Published in final edited form as:

Int Rev Neurobiol. 2018 ; 138: 1–15. doi:10.1016/bs.irn.2018.02.001.

Placebo Analgesia in Rodents: Current and Future Research

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Abstract

The investigation of placebo effects in animal pain models has received less attention than human research. This may be related to a number of difficulties, including the fact that animals lack the ability to use language and establish expectancies verbally, that animals cannot report and rate the extent to which they experience pain, and the inadequacy of current models of pain. Here, we describe the relatively small number of studies that have been published, communicating the opportunities and excitement of this research. We critically discuss pitfalls and limitations with the hope that this will advance future animal placebo-related research.

1. INTRODUCTION

Unlike many other fields of biomedical sciences, the field of placebo analgesia has been led primarily by advances in human research (Colloca & Benedetti, 2005; Colloca, Enck, & DeGrazia, 2016; Wager & Atlas, 2015). Animal studies—that provide critical synergy to human studies—have lagged in this field. Indeed, the first systematic studies of placebo analgesia in rodents have appeared only in the current decade. This may simply reflect the historical origins of placebo research that emerged from attempts to better control clinical trials. However, it is also likely that prejudices against the capacity of nonhuman animals to display placebo analgesia have contributed to the paucity of research in this field (McMillan, 1999).

This deficiency is unfortunate because of the distinct advantages of animal research for revealing the underlying mechanisms of clinical phenomena, in particular those related to pain (Burma, Leduc-Pessah, Fan, & Trang, 2017; Mogil, 2009; Mogil, Davis, & Derbyshire, 2010; Munro, Jansen-Olesen, & Olesen, 2017). These advantages include the ability to produce reproducible models of pain, the use of transgenic animals to probe-specific mechanisms and brain circuits, and the ability to perform experimental manipulations that cannot be performed in humans for technical or ethical reasons.

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The authors report no conflict of interest.

Nevertheless, recent progress suggests that animal models of placebo analgesia with face validity can be developed and utilized to understand the mechanisms underlying this clinically important phenomenon. In this review, we illustrate the most relevant contributions on pain modulation induced by pharmacological conditioning in which the unconditioned stimulus (UCS) (e.g., the drug) is replaced by a placebo to elicit a conditioned analgesic response in both acute and chronic pain. We critically review pitfalls and strengths of published studies with a careful description of the methodology and discussion of the findings. We trust that this critical review of animal studies may help advance future methodology and approaches to explore placebo effects in animals with sophisticated molecular, behavioral, and brain imaging techniques.

2. PLACEBO ANALGESIA AND ACUTE PAIN IN RODENTS: PIONEERING ATTEMPTS

One of the earliest attempts to induce placebo analgesia in rodents achieved the opposite effect. Krank, Hinson, and Siegel (1981) reported that pairing morphine administration with an environmental cue resulted in significantly *more* reactivity to an aversive heat stimulus.

In contrast, Bardo and collaborators have shown that when a gustatory cue is used as a conditioned stimulus and paired with morphine as an UCS, rats exhibit placebo analgesia (Bardo & Valone, 1994; Miller, Kelly, Neisewander, McCoy, & Bardo, 1990). Bardo and colleagues tested rats responses on a hot plate test by administering morphine (UCS) paired with an aversive taste cue (conditioned stimulus, CS). First, they showed a decrease in CS intake across repeated testing, as expected. After three pairings, subsequent presentations of the aversive taste cue increased paw-lick latencies. They confirmed that the placebo response was not due to stress-induced analgesia from the injections because similar injections using lithium as the CS produced conditioned aversion, but not placebo analgesia.

Bardo et al. subsequently demonstrated that repeated applications of an odor (every other day for 4 days) can be used as the CS with morphine to produce placebo heat analgesia (Valone, Randall, Kraemer, & Bardo, 1998).

Surprisingly, Bryant et al. reported that a *single* association of a novel context with fentanyl resulted in a placebo effect (Bryant et al., 2009). The authors tested the prediction that mice conditioned with an opioid in a novel context would exhibit multiple, conditioned opioid-like behaviors, such as hyperlocomotion, Straub tail, and analgesia. Naïve C57 male mice in the conditioned group were administered fentanyl in a novel context (an unheated hot plate) and saline in the home cage, while control animals received saline in both locations. A third, “unconditioned” group of animals received fentanyl in the home cage and saline in the novel context. On test day, after receiving a vehicle injection, the conditioned mice showed an increased latency to lick, compared to controls, when placed on the heated hot plate. This result is somewhat difficult to assess because the contexts used for the conditioned and control groups were different, and because sample sizes used are not apparent.

