Research report

Behavioural analysis and susceptibility to CNS injury of four inbred strains of mice

Stephen J. Royle, Fiona C. Collins, H. Tom Rupniak, Julie C. Barnes, Richard Anderson

Neuroscience Unit, Glaxo Wellcome Research and Development, Medicines Research Centre, Gunnels Wood Road, Stevenage, Herts, SG1 2NY, UK

Accepted 20 October 1998

Abstract

Interpretation of data from gene targeting studies can be confounded by the inherent traits of the background inbred strains used in the generation of transgenic and null mutant mice. We have therefore compared the behaviour and response to CNS injury of four inbred strains commonly used in molecular genetic studies to produce models of neurological disease. Adult, male 129/Ola, BALB/c, C57BL/6 and FVB/N mice (2–4 months) were initially subjected to behavioural tests that comprised a neurological examination, determination of motor function and cognitive testing in the Morris water maze. Also the response to CNS injury following an acute kainic acid (KA) challenge (30 mg kg\(^{-1}\), i.p.) was determined. The 129/Ola and BALB/c strains showed significant motor deficits when compared with the C57BL/6 and FVB/N strains. In contrast, only the FVB/N strain showed evidence of apparent cognitive impairments in the water maze as evidenced by increased pathlengths to locate the escape platforms and impaired performance in a probe trial. In addition, the FVB/N strain showed the most severe seizure response and mortality rate (62%) following administration of KA (30 mg kg\(^{-1}\), i.p.). These behavioural changes were also associated with a greater degree of cell body and synaptophysin loss in the pyramidal CA3 hippocampal cell layer and astrogliosis 72-h post-dose. These data suggest that the FVB/N strain may not be the most suitable background strain for the development of new transgenic mice for the study of genes implicated in the learning and memory process.

Keywords: Mouse; Inbred strain; Cognition; Kainic acid; Excitotoxicity

1. Introduction

Genetic targeting technologies provide a powerful means with which to study genes associated with disease processes [1,2]. For example, these techniques have been employed to create transgenic and null mutant mice in order to facilitate study of the roles of amyloid precursor protein [16,44] presenilin-1 [20] and apolipoprotein E [36,51] in the pathogenesis of neurodegenerative disease. In addition, transgenic mice expressing mutant forms of the Cu/Zn superoxide dismutase (SOD-1) gene have been used to provide an animal model of peripheral motor neurone degeneration characteristic of amyotrophic lateral sclerosis (ALS) [11,38]. The majority of gene targeting is undertaken in embryonic stem (ES) cells derived from the 129 strain and the modified cells injected into a distinct strain of host blastocyst. To aid the identification of ES-derived offspring, the resulting chimeras are subsequently backcrossed to an inbred strain carrying an alternative coat colour marker. Problems can arise when the offspring from such matings (F1 generation) are intercrossed, since the resultant F2 mice have recombinant genotypes derived from the two parental mouse strains [17] and several recent reports have discussed problems associated with this technique [10,17,18]. The main difficulty arises in the interpretation of many transgenic phenotypes due to background genetic polymorphism in the inbred strains used to generate the mutants [10,17]. For example, behavioural deficits [7] and susceptibility to neurodegeneration following both a kainic acid (KA) challenge [32] and middle cerebral artery occlusion [8,15] have been reported in transgenic mice but a contribution from background parental genes may, in part, explain these phenotypes. Strategies adopted to facilitate definition of transgene effects over genetic background include the use of wild type littermate controls [52], multiple backcrossings into a preferred host strain and more recently, in order to accelerate this process, the development of marker assisted ‘speed congenic’ breeding methods [3,27].
Despite these measures, if inappropriate host strains are selected, a transgenic model may be of limited value since mutations can have markedly different phenotypes in different genetic backgrounds [41,47]. For example, overexpression of APP on an inbred FVB/N or C57BL/6 background proves lethal whereas the mutation is well tolerated on outbred lines which facilitate the development of amyloid plaques [7]. The development of new transgenic mouse models of CNS disorders will therefore critically depend on the correct selection of the most suitable background parental strains and an informed choice will require a thorough understanding of the endogenous phenotypes of the inbred strains commonly used in molecular genetics [10]. Accordingly, we have characterised the phenotype and response to CNS injury of four common laboratory mouse strains (129/Ola, BALB/c, C57BL/6 and FVB/N) in order to investigate the impact of inbred parental genotypes on behaviour and response to CNS injury.

