Non-invasively Assessing Disturbance and Stress in Laboratory Rats by Scoring Chromodacryorrhoea

Georgia Mason,[1] David Wilson,[1] Charlotte Hampton[1] and Hanno Würbel[2]

[1]Oxford University, UK; [2]University of Giessen, Germany

Summary — In rats, like many rodents, Harderian glands next to the orbits secrete porphyrins, lipids and other compounds. High levels of secretion lead to chromodacryorrhoea (red or "bloody" tears), often taken as a sign of stress or disease. Here, we developed a scoring system for recording chromodacryorrhoea in a quantitative way, and investigated whether the low-level, transient Harderian secretions of normal, healthy rats correlate with low to moderate levels of stress or disturbance. Rather than exposing our subjects (24 Lister Hoodeds, housed in 11 single-sex cages) experimentally to stressors, we made opportunistic use of three likely sources of low-level stress within the unit: 1) building maintenance work, taking several hours and involving several potential stressors; 2) visits by unfamiliar humans, and the other mild sources of disturbance normal in an animal unit; and 3) social status within the cage. The mean daily chromodacryorrhoea score increased most with the severe disturbance of building maintenance work ($F_{1,9} = 602.67$, $p << 0.0001$), and also increased — though to a lesser extent — with the mild disturbance of visitors and similar ($F_{1,9} = 8.77$, $p = 0.008$), while being the subordinate member of a cage-group had a smaller effect still ($F_{1,6} = 7.86$, $p = 0.03$). Individual rats scored consistently across treatment conditions, and there was also significant inter-observer reliability between independent scorers. We therefore suggest that scoring chromodacryorrhoea could be a simple, practical and non-invasive way of sensitively assessing the impact on rats of housing, husbandry, or procedures.

Key words: *bloody tears, chromodacryorrhoea, dominance, non-invasive, rat, red tears, social status, stress, welfare.*

Address for correspondence: *G. Mason, Department of Zoology, Oxford University, South Parks Road, Oxford OX1 3PS, UK.*
E-mail: *georgia.mason@zoology.oxford.ac.uk*

Introduction

Refining the husbandry and use of laboratory animals requires usable, well-validated methods for assessing stress and distress. Non-invasive techniques are advantageous, generally being less aversive than invasive methods, and usually requiring no licensing or other form of monitoring (Gimpel & Mason, submitted). Methods that require little technical expertise or equipment are also particularly valuable, especially if cheap and/or readily used by animal care personnel (1). The aim of the experiments presented here was therefore to evaluate the validity and practical usefulness of a novel, potentially simple method of non-invasively assessing disturbance and stress in laboratory rats: the quantitative scoring of chromodacryorrhoea (red or "bloody" tears). Chromodacryorrhoea is the term for an overflow of reddish secretions from the Harderian gland, a secretary body medial to and behind each orbit. In rats, these secretions can stain the fur around each eye, or appear around the nose after reaching the nostrils via the lacrimal ducts (2, 3). The Harderian gland is widespread in vertebrates, and is well-developed in most rodent species (4, 5). Here, its secretary activity is primarily under parasympathetic, cholinergic control (4, 6), but the gland is also affected by endocrine factors, including a range of gonadal, thyroid and pituitary hormones (4), and possibly also adrenaline (7, though see 3). The gland's secretory products contain lipids, indoles such as melatonin, and, in rodents, porphyrins, responsible for the dark red colour (3–5, 8). On release, secretions are typically spread over the fur of the rodent's face and other parts of the body via grooming (9, 10). They have a very diverse array of functions, ranging from thermoregulation in Mongolian gerbils (4), to aggression-inhibitors in blind mole-rats (11, 12), although their functions in the Norway rat itself seem not to have been investigated.