Moreover, it has been demonstrated that repeating associations between conditioning stimuli and unconditioned stimuli is critical for the emergence of learned placebo responses (Colloca, 2014; Pacheco-Lopez, Engler, Niemi, & Schedlowski, 2006).

3. PAVLOVIAN CONDITIONING AND ACUTE PAIN

Some of the seminal rodent work on placebo analgesia in acute pain was performed by Guo's group at the Chinese Academy of Sciences. The authors paired tactile and visual cues with morphine administration for 4 days in female mice placed on a hot plate, while measuring the animal's latency to respond to the noxious heat (Guo, Wang, & Luo, 2010). Animals were exposed to noxious heat (55°C) on a hot plate 30min after receiving an injection of either morphine or saline. During this 30min window, the mice were placed in the conditioning chamber that included a grid floor (tactile) and light (visual) cue only after they had received 10mg/kg of morphine. On day 5, the mice were administered only saline and then placed in the conditioning chamber. As expected, morphine conditioning increased the latency of the nocifensive responses (paw lick or jump). Response latencies after saline injection on the 5th day were longer than those recorded before the contextual pairing in the morphine-conditioned group, suggesting that these mice developed placebo analgesia. Importantly, this effect was prevented by the opioid receptor inverse agonist, naloxone, when it was administered in place of saline on the 5th day.

Similarly, Guo et al. (2010) reported that placebo analgesia was evoked by an exposure to the conditioned cues previously paired with aspirin (400 mg/kg), but that this effect was *not* blocked by naloxone. This study replicated human findings on naloxone that blocked placebo analgesia only when conditioning with opioids was performed (Amanzio & Benedetti, 1999). However, despite the fact that aspirin reduces pain, it is somewhat surprising that aspirin produced heat analgesia in naïve, inflammation-free, and unsensitized animals, a necessary condition for evoking the placebo reported in this chapter. Indeed, higher doses of NSAIDs are reported to be ineffective in modulating responses to heat (Zelcer, Kolesnikov, Kovalyshyn, Pasternak, & Pasternak, 2005). There is also a concern that the use of a multiple comparison statistical test—in analyzing both the morphine and aspirin data—that violates the assumption of independence of observations, and that is prone to Type I errors, may have complicated the interpretation of the results (Guo et al., 2010).

In a follow-up study, Guo et al. (2011) used the same paradigm to associate cues with administration of morphine, with the aim to examine the effects of placebo analgesia on depressive behaviors. As before, the mice developed robust placebo heat analgesia after pairing morphine with contextual cues (based on their published data, the effect size is large; Cohen's $d = 1.2$). Mice trained to associate the contextual cue with an increased paw-lick latency were subsequently tested on the tail suspension test and forced swim test. Interestingly, this contextual paradigm was reported to also produce antidepressant effects on test day, evidenced by a modest reduction in the duration of immobility measured with both tests. Indeed, the opioid system involved in the production of placebo analgesia is also implicated in mood disorders (Ide et al., 2010). Thus, this finding is consistent with their hypothesis that the placebo effect could be transferable from one domain to another, that is, from pain to emotion. These placebo effects were similar in magnitude to those obtained by

administering to mice the serotonin–norepinephrine reuptake inhibitor, clomipramine, which has both antidepressant and direct antinociceptive actions (Guo et al., 2011). These behavioral findings were also accompanied by reductions in levels of the stress-related hormone, adrenocorticotrophic hormone during the placebo testing, providing additional support for the interpretation that placebo analgesia reduced stress levels.

In their third study, Guo's group asked whether placebo analgesia could be expressed also in male rats (Zhang, Zhang, Wang, & Guo, 2013). Consistent with their results in mice (Guo et al., 2010, 2011), they demonstrated placebo analgesia after 4 days of preconditioning with morphine. On day 5, naloxone, delivered systemically at relatively high dosage (5 mg/kg), produced a small attenuation of the placebo effect. Injection of naloxone in the rostral anterior cingulate cortex (rACC) was reported to produce a more robust, and dose-dependent reversal of the placebo effect. They also attempted to determine which opioid receptors were involved in the placebo analgesic response with microinjections of μ -, δ -, or κ -opioid receptor antagonists into the rACC. Curiously, Zhang et al. (2013) do not appear to have included in the statistical analyses data from the important saline injection group. These saline-control data were also not included in their analyses of the effects of antagonists of μ -, δ -, or κ -opioid receptor antagonists. Nevertheless, they conclude from these experiments that the placebo effect is mediated exclusively via the μ -opioid receptor.

