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The placebo effect: from concepts to genes

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Abstract

Despite its initial treatment as a nuisance variable, the placebo effect is now recognized as a powerful determinant of health across many different diseases and encounters. This is in light of some remarkable findings ranging from demonstrations that the placebo effect significantly modulates the response to active treatments in conditions such as pain, anxiety, Parkinson's disease, and some surgical procedures. Here, we review pioneering studies and recent advances in behavioral, neurobiological, and genetic influences on the placebo effect. Based on a previous developed conceptual framework, the placebo effect is presented as the product of a general expectancy learning mechanism in which verbal, conditioned and social cues are centrally integrated to change behaviors and outcomes. Examples of the integration of verbal and conditioned cues, such as instructed reversal of placebo effects are also incorporated into this model. We discuss neuroimaging studies that using well-established behavioral paradigms have identified key brain regions and modulatory mechanisms underlying placebo effects. Finally, we present a synthesis of recent genetics studies on the placebo effect, highlighting a promising link between genetic variants in the dopamine, opioid, serotonin, and endocannabinoid pathways and placebo responsiveness. Greater understanding of the behavioral, neurobiological, and genetic influences on the placebo effect is critical for evaluating medical interventions and may allow health professionals to tailor and personalize interventions in order to maximize treatment outcomes in clinical settings.

Keywords

Learning; Expectancy; Conditioning; Genes; Pain

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The placebo effect is a fascinating and important psychobiological phenomenon whereby treatment cues trigger improvement. While traditionally viewed as a nuisance variable to be controlled for, the past three decades have seen a surge in interest in the placebo effect in light of some remarkable clinical and laboratory discoveries that have demonstrated its potential power to improve patient outcomes. Furthermore, recent advances in neuroimaging and genetics have allowed researchers to begin to understand the brain mechanisms underlying the placebo effect as well as to explore its genetic bases. In this review, we highlight some historical and pioneering studies on the placebo effect, present a recent developed conceptual framework for understanding the placebo effect in which verbal, contextual, and social cues elicit expectancies that drive the placebo effect via learning, outline behavioral studies that demonstrate how new forms of learning shapes the placebo effect, and review what is currently known about neurobiological and genetic bases of the placebo effect. The possibility that genetic variations could be used to predict individual placebo and nocebo responses is particularly exciting as it suggests a way that future placebo interventions could be individually targeted to patients to maximise their benefits.

Historical and pioneering studies on the placebo effect

Many researchers have proposed that the history of prescientific medicine is in fact the history of the placebo effect (Wolf, 1950, Moerman, 1997, Shapiro and Shapiro, 1997). However, it was not until placebos began to be used as controls in clinical trials that they became a mainstay of modern medicine. One of the first documented uses of placebos as controls was a trial conducted by Benjamin Franklin and Antoine Lavoisier who were commissioned by Louis XVI in 1784 to test Franz Mesmer's claim to have uncovered "animal magnetism" – a supposed invisible force that Mesmer believed contained healing properties (Kaptchuk, 2009). Franklin and Lavoisier exposed patients to supposedly "mesmerised" objects or untreated objects (i.e. placebos) without telling the patients which ones they were being exposed do. They found that patients' responses to the objects were entirely unrelated to whether or not the object had been mesmerised and concluded that animal magnetism had no scientific basis.

While the advent of the double-blind placebo-controlled trial was undoubtedly a critical step in the advance of scientific medicine, an unfortunate side effect was that it meant that despite being commonly used in clinical trials, the placebo effect was relegated to being considered only a nuisance variable to be controlled for. It was not until the mid-1900's that interest in the placebo effect as an interesting phenomenon in its own right emerged. Probably the most influential piece of research to this end was a meta-analysis by Beecher (1955). Here, Beecher combined the data from the placebo groups of 15 studies on different maladies including pain, seasickness, cough, and anxiety, and calculated that on average, placebos led to a 35% improvement in symptoms – leading him to argue that the placebo effect was powerful and worthy of study. Despite Beecher's methodology later being criticized (Kienle and Kiene, 1997), his research sparked great interest in the placebo effect's potential power to heal. There are now over 5,000 research articles in the PubMed database that make specific reference to the placebo effect, which include demonstrations of placebo effects for pain, depression, anxiety, insomnia, immunosuppression, Attention Deficit Hyperactivity

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Disorder (ADHD), and even Parkinson's disease, to name a few (Colloca et al., 2013, Benedetti, 2014). In this section, we highlight some of the most important pioneering studies on the placebo effect conducted to date, which demonstrate the broad range of effects that placebo interventions can induce and their clinical relevance. These include evidence that placebo effects modulate active treatment outcomes, placebo surgery can be just as effective as real surgery, placebo effects may occur even without deception, and placebo effects are not always beneficial.

Placebo effects for active treatments

One of the most pivotal findings for demonstrating the clinical relevance of the placebo effect were the studies demonstrating that it contributes to the responses to active treatments, not just inert ones. Wolf (1950) was one of the first to report this. He showed that the effect of emetic treatments could be moderated by the instructions accompanying them. In a patient suffering from nausea, Wolf administered the emetic ipecac but told the patient it was an anti-emetic. Remarkably the patient's nausea was alleviated, both in terms of subjective and objective indices. More systematic analysis of these effects followed. Notably, Levine and colleagues (1981 Notably, Levine and colleagues (1984) compared open administration of placebos (i.e. administration in the presence of a nurse) with hidden administration of placebos and analgesics (i.e. via an automated intravenous pump) for pain relief post-dental surgery. They found that the open administration of placebo produced equivalent pain relief to hidden administration of 6–8mg of morphine and claimed that a substantial component of treatment responses to open treatments could be attributed to the placebo effect. Perhaps the clearest demonstration of placebo effects modulating active treatment effects, however, was provided by Benedetti et al (2003a). Benedetti and colleagues directly compared the effect of open versus hidden administration of active treatments across four different conditions, namely morphine for postoperative pain, diazepam for anxiety, subthalmic stimulation for Parkinson's disease, and beta-blockers for cardiovascular function. Across each of these treatments, they found that open treatment led to significantly larger improvement than the same hidden dose. This provided unambiguous evidence that the placebo effect was not confined to inert agents and that many active treatments involve a placebo component that substantially contributes to the overall treatment response, demonstrating the importance of considering the placebo effect in any treatment setting.

Placebo surgery

Another important discovery was that placebo effects also exist for surgery. In one of the first such studies, Cobb et al (1959) compared internal mammary artery ligation with placebo surgery for angina. The ligation of the mammary artery was believed to reduce angina by facilitating coronary flow in the adjacent channels. In both the real and the placebo surgery groups, patients were anesthetized and an incision was made in their chest, however, only in the real surgery was the mammary artery actually ligated. Remarkably, the response to the placebo surgery was just as strong as it was to the real surgery. However, Cobb et al.'s study was fairly small, involving only 17 patients, and there were already some significant doubts about the effectiveness of the procedure, which was subsequently discontinued. Moseley and colleagues (2002) provided a more recent and compelling demonstration of the power of placebo surgery. They randomized 180 patients with

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osteoarthritis of the knee to arthroscopic débridement, arthroscopic lavage, or placebo surgery, under double-blind conditions. All participants underwent general anaesthesia and had incisions made on their knees to maintain the blinding. However, only in the two real surgery conditions was any genuine procedure implemented, i.e. débridement or lavage. Fascinatingly, for the entire two year follow-up period, placebo surgery proved just as effective as the real surgeries. That is, the simple belief that surgery had been performed was sufficient to produce as much relief from knee osteoarthritis as real surgery produces. Given that according to the authors, in the United States alone, approximately 650,000 patients undergo this surgery each year, costing approximately $5,000 per patient, and half of all patients report a significant benefit, their finding demonstrated the significant potential of the placebo effect to generate improvement even for debilitating disease such as osteoarthritis. Furthermore, the finding raises interesting ethical considerations regarding the use of placebos in surgical trials (see Wartolowska et al., 2014 for a review). On the one hand, without such controls it is very difficult if not impossible to determine whether equivalent improvement to the real surgery could be achieved via the placebo effect, which may entail substantially less risks and cost to the individual and health care system. On the other hand, using placebo surgery as a comparator condition in surgical trials is ethically questionable because it involves invasive procedures and patients' deprivation of a potentially more effective treatment.

Placebos without deception

Perhaps one of the most intriguing recent discoveries is that placebo effects may exist even when there is no deception. The received wisdom has been that by their very nature, the placebo effect should only exist when participants have been deceived into believing that they have been given a real treatment. Why else would patients expect improvement? A handful of open-label placebo studies where patients know a placebo is being administered challenge that conception (Park and Covi, 1965, Sandler and Bodfish, 2008, Kaptchuk et al., 2010, Kelley et al., 2012). An early study by Park and Covi (1965) involved administering open-label placebos to 15 "neurotic" outpatients, many of whom reported being satisfied with the placebo treatment and at least five of whom wished to continue taking the placebo after the study's completion. However, that study suffered from a lack of a natural history group, making it impossible to determine whether improvement would have occurred without the placebo treatment. Perhaps the most influential study on placebo effects without deception is that of Kaptchuk and colleagues (2010), who compared standard care with and without the addition of open label placebo treatment for irritable bowel syndrome (IBS). They found that the open-label placebo significantly improved IBS symptoms, despite the fact that the patients had been told that the treatment was a placebo and that any benefit should be attributable to the placebo effect. While Kaptchuk et al. (2010) study included fairly strong information presenting placebo effects as a powerful treatment that should improve IBS symptoms, the critical component was that this information was non-deceptive, which challenges whether deception is necessary to elicit a placebo effect. An important implication of placebo effects without deception is that they maye circumvent many of the potential ethical issues to do with using the placebo effect in the clinic (Miller and Colloca, 2009), particularly in terms of maintaining patient autonomy, provided that the information is accurate and supported by evidence.

