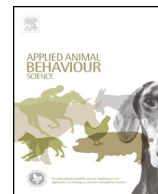




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Conditioned placebo effect in dogs decreases separation related behaviours

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ABSTRACT

In humans, placebo effect can be produced by giving verbal information and also by conditioning when, after repeated administration of an active substance, an inactive compound that just looks like the drug administered before, can produce the effect of the active substance. Conditioned placebo effect has been reported in rodents, however, the dog (*Canis familiaris*) may also provide a promising model species. In our study dogs' behaviour was observed while they were repeatedly separated from their owners in the same unfamiliar room. First, subjects did not receive any pre-treatment (Baseline trial), then they participated in either of two different conditioning contexts: after having received either sedative drug (Conditioned group) or non-sedating vitamin (Control group) treatment, subjects participated in 3 conditioning trials on consecutive days. Finally, in the 'Test trial', both groups were separated from their owners after receiving placebo (non-sedating vitamin). Results show significant effect of the sedative drug conditioning; when comparing the change from Baseline to Test trials in the *Conditioned* and the *Control* groups, conditioned subjects showed less active signs of distress ($U_{(26)} = 48, p = 0.021$) and more passive behaviours ($U_{(26)} = 50, p = 0.027$). We also investigated the association between dogs' susceptibility to conditioned placebo effect and their expectancy bias towards positive outcomes and found a positive correlation ($r_{(12)} = 0.697, p = 0.008$), suggesting that dogs with more positive expectations are more responsive to placebo treatment. Considering previous human findings about stronger responsiveness to placebo in optimistic people, our results support the validity of the application of a dog model towards a better understanding of some aspects of the placebo phenomena in humans.

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1. Introduction

Investigation of the mechanisms as well as the behavioural and psychological dimensions of the placebo effect has become a burgeoning field of life sciences in the last few decades. According to the widely accepted definition, placebo is a substance or procedure that has no inherent power to produce an effect that is sought or expected (Stewart-Williams and Podd, 2004). The effect that placebos have can be highly variable involving both

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psychological and physiological changes (e.g. endogenous opiate release [Petrovic et al., 2002](#); [Wager et al., 2004](#)).

Nevertheless, placebo effect is often conceptualised as a psychosocial context effect ([Benedetti and Colloca, 2004](#)) involving the formation of cognitive expectancies, a process driven by verbal information from a trustworthy, certified person ([Benedetti et al., 1999, 2003](#)). Although this view would strongly suggest that placebo effects are limited to humans, experimental evidence indicates that this complex phenomenon stems from both higher mental functions and lower conditioning effects, and thus can also be studied in nonhuman subjects (see [Price et al., 2008](#) for a review).

Increasing evidence suggests that placebo responses can be formed by classical conditioning in both humans ([Voudouris et al., 1990](#)) and different species of animals ([McMillan, 1999](#)). This process is based on the association between an active substance (unconditioned stimulus) and some characteristic property of the substance (smell, taste, colour) and/or some environmental cues (places, persons, procedures, rituals) surrounding the treatment (conditioned stimuli). After repeated experience of the specific effects of the treatment, a procedure with the same features but without the active substance can produce the very same physiological and/or behavioural effects evoking a conditioned response. The induction of a placebo effect via conditioning is possible even when the effect of the treatment is unconscious and imperceptible to the subject (e.g. change in hormone level – [Benedetti et al., 2003](#) or immune response – [Goebel et al., 2002](#)).

In addition to rats and other laboratory rodents that are often used to demonstrate the conditioned placebo effect (see [Stewart-Williams and Podd, 2004](#) for a review), some evidence suggests a placebo-like effect in pet dogs that have undergone veterinary treatment. However, it is important to note that in all placebo studies on dogs, assessment of the magnitude of placebo responses has been based solely on the owners' subjective evaluation; therefore, the results could be strongly influenced by the owners' expectations ([Muñana et al., 2010](#); [Jaeger et al., 2005](#)). Although the mechanism mediating the effects of placebo treatment in dogs is still unclear, [Cracknell and Mills \(2008\)](#) investigated the role placebo treatment plays in overcoming fear and anxiety. They found a significant anxiolytic effect in dogs that showed excessive fear response to fireworks. This result was also based on owners' reports, so further confirmation of conclusions about the role of placebo in alleviating fear or relieving pain would require the collection of behavioural data through direct observations.

