COMMUNICATION BETWEEN RATS OF EXPERIMENT-INDUCED STRESS AND ITS IMPACT ON EXPERIMENTAL RESULTS

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Abstract

Animals can communicate with one another through various types of signals. Evidence is presented that in certain experiments with rats, stress as induced by experimental treatment may give rise to the production of signals that affect non-treated animals housed nearby. Such communication between test and control animals may cause biased results and disturbed welfare of the latter. Communication of stress may be prevented by separate housing of control and test animals, but this could introduce another source of bias.

Keywords: *animal welfare, biased results, communication, rats, stress*

Animal welfare implications

In animal experiments, stress in test animals as induced by experimental treatment may be signalled to non-treated control animals. This could cause disturbed welfare in control animals, which may be prevented by housing control and test animals separately.

Introduction

It is well-known that animals communicate with one another through the production and decoding of auditory, olfactory, gustatory and visual signals. This communication plays an important role in the regulation and maintenance of social behaviours among conspecifics, such as sexual, fear and aggressive behaviour. Communication between animals in the absence of physical contact could be important from the point of view of the quality of animal experimentation and the welfare of laboratory animals. It is possible that in certain experiments, non-treated control animals can be influenced by communication with test animals in the same room. Signals from test to control animals may be triggered by discomfort stress caused by the experimental treatment. In this paper the evidence for intraspecific communication of stress and its consequences are discussed.

Theoretical considerations

The hypothetical effect on experimental results of communication between test and control animals is illustrated in the Figure. It is assumed that in the course of the experiment the animals do not become fully habituated to the stress related to experimental treatment, and that stress associated with the experimental treatment induces increased parameter values in test animals. The stressed test animals influence the control animals which leads to increased levels of stress and values of parameters in the latter. This in turn results in decreased differences between group mean values of parameters determined in control and test animals.

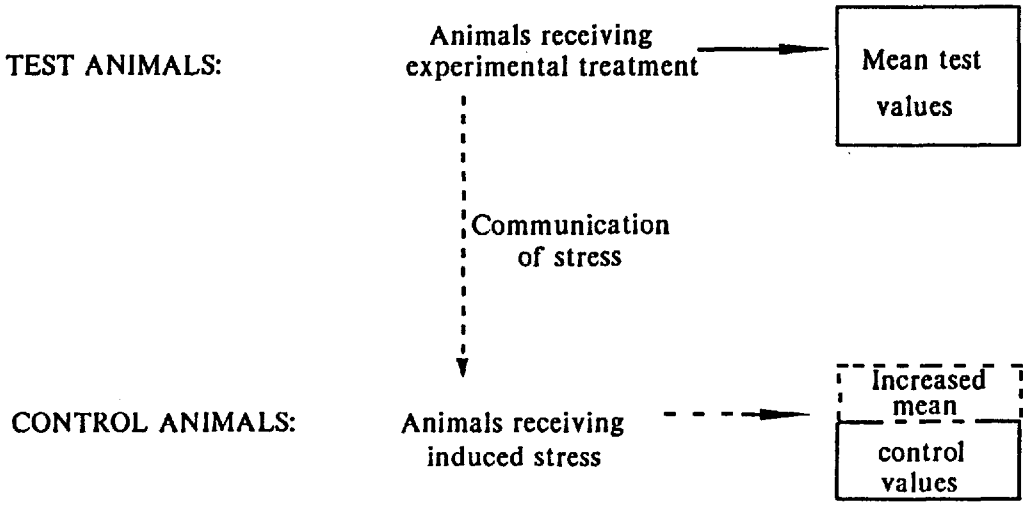

Figure Hypothetical effect of communication between test and control animals on group mean values of measured parameters. Communication may occur in the absence of physical contact. Communication of stress causes a decreased difference between group mean values of parameters measured in control and test animals.

Theoretically on the other hand, experimental stress could mediate a decreasing effect on parameter values which would result in increased differences between group mean values of parameters in control and test animals.

The following consequences of communication between test and control animals can be envisaged. First, the experimental data, ie differences between control and test animals, can be biased and therefore interpreted falsely. From a scientific point of view, this is undesirable. If experimental results contain systematic errors, this also implies that the results of a given experiment are often not comparable with those of other studies. This will increase the need to repeat experiments, which will not contribute to reduction of animal use. Obviously, the chances that this occurs will depend strongly on the nature of the measured parameters, especially their sensitivity to stress associated with experimental treatment.

