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Early behavioural changes in mice infected with BSE and scrapie: automated home cage monitoring reveals prion strain differences

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Abstract

Mice inoculated with transmissible spongiform encephalopathies (TSE) show behavioural abnormalities well before the appearance of clinical signs. TSE strains are obtained by serial re-infection of infectious brain homogenates in laboratory rodents. They are characterized by strain-typical brain lesion profiles, which implies that they might be differentiated behaviourally as well. Seventy female C57BL/6 mice were tested, 14 per group. Controls received no or sham inocula, two other groups received scrapie strains adapted to mice (139A, ME7) and one group a mouse-adapted BSE strain (301C). From week 7 until the end of the incubation period, 8 mice per group were subjected once every 2 weeks to open-field and hot-plate tests. Assessment of clinical signs, and measuring of body weight, food and water consumption were carried out weekly on the remaining animals kept in single cages. In addition, locomotor activity was recorded continuously in these mice by means of infrared detectors. Monitoring of circadian activity revealed early significant TSE strain differences, most pronounced during the nocturnal active phase. Behavioural changes in open-field tests also occurred before the appearance of clinical signs, and differences in rearing, wall rearing and sniffing were strain-specific, however, such differences varied according to the period of testing. Hind paw lick latencies increased equally in all groups after week 19, jump latencies also increased in the two scrapie groups but not in the BSE group. It was at this time that clinical signs first appeared consisting of ataxia, lack of balance, motor dyscoordination, and lordosis. These data imply that automated assessment of circadian activity in mice is a powerful and economical tool for early behavioural typing of TSE strains.

Introduction

Scrapie is a naturally occurring transmissible spongiform encephalopathy (TSE) that affects sheep and goats. Several strains of scrapie have been isolated in mice and hamsters after repeated inoculations (serial passages) with brain-infected homogenates (Bruce & Fraser, 1991; Kascsak *et al*., 1991). Bovine spongiform encephalopathy (BSE) has been adapted similarly to laboratory mice (Bruce *et al*., 1994). These species-adapted TSE strains are characterized by much shorter incubation times than obtained after inoculation with homogenates of sheep or bovine brains. By means of processes not yet fully understood, they differentiate during passaging in mice and hamsters and maintain transmissible characteristics. The differences among these adapted strains and, more generally, the characterization of other TSEs in the mouse are primarily based on the experimental assessment of two parameters: (i) the 'incubation period', the time between inoculation of the infective agent and the appearance of clinical signs (Prusiner, 1998) and (ii) the presence and distribution of grey matter 'vacuolation' in the brain, the so-called lesion profile (Fraser & Dickinson, 1973) which can vary considerably between TSE strains.

Mice infected with scrapie may show behavioural abnormalities before the appearance of clinical signs (McFarland & Hotchin, 1980; Betmouni *et al*., 1999). In some cases, changes are specific, such as in spatial reversal learning (Lysons & Woollard, 1996) or in displacing food pellets from a tube (Deacon *et al*., 2001). In other cases, the effects appear to be more generalized, such as changes in locomotion (Suckling *et al*., 1976) and altered performance in motor ability tests (Guenther *et al*., 2001). The behavioural abnormalities are also accompanied by changes in body weight, feeding and drinking (Outram, 1972; Guenther *et al*., 2001).

The above studies used a single scrapie case and did not investigate whether different scrapie strains or BSE would have produced different effects. Other behavioural studies that compared different strains searched for differences at the time of appearance of early clinical signs and did not include BSE (McFarland *et al*., 1980; Hunter *et al*., 1986). Since the severity of the damage in specific brain areas (lesion profile) can vary widely between scrapie strains, it is likely that behaviours depending on the integrity of these areas can be affected too.

The present study tested the hypothesis that different mouse-adapted TSE strains characterized by different lesion profiles will

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show different early behavioural effects. The C57Bl/6J mice were inoculated with the two scrapie strains most frequently used in previous studies (ME7 and 139A), and with one mouse-adapted BSE strain (301C). In order to assess the dynamics of behavioural changes, one sample of mice was monitored continuously for circadian activity from 7 weeks postinoculation onwards, another sample was investigated by biweekly assessment of open-field activity, complemented by hot-plate tests to detect possible effects on pain reactivity. This measure has never been considered in previous studies with scrapie and other TSEs, although brain areas usually affected by vacuolation can play a role in the processing and modulation of pain (Schnitzler & Planer, 2000).

Materials and methods

Subjects

Laboratory mice were C57BL/6J virgin females, aged 60 days. Fourteen animals were assigned to each of five groups, namely, one control group receiving no inoculation (Control 1), one group receiving a control inoculum (Control 2), and three groups injected with the mouse-adapted scrapie strains ME7 and 139A, and the mouse-adapted BSE strain 301C (see below). The experiments started in December and lasted about 6 months. Mice were kept in two separate rooms with identical conditions of temperature (21°C). The rooms had a large window on one wall and the illumination inside followed the natural daylight cycle characterized by a progressive increase of the illumination period at the latitude of Rome. Mice in one room were assigned to continuous monitoring of activity in their home cage and were kept in individual cages. The mice in the other room were assigned to open-field and hot plate tests and were kept together in the same-treatment groups. Testing was carried out between 09:30 and 13:00 h. For all mice, pellet food and water were available *ad libitum*. The study was carried out in accordance with the Italian law on animal experimentation.

