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Refining dosing by oral gavage in the dog: A protocol to harmonise welfare

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ABSTRACT

Introduction: The dog is a frequently-used, non-rodent species in the safety assessment of new chemical entities. We have a scientific and ethical obligation to ensure that the best quality of data is achieved from their use. Oral gavage is a technique frequently used to deliver a compound directly into the stomach. As with other animals, in the dog, gavage is aversive and the frequency of its use is a cause for welfare concern but little research has been published on the technique nor how to Refine it. A Welfare Assessment Framework (Hall, 2014) was previously developed for use with the laboratory-housed dog and a contrasting pattern of behaviour, cardiovascular and affective measures were found in dogs with positive and negative welfare. **Methods:** Using the framework, this study compared the effects of sham dosing (used to attempt to habituate dogs to dosing) and a Refined training protocol against a control, no-training group to determine the benefit to welfare and scientific output of each technique. **Results:** Our findings show that sham dosing is ineffective as a habituation technique and 'primes' rather than desensitises dogs to dosing. Dogs in the control group showed few changes in parameters across the duration of the study, with some undesirable changes during dosing, while dogs in the Refined treatment group showed improvements in many parameters. **Discussion:** It is recommended that if there is no time allocated for pre-study training a no-sham dosing protocol is used. However, brief training periods show a considerable benefit for welfare and quality of data to be obtained from the dogs' use.

1. Introduction

1.1. The 3Rs and toxicology

There are good reasons why positive welfare should lead to good quality of data output in laboratory-housed animals (see Poole, 1997, for review). Improvements in welfare, which has been defined as "an individual's state in relation to its attempts to cope with its environment" (Broom, 1986), have shown corresponding improvements in data output (as measured by repeatability, sensitivity and validity) in species from mice (Wurbel, 2001) to macaques (Tasker, 2012). Such research has been largely lacking in the dog, with some exceptions (e.g. Hubrecht & Serpell, 1993).

The guiding principles of humane research with animals are the 3Rs: Replacement, Reduction and Refinement (Russell & Burch, 1959). The dog is a common non-rodent model in safety assessment and other research, with >3200 dogs used in the UK (Home Office, 2013) and >72,000 used in the USA (USDA, 2014) in 2012. We have an obligation to ensure that the use of the dog is Refined where its use in toxicology cannot be Replaced or Reduced. Refinement is defined as "any approach which avoids or minimises the actual or potential pain, distress and other adverse effects experienced at any time during the life of the animals involved, and which enhances their wellbeing" (Buchanan-Smith et al., 2005, p.381). Our recent research (Hall, 2014) has led to the development of a framework used to identify dogs with negative welfare and producing lower quality data (defined as reduced sensitivity and repeatability, and increased unwanted variation). Another application of this framework is to monitor the effects of planned Refinements and provide empirical evidence for the implementation of changes to housing, husbandry and regulated procedures. The physical and behavioural effects of stress introduced by a dosing technique are undesirable for ethical and scientific reasons.

1.2. Oral gavage as a dosing technique

Oral gavage is a technique for delivering a substance directly into the stomach and is frequently used to administer test compounds in research and toxicity testing. It is recognised as an invasive and aversive event in the life of a laboratory animal (Wallace, Sanford, Smith, &

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Spencer, 1990), and therefore a negative impact on welfare and quality of data output. In a standard one- or three-month toxicology study, dogs may experience daily oral gavage, while other study types may require multiple doses in a day. We estimate that most laboratory-housed dogs which are subject to regulated procedures will experience oral gavage, and the potential impact of oral gavage on welfare may be significant given the regularity of its use. While it is recommended that dogs are introduced to the technique and habituated (commonly referred to as Sham Dosing, ShD) before a study begins (Prescott et al., 2004), there is little standardisation in the method for doing this.

In addition, there is no robust scientific evidence demonstrating a welfare benefit from the procedure of ShD. Apparent cooperation may be a 'freezing' response to fear. A proficient technician is able to deliver a dose of a compound quickly and without physical trauma. However, a technique which is invasive, which happens at potentially unpredictable intervals, and is beyond the control of the dog always has the potential to be highly aversive (Laule, 2010). It is unclear whether the practice of ShD has any welfare benefit, although it is widely used.

There is comparatively little guidance published on training of the laboratory-housed dog for procedures (i.e. organisations such as NC3Rs and IAT produce guidance for procedures in rodents) and almost nothing specifically for the Refinement of oral gavage in the dog. However, there is a wealth of literature available (Laule, 2010; McKinley, Buchanan-Smith, Bassett, & Morris, 2003; Prescott, Buchanan-Smith, & Rennie, 2005) supporting the benefits of positive reinforcement training (PRT) for various aspects of husbandry and procedures for many species in the laboratory environment. PRT is also used extensively in the training of dogs in other situations (e.g. (Hiby, Rooney, & Bradshaw, 2004; Batt, Batt, Baguley, & McGreevy, 2008; Fjellanger, Andersen, & McLean, 2002), pet, guide and sniffer dog respectively).

