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Training pair-housed Rhesus macaques (*Macaca mulatta*) using a combination of negative and positive reinforcement

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ABSTRACT

When training animals, time is sometimes a limiting factor hampering the use of positive reinforcement training (PRT) exclusively. The aim of this study was to evaluate the effects of a combination of negative and positive reinforcement training (NPRT). Twenty naïve female Rhesus macaques (*Macaca mulatta*) were trained in 30 sessions with either PRT ($n = 8$) or NPRT ($n = 12$) to respond to a signal, move into a selected cage section and accept confinement. In the NPRT-group a signal preceded the presentation of one or several novel, and thus aversive, stimuli. When the correct behaviour was performed, the novel stimulus was removed and treats were given. As the animal learned to perform the correct behaviour, the use of novel stimuli was decreased and finally phased out completely. None of the PRT-trained animals finished the task. Ten out of 12 monkeys in the NPRT-group succeeded to perform the task within the 30 training sessions, a significant difference from the PRT-group ($p = 0.0007$). A modified approach test showed no significant difference between the groups ($p = 0.67$) in how they reacted to the trainer. The results from this study suggest that carefully conducted NPRT can be an alternative training method to consider, especially when under a time constraint.

1. Introduction

Training methods should ideally be practical and efficient, yielding fast responses without compromising the welfare of the animal being trained. Positive reinforcement training (PRT), the addition of a reward following a desired behaviour (Laule, 2003; Skinner, 1938). is often considered to be better for the welfare of animals compared with other training methods and is therefore the main method used in animal training today (Prescott et al., 2005; Ramirez, 1999) (see Table 1 for more explicit definitions and explanations of training methods).

In our facility, we use PRT as our standard training procedure. Nonetheless, we have had limited success in using PRT to obtain full cooperation in capture procedures on newly arrived monkeys within the time available. It is known that training with PRT often initially requires some time investment before becoming efficient (Perlman et al., 2012), and in our situation there is not always enough time available to rely solely on PRT. We thus need to consider alternative training methods to obtain the desired behaviour without compromising the animals' welfare. One such alternative is negative reinforcement training. This involves the removal of an aversive stimulus contingently on the animal displaying the correct behaviour (Vargas, 2009 Table 1). By performing the behaviour again, the animal can avoid aversive stimulation. It is the termination of the negative reinforcer that acts reinforcing on the correct behaviour and will influence its future recurrence – timing is therefore crucial (Kazdin, 2001), as in all training.

Negative reinforcement is often misunderstood by animal trainers (McLean, 2005). In addition, some authors have advised against using negative reinforcement, since it involves exposing the animals to aversive stimuli (Reinhardt, 1992; Laule et al., 2003). This may give the animal a potential unpleasant experience of the training. However, combining such training with PRT is suggested to reduce the potential aversiveness of the situation (McKinley, 2004; Warren-Smith and McGreevy, 2007). Using combined reinforcement, NPRT, results in both the removal of the aversive stimulus and the subsequent addition of a reward contingent on the correct behaviour. Since an aversive stimulus is followed by the presen-

Abbreviations: NPRT, Negative and positive reinforcement training; PRT, Positive reinforcement training.

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Table 1
Definitions of terminology found in this paper.

Positive reinforcement training (PRT)	As the animal responds correctly a desired reward is delivered; the response thus becomes more likely to recur. The animal repeats the behaviour in order to obtain the reward (Laule et al. 2003).
Primary reinforcer	An inherently rewarding stimulus that satisfies biological drives, such as e.g. food(Egger and Miller, 1962).
Secondary reinforcer	A stimulus that has gained significance to the animal through association with primary reinforcers (Egger and Miller, 1962), e.g. a clicker.
Negative reinforcement training (NRT)	By removing an aversive stimuli contingent upon the animal performing a specific behaviour, that specific behaviour is reinforced and the probability of it occurring again will increase. The repetition of behaviour occurs as the animal tries to avoid the aversive(Ramirez, 1999; Vargas, 2009).
Aversive stimuli	An aversive stimulus is anything the animal moves away from, i.e. wants to avoid or escape from. They may range from low-intensity to painful stimuli, including conditioned stimuli (Carter and Wheeler, 2005) and, as in this study, novel stimuli to which animals inherently tend to keep a distance (Misslin and Ropartz, 1981).
Combination of negative and positive reinforcement training (NPRT)	When combining positive and negative reinforcement, correct behaviour is followed by the removal of the aversive stimulus and the subsequent addition of a reward. Behaviour is reinforced, however it is unclear whether the behaviour change is driven by negative or positive reinforcement - or a combination.
Approximation step	The progressive steps in training,reinforcing behaviour incrementally one step at a time until the desired behaviour is completed (i.e. shaping, McMillan et al., 2014).
Desensitisation	A process in which the animal's perception of a certain event is changed to a more neutral one with the help of time and/or experience (Ramirez, 1999).
Counter conditioning (active desensitisation)	An active desensitization technique where the trainer associates the aversive stimulus or event with something the animal desires, thus lessening the impact of the aversive stimulus (Chance, 2009).
Habituation (passive desensitisation)	A process in which the animal is repeatedly exposed to a stimulus in order to decrease its response when exposed. No reinforcement is involved in the process (McMillan et al., 2014).
Systematic desensitisation	Gradual exposure to the aversive stimulus, always below response threshold, enabling the animal to gradually get used to the stimulus (Wolpe, 1961). Systematic desensitization is a type of habituation procedure, often combined with counter conditioning (ref).
Signal (predicting or response eliciting)	A sound or any other distinct stimulus that is presented in order to inform the animal that something is either going to happen (predicting signal; Bassett and Buchanan-Smith, 2007) or to elicit a certain response as a result of a learned association (also known as cue, Ramirez, 1999).
Least reinforcing scenario (LRS)	If the animal performs an incorrect behaviour the trainer pauses for 3?5 seconds before continuing the training session, i.e. the least reinforcing scenario has been provided (Ramirez, 1999). This procedure may reduce the likelihood of the unwanted behaviour being repeated.