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The nocebo effect: the 'bad' side of the placebo effect

Another critical finding was that in addition to beneficial effects, placebos can also produce aversive outcomes, referred to as the nocebo effect. While there has been less research on the nocebo effect historically, a growing body of evidence indicates that they exist and can be powerful. In fact, in his meta-analysis, Beecher (1955) assessed adverse effects following placebo treatment and found that placebo treatment led to a substantial number of side effects, including headaches (25%), fatigue (18%), and nausea (10%). In one of the most striking early experimental demonstrations of the nocebo effect, Ikemi and Nakagawa (1962) found that Japanese men who were allergic to lacquer trees reacted to resin from harmless trees when they were told that the resin was from a lacquer tree. These nocebo-induced adverse reactions were quite severe, with participants developing skin irritation and rashes that lasted for up to 11 days. While that particular paradigm has not been replicated, a number of studies have found similar effects whereby informing individuals with asthma that they are inhaling an allergen leads to bronchoconstriction even when they are actually given nebulised saline (Luparello et al., 1968, McFadden et al., 1969). Various studies have since confirmed nocebo effects for pain, nausea, and many other conditions (see Benedetti et al., 2007, Colloca and Miller, 2011b for reviews). Nocebo effects are a particular concern in terms of treatment side effects, with experimental models demonstrating that warnings about side effects can increase side effect occurrence (e.g. Colagiuri et al., 2012, Neukirch and Colagiuri, 2015), thereby raising questions about informed consent and the best way to frame side effect warnings (Colloca and Miller, 2011b, Colloca and Finniss, 2012). Furthermore, the clinical relevance of these nocebo-induced side effects is highlighted by the fact that expectancies often predict the severity of side effects, even for invasive treatments such as chemotherapy (Montgomery and Bovbjerg, 2000, Olver et al., 2005, Zachariae et al., 2007, Colagiuri et al., 2008, Colagiuri and Zachariae, 2010, Roscoe et al., 2010, Colagiuri et al., 2012). While the primary focus of the current review is on the placebo effect, the accumulating evidence for the nocebo effect highlights the that it is not only important to understand how these effects can enhance beneficial clinical outcomes, but also to understand the mechanisms of negative effects in order to minimise the harmful effects they can induce.

Conceptual framework for understanding the placebo effect

Not surprisingly, the pioneering placebo studies just described have led to great interest in understanding how the placebo effect is formed. Many attempts have been made to conceptualize the placebo effect, including expectancy theory (Kirsch, 1985), classical conditioning accounts (Wickramasekera, 1980), context effects (Di Blasi et al., 2001), and the meaning response (Moerman and Jonas, 2002). In the current review, we adopt Colloca and Miller's (2011a) recently proposed framework based on Integrative Framework Theory (Peirce, 1940). Essentially, they propose that the placebo effect is a learned response, whereby various types of cues (verbal, conditioned, and social) trigger expectancies that generate placebo effects via the central nervous system. That is, they argue that while verbal, conditioned, and social cues differ in terms of their nature, these cues are integrated in order to generate central expectancies about treatment responses that drive the placebo effect. One advantage of this framework is that it allows integration of empirical findings for placebo

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effects established via verbal suggestion, direct and observational conditioning, and other social cues into a single conceptual model, rather than appealing to dual mechanisms.

Various formal leaning models outline the way in which cue-outcome associations form and can give rise to expectations, including highly influential error prediction models and more recent Bayesian models (e.g. Kruschke, 2006). The specifics of these models are beyond the scope of the current review, but in general they propose that cues provide information about the likelihood of future events based on the events experienced following those cues in the past. In the context of the placebo effect, when a patient encounters a treatment (whether active or placebo), the verbal, contextual, and social cues present cause the individual to recollect the sensations experienced in prior situations, which in turn develops into an expectancy for what is likely to be experienced in response to the current treatment (Colloca and Miller, 2011c). These expectancies drive the placebo effect via their influence on the central nervous system (see 'Neurobiology of the placebo effect' section below for more details). Importantly, one very adaptive feature of learning is generalisation – whereby learning about a specific cue can generalize to other similar cues (see Ghirlanda and Enquist, 2003 for a review of generalisation, and Guo et al., 2011 for a specific placebo-related example). This means that the cues that trigger placebo effects do not need to be identical to those that have previously been experienced, but only need to share some features – i.e. participants generalize their previous experiences with treatment to similar situations they encounter in the future. Furthermore, verbal suggestion may be one quite flexible way of facilitating generalisation, if for example two treatments vary in terms of their physical characteristics, but the patient is told that the two treatments have similar mechanisms and outcomes. As such, verbal suggestion may be a more unique type of cue in terms of its ability to more flexibly evoke memories of prior experiences and elicit the associated expectancies.

A critical question is whether the expectancies that drive the placebo effect require conscious awareness. While in lay-language expectancy typically conjures the idea of a conscious anticipation of a future event, we do not consider expectancy to necessarily entail conscious awareness. Instead, we consider it to be a more general predictive/anticipatory state that may or may not be consciously accessible depending on the specific process involved. For example, the expectancies that govern placebo effects for pain appear open to conscious interruption whereas those that govern placebo effects for hormonal responses appear unaffected by conscious processes (Benedetti et al., 2003b). A benefit of not confining expectancy to being conscious is that it prevents the potential disconnect between animal and human research that would occur if expectancies were only considered consciously accessible, or in the extreme case, mediated by language. This is consistent with various general conceptualizations of expectancies not entailing awareness (Dennett, 1991, Evans, 2003) as well as with evidence that non-human animals learn to predict and expect outcomes both in general (e.g. honeybees, (Gil, 2010)) and in the context of placebo manipulations (Herrnstein, 1962, Ader and Cohen, 1982). Of course, generally the higher the phylogenetic level, the larger the role of conscious cognition will have in forming expectations (Colloca and Miller, 2011a, c), but a broad approach allows for much better continuity across species.

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A similar broad approach can be used to conceptualising the relationship between placebo and nocebo effects. That is, while there are differences in the psychobiological mechanisms that are involved in producing nocebo effects compared with placebo effects (Benedetti et al., 2007, Enck et al., 2008), we consider the general conceptual framework for understanding both types of effects to be the same. That is, that both placebo and nocebo effects are driven by the expectancy elicited by the available cues when treatment is encountered, with the expectancies determined by prior experience.

In the following sections, we outline the learning mechanisms, neurobiology, and genetic influences on placebo effects. We focus on studies involving pain, given that pain is by far the most studied condition in these areas, but we predict that these results will likely generalize to other conditions.

Learning mechanisms

In this section, we describe various types of learning phenomena that give rise to placebo effects and explain how these findings can be integrated within an integrated framework (Colloca and Miller, 2011a, Colloca, 2014).

Classical Conditioning

Classical conditioning is the learning mechanism most frequently invoked to explain the placebo effect. In addition to his better known conditioned salivation experiment, Pavlov (1927) demonstrated that pairing a bell with the delivery of morphine, which induces restlessness in dogs, led the dogs to become restless when they later heard the bell alone, providing early evidence that drug-like (placebo) effects can be conditioned. In terms of the placebo effect, the contextual cues (e.g. syringe, treatment room) are considered the conditioned stimuli, which through pairings with an active treatment (e.g. morphine; the unconditioned stimulus) can produce conditioned placebo effects by themselves (e.g. pain relief; the conditioned response).

Numerous studies provide evidence that both pharmacological and non-pharmacological classical conditioning can lead to placebo effects (see Colloca, 2014 for a review). Amanzio and Benedetti (1999) conducted one of the most interesting pharmacological conditioning studies. Participants underwent two-days of conditioning with injections of either morphine (an opioid-based analgesic) or ketorolac (a non-steroidal anti-inflammatory analgesic). On the placebo test day, participants were either given a saline injection or naloxone – an opioid antagonist – and were told that it was a painkiller. Participants tested with saline showed significant placebo analgesia compared with a natural history group, irrespective of the type of drug that they were trained with. However, those given naloxone only demonstrated placebo analgesia if they had been conditioned with ketorolac and not with morphine. Blockade of placebo analgesia by naloxone in the morphine group indicates that morphine-induced placebo analgesia involves the opioidergic system and is consistent with morphine's pharmacodynamics. The fact that naloxone failed to block placebo analgesia indicates ketorolac-induced placebo analgesia is independent of the opioidergic system, and must have involved another system (possibly cyclo-oxygenae inhibition) consistent with

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ketorolac's pharmacodynamics. This demonstrates that classically conditioned placebo effects operate via the specific biological system activated by the pharmacological agent.

However, pharmacological conditioning limits the types of manipulations that can be implemented because it usually only allows one conditioning trial per day. A key development in placebo research was Voudouris and colleagues' design (Voudouris et al., 1985, Voudouris et al., 1989, 1990) in which they simulated drug conditioning via surreptitious manipulations of pain stimulation with and without a placebo applied during training and then tested pain in response to identical pain stimulation with and without the placebo in a test phase. Importantly, this meant that repeated stimulation with and without the placebo could be delivered in a single session, thereby paving the way for deeper analysis of the behavioral conditions that produce the placebo effect, not to mention the underlying neurobiology, as described further below. As a result, Voudouris et al.'s design has been used extensively to demonstrate several important features of placebo and nocebo effects.

One such important finding is how the length of training affects the placebo effect. Colloca et al (2010) tested how the length of training influenced placebo and nocebo effects. Participants were randomized to receive short (10 trials) or long (40 trials) training involving supposed activation of a sham electrode being paired with either a surreptitious decrease (placebo conditioning) or increase (nocebo conditioning) in pain relative to when the sham electrode was 'inactive'. As predicted by learning theories (e.g. Rescorla and Wagner, 1972), the longer the training period the larger the placebo and nocebo effect. This indicates that the amount of prior experience is a key determinant of the magnitude of both placebo and nocebo effects.

