Animal Science Papers and Reports vol. 37 (2019) no. 2, 209-226
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Behaviour as direct measure of animal welfare – the effect of strain, social stressors and stroboscopic illumination on rat (*Rattus norvegicus*) behaviour under high- versus low-anxiety conditions*

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(Accepted April 12, 2019)

Animal welfare is associated with many factors, both environmental and genetic, which are reflected in animal behaviour. The aim of this study was to find out how different welfare levels affect behaviour of genetically different strains of rats subjected to long-term social stressors and short-term stroboscopic illumination. We performed a battery of behavioural tests to measure rat (*Rattus norvegicus*) response to high (Open Field/Elevated Plus Maze) and low anxiety conditions (chamber for self-exposure to light-stimuli). The results of the study confirmed the importance of genetic factors. Brown Norway (BN) rats showed a lower level of explorability under high anxiety conditions and activity under low anxiety conditions than Wistar Albino Glaxo (WAG). As expected, the social conditions have a major influence on rats' behaviour. Single housed rats display a lower level of exploratory behaviour. The most interesting finding in WAG rats (but not in BN rats) is that the animals, kept in isolation and over-crowding, also display lower levels of emotional response during behavioural testing. The short-term illumination stressor proved to have little effect on rats' behaviour only in the case of stimulability. Stressed rats displayed lower stimulability than non-stressed rats.

KEYWORDS: animal welfare / social environment / stressor / rat

In 1979 the UK Farm Animal Welfare Council (FAWC) issued a document referred to as "*Five Freedoms"*. The document was then adopted as the main checklist for

*The research was supported by grant BW 1735/19/2006 and the manuscript was supported by grant BST 0181400-17/2017 from the Faculty of Psychology, the University of Warsaw.
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all scientific and non-scientific personnel dealing with animal welfare. It was rooted mainly in the earlier "Brambell Report" published as early as 1965 [Brambell 1965]. Despite the fact that over 50 years passed since Brambell's seminal publication, the exact definition of welfare is still lacking. While the first definitions of welfare focused on the description of factors causing diseases, thus leading to harm and impairment of the body condition, the more elaborate definitions also included mental health, as a factor enabling the animal's harmonious co-existence with the surrounding environment [Hughes 1988]. Subsequently also the role of the subjective individual cognitive state and emotions [Dawkins 2006] were put into focus. Finally, according to Webster [2001], to attain welfare the animal needs to keep its physical integrity intact, at the same time being devoid of any mental distress. The author then pays attention to both mental and physical factors that both may affect welfare of the individual.

Just as there is no single definition of welfare, no objective method is available to accurately assess its level. Therefore, evaluation of welfare should include both biological and behavioural indicators. The latter ones seem to be especially appropriate sources of information on animal welfare, as they can often be non-invasive for the animals [Reinholz-Trojan and Stępniewska 2009].

The laboratory rat is a very popular animal model in science. It is the first animal ever to be domesticated purely for scientific purposes [Prusky *et al*. 2002]. According to the European Commission, rats score second after mice in terms of the laboratory animal model tested in 2011 (13.9%) [Commission of European Communities 2018]. The vast array of strains and stocks within the modern "laboratory rat" represent a huge variety of the behavioural characteristics expressed. Especially exploratory behaviour depends highly on genetic factors [Bolivar *et al*. 2000]. Among inbred strains and stocks the albino strains prevail, thus pigmentation should be taken into account during selection of animals for experiments. Non-pigmented rats exhibit a decreased sensory performance compared to pigmented rats [Hupfeld and Hofman 2006]. Especially the sense of sight is negatively affected [Prusky et al. 2002]. Also strong lightning is a much more aversive stimulus for the albino rats than for the pigmented strains. It can also lead to illnesses such as retina degeneration [Safa and Osborn 2000].

As we can see, the selection of the strain/stock for research may carry serious welfare consequences, as different lineages of laboratory rats may exhibit differentiated caging and lightning requirements that could introduce considerable erroneous "noise" to the data if not taken into account. What is worth noting, despite only 300 generations that divide the wild Brown rat from its laboratory dwelling counterpart, the high selective pressure applied by human manipulation resulted in a profound reconstruction of the laboratory rat phenotype, behavioural characteristics and huge interstrain variation [Stryjek and Pisula 2008].

Rats in nature live in strictly territorial family groups. The area occupied by a single family may reach up to 50 m^2 in range. Thus the caging area and the number of animals may determine the social structure within a rat group in captivity [Russel 2002]. However, whether this is the size of the group or rather the cage size that has a

greater effect on the welfare state of caged rats remains elusive. According to Lawlor [2002], the group size of 3-4 individuals is the optimal one. Nonetheless, Patterson-Kane [2002] claimed that familiarity of the group decreases the impact of the group size on the welfare, as it usually generates preference for a bigger living space in the animals.