In an attempt to avoid the potential distress associated with injections of analgesics during the conditioning phase of inducing placebo analgesia, Lee et al. (2015) developed an alternative conditioning approach, employing a conditioned place preference paradigm—a form of Pavlovian conditioning that allows assessing the motivational effects associated with experiences. The authors conditioned rats to develop preference for a chamber that was associated with a subsequent exposure to low heat (45°C), and to develop aversion to a chamber associated with a subsequent exposure to noxious heat (50°C). We note that the change in preferences reported, about 10%, was modest, and their interpretation complicated by the authors' inexplicable decision to exclude from analysis periods in which the rats were in neither chamber, and by their use of a data normalization approach that may inappropriately amplify observed differences. Injections of haloperidol (a dopamine receptor antagonist), but not of naloxone (an opioid receptor antagonist), after the conditioning phase and immediately before testing abolished the expression of the learned preferences. Sample sizes (three animals per group) were rather small.

Using a similar analysis, the authors found that this drug-free conditioning process produced a reduction in nociceptive responses to heat in animals that learned to associate their conditioned chamber with lower heat exposure. This effect was small, about 5% in magnitude, and was unaffected by prior treatment with either naloxone or haloperidol.

Replication, and more rigorous analyses, is necessary before this novel approach can be evaluated as a method for producing rodent placebo analgesia.

4. AN OPERANT CONDITIONING MODEL

An important advance in developing a rodent model of placebo analgesia was the demonstration that placebo can be conditioned with an operant learning paradigm with pain. Neubert and collaborators took advantage of an operant conflict paradigm they have previously adapted to assess orofacial pain without experimenter initiated stimuli, to reduce handling related stress, and to produce cortically dependent behavior that is indicative of pain intensity (Neubert et al., 2005; Nolan, Price, Caudle, Murphy, & Neubert, 2012). The paradigm establishes a behavioral outcome whereby an animal decides between receiving a food reward and escaping a mildly aversive thermal stimulus. Thus, unlike prior preclinical models of placebo analgesia that relied on purely reflexive responses to relatively intense noxious stimuli as pain assays, Nolan et al. (2012) were able to address the emotional aspects of placebo-induced analgesia and executive cortical control placed over them.

Specifically, Nolan et al. (2012) demonstrated that associating, in hairless male rats, a simple placebo manipulation—handling and a subcutaneous injection—with the analgesic effect of morphine (1mg/kg) for only two sessions produced a significant placebo effect that was reversed by naloxone. As the authors readily admit, their study included a relatively small number of subjects, and, as they correctly state, this “may have impacted the reliability of the statistical analyses.”

Nolan and colleagues report also a large variability in response size among their rats and suggested that some rats may have a higher propensity to behave as placebo responders (Nolan et al., 2012). This is consistent with human studies demonstrating large variations in placebo effects among individuals, opening up new research avenues such as the investigation of critical phenotypes that characterize placebo responders and nonresponders (Belcher, Ferre, Martinez, & Colloca, 2017; Colloca & Benedetti, 2005; Colloca & Miller, 2011).

5. PLACEBO-CONDITIONED RESPONSES IN CHRONIC PAIN MODELS

To our knowledge, the first published study on an attempt to develop a model of placebo analgesia in chronic pain was by McNabb, White, Harris, and Fuchs (2014). They used a well-established and characterized model of persistent peripheral neuropathic pain—unilateral spinal nerve ligation in female rats (Kim & Chung, 1992). Pain was defined, in this study, as significant reduction in thresholds to withdrawal from tactile stimuli applied to the hind paw affected by the nerve ligation. The authors explain that they chose this model because the resulting hypersensitivity to mechanical stimulation is long lasting, stable over time, and responsive to treatment with analgesics (gabapentin, loperamide, or morphine) that were used in this study as unconditioned stimuli. The conditioning stimuli were contextual cues, including environmental (a novel testing room), olfactory, visual, tactile (restraint and drug injections), and temporal (fixed time of day and injection-test latency). In each experimental group of rats, these cues were paired with one of the analgesics for 4 days. On the 5th day, the cues were paired with saline administration to determine if placebo analgesia had developed. It did not. No significant differences were found on test day, when saline was

administered, between control and placebo groups, indicating a lack of a conditioned placebo analgesic response.