Consistent with this, Colloca and Benedetti (2006) found that prior experience with an ineffective treatment attenuates the placebo effect. In their study, a group of participants received training in which activation of a sham electrode led to no change in pain before undergoing a placebo conditioning phase in which the sham electrode was paired with a surreptitious reduction in pain. This group of participants demonstrated weaker placebo analgesia on test compared with a group who had only ever experienced the sham electrode being paired with a surreptitious pain reduction. This attests to the importance of considering an individual's prior experience with treatment, both positive and negative, in terms of predicting the likelihood of them experiencing a placebo effect.

Another important recent discovery is that both placebo and nocebo effects can be established following partial reinforcement (Au Yeung et al., 2014, Colagiuri et al., in press). Placebo research involving conditioning has almost exclusively involved training with continuous reinforcement, i.e. when the placebo is always paired with a reduction in pain. The same applies to nocebo research. Partial reinforcement refers to when the cue is paired with the relevant outcome on some, but not all trials (Bouton, 2007). In two recent studies, we compared partial reinforcement with continuous reinforcement for placebo analgesia (Au Yeung et al., 2014) and nocebo hyperalgesia (Colagiuri et al., in press). Partial reinforcement involved pairing activation of a sham electrode with a surreptitious decrease (placebo) or increase (nocebo) in pain during only 62.5% of the training trials and keeping it constant for

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the remainder. As shown in Figure 2, we found that partial reinforcement leads to weaker initial placebo analgesia than continuous reinforcement, but that the placebo effect established under partial reinforcement was more resistant to extinction.

Interestingly there was some asymmetry in terms partial reinforcement's effect on the nocebo effect (Colagiuri et al., in press), whereby partial reinforcement also led to a weaker nocebo effect than continuous reinforcement, but where both nocebo effects were equally resistant to extinction. This suggests that the same conditioning manipulation can differentially affect placebo effects and nocebo effects. In general, the evidence that placebo and nocebo effects can be established under partial reinforcement is critical in terms of ecological validity, because outside of the laboratory it is likely that patients experience some level of variability in the efficacy of their treatments. Thus, these studies demonstrate that placebo and nocebo effects can be established even when there is some variability in treatment effectiveness. Furthermore, the increased resistance to extinction of placebo effects established under partial reinforcement suggests that partial reinforcement could be used to extent beneficial placebo effects in clinical settings. Conversely, the apparent resistance to extinction of nocebo effects irrespective of the training schedule suggests that partial reinforcement could be useful for reducing the net level of nocebo hyperalgesia experienced in the clinic, with it leading to weaker overall hyperalgesia.

Integrating verbal suggestion with conditioning

Given that it is clear that both verbal suggestion and conditioning can elicit to placebo effects, an important issue in placebo research has been trying to understand how verbal suggestion interacts with conditioning. As above, we simply consider verbal suggestion to be one type of cue that can generate expectancies. One of the best examples of this is a study by Montgomery and Kirsch (1997), who compared open placebo conditioning with surreptitious conditioning. All participants received conditioning involving pairings of a placebo cream with a reduction in pain stimulation. For one group the reductions were surreptitious, however for the other group they were open, with participants receiving (accurate) verbal suggestion that the researchers were reducing the pain stimulation when the cream was applied. Despite both groups of participants receiving identical cream-pain reduction pairings, only the surreptitious group exhibited placebo analgesia on test. The verbal suggestion that the pain stimulation was being reduced in the open group blocked placebo analgesia. While it may be tempting to interpret such an effect as demonstrating separable verbal suggestion and classical conditioning mechanisms, this finding can easily be incorporated into the described framework via 'cue competition' (Rescorla and Wagner, 1972, Rescorla, 1988, Balsam and Gallistel, 2009). In cue competition, two or more cues compete for associability with a given outcome. If one is already predictive, then no learning occurs to the other cues (cf blocking; (Kamin, 1968)). In Montgomery and Kirsch's (1997) study, the verbal instructions in the open group perfectly predicted reduced pain during training, meaning that the placebo cream was a redundant cue to which no new expectancy learning occurred that would produce a placebo effect. This type of finding indicates that individuals make use of all available cues – whether verbal or contextual - when learning what to expect from a treatment, which in turn influences the likelihood of them experiencing a placebo effect. A similar approach can be used to explain reversal of

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conditioned placebo effects by verbal suggestion, for example blockade of conditioned placebo analgesia by suggestion that the i.v. injection contains an antibiotics (Benedetti et al., 2003b). This kind of reversal effect can be explained by viewing the verbal suggestion as a stronger cue that overpowers the contextual cues to produce a placebo effect in a way that is consistent with the direction to the suggestion.

Are placebo effects always consciously mediated?

A related issue concerns whether or not the learning that drives the placebo requires conscious mediation. This has been discussed at length elsewhere (Stewart-Williams and Podd, 2004). As above, the conceptual framework is intentionally broad and allows for non-conscious expectancies. Three lines of evidence suggest that placebo effects may occur in the absence of conscious awareness. First, counter-instructions following placebo conditioning, such as ‘this treatment is a placebo’, fail to completely eradicate the placebo effect (Schafer et al., 2015). Second, pharmacological conditioning of non-conscious processes, such as hormonal responses, appears capable of inducing placebo effects (Benedetti et al., 2003b). Third, at least two studies suggest that conditioning with supposedly subliminal cues can lead to placebo analgesia and nocebo hyperalgesia (Jensen et al., 2012, Jensen et al., 2015). However, some caution is required here, as conditioning without awareness has been a somewhat contentious issue in the broader learning literature (see Mitchell et al., 2009 and associated comments for a detailed review). Lovibond and Shanks (2002) critique of studies claiming learning without awareness may be particularly relevant for research on the placebo effect. They argue that most studies claiming learning without awareness involve much more sensitive tests of learning than of awareness, which according to them leads to false evidence of learning without awareness. As such, future placebo research investigating the role of awareness should incorporate more detailed tests of awareness. In addition, given the small number of total studies in this area and their somewhat controversial nature, replication of those studies claiming placebo effects without awareness would also prove useful for advancing this debate.

Social learning

All of the above examples of placebo effects involve direct first-hand experience. However, placebo effects can also be established via social learning. Colloca and Bendetti (2009) were the first to demonstrate this. They had participants observe a demonstrator reporting less pain when a placebo electrode was supposedly activated compared with when it was ‘inactive’. Participants were then exposed to the pain stimulation with and without the same placebo electrode being activated. As shown in Figure 3A, despite the intensity of the pain stimulation being equivalent, participants reported less pain with the placebo applied than without it. The fact that the participants never directly experienced pairings of the placebo with a reduction in pain indicates that their placebo analgesia was learned socially. Notably, the magnitude of socially-driven placebo analgesic effects was comparable to conditioned responses and substantially larger than verbally-induced analgesia (Fig. 3B).

Recently, this evidence has been extended to video demonstrations, whereby viewing a video of a confederate reporting less pain when a placebo is applied can also induce placebo analgesia in the observer (Hunter et al., 2014). Vogtle and colleagues (2013) have also

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shown that nocebo effects can be established via social learning, such that observing a treatment leading to hyperalgesia in a demonstrator can lead to nocebo hyperalgesia when that treatment is later encountered. Socially-induced nocebo effects may be particularly relevant to various practical situations. For example, many individuals report adverse effects as a results of exposure wind turbines, however evidence suggests that these effects are driven by negative expectancies induced by others' reports of adverse effects, i.e. the nocebo effect, rather than any unconditioned adverse effects of the turbines (Crichton et al., 2014).

Overall, these behavioral studies demonstrate the central role that learning plays in the placebo effect. The fact that the length of training, prior experience with an ineffective treatment, and the training schedule (continuous versus partial reinforcement) affect the strength of the placebo effect emphasises the importance of considering each individual's treatment history in terms of predicting placebo effects as well as suggesting ways of using learning manipulations to maximise the placebo effect, such as using partial reinforcement to prevent extinction of the placebo effect. Furthermore verbal, classically conditioned, and social cues compete for learning during training and the resulting placebo effects depend on the integration of all of the available information present at the time of treatment as routinely occurs in clinical practice.

Neurobiology of placebo effects

A deeper understanding of the behavioral mechanisms underlying the placebo effect has produced excellent experimental models for examining the neurobiological systems involved in producing placebo effects. In particular, neuroimaging techniques such as fMRI and PET have led to significant advances in our understanding of the neurobiological mechanisms of placebo effects (Colloca et al., 2008). As with behavioral studies, the majority of neuroimaging studies investigating placebo effects are on pain (placebo analgesia).

Neural correlates of placebo and nocebo effects

Pain processing has been associated with several brain regions including thalamus, primary and secondary somatosensory cortex (S1/S2), anterior cingulate cortex (ACC), and the insula (Peyron et al., 2000, Price and Barrell, 2000, Apkarian et al., 2005). Several studies have investigated whether placebo analgesia reduces the fMRI activity in pain-responsive regions. For example, during painful stimulation with electric and thermal stimuli, Wager et al. (2004) compared fMRI activity in a placebo cream condition with a control condition. They observed reduced activity in several pain related areas, including the ACC, insula, and thalamus. This is consistent with several other studies that have found reduced fMRI signals in pain-relevant regions during placebo analgesia, including reductions in insula, S1, S2, ACC, amygdala, and basal ganglia (Price et al., 2007, Eippert et al., 2009a). Further, a recent meta-analysis of fMRI studies on placebo analgesia identified the insula, dorsal ACC, thalamus, amygdala and right lateral prefrontal cortex as consistently less activated during placebo analgesia (Atlas and Wager, 2014).

These studies provide evidence that placebo analgesia is accompanied by reductions in activation in pain responsive regions. Although, it is worth noting that a higher fMRI activity in the ACC and the anterior insula during placebo analgesia has also been reported (Kong et

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al., 2006). Similarly, a meta-analysis reported evidence of both activations and deactivations in these brain regions during placebo analgesia (Amanzio et al., 2013). However, it is these regions are not exclusively responsive to pain processing, but are also likely to be involved in several more general placebo analgesia related mechanisms, which could explain the apparent discordance.