These findings are in line with the increasing evidence of dogs' human-tuned social cognitive skills ([Kaminski, 2008](#)) and support the idea that the fear/anxiety-alleviating effect of placebo treatment in dogs is a phenomenon worth investigating within the context of the dog–human social bond. It has been suggested that dogs possess a specific behaviour organising mechanism (called interspecific attachment), which evokes specific responses in stress situations related to separation from the attachment figure (see [Topál and Gácsi, 2012](#) for a review). Separation related behaviours, the fear or dislike of isolation from

the owner even in familiar environments, are frequently reported problems in pet dogs ([Wright and Nesselroete, 1987](#)). Behaviour symptoms associated with physiological changes ([Palestrini et al., 2010](#)) can be reduced by medication or behaviour therapy ([Butler et al., 2011](#); [Appleby and Pluijmakers, 2004](#)). Concerning the medication to treat anxiety disorders in dogs, Sedalin is one of the widely used psychoactive drugs. Its active substance is acetylpromazine, which has a tranquilising effect ([Booth and Mc Donald, 1991](#)) as it causes a general depression of the nervous system characterised by both neuronal and behavioural changes ([Tontodonati et al., 2007](#)).

The most widely used experimental paradigm to study dog–human attachment and separation anxiety is the Strange Situation Test (SST), which capitalises on the tendency of dogs to show specific behaviours when separated from the owner in an unfamiliar room ([Topál et al., 1998](#)). In this context, efforts to re-establish the proximity (scratching the door, orientation to the door, vocalisation) are typical characteristics of dogs' behaviour (e.g. [Prato-Previde et al., 2003](#); [Palmer and Custance, 2008](#)).

Although behavioural manifestations of separation anxiety in dogs are easy to observe and behavioural symptoms of anxiety can be reduced by tranquilisers, placebo conditioning studies are missing. Thus, in the first experiment of the present study we aimed to investigate the role of placebo in reducing dogs' separation related distress behaviours and to determine whether it is possible to produce a conditioned placebo-effect after repeated experiences of the anxiolytic effects of psychoactive drug (Sedalin) treatment in the experimental situation.

Moreover, since responsiveness to expectancy based placebo treatment in humans is positively affected by subjects' dispositional optimism ([Geers et al., 2005, 2007, 2010](#); [Morton et al., 2009](#)), in a follow up study (Experiment 2) we aimed to test whether individual differences in dogs' susceptibility to the placebo effect are linked to the subjects' tendency to form positive expectations about upcoming events.

Discrimination learning tasks are standardly used to assess positive expectation bias in non-human animals ([Harding et al., 2004](#)) including rats ([Burman et al., 2009](#)), sheep ([Doyle et al., 2010](#)), starlings ([Bateson and Matheson, 2007](#)), and honeybees ([Bateson et al., 2011](#)). After the subjects have learned that one stimulus (sound, colour, location, etc.) is negative (non-reinforced), while another one is positive (reinforced) they typically respond with higher latency to the negative stimulus. When subjects are presented with an ambivalent stimulus (transition between negative and positive stimuli), "optimistic" subjects respond as if they were presented with the positive stimulus ([Mendl et al., 2009](#)). This method was successfully applied for dogs with location cues ([Mendl et al., 2010](#); [Müller et al., 2012](#)) and in colour discrimination contexts ([Burman et al., 2011](#)).

In the present study we hypothesised that there would be a significant positive correlation between dogs' susceptibility to placebo conditioning (measured by the relative change in behaviour signs of distress – Experiment 1), and their positive expectation bias scores (measured by [Mendl et al.'s \(2010\)](#) discrimination learning task – Experiment 2).

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