Scrapie inocula and inoculation procedure

The ME7 and the 301C strains were provided by the Institute of Animal Health, Compton, Newbury, Berkshire, UK. The mouse-adapted scrapie strain 139A was donated by the Laboratory of Virology of the Istituto Superiore di Sanità and passaged three times in our mice before being used for the present study. The inocula were obtained from infected brains of mice euthanased with CO_2 at the terminal stage of the disease. Brain tissue was homogenized in sterile phosphate buffer and prepared following a standard procedure (Bruce *et al*., 1991). The control inoculum (Control 2) was prepared from the brain of a healthy mouse following the same procedure used for the TSE inocula. All inocula were at 1% w/v. Before injection the inocula were re-suspended and vortexed in their vials for 30–40 s. Mice were lightly anaesthetized with methoxyflurane and injected intracerebrally with 30 μL of inocula into the left striatum using a hand-held syringe with a stop at 3.7 mm.

Activity in the home cage

For each group, six mice were assigned to the continuous monitoring of activity and were kept in individual cages in one single rack. The position of cages in the rack was such that mice for each group of inocula were equally distributed in rows and columns. The assessment of daily activity was carried out by means of an automated system that used small passive infrared sensors positioned on the top of each cage (ACTIVISCOPE^{TM}, NewBehaviour Inc., Zürich, Switzerland, *http://www.newbehaviour.com*). The system was activated on week 7 postinoculation (PI) and operated continuously until the end of the experiment. The sensors detected any movement of the mice and transmitted the signals through an interface to a recording computer with dedicated software. No movements were detected by the sensors when mice were sleeping, inactive, or performed moderate self grooming. An additional sensor was installed in proximity of the experimental rack containing the cages, for recording human activity in the animal room. Scores were processed and counts were cumulated over a 1-h period in order to produce a 24-h profile. Weekly profiles of daily activity were obtained by averaging the scores over 7 days. The access of the personnel to the animal room was not restricted and followed the routine schedule (between 08:00 and 11:00 h).

Open-field tests

Eight mice from each group were assigned to behavioural observations in the open-field and were kept together in same group cages in a separate experimental room. Prior to inoculation these mice were tagged with passive integrated transponders for allowing individual identification throughout the experimental phases. Open-field tests started on PI week 7 and were repeated once every 2 weeks until week 21 or until the mice were severely impaired by the disease. Mice were tested in white opaque open-field arenas ($50 \times 30 \times 30$ cm) made of plexiglass that were cleaned by a moist towel after each trial. Each mouse was gently placed in the centre of the arena for an 18-min test. Four mice belonging to different groups were recorded at the same time in separate open-fields by two video cameras, each suspended on the ceiling above two open-fields. An automatic scanner allowed to record from each video camera five two-minute periods, namely 0–2, 4–6, 8–10, 12–14 and 16–18 min. Time of individual testing was balanced as much as possible between groups in subsequent tests. Behaviour was then analysed offline with WINTRACK, a software package for spatial analysis that also allows quantification of frequency and duration of behavioural events, freely available at *http://www.dpwolfer.ch/wintrack*. The parameters considered included grooming, rearing, wall rearing, sniffing, and walking (duration of horizontal movements).

Assessment of analgesia

At the end of each open-field test, starting from PI week 11, mice were tested for their analgesic sensitivity using a hot-plate apparatus. The temperature was set at 51 ± 0.1 °C and cut-off time was 60 s. the plate was cleaned with a moist towel between trials. The parameters recorded were the latencies to front-paw licking, hind-paw licking and to jump (all four paws off surface simultaneously).

Monitoring of body weights and food and water consumption

From PI week 7 onwards body weight was measured weekly in mice assigned to the individual monitoring of activity. At the same time, water and food consumption was also determined by weighing the bottles and the amount of pellets left before refilling. It was assumed that eventual spillage was similar among groups and therefore no correction was applied for it.

Assessment of clinical signs

In addition to the procedures described above, mice were also monitored according to standard clinical protocols to detect the appearance of the first signs of disease. The clinical scoring was carried out by an experienced technician not involved in the behavioural monitoring and consisted of a weekly assessment of the health conditions of the mice according to the criteria listed by Prusiner (1998). When mice appeared to show the first signs of disease the inspection was carried out daily. The first signs consisted of disturbed balance, impaired motor coordination, and lordosis. Mice

were killed with carbon dioxide when they reached the terminal stage of the disease and their brain excised. The terminal stage was the time preceding natural death, the signs included pronounced ataxia, severe hypo-activity and hypo-reactivity and inability to adopt an upright position after having been turned on their back. According to our experience, these signs precede death by a few hours, and at most 2 days. Automated monitoring signalled approaching death when activity was 90% below that of controls for >12 h. Then, the incubation time (interval time between inoculation and the appearance of first signs) and the survival time (interval time between inoculation and euthanasia) were calculated.