Secondly, communication could cause decreased differences of group mean parameter values between test and control animals (Figure). Thirdly, it could be suggested that the variance of parameter values in control animals might be increased. In general, stress causes increased inter-individual variation. Both a negative bias and decreased precision of results will lower the statistical power. Thus, more animals are needed to obtain statistically significant differences between control and test animals.

Fourthly, it could be suggested that the communication of stress between test and control animals causes disturbed welfare of the latter. This would imply that non-treated animals in certain experiments experience discomfort because they receive signals that are emitted by stressed test animals.

It should be emphasized that the aforementioned consequences of communication between test and control animals are based on theoretical considerations. Below, I present evidence that communication does occur under certain conditions.

Intraspecific communication of stress

Iimori *et al* (1982) measured plasma corticosterone concentrations in rats that were exposed to other rats receiving electric foot shocks. For this purpose, a box was subdivided into various compartments with the use of transparent plastic walls. The floor consisted of grids through which a scrambled electric shock was delivered; in some compartments plastic plates were placed on the grids so that no shock was received.

There were three experimental groups of rats which had been habituated to the compartments of the box in the absence of electric shocks: control rats that were placed in the non-shock compartments while the shock compartments were empty; shocked rats that were placed in the shock compartments, and a 'psychological stress' group that was placed in the non-shock compartments at the same time that the shock rats were in the box. Immediately after the experimental treatment, all the rats were decapitated and trunk blood was collected.

Table 1 shows that foot-shock stress caused drastically increased concentrations of corticosterone in plasma. Rats of the psychological stress group showed corticosterone levels that were increased by 50 per cent when compared with the non-shocked controls. This suggests that the rats in the psychological stress group were influenced by the shocked rats. The shocked rats jumped, squeaked, defecated and urinated while being shocked. Thus the signals received by the so-called psychologically stressed animals could be of a visual, auditory or olfactory nature.

It should be mentioned that the corticosterone concentrations of the non-shocked controls might be biased because these animals were placed in the box while the shock compartments were empty. Ideally, the compartments should have contained rats that were not electrically stimulated. Thus, it cannot be excluded that the psychological stress group was influenced by the presence of the other rats *per se* rather than by their reactions to electric shocks.

Table 1 **Plasma corticosterone concentrations of male rats exposed to counterparts receiving electric foot shocks.**

Treatment	Plasma corticosterone (μg/dl)
Non-shocked controls	13.9 ± 3.8
Non-shocked 'psychological stress' group	21.1 ± 2.4
Foot-shocked rats	33.9 ± 1.1

Means ± SE; n = 8 (*after Iimori et al 1982*)

Table 2 **Plasma corticosterone concentrations of male rats placed in buckets and daily exposed to immobilized counterparts.**

	Experiment 1		Experiment 2		
Day	Bucket rats	Four limb prone restraint	Bucket rats	Tube restraint	Sampling controls
	Plasma corticosterone (μg/dl)				
1	37	37	18	22	4
3	25	30	4	21	4
5	35	35	6	19	3
7	26	30	4	20	3
14			6	23	3
21			4	20	3

Means for 6-12 rats per group (*after Pitman et al 1988*)

Table 3 **Influence of transportation stress in rats on sniffing behaviour of non-transported rats.**

Control pairs		Experimental pairs	
Non-transported	Non-transported	Non-transported	Transported
Sniffing frequency (times/5min)			
31.9 ± 5.9	31.1 ± 6.2	36.4 ± 5.8	29.4 ± 4.4

Means ± SD; n = 14 (*after De Laat et al 1989*)