The dorsolateral prefrontal cortex (DLPFC) has also repeatedly been shown to be involved in the processing of placebo effects (Wager et al., 2004, Zubieta et al., 2005, Watson et al., 2009, Krummenacher et al., 2010, Lui et al., 2010). Several studies have found greater activity of the DLPFC in anticipation of pain relief, and that the fMRI signal in the DLPFC during anticipation of analgesia correlates with the strength of the placebo effect across participants (Wager et al., 2004, Lui et al., 2010). In a study where fMRI data were collected both during a conditioning phase (surreptitious pain reduction) and a test phase, stronger modulation of anticipatory brain activity in the DLPFC and the ACC was observed in the placebo group compared with a control group during conditioning, consistent with the surreptitious pain reduction the placebo group was receiving. Critically, the same areas were modulated during the anticipation of analgesia in the placebo group in the subsequent placebo test phase (Watson et al., 2009) – a result that has since been replicated (Lui et al., 2010).

Further evidence of the involvement of the DLPFC stems from an experiment using transcranial magnetic stimulation (TMS) to disrupt the function of left and right DLPFC. TMS over the DLPFC reduced placebo effects while sham TMS had no effect (Krummenacher et al., 2010). Consistent with this, placebo effects appear weaker in patients with Alzheimer Disease, with the loss of prefrontal executive function negatively correlating with the strength of placebo effect (Benedetti et al., 2006). These studies provide strong evidence that the DLPFC is crucial in the processing of placebo and nocebo effects and that it is feasible to modulate conditioned placebo and nocebo effect by changing the excitability of the rDLPFC using tDCS (Egorova et al., 2015). This is perhaps not surprising given that the DLPFC has been associated with a wide range of cognitive processes, including emotion regulation (Ochsner and Gross, 2005), working memory (Petrides, 2000), and cognitive control (Miller and Cohen, 2001). As such, it has been proposed that the DLPFC is involved in maintaining and updating the expectancies that drive the placebo effect and that the DLPFC exerts active control on pain perception by modulating corticosubcortical and corticocortical pathways (Lorenz et al., 2005).

Neuroimaging has also provided some insights on the neural processing of nocebo effects, however, there are considerably less studies investigating the nocebo effect. Kong et al. (2008) combined a verbal suggestion manipulation with heat pain and investigated the neural processing of nocebo effects using fMRI. In the nocebo condition, they found stronger activation of affective-cognitive pain regions including the ACC, insula, operculum, orbitofrontal cortex, and lateral prefrontal cortex. This is consistent with other studies investigating nocebo hyperalgesia that also found increased activity in pain processing regions after negative verbal suggestion (Sawamoto et al., 2000, Koyama et al., 2005, Keltner et al., 2006, Rodriguez-Raecke et al., 2010, Schmid et al., 2013). In addition, a stronger fMRI signal in the hippocampus has been observed during negative verbal

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suggestions about pain (Bingel et al., 2011). This finding is particularly interesting as the hippocampus has been associated with increased anticipatory anxiety during the processing of painful stimuli (Ploghaus et al., 2001). Together, these studies indicate that nocebo hyperalgesia tends to be associated with increased activity in pain responsive regions, which is consistent with decreases in these areas during placebo analgesia. However, the evidence of involvement of the hippocampus in nocebo hyperalgesia suggests that anticipatory anxiety is involved in the neural processing of nocebo effects, but not in placebo effects.

Recently, fMRI studies have also shown that placebo effects are modulated even at the level of the spinal cord. Eippert et al. (2009b) observed reduced fMRI signal at the ipsilateral side of the dorsal horn during placebo analgesia. The modulation of pain perception at the spinal level has also been observed in a recent nocebo study in which a conditioning manipulation was applied to reinforce verbal suggestion of hyperalgesia (Geuter and Buchel, 2013). Taken together, these studies suggest that the descending modulatory network evoked by placebo treatment can influence pain processing as early as the spinal cord in both a positive and negative manner.

Drug and placebo effects

Another interesting application of neuroimaging studies is to add to debate regarding the relationship between drugs and placebo effects. The relationship between drug treatment and placebo effects is highly relevant for clinical practice and research. In clinical practice, it is desirable to maximize treatment outcome by using the positive contributions of the placebo effect to enhance the outcomes of the active treatment. In research, the relationship between drug effects and placebo effects is fundamental given that clinical trials involving placebo control are predicated on the assumption that drug effects and placebo effects are additive (see Colagiuri, 2010 for a review). Thus a central question is whether or not drug and placebo effects are additive.

Several studies have attempted to explore the relationship between drug effects and placebo effects using the balanced placebo design. The balanced placebo design uses a 2 × 2 design with instruction about the drug (told drug versus told placebo) as one factor and actual drug (given drug versus given placebo) as the other factor, which allows tests of interactions between drug and placebo effects. Combining this design with neuroimaging, a handful of studies have found evidence of both additive and interaction effects between behavioral reports and neural signals (Volkow et al., 2003, Keltner et al., 2006, Volkow et al., 2006).

In the field of pain, both additive effects and interactions have been found. Kong et al. (2009) combined verbal instruction (positive instruction vs neutral instruction) with acupuncture treatment (real vs sham). Pain ratings were significantly lower in the positive instruction groups compared with the neutral instruction groups, with no evidence of an interaction between instructions and treatment. On the neural level, however, they observed significant fMRI activity associated with the main effect of instruction as well as an apparent interaction with treatment in terms of activity in the bilateral inferior frontal gyrus and left medial frontal gyrus. Atlas et al. (2012) combined an opioid agonist remifentanil with the instruction of pain relief, which is associated with endogenous opioid signaling as described above. They investigated behavioral pain ratings using a balanced placebo design and neural

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signals using an open-hidden design, separating the effect of remifentanil and instruction with a pharmacokinetic model. Remifentanil and instructions both reduced pain ratings, but the effect of remifentanil on pain reports and fMRI activity did not interact with the placebo effect. In contrast to that, Schenk et al. (2014) investigated pain ratings and neural signals using fMRI in a within-subject balanced placebo design by combining topical treatment (received lidocaine/prilocaine versus received control cream) with an instruction manipulation (told lidocaine/prilocaine versus told control cream). The authors observed a treatment effect of lidocaine/prilocaine on pain ratings and in the anterior insula, as well as interaction effect on pain ratings and in the rACC, anterior insula, and the ventral striatum (Fig. A–C).

Thus, even though Atlas et al. (2012) provide some evidence that placebo and opioid treatment do not interact, it remains unclear to what extent endogenous and exogenous opioids may influence each another because of a lack of in vivo receptorial studies (e.g. PET studies with carfentanil radiotracers). Further, there is at least some evidence of interactions between placebo and drug effects in terms of behavioral reports and neural signal changes for pain (Schenk et al., 2014) as well as some clinical findings (Colloca et al., 2004) that suggest that placebo and drug effects may not be merely additive. Clearly, additional research is necessary to further elucidate the behavioral, clinical and neural mechanisms of interactions between drug and placebo effects across different receptor systems.

Individual differences in placebo responding

Understanding the neural mechanisms underlying individual differences in placebo effects is of high interest, with several studies attempting to associate brain characteristics with the size of the placebo effect, individuals display. Wager et al. (2011) used data from two previous fMRI placebo studies to predict the placebo analgesia of individual participants using neural activity patterns. A number of interesting findings emerged. First, increased activity in a frontoparietal network and decreased activity in a posterior insula/temporal network predicted placebo analgesia during anticipation. Second, decreased activity in limbic and paralimbic regions predicted placebo analgesia during pain. Third, regions associated with emotional appraisal and not cognitive control or pain processing, were most predictive, suggesting that the engagement of emotional appraisal circuits is important for individual variation in placebo analgesia. In a similar study, Kong et al. (2013) manipulated pain expectancy with high and low pain cues and correlated the difference in pain ratings with pre-test resting state functional connectivity. Here, placebo analgesia was associated with functional connectivity between a frontoparietal network and the left and right prefrontal cortex / left rACC as well as between the sensory motor network and the left and right cerebellum.

Resting state fMRI also seems to predict placebo effects in chronic pain patients. In two recent studies, Hashmi et al. (2012), (Hashmi et al., 2014) used resting state functional connectivity or topologic network synchronizations involved in placebo effects in response to both real and sham acupuncture. In patients with chronic back pain (Hashmi et al., 2012), functional connectivity between left medial prefrontal cortex and bilateral insula accurately differentiated between placebo responders and non-responders. Additionally, left

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dorsolateral prefrontal cortex high-frequency oscillations also predicted placebo responding. Notably, by combining both measures, placebo responders could be predicted with a very high level of accuracy. In their other study in patients with chronic knee pain (Hashmi et al., 2014), placebo responders and non-responders were differentiated by resting state topologic network synchronizations during the previously measured baseline period, an indirect measure of how efficiently a given individual's brain transmits information between local and segregated networks. In particular, regions involved in cognitive modulation of pain, emotion, motivation, memory, and visual processing were associated with placebo analgesia.

Using diffusion tensor imaging, Stein et al. (2012) determined white matter integrity with fractional anisotrophy (FA) and correlated white matter integrity with the individual placebo analgesic effect. Voxel-wise FA values in the DLPFC, the rACC and the area of the PAG were associated with the magnitude of individuals' placebo analgesia. Using tractography, they observed that higher placebo responses correlated with increased FA values within the white matter tracts connecting the PAG with the rACC and the DLPFC. Another study assessed the association between gray matter density (GMD) using voxel-based morphometry and placebo effects Schweinhardt et al. (2009). Here, placebo analgesia correlated with the individuals' GMD clusters in the bilateral ventral striatum, the insula/temporal cortex, and the medial frontal gyrus.