Social animals tend to maintain constant contact with the members of their group [Wilson 2000]. It is described in literature that in rats distress resulting from isolation leads to the so-called isolational stress [Hansen and Baumans 2007]. According to Russel [2002], isolation also increases the risk of auto-aggressive behaviours. Additionally, isolated animals may exhibit stereotypies and aggressive behaviours more often, they tend to fall to some specific types of diseases such as scaly tail and show increased vulnerability to stress and toxicity [Van Loo *et al*. 2003]. Isolated individuals become more anxious and emotionally reactive [Van den Berg *et al*. 1999] with highly increased neophobia [Kaliste and Mering 2007]. Also, the hypothalamic-pituitary-adrenal axis becomes activated in isolated animals more frequently [Weiss *et al*. 2003].

However, isolation is not the only social stressor applied under experimental conditions, with overcrowding being often used as well to induce socially mediated stress. Group maintenance of rats can result in a stressful response in individuals with a lower group rank. It can also affect the group dominants, especially when the group contains many aggressive individuals [Blanchard *et al*. 1995]. Also despite the fact that hierarchy often does not arise within same-sex groups, over-crowding may increase a tendency for hierarchy arising [Hurst 1999].

Although many experiments concerning welfare of laboratory animals are conducted on mice and rats, relatively few compare different strains. Therefore the purpose of our study was to evaluate how different levels of welfare (stressor manipulation – long-term social stress and stroboscopic illumination) would affect different rat strains (pigmented and non-pigmented) examined under high- and low-anxiety conditions.

Material and methods

Animals and maintenance

The animals came from two homozygous strains: pigmented Brown Norway (BN, n=48) and albino Wistar Albino Glaxo (WAG, n=48). Animals were provided by a certified breeding centre, the Animal House of the Mossakowski Medical Research Centre, the Polish Academy of Sciences. The two strains were selected to compare susceptibility to social and physical stressors with special focus on light in pigmented versus non-pigmented rats. Additionally, both strains are commonly used in behavioural science. Only males were tested, due to the modulatory effect of oestrogen on anxiety and stress-related behaviours [Milian 2003] as well as cognition [Galea 2008, Spencer *et al*. 2008]. Therefore stress-related research should only be performed under strict control of the menstrual cycle, which imposes an additional

experimental constraint. Moreover, females were reported to show increased tolerance to social stress, i.e. isolation and overcrowding, which were focused on in our study [Brown and Grunberg 1995]. The maintenance conditions in our laboratory were as follows: 12:12 L:D photoperiod (light switch-on time 8 am); light intensity throughout the light period varying between 15 and 30 lx; constant T = 21°C, humidity 55+/-10%. Air-conditioning operation produced background noise at 45 dB. The animals were fed *ad libitum*.

Experimental procedures and set-up

Animals of two different inbred strains (pigmented Brown Norway versus albino Wistar Albino Glaxo) arrived to our laboratory at the age of 42 days. Subsequently animals were habituated to the maintenance room for 14 days in standard cages (Eurostandard Type IV with raised grate – 595x380x200/+70 mm, floor area 1820 cm^2). Next animals of both strains were randomly assigned to one of the three experimental groups designated as social environment (isolation - social stress, optimal group size (n=4) – no social stress, over-crowding (n=8) – social stress). The cage size remained identical, only the number of animals kept in one cage differed. The social conditions and individual set-up of each group were kept constant throughout the experimental procedures. After 30 days, 24 h before the first behavioural test, half of the animals from each social condition variant were transported to a separate room and subjected to 1 day of stroboscopic illumination (of 0.5 Hz frequency). After that the testing procedure began. All rats were examined first in an Elevated Plus Maze, the next day in an Open Field test, and finally on the third day in the Chamber for self-exposure to light-stimuli test. Subsequent tests were conducted with 24-hour intervals. The first to be tested were the animals subjected to stroboscopic illumination. Within the groups subjected and not subjected to the light stressor, cages housing were 1 (isolated), 4 (standard) or 8 rats (overcrowded) of either of the strains (BN or WAG), were assigned alternately. Every rat allocated for the study was put by the experimenter (a person previously known to the animals) to the transport cage and then transferred to the experimental room. The light in the corridor was dimmed (about 20 lux). After the test every rat was placed in a temporary cage, so that other individuals from the home cage would not make contact before the procedure with the animal already tested. Once all the rats from a given cage were tested, animals returned to the animal house and their home cages.

The experiments tested four behavioural traits. Exploratory behaviour (denoted as explorability) and emotional arousal (denoted as emotionality) were both tested under high anxiety conditions (Open Field/Elevated Plus Maze), whereas need for light stimulation (stimulability) and the animal's activity while seeking for light stimulus (activity) were both tested in a chamber for self-exposure to light-stimuli, serving as a low anxiety environment. Experimental procedures and set-up are presented in Fig. 1a below.