Conspicuous chromodacryorrhoea has long been used as a qualitative sign of stress and/or respiratory disease (2, 3). Stress causes the rapid production and/or release of Harderian compounds — one study showed that this occurred within, on average, 16 minutes of limb restraint (13) — and in long-term conditions, the build-up may be exacerbated by reduced levels of grooming (V. Baumans, personal communication). The result can be areas of secretion several millimetres across, which dramatically alter the animal's appearance, often by ringing the eye with red (2, 14, 15). Such chromodacryorrhoea appears, for example, in rat models of arthritis, alongside rises in plasma corticosteroid levels and

decreases in body weight (16); in rats withdrawing from morphine (17); and in rats deprived of REM sleep (18). It is also a common sign of poisoning in toxicity studies (19), as well as appearing in less severe, less chronic situations such as fright (14). In studies of cholinergic functioning, chromodacryorrhoea has been scored quantitatively, on a rating scale (6).

Our interests here were twofold: 1) could a similar scoring system be developed for stress-related secretion? 2) Do inconspicuous manifestations of chromodacryorrhoea similarly convey information about welfare? We were alerted to this possibility by observations that several rats in our unit showed very small, transient spots of dark red secretion around their nostrils, approximately 1mm across, after the timer controlling their room's light cycle had malfunctioned for a day.

Our research was not conducted under a Home Office project licence, so we were unable to experimentally stress our animals. However, we could opportunistically make use of three likely sources of low-level stress within the unit:

1. the performance of maintenance work within our subjects' room;

2. other sources of potential disturbance, such as visits to the unit by unfamiliar humans; and

3. social ranking within each cage.

Experiment 1: Can Chromodacryorrhoea be Quantitatively Scored, and Does It Significantly Increase in Response to a Disturbing Event?

Methods

The subjects for this experiment were 24 young adult (*c.* 6 months old), healthy Lister Hooded rats of both sexes. They were housed in single-sex pairs or triplets in 11 cages for the purpose of another research project. These cages were large — 0.6m (W) × 0.6m (L) × 0.3m (H) — and enriched, with solid plastic walls, a wire mesh lid, and a wire mesh window at the front. Cagemates were identified and distinguished by their throat and tail markings. All the rats had been born within the unit, which had only previously housed their mothers — females brought in pregnant from a disease-free commercial barrier unit. Three subjects were also health-screened at the end of the three experiments, to confirm their disease-free status.

The rats were observed every day for 44 days, always during the light phase, and typically during the morning. Data were collected only once each animal had approached the front mesh window, so that it could be seen clearly. Any chromodacryorrhoea was scored on a five-point scale, as illustrated in Table 1. The scoring system was primarily based on the secretions visible on noses, because only one rat was ever observed with chromodacryorrhoea around an eye. At the end of the six weeks, planned maintenance work was conducted in the unit. This occurred on one day, between 1400 and 1630, and involved: an unfamiliar human visitor to the unit; the rats' cages being moved into a neighbouring room; power-tool use and other noisy maintenance work in the animals' original room, audible from their new location; being carried back to the unit; and one brief use of an electric drill within the unit itself (which precipitated a panicked flight response from the nearest rats). At 1800, the rats were scored for chromodacryorrhoea. All the data were then converted into mean scores/cage/day, for both baseline and treatment conditions (cagemates' values were meaned as cagemates were not statistically independent). They were analysed with a General Linear Model (Minitab 13.1) of the form Score = cage (sex) + sex + treatment + sex*treatment, with cage as a random factor, and chromodacryorrhoea scores logged due to heterogeneity of variance. Individual consistency in baseline and disturbance scores was assessed with the model: Disturbance score = baseline score + sex, with baseline score being a covariate.

Results

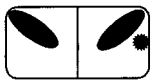
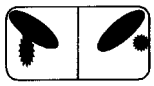
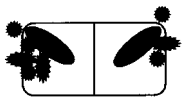
Chromodacryorrhoea was successfully quantitatively scored and showed a highly significant treatment effect ($F_{1,9} = 602.67$, $p << 0.0001$; see Table 2), scores being much higher on the disturbance day than during baseline conditions. There was no sex difference, even though Harderian glands are heavier in male rats than in female rats (20).

