cJ (red) vs BALB/cByJ (blue) mice.

Results – As shown in Fig. 1, increased pathological aggression (reduced attack latency ($p<0.01$), atypical pattern ($p<0.001$) and increased number of bites ($p<0.01$) were observed in the BALB/cJ mice. Phenotyping of anxiety demonstrates increased freezing behavior (not shown), a marker for anxiety, in the open field test ($p<0.01$) and increased preference (not shown) for the closed arms in the elevated plus maze ($p<0.01$), a marker for increased anxiety in BALB/cJ versus BALB/cByJ mice. No deficits were found in impulsivity and compulsivity, as measured by respectively the number of premature responses and the number of time-out responses. Interestingly, the aggressive BALB/cJ mice were associated with global attention-deficits ($p<0.001$) as measured by an increased number of omissions (not shown). DTI of the BALB/cJ mice showed a reduced directional diffusivity measured by fractional anisotropy (FA) in both the ACC ($p<0.01$) and the DMS ($p<0.05$) with no change in the mean diffusivity (Fig. 2). No change was seen in cerebral blood flow (CBF, Fig. 3) measured by ASL. A representative spectrum acquired in the DMS is shown in Fig. 4. We found that BALB/cJ exhibited a significant decrease in GABA ($p<0.01$) and total choline (tCho, $p<0.05$) in the ACC compared to the BALB/cByJ (Fig. 5). No significant change in the DMS metabolites was observed. The aforementioned alterations in the brain structure and metabolism did not alter cerebral blood flow measured by the ASL.

Discussion – The results from the resident-intruder test proved that BALB/cJ mice have elevated levels of aggression which might be related to the more anxious phenotype observed in the open field and elevated plus maze. Significant differences observed in global attention processing suggest possible comorbidity of aggression and inattention. Reduced FA and changes in the white matter (WM) organization is likely related to the aggression, which also has been reported in traumatic brain injury patients possessing aggressive behavior [2]. Reduced inhibitory GABA tone in the ACC seems to be linked to aggression, which confirms similar findings in aggression in humans. In addition, prefrontal GABA tone is associated with anxiolytic effects, therefore the increased anxiety in the BALB/cJ mice may be explained by decreased GABA tone. Therefore understanding GABAergic tone might be important in a fuller understanding of aggression linked to anxiety. tCho levels are a precursor for the neurotransmitter acetylcholine and were also significantly decreased in the ACC in the BALB/cJ mice. Interestingly, reduced acetylcholine tone has been reported to be linked to aggression [3]. Whether this is linked to inattention (also observed in the BALB/cJ mice) remains an open question.

Conclusion – The BALB/cJ mouse model shows marked aggression coupled to increased anxiety and a global attention deficit with no evidence for increased impulsivity and compulsivity when compared to the controls, BALB/cByJ mice. Our data indicates that pathological aggression observed in the BALB/cJ mice is likely related to structural and neurochemical changes leading to inattention and anxiety; such that BALB/cJ mice are insensitive to environmental cues resulting in inappropriate behavior such as antisocial behavior and aggression. In summary, BALB/cJ mice may serve as a suitable model to investigate potential therapeutic approaches for CD such as modulation of anterior cingulate cortical GABA and acetylcholine tone.

References – [1] Buitelaar J. K., et al., Eur. Child Adolesc Psychiatry, 2013 Feb; 22 Suppl 1:S49-54. [2] Wozniak J. R., et al., Arch Clin Neuropsychol. Jun 2007; 22(5): 555–568. [3] Chan A., et al., J Nutr Health Aging,2008 Apr;12(4):252-61.