In a paradigm using high and low pain cues to manipulate placebo expectancy, Yu et al. (2014) used regional homogeneity to measure the local synchronization of resting state fMRI signals. They observed that the regional homogeneity in the ventral striatum was significantly associated with conditioning effects on pain rating differences. Together with an exonic single-nucleotide polymorphism (SNP, rs4680) in the in the catechol-O-methyltransferase (*COMT*) gene and openness personality score, regional homogeneity in the ventral striatum accounted for 59% of the variance in the change in pain ratings.

These findings suggest that individuals' neurochemistry, as measured by neuroimaging methods, can in part explain individual differences in placebo responses. However, as this is a relatively new domain of research with a limited number of studies, the area would benefit from independent replication of these findings.

Endogenous opioid- and nonopioid-activations underlying placebo analgesic effects

Placebo analgesic effects are related to the activation of endogenous brain modulatory systems and the release of endogenous opioid- and nonopioid neurotransmitters (Levine et al., 1978, Amanzio and Benedetti, 1999, Eippert et al., 2009a, Pecina et al., 2015), Evidence of the role of endogenous opioids in placebo analgesic effects comes from pharmacological approaches demonstrating that placebo analgesia can be partially blocked by the opioid antagonist naloxone (Levine et al., 1978, Amanzio and Benedetti, 1999, Eippert et al., 2009a). By combining pharmacological and fMRI approaches, Eippert and colleagues demonstrated a strong functional coupling of the rACC and the PAG during the testing phase and the magnitude of functional coupling was positively correlated with the placebo effect, in line with previous results (Bingel et al., 2006, Wager et al., 2007). Naloxone (0.15 mg/kg) given before the placebo test phase, significantly reduced the placebo effect and the functional coupling between the rACC and the PAG. In addition, there was significantly

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stronger activation of the DLPFC and the rACC in the saline group compared with the naloxone group. This indicates that, via opioid dependent signaling, the DLPFC recruits regions such as the rACC that can engage other regions such as the PAG to modulate pain, thereby confirming notions about the descending modulatory networks for pain in humans (Fields, 2000).

Opioid signalling during placebo analgesia has also been confirmed by Petrovic et al. (2002) using a $H^2[^{15}O]$ PET approach. Participants received painful thermal stimuli and an opioid, placebo, or no treatment. The authors observed a stronger activation of the rostral ACC (rACC) and the orbitofrontal cortex and an increased functional coupling between the rACC and the brainstem in both the verum opioid and placebo conditions compared with no treatment, indicating that placebo analgesia act on similar neural mechanisms to verum opioid-induced analgesia. The central role of endogenous opioids in placebo analgesia has been also supported by studies using PET and the μ–opioid receptor-selective radiotracer $[^{11}C]$carfentanil (Zubieta et al., 2005, Wager et al., 2007, Scott et al., 2008) - a method allowing measurement opioid ligand displacement and therefore the in-vivo release of endogenous opioids. In these studies, placebo-induced activation of μ-opioid receptor-mediated neurotransmission has been found in brain areas such as the ACC, insula, DLPFC, orbitofrontal cortex, amygdala, periaqueductal grey (PAG), and thalamus.

However, placebo analgesic effects are not merely modulated by the opioid system and its receptors. Other systems such as the dopamine, cannabinoid, and cholecystokinin (CCK) systems are involved in the enhancement and reduction of placebo analgesia in humans. For example, the activation of dopamine (and opioid) neurotransmission was explored during a placebo manipulation with changes in the binding potential of carbon 11 [11C]-labeled raclopride (and [11C] carfentanil) in a PET study (Scott et al., 2008). A significant dopaminergic activation was found in the ventral basal ganglia, including the nucleus accumbens. Regional dopamine (and opioid) activity was associated with the individual perceived effectiveness of the placebo and reductions in pain ratings. Higher placebo responses correlated with larger dopamine (and opioid) activity in the nucleus accumbens that accounted for 25% of the variance in placebo analgesic effects. Interestingly, nocebo hyperalgesia was linked to a deactivation of dopamine and opioid release (Scott et al., 2008).

When placebo analgesia is elicited by a non-opioid pharmacological conditioning with the non-steroidal anti-inflammatory drug (NSAID) ketorolac, the CB1 receptor antagonist, rimonabant blocks the conditioned analgesic effects, thus indicating an involvement of the endogenous cannabinoid system. Placebo analgesia is negatively shaped by the CCK system that is involved in the modulation of anxiety and hyperalgesia. By blocking the CCK A and B receptors with the nonselective A/B receptor antagonist proglumide, nocebo hyperalgesia can be reversed

Recently, it has been demonstrated that oxytocin agonists given intranasally, enhance placebo analgesia in men (Kessner et al., 2013). The brain distribution of oxytocin receptors overlaps with those of arginine vasopressin (Donaldson and Young, 2008, Kogan et al., 2011). Avp1a and Avp1b vasopressin receptors are largely expressed within the central nervous system and regulate social and stress behaviors. In humans, vasopressin regulates

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conciliatory behaviors (Feng et al., 2014, Rilling et al., 2014) and social communication (Thompson et al., 2004, Thompson et al., 2006) prompting women to display 'tend-and-befriend' response patterns toward other women, and 'fight-or-flight' responses in men (Thompson et al., 2006). Using a double-blinded randomized approach, Colloca and colleagues (Colloca et al., 2015) tested the role of vasopressin agonists on placebo analgesia while controlling for sexually dimorphic influences. They found that the nonselective vasopressin agonist for both Avp1a and Avp1b receptors enhanced placebo effects in women but not in men. The modulatory action of vasopressin was highly significant when compared with the no treatment, oxytocin and saline groups (Fig. 5). A 24 IU of intranasal oxytocin did not enhance placebo effects in either sex as compared to 40 IU dose (Kessner et al., 2013) suggesting that oxytocin modulation of placebo effects can be dose-dependent. Interestingly, Colloca and colleagues also found that the role of cortisol and sexually-dimorphic influenced placebo analgesia. Specifically, women with lower dispositional anxiety and cortisol levels showed the largest vasopressin-induced modulation of placebo effects, suggesting a moderating interplay between pre-existing psychological factors and cortisol changes (Colloca et al., 2015).

Overall pharmacological studies have illustrated an intriguing 'inner pharmacy' that is in turn activated to create and shape placebo analgesic effects. This knowledge has been pivotal in guiding research on the putative role of distinct genetic variants as described in the next section. Future efforts should be made to understand the contribution of specific receptor expressions and functions within each system using selective antagonists and agonists.

Genetic influences on the placebo effect

Genetic variation is another important factor that may influence (and help predict) placebo effects. While the study of "the placebome", or the genes that influence the placebo effect (Hall et al., 2015), is only just emerging, its potential to improve our understanding of the mechanisms underlying the placebo effect is promising. Importantly, greater understanding of how different genes influence the placebo effect may allow researchers and clinicians to tailor treatment settings to individuals in order to maximize their treatment outcomes via the placebo effect. Further, better understanding genetic influences on the placebo effect may also help researchers disentangle active treatment effects from placebo effects, which is critical for evaluating the efficacy of interventions. By combining behavioral, neurobiological, and genetic research on the placebo effect, recent studies have begun to uncover genetic variants that significantly influence the placebo effect. In particular, with advances in knowledge of the neural pathways and neurotransmitters that influence the placebo effect has continued to grow, this has provided specific candidate genes to focus on.

The analysis of the genetic variants has centered around four systems, namely the dopamine, opioid, serotonin, and endocannabinoid systems, which we review here. These systems have been found to influence cognitive and neural aspects of the placebo effect, and are seen as important pathways in the subjective experience of the symptom relief associated with the placebo effect (e.g. placebo analgesia).

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Dopaminergic pathways

One of the gene variants with the most compelling support for being a key determinant of the placebo effect in patient populations is an exonic SNP in the catechol-O-methyltransferase (*COMT*) gene, rs4680. This polymorphism encodes a valine to methionine amino acid substitution at codon 158 (val158met) that is reported to reduce the enzymatic activity of this genes' protein by 3–4 fold (Lotta et al., 1995). The less active met allele, particularly in the homozygous form, has been associated with reduced dopamine in the prefrontal cortex, with dopamine implicated in the placebo effect as discussed above. Such biological plausibility combined with the fact that rs4680 is a common SNP with an estimated Minor Allele Frequency (MAF) of 0.37 in Caucasians, fits with recent studies that have supported its role in predicting the placebo effect (Hall et al., 2012, Yu et al., 2014). So far, the rs4680 polymorphism has been implicated in affecting the outcomes in both the placebo and drug treatment arms of several studies investigating diseases ranging from schizophrenia and general mental health to cardiovascular disease and IBS (Tammimaki and Mannisto, 2012). In the context of the placebo effect, this SNP has been associated with better outcomes in patients with IBS (Hall et al., 2012) and placebo analgesia in healthy subjects (Yu et al., 2014). The IBS study may be particularly important because it included a no-treatment control group. This allowed the researchers to examine responses specific to the placebo effect, while ruling out natural progression phenomena that may influence responses to a placebo treatment, such as regression to the mean, spontaneous recovery, and the natural fluctuations of illness. Specifically, the IBS study examined the relationship between the rs4680 *COMT* polymorphism and IBS symptoms for patients randomized to no-treatment, placebo treatment (placebo acupuncture), or placebo treatment with a supportive doctor-patient relationship (placebo acupuncture + supportive). The number of val158met alleles in the *COMT* genes had a statistically significant positive association with reduction in IBS symptom severity. Additionally, there was a significant interaction between *COMT* genotype, the supportive doctor-patient relationship augmented placebo treatment arm, and IBS-related pain/quality of life which suggests that this effect was somewhat specific to the placebo effect, rather than natural progression in general. However, the fact that this study focused primarily on Caucasian women could limit its generalizability.

Dopamine neurotransmission pathways are associated with pain syndromes, such as headache, post-operative pain, and fibromyalgia. Accordingly, additional polymorphisms in dopamine pathways and associated neural areas have been linked to placebo analgesia. While these variants currently have less evidence supporting their role in the genetics of placebo (Hall et al., 2015) than *COMT,* they are important potential candidates and are consistent with the notion that low dopaminergic activity is associated with higher pain sensitivity in general.