The observed chromodacryorrhoea was always transient — during the baseline scoring, even the rare animal scoring a "4" (see Table 1) would typically be completely clear by the next day, and indeed sometimes the secretions disappeared within a matter of hours. Rats showed individual consistency, animals with relatively high baseline scores also showing the highest scores in the treatment condition ($F_{1,8} = 13.74$, $p = 0.006$).

Discussion

This experiment indicates that low-level, generally inconspicuous chromodacryorrhoea can be quantitatively scored. It also tracked environmental changes, increasing in response to a presumably stressful event, although to far lower levels than the red tears and stained muzzles typical of more-severe and chronic stressors or illness. (The mean score after the treatment was still less than

Table 1: A scoring system for quantifying chromodacryorrhoea

Score	Description	Example of appearance of nose (diagrammatic)
0	No chromodacryorrhoea	
1	Very slight, inconspicuous chromodacryorrhoea, e.g. one small spot < 1mm across (often just lateral to a nostril), only visible if rat pushes nose out between the cage bars	
2	Slight, rather inconspicuous chromodacryorrhoea, e.g. one spot *c.* 1mm across, or several spots < 1mm across, only visible if rat pushes nose out between the cage bars	
3	Noticeable chromodacryorrhoea, e.g. one spot *c.* 2mm across, or several spots *c.* 1mm across, often around nostrils and surrounding fur	
4	Relatively severe chromodacryorrhoea, e.g. several patches 2–3mm across or more, around nostrils and on surrounding fur, and visible even in an animal at the back of the cage. Rare secretions around eyes were also given this score	

The very conspicuous chromodacryorrhoea typically used as a qualitative sign of stress, illness or intoxication (see 2, 14, 15) would score approximately 10–12 on this scale.

a "2"; see Table 1.) We should emphasise that the subjects were healthy; and that the occurrences of chromodacryorrhoea were generally very transient, as well as quite subtle. Thus baseline levels of chromodacryorrhoea were, happily, very low in the unit: the median and modal scores were zero, and the mean was equivalent to just one score of "very slight chromodacryorrhoea" per rat (see Table 1) every five days. We are thus confident that we were recording the responses of normal animals. Furthermore, the transient nature of the secretions suggested that they were tracking external events in a sensitive manner. This sensitivity could, perhaps, explain why they showed a response to the disturbing event, while urinary corticosteroids did not (G. Mason, unpublished data).

Overall, the data thus suggested that close inspection for Harderian secretions might be a useful way of monitoring rat welfare. However, many aspects of this opportunistic experiment were not ideal: baseline and treatment scores were obtained at different times of the day; there was a potential for order effects, due to lack of a second, post-disturbance baseline; and data were not collected blind, as the scorer on the disturbance day — GM — knew the treatment the rats were exposed to, and also the hypothesis under test. A second experiment was therefore conducted.

Table 2: The chromodacryorrhoea scores of rats on standard, non-experimental days (n = 44) in the colony, compared with scores on a day when moved to a new room and exposed to both unfamiliar humans and the sounds of building maintenance work

Sex (no. of cages)	Standard day (mean score/day ± SD)	Severe disturbance day (mean score/day ± SD)
F (n = 6)	0.19 ± 0.06	1.47 ± 0.62
M (n = 5)	0.22 ± 0.07	1.33 ± 0.61

*These are raw data, broken down by sex for interest, but note that there was no significant sex or sex*treatment effect, only a significant main effect for treatment (on logged data, to correct the non-homogeneous variances).*

Experiment 2: Does Chromodacryorrhoea Respond to Mild Disturbance?