The authors carefully concluded that placebo analgesia of chronic pain is “not particularly robust.” They also postulated that the failure to evoke placebo analgesia may reflect: (1) the severity of the spinal nerve injury model, suggesting that intense, chronic pain may not be subject to placebo analgesia; (2) the relatively slow time course of the analgesics used, resulting in inadequate temporal alignment of the conditioning cues; and (3) the reliance on only a reflexive measure of tactile sensitivity, suggesting that addressing these issues might reveal placebo analgesia in animal models of chronic pain (McNabb et al., 2014).

6. FUTURE DIRECTIONS

The demonstration of placebo analgesia in the studies described earlier is valuable and exciting. However, these studies present at least two constraints. First, many of them rely on analyses of nociceptive tests, such as the tail-flick test. These tests reflect, at least in part, spinal reflexes, as they persist after section or cold block of upper parts of the spinal cord (Irwin, Houde, Bennett, Hendershot, & Seevers, 1951; Sinclair, Main, & Lo, 1988). Although spinal mechanisms are involved in placebo analgesia (Matre, Casey, & Knardahl, 2006), clearly supraspinal—and most likely cortical—structures orchestrate these effects, indicating that studies of placebo analgesia need to incorporate nonreflexive nociceptive measures as well. The study by Nolan et al. (2012), described earlier, is of particular importance in this regard.

Equally important, studies of placebo analgesia in animals have focused primarily on models of acute pain. Although clearly informative, these studies may not have immediate relevance to chronic pain conditions that present a far more intractable, intolerable, and complex clinical problem. Chronic pain affects over 100 million Americans—more than affected by heart disease, cancer, and diabetes combined and is the most common complaint of patients in outpatient clinics (Upshur, Luckmann, & Savageau, 2006). Chronic pain is also an important contributor to the opioid epidemic ravishing many communities, especially in the United States (Skolnick & Volkow, 2016; Volkow & McLellan, 2016).

It is, therefore, desirable to develop models of placebo analgesia in chronic neuropathic pain to determine the possibility that a placebo given after pharmacological conditioning elicits relief of pain intensity and pain unpleasantness.

Whereas McNabb et al. (2014) used 4 days of conditioning, it would be important to increase the number of conditioning days, because the strength of the conditioned placebo response may increase with the number of cue associations (Ader & Cohen, 1982; Niemi et al., 2007).

Finally, it would be of interest to include a behavioral assay of neuropathic pain as well as quantitative measurements of facial expressions, as measurements of the evolutionarily conserved ability to express emotions related to pain (Crook, Dickson, Hanlon, & Walters, 2014; Darwin, 1872; Williams, 2002). To allow reliable and reproducible quantification of pain-related expressions, Mogil and collaborators developed facial grimace scales for mice

(Langford et al., 2010) and for rats (Sotocinal et al., 2011) and demonstrated that these objective metrics have high accuracy and reliability for detecting ongoing pain of relatively short duration (minutes to hours). This approach has been shown to be a reliable and sensitive metric for the assessment of ongoing pain in a rodent model of chronic, trigeminal neuropathic pain in both rats and mice (Akintola et al., 2017). With these experimental components in hand, it would be possible to set out to test the hypothesis that placebo analgesia can be conditioned in animals suffering from chronic pain.

6.1 Conditioned Compensatory Responses

Another relevant aspect to be considered is represented by learning paradigms that are the applicable ways to induce placebo effects in animals. When learning paradigms are adopted (e.g., classical conditioning), conditioned compensatory responses can occur. These responses are opposite to those induced by the pharmacological drug administered during the acquisition phase (exposure period). Compensatory mechanisms also underpin tolerance, defined as a decreased response to a drug within the course of administration (Siegel, Baptista, Kim, McDonald, & Weise-Kelly, 2000). The literature on pharmacological conditioning indicates that opposite behavioral responses may happen. For example, dogs treated with epinephrine every few days developed tolerance and *tachycardic* responses that tended to decrease over time. When placebo (e.g., an inert solution) replaced the epinephrine, an opposite *bradycardic* response was noted (Subkov & Zilov, 1937). As mentioned earlier, Krank and colleagues observed that rats that received morphine in the presence of the environmental cue showed an increased sensitivity to the heat stimulus, suggesting conditioned hyperalgesia (Krank et al., 1981). They performed a study in an experimental model of acute pain—response to thermal heat sensitivity on a hot plate test. Rats were administered saline (placebo) and tested on the hot plate test in the presence of an environmental stimulus which had either been paired or unpaired with either morphine or saline. Rats were divided into four groups: receiving morphine injection with and without the cue or saline injections with and without the cue. Rats conditioned with morphine and the cue showed hypersensitivity, whereas rats conditioned with morphine without the cue were not significantly different from both control groups (Krank et al., 1981). These paradoxical responses in relationship with pharmacological exposure indicate the initiation of adaptive responses that aim at compensating body responses for the primary action of drug.