The monoamine oxidase A (*MAO-A*) X-linked gene plays a role in the oxidation of monoamines, including dopamine, as well as metabolizing serotonin and affecting serotonergic availability and signaling (Mickey et al., 2008). A common SNP (rs6323) in *MAO-A* leads to a 75% reduction in enzymatic activity in individuals who carry only this allele (homozygotes in females and hemizygotes in males), and represents an interesting potential target for predicting placebo effects (Hotamisligil and Breakefield, 1991). A study

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looking at the association of *MAO-A* genotypes with the placebo effect for clinical depression found that individuals with high-dopamine-activity genotypes had greater placebo-induced reduction in depressive symptoms (Leuchter et al., 2009). When examining additional candidate genes, this study found that SNPs in the *COMT* gene were significant predictors of the placebo effect when controlling for baseline depression, although *COMT* alone in the model was not a significant predictor. However, the direction of the effect was opposite to what would be predicted, with participants carrying Met alleles showing the smallest placebo-induced reduction in depressive symptoms. Those with Met alleles were more likely to have previously received anti-depressant treatment, which could explain the unexpected direction of the effect. While it is not clear whether the result that *COMT* is due to low power, study population differences, or other limitations, the influence of *COMT* in placebo responsiveness cannot be excluded (Tab. 1).

One of the largest studies of genetic variation in patients randomized to placebo treatment (n=257) looked at 34 candidate genes and 532 single nucleotide polymorphisms (SNPs) in data combined from four RCTs of bupropion treatment for major depressive disorder (Tiwari et al., 2013). This study found significant associations between two variants and placebo-induced improvement in depression; rs1048261 in the glucocorticoid receptor gene *NR3C1* and rs6609257 in the *MAO-A* gene (p=0.046). A study of this magnitude (532 SNPs) sacrifices power and significant associations become very difficult to detect.

A recent study investigated the effects of dopaminergic variation on placebo effects in a double-blind placebo-controlled RCT for schizophrenia (Bhathena et al., 2013). Participants in the placebo group who were homozygous for rs6280, a serine-to-glycine coding polymorphism that increases the affinity for dopamine of the *DRD3* dopamine receptor, showed significantly better outcomes than when treated with the novel drug ABT-95. Further, a genotype-by-treatment group interaction was detected, which may be due to the effect of *DRD3* genotype on the placebo effect, and that such interaction terms in general, which are often interpreted as resulting from the effect of genotype on treatment, are actually a result of the effects of genotype on placebo effect. Such findings may be important for understanding outcomes in placebo groups of RCTs, whereby the placebo is not simply an inert intervention, as traditionally conceptualized. Interestingly, this study also showed that patients who were homozygous for the rs4680 met/met *COMT* allele had larger placebo effects, adding to the somewhat conflicting evidence regarding the role of *COMT*, which may depend on the specific condition being treated.

Another candidate is the dopamine beta-hydroxylase (*DBH)* gene. Alcohol addiction studies have shown that individuals homozygous for rs1611115 C allele in *DBH* seem to do better when receiving a placebo than when treated with naltrexone (Arias et al., 2014). While this result is only suggestive of a specific role in the placebo effect, further support for *DBH* can be seen in a 34-candidate gene analysis in buproprione RCTs for depression mentioned above, where the *DBH* SNP rs2873804 was significantly associated with the placebo effect (Tiwari et al., 2013).

The brain-derived neurotrophic factor (*BDNF*) gene represents another interesting candidate, despite a lack of direct evidence of its association with the placebo effect. The rs6265 SNP

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(val66met) in the *BDNF* gene reduces *BDNF* trafficking and secretion, and despite not being associated directly with placebo analgesia, was associated with greater placebo mediated dopamine D2 and D3 receptor activation in individuals homozygous for the valine allele (Peciña et al., 2014). On the whole, these findings of associations between dopamine related variants and placebo effects in healthy and patient populations provide support for dopamine pathway genes/SNPs as effectors and/or markers of the placebo effect.

Opioidergic pathway

In addition to, and in conjunction with, dopaminergic pathways, opioid signaling pathways have long been implicated in the formation of placebo effects (Pecina et al., 2015), especially for placebo analgesia and pain perception in general. In terms of genetic influences, the functional rs1799971 polymorphism in the μ-opioid receptor gene (*OPRM1*) has been found to associate with placebo-mediated activation of dopamine neurotransmission in the nucleus accumbens during placebo analgesia (Pecina et al., 2015). This association might relate to the fact that rs1799971 aspartic acid (G) allele carriers, who in this study had lower placebo-related dopamine neurotransmission activation, have been shown to have reduced opioid receptor expression, function, and density (Zhang et al., 2005). In that same study, Pecina et al explored the role of genetic variation within the opioid system in general pain sensitivity and placebo analgesia further by examining the association of the rs1799971 *OPRM1* SNP with pain and placebo-induced changes in mood and neurotransmitter activation across the brain. Using positron emission tomography (PET) and selective radio tracers to label μ-opioid and dopamine receptors (D2/D3), they found that AA homozygotes showed an increase in baseline μ-opioid receptor availability in brain areas associated with pain and mood compared with G allele carriers. While rs1799971 genotype showed no effect on pain-induced endogenous opioid release, AA homozygotes exhibited reduced dopamine release in the nucleus accumbens in response to pain. Following a placebo treatment, individuals with G alleles demonstrated lower mood, lower μ-opioid system activation in the anterior insula, amygdala, nucleus accumbens, thalamus, and brainstem, and lower dopamine receptor activation levels (D2/3). In addition, higher NEO-Neuroticism personality scores were correlated with G allele carriers. While it is hard to determine clearly from these results which genotype predicts placebo effects and why, these findings show clearly implicate the involvement of *OPRM1* variation in the inter-individual differences in neurotransmitter response to pain and placebo-induced modulation.

Endocannabinoidergic and serotoninergic pathways

An earlier study by Pecina and colleagues (2014) investigated the role of a functional variant in the fatty acid amide hydrolase (*FAAH*) gene in neurotransmitter response to pain and placebo analgesia. In addition to reporting that individuals homozygous for the common Pro129/Pro129 *FAAH* genotype reported larger placebo analgesia and improved mood, they were also able to directly link the opioid system with the cannabinoid system in the context of placebo analgesia (Pecina et al., 2014). These two systems were already thought to act together in pain relief and reward mechanisms and, while endocannabinoid mediated placebo analgesia has been shown in antagonist based ketorolac-conditioned placebo analgesia studies (Benedetti et al., 2011), this additional link in the context of placebo widens the candidate genes net to include endocannabinoid genes such as *FAAH*.

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Genetic variations in serotoninergic pathway genes have also been assessed for their role in the placebo effect. While there is some evidence in studies of depression and social anxiety that serotonin pathway genes (*TPH2*, *5-HTTLPR*) associate with variation in the placebo effect (Furmark et al., 2008, Faria et al., 2012), the large 34 candidate gene analysis of placebo effects for depression mentioned above (Tiwari et al., 2013) did identify several serotonin genes that associated with the placebo effect (5-hydroxytryptamine transporter *SLC6A4* SNP rs4251417, *HTR2A* SNPs rs2296972 and rs622337), the necessary data does not yet exist to conclusively address the question of the role of serotonin in the genetics of placebo effects. As such, it remains only a potential candidate.

Overall, while these genes are very promising (Table 1), it is important to remember how multifaceted placebo effects are. With evidence of multiple mechanisms underlying placebo effects (Benedetti, 2013), it would be unrealistic to expect single polymorphisms and other isolated genetic variants alone to explain a substantial proportion of the placebo effect. The last decade of genome-wide association studies have demonstrated that complex traits are often influenced by a large number of common alleles with very small effect sizes (Gibson, 2012). Therefore, although certain genetic variants with the largest effect sizes can potentially provide insight into predicting placebo responders vs non-responders, it is important to keep in mind that such a complex phenomenon is unlikely to be explained on the basis of genetics alone. Further, one constant issue with exploring genetic influences on the placebo effect and behavior more generally is the trade-off between power and Type I errors. Assessing more genes in a single study substantially increases the risk of Type I errors if no control for multiple comparisons is implemented. However, controlling for multiple comparisons means that power is substantially reduced leading studies to require significantly larger sample sizes. Hence, with the relatively small number of participants included in the studies above, both under- and overestimation of the role of some polymorphisms is quite plausible. As genetic sampling becomes more common, one way to circumvent this problem in the future may be to develop a central bank of data on genetics and the placebo effect, which would allow for greater precision in detecting genuine genetic influences. In addition, one potential way to help advance knowledge on the neurobiological basis of the placebo effect to increase power, reduce noise, and isolate variables would be to use twin and/or sibling studies to investigate the contribution of genetics to placebo proneness. Depending on the exact study designs and hypotheses, such an approach could control for genetic background and/or environment in unique ways, and therefore help shed new light on both the genetics and evolutionary meaning of the placebo effect and clearly define its clinical relevance.

Conclusions

The placebo effect is a robust phenomenon that influences responses to both active and placebo treatments across many diseases and health settings. By viewing the placebo effect as a learned response triggered via the expectancies elicited by verbal, contextual, and social cues, research on the placebo effect can be integrated into a single conceptual framework. Advances in neuroimaging have greatly increased our understanding of the neurobiology of the placebo effect, particularly for placebo analgesia, where the ACC, insula, thalamus, amygdala, and DLPFC appear to play a key role. Similarly, recent developments in genetics

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have allowed researchers to begin to unpack genetic influences on the placebo effect, with genetic variations in the dopamine, opioid, serotonin, and endocannabinoid pathways appearing to be the most promising. These substantial theoretical and neurobiological advances in knowledge of the placebo effect are essential for disentangling drug effects from placebo effects and may allow clinicians to maximize therapeutic outcomes by accurately taking the placebo effect into account when tailoring treatments to every individual.