Tissue processing and Western blotting

The diagnosis of successful transmission of the TSEs was carried out by histopathology (presence of spongiosis in the brain) and by PrP^{Sc} analysis in Western blotting. After excision each brain was cut parasagitally in two. One part was fixed in 10% formalin buffer for histopathological evaluation (data not shown in this study), the other part, a lateral third, was immediately frozen and kept at –80 C until use. For the preparation of homogenates, brain tissues from individual mice were weighed and homogenized (10% w/v) with Omni GLH tissue homogenizer in 10% Sarcosyl (Sigma, USA). Brain homogenates (1 mL) were left for 30 min at room temperature and then cleared by ultracentrifugation in a bench-top Beckman Optima TL ultracentrifuge equipped with a TLA 100.3 rotor at 23 000 r.p.m. (22 000 *g*) for 20 min at 10 °C. The supernatants were collected and proteinase K (50 μg/mL) was added. Proteins were hydrolysed for 1 h at 37 C with continuous stirring, and the digestion was stopped with 5 mM phenyl-methyl sulphonyl fluoride. Insoluble proteins were spun down by ultracentrifugation at 72 000 r.p.m. (215 000 *g*), the pellets were collected, resuspended in 100 μL 1 × NuPAGE LDS sample buffer (Novex, Invitrogen, UK) and finally heated at 90 C for 10 min before loading in NuPAGE 12% Bis-Tris Gel (Novex). The electrophoretic separation of proteins was performed under constant voltage at 200 V for ~40 min proteins were then electroblotted onto PVDF membranes by use of a semidry blotting unit (Bio-Rad, UK) under constant current (100 mA for 40 min). After blocking in PBS containing 0.1% Tween 20 and 5% nonfat milk powder, the membranes were incubated with the monoclonal antibody SAF 84 (CEA, France) at a concentration of 1 μg/mL, then with HRP-conjugated antimouse IgG (Sigma). The membranes were developed using ECL chemiluminescent substrate (Amersham) and exposed to hyperfilm ECL autoradiographic films (Amersham).

Statistical methods

Between-group differences in the weekly scores of mean activity per hour were analysed by analysis of variance (ANOVA) considering treatments as grouping factor and weekly scores as repeated measures. Treatment × repeated measures ANOVA were also performed to analyse the daily profiles of activity and changes in body weight, food and water consumption. Behaviours recorded in the open-field were also analysed with ANOVA, with treatment as a within-group factor and the five 2-min blocks of the 18-min open-field as repeated measures. Total duration of behaviours during each test was also computed and analysed by separate one-factor ANOVA. When needed, posthoc comparisons were performed using Tukey's honest significant difference (HSD) test.

Results

All mice injected with the infectious inocula developed the disease and were euthanased, whereas none of the mice in the two control groups died and were still alive after 400 days. All infected mice showed typical brain spongiform lesions as verified by histopathology and gave a positive Western blot, showing the expected PrP^{Sc} banding pattern for the three different strains (data not shown). The survival times (± SEM) were 155.5 ± 0.5, 159.8 ± 0.7 and 171.4 ± 0.7 days for the ME7, 139A and 301C strains, respectively, revealing the expected differences between the TSE strains. Clinical signs were first detected at 128.2 ± 3.2, 132.7 ± 4.6 and 148.4 ± 4.1 days for the three inocula, respectively, which was about 2–3 weeks before the mice were euthanased. There were no significant differences between the two control groups for any of the parameters analysed. Thus, although statistical information provided in the text refers to comparisons of the five experimental groups, for a better understanding of the figures the two control groups are graphically pooled.

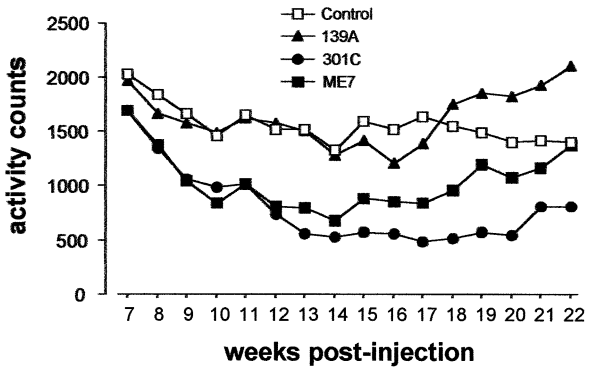

FIG. 1. Time course of the activity scores of control mice (in this and the following figures, the two control groups combined) and mice inoculated intracerebrally with two mouse-adapted scrapie strains (139A and ME7) and one mouse-adapted BSE strain (301C). Mice were kept singly in their home cages and monitored with ACTIVISCOPE™. Scores represent the mean weekly values of the number of scores per hour. See Results for statistical information about between-group comparisons.

Activity in the home cage

Figure1 shows the time course of the weekly averaged values of the mean activity scores per hour. Starting from PI week 7, mice inoculated with the ME7 and 301C were significantly less active as compared to the other three groups (main effect of treatment $F_{4,375} = 3.87$, $P < 0.01$; repeated measures $F_{15,375} = 12.72$, $P < 0.01$, in the absence of a significant interaction all values of $P < 0.05$ or less in posthoc comparisons vs. controls from 8 to 18 weeks PI for ME7, and throughout the whole period for 301C). On PI week 13, the 301C-inoculated mice reached the lowest level of activity and this was maintained until PI week 20, then, activity slightly raised but to levels still significantly below that of controls when mice reached the terminal state of the disease. Mice injected with ME7 showed decreasing activity similar to that in the 301C group until week 14, thereafter, however, the activity progressively increased, at levels significantly higher than that in the 301C group but lower than controls until week 18. The activity of mice injected with 139A was similar to that of controls until PI week 14, after this the activity was slightly (but not significantly) lower than controls for 3 weeks and then after week 18 started to rise to levels above controls ($P < 0.05$ in posthoc comparisons at weeks 19 and 20).

