Behavioural and cognitive alterations of the young megencephaly (BALB/cByJ-Kv1.1 $^{mceph/mceph}$) mouse

Sarah Holst[1], Elin Åberg[2], Therese M Eriksson[1, 3], Catharina Lavebratt[2] and Sven Ove Ögren[1]

Background and Aims

The epileptic mouse model BALB/cByJ-Kv1.1mceph/mceph (mceph/mceph) is homozygous for a spontaneous mutation truncating the Shaker-like voltage gated potassium channel, Kv1.1 (KCNA1), which causes defect potassium channels and gives rise to epileptic seizures. The mceph/mceph mice are normal at birth but from 3 weeks of age the defect of Kv1.1 results in a pathological overgrowth (megencephaly) of primarily the hippocampus, crucial for declarative types of spatial and emotional memories.

The aim of the present study was to determine the influence of this overgrowth on emotional memory. In order to increase the liability of the interpretation, we also performed a phenotypical characterisation of the mceph/mceph mouse. In addition, the number of Neu-postive cells in the dentate gyrus was determined in the heterozygous and the wild type mice.

Conclusions

The mceph/mceph mice had in the Passive Avoidance test unchanged step through latency and a lower frequency of risk assessment behaviour with more time spent in the dark compartment, suggesting that they prefer the dark compartment despite the aversive cue received 24 h earlier indicating an impaired memory.

The mceph/mceph mice had decreased behavioural activity including ambulation, exploration, displacement behaviour, shelter seeking, defensive and anxiety-related behaviour in the Novel Cage test.

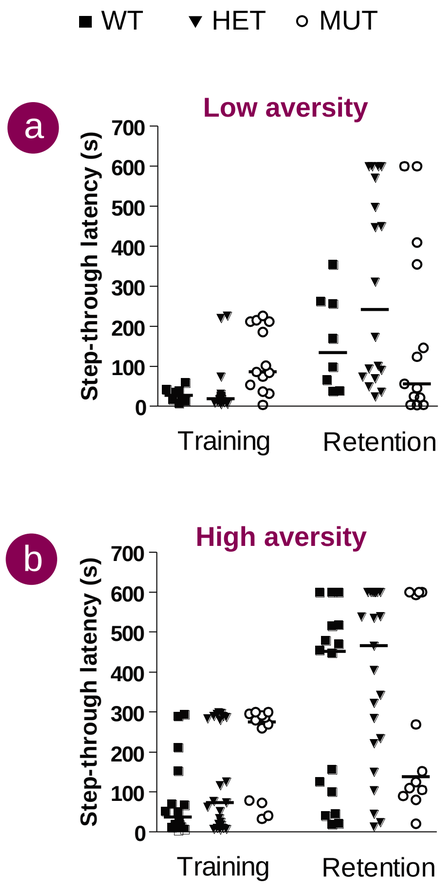
The mceph/mceph mice had temporally minor seizures, low body weight, reduced number of vocalizations and more individuals with teary eyes.

The number of Neu-postive cells in the dentate gyrus did not differ in heterozygous compared to wild type mice.

Sarah Holst
PhD, Post Doc
Department of Neuroscience, Division of Behavioural Neuroscience, Karolinska Institutet

Results

Passive Avoidance test



Step-through latency (s) of training and retention with low (0.30mA; a) and high (0.50mA; b) aversity cue in the Passive Avoidance (PA) test. The retention step-through latency was significantly increased compared to the training step-through latency in the wild type (low aversity : p<0.05; high aversity; p<0.01) and heterozygous (low aversity : p<0.01; high aversity; p<0.001) mice, while it was unchanged in the mceph/mceph mice.