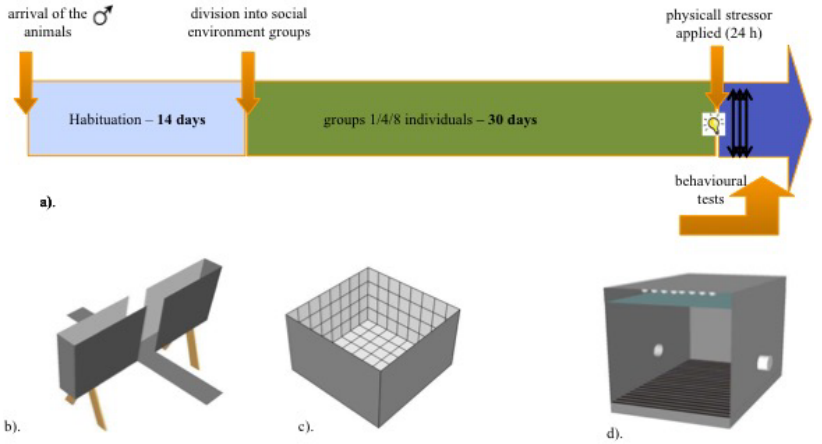

Fig. 1. The scheme of experimental apparatuses and procedures: a). arrival of 48 WAG and 48 BN male rats to the laboratory is followed by their subsequent division into social environment groups (30 days), application of the physical stressor (24 h) and behavioural testing. Elevated Plus Maze b.) and Open Field c.) apparatuses provide high-anxiety conditions for testing of the animal welfare state and behaviour, whereas Chamber for self-exposure to light stimuli d). provides low-anxiety conditions.

Open field

The Open Field test is often employed as the initial testing tool providing variables that can be subsequently used as Factorial Analysis components in various rodent model systems [Ramos *et al*. 2008]. It mostly quantifies the animal's exploratory behaviour and the level of emotional arousal. In our experiment the apparatus consisted of a square floor (75x75 cm), 4 wooden walls of 60 cm in height, and a Plexiglas lid covering the floor to facilitate inter-test cleaning. The floor and walls were covered with a white composite material divided with a grid (1.5 cm thick) into equal square fields (15x15 cm). It was lit with a single centrally located source of light (100 lx as measured immediately above the apparatus floor). The animals were released in one of the corners of the apparatus and the release corner was rotated clockwise between the trials. The experimenter was not present in the experimental room while testing. The test was cam-recorded and proceeded for 3 minutes. Subsequently the behavioural categories were scored manually upon watching the recorded material. Each defecation and urination was counted immediately after the animal's removal.

Behavioural categories scored included rearing bouts (the number of rearing events), maintained rearing bouts (against the apparatus walls), jumping bouts, locomotion (bouts of square entering), self-grooming bouts, urination (drops count) and defecation (faeces count).

Elevated Plus Maze

Similarly to the Open Field, the Elevated Plus Maze is a behavioural test used to examine the animal's emotional state and exploratory behaviour [Ramos *et al*. 2008].

In our set-up the test apparatus was made of wooden material covered with a white composite material. The apparatus consisted of two platforms 1 m long and 10 cm wide each. The platforms were crossed to divide each into equal halves. Two opposite arms of the apparatus were enclosed with 40cm high walls, while the other two arms remained open. All the four arms of the apparatus were elevated 1 m above the floor level. Underneath the apparatus there was an isomat spread on the floor to protect the animals, which could accidentally fall off the apparatus' open arms. The apparatus was situated in an experimental room with a single centrally located source of light (100 lx). Animals were released onto the middle platform created in the place, where the two long platforms crossed. The test was cam-recorded and proceeded for 3 minutes. The experimenter was present during the experiment to monitor possible falls of the test animal while in the open arm of the Maze. Urination and defecation were counted immediately after removal of the test animal. The other behavioural categories were scored manually upon analysis of the recorded material.

Behavioural categories scored included closed arm entries bouts, open arm entries bouts, falls from open arm bouts, rearing bouts, maintained rearing bouts (against the apparatus walls), self-grooming bouts, urination (drops count) and defecation (faeces count).

Chamber for self-exposure to light-stimuli

The chamber for self-exposure to light-stimuli is an experimental device constructed on the basis of the modified Skinner box. Briefly, the boxes used in our experiment (5 boxes of 33x30x27 cm each) consisted of side walls made of aluminium, front and rear walls made of Plexiglas (with an opening situated in the front wall) and a floor consisting of parallel metal bars with a removable drawer filled with woodchips. The ceiling of each box consisted of the dimmed Plexiglas cover with 6 light bulbs of 1.5 W mounted above the cover. Two rounded holes of 3 cm in diameter each were situated 10 cm above the floor on the two opposite side walls. The animal tested in our device could self-expose itself to an ambient light stimulus (28 lx light for 2 s) by inspecting an experimental (active) hole in the apparatus, whereas inspection of the control hole (non-active) was not followed by stimulus exposure. The inspection of the active hole triggered stimulus loading via an infrared beam operated device connected to a computer running on custom-made software. The software also recorded the number of inspections for each of the holes.