tation of a desired reward, this procedure can be construed as counter conditioning (Table 1; Yin, 2009), and Chance (2009) purports that this active pairing of an aversive event followed by a rewarding stimulus gradually decreases the ability of the aversive event to adversely affect the animal. Thus, we propose that there is a potential difference between using negative reinforcement solely and a combination of reinforcement, in terms of how the aversive stimulus is perceived by the animal. Stacey et al. (1999) included negative reinforcement in their normal PRT to successfully train a common bottlenose dolphin (*Tursiops truncatus*) to be restrained and injected for medical reasons. In this case, they positively reinforced the dolphin as long as it participated in the session, but if it refused a net was used to guide the dolphin to the selected area where it was once again positively reinforced. Thus, the desired behaviour resulted in both the removal of the aversive stimulus and the addition of a reward.

The choice of aversive stimulus in negative reinforcement is delicate and warrants ethical consideration. The negative reinforcer may range from a light aid (McLean, 2005) to highly intense, painful stimuli (McGreevy and Boakes, 2011). Sometimes it is difficult to foresee whether the addition or removal of a specific stimulus will reinforce a behaviour. From a trainer perspective, the behaviour of the animal will indicate whether or not a stimulus functions as a reinforcer. If the animal increases a targeted behaviour in order to avoid an object, that object is negatively reinforcing the behaviour (Vargas, 2009). "Aversive" denotes something the animal wants to avoid (Ulrich et al., 1964), and it does not have to be frightening or painful (Innes and McBride, 2008). Novel objects are often initially aversive (Misslin and Ropartz, 1981), inducing neophobic reactions (Misslin and Cigrang, 1986) a phenomenon demonstrated in e.g. rodents, humans and non-human primates (Corey, 1978). This suggests that novel objects could potentially be used as negative reinforcers.

Moreover, a signal preceding an aversive event can be used to further decrease potential discomfort, as aversive events become predictable and even avoidable if the animal performs the correct behaviour (Bassett and Buchanan-Smith, 2007). When using negative reinforcement on horses, McGreevy and Boakes (2011) suggest the use of a signal before the pressure of the bit in the horse's mouth is increased, thus giving the horse the chance to respond correctly before the negative reinforcer even is applied.

A training regime that involves aversive elements may negatively affect the relationship between the animals and the trainer, which could cause problems for future interactions (McKinley, 2004). Since combined reinforcement training, i.e. NPRT, involves the avoidance/removal of an aversive stimulus, the aversive events could potentially be associated with the trainer. To test if the aversiveness of the training situation becomes associated with the trainer, a modified approach test may be used (for a review of human fear tests see Forkman et al., 2007). If the reaction of the monkeys towards the trainer is not affected, we propose that the level of aversion experienced in the training situation is small enough not to contaminate the overall interaction with the trainer.

As mentioned, in laboratory settings it is sometimes crucial to obtain results within a limited time period, as the animals are predestined for biomedical experiments. We had the possibility to use the monkeys quarantine period of three months, a short and valuable opportunity, to prepare them for their participation in the upcoming experiments. The aims of this study were therefore firstly to investigate if NPRT, using novel objects as negative reinforcers, was more efficient than PRT alone when training monkeys to perform a specific behaviour, and secondly to investigate if such training methods affected the response towards the trainer. We did this by comparing two groups of Rhesus macaques *(Macaca mulatta)*, one group being trained solely with PRT and one with NPRT. The central task for the monkeys was to move into a selected section of their cage and accept the gate being closed. For each of the two training groups we evaluated (1) how many individuals that performed the behaviour within a given time frame and (2) if the monkeys' response towards the trainer was affected by the training methods used.