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Influence of husbandry procedures on mouse locomotor activity

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Introduction

Stress may be defined as: "A state of stress occurs when an animal encounters adverse physical or emotional conditions which cause a disturbance of its normal physiological and mental equilibrium" (*Manser*, 1992).

Stressors can be emotional or physical, very often overlapping each other. Due to experimental or husbandry procedures physical stressors applied to laboratory animals are likely to provoke a certain level of discomfort and perhaps fear in animals not used to being handled indicating emotional and psychological responses to physical stimuli. Short lasting and unpredictable, or predictable and longer lasting conditions (stimuli) have been demonstrated to be associated with the induction of significant physiological stress (*Abbot et al.* 1984). The perception of pain or acute fear is very often associated with an immediate reduction in locomotor activity (*Smith*, 1994).

Genetic predisposition, individual variations and the fact that sometimes stress in laboratory animals can be difficult to recognize, makes it complicated to quantitate stress. Comprehensive knowledge of normal behavioural characteristics and how and when stress occurs is needed for accurate assessment of well-being and welfare of laboratory animals. A number of stress indicators have been used to measure diminished well-being of animals. Acute stress raises circulatory glucocorticoids and catecholamines (*Livezey et al.* 1985) and changes in plasma levels of hormones such as, prolactin can be useful indicators of acute stress (*Kant et al.* 1986). Chronic stress suppresses the humoral (*Griffin* 1989) and cellular immune system such as T-cell function (*Okimura & Nigo* 1986). Biochemical markers of stress usually require invasive methods for monitoring and are thus, themselves a source of stress which renders them less desirable from a welfare point of view. Behavioural studies have been carried out for safety evaluation of commercial products and in the assessment of animal welfare (*Barclay et al.* 1988) and different behavioural sampling and recording methods have been used, including continuous and time sampling (*Saibaba et al.* 1995a). In this study we were interested in overall locomotor activity and not detailed studies of different behavioural characteristics. The equipment available to monitor and analyse locomotor activity tends to be very expensive and is predominantly used by pharmaceutical companies. The "Jiggle cage" is an old method which detects an animals locomotor activity by monitoring mechanical vibrations in the floor of the cage caused by the animals' movement. This is a complex method and difficult to set up for routine use and has been largely superseded by the equally expensive but more commonly used array of infrared beams and photo cell apparatus (*Hansen & Hubner* 1976). In our study the circadian fluctuation in spontaneous locomotor activity of female mice was recorded by capacitance technique/equipment and video recording as previously described (*Stodulski et al.* 1994). The capacitance method has been successfully used by *Barclay et al.* (1988) in the development of the "Disturbance index" and to study locomotor activity in both rats and mice (*Stodulski et al.* 1994, *Saibaba et al.* 1995b). The main advantages of the capacitance method are that the equipment is inexpensive and monitoring is carried out in the animals home cage thereby introducing fewer novel variables which may act as uncontrollable stimuli and additional stressors to the animal. The disadvantages are that capacitance recording equipment has been underdeveloped, and the hardware and software are not readily available.

The aims of the present study were: i) to examine the diurnal activity of individual CD-1 mice and ii) to examine the effects of simple procedures (move

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cage, open cage, handle animal, restrain animal) on the activity of female CD-1 mice.
In the present study we hypothesized that the effects of disturbing events could be measured by monitoring changes in the locomotor activity of a mouse in its home cage.

Materials and Methods – Equipment
The capacitance detector was originally adapted and developed from a design for a metal detector (*Anon* 1981) and described in detail in *Stodulski et al.* (1994). The equipment was cordoned off so that it would not suffer interference from the movement of personnel working nearby. This signal is fed to the calibrated analogue input of the Logit data logger (*Griffin and George,* Loughborough, UK). The data were downloaded from the data logger to a PC computer spreadsheet (MS Works) in "SID" format and then plotted in arbitrary activity units (AU) with standard error means (SEM). The Logit was programmed to record at a 'periodic' 'interval' of 15 seconds in 'count' mode with 'high resolution' off, using all the memory available. The 'marker' channel was left on to record the signal indicating that a procedure had been completed. A synchronised video camera recording with capacitance monitoring was made to establish the correlation, reliability and accuracy of the capacitance monitor. The video recording was analysed by 1/0 sampling at 15 second intervals and recordings made in a chart.