2. Materials and methods

2.1. Animals

Test groups comprised of male 129/Ola (Harlan, UK) BALB/c, C57BL/6 and FVB/N mice (all originally from Charles River, USA) at 2–4 months of age. The strains were incorporated into our breeding facility in the UK and bred in specified pathogen-free conditions. Following transfer of the experimental groups to Stevenage, the mice were allowed 2 weeks to acclimatise prior to the study. All animals were housed individually in a temperature-controlled environment (21 ± 2°C) under a 12-h light/dark cycle and allowed free access to standard laboratory food and water. The research complied with national legislation and with company policy on the Care and Use of animals.

2.2. Behaviour

2.2.1. Neurological assessments

All mice were subjected to a battery of tests to probe spinal cord (flexion, medulla (corneal reflex), pons (righting reflex), and autonomic (salivation) function as described in Table 1. Motor coordination was assessed on a mouse rota-rod (Hugo-Basile) and forelimb grip strength determined with a grid floor and pressure transducer.

2.2.2. Locomotor activity

Mice were individually placed in activity monitor cages (35 cm long, 21 cm wide and 18 cm deep) and locomotor activity was recorded for 1 h. There was no prior habituation. Locomotor activity was quantified by measuring the number of beam breaks.

Table 1 Neurological examination methods

<table>
<thead>
<tr>
<th>Title</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexion reflex</td>
<td>Animal lifted by nape and toes lightly pinched with forceps. A response is registered by a rapid foot withdrawal (present/absent).</td>
</tr>
<tr>
<td>Righting reflex</td>
<td>Animal placed on back will resume normal position. In addition, the animal is dropped upside down from 30 cm above a soft surface. The animal should land on all four legs (present/absent).</td>
</tr>
<tr>
<td>Corneal reflex</td>
<td>The cornea of the restrained animal is lightly touched with a hair. The animal should respond with a blink reflex (present/absent).</td>
</tr>
<tr>
<td>Secretary signs</td>
<td>The animals were examined for secretary signs around the mouth (salivation) and eyes (lacrimation), (present/absent).</td>
</tr>
</tbody>
</table>

In addition, measures of body weight and body temperature were taken.

2.2.3. Morris water maze

The water maze consisted of a white Perspex circular pool (100 cm diameter, 30 cm deep). The pool was filled with water (21 ± 1°C) to a depth of 25 cm and rendered opaque by the addition of a liquid latex solution (Warner Jenkinson). The cued escape platform was painted black, flagged and protruded 1 cm above the water surface. The hidden escape platform (6.5 cm diameter) was painted white and positioned just below the water surface. The cued task trains the mice to associate the platforms with escape from the pool and was therefore conducted prior to the hidden platform task. The maze was isolated with screens and distal cues placed prominently around the pool.

All mice were initially trained over four sessions (two per day, three trials per session with a 10-min intertrial interval (ITI)) using the cued escape platform (cued task). In this case both the starting position of the mouse and the cued platform position were both varied to avoid habituation to a particular quadrant. Each trial was commenced by placing the mouse gently in the pool facing the perimeter and activating an overhead camera connected to a computerised tracking system (HVS Image; Hampton, UK) that recorded the swim paths of the mice to locate the platform. If a mouse failed to locate the platform within the maximum trial period of 60 s it was led to and placed on the platform for 10 s.

Following the cued task, the mice received eight sessions (three trials per session, 10-min ITI) over 4 consecutive days with the hidden platform, the position of which was fixed for each individual mouse (hidden task). After the eighth hidden session the mice underwent a probe trial, which consisted of a 60-s trial without the platform present, during which the search pattern was analysed. The time spent searching the quadrant that formerly contained the hidden platform was recorded as a percentage and compared with that spent searching in the other quadrants.
Table 2
Neurological assessment of four inbred mouse strains

<table>
<thead>
<tr>
<th>Inbred strain</th>
<th>n</th>
<th>Mean weight (g)</th>
<th>Mean temperature (°C)</th>
<th>Righting reflex</th>
<th>Corneal reflex</th>
<th>Salivation</th>
<th>Grip strength (g)</th>
<th>Flexion</th>
<th>Rota-rod</th>
</tr>
</thead>
<tbody>
<tr>
<td>129/OLA</td>
<td>12</td>
<td>32.3 ± 0.5</td>
<td>36.4 ± 0.3</td>
<td>(100%) 12/12</td>
<td>(100%) 12/12</td>
<td>(0%) 0/12</td>
<td>77.6 ± 3.7</td>
<td>57.8 ± 16.3*</td>
<td></td>
</tr>
<tr>
<td>BALB/c</td>
<td>12</td>
<td>30.9 ± 0.7</td>
<td>37.4 ± 0.2</td>
<td>(100%) 12/12</td>
<td>(100%) 12/12</td>
<td>(8%) 1/12</td>
<td>73.4 ± 5.2</td>
<td>47.3 ± 4.5*</td>
<td></td>
</tr>
<tr>
<td>C57BL/6</td>
<td>12</td>
<td>33.0 ± 1</td>
<td>36.5 ± 0.3</td>
<td>(100%) 12/12</td>
<td>(100%) 12/12</td>
<td>(0%) 0/12</td>
<td>73.1 ± 3.8</td>
<td>96.5 ± 10.9</td>
<td></td>
</tr>
<tr>
<td>FVB/N</td>
<td>12</td>
<td>34.5 ± 1</td>
<td>37.5 ± 0.2</td>
<td>(100%) 12/12</td>
<td>(100%) 12/12</td>
<td>(0%) 0/12</td>
<td>62.9 ± 3.6</td>
<td>89.3 ± 16.3</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05 vs. C57BL/6 and FVB/N strains.

2.3. Drug

Kainic acid (KA, Sigma, Lot 94H0230) was dissolved in 0.9% (w/v) phosphate buffered saline (PBS). The dose (30 mg kg⁻¹) is expressed in terms of free base and was administered as an intraperitoneal (i.p.) injection in a dose volume of 10 ml kg⁻¹. Vehicle-treated mice were injected with PBS.

2.4. Behavioural response to KA

Following administration of KA, seizure severity was quantified using a 5-point observer rating scale (0 = normal behaviour, 1 = rare wet dog shakes (WDS) and rare type A convulsions; 2 = frequent WDS and type A convulsions (no rearing/salivation); 3 = frequent type A convulsions with appearance of type B convulsions with rearing/salivation; 4 = frequent type B convulsions with falling and salivation; 5 = continuous, generalised limbic seizures and death within 3 h). Type A convulsions comprised focal convulsions affecting the head and extremities, typically starting within 30 min of KA administration. Type B comprised generalised tonic-clonic seizures usually starting between 60–90 min post-KA administration. In addition, mean latency to onset of seizures, total seizure duration and mortality were recorded for each treatment group. Mice were returned to their cages after 6 h or when seizure activity had ceased.

2.5. Histology and immunohistochemistry

Seventy-two hours following administration of KA, the mice were sacrificed by cervical dislocation and the brain hemisected. The left hemisphere was fixed in 2% paraformaldehyde for 18 h, paraffin embedded and serial 8 μm coronal sections obtained at the level of the anterior hippocampus for histochemical analysis. In order to study gross hippocampal damage sections were stained with Cresyl violet for 10 min. For glial fibrillary acidic protein, (GFAP), staining slides were dewaxed in xylene prior to a 30 min incubation with 3% H₂O₂ in PBS. Following a 10 min wash in PBS, the sections were incubated for 1 h with

![Graph](image URL)

Fig. 1. Time course of locomotor activity (LMA) in 129/OLA ( ), BALB/c ( ), C57BL/6 ( ) and FVB/N ( ) mice. Data points are expressed as the mean ± S.E.M. of 12 mice per strain. Data were analysed by two-way ANOVA with strain as between-subjects factor and time as within-subjects factor. Post-hoc comparisons were made by Dunnett’s test assuming the C57BL/6 group as control. The 129/OLA and BALB/c strains exhibited reduced LMA, with the 129 strain further impaired in comparison with the BALB/c mice.
Fig. 2. (a) Performance of 129/Ola (●), BALB/c (□), C57BL/6 (■) and FVB/N (○) strains in water maze learning in both cued (sessions 1–4) and hidden platform (sessions 5–12) tasks. Data are expressed as mean pathlengths (n = 12 per group). Statistical significance was determined by two-way ANOVA followed by Dunnett’s test assuming the C57BL/6 group as control. (b) Performance of the same mice in a 60-s probe trial conducted 10 min following the 12th session. Each strain is represented by four histobars corresponding to each quadrant of the water maze (adjacent left, island quadrant, adjacent right and opposite). Note that the 129/Ola, C57BL/6 and BALB/c strains show good evidence of spatial learning whereas the FVB/N mice fail to show a quadrant preference. (c) Median probe data path plots of the four inbred strains.
Table 3
Seizure response to kainic acid (30 mg kg⁻¹, i.p.) of four inbred strains of mice

<table>
<thead>
<tr>
<th>Inbred strain</th>
<th>n</th>
<th>Treatment</th>
<th>Median peak seizure score</th>
<th>Mean latency to seize (min)</th>
<th>Total seizure time (min)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>129/Ola</td>
<td>8</td>
<td>KA 30 mg kg⁻¹</td>
<td>0.5 (0–1)</td>
<td>50 ± 4</td>
<td>0.3 ± 0.2</td>
<td>0</td>
</tr>
<tr>
<td>BALB/c</td>
<td>7</td>
<td>KA 30 mg kg⁻¹</td>
<td>0 (0–1)</td>
<td>16 ± 3 **</td>
<td>7 ± 5</td>
<td>29</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>8</td>
<td>KA 30 mg kg⁻¹</td>
<td>4 (3.8–4.3)</td>
<td>28 ± 2</td>
<td>89 ± 44 **</td>
<td>12.5</td>
</tr>
<tr>
<td>FVB/N</td>
<td>8</td>
<td>KA 30 mg kg⁻¹</td>
<td>4 (3.8–4.3)</td>
<td>24 ± 3 **</td>
<td>97 ± 44 **</td>
<td>62+</td>
</tr>
</tbody>
</table>

Seizure severity was quantified on a 5-point observer rating scale (see text). Note the increased susceptibility to seizures observed in FVB/N strain as evidenced by a significantly reduced latency to onset \( F(3,28) = 5.6, p < 0.05 \) and increased duration of seizures \( F(3,28) = 4, p = 0.01 \) and mortality rate \( (p = 0.04) \).

5% normal horse serum before overnight application of 1:500 diluted GFAP antibody (Sigma, G-9269) at 5°C. The following day sections were washed in PBS before incubation for 30 min with biotinylated anti-mouse antibody (Vectorstain elite ABC-peroxidase kit). Following a further wash, the slides were then incubated for 30 min with ABC reagent before a final wash and a 4.5 min incubation with DAB peroxidase substrate kit (SK-4100, Vector labs).

In order to determine synapse loss, sections were dewaxed in xylene prior to overnight incubation with undiluted primary synaptophysin antibody (MCA860, Serotec, UK) at 5°C. The following day slides were washed in PBS before incubation for 30 min with biotinylated anti-mouse antibody (Vectorstain elite ABC-mouse kit). Following a further wash, the slides were then incubated for 30 min with streptavidin-fluorescein (2% in PBS, Vector labs) before a final wash and mounting with vectorshield mounting medium (Vector labs). All sections stained with either the GFAP or synaptophysin antibody were processed at the same time and in a random fashion.

2.6. Image analysis

Gross hippocampal cell damage was assessed by light microscopy using a Leica Q600 Image Analyser and DM microscope (Leica UK, Milton Keynes) and quantified by counting viable neurones, within a counting frame (1500 pp²), where both nucleus and cell membrane were intact. For GFAP staining, background levels were determined from negative controls and all sections normalised. GFAP immunoreactivity was quantified using an image analysis program. Positively stained structures were counted within a 0.21-mm × 0.17-mm field (0.036 mm²).

Images of synaptophysin stained sections were taken with a Leica TCS4D confocal microscope (Leica Lasertechnic, Heidelberg, Germany) with 488 nm fluorescence excitation and 530 nm emission. A 10 × objective lens was used and the height of the microscope stage was adjusted to give the brightest image from each section, and the gain of the microscope was set for the section with the brightest fluorescence. Images were stored on optical disk (Maxell, MA-M128) and transferred to the image analyser for measurement.

Analysis of confocal images was made with a Leica Q600MC image analyser (Leica UK, Cambridge, UK). The analysis was automated using a QUIPS macro that performs a sequence of functions as follows: For each image, the image analyser was first used to threshold the fluorescent staining from the background, and then to define sample areas within the hippocampal subfields. Binary image arithmetic was used to display the three areas in colour overlying the grey image of fluorescence, and to avoid overlapping between the areas. The image analyser then measured the mean grey level for each of the areas (grey level is proportional to intensity of fluorescence). Mean grey levels from negatively stained sections were then subtracted from each result to obtain intensity of fluorescence without background staining.

2.7. Lipid peroxidation

The left cortical hemisphere of each mouse was homogenised in Ripa Lysis buffer (50 mM Tris–HCl, 150 mM NaCl, 1% deoxycholic acid, 0.1% SDS, 1% Triton X-100 adjusted to pH 7.5), aliquoted, and stored at −80°C. Basal lipid peroxidation was determined by measuring malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) concentrations using a standard kit (R&D Systems).

![Fig. 3. Time course of seizure severity in 129/Ola (●), BALB/c (□), C57BL/6 (■) and FVB/N (○) strains following KA (30 mg kg⁻¹, i.p.)](image-url)
Fig. 5. Normal pyramidal cell counts in sections following vehicle (■) or 72 h post-KA (30 mg kg⁻¹) (●). Note the significantly decreased number of cells with intact nuclei and plasma membrane in the CA1 (69%) and CA3 (91%) subfields of the FVB/N sections ∗ ∗ p < 0.01 vs. vehicle (unpaired t-test).

2.8. Data analysis and statistics

The four inbred strains were analysed for strain differences using either parametric (one-way ANOVA for body weight and temperature, grip strength, rota-rod and water maze probe data) or non-parametric (all other neurological data) tests. The water maze acquisition and LMA studies were analysed by two-way ANOVA with session/time bin as the within-subjects (repeated measures) variable and strain as the between-subjects variable. In all cases when a significant main effect and/or significant interaction terms were found, subsequent post-hoc comparisons were made by Dunnett’s test comparing all strains to C57BL/6 and FVB/N mice. Lipid peroxidation and seizure measures were analysed by one-way ANOVA except mortality, which was determined by unpaired t-test.

3. Results

3.1. Behaviour

3.1.1. Neurological assessment

Table 2 shows a neurological assessment of the four inbred mouse strains. No obvious differences were seen between strains in body temperature, righting and corneal reflexes, secretary signs or grip strength. However, the BALB/c strain showed a significantly reduced body mass compared with the other strains [F(3,44) = 4, p = 0.02] and in addition to the 129/Ola mice, impaired flexion reflex [BALB/c (p = 0.01) and 129/Ola (p = 0.04)] and reduced rota-rod latency scores in comparison with C57BL/6 and FVB/N mice [F(3,44) = 3, p = 0.02].

Total locomotor activity scores clearly revealed a marked hypoactivity in the 129/Ola and BALB/c strains compared with the C57BL/6 and FVB/N strains, i.e., total activity scores for 129/Ola, BALB/c, C57BL/6 and FVB/N mice were 426 ± 63 * *, 788 ± 109 *, 1221 ± 150 and 2191 ± 681, respectively [F(3,44) = 26, * * p < 0.001)]. Time course analysis (Fig. 1) showed a significant main effect of strain [F(3,44) = 24, p < 0.001] and strain x time interaction [F(3,44) = 91, p < 0.001] with the 129/Ola and BALB/c strains exhibiting a lower rate of initial exploratory activity.

3.1.2. Morris water maze

Fig. 2a (sessions 1–4) shows pathlength to locate the visible platform in the cued task and Fig. 2a (sessions 5–12) pathlength to locate the hidden platform. A main effect of strain was found in the cued [F(3,44) = 74, p < 0.01] and hidden [F(3,44) = 50, p < 0.01] tasks with the FVB/N strain showing significantly increased pathlengths to locate the escape platforms in comparison with the other three strains. In addition, the FVB/N strain exhibited significantly elevated swim speeds at each session [F(3,44) = 9, p < 0.001]. For example, swim speeds at the 12th session were 13 ± 0.6, 13 ± 1, 16 ± 1, 26 ± 0.4 * * cm s⁻¹ for 129/Ola, BALB/c C57BL/6 and FVB/N mice, respectively. Analysis of probe data following the 12th session showed significant spatial learn-
ing in the 129/Ola, BALB/c, and C57BL/6 mice (all \( p < 0.001 \)) as evidenced by a significantly greater time spent in the former platform quadrant. In contrast the FVB/N mice failed to show a significant quadrant preference (Fig. 2b) \( [F(3,44) = 0.6, \ p = 0.6] \). Median probe path plots for each of the strains following the 12th session are shown in Fig. 2c.

### 3.2. Behavioural response to KA

Administration of KA (30 mg kg\(^{-1}\), i.p.) produced a marked strain-dependent seizure response with the following rank order: C57BL/6 = FVB/N > BALB/c > 129/Ola (Table 3). The behavioural changes initially manifest as reduced LMA, catatonia, head bobbing and face washing progressing to wet dog shakes, tremors and finally rearing, generalised seizures and in some instances death. Whilst all strains developed seizures, the C57BL/6 and FVB/N strains exhibited a greater seizure severity score and seizure duration (Fig. 3). In addition, the FVB/N mice showed the highest mortality rate (62%).

### 3.3. Immunohistochemistry

Cresyl violet staining of hippocampal sections 72 h following KA treatment revealed marked pyramidal cell loss in the CA3 subfield when compared with vehicle-treated sections (Fig. 4) Damaged neurones appeared shrunken with dense pyknotic nuclei. There was marked disruption of the cytoarchitecture and increased spacing of the pyramidal cell layer. Sections from the 129/Ola, BALB/c and C57BL/6 mice showed a similar degree of damage but those from the FVB/N mice showed a more pronounced cell loss with almost complete destruction of the CA3 cell layer (Fig. 5).

#### 3.3.1. GFAP immunoreactivity

Vehicle-treated sections showed a low level of GFAP immunoreactivity distributed evenly throughout the hippocampus. In contrast, immunostaining of KA-treated tissues revealed a marked astrogliosis in all strains as evidenced by increases in the size, density and ramification of GFAP expressing cells (Fig. 6). Reactive astrocytes were found throughout the hippocampal area but none were observed in the pyramidal cell layer. Image analysis techniques revealed that the FVB/N strain displayed a more intense astrogliotic reaction following KA administration when compared with the other three strains (Fig. 7).

#### 3.3.2. Synaptophysin immunoreactivity

Immunocytochemical analysis revealed characteristic punctate immunostaining of presynaptic terminals using an antibody against the presynaptic vesicle protein, synaptophysin. Quantitative image analysis of synaptophysin immunolabelled sections failed to reveal a significant reduc-

---

**Fig. 7. KA-induced increases in hippocampal GFAP immunoreactivity following vehicle (□) or 72 h post-KA (30 mg kg\(^{-1}\), i.p.) (■). Histobars show mean ± S.E.M. positively stained structures per area (see text). Note the significantly increased number of positively stained structures in the FVB/N sections. * \( p < 0.05 \) vs. vehicle (unpaired \( t \)-test).**

---

**Fig. 6. GFAP staining of adjacent sections revealing astrogliosis in: (a) 129/Ola, (b) BALB/c, (c) C57BL/6, (d) FVB/N [×200] 72 h following injection of KA 30 mg kg\(^{-1}\), i.p. (right panels). Note the more intense GFAP labelling, thicker, shorter processes and swollen soma of astrocytes in the KA-treated sections compared with respective vehicle-treated sections (left panels).**
tion in the level of immunoreactivity in the inner, middle and outer molecular layers of the hippocampus in KA-treated sections from the 129 and outer molecular layers of the hippocampus in KA-treatment in the level of immunoreactivity in the inner, middle and outer molecular layers in vehicle (■) and KA (30 mg kg⁻¹, i.p.) (□) treated FVB/N sections as determined by laser scanning confocal microscopy and subsequent image analysis. Data are presented as mean ± S.E.M. * p < 0.05 vs. vehicle (unpaired t-test).

3.4 Lipid peroxidation

The effect of KA on lipid peroxidation in cortical homogenates was assessed by measuring the change in malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) concentrations 72 h post-dose. The degree of lipid peroxidation measured in vehicle-treated tissues of the 129/Ola, BALB/c and C57BL/6 mice (data not shown). In contrast, the FVB/N mice showed a 50.4% and 49.8% loss of synaptophysin immunoreactive presynaptic terminals in the middle and outer layers respectively compared with vehicle-treated mice (Fig. 8). Synaptophysin levels in the dentate gyrus remained unchanged by KA treatment in all strains. Omission of the GFAP and synaptophysin antibodies resulted in a complete absence of signal (data not shown).

4. Discussion

In order to avoid over-interpretation of a mutant phenotype a comprehensive understanding of the traits of the host strains in which the phenotype will be studied is essential [10,17]. The aim of the present study was therefore to investigate further the endogenous characteristics of four inbred mouse strains commonly used in molecular genetics.

A comparison of neurological function in the four inbred strains revealed an absence of the flexion reflex in approximately 50% of the 129/Ola and BALB/c mice which suggested impaired cerebellar/spinal cord function [48]. Further evidence to support motoric deficits in these strains was obtained from analysis of LMA and rotarod studies, which showed clear reductions in exploratory behaviour and coordination respectively. Low exploratory behaviour has previously been reported in BALB/c mice [25] and inter-strain variation in the ‘emotional’ status of the mice may play a role in this phenotype since anxiety and stress have been shown to depress LMA in mice [9,28]. The marked hyperactivity observed in the FVB/N strain in this study may provide a favourable background upon which to produce mutations designed to mimic the motor impairments that characterise ALS, since a high level of activity would be desirable to define clear trans-gene-induced deficits.

Analysis of acquisition and probe data from the Morris water maze study revealed an apparent cognitive deficit in the FVB/N strain. However, since pathlengths to locate the cued platform were significantly elevated in addition to those in the hidden task, these data suggest that the deficits in FVB/N performance may not be due to learning and memory impairments alone. Rather, since the retinal degeneration gene (rd) has been identified on chromosome 5 of the FVB/N strain [46] deficits in visual acuity are likely to underlie this phenotype. Perhaps surprisingly, the behaviour of the 129/Ola mice was indistinguishable from that of the BALB/c and C57BL/6 strains in that all showed evidence of good spatial learning and memory. The 129 strain is widely used in genetic targeting research as it is one of the most valuable donor strains of ES cell lines but the majority of cognitive studies have characterised the 129 strain as a poor learner [17,35,50]. However, there are many substrains within the 129 family [40] and evidence is accumulating to support behavioural heterogeneity within the substrains. For example, the 129 SvHsd substrain (also known as the 129 Sv-ter) differs from other 129 substrains at many genetic loci [12,40] and this may account for the variation in water maze performance. Indeed, Montkowski et al. [31] recently demonstrated heterogeneity in water maze learning in four 129 substrains with the 129/Ola substrain showing good evi-

<table>
<thead>
<tr>
<th>Inbred strains</th>
<th>n</th>
<th>Treatment</th>
<th>MDA + 4-HNE concentration (nmoles [mg protein]⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>129/Ola</td>
<td>4</td>
<td>Vehicle</td>
<td>6.2 ± 0.5</td>
</tr>
<tr>
<td>8</td>
<td>KA 30 mg kg⁻¹</td>
<td>7.0 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>KA 30 mg kg⁻¹</td>
<td>6.7 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Vehicle</td>
<td>8.5 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>KA 30 mg kg⁻¹</td>
<td>5.6 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>KA 30 mg kg⁻¹</td>
<td>5.5 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>FVB/N</td>
<td>4</td>
<td>Vehicle</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>3</td>
<td>KA 30 mg kg⁻¹</td>
<td>6.1 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.E.M. MDA + 4-HNE concentrations (nmoles [mg protein]⁻¹). * p < 0.05 vs. vehicle (unpaired t-test).
KA reliably induces seizures in both rats and mice [23,42] and is widely used to model epilepsy [33]. The seizures are associated with characteristic neurodegeneration in the hippocampus with the CA1–CA3 subfields proving particularly vulnerable [43]. Analysis of KA-induced seizure activity revealed a marked strain-dependent response with the 129/Ola mice proving relatively resistant to the convulsive effects of KA. In contrast, intense and protracted seizure activity was observed in the FVB/N and C57BL/6 strains with the FVB/N mice exhibiting the highest mortality rate. The increased vulnerability of the FVB/N strain to seizures was paralleled histologically by a greater degree of cell loss observed predominantly in the CA3 pyramidal cell layer and significant loss of synaptic immunoreactivity throughout the hippocampal fields. In addition, this cell damage was also associated with an intense astrogliosis. This suggests that the FVB/N strain is more susceptible to neurodegeneration and impaired neuronal function following KA induced excitotoxicity. One of the mechanisms thought to underlie KA mediated excitotoxicity is via the generation of free radicals [4,6] which subsequently lead to lipid peroxidation [45] and neuronal membrane damage [29,30,37]. In agreement with the histological findings measurements of cortical lipid peroxidation revealed a significant increase in the FVB/N strain following KA that was not observed in the other strains. Thus the behavioural, immunohistochemical and biochemical data all suggest that the FVB/N strain is more susceptible to KA-induced seizures and neurodegeneration.

It is unlikely that the inter-strain variation in response to KA is due to pharmacokinetic reasons since previous studies have shown no evidence for differential uptake of the compound into the murine CNS [13,39]. Rather, interstrain differences in kainate receptor densities [24] and/or the presence of genes that either mediate neuroprotection or predispose to excitotoxic damage may govern the level of injury. The contribution of background genetic factors was explored recently in a quantitative trait loci (QTL) study that identified as many as eight chromosomal loci that are strongly linked to the differential response of inbred strains to KA-induced seizures [14]. The locus present on chromosome 1 accounted for 17% of the genetic variance between strains and candidate genes at this locus include the α- and β-subunit of the Na+/K+-ATPase pump [22]. Clearly, compromised function of this ion transporter could lead to perturbed neuronal polarisation and render the FVB/N strain more susceptible to ectopic discharge. Indeed, pharmacological inhibition of this enzyme potentiates excitotoxic injury of adult rat hippocampal neurones [5] and such an effect may explain the previously reported appearance of spontaneous seizures in this strain [19] and the increased susceptibility to KA-induced seizures in the present study. In addition, it is interesting to note that the high mortality rate observed in this strain is consistent with poor survival of transgenic mice produced on this background [7,21,26].

The present data are in conflict with those of Schauwecker and Steward [39] who reported comparable seizure activity in the same strains following administration of KA at the same dose used in the present study. Nevertheless, Table 1 (p. 4104 of Ref. [39]) which summarised seizure type in each strain, stated that 85% of FVB/N and only 20% of 129/SvEMS mice exhibited ‘severe seizures’. The authors also reported relatively little excitotoxic cell loss in C57BL/6 and BALB/c strains. Whilst our data is in agreement with the observation that C57BL/6 mice are less susceptible to cell loss, we observed a 34.7% reduction in normal cell number in the CA1 region of BALB/c mice. However, differences in the route of administration, the substrains of mice used (for example, 129/SvEMS vs. 129/Ola) and time points used to study histological changes may account for these discrepancies.

FVB/N mice have proven useful in transgenic work due to their vigorous reproductive performance and the presence of prominent pro-nuclei in fertilised eggs that facilitate microinjection of DNA [46]. However, taken together the data from the present study suggest that this strain may not be the most suitable for the development of new transgenic mice to aid the study of genes implicated in CNS disorders. This may be particularly relevant for longitudinal studies where inbred traits such as spontaneous seizure activity and retinal degeneration may be exacerbated with age. The FVB/N strain will however, remain useful in gene targeting studies designed to interrogate genes involved in peripheral disease, for example, in immunological studies [34,49].

Acknowledgements

We gratefully acknowledge Dr. Neil Clarke for expert advice on transgenic technology.

References

17 R. Gerlai, Gene-targeting studies of mammalian behavior: is it the  
16 D. Games, D. Adams, R. Alessandrini, R. Barbour, P. Berthelette,  
18 R. Gerlai, K.J. Millen, K. Herrup, K. Fabien, A.L. Joyner, J. Roder,  
15 R.M. Friedlander, V. Gagliardini, H. Hara, K.B. Fink, W. Li, G.  
12 W.F. Dietrich, J. Miller, R. Steen, M.A. Merchant, D. Damron-Boles,  
19 M.F. Goelz, J. Mahler, J. Harry, P. Myers, J. Clark, J.E. Thigpen,  
21 K.K. Hsiao, D.R. Borchelt, K. Olson, R. Johannsdottir, C. Kitt, W.  
Yumis, S. Xu, C. Eckman, S. Younkin, D. Price, C. Iadecola, H.B.  
Clark, G. Carlson, Age-related CNS disorder and early death in  
transgenic FVB/N mice overexpressing Alzheimer amyloid precursor  
Lalley, R. Levenson, D.E. Houseman, Genes encoding α and β  
subunits of Na, K ATPase are located on three different chromo-  
5373.  
23 T.G. Kokate, A.L. Cohen, E. Karp, M.A. Rogawski, Neuroactive  
stEROIDS protect against pilocarpine- and kainic acid-induced limbic  
seizures and status epilepticus in mice, Neuropharmacology 35  
Ferraro, Kainate and AMPA receptor binding in seizure-prone and  
of locomotor activity, acoustic and tactile startle, and prepulse  
inhibition of startle in inbred mouse strains and F1 hybrids: implica-  
tions of genetic background for single gene and quantitative trait loci  
26 J.F. Mahler, W. Stokes, P.C. Mann, M. Takaoka, R.R. Maronpot,  
Spontaneous lesions in aging FVB/N mice, Toxicol. Pathol. 24  
Smutko, K.J. Moore, Theoretical and empirical issues for marker-as-  
sisted breeding of congenic mouse strains, Nat. Genet. 17 (1997)  
280–284.  
28 C. Mathis, P.E. Neumann, H. Gershonfeld, S.M. Paul, J.N. Crawley,  
Genetic analysis of anxiety-related behaviors and responses to ben-  
zodiazepine-related drugs in AXB and BXA recombinant inbred  
29 M. Miyamoto, J.T. Coyle, Idebenone attenuates neuronal degenera-  
tion induced by intrastriatal injection of excitotoxins, Exp. Neurol.  
30 M. Miyamoto, T.H. Murphy, R.L. Schnaar, J.T. Coyle, Antioxidants  
protect against glutamate-induced cytotoxicity in a neuronal cell  
31 A. Montkowski, M. Poettig, A. Mederer, F. Holsboer, Behavioural  
performance in three substrains of mouse strain 129, Brain Res. 762  
Ballas, H. Choi, W.H. Berrettini, Mapping murine loci for seizure  
33 J.V. Nadler, Mini-review: kainic acid as a tool for the study of  
34 A. Nii, D.A. Reynolds, H.A. Young, J.M. Ward, Osteochondrodys-  
plasia occurring in transgenic mice expressing interferon-gamma,  
of learning by the Morris water task and fear conditioning in inbred  
mouse strains and F hybrids: implications of genetic background for  
single gene mutations and quantitative trait loci analyses, Neuro-  
36 J.A. Pedralta, S.H. Zhang, J.R. Hagaman, P.M. Oliver, N. Maeda,  
Generation of mice carrying a mutant apolipoprotein E gene inacti-  
37 P.S. Puttfarcken, R.L. Getz, J.T. Coyle, Kainic acid-induced lipid  
peroxidation: protection with butylated hydroxytoluene and U78517F  
in primary cultures of cerebellar granule cells, Brain Res. 624 (1993)  
223–232.  
38 M.E. Ripp, G.W. Huntley, P.R. Hof, J.H. Morrison, J.W. Gordon,  
Transgenic mice expressing an altered murine superoxide dismutase  
gene provide an animal model of amyotrophic lateral sclerosis, Proc.  
39 P.E. Schauwecker, O. Steward, Genetic determinants of susceptibil-