Our previous research using other groups of dogs in the same facility identified convergent validity in patterns of behaviours, cardiovascular parameters, affective state (free-floating mood states, not directed at an object, requiring a lesser degree of information processing, as determined by cognitive bias testing Paul, Harding, and Mendl (2005)) and mechanical pressure threshold (MPT). These factors distinguished welfare states between dogs (see Hall, 2014). Those with more negative welfare showed higher levels of undesirable behaviours (and often more 'reactive' behaviours) at baseline in the home pen and in response to behavioural challenges. Dogs also had higher blood pressure at baseline, exhibited a greater cardiovascular response to a brief physical restraint on the procedure table, exhibited a negative affective state and had a lower threshold for mechanical pressure. It is likely that these dogs adapt less well to aversive techniques such as gavage. Anecdotally, technical staff report that some dogs in any study will consistently fail to adapt, which is likely to produce unwanted variation and lower quality data output. This is concerning given the numbers of dogs subject to oral gavage. Understanding the link between positive welfare and high quality of data output is critical for ethical and scientific reasons. The framework is designed to identify those dogs most at risk of negative welfare and highlights the need for harmonisation of training and desensitisation.

The response to a brief physical restraint by a handler (on the procedure table, mimicking that used in regulated procedures) highlighted it as an aspect of study protocol particularly in need of Refinement. This was due to the undesirable changes in behavioural and cardiovascular parameters seen in the absence of a regulated procedure.

1.3. Training for procedures through habituation, desensitisation, predictability and control

While habituation may be the most common form of training for aversive events such as restraint, desensitisation is more desirable when positive welfare is to be promoted. Habituation is the process by which the response to a stimulus diminishes by repeated exposure to the stimulus, while desensitisation is the process of reducing the response to an aversive stimulus by pairing a reward (usually food) with the presentation of the stimulus (Laule, 2010). Habituation may be common practice for regulated procedures in a laboratory setting and may result in a decreased behavioural response to the aversive stimulus or event. However, this may not represent actual habituation but rather a "freezing" response and cooperation, while internal arousal has not decreased (e.g. Ruys, Mendoza, Capitanio, & Mason, 2004). It is commonly recommended that some form of "habituation" take place before a study (e.g. Laule, 2010), however the interpretation of its use varies, and there is currently no standardisation in the use of desensitisation within the laboratory environment for the dog (Prescott et al., 2004). Sham dosing (dosing with no compound administered) twice before a study begins is, in our experience, the most common form of habituation used for oral gavage.

Desensitisation or PRT may not be implemented in the laboratory environment because of a lack of understanding of the methodology or benefits of the techniques. Additionally, PRT usually involves giving a food reward which is perceived as undesirable and a source of unwanted variation in safety assessment. The interaction between perceived non-standardised food and the test substance is commonly given as the reason for not standardising desensitisation in the laboratory setting. Instead, negative reinforcement training (NRT) is more commonly used than PRT. NRT is by definition the removal of a stimulus to increase the expression of a behaviour (animal removed from stimulus upon compliance), however in practice it often involves the use of an unpleasant stimulus and as such instils fear, resistance and avoidance ("priming" a strongly negative response to the event), all of which are undesirable states in an in vivo model of a healthy human.

As PRT is likely to have a more positive impact on welfare than NRT, and is also likely to increase rather than decrease cooperation, it should be the preferred training method in the laboratory environment. PRT also increases the animal's ability to control its environment (Bassett & Buchanan-Smith, 2007).

Overmier, Patterson, and Wielkiewicz (1980) found that this ability to exert control increases the positive effects and decreases the negative effects of an event. Therefore, control may reduce the negative effects of an aversive event. Control and predictability are also interlinked, as increased control leads to increased predictability over the occurrence of an event, while increased predictability can lead to an increased ability to exert control, although some aversive events may never be controllable. For a review of the benefits of predictability and perceived control, see Bassett and Buchanan-Smith (2007). A combination of desensitisation, PRT, control and predictability provides a robust method of mitigating the effects of aversive events.

1.4. Aims

The first aim of this study was to compare the current sham dosing procedure (ShD group) with a group receiving no sham dosing (Control group) to determine whether the sham dosing procedure alone has a benefit for the dogs' welfare. The second aim was to compare both of these groups with a third group receiving Refined desensitisation and handling (RP group) to determine whether additional training and Refinements to the sham dosing technique have any benefit to dogs' welfare and quality of scientific output.

2. Methodology

2.1. Overview of study design

Table 1 illustrates the treatment given to each of the three groups in each aspect of the study. There were three phases to the study: Training, Sham Dosing and Dosing. Each of the three groups received different