Pitman *et al* (1988) measured plasma corticosterone concentrations in rats during repeated daily presentations of two intensities of restraint stress. In the first experiment, rats placed in large opaque plastic buckets with bedding material were exposed to rats restrained in a prone position by taping their outstretched limbs to a board. In the second experiment, bucket rats were exposed to rats placed in tubes with their forward and backward movement restricted by plastic partitions. After one hour of restraint, blood samples were taken by tail clip. After another hour of restraint, the restrained rats were released, and all rats were returned to their home cages. In experiment 2, there was a bucket control group not exposed to restrained counterparts. Table 2 summarizes the results, and shows that limb restraint caused somewhat higher plasma corticosterone concentrations than did tube restraint when compared with control rats. The corticosterone response in immobilized rats did not habituate. Likewise, bucket rats exposed to rats stressed by limb restraint in experiment 1 showed increased levels of corticosterone relative to control rats. The corticosterone levels remained stable during the course of the experiment. In contrast, the corticosterone concentrations in bucket rats exposed to tube restraint rats in experiment 2 habituated completely within three days. These results point to communication between rats of the intensity of stress. This conclusion should be qualified. Experiments 1 and 2 (Table 2) were not carried out concurrently, and thus direct comparison is not fully justified. Theoretically, it cannot be excluded that the bucket controls were stressed by novelty, or in other words, by the mere presence of the restrained rats. This possibility could be excluded only by showing that exposure of bucket rats to other bucket rats does not affect plasma corticosterone levels.

De Laat *et al* (1989) have studied the effect of transportation stress in rats on the behaviour of other, non-transported rats in the absence of physical contact. For this purpose an open box consisting of two adjacent small compartments was used. The compartments were separated by a perforated, transparent, plastic partition. A rat was placed in each compartment and its behaviour was assessed by scoring the following activities: ambulation, grooming, sniffing, rearing of forelegs, standing upright with elevated heels, defecation and urination. The control measurement involved pairs of non-transported rats and the experimental pairs consisted of a transported and a non-transported rat. The transported rat was put in a cage on top of a trolley which was pushed through the animal house for 2-3min.

Transported rats of the experimental pairs showed significantly decreased sniffing and rearing when compared with non-transported rats, whereas grooming was increased significantly. Behaviour of the two non-transported groups of the control pairs was almost identical. In contrast, the non-transported rats of the experimental pairs displayed significantly increased sniffing (Table 3) and tended to urinate more frequently when compared with rats of the control pairs. Thus this study suggests that transportation stress can be communicated from one rat to another. Possibly, transportation of rats causes the emission of odours which trigger increased sniffing in other rats. Mackay-Sim and Laing (1981) reported evidence that body odours released by rats upon electric shock induced increased frequencies of sniffing in rats receiving the odours.

Intraspecific communication and welfare

Rottman and Snowdon (1972) have provided evidence that mice demonstrated an aversion to the odour of mice stressed by intraperitoneal injection of hypertonic saline. These authors studied the response of recipient mice to the odour of stressed counterparts. The communicator mouse was placed in one of the two compartments of a chamber, the recipient mouse in the other, and an airstream was directed from the communicator's to the recipient's compartment. For 5min (300s) the airflow passed through the communicator's chamber into the recipient's chamber. The communicator was then treated as indicated in Table 4, and the movements of the recipient were recorded for a further period. The measure of aversion to an odour was the time spent by the recipient mouse on the output half of its compartment following a stress to the communicator, minus the time spent on the output side prior to any stressing of the communicator (see Table 4).

Table 4 Increased times spent by recipient mice on the side in the test compartment furthest from the air input whilst odour of stressed counterparts was passed for 300s.

Treatment of communicator	Mean increase in time spent away from air input relative to base-line measure (s)	Incidence of mice showing aversion responses on 100% of their trials
Handling	- 1.3	0/18
Insertion of empty needle in abdominal cavity	+ 9.2	3/31
Intraperitoneal injection of 1 M NaCl	+ 27.8	7/14

(after Rottman & Snowdon 1972)

Only the injection of hypertonic saline was clearly disruptive and painful. The mice squealed, hunched their backs, and groomed their abdomens vigorously for several minutes after injection. Exactly 1min after each treatment of the communicator, it was placed back in its chamber for another 5min. During this period the airflow again passed from the communicator's to the recipient's chamber. Table 4 shows that mice exposed to the odour of stressed conspecifics demonstrated an aversion response, that is they spent more time on the side furthest from the air input when the communicator was injected with hypertonic saline compared with the other treatments. This suggests that welfare of recipients was reduced upon receiving the odours of their stressed counterparts.

Conclusions

There is suggestive evidence that intraspecific communication of stress can occur in rats. Possibly, this may also take place among animals housed at different locations in the same room. This phenomenon requires further investigation as it might imply that in certain experiments control animals can be affected by stressed test animals. As outlined, this communication between test and control animals may cause biased results and disturbed welfare of control animals. Separate housing of control and test animals may be considered to prevent communication of stress. However, separate housing may introduce another source of bias.

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