The described chamber was intensively used in our laboratory in the last three decades to test for stimulus seeking behaviour in relation to factors as diverse as social experience, physical environment, sex and genotype [Matysiak 1993, Osinski 2003, Ostaszewski and Pisula 1994]. The test may be used to measure two basic types of animal behaviour: need for light stimulation (stimulability) and exploratory behaviour shown during stimulus seeking (activity).

The stimulability can be calculated using the formula [Matysiak 1993]:

$$S = Ie/(Ie + Ic)$$

where: Ie – count of inspections of the experimental hole; Ic – count of inspections of the control hole.

Activity, in turn, can be calculated based on the formula [Matysiak 1993, modified]:

$$A = It \times (Ia + 1)$$

where: It – total count of inspections of any of the holes (Ie + Ic); Ia – count of alternation between experimental and control holes.

The design of all the three above-mentioned experimental apparatuses is presented in Fig.1b, c and d.

The Elevated Plus Maze and Open Field tests are popular tools in anxiety research in rodents [Gonzalez and File 1997]. Additionally, animals in our tests were subjected to high light insensitivity of 100 lx, which according to many studies represents a highly aversive stimulus for rats, whereas darkened conditions provide safety [Devan *et al.* 1999].

Conversely, the light-stimuli self-exposure box represented low anxiety conditions, as the boxes are tight small spaces enabling rats to experience thigmotaxis, which is consistent with their natural needs [Devan *et al*. 1999, Stryjek and Pisula 2008]. Also, the animals were placed in the above-mentioned apparatus under darkened conditions to reduce stress.

Statistical analysis

The Open Field and The Elevated Plus Maze data (superficial variables) were subjected to Exploratory Factor Analysis (Principal Component Analysis employed as the method of extraction) to extract factors that cluster superficial variables into latent interrelated variables. To obtain final factorial solutions, we used the orthogonal Varimax rotation and the Kaiser-Meyer-Olkin test to check for sample adjustment. Data from the chamber for self-exposure to light-stimuli were analysed separately, as they were supposed to test for the low-anxiety environment. The results were analysed using three-way analysis of variance. The effect size was measured by Eta-squared ($\eta 2$). All variables were tested for normality with the use of standard tests available in the SPSS statistical analysis software [IBM SPSS Statistics, 2016] and subsequently tested for homogeneity variance using Levene's test. Non-parametric tests, i.e. the Kruskal-Wallis test and the Mann-Whitney U test were applied when normality and treatment homogeneity criteria were not met.

Results and discussin

Open Field and Elevated Plus Maze – Exploratory Factor Analysis

The behavioural categories (superficial variables) scored during the Open Field (OF) and Elevated Plus Maze (EPM) tests were subjected to Exploratory Factor Analysis yielding Principal Components (PC) that correlated at the stated level with each of the initial superficial variable entered to the model. Only superficial variables

with factorial loadings of 0.4 or more were included in further analysis.

PC1 extracted by means of our analysis included entries to the closed arm of the maze (EPM, bouts, factorial load 0.852), entries to the open arm of the maze (EPM, bouts, factorial load 0.845) and open field locomotion (OF, square entries bouts, factorial load 0.599).

PC2 extracted by means of our analysis included open field grooming (OF, bouts, factorial load 0.661), elevated-plus-maze grooming (EPM, bouts, factorial load 0.648), elevated-plus-maze defecation (EPM, number of faeces, factorial load 0.463) and urination in the open field (OF, number of urine drops, factorial load 0.439).

Based on literature we renamed the extracted PCs as the level of exploratory behaviour (explorability - PC1) and the level of emotional arousal (emotionality - PC2) [Fernandes *et al*.1999]. Subsequent analysis took into account individual scores counted for each animal with the use of the calculated factorial loads obtained for the respective superficial variables. Individual scores can take both negative and positive values. A negative value means that the specific animal scored below the average for all of the analysed animals, whereas a positive value means that the animal scored above the average for all of the tested animals. Individual PC values (PC1 and PC 2) were analysed separately as dependent variables.

The following independent variables were included in the two respective models: genetic strain (Brown Norway, Wistar Albino Glaxo), social environment (isolation - stress, optimal group size (n = 4), overcrowding (n = 8) - stress) and illumination stress (present, absent).

The analysis of variance for explorability showed a statistically significant main effect of strain $p<0.001$; $\eta^2=0.32$, with Brown Norway rats showing a lower level of explorability: mean(M)=-0.540; SD=0.768) when compared to Wistar Albino Glaxo rats (M=0.540; SD=0.915) (Fig. 2a).

The analysis of variance showed also a near significant trend for the main effect of social environment, $p=0.054$; $\eta^2=0.07$, with isolated rats showing lower explorability (M=-0.261; SD=1.075) than the ones maintained under standard conditions (M=0.255; SD=0.937) (Bonferroni post-hoc test; p=0.049). Isolated rats did not show significantly different values of explorability than the ones observed among animals maintained under over-crowding conditions (M=0.006; SD=0.946) (see Fig. 2b).