6.2 Evolutionary Perspective

Are placebo effects regulated by natural selection? Why do some animals (as well as humans) respond to placebo manipulations, while others do not respond? Placebo effects elicited in animals predominantly rely on learning principles and indicate that the capability to release endogenous substances may have promoted survival (Colloca & Miller, 2011). Exploring the evolution of placebo effects is relevant to understanding the foundation of body pharmacological memories and, perhaps, biological functions and factors that have favored survival in the face of threats to life and ameliorated symptoms that are modulated by self-healing mechanisms (Miller, Colloca, & Kaptchuk, 2009). The studies discussed in this review suggest that there exists an innate capacity to enhance adaptation and mimic drug responses. The modulation of endogenous systems to promote healing by learning would,

presumably, enhance survival. Human research suggests that the genetic makeup may shape the vulnerability to environmental features and situational factors. Despite the high complexity of the placebo phenomenon, recent evidence supports the idea that placebo responses may be associated with genetics (Colagiuri, Schenk, Kessler, Dorsey, & Colloca, 2015). Further animal research is needed to understand whether genetic polymorphisms—that have been linked to the neurobiology of the placebo effects—may also account for variations among animals in placebo studies.

The propensity to be conditioned to placebo manipulations to modify behaviors and disease may also be part of the biological heritage across species. An organism as simple as *Aplysia californica* responds to classical and instrumental conditioning (Carew, Hawkins, & Kandel, 1983). However, associative learning cannot explain the full range of placebo effects. The ability to experience placebo effects as a by-product of the interaction among peers, in group solidarity behaviors and prolonged nurturance adds, from an evolutionary perspective, a social dimension to the placebo effect (Humphrey, 2002). Internal healing mechanisms may counteract the otherwise biologically useful defense mechanisms, such as emotions associated with pain and anxiety reactions. Placebo effects are based on an innate ability to learn from direct and vicarious experience with a potential survival value that, in humans, is mingled with cultural features.

7. CONCLUSIONS

Recent research efforts in humans are targeting distinct pain disorders, including irritable bowel syndrome (Kaptchuk et al., 2008; Vase, Robinson, Verne, & Price, 2003, 2005), idiopathic and neuropathic pain (Petersen et al., 2012, 2014; Vase, Petersen, & Lund, 2014), low back pain (Carvalho et al., 2016; Hashmi et al., 2012), migraine (Kam-Hansen et al., 2014), and knee osteoarthritis (Gollub et al., 2018; Tetreault et al., 2016). Studying placebo analgesia in models of chronic pain is important because the pathogenesis of chronic pain involves brain structures and mechanisms that differ substantially from those related to acute pain (Baliki & Apkarian, 2015; Tracey, 2016). This suggests that mechanisms underlying placebo analgesia in chronic pain may differ substantially from those related to placebo analgesia in acute pain, emphasizing a need for further research in animal models of chronic pain.

This chapter reviews current published studies in rodents with the scope to provide critical thoughts for future animal research that offers the unique possibility to investigate new molecular mechanisms and CNS pain modulation systems involved in pharmacological (conditioned) placebo effects. In particular, the ability to create pharmacological memories with behavioral and neurobiological drug-like effects throughout conditioning points to a potential innovative approach to clinical pain management. Combining drugs and placebos in conditioning paradigms (e.g., a dose-extending placebo regimen) is clinically relevant because it may provide us with strategies to optimize clinical outcomes, while dependence, cost, and other unwanted side effects of opioid and nonopioid therapeutics are minimized (Colloca et al., 2016).

The current widespread epidemic of opioid addiction and annual deaths driven by the misuse of opioids adds clinical translational value to this research approach (Skolnick & Volkow, 2016; Volkow & McLellan, 2016). The development of novel animal placebo models for acute and chronic pain is relevant for addressing issues ranging from the optimization of placebo-conditioned paradigms (e.g., number of conditioning trials; occurrence of extinction) to the development of biological markers for responders and nonresponders.

Acknowledgments

Work reported here was supported by grants from the National Institute of Neurological Disorders and Stroke (R01NS099245 to A.K.), and the National Institute of Dental and Craniofacial Research (R01DE025946 to L.C.) of the National Institutes of Health. This work was supported also by the MPowering the State Grant from the State of Maryland (to A.K. and L.C.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies. The funding sources had no role in study design; the collection, analysis, and interpretation of data; the writing of the report; or in the decision to submit the chapter for publication.

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