In order to analyse the circadian activity patterns in more detail, hourly activity scores were averaged per week (Fig. 2). This permitted visualization of progressive changes in activity peaks and

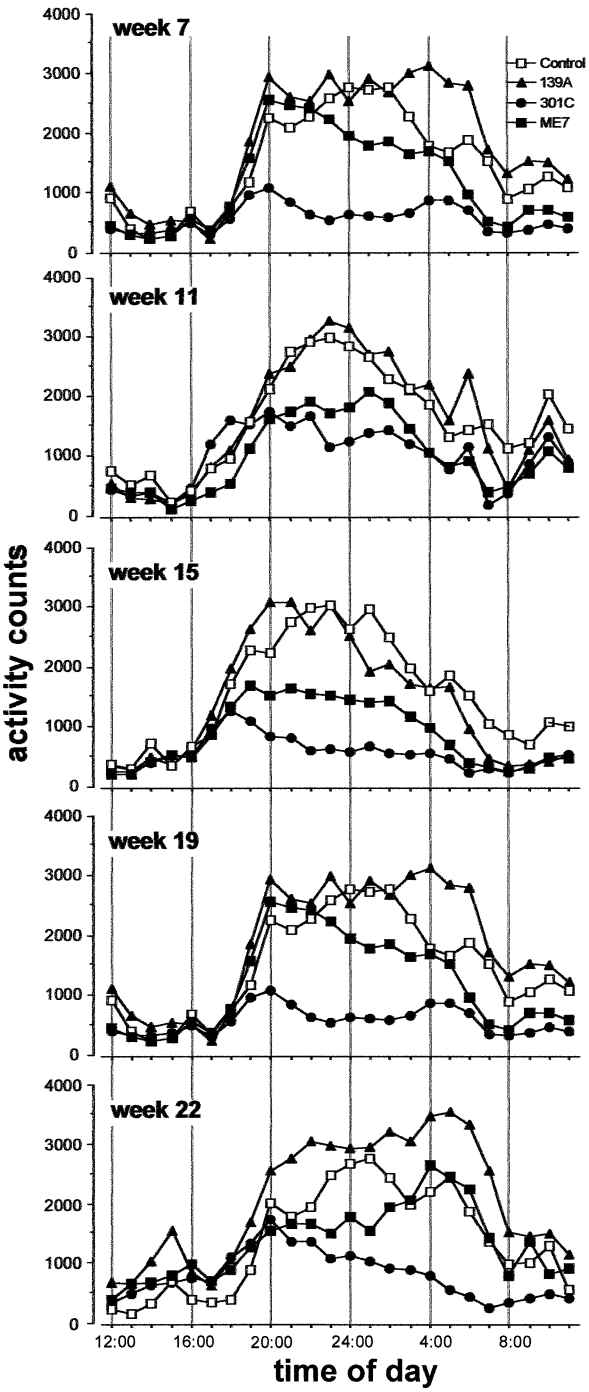

FIG. 2. Daily activity profiles of control mice and mice inoculated intracerebrally with two mouse-adapted scrapie strains (139A and ME7) and one mouse-adapted BSE strain (301C) at selected weeks post inoculation. Mice were kept singly in their home cages and activity scores were computed by ACTIVISCOPE™ at 1-h intervals. The mean activity profile was calculated on a weekly basis. See Results for statistical details.

to recognize those time windows with the largest TSE strain differences. Specifically, on week 7 (when treatment effects were small for the averaged values), the nocturnal peak of activity was lower in ME7 and 301C mice with respect to controls and 139A mice ($P < 0.05$ in comparisons at 22:00, 23:00 and 24:00 h for 301C, and at 24:00 h for ME7). A main effect of repeated measures ($F_{23,575} = 52.71$, $P < 0.01$) was also present but there were no interactions between the two factors. From week 11 onwards, in addition to nearly significant treatment effects ($F_{4,575} > 2.45$, $P < 0.07$) and a significant effect of repeated hourly measures ($F_{23,575} > 30.34$, $P < 0.01$), an interaction between the two factors ($F_{92,575} > 1.40$, $P < 0.01$) was always present. Posthoc comparisons showed that most of the differences between groups did indeed occur during the nocturnal phase. From week 11 onwards both ME7 and 301C mice were less active than the other groups but there were now also differences in the activity level between these two strains, the 301C mice being significantly less active than the ME7 mice. The increase in activity of 139A mice on week 19 mainly occurred during the second half of the dark phase whereas on week 22 it encompassed the whole activity period.

Open-field tests

Because of the many sessions carried out and the large amount of data gathered, the behavioural results from the open-field are presented only for selected weeks and are given as duration, as most of the results considering frequency scores were, in general, very similar. Figure 3 shows the total duration of selected behaviours from week 7 at 4-week intervals, revealing a mixed and changing pattern of TSE strain differences, depending on behavioural measure and time of testing. Week 7 coincided with the first test session. During the first session, 139A and ME7 mice showed less rearing and more sniffing than controls whereas in the last session this picture slightly changed in that sniffing in 139A mice did not differ from controls (all $F_{4,35} > 2.67$, all $P < 0.05$). In addition, in the last session ME7 mice showed increased wall rearing and both 139A and ME7 mice also showed increased walking (all $F_{4,35} > 2.97$, all $P < 0.05$). In the intermediate session at week 15, ME7 mice showed a reduction in rearing and an increase in sniffing (all $F_{4,35} > 4.01$, all $P < 0.01$), whereas no differences emerged for the other groups for any behaviour with respect to controls.