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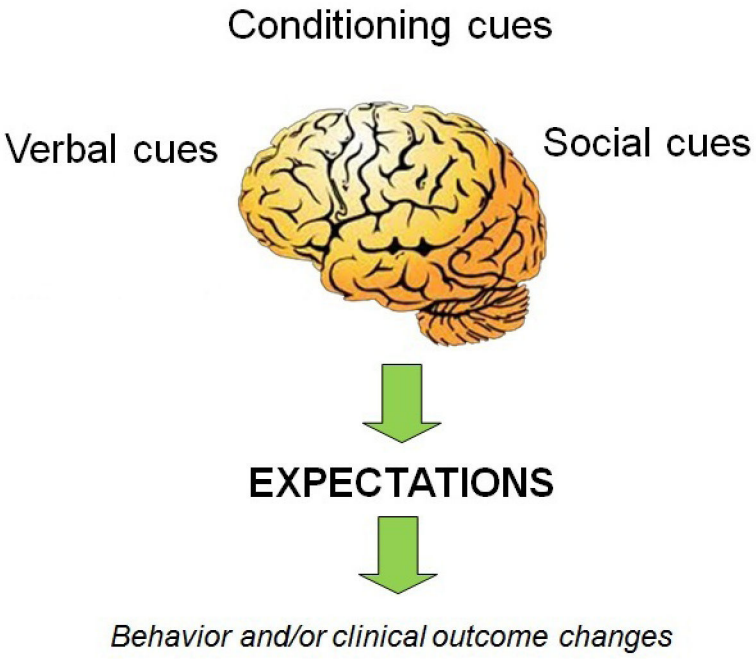

Fig. 1.

A learning framework. The integrated conceptual framework posits that the placebo effect is a learned response, whereby various types of cues - verbal, conditioned, observational, and social - trigger expectancies that generate behavioral and clinical outcome changes via central nervous system mechanisms. Adapted from Colloca and Miller, 2011.

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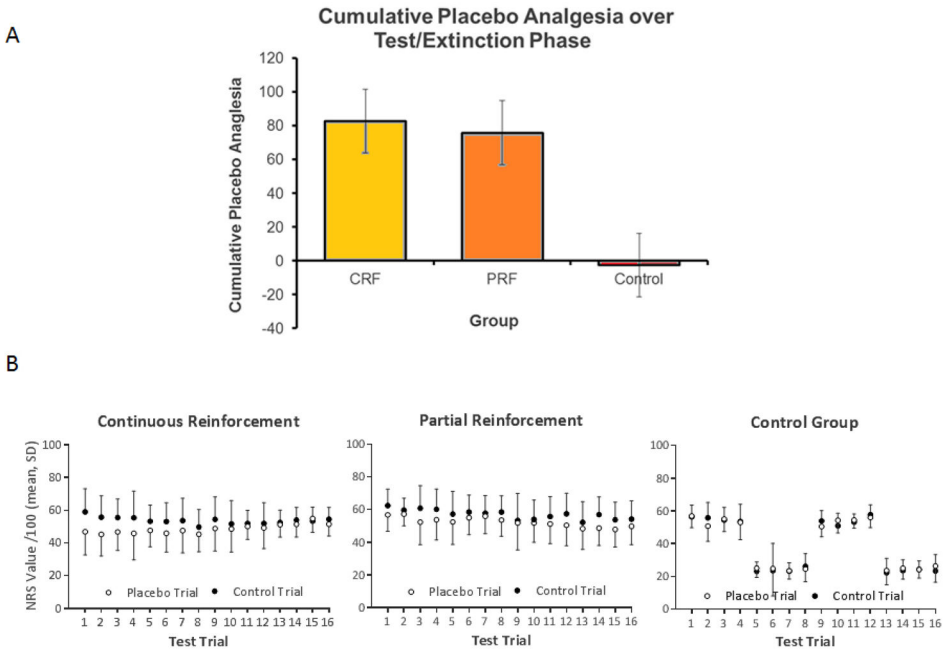

Fig. 2.

Placebo analgesia elicited by continuous (CRF) and partial (PRF) reinforcement paradigms. The cumulative placebo analgesia (+SE) over the entire test/extinction phase was comparable in magnitude for CRF and PRF groups with no significant differences between the two conditions (A). However, the trial-by-trial graphs show that placebo analgesic responses induced by CRF extinguished while those evoked by the PRF did not extinguish over the entire test phase. The Control group received neither conditioning nor verbal suggestion and showed no difference between placebo and control trials (B). Data are presented as mean pain reports ± S.D. The black dots indicate the Control trials and the white dots represent the placebo trials. The pain intensity was set at the same level to test for placebo-induced pain modulation. Data from Au Yeung et al., 2014.

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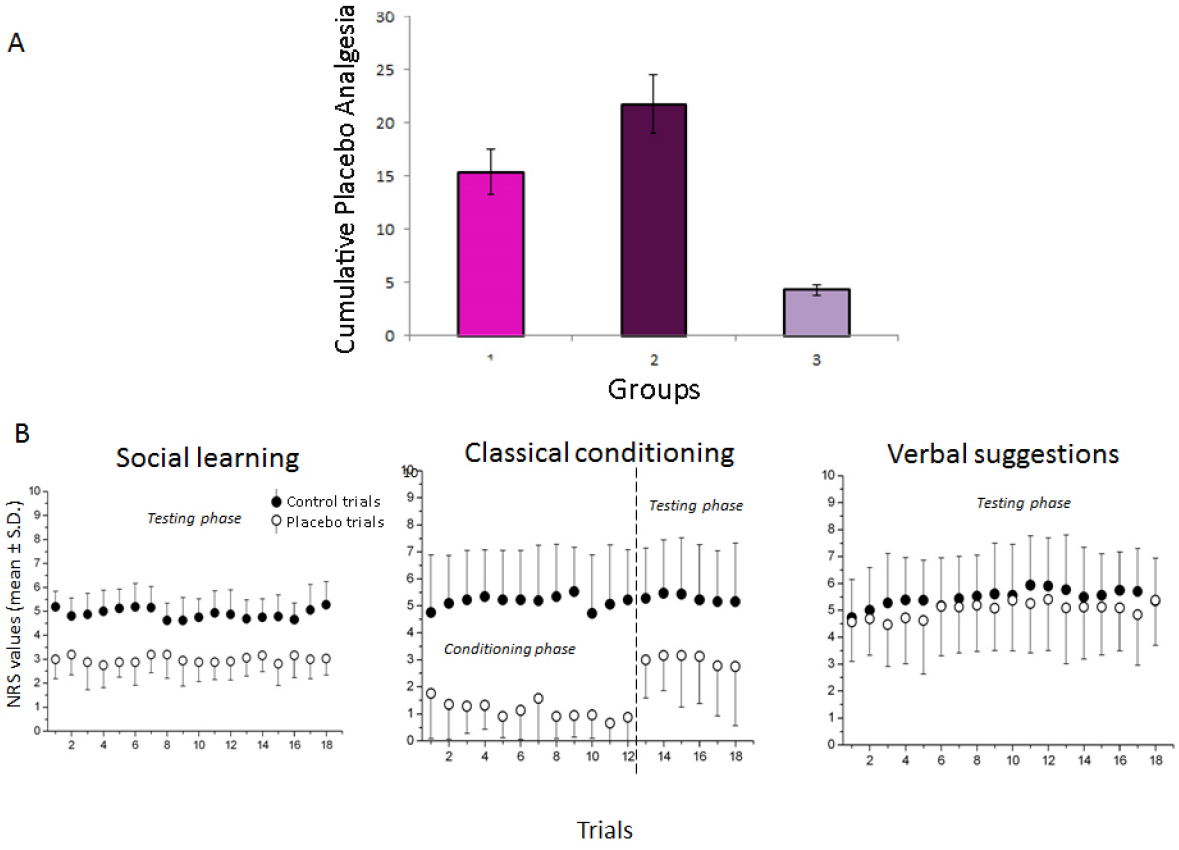

Fig. 3.

Placebo analgesia elicited by social learning, conditioning and verbal suggestions. Placebo analgesia was comparable in magnitude in the social learning and classical conditioning groups without a significant difference between the two conditions. Both the learning paradigms produced significantly larger effects than verbal suggestions (A). The graphs show the placebo responses following prior observation, first-person experience via classical conditioning (acquisition and testing phase), and verbal suggestions of benefit (B). Social observational learning and classical conditioning induced significant effects that did not extinguish over the entire experimental session. Conversely, verbal suggestions alone produced smaller and more variable placebo responses. Data are presented as mean pain reports ± S.D. with the black dots indicating the Control trials and the white dots representing the placebo test trials. Data are from Colloca and Benedetti 2009