Emotionality data showed non-equal variance distribution among the experimental groups when assessed with Levene's test, thus we used non-parametric tests.

We examined the impact of the social situation separately for each of the strains. The social situation significantly affects emotionality only in the group of WAG rats, p=0.002 (Fig. 3a), while it has no effect in the BN group p=0.137 (Fig. 3b). The rats from the standard group scored higher in terms of emotionality (M=0.890; SD=1.254) and differed significantly from both isolated, p=0.002, and over-crowded rats p=0.002. The over-crowded and isolated WAG rats did not differ in terms of their levels of emotionality, p=0.734.

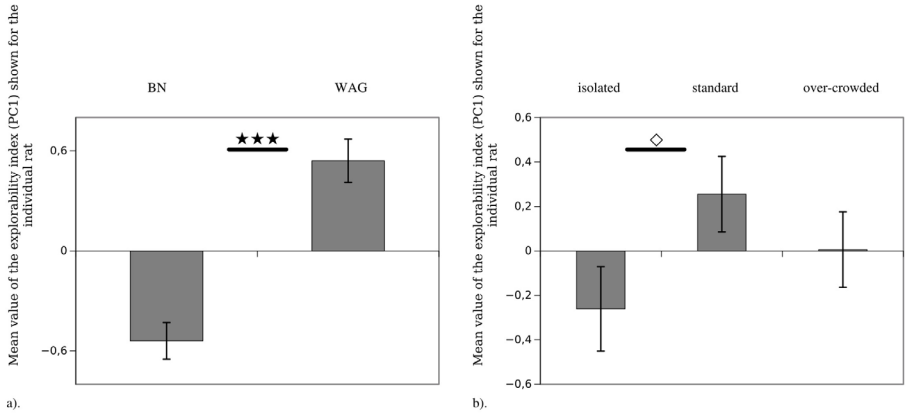

Fig. 2. Explorability index (PC1) for the rats of both strains tested under high-anxiety conditions: a) explorability differs between the two tested strains of rats ($p<0.001$; BN vs. WAG; $p<0.001$); b) mean level of explorability shows near significant trend for the social environment as the main effect ($p=0.054$; social isolation vs. standard conditions; Bonferroni post-hoc $p=0.049$). Means and the standard error of the mean are shown. Empty diamond - near significant trend, three full stars - $p<0.001$

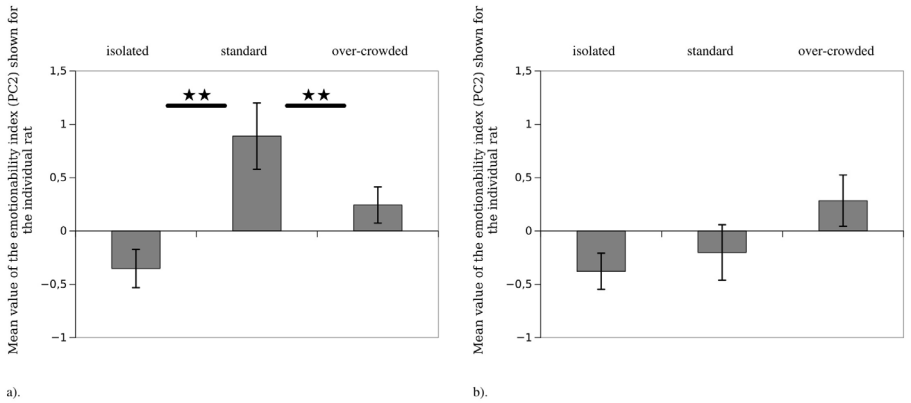

Fig. 3. Emotionality index (PC2) for rats of both strains tested under high-anxiety conditions: a) mean level of emotionality differs significantly between different social environment groups only among WAG rats (Kruskall-Wallis test $p=0.002$; WAG rats: I<S and OV<S; b) mean level of emotional arousal is not affected by social environment among BN rats (Kruskall-Wallis test $p=0.137$). I: isolation, S: standard conditions, OV: over-crowding; post-hoc U Mann-Whitney test, $p=0.002$). Means and standard error of the mean were shown. Two full stars - $p<0.01$.

Chamber for self-exposure to light-stimuli

Stimulability. Stimulability (S) in the present study is the measure of the animal's internal need for light stimulation. The S indices collected during all our experiments were subjected to three-way analysis of variance with the same independent variables as in the case of the Exploratory Factor Analysis (namely strain, social environment and presence of stress). The analysis revealed only a significant effect of the illumination

stressor ($p=0.048$; $\eta^2=0.04$). Stressed rats displayed lower mean stimulability (M=0.562; SD= 0.169) than non-stressed rats (M=0.632; SD=0.163) (Fig. 4a).