Methods

After a break of two weeks, the subjects continued to be scored every day as in Experiment 1, for 116 data collection days. Previously, day-to-day variation had been noted during the baseline scoring period, and this experiment now capitalised on and sought to explain this variation. Each day, any events that might disturb the animals were noted, namely: unfamiliar visitors to the unit; maintenance work conducted outside the building; evidence of fighting within any of the cages in the unit; and, on one occasion, signs that a football had been kicked onto the unit roof from a neighbouring playing field. At the end of the experiment, each day was categorised as to whether or not such an event had happened in the 24 hours before chromodacryorrhoea-scoring. Disturbance days totalled 28; undisturbed days totalled 88. Daily means per cage were then calculated as before, for each of the two conditions, and statistical analyses were also as before. Disturbance scores from this dataset were then also compared with those from Experiment 1, by using the model: Mean score = experiment + sex + experiment*sex.

Note that, in Experiment 2, scoring was largely blind with respect to treatment, in that the scorer collecting data on 75% of the days (DW) was unaware of the hypothesis under test; GM, who collected data on the remaining 25% of the days, was aware of it.

Results

There was a significant treatment effect ($F_{1,9} = 8.77$, $p = 0.008$; see Table 3), although the increases observed were much smaller in magnitude than those seen in Experiment 1 ($F_{1,9} = 48.29$, $p < 0.0001$). Thus, scores were higher on days in which a "disturbing event" had happened in the previous 24 hours. Again, there was no sex difference in the mean chromodacryorrhoea score; and there was a correlation between scores on undisturbed and disturbed days ($F_{1,8} = 5.94$, $p = 0.041$), showing individual consistency in rats' levels of chromodacryorrhoea.

Discussion

Chromodacryorrhoea scoring, now conducted semi-blind and always at approximately the same time each day, confirmed that low-level transient Harderian secretions occur in response to disturbance. Compared with the results from Experiment 1, they also indicate that mild disturbance leads to a smaller response than does severe disturbance. An additional point of note is that the overall mean values (pooling all days' observations regardless of what was happening in the unit) were, at *c.* 0.25/rat/cage/day, slightly higher than the baseline scores recorded in Experiment 1, perhaps because the rats were now older (glandular porphyrin and Harderian cholinergic responsiveness increases with age: 6; 21). As in the previous experiment, however, individual responses were still consistent between disturbed and undisturbed conditions, some rats always showing more chromodacryorrhoea than others.

Over thirty years ago, Lane-Petter & Pearson (22) suggested that the regular recording of chromodacryorrhoea would be a useful way to monitor health and the adequacy of husbandry, and perhaps to anticipate more severe problems like disease. Our results suggest that this idea was a good one, as chromodacryorrhoea seems to sensitively reveal the rats' responses, even to the types of disturbance typical in any animal unit. However, this experiment is still open to the criticism that it was not conducted completely blind. Thus, one further analysis was performed to investigate the relationship between social status within the cage and the mean chromodacryorrhoea score. Social status does not have a simple relationship with stress (23, 24), but in general, if it relates to stress at all, subordi-

Table 3: The mean daily chromodacryorrhoea scores of rats in their home cage on apparently undisturbed days (n = 88) and on days when exposed to mild disturbances, such as unfamiliar human visitors (n = 28)

Sex (no. of cages)	Undisturbed day (mean score/day ± SD)	Disturbed days (mean score/day ± SD)
F (n = 6)	0.23 ± 0.11	0.38 ± 0.12
M (n = 5)	0.18 ± 0.09	0.48 ± 0.15

As for Table 2, these are untransformed data, broken down by sex solely for interest.

nates are the most affected. Thus in small, stable groups of rabbits, subordinates tend to have higher levels of plasma corticosteroids (25), while in single-sex groups of rats, subordinate individuals sleep less and spend more time performing the intention movements of escape (24). Subordinate status could therefore be another potential predictor of stress, and thence chromodacryorrhoea.

Experiment 3: Do Chromodacryorrhoea Scores Vary with Social Status?