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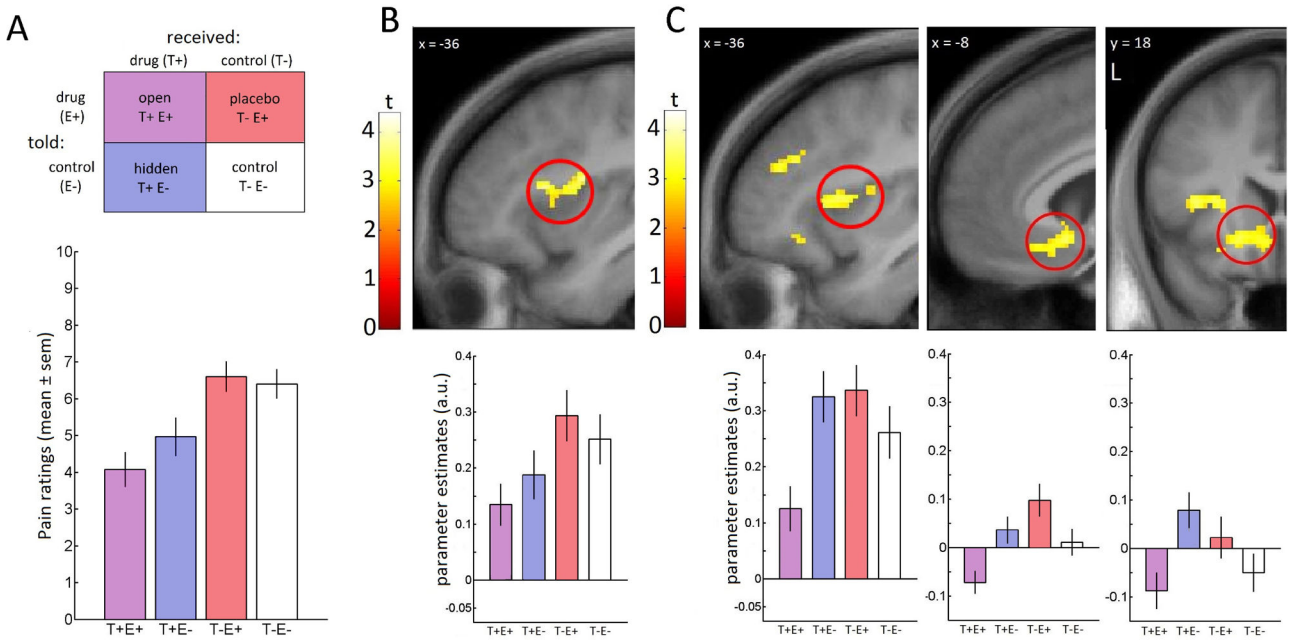

Fig. 4.

Expectancy-drug interactions in a balanced placebo design. Behaviorally, participants who received treatment experienced significantly less pain. Open treatment led to significantly less experienced pain compared to hidden treatment. Most importantly, there was a significant interaction between expectancy and drug treatment: The effect of expectancy was significantly larger in the treatment conditions compared to the no treatment conditions (A). At the level of neural responses, the treatment effect was associated with BOLD signal decreases in the anterior insular cortex (B). The interaction between expectancy and drug treatment was associated with BOLD signal changes in the insular cortex, the rACC and the ventral striatum (C). (Data from Schenk et al., 2014).

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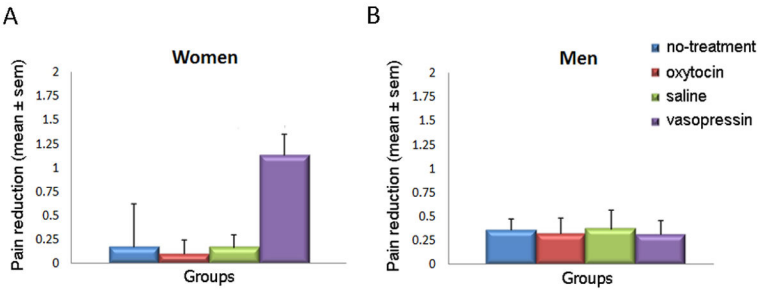

Fig. 5.

Arginine vasopressin increased placebo analgesia significantly as compared to no treatment, oxytocin, and saline in women but not in men. Participants were instructed to self-administer intranasal oxytocin, vasopressin or placebo. A no treatment group (nor drugs neither saline) was included to control for effects related to the mere administration of drugs. Forty minutes after the acute administration of one of the three agents or watchful waiting, the placebo manipulation took place and participants were tested for placebo analgesic effects while receiving red- and green-paired stimuli set at a painful level. Data are presented as differences between red- and green-pain reports. (Data from Colloca et al. 2015).

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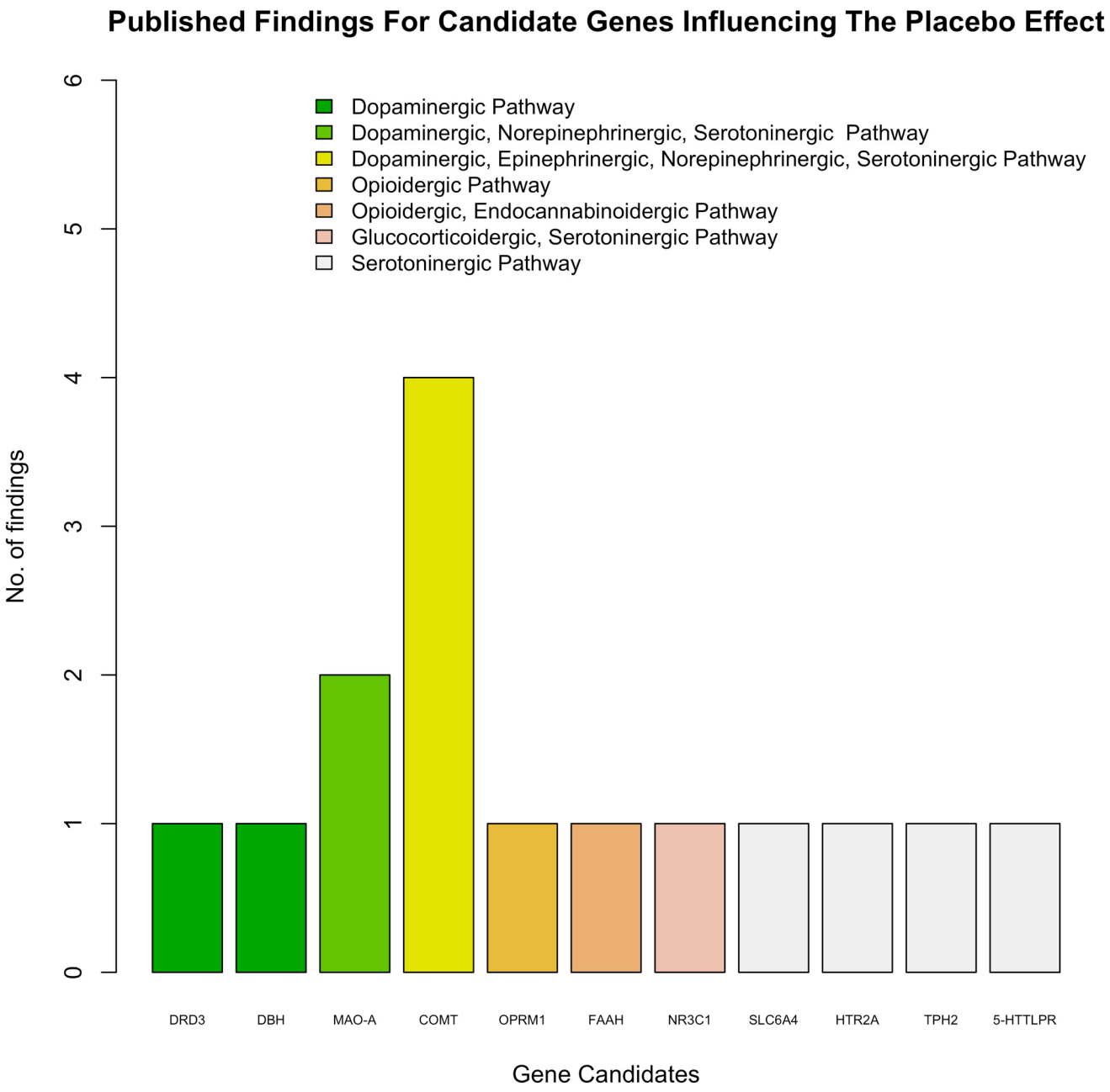

Fig. 6.

Genetic variant findings. The bar-plot summarizes current results in genetics of placebo effects. Number of publications (y axis) for each published gene (x axis) are presented. Color represent distinct gene pathways (e.g. opioidergic pathway) associated with placebo effects.

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Table 1

Summary of studies linking placebo responsiveness to genetic variants

Gene Symbol	SNP	Pathway	Sample Size	Associated Placebo Outcomes	Refs
COMT	rs4680	Dopaminergic Serotoninergic Epinephrinergic Norepinephrinergic	**1** 104 **2** 48 **3** 62 **4** 52	**1** Reduction in IBS-SSS and pain rating **2** Suppression of pain **3** Increase in drug-specific and general side-effects **4** Reduction in depression scale ratings	**1** Hall et al., 2012 **2** Yu et al., 2014 **3** Wendt, Albring et al. 2014 **4** Leuchter et al., 2009
MAO-A	rs6323 rs6609257 rs2235186	Dopaminergic Serotoninergic Norepinephrinergic	**1** 52 **2** 246	**1** Reduction in depression scale ratings **2** Reduction in depression scale ratings	**1** Leuchter et al., 2009 **2** Tiwari et al., 2013
NR3C1	rs1048261	Glucocorticoidergic Serotoninergic	257	Reduction in depression scale ratings.	Tiwari et al., 2013
DRD3	rs6280	Dopaminergic	117	Improvement in schizophrenia scale	Bhathena et al., 2013
DBH	rs1611115	Dopaminergic	254	Improvement in alcoholism	Arias et al., 2014
BDNF	rs6265	Dopaminergic	72	Increased dopamine D2/D3 receptor activation	Peciña et al., 2014
OPRM1	rs1799971	Opioidergic	50	Activation of mood response and neurotransmission	Pecina et al., 2015
FAAH	rs324420	Opiodergic, Endocannabinoidergic	42	Improved analgesia and affective state	Peciña et al., 2013
SLC6A4	rs4251417	Serotoninergic	257	Remission from major depressive disorder	Tiwari et al., 2013
HTR2A	rs2296972, rs622337	Serotoninergic	257	Remission from major depressive disorder	Tiwari et al., 2013
TPH2	rs4570625	Serotoninergic	25	Reduced stress-related activity in amygdala, reduced anxiety symptoms	Furmark, Appel et al. 2008
5-HTTLPR	Variable Number Tandem Repeats (VNTR)	Serotoninergic	**1** 25 **2** 191	**1** Reduced stress-related activity in amygdala **2** Increased pain inhibition in CPM nonpainful paradigm	**1** Furmark, Appel et al. 2008 **2** Treister, Pud et al. 2011