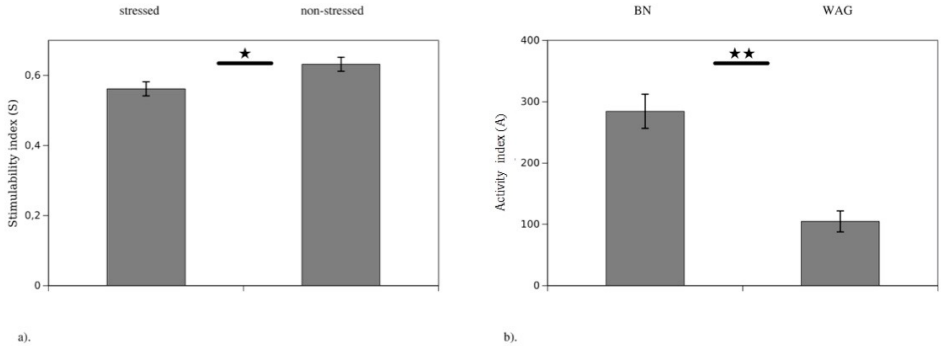

Fig. 4. Stimulability and activity indices show respectively the animal's need for light stimulation (emotional state) and explorative behaviour shown under low-anxiety conditions tested in a chamber for self-exposure to light stimuli: a) the stimulability index (S) differs among stressed versus non-stressed animals subjected vs. not subjected to stroboscopic light (24 h of strobe light) ($p=0.048$); b) activity shows significant differences among the two rat strains tested ($p<0.005$, BN<WAG). Means and the standard error of the mean are shown. One full star - $p<0.05$, two full stars - $p<0.01$.

Activity. The activity data set did not meet the normality criteria, thus we analysed it with the use of the non-parametric tests. The between strain and stressed versus non-stressed comparisons were performed with the use of the Mann-Whitney U test, whereas differences between the three different social environment conditions (isolation, standard and over-crowding) were compared with the use of the Kruskal-Wallis test. Only inter-strain differences proved to be significant ($p<0.005$), with BN rats showing lesser activity in the chamber for self-exposure to light-stimuli (M=104.735; SD=118.768) than WAG rats (M=284.417; SD=193.238) (Fig. 4b).

Role of genetic factors

A vast majority of research on laboratory animal welfare is conducted on mice and rats. However, few experiments examined inter-strain differences in this context. Also the predominant body of research focuses on albino rat strains that can be characterized by impaired sensory, cognitive and locomotor abilities [Hupfeld and Hoffman 2006, Prusky *et al*. 2002, Widshaw *et al*. 2003]. Albino rats exhibit lower propensity for emotional arousal and thus are thought to be easier to maintain under laboratory conditions than pigmented strains [Tyl-Bielecka, 2008].

Brown Norway rats explored less in high anxiety conditions (OF+EPM) and displayed lower activity in low anxiety conditions, thus their general propensity for exploration was lower than in the case of WAG rats in either of the anxiety environments. Thus our results seem to contradict to some extent another study, in which WAG rats during a similar battery of tests displayed lower explorability and activity than the three tested pigmented strains, namely DA/Han (agouti), Long Evans

(black hooded) and August (dilute hooded) [Ostaszewski and Pisula 1994]. Thus it seems that the array of exploratory behaviour shown by BN rats may be much different from the one shown by the other pigmented strains. Also, it is noteworthy that the above-mentioned study included both sexes and explorability shown in the OF test proved to be highly sex-dependent, especially in WAG rats with male WAG rats scoring as second among all of the groups tested for locomotion in the OF test.

Inbred Wistar male rats showed also higher locomotion in the OF in another study [Ostaszewski *et al*. 1992] when compared to BN rats, which proves that BN rats may generally display lower propensity for exploration than both male WAG rats and other male pigmented strains. In a different study [Tyl-Bielecka 2008] BN rats showed higher general activity in EPM test than WAG and August rats, at lower exploration in the OF test. In our study, however, the EPM and OF scores were subjected to Factorial Analysis, so the exact comparison as for the differences between BN rats and the other two strains tested cannot be compared in the one-to-one measure. Also, the design of the OF apparatuses in the two studies was different. In the study by Tyl-Bielecka BN rats tended to jump out of the OF apparatus often, which might have altered the exploration results for that strain. This in turn sheds light on the importance of test standardisation among different studies, which could potentially account for most of the erroneous "false" differences introduced. The influence of different laboratory conditions can also be meaningful, as it may be a source of variability in results between studies [Chesler *et al.* 2002, Lyte *et al*. 2005].

