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Stress in Laboratory Animal Studies: Preconceptions Misconceptions

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The purpose of this paper is to outline how some common laboratory procedures can effect what is commonly known as the animals' stress response. All too often we do not take into consideration the stressfulness of some of our experimental or *maintenance procedures*, or the animals' previous experiences with stress. We perform experiments on animals which have differed in their earlier experiences, or even whose mothers have differed markedly in this respect. These differences can have a marked effect on experimental data collected at a later time. As an experimental psychologist, I am only too aware of these problems, but it is my feeling that most animal experimenters tend to forget that the animal has a head, within which lies a brain, which retains memories and can be conditioned, and which influences all body systems. At least within animal behavior studies, it is my contention that a large proportion of the error variance arises from differences in animals' earlier experiences. Use of similarly treated animals will thus reduce this error variance, and thereby reduce the number of animals required in laboratory studies.

There are a number of preconceptions both within and without the laboratory world about what is and what is not stressful for the animal. For example, we know that subjecting an animal to electric tail- or foot-shock is stressful. It leads to marked increases in pituitary-adrenocortical activity, and if prolonged will give the animal stomach ulceration. What is less known is that the stressfulness of the shock depends not only on the physical parameters of the stimulus (mA), but also on the way in which the animal might perceive the shock. There is sufficient evidence to fill a whole volume that the animal which can predict, or even better, control shock, is by all measures less stressed than other animals given exactly the same shock, but which are unable to predict or control it (*Weiss*, 1968;*Weiss* 1970).

Of course, some might argue that we cannot measure "stress". These I would refer to a discussion by *Ursin* and *Murison* (1984). Generally it would seem reasonable to me to say that a stimulus or situation is stressful if it can eventually lead to pathology of some kind, behavioral or somatic. Since plasma corticosterone levels in rodents seem to correlate with this property, adrenocortical activity has been taken as a measure of the animals' stress response. We know that situations which lead to sustained high levels of adrenocortical activity also eventually lead to pathology. However, the animals' initial adrenocortical response is not indicative of what might happen later. Animals exposed to shock exhibit high corticosterone levels, but animals which are able to control (avoid/escape) the shock show lowering of this response to the shock situation on repeated exposures.

Another clearly stressfull situation is restraint or immobilisation, but this is a technique often used by non-psychologists for controlling the animals' movements during experimental manipulations. Restraint in itself is powerfully ulcerogenic, also giving high adrenocortical activation and lowering of brain norepinephrine. Researchers using this technique should be aware of what they are doing to the animal. It is not enough to assume that be-

cause the "control group" is also restrained that this is unimportant.

There are in all likelihood some nasty interactions between stress and the independent variable in any given experiment. As an example of this, let us take the relationship between earlier pre-shock experience and restraint-induced gastric pathology. Single-housed animals given pre-shock experience develop more pathology (ulceration) under later restraint stress than do animals without the pre-shock experience (*Murison & Isaksen*, 1980). However, housing variables, which are often described as stress variables, interact with this phenomenon. Group housed animals subjected to the *pre-shock procedures* show *less* gastric pathology under later restraint stress than do group housed animals not exposed to the pre-shock (*Murison & Isaksen*, 1981).

One of the preconceptions about stress in the laboratory has been that it is best for animals to be housed in an isolated room and only brought to the main laboratory area on the day of the experiment. In a recent study, we compared adrenocortical activity of animals kept isolated with those brought into the laboratory area every day. The results showed that movement of the animals into the laboratory each day was clearly a stressor – with a tenfold increase in corticosterone accompanying the move. However, after five days of this, the corticosterone levels returned to basal levels within two hours of being brought into and being allowed to stand in the laboratory corridor. The message is that moving animals around is a stressor, but that repeated exposure to movement modulates the stressfulness. Similarly, I was once asked by a colleague to take "basal" blood samples from his rats. On the day in question I was surprised to find the animals standing in an open laboratory with the persistent noise of an electric stapling machine in the background, which was too much even for me. Despite this, these animals exhibited extremely low levels of corticosterone. It appeared that they were well habituated to the laboratory and were not in the least stressed by the noises and activities around them.

A common misconception is that animals respond negatively (i.e. with negative affect) to the sight, sound and smells of other animals which are frightened or stressed. In rats at least this does not appear to be the case. Rats exposed to the smells of other stressed rats show exploratory activity, but no increase in the adrenocortical response (*Mackay-Sim & Laing*, 1980). In a recent study, we exposed rats to the sight, sounds and smells of other rats exposed to electric foot-shock over several minutes, exactly to demonstrate why we should not do this! To summarise the results, the observer rats showed no distress or increase in corticosterone associated with the stimuli emanating from their stressed conspecifics. In fact they reacted more to being placed in a new laboratory environment.

Despite the above, we must be cautious. This lack of effect might be peculiar to rats. The case of pigs awaiting slaughter might be different, and we should not assume anything else. The only way we have of being able to discuss the role of stress in either laboratory rats or pigs awaiting slaughter is to actively measure the stress response. It seems that we very often both underestimate and overestimate the stressfulness of situations, and for the sake of the animals and ourselves it is time that we studied this area empirically. Intuition might not give us the right answer, and is often an unjustified cross-species generalisation.

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Sammendrag ved O. M. Poulsen

Denne artikkel skisserer, hvorledes alminnelige laboratorierutiner kan virke på stressreaksjoner hos dyr. Jeg vil påstå, at en stor del av metodefeilene ved adferdsstudier skyldes ulike tidligere opplevelser hos dyrene.

Kortikosteroid-nivået i plasma kan benyttes som et godt mål for stressreaksjonen hos dyrene. Dyr som utsettes for sjokk viser høye kortikosteronverdier, men dyr, som kan kontrollere situasjonen ved å avverge eller unvike, viser avtagende reaksjon, når de siden utsettes for samme situation.

Fengsling eller immobilisering er en fremgangsmåte, som brukes ofte for at få herredømme over dyrets bevegelser. Fengsling har i seg selv en sterk tendens til å gi magesår samt høyt aktivitetsnivå i binyrebarken. Virkningen av fengsling av dyr, der har vært utsatt for sjokkforberedelser, er afhengig av, om dyrene har vært opstallet alene eller i grupper.

Vi har vist, at flyttning mellem stallrum og forsøksrum er stressfremkallende hos dyrene, men at stadig gjentagelse avdemper virkningen. Endelig har vi vist, at rotter ikke viser noen klinisk reaksjon eller økning i kortikosteronverdiene som følge av at være tilskuer til (synet, lyden og lukten), at artsfrender utsettes for elektrisk støt i flere minutter.