Methods

The dominance status of the rats was assessed for all subjects as part of another project, midway through the baseline scoring period of Experiment 1. These data were collected by one of us (CH) completely independently from the chromodacryorrhoea scores collected by GM and DW. Dominance was assessed via a food competition test (cf. 26, 27), conducted three times a day for six consecutive days. Each test involved offering three small items of food (a raisin or piece of sugar-coated breakfast cereal) one at a time, and observing which rat obtained it. Within most cages (8/11), one animal clearly emerged as winning the treat in more than 80% of the tests, while another obtained it only rarely. Such animals were categorised as Dominant and Subordinate, respectively. In cages with three animals, two generally emerged with similar scores in the test, indicating shared dominance or subordinancy, and these rats' chromodacryorrhoea scores were meaned before analysis. The data were then analysed by general linear models, again treating the cage as a random effect.

Results

The analysis showed that, on any typical day in the unit, subordinate animals had slightly, but significantly, higher chromodacryorrhoea scores than their dominant cage-mates ($F_{1,6} = 7.86$, $p = 0.03$; see Table 4).

Discussion

A third potential source of stress, being subordinate, thus also predicted increased levels of Harderian output. Although the effect of size was smaller still than that seen in Experiments 1 and 2, these data are valuable, because no recording bias could have underlain this finding. They therefore validate the conclusions of Experiments 1 and 2.

If chromodacryorrhoea is a general response to all stressors, then this result could indicate that subordinates are indeed stressed by their status, their higher scores being a response to losing competitions. Alternatively, subordinate animals may be more generally stress-sensitive, and responding more to the external events in the unit (although no such effect could be seen statistically). A further possible interpretation is that rat chromodacryorrhoea is, as in mole-rats (12), a signal of appeasement, which then becomes generalised to a range of non-social aversive situations. There is, after all, considerable evidence that these secretions have social functions in other myomorphic rodents. As reviewed by Brown (28), the Harderian secretions of hamsters are sexually dimorphic, and in females, change in composition throughout the oestrous cycle, while those of gerbils are also involved in the signalling of sex, as well as being important for status. However, future research is needed to resolve these issues for rats.

One other issue still needing to be resolved for the purposes of this project was the reliability of the scoring system. Although the detection of subtle treatment effects, and the finding of individually consistent levels of responding, both argued for this, inter-observer reliability still needed to be measured formally. This was the objective of the final experiment.

Experiment 4: Is There Inter-observer Reliability in Chromodacryorrhoea Scoring?

Methods

A new population of 59 Lister Hoodeds was used for this study, containing both males and females, and

Table 4: The relationship between rat social status and mean chromodacryorrhoea scores on standard, non-experimental days in the colony

Sex (no. of cases in which there was a clear ranking within the cage)	Dominant (mean score/day ± SD)	Subordinate (mean score/day ± SD)
F (n = 5)	0.17 ± 0.06	0.23 ± 0.04
M (n = 3)	0.10 ± 0.08	0.33 ± 0.08

housed in 17 cages in groups varying between two and six in size. The new subjects were nine months old at the start of this study. Over a period of eight weeks, on 12 occasions, all 59 rats were scored twice within a 90-minute period, once by each of two independent scorers. One was always GM; the other was DW 10 times and a trained graduate student on the other two occasions. A mean score for each rat was obtained for the observer GM, and for the other observers' pooled values, and the relationship between the two sets of scores was tested with a regression.

Results

The mean daily scores for each rat as assessed by one observer correlated significantly with those assessed independently by another; $F1,57 = 45.22$, $p < 0.0001$, $R^2 = 0.43$.

Discussion

These data confirmed that when the scoring method was used independently by two trained observers, it yielded similar results.

Despite the high significance, the scores from one observer admittedly explained less than half the variance in the scores by the second observer. However, some informal comparing of results suggested that this was because within each 90-minute interval, rats' real levels of chromodacryorrhoea could actually change by the equivalent of a score or two. It was thus felt that the concordance between two observers would have reached much higher levels if the animals could always have been double-scored within a much shorter time interval. Again informally, it was also noted that, perhaps unsurprisingly, observers tended to score faster, and to agree with more certainty, when scores were 3 or 4, i.e. when any chromodacryorrhoea present was fairly conspicuous.

Conclusions

Severe, conspicuous forms of chromodacryorrhoea have long been used as qualitative signs of stress. Our data show that subtle, low-level, transient Harderian secretions in normal, healthy rats similarly convey information about stress and disturbance. Furthermore, they can be scored in a way that allows ready statistical analysis and that gives significant inter-observer reliability.

Clearly, some more work is now needed to more fully validate this approach. We need a more fundamental understanding of the control and the function of Harderian secretions in rats, and we need fuller data on the factors that elicit a response and those that do not to assess the specificity of its relationship with stress. The scoring methods could also, perhaps, be refined. For example, as long as they did prove aversive to the rats, ultra-violet "Wood's lamps" could possibly be used to increase the detectability of the Harderian secretions, as porphyrins fluoresce coral pink under long-wave UV (10, 29, 30). Overall, however, our results suggest that, even without this, chromodacryorrhoea scoring has real potential for non-invasive stress assessment in rats to monitor subtle changes in housing and husbandry as well as the impact of experimental procedures.

Acknowledgements

Thanks to: Naomi Latham for first noticing very low-level chromodacryorrhoea in the unit; Peter Thornton for identifying it and suggesting its links with stress; Charlotte Burn for helping collect inter-observer reliability data; and the BBSRC for funding.

References

1. Roughan, J.V. & Flecknell, P.A. (2003). Validation of a behaviour-based post-operative pain-scoring system in rats. Proceedings of the Association of Veterinary Anaesthetists Spring Meeting, Dublin, May 2002. *Journal of Veterinary Anaesthesia* **30**, 54.
2. Wolfensohn, S. & Lloyd, M. (1998). *Handbook of Laboratory Animal Management,* second edition, 344pp. Oxford, UK: Blackwell Scientific.
3. Anon. (date unspecified). Chromodacryorrhoea. Website http://www.labanimal.com/rel/rel.htm. *Lab Animal*: US (accessed 30.10.02).
4. Payne, A.P. (1994). The Harderian gland — a tercentennial review. *Journal of Anatomy* **185**, 1–49.
5. Buzzell, G.R. (1996). The Harderian gland: perspectives. *Microscopy Research and Technique* **34**, 2–5.
6. Espinola, E.B., Olieira, M.G.M. & Carlini, E.A. (1999). Differences in central and peripheral responses to oxotremorine in young and aged rats. *Pharmacology, Biochemistry and Behavior* **62**, 419–423.
7. Rubio, A., Guerrero, J.M., Gonzalez, M.A., Lopez-Gonzalez, M.A. & Osuna, C. (1991). Beta- and alpha-adrenergic receptors are involved in regulating type II thyroxine 5´-deiodinase activity in the rat Harderian gland. *Life Sciences* **49**, 1523–1530.
8. Djeridane, Y. & Touitou, Y. (2001). Chronic diazepam administration differentially affects melatonin secretion in rat pineal and Harderian glands. *Psychopharmacology* **154**, 403–407.
9. Shanas, U. & Terkel, J. (1995). Grooming expressed Harderian gland materials in the blind mole-rat. *Aggressive Behavior* **21**, 137–146.
10. Coulson, J.O & Pinter, A.J. (1996). The Harderian gland of the northern grasshopper mouse, *Onychomys leucogaster*. *Canadian Journal of Zoology* **74**, 1220–1228.

11. Shanas, U. & Terkel, J. (1996). Grooming secretions and seasonal adaptations in the blind mole-rat (*Spalax ehrenbergi*). *Physiology and Behavior* **60**, 653–656.
12. Shanas, U. & Terkel, J. (1997). Mole rat Harderian gland secretions inhibit aggression. *Animal Behaviour* **54**, 1255–1263.
13. Harkness, J.E. & Ridgeway, M.D. (1980). Chromodacryorrhoea in laboratory rats (*Rattus norvegicus*): etiologic considerations. *Laboratory Animal Science* **30**, 841–844.
14. Animal Health Center (2002). Miscellaneous conditions. Website http://www.caringtogether.com/exotics/micerats3.html Valdosta, US: Animal Health Center (accessed 30.10.02).
15. Porter, S.L. (2002). *Rattus norvegicus*. Website http://www1.br.cc.va.us/porter/vet217/rattus/tsld001.htm. Weyers Cave, US: Blue Ridge Community College (accessed 22.01.02).
16. Harper, R.P., Kerins, C.A., McIntosh, J.E., Spears, R. & Bellinger, L.L. (2001). Modulation of the inflammatory response in the rat TMJ with increasing doses of complete Freund's adjuvant. *Osteoarthritis Cartilage* **9**, 619–624.
17. Delle, M., Ricksten, S.E. & Thoren, P. (1988). Renal sympathetic activity during morphine abstinence in sino-aortic baro-receptor-denervated rats. *Acta Physiologica Scandanavica* **134**, 479–491.
18. Hipolide, D.C. & Sergio, T. (1995). Paradoxial sleep deprivation in female rats alters drug-induced behaviors. *Physiology and Behavior* **57**, 1139–1143.
19. Extonet (2001). Allethrin. Website http://pmep.cce.cornell.edu/profiles/extonet/24d-captan/allethrin-ext.html. New York, USA: Pesticide Management Education Programme, Cornell University (accessed 30.10.02).
20. Sashima, M., Hatakeyama, S., Satoh, M. & Suzuki, A. (1989). Harderianization is another sexual dimorphism of rat exorbital lacrimal gland. *Acta Anatomica* **135**, 303–306.
21. Rodriguez, C., Mernendez, P.A., Howes, K.A. & Reiter, R.J. (1992). Age and food restriction alter the porphyrin concentration and nRNA levels for 5-aminolevulinate synthase in rat Harderian gland. *Life Sciences* **51**, 1891–1897.
22. Lane-Petter, W. & Pearson, A.E.G. (1971). *The Laboratory Animal — Principles and Practice,* 293pp. London, UK: Academic Press.
23. Mendl, M. (2002). How do animals cope with social problems? In *Coping with Challenge*: *Welfare in Animals including Humans* (ed. D.M. Broom), pp. 211–218. Freie Universität Berlin, Berlin: Dahlem Workshop Reports.
24. Hurst, J.L., Barnard, C.J., Hare, R., Wheeldon, E.B. & West, C.D. (1996). Housing and welfare in laboratory rats: time-budgeting and pathophysiology in single-sex groups. *Animal Behaviour* **52**, 335–360.
25. Von Holst, D. (1998). The concept of stress and its relevance for animal behavior. *Advances in the Study of Behavior* **27**, 1–131.
26. Militzer, K. (1995). Social dominance and bodily condition in small groups of male and female laboratory rats of known familiarity. *Zeitschrift für Saugetierkunde* **60**, 97–111.
27. Boreman, J. & Price, E.O. (1972). Social dominance in wild and domestic Norway rats. *Animal Behaviour* **20**, 534–542.
28. Brown, R.E. (1985). The rodents II: Suborder Myomorpha. In *Social Odours in Mammals, Volume 1* (ed. R.E. Brown & D.W. Macdonald), pp. 345–457. Oxford, UK: Clarendon Press.
29. Williams, R. & Williams, C. (2002). Fluorescence photography. Website http://msp.rmit.edu.au/Article_02/. Melbourne, Australia: Royal Melbourne Institute of Technology (accessed 31.10.02).
30. Kamel, M.N. (1998). The Porphyrias. Website http://www.geocities.com/stantonios/mc/porphyria.html. Cairo, Egypt: MediCAD Multi media (accessed 31.10.02).