It needs to be stressed that both activity and explorability are defined differently in various studies. Higher activity may account for higher curiousness of the animal towards novelty, but may also correspond to fear and lead to attempts to escape from the unfriendly environment [Matysiak, 1993, Commissaris *et al*. 2000]. Both cases are linked to exploratory behaviour; however, the emotional basis is different here. The brief analysis of the single superficial variables relating to the two above-mentioned different aspects of exploratory behaviour sheds more light on the phenomena discussed. As expected, WAG rats show higher locomotion in the OF test than BN rats; however, other behaviours concerning exploration such as rearing, rearing against apparatus walls and jumps show an opposite pattern. WAG rats score lower at these behaviours. What is worth stressing here, jumping behaviour is entirely absent in WAG rats (Tab. 1a).

The examples reported above prove that exploratory behaviours in the case of WAG rats contain more of the locomotory component, whereas in the case of BN rats exploration is rather related to the escape component related to aversive behaviours. Also, BN rats showed more rearing and maintained rearing behaviour than WAG rats during the EPM test, whereas WAG rats showed more locomotory activity (Tab. 1b).

Under low anxiety conditions WAG rats showed more activity than BN rats (compare Tab. 2), which additionally highlights a more aversive role of exploratory behaviour in the case of BN rats that showed more of the exploratory behaviour only under high anxiety conditions and only in the case of behaviours that were in fact attempts to escape rather than curiosity-driven exploratory behaviour.

Table 1a. Basic behaviours (mean and standard deviation - SD) scored during the Open Field test

Behaviour	Strain	Mean	SD
Urination (drop count)	BN	5.80	5.12
	WAG	3.60	3.42
Defecation (faeces count)	BN	3.81	1.70
	WAG	1.32	1.93
Locomotion (square entries bouts)	BN	48.94	17.82
	WAG	62.46	21.20
Jumps (bouts)	BN	0.21	0.50
	WAG	0	0
Grooming (bouts)	BN	0.30	0.65
	WAG	0.67	0.86
Freezing (bouts)	BN	0.50	0.85
	WAG	0.79	1.03
Rearing (bouts)	BN	3.55	2.24
	WAG	1.58	1.77
Rearing against apparatus walls (bouts)	BN	10.58	3.85
	WAG	8.23	3.15

Table 1b. Basic behaviours (mean and standard deviation- SD) scored during the Elevated Plus Maze test

Behaviour	Strain	Mean	SD
Urination (drop count)	BN	4.92	4.04
	WAG	7.42	5.24
Defecation (faeces count)	BN	1.67	1.76
	WAG	1.33	1.81
Rearing (bouts)	BN	1.60	1.85
	WAG	0.58	1.05
Rearing against apparatus walls (bouts)	BN	6.76	3.20
	WAG	4.29	2.53
Grooming (bouts)	BN	0.09	0.28
	WAG	0.23	0.56
Open arm falls (bouts)	BN	0.28	0.49
	WAG	0.33	0.48
Open arm entries (bouts)	BN	0.76	0.55
	WAG	1.79	1.07
Closed arms entries (bouts)	BN	1.30	0.58
	WAG	1.83	0.86

Stroboscopic illumination should in theory affect more albino rats, as they are more vulnerable to light [Kaliste and Merling, 2007]. However, no interaction between strain and illumination stressor effects was observed in this study. As we can see, classification of rat strains in terms of their pigmentation as far as vulnerability to light is concerned might be far-fetched. Also, it can be drawn from literature that within both pigmented and albino strains there exists huge variability in terms of

Table 2. Basic behaviours (mean and standard deviation - SD) and indicators scored during the chamber for self-exposure to light stimuli test

Behaviour	Strain	Mean	SD
Stimulability (index)	BN	0.61	0.19
	WAG	0.59	0.15
Inspection of inactive hole (count)	BN	5.68	2.99
	WAG	12.67	5.85
Inspection of active hole (count)	BN	11.08	9.68
	WAG	19.71	11.59
Activity (index)	BN	104.74	118.77
	WAG	284.42	193.24
Active vs. inactive hole alternation (count)	BN	4.28	2.53
	WAG	7.06	2.83

vulnerability to physical stressors, behavioural characteristics and need for tactile-kinetic stimulation [Ostaszewski and Pisula 1994].

Role of long-term social stressor

Both isolation and over-crowding are supposed to provide long-term social stress for animals maintained under laboratory conditions [Honga *et al*. 2012, Panksepp and Lahvis 2007]. Interestingly, over-crowding is often avoided under current laboratory regulations, which stems from current EU legislation. Nonetheless, isolation is often applied during experimental procedures, as it enables strict body mass control and prevents aggression [Van Loo *et al*. 2002, Reinhardt and Reinhardt 2001]. Individual caging is actually in some cases recommended when working with especially aggressive strains [Committee on Infectious Diseases of Mice and Rats 1991, Mouse Genome Database 1998].

In our study we observed the influence of the social situation on exploration behaviours. Isolated animals differed only from animals kept under standard conditions, and they explored less than them. This result was in line with expectations [e.g. Arakawa 2005]. However, we observed no differences between standard and over-crowding conditions. Perhaps the effect of over-crowding was not found, because over-crowding was insufficient. Botelho *et a*l. [2007] showed that only extreme over-crowding ranging from 16 to 24 animals caged in conditions recommended for 6 animals results in an altered pattern of behaviour during an EPM test. The animals featured in the mentioned study explored open arms of the maze less often upon extreme over-crowding.

According to other authors [Honga *et al*. 2012, Panksepp and Lahvis 2007], social isolation is an extremely aversive experience for rats. In turn, the opportunity to maintain social contact is highly rewarding and brings welfare benefits. Some authors claim that a bigger group size is more rewarding than a large cage area, constituting a more important factor for the animal well-being under laboratory conditions than the latter one [Lawlor 2002]. Also, there is a report claiming that group familiarity is

the key factor for the rat well-being in laboratory rather than group size and cage area [Patterson-Kane 2002].

It is worth stressing that isolation in the present study was in fact not absolute. It should rather be referred to as individual housing, as denoted by Van Loo *et al.* [2003]. Even in such a set-up, the ability of visual, olfactory and auditory contact with other animals is still provided. Thus isolation in this case means predominantly depriving of the tactile stimulation coming from conspecifics. Some studies point out the increased effect of partial versus complete isolation in boosting welfare of rats maintained in captivity [Hawkins *et al.* 2011].

It is interesting that extreme social situations do not seem to affect emotionality within one strain, while their influence is revealed in the case of another strain. It should not come as a surprise if we take into account findings in the field of behavioural genetics. Our results show rather that one should be particularly cautious in generalizing the conclusions from a study of one strain to other strains or rats in general. As we analysed emotionality, we found a significant impact of the social environment for the WAG strain, while emotional arousal did not prove to be significantly affected by the social environment in the case of BN rats. Instead, BN rats seem to be emotionally affected neither by isolation, nor over-crowding conditions, which were supposed to act as social stressors. Within the WAG strain, isolated and over-crowded animals had lower emotionality than animals kept under standard conditions, which is surprising.

Most studies report higher emotional arousal in isolated animals versus non-isolated ones, which seemingly contradicts our results in the WAG strain [Kaliste and Mering 2007]. This observation may be explained by the study on the effect of within-cage order of testing animals [Lyte *et al*. 2005]. The animals in our study did not return to their companions from the home cage, so the emotional contagion from the already tested animals can be excluded. However, it is possible that the removal of individual animals from the cage influenced the initial level of arousal of animals in behavioural tests in individual groups. Isolated animals were always first to be taken from cages and they were tested probably immediately after waking up (tests were performed during the day, when rats usually sleep), which could explain the low level of stimulation of isolated WAGs. In the case of animals kept in the group, the arousal may depend on the number of animals in the cage. After taking the first cage mate, the emotional arousal of the remaining rats increases. However, with each subsequent rat taken, the other animals habituate to the researcher's activity and their arousal becomes low again. This would also explain the fact that in our study WAG rats from the over-crowded group (i.e. from the cages with the largest number of animals – twice as high as in the standard group) also had a lower level of emotionality than in the standard group. Therefore the effect we observed in WAGs is perhaps not so much related to the welfare of the animals, as to the procedures immediately preceding the testing. As indicated by Chesler *et al.* [2002], there are strains of mice that are particularly susceptible to the effect of sequence, and perhaps among rats WAG is such a strain. Although in most studies the factor related to the effect of within-cage

order of testing is routinely considered neutral, it seems advisable to consider it also in the context of our emotionality results.

Role of illumination stressor

Research shows that short-term environmental stressors have a relatively low impact on long-term behaviour and only long-term and/or repetitive stress deteriorates the animal's welfare [Broom 1997]. Our research seems to confirm these observations. If the light stressor used affected the behaviour of animals, it would be revealed in the behavioural tests that were used. However, the effect of using strobe light was observed only in the case of stimulability. The animals subjected to 24 hours of stroboscopic light illumination showed a lower need for light stimulation. This could potentially arise from the over-stimulation effect, as the stressor and the stimulus used in the test apparatus were of the same type. This explanation is consistent with the hypothesis, which follows from the classical drive theory [Hebb 1955] that the hyper-stimulated group shows a lower stimulating light activity. Stroboscopic illumination was supposed to be a short-term environmental stressor. It seems sensible to use a different type of stressor in order to answer the question whether a low level of stimulability recorded in the test in the chamber for self-exposure to light-stimuli results from overstimulating, or stressing, the animals.

Conclusions

The short-term illumination stressor affects rat behaviour in a negligible way. The social environment affects rat behaviour in a crucial way; however, its role is revealed only under high anxiety conditions. The group composition factor affects rat behaviour; however, the effect of its operation may be strain-dependent. The strain of rats selected for this study has a strong effect on animal welfare and behaviour under both high and low anxiety conditions.

***Acknowledgements**. We would like to express our deep gratitude to Professor Jan Matysiak for his valuable and constructive suggestions during this study*.

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