The within-session time course of the open-field behaviour is shown for weeks 15 and 19 in Fig. 4. By week 15, a low level of rearing was maintained throughout the entire session by ME7 mice with respect to the other groups. By week 19 this behaviour was even more depressed and was also lower in the other infected mice than in the controls. Wall rearing in the same week showed a reverse pattern, but there was no difference between 139A and 301C mice. Sniffing scores were higher in ME7 mice on weeks 15 and 19 at the beginning of the session (main effect of treatment all $F_{4,140} > 4.01$, $P < 0.01$; repeated measures all $F_{4,140} > 4.01$). Owing to large individual variability, significant differences with respect to controls in the amount of walking emerged only in ME7 mice on week 19 and were limited to the very beginning of the session (treatment × repeated measures, $F_{16,140} = 2.02$, $P < 0.05$; $P < 0.05$ in the first two points).

Hot-plate test

The time course of the latency to hind-paw licking from 11 to 23 weeks is shown in Fig. 5 (top). All the TSE-infected mice had longer latencies than both control groups, yet only on weeks 21 and 23 (treatment–repeated measures interaction, $F_{24,210} = 2.86$, $P < 0.01$; $P < 0.05$ in posthoc comparisons). No differences between groups emerged in the profiles of front-paw licking whereas for jumping (Fig. 5, bottom) ME7 mice, from week 17 onwards, and 139A mice on week 19 and 21, had longer latencies than controls (treatment–repeated measures interaction, $F_{24,210} = 1.78$, $P < 0.01$; $P < 0.05$ or less in posthoc comparisons).

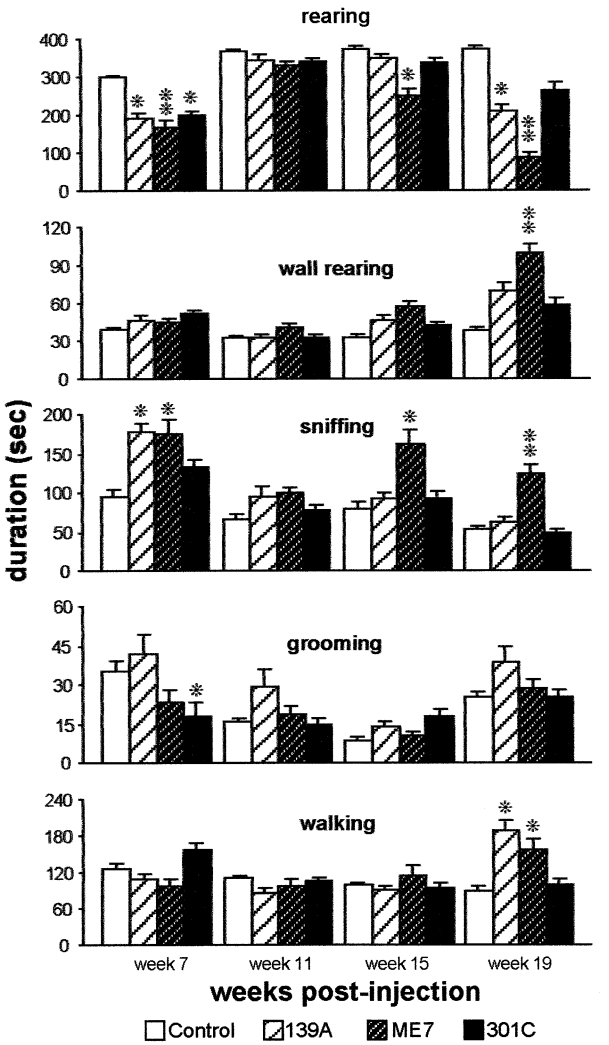

FIG. 3. Total duration (mean + SEM) of behavioural parameters in the open-field of control mice and mice inoculated intracerebrally with two mouse-adapted scrapie strains (139A and ME7) and one mouse-adapted BSE strain (301C). Behaviours were recorded at 2-min intervals during an 18-min session repeated at 2-week intervals. The total was summed over the five 2-min bins each spaced 2 min apart. Week 7 coincided with the first test session. *$P < 0.05$, **$P < 0.01$ compared with controls.