Principal Component Analysis based on behaviour in Passive Avoidance

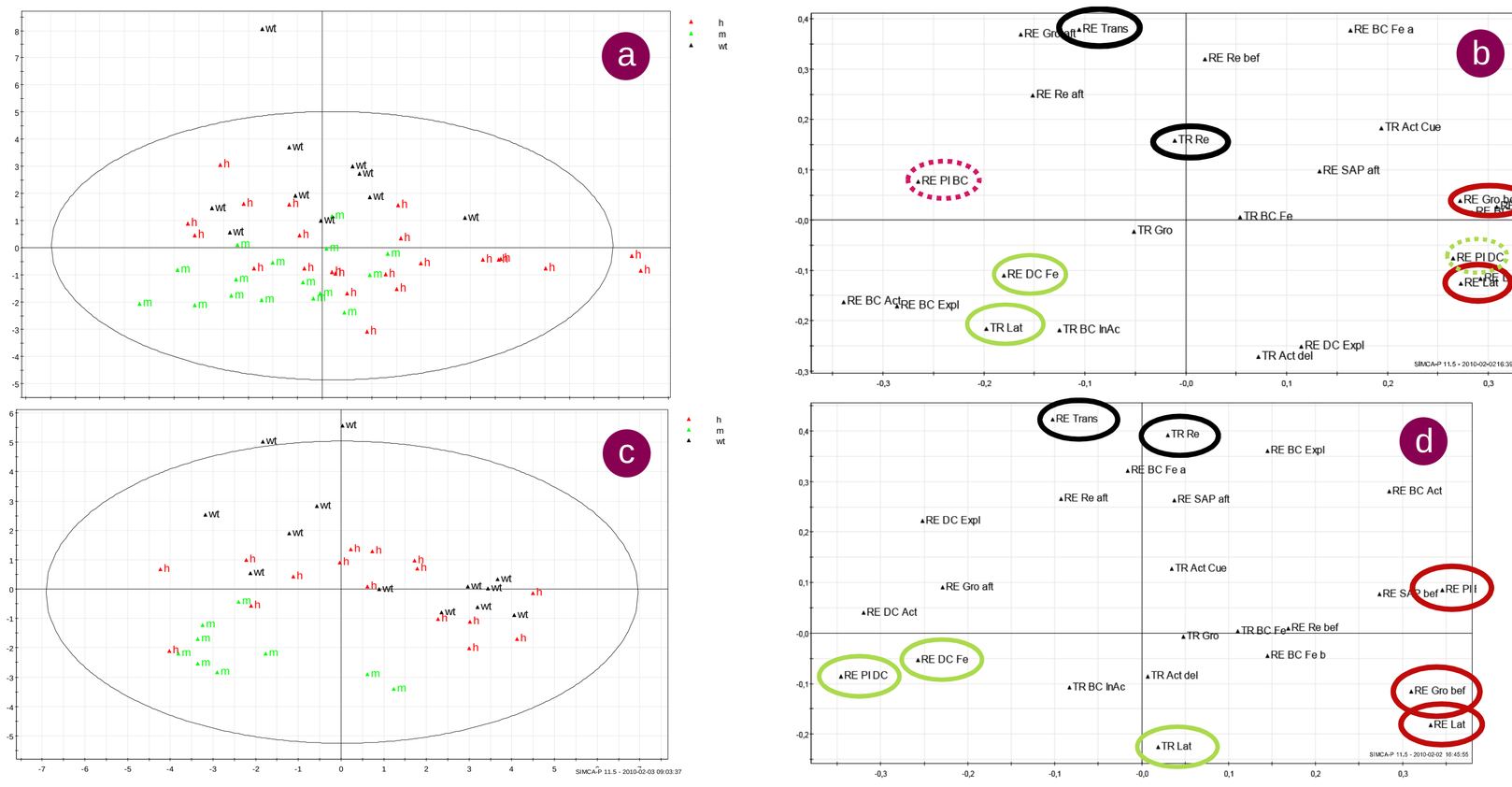


Fig. 1. The Principal Component Analysis (PCA) from the PA low aversity (a & b) and high aversity (c & d). The score plots (a &c) illustrate the individuals and the separation of the groups. The loading plots (b & d) illustrate the PA variables that were included in the analysis. Variables located further away from the origin are most important for differentiation between the groups. Green circles represent variables that characterise the mceph/mceph mice. Red circles represent variables that characterise the group of mice responding to the aversive cue with a reactive coping style-.Black circles represent variables that characterise the group of mice responding to the aversive cue with a proactive coping style. Both coping styles indicated memory retention since the characteristic variables were located near the risk assessment behaviour SAP in the loading plot (b & d), reflecting the remembrance of the earlier presented aversive cue .In contrast, the characteristic variables of the mceph/mceph mice were located in the quadrant opposite to the SAP.

The principal components analysis (PCA; SIMCA-P+11 software (Umetrics®) transforms the number of possibly correlated variables into a smaller number of uncorrelated variables that are called principal components. The PCA was based on the PA results and used to identify relationships between the behavioural patterns and the genotype of the mice.