Animals
The mice used in the study were ten 20 g female CD-1 (Charles River UK Ltd, Margate, UK), singly housed in 16x32x12cm Macrolon cages with chromed metal grid lids. They were fed (RM3, SDS, Witham, UK) and watered (tap water) *ad lib.* The cages were cleaned 12 hours before an animal was subjected to the procedures. The bedding used was dust-free sawdust (RS Biotech, Northampton, UK). The cage was placed in a room with natural daylight close to a window but out of direct sunlight so it would be exposed to natural day light conditions during the third and fourth weeks of August. The temperature was continuously monitored and varied between 19-24°C. The relative humidity was not monitored, but was known to vary between 55 and 70%.

Procedures
Data, already available in the laboratory for BALB/c mice (*Stodulski et al.* 1994), were used as a reference to compare with 24 hour diurnal locomotor activity fluctuations in CD-1 mice. A female CD-1 mouse was monitored by the capacitance method for 8 days continuously to establish a 24 hour pattern of activity.
Each of ten mice were subjected to a series of four consecutive procedures carried out with half-hourly intervals once during the day time and once during the night time. These were:
Procedure 1. moving the cage for thirty seconds
Procedure 2. opening the cage for thirty seconds
Procedure 3. handling the mouse for thirty seconds
Procedure 4. restraining (scruffing) the mouse, as if carrying out an i.p. injection, for 15 seconds

Data logging
The Logit was programmed to start monitoring at 11.00 am. The first procedure (moving cage) was carried out at 1.00 pm, and the three remaining procedures (opening cage, handling mouse, restraining mouse) at half hourly intervals. Immediately after each procedure the cage was returned to the monitoring position and a signal button on the Logit was pressed to indicate that the procedure had been completed and monitoring initiated. This signal was necessary so that the disturbance due to the procedure itself could be eliminated from the graph when the data was analysed. The data in the Logit from the daylight recording were retrieved and stored before reprogramming the Logit for night-time monitoring starting at 9.00 pm. The first night-time procedure was carried out at 11.00 pm and continued as before at half hourly intervals. Half an hour after the final night-time procedure the data were retrieved from the Logit and stored. The Logit was reprogrammed ready for the next mornings daylight session.

1/0 time sampling analysis
The video recording was analysed by 1/0 time sampling (*Martin & Bateson* 1993). The behavioral characteristics table (ethogram) used for the mice was identical to the one used in studies of the rat by *Saibaba et al.* (1995b). An interval length of 15

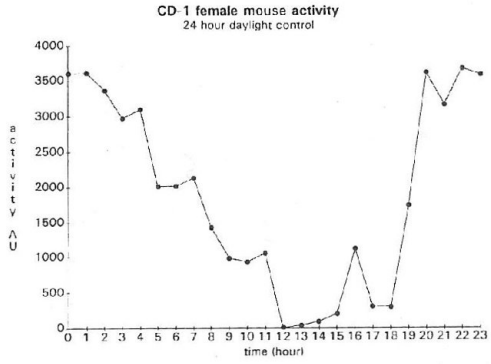

Figure 1. Graph of 24 hour capacitance activity measurement for a female CD-1 mouse performed under natural light conditions.

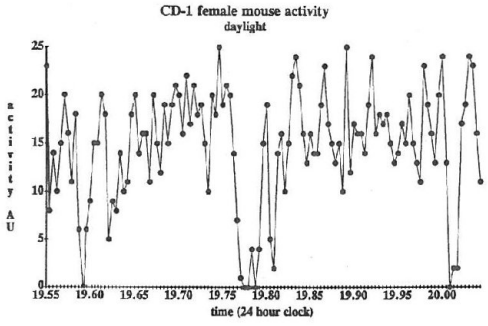

Figure 2. Graph of CD-1 female mouse activity performed by the capacitance method for 1/2 hour from 19.30 to 20.00.

seconds was used. The shorter the interval the closer the measurements become to continuous sampling which is the absolute reference method of behaviour measurement. The 1/0 measurements can be optimised to save time by increasing the intervals studied until there is a significant inaccuracy, and then using the longest interval whose accuracy is acceptable.

Statistics

Linear regression analysis (Quattro Pro Windows) was performed on the first 15 minutes of data for day time and night time procedures and controls as recorded by the capacitance method. Areas under the curves were calculated for 0-15, 0-10, 0-5, 5-10, 10-15 minutes for all the curves following each procedure. Differences were considered significant when p values lower than 0.05 were calculated using ANOVA statistics.

Results

Comparison of diurnal activity patterns.

The pattern of diurnal activity of CD-1 mice (Figure 1) exhibited increased activity during dark periods compared with day-time. Very little activity was recorded between 12 and 15.

Comparison of activity measured by Capacitance recording and 1/0 sampling from Video recordings.

A typical example of the activity of a mouse monitored for an 1/2 hour by the capacitance method, from 19.50 to 20.00 hours (times are expressed as hours and decimals of an hour throughout) is shown in Figure 2. The activity of the same mouse was analysed from a simultaneous video recording by 1/0 sampling for the half hour from 19.50 to 20.00 hours (Figure 3). The low peaks of Figure 2 between 20.37 and 20.50 hours were due to slight head movements and tail flicks while the mouse was asleep.

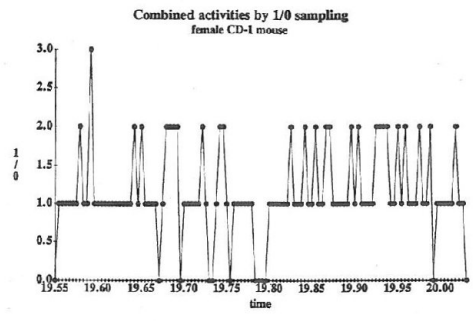

Figure 3. Graph of CD-1 female mouse activity (combined foraging, grooming and rearing/climbing) measured by 1/0 sampling method for the same 30 minutes interval as capacitance method (Figure 2) between 19.30 and 20.00 pm. Any locomotor activity is scored and each recording represents a 15 s interval.