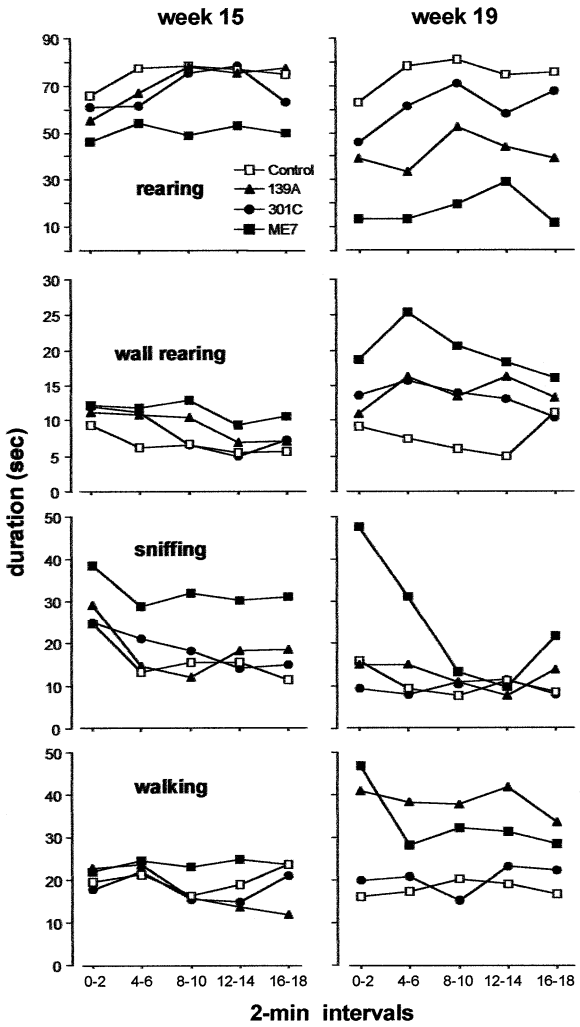

FIG. 4. Time course of selected behaviours observed during an 18-min open-field session in control mice and mice inoculated intracerebrally with two mouse-adapted scrapie strains (139A and ME7) and one mouse-adapted BSE strain (301C). On the left tests carried out at week 15. On the right tests carried out at week 19. See Results section for statistical information.

Body weight, food and water consumption

Figure 6a shows the body weight profiles of mice during the experimental phase. No main effect of treatment emerged from the ANOVA, however, a main effect of repeated measures indicated a general increase with time of body weight ($F_{13,325} = 33.68$, $P < 0.01$). After week 17, body weight started to decrease in all scrapie-infected mice and posthoc comparisons indicated that the average weight of ME7 and 139A-infected mice was lower than controls on weeks 19 and 20 (treatment × repeated measures $F_{52,325} = 4.76$, $P < 0.01$; $P < 0.05$ or less in posthoc comparisons). Food consumption was more or less constant in all mice until week 14 (Fig. 6b). Afterwards the food consumption of 301C mice, and that of ME7 mice after week 19, was significantly lower than that of controls (treatment ×repeated measures, $F_{52,325} = 3.02$, $P < 0.01$; $P < 0.05$ or less in posthoc comparisons). Mice injected with these two strains also showed significantly reduced water consumption as compared to controls during the central part of the incubation period ($P < 0.05$ or less in posthoc comparisons, with no significant interaction between treatment × repeated measures; Fig. 6c). 139A-infected mice drank less as well, albeit not significantly so.

Discussion

This study confirmed that changes in activity and other behaviours occurred in mice inoculated with TSEs much earlier than the appearance of clinical signs, and showed, as predicted, different patterns according to TSE strains.

Home cage activity

The most robust effects were revealed by the automated assessment of circadian activity in the home cage. Each strain showed a different profile of the weekly average scores and of the circadian activity during the incubation period. From week 7 onwards the BSE strain 301C and the scrapie strain ME7 produced a persistent depression of activity with an upsurge at the time when clinical signs of disease first developed. By contrast, the 139A-infected mice remained similar to

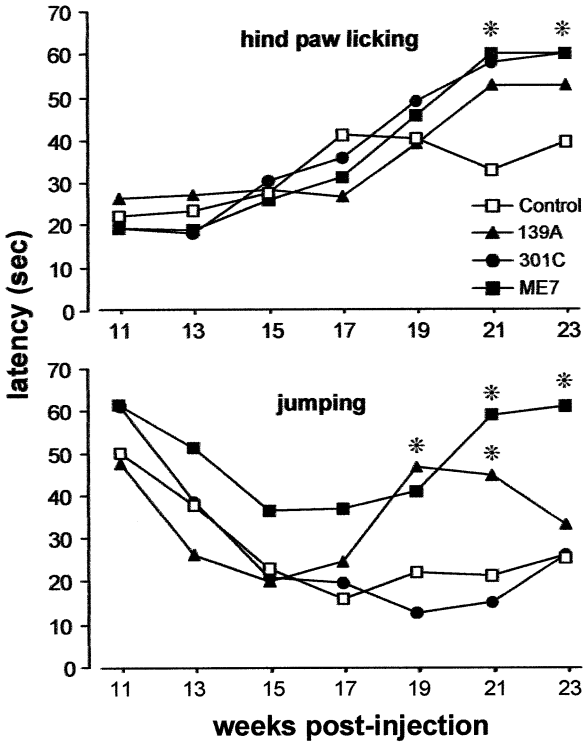

FIG. 5. Time course of the latency to hind paw licking (top) and jumping (bottom) in control mice and mice inoculated intracerebrally with two mouse-adapted scrapie strains (139A and ME7) and one mouse-adapted BSE strain (301C). See Results section for statistical information.