For low aversity, the two principal components explained 30% of the variance (R2X=0.303; Q2X=-0.105 respectively) and for high aversity it explained 47% of the variance (R2X=0,469; Q2X=-0.172 respectively) and values of explained variation and predicted variation were within an appropriate range.

Abbreviations: TR=Training day, RE=Retention day, BC=Bright Compartment, DC=Dark Compartment, Lat=Latency time to step through; InAct= InActivity before the door is opened; Act Cue= Activity during aversive cue.; Act delay= Activity during delay after aversive cue.; Act=Activity after the door is opened,; Expl= Exploring after the door is opened.; Re = the number of rearings; bef=before step through.; aft=after step through.; SAP=the number of stretch attend postures; Gro= the number of self groomings; Trans= the number of transfers after the door opened.; Pl=Place preference (the total duration in a region after the door opened); Fe= the number of feces pellets, wt=wild type, h=heterozygotes, m= mceph/mceph.

Methods

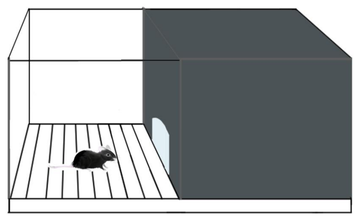
In Experiment 1 the same individual was tested in the Novel Cage test (NCT) test at 21-22 days of age, PA with low aversity (current 0,30 mA) at four weeks of age and the NCT test at six weeks of age. Body weight, eye condition and vocalizations were recorded after the NCT, the mceph/mceph mice had reduced body weight and vocalization frequency, higher frequency of teary eyes. Data not shown.

In Experiment 2 the same individual was tested in three tests during their fourth week of age. Two days elapsed between the tests. First they were tested in the PA with high aversity (current of 0,5 mA), then the Open Field. The Open Field results were not significant, data not shown.

Experiment 3, Five weeks old mice were deeply anaesthetized and their brains perfused and the number of NeuN-immunoreactive cells in the entire dentate gyrus was counted in wt and h. There was no significant difference. Data not shown.

In Experiment 4 the motor coordination of two sets of heterozygous and mceph/mceph mice, age 28-38 days, were examined in the Rotarod. There was no significant difference. Data not shown.

The PA test was used to test emotional memory. The mouse was put in a brightly lit compartment with free access to a dark compartment. Upon entering the dark compartment the door closed and the mouse received a foot shock (duration 1s). Two intensities of the electrical current, 0,3 mA (low aversity) and 0,5 mA (high aversity), of the shock were tested. The retention was tested 24 h after the shock delivery, by measuring the step-through latency time to the first transfer from the light compartment to the dark compartment.



Functional analysis of the tests PA and NCT

Test	Functional categories	m≠wt h?	wt≠h?
PA	General Activity	m<h wt	-
NCT		m<h wt	-
PA	Exploratoration	m<h wt	wt>h
NCT		m<h wt	-
PA	Risk assessment	m<h wt	-
NCT		m≤h wt	wt<h
PA	Displacement behaviours	-	-
NCT		m<wt	-
PA	Defensive behaviours	n m	n m
NCT		m<h wt	-
PA	Open-shelter	n m	n m
NCT		m<h	-
PA	Anxiety-related behaviour	m<h	-
NCT		-	-

Table is showing differences recorded from the PA and the NCT. Arrows indicate the direction of the difference. Abbreviations: , PA=Passive Avoidance, NCT=Novel Cage test, n m=not measured, wt=wild type, h=heterozygots, m= mceph/mceph.

The NCT evaluates emotional reactivity by quantifying exploration and risk assessment behaviour. The mouse is placed in the center of a clean Macrolon type III cage with fresh bedding under a light intensity of approximately 200 lux. The locomotor, explorative and risk assessment behaviours were video-recorded for 5 min using a digital camera placed above the cage. The latency time, frequency and duration of the behaviours were calculated.

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[1] Department of Neuroscience, Karolinska Institutet, Retzius väg 8, 171 77 Stockholm, Sweden; Tel: +46 8 524 8 7232, Fax: +46 8 302875, e -mail: Sarah.Holst@ki.se
[2] Department of Molecular Medicine and Surgery, Karolinska University Hospital, Stockholm, Sweden.
3 Center for Molecular Medicine. Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden.