

Appendices

Appendix 1: Framework for best practices

This framework is an overarching document that can be applied to all animal species and is designed to act as a guideline for research in which the motivation of animals is required for the execution of a behavioural task. A highly common method within neurocognitive research, yet not limited thereto, is the restriction of fluid and/or food. This framework also outlines a number of other training methods. Researchers are required to make a reasoned choice for a particular training method, where, from an animal welfare perspective, in the event of equal effectiveness, methods of reward without restriction should be given preference over methods of reward involving restriction.

Definition of terms

Methods that involve animals being motivated to carry out behavioural tasks by way of fluid or food restriction are categorised into a number of gradations according to the degree to which the amount of fluid or food is restricted and/or the duration within during which the fluid or food is made available to the animal. A number of concepts and definitions are used in these research methods. However, there is inconsistency in the interpretation of these concepts in the public debate on this subject, as well as in the literature and in existing Codes of Practice. The following section outlines what interpretations and definitions will be used for the

relevant concepts in this opinion and in the corresponding framework and the Codes of Practice.

- *Ad libitum fluid/food supply*: the supply of an unlimited amount of fluid or food of homogeneous quality and composition for a period of 24 hours a day, 7 days a week.
- *Restricted fluid/food supply*: the reduction of the **amount** of fluid or food made available to an animal and/or the **time units**¹² in which an animal is able to consume the fluid or food. Restriction may be applied in gradations, ranging from very limited to very severe restriction. Restriction may result in physiological changes, such as a decrease in body weight or decreased urine production. In cases of severe or long-term restriction, pathophysiological changes (physical and/or behavioural) may occur.

The terms restriction and deprivation are both used in the literature and in the field. Occasionally these concepts are used interchangeably, with the same definition, while on other occasions they are defined differently. Restriction is almost always interpreted as a reduced amount of fluid/food that is offered to the animal or a restriction of the duration during which the fluid/food is offered. Deprivation is sometimes used as a synonym for restriction, but is also defined as a total withdrawal of fluid/food (associated with a specific length of

time, e.g. 24 hours) or the omission of a specific nutrient, for example. Due to the various interpretations of the concept of deprivation, this opinion will use the term restriction.

Restriction may be necessary to sufficiently stimulate animals to carry out (complex/aversive)¹³ behavioural tasks. The extent to which the animal has to be stimulated to carry out a particular task determines the extent and duration of the restriction applied by the researcher. The extent and duration of the restriction, in turn, determine the extent to which animal welfare may be impaired. This entails a delicate balance: too severe a restriction may impair the welfare of the animal to such an extent as to inhibit the stimulation of the animal to carry out the behavioural task, as it becomes lethargic (anhedonia) or overly tense (“over-motivation”), which adversely affects its learning ability. In such cases, an animal will no longer be capable of learning or carrying out the requested task. In extreme cases, the animal may even present symptoms of apathy. As such, restriction, dependent on the extent to which it is applied, may result in physical and/or behavioural changes such that this may result in a decrease in animal welfare.

A limited degree of restriction and the possibility of carrying out a task in order to obtain food may, however, have a positive impact on the animal’s welfare, both physically and mentally. For that reason, an optimal protocol, in which the intended scientific objectives are achieved with minimal infringement of the animal’s welfare, requires a carefully established balance between restriction, the animal’s needs and motivation.

General

Selection of research method

The NCad does not deem the means but rather the objective of the research crucial to selecting a research method, with the 3Rs as guiding principles. Research that involves the motivation of animals to perform a particular behavioural task must, in principle, apply a method without restriction, unless well-founded scientific arguments or compelling public interests have been demonstrated, or unless this would lead to a disproportionate increase in the number of laboratory animals being used. The selection of the research method must be well substantiated, with the foregoing aspects taken into consideration.

In order to guide this selection or decision process, a decision tree was included on page 26 which outlines the various decision points and options.

Animal species selection

In general, neurocognitive research is carried out using rodents and primates (NHPs: non-human primates). In addition, as a general principle, the “lowest possible”, but nevertheless the most suitable animal species must be selected. Within this context, rodents are classified as lower animals than NHPs. The choice for NHPs is determined by a number of factors:

- Complexity of the task: the complexity of tasks that can be taught varies per animal species. The higher the animal species, the more complex the tasks will be that the animal is able to learn.¹⁴
- Translation to humans: the structure and functioning of the brain

in NHPs bears more similarities to that in and of humans than is the case with rodents. In addition, NHPs make eye movements and hand movements that are similar to those of humans.

In research into higher cognitive functions, the decision to use NHPs is considered.

Roles and responsibilities

The roles of the various parties involved are laid down in the Experiments on Animals Act (Wod). Appendix 7 provides an outline of these roles and responsibilities, where possible specified according to research that requires animals to be motivated to carry out behavioural tasks within the context of neurocognitive research.

Animal welfare considerations

The nature of the research and the competence of all parties involved in the implementation of the protocol in part determine the extent of discomfort. In order to guarantee animal welfare when these research methods are used, regular monitoring must take place of a number of key parameters related to physical health and behaviour. The findings must be recorded and assessed per individual animal. In the event of abnormalities, appropriate measures must be put in place to safeguard the welfare of the animals. Moreover, the documentation of the findings serves to build a dossier on the basis of which a welfare assessment can be drawn up that can lead to improvements in the research or in animal welfare. This should also result in a substantiated discomfort score at the end of the experiment for the benefit of the records. In addition, these notes are required for the

transfer of knowledge between the various stakeholders (researchers, analysts, biotechnicians, animal handlers, veterinarians). Finally, these notes are necessary to enable internal and external (NWWA) monitoring.

- Physical health parameters:
 - Fluid/food intake: daily
 - Condition of the animal:
 - (Deep-set) eyes, activity and coat grooming: daily; in addition, a more extensive observation during the weekly clean of the animal housing
 - Body condition score:¹⁵ weekly, during cleaning activities or, if necessary, more frequently in the case of any deterioration in condition
 - Weight: registered at least weekly (rodents) or if the animal is removed from the cage and placed in the primate chair (NHP). More frequent monitoring may be required on indication of condition deterioration
 - On indication and if possible: urine production, specific weight, colour. In the event of any obvious deviations or on indication in combination with appropriate treatment or determinations of Humane Endpoint (HEP)
 - Dehydration (turgor): on indication
- Behavioural parameters:¹⁶
 - alertness
 - behavioural changes such as stereotypical behaviours, aggression and/or automutilation behaviour
 - social interaction within the group

- activity in the home cage
 - urine licking (in relation to NHPs)
 - body posture
- Housing parameters:¹⁷
 - humidity
 - temperature
 - light/dark rhythm

Access to fluid and food must meet the physiological and ethological needs of the animal as much as possible. This means meeting the nutritional, energetic and natural behavioural needs of the animal. The provisions in the CoPs Monitoring Welfare of Laboratory Animals issued by the Inspectorate for Health Protection, Commodities and Veterinary Public Health (predecessor to the NVWA; *Inspectie W&V: Inspectie Gezondheidsbescherming, Waren en Veterinaire Zaken*), if updated, constitute a guideline for welfare monitoring.¹⁸

Humane endpoints

On the basis of the data collected through monitoring activities, a decision may be taken regarding whether it is necessary to intervene in the experiment. There are several types of possible intervention: treatment of an animal, modification of the degree of restriction, removing an animal from the procedure or killing the animal. The working protocol approved by the Animal Welfare Body should determine when an animal should be treated or whether the extent of restriction should be adapted or in the case of what severity or

combination of deviations (based on the foregoing parameters) a Humane Endpoint (HEP) can be established. In the event of a HEP, an animal must be removed from the procedure immediately or killed, depending on the severity of the deviation. If there are any doubts in this regard, the person referred to in Article 14 of the Experiments on Animals Act (WOD) or the Animal Welfare Body must be contacted immediately. These parties will determine whether – and if so what type of – intervention is required.

Animal training

Animal training prior to their being used in the actual research must meet a number of requirements.

Challenging animals to make choices

Experience has shown that animals that have grown up in an environment rich in stimuli which challenges them to make choices are frequently easier to train at a later stage. The best high-stimulus environment is created by providing (large-scale) group housing from a young age – social challenges and variation. For primates this means growing up in a breeding colony including family members. A high-stimulus environment can also potentially be created through the use of diet plates,¹⁹ intelligence puzzles or other more complex types of environmental enrichment in addition to standard cage enrichment.

Uncooperative animals

Should the experimental group include any animals that are difficult to train, then these animals may not be subjected to more stringent restriction than is outlined in the working protocol. In such cases, from an animal welfare perspective, the trainers must find other training methods/rewards that the animals respond to better, design the activity to be less complex/aversive or they must decide to remove these animals from the experiment.

Group versus individual

Animals preferably should be trained individually and assessment take place on an individual basis regarding which training method/reward works best for that specific animal. A learning curve difference should be taken into account prior to an individual animal fulfilling a specific criterion (as such, an animal need not immediately be excluded if it demonstrates a slightly deficient learning curve). This, however, does not mean that this should result in the solitary housing of an animal during the training period.

Trainer requirements

The trainer must be demonstrably qualified and must have knowledge of the species-specific behaviour and the needs of the animal species/strain they are working with. The trainer should have demonstrable knowledge of the various training methods and specifically of the training methods that are used. In addition, trainers are expected to keep their knowledge up to date.

Incorporating pilot study

The execution of a Synthesis of Evidence²⁰ (SoE) is required prior to the study. The researcher and the Animal Welfare Body will subsequently discuss the information obtained from the SoE and the available knowledge and experience of the research group, taking into account the objective of the research, with a view to ascertaining whether it would make sense to incorporate a pilot prior to the actual research. This pilot could, for example, be used to explore whether a training method based on reward without restriction would be feasible within the framework of the research. If restriction is necessary, a pilot might be used to ascertain how the working protocol could be optimised. The pilot could be used to ascertain the minimum degree of restriction required to have a behavioural task be conducted with sufficient frequency.

Training structure

The rate at which animals are able to learn varies per species, per strain and per individual animal. Therefore it is impossible to conceive a uniform training schedule that would function optimally for all animals. Gradual increase and build-up is crucial in any training method, allowing the animals to have time to learn what is required of them. If training is taking place using a restriction scheme, then this must be progressively increased, meaning that complex/aversive tasks should be taught in progressive steps. This also means that animals must initially be taught simple steps, such as getting used to the test design, using reward without restriction.²¹ As the tasks become more extensive, food and fluid restriction can gradually be introduced. Based on a predetermined ad lib intake of fluid and/or food.

Training methods

Animals are able to learn behavioural tasks through a variety of training methods. There is no generic best method, as the method depends on the research question. The section below provides a brief outline of some common training methods.

Positive reinforcement

Positive reinforcement training (PRT) is usually used to teach a task.

This means that desired behaviour is rewarded with fluid or food and that undesired behaviour is ignored. This positive reinforcement will result in an increase in desired behaviour.

Reward without restriction

In cases where the behavioural task permits, the animal will be trained without the use of restriction. Once the animal carries out its behavioural task, it is rewarded immediately, for example, with fruit juice, or with a “treat” (sucrose pellet; for macaques this may be a raisin or a peanut or, in any case, an item of food that is appealing compared to the daily diet). This method uses rewards that supplement the normal supply of fluid and food. This means that the animal will be saturated at a certain point and will no longer be prepared to carry out the task.

For that reason, the method is especially suited to tasks that are relatively simple and require little repetition. Reward without restriction is the preferred method for tasks that are less complex/aversive and as basic training for complex/aversive tasks.

Clicker training

Positive reinforcement training may use a clicker (clicker training). During the acquisition phase²² of clicker training, the animal is offered a reward at the same time or prior to a click (classical conditioning).

Over time, the animal will associate the sound of the clicker with receiving a reward, with the clicker acting as a bridge between the desired behaviour and the actual reward, which could be fluid or food (operant conditioning). As clicker training progresses, the click functions as a reward to the animal and a click need not necessarily be followed by a food reward every time. This will result in saturation occurring less quickly than in the case of animals subject to immediate reward without restriction. Clicker training can be combined both with a restriction regime as well as a regime without restriction.

Reward with restriction

Once the task the animal must carry out becomes more complex/aversive, and more repetitions are required, training can be carried out using a restriction protocol. Here too the animal will be offered fluid or food immediately after it displays the desired behaviour (or carries out the behavioural task). However, the animal is restricted in the amount of fluid/food it is offered prior to the experiment. This makes the animal more thirsty or more hungry when the training (or task) starts and, as such, the animal will be more motivated to carry out its behavioural task than when it is not subject to restriction.

This method is currently widely used in teaching complex/aversive behavioural tasks and for tasks that require a large number of repetitions (for example, for over 100 repetitions). The Decision Tree and the Codes of Practice (*appendices 2 and 3*) outline the requirements for the execution of this training method.

Punishing undesirable behaviour

Instead of rewarding desired behaviour, trainers may also choose to punish undesirable behaviour, which is referred to as “positive punishment” in the literature.²³ In this instance, the animal experiences a certain negative consequence as soon as it exhibits undesirable behaviour (for example, an air puff or a light electric shock). “Positive punishment” is unpleasant, but generally not painful. The time-out is a variant of “positive punishment”, which involves undesirable behaviour punished with a time penalty. The subsequent trial of the task will, for example, begin half a second later, which prolongs the interval until the next opportunity of reward occurs. This variation of the punishment is used both for rodents and NHPs. As such, positive punishment can also be used in combination with positive reinforcement and restriction.

In summary, there are various training methods that can be used to teach animals behavioural tasks, either based on a system of rewarding desirable behaviour or based on a system of punishing undesirable behaviour, or a combination of the two.

As soon as an animal has learned the task and has mastered it, the trainers may choose to apply variable ratio and interval schedules:

Variable ratio and interval schedules

Variable ratio (VR) and variable interval (VI) schedules are methods of operant conditioning in which a reward is issued following a variable number of responses or following a response after a variable time interval. The absence of a fixed reward pattern results in a factor of unpredictability, which leads a stable and high response ratio. VR and VI can also be used regarding the nature of the rewards: by applying variation the animal is unable to predict which reward is to follow after correctly carrying out the task and will remain more motivated to carry out the task. The researcher is able to regulate the time, the amount and the nature of the reward. VR and VI can be used in all of the described training methods.

Working protocol

In addition to the research objective and a substantiation of the elected procedure, the working protocol²⁴ must at least include the following information. This information may vary per animal species:

- duration of the research, starting from the training period
- species, strain, number of animals, age, sex, origin
- training of animals:
 - training method
 - training structure
 - selection of animals: age, sex, condition
- species-specific traits that should be taken into account (e.g. day/night rhythm, stress sensitivity)
- housing (with particular focus on group versus individual and cage enrichment)
- reduction: how to ensure that the minimum number of animals is used that is required in order to answer the scientific question
- refinement: a specific design of the protocol that limits discomfort to an unavoidable minimum
- restriction schedule if restriction is required
 - restriction schedule:
 - week regime
 - weekend regime
 - amounts per individual animal and dependency relationship, e.g. with body weight/natural intake per individual animal
 - minimum amount of fluid/food that is offered during training and during the experimental phase
 - times at which fluid/food is made available

- monitoring during the entire study (training, research)
 - in accordance with earlier description of “Welfare aspects” (fitness, body weight, etc.)
 - humane endpoints: criteria to adapt the protocol in the interim, suspend the procedure or prematurely terminate the procedure. Examples include:
 - criteria for maximum weight loss with regard to the reference weight²⁵
 - nature and extent of physiological and behavioural changes
 - indication of dehydration
 - supplemental and experiment-specific endpoints
 - communication and agreements regarding monitoring
 - communication between researcher, appointed veterinarian, responsible biotechnician/animal handler and Animal Welfare Body (IvD)
 - monitoring times by supervisory bodies
 - reporting of monitoring findings
 - score sheets
 - welfare assessments

Decision tree

All research must include a preliminary review of which method suits the purpose of the research best, taking into account the 3Rs. Researchers and assessors can use a decision tree²⁶ to establish which training method would best suit their research.

Decision tree