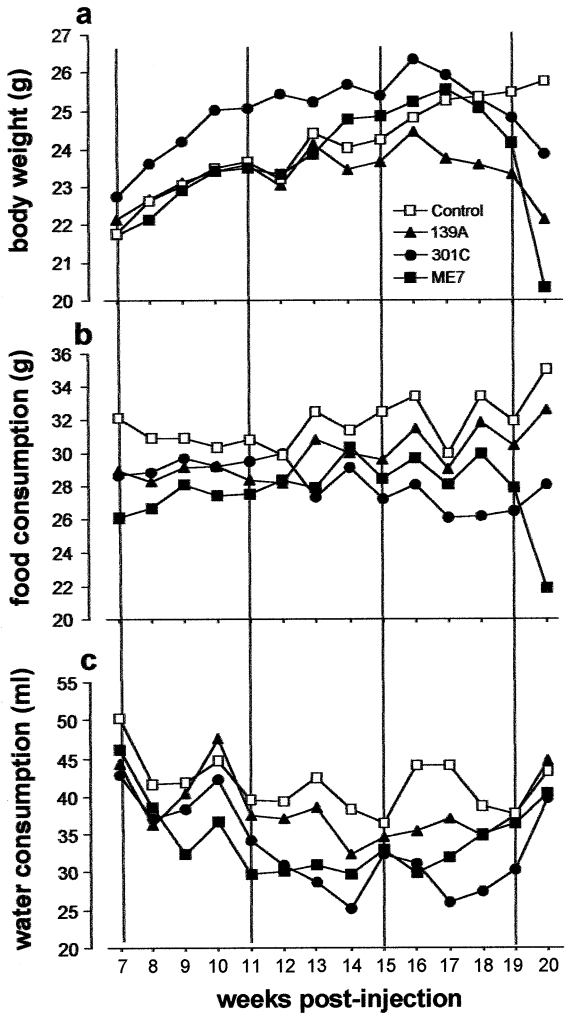

FIG. 6. Time course of body weight and food and water consumption in control mice and mice inoculated intracerebrally with two mouse-adapted scrapie strains (139A and ME7) and one mouse-adapted BSE strain (301C). Food and water consumption are weekly means of the daily amount consumed by individual mice: (a) body weight; (b) food consumption; (c) water consumption. See Results section for statistical information.

controls and then showed hyperactivity in the late stage of the disease. The activity pattern of the first two inocula, although measured using a different method, was similar to that described earlier (Suckling *et al.*, 1976). However, in the case of 139A the effects were different. This difference was not due to the infective agent because these authors used an inoculum derived from the so-called Chandler strain, from which the 139A inoculum originated (Bruce & Fraser, 1991). The differences were probably due to the fact that the older study was based on Swiss/A_2G mice as it is known that mouse strains can have different reactions to the same inoculum (McFarland *et al.*, 1980). It is interesting to note that in both this study and previous work, the differences occurred mainly during the nocturnal activity phase, while during day time normal activity levels were too low to discover reductions. A general small increase in activity of all mice during mid-morning was due to the operations of the caretakers. However, from week 15 to 19, the treated mice (except for 139A-infected animals) no longer reacted.

Open-field behaviour

A detailed analysis of behavioural parameters revealed general treatment effects that were also partially dependent on the TSE strains. However, effects varied greatly with week of testing and time windows within sessions, which made it difficult to judge their significance.

A recent paper by Guenther *et al.* (2001) reported increased open-field locomotor activity in the same mouse strain (C57BL/6) upon inoculation with ME7. These findings paralleled our results in the initial minutes of the open-field and should not be regarded as in contrast with the pattern of decreased activity in the home cage discussed above. Given the very short tests (3 min) carried out by Guenther and colleagues it is likely that the measures obtained can be attributed partially to differences in hyper-reactivity to novel stimuli rather than to levels of genuine spontaneous activity; as scores from the initial minutes in the open-field do not only reflect spontaneous activity but also reactivity to novel or threatening stimuli (Choleris *et al.*, 2001). This would be in agreement with their observation of increased reaction to shock shown by the scrapie-infected mice in a passive avoidance test. Such initial hyper-reactivity would also explain the decrease in activity we observed after 4 min in the open-field, eventually leading to equal scores between control and ME7 mice. Activity increased however, both in the open-field and in the home cage in the case of 139A mice. The paradoxical increase in wall rearing shown by ME7 mice, accompanied by a decrease in rearing distantly from the walls, resulted possibly from impaired motor coordination: the mice preferred to rear against the wall because they were ataxic and needed the support. Such an effect of scrapie on motor coordination was shown by Guenther *et al.* (2001). Yet older reports using open-field tests also revealed somewhat inconsistent changes in scrapie-infected mice, and the assessment of reactivity to

stimuli without the manifold subtle confounds of the open-field appeared to be more informative as early diagnostic parameters (Savage & Field, 1965; Heitzman & Corp, 1968). Finally, one may note that the location of injection sites might complicate interpretations additionally. Guenther and colleagues injected into the hippocampus, while our injections were aimed at the striatum. Hippocampal but not striatal lesions are known to entail hyperreactivity to distracting stimuli. However, the interactions between spread of PrP^{Sc}, scrapie-strain specific lesion profiles, and differential functional deficits depending on injection site remain a matter of investigation (see also below).

Pain sensitivity

This measure proved to be less informative than expected, as hotplate tests did not reveal any differences between the TSE-infected and control mice during the preclinical period. Delayed reactivity to pain occurred eventually in all infected animals, regardless of the TSE strain, but only at a time when the mice were suffering from other clinical impairments. At these stages, mice infected with scrapie strains 139A and ME7 showed prolonged jump latencies but it is difficult to judge *a posteriori* whether this reflects decreased pain sensitivity or impaired motor abilities.

Food and water intake

Among these measures, a decrease in water consumption was clearly the most robust sign of TSE infection although it did not emerge prior to week 10 post-inoculation, and did not discriminate well between TSE strains. These findings are in agreement with the older literature (Outram, 1971) and indicate that this measure is useful not only for scrapie but also for BSE strains. With respect to body weight and food consumption we obtained mixed results, generally congruent with earlier reports (for 139A and ME7 see Carp *et al*., 1984; Kim *et al*., 1987). The 301C-infected mice were slightly heavier than controls and this was not paralleled by an increase in food consumption. Perhaps this reflects a lower energetic demand due their reduced home cage activity.