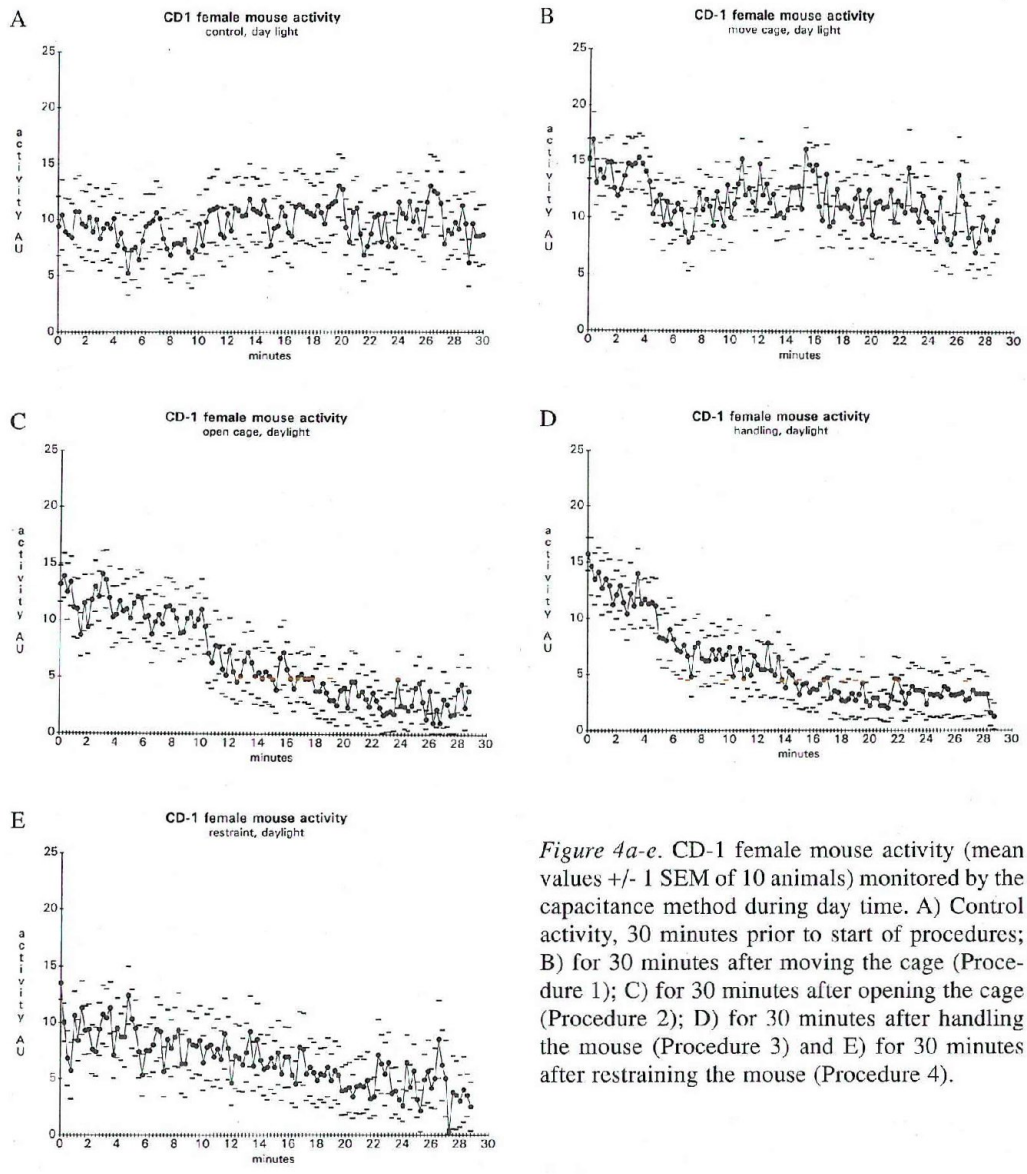

Figure 4a-e. CD-1 female mouse activity (mean values +/- 1 SEM of 10 animals) monitored by the capacitance method during day time. A) Control activity, 30 minutes prior to start of procedures; B) for 30 minutes after moving the cage (Procedure 1); C) for 30 minutes after opening the cage (Procedure 2); D) for 30 minutes after handling the mouse (Procedure 3) and E) for 30 minutes after restraining the mouse (Procedure 4).

Effect on activity of 4 simple animal handling procedures. The average locomotor activity of the female CD-1 mice (n=10) from 12.30-13.00 am was found to be rather stable throughout the period (Figure 4a). The subsequent procedures carried out with half-hourly intervals resulted in initial increases in activity followed by a decrease (Figures 4b-e). A similar activity pattern was observed when the procedures were carried out in the evening. Table 1 shows linear regression data for the first 15 minutes after each day time procedure. The procedure of moving the cage, opening the

Table 1. Linear regression results for the first 15 minutes of day time activity data after the procedures and for control data.

Day time	Procedure				
Regression constant	control	1	2	3	4
Constant	8.57	13.5	13.4	13.3	9.75
Slope	0.08	- 0.18	- 0.53	- 0.67	- 0.22

Table 2. Linear regression results for the first 15 minutes of night time activity data after the procedures and for control data.

Night time	Procedure				
Regression constant	control	1	2	3	4
Constant	11.4	16.8	14.2	13.2	8.84
Slope	0.08	- 0.64	- 0.33	- 0.21	- 0.27

cage and handling during day time each resulted in a higher intercept on the curve indicating an initial increase in activity followed by a marked decrease. Restraint and the control had similar intercepts, but significantly different slopes. A similar activity fluctuation pattern was obtained during the night time studies (Table 2). The areas under the activity curves during day time (Table 3) and night time (Table 4) procedures demonstrated significantly lower activity levels following the restraint procedure compared with all other procedures. The areas for day time restraint and control procedures were markedly lower that recorded for the other procedures.

Discussion

BALB/c mice have been used in the laboratory to study locomotor activity, and data obtained by capacitance methods are available for both males and females in natural and artificial light. BALB/c mice have a diurnal pattern of activity which is almost continuous activity during hours of darkness with corresponding inactivity during the hours of light (*Stodulski et al.* 1994). In the present study it was decided to study the CD-1 strain in order to analyze inter strain variation. The diurnal pattern of locomotor activity for female CD-1 mice was found to be similar to that of female BALB/c mice (*Stodulski et al.* 1994) (Figure 1).

In order to assess the sensitivity of the capacitance equipment capacitance locomotor activity (Figure 2) was compared directly with the 1/0 time sampling observations (Figure 3) and found to be very similar but having more fine detail. It was possible to synchronise the video recording of the mouse in the cage to within 2 or 3 seconds by using tail flicks and minor head movements of the mouse during its sleep periods as markers, which appeared as discrete peaks on a flat baseline (Figure 2, between 19.55 and 20.00 hours).

Figure 3 is a composite curve of all the behaviour characteristics observed in the half hour period between about 7.30 and 8.00 pm of 1/0 time sampling. The mouse spend long and continuous periods of time in grooming and climbing/rearing (which is sometimes referred to as exploring), while foraging tended to be intermittent in nature. These three behaviour characteristics are responsible for most of the time the mouse spend in locomotor activity. This is reflected in the similarity to the capacitance curve which is obtained by the movement of the animal in the cage only.

Table 3. Areas under the curve for 15 minutes of control data, and 15 minutes after the procedures during the day time.

Day time	Procedure				
Time window (minutes)	control	1	2	3	4
0-15	558	891	577	506	494
0-10	355	497	454	399	357
0-5	191	289	246	257	199
5-10	164	208	208	142	158
10-15	203	394	123	107	137
5-15	367	602	331	249	295

Table 4. Areas under the curve for 15 minutes of control data, and 15 minutes after the procedures during the night time.

Night time	Procedure				
Time window (minutes)	control	1	2	3	4
0-15	737	734	714	708	419
0-10	475	551	519	493	292
0-5	257	324	277	266	180
5-10	218	227	242	227	112
10-15	262	183	195	215	127
5-15	480	410	437	442	239

The choice of procedures in this study was based on the study of *Kvetnansky et al.* (1978). They used 'move cage', 'open cage', 'handle animal' and 'restraint' as well definable individual procedures to compare effects of stress on rats associated with these common husbandry procedures by measuring classical stress hormones such as plasma corticosterone and catecholamines. Restraint had the greatest influence on raising these hormones on rats, while removal of the lid and moving the cage had little effect. The effect of handling on plasma levels of stress hormones was intermediate between restraint and moving the cage.

In the present study it was decided to use the four procedures described by *Kvetnansky* and coworkers (1978) although we studied mice and not rats. It was decided not to measure stress hormones such as corticosterone because this would required either blood sampling, which is more disturbing than the procedures studied, or housing the animals in metabolic cages to make noninvasive collection of suitable urine volumes possible (*O'Brien et al.* 1994). This would counteract the objective of the study namely to study the animals in their home cage.

The activity curves for the four Procedures (1 to 4), moving the cage, opening the cage, handling the mouse and restraining the mouse, as well as the control curve had encouragingly small SEMs (Figures 4a-e) despite the fact that some of the animals

spent relatively long periods of time asleep while others were active almost continuously.

The time selected for the night time study was between 11.00 pm and 1.00 am, with two hours before this to obtain a stable control baseline of activity. The day time hours used were between 1.00 and 3.00 pm, again with 2 hours monitoring before the procedures were carried out to establish a baseline control. The control activity plots were obtained from data in the half hour immediately preceding the start of the first procedures, 10.30-11.00 pm for the night time studies, and 12.30-1.00 pm for the day time studies (Figure 4a). Both these have a level baseline as expected with the day time one being lower than the night time.

The day time and night time activity curves (Figure 4a) were both horizontal (both slope = 0.08) as expected, but the daytime curve was at a higher activity level than expected. For a truly comparable 24 hour diurnal activity curve the mice should have been placed in fresh cages 12 hours before a 24 hour monitoring, and replaced with new mice for each 24 hour period. This probably explains the higher than expected control base line for the day time activity curve, as the mice were possibly still exploring their new environment and the 24 hour diurnal activity curve was obtained from a single mouse in a cage cleaned twice a week. We have recently compared mouse locomotor activity in artificial light and normal day light (*Saibaba et al.* 1995). In the present study we chose natural daylight because many procedures are carried out in laboratories with natural daylight. The four curves for both day time and night time are continuous in procedure order 1 to 4, with the control curve preceding procedure 1. Although there is a drift down of activity after some of the procedures (very marked in Figures 4b-d) to below that of the control there is in every case an increase in activity immediately after the start of the next procedure.

The regression data for night time activity (Table 4) are most notable for the linear regression constants (the y axis intercept when time is zero) which decrease in the order Procedure 1 to 4. This may be because procedure 1 was perceived the least stressful to the mouse and 4 the most. Only procedure 4, restraint, showed a Constant lower than the control (reduced activity immediately after the procedure), suggesting that this was a distressful experience reducing activity and inhibiting exploratory behaviour. A cumulative effect of the four procedures can not be excluded, but Procedures 1 to 3 are probably not perceived stressful by the mice in the present study as they were used to these being normal recurring husbandry associated procedures and the mice elicited an increase in activity immediately after the procedures. Daytime regression constants (Table 3) were similar for procedures 1 to 3, but that for restraint is lower and similar to that of the control. This indicates that procedures 1 to 3 rouse exploratory behaviour in the mice while restraint does not. These findings agree well with the hormone results of *Kvetnansky* and coworkers (1978) which demonstrated that restraint was associated with a dramatic increase in plasma levels of stress hormones. In the present study restraint is clearly perceived differently by the animals than the other three procedures. In addition, handling in this study seems to be a less stressful experience than in the study of *Kvetnansky et al.* (1978), and this may be due to the fact that the mice used in our study were used to being handled, so that handling seemed to be an emotionally neutral experience.

Measuring areas under the curves integrates the total locomotor activity for the time interval measured. The area for daytime activity measurements (Table 3) for the interval 0-15 minutes (the 15 minutes immediately after the procedures) decrease in the order Procedure 1 to 4, quite markedly. The differences in areas for the first 5 minutes after the procedures (0-5 minutes) are even more pronounced (Table 4) in their decrease. Ideally each animal should be used only once because of the learning influence of animals which had been handled/gentled before, but the marked reaction to procedure 4 in pilot experiments suggested that this precaution was not necessary.

Further studies involving larger numbers of animals and well defined procedures with respect to their stress effect on the animals are needed in order to examine the usefulness of reduced locomotor activity as a potential measure of stress associated with different experimental procedures. However, the present study indicates that measurements of locomotor activity may be a simple method to examine and compare stressfulness associated with different experimental procedures.

Summary

The circadian fluctuation in spontaneous locomotor activity of CD-1 female mice was recorded by capacitance monitoring and video recordings. The capacitance recording system was found to correlate accurately with the video recording system and with 1/0 time sampling studies (15 seconds intervals). The activity of 10 female mice was monitored, during daylight and night-time conditions consecutively. The impact on locomotor activity following four procedures commonly used in animal husbandry and handling (move cage, open cage, handle animal, restrain animal) was recorded for 30 minutes following each procedure on 10 female mice during different stages of the diurnal activity rhythm (11am-3pm and 9pm-1am). All activity recordings following the procedures showed an initial increase in activity followed by a decrease over the next 30 minutes. The mice had previously been handled, and restraint was considered the only negative stressor assessed. The results indicate that restraint consistently results in a decrease in activity over the following 30 minutes after the mouse is returned to its home cage.

Acknowledgments

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