Are the behavioural changes linked to differential lesion profiles?

A correlational analysis of strain-typical lesion profiles and occurrence of strain-typical behavioural impairments would require many cohorts of infected mice in order to document the stages of pathological changes in specific brain regions, and might even include assessment of different injection sites (see above). In this study, lesion profiles were assessed in the terminal stages of the disease only. However, it has been suggested that the brain areas that are more affected by spongiosis at the time the animals were euthanased are also those in which vacuolation develops earlier (Fraser & Dickinson, 1968). Thus, early changes in these areas are likely to be accompanied by changes in behaviours that depend on their integrity. Abnormal behaviour and motor deficits in the ME7-infected mice, for example, have been tentatively related to changes occurring in the hypothalamo-pituitary-adrenal (HPA) axis (Ye & Carp, 1995). Thus, the decrease in the home-cage activity observed in our mice infected with the ME7 and 301C strains, but not in mice infected with 139A, could be a consequence of hypothalamic damage. The strains ME7 and 301C, in fact, produce severe lesions in this structure (Fraser & Dickinson, 1973; Bruce *et al*., 1991; Bruce *et al*., 1994). Water metabolism is also controlled by hypothalamic mechanisms (Beck & Daniel, 1971), and changes in water consumption developed earlier and were more pronounced in ME7 and 301C mice with respect to mice infected with 139A. Lastly, it should be noted that circadian activity is regulated by the suprachiasmatic nucleus within the hypothalamus. On the other hand, scrapie strain 139A is known to produce only mild hypothalamic spongiosis, and this appears to be in agreement with the moderate decrease in water intake and the lack of hypoactivity seen in those infected mice. The consistent hyperactivity of 139A-infected mice, both in the home cage and in the open-field, however, eludes interpretation in terms of CNS damage at present.

As far as the open-field responses are concerned (decreased rearing and increased sniffing in the first open field test), these emerged only in mice infected with the two scrapie strains ME7 and 139A. These strains, as opposed to the BSE-related strain 301C, produce severe vacuolation in the thalamus and hippocampus (Fraser & Dickinson, 1973; Kim *et al*., 1987; Bruce *et al*., 1997).

Why is early and robust differentiation of TSE strain symptomatology important?

The detection of the first signs of TSE disease is important for two reasons. Firstly, they are used for calculating the incubation period, a parameter of obvious clinical interest. In many studies it is determined when the mouse has shown clinical signs of disease for up to 3 weeks (Dickinson *et al*., 1968). In other studies, traditional neurological assessment includes an evaluation of motor co-ordination and reactivity capabilities of mice, and the assignment of individual scores based on the severity of the handicap observed (Carp *et al*., 1984; Prusiner, 1998). The procedure for determining the incubation time can vary widely between studies and even when the same procedures are used, differences in incubation time for the same scrapie-host combination can occur. For example, the incubation times in C57 mice for ME7 and 139A ranged between 122 and 171, and 106 and 166 days, for the two strains, respectively (Kim *et al*., 1987; Bruce *et al*., 1991; Kascsak *et al*., 1991). Hence, clinical observations are insufficient for determining the incubation period (Silverman, 1987).

Secondly, early behavioural signs of infection by particular TSE strains are of epidemiological importance. For example, it is possible that some recent outbreaks of TSE in sheep are due to the BSE agent (Butler, 2000). Thus, our findings that early behavioural changes caused by mouse-adapted BSE strains can be reliably differentiated from effects caused by scrapie strains suggests that a behavioural assessment may offer a useful tool for early discrimination of differential sources of infection. This may apply not only to laboratory rodents but likely also to larger animals, for example to sheep. Because typing of TSE strains requires extensive neuropathological and biochemical assessment *post mortem*, a behavioural test could be included when screening for potentially infected animals. The advantages of automated early behavioural characterization are obvious, and can accompany recent biochemical improvements in the early detection of PrP^{Sc} (Schulz-Schaeffer *et al*., 2000).

Assessment of circadian activity vs. serial longitudinal testing

Our study demonstrates that automated assessment of activity in the home cage represents a robust, noninvasive and objective criterion for characterizing TSE strains. Circadian activity is linked to, and expresses, a synthesis of the animal's physiological, metabolic, and neurological processes. It is also worth noting that, it also appears disturbed in mouse strains deficient for the prion protein (Tobler *et al*., 1997). Nowadays, the continuous assessment of circadian activity can be performed with relatively simple and inexpensive electronic methods, and appears most suitable for comparative studies, even in larger species such as sheep, as it requires little standardization and special set-ups. For mice, assessment of circadian activity in group-caged animals is now also possible, reducing the potential effects of

isolation stress. We do not deny the usefulness of repeated behavioural testing in order to obtain additional criteria. However, the large number of animals required to run longitudinal studies and the manpower needed to conduct repeated behavioural tests makes them costly for diagnostic purposes. Moreover, tests of spontaneous activity are notoriously difficult to standardize (Crabbe *et al*., 1999).

Conclusions

Behavioural methods, particularly the automated assessment of circadian activity, are useful tools for anticipating a TSE diagnosis and can provide additional and economical criteria for the characterization of natural and adapted TSE strains.

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