Neuron
Review

New Insights into the Placebo and Nocebo Responses

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DOI 10.1016/j.neuron.2008.06.030

In modern medicine, the placebo response or placebo effect has often been regarded as a nuisance in basic research and particularly in clinical research. The latest scientific evidence has demonstrated, however, that the placebo effect and the nocebo effect, the negative effects of placebo, stem from highly active processes in the brain that are mediated by psychological mechanisms such as expectation and conditioning. These processes have been described in some detail for many diseases and treatments, and we now know that they can represent both strength and vulnerability in the course of a disease as well as in the response to a therapy. However, recent research and current knowledge raise several issues that we shall address in this review. We will discuss current neurobiological models like expectation-induced activation of the brain reward circuitry, Pavlovian conditioning, and anxiety mechanisms of the nocebo response. We will further explore the nature of the placebo responses in clinical trials and address major questions for future research such as the relationship between expectations and conditioning in placebo effects, the existence of a consistent brain network for all placebo effects, the role of gender in placebo effects, and the impact of getting drug-like effects without drugs.

Introduction

Recent experimental work clearly demonstrates that a better understanding of the neurobiology and psychology of the placebo and nocebo responses is of great importance, as it might have profound implications for basic and clinical research and clinical practice. In basic research, we can learn more about how psychological processes affect CNS neurochemistry and how these alterations subsequently shape peripheral physiology and end organ functioning. The growing knowledge on the neurobiology of the placebo/nocebo response will also affect the design of clinical trials in which treatment is tested against a placebo. Finally, it might affect our health care system not only by initiating a discussion on the ethical dimension of placebo treatment but also by forcing us to reconsider the significance of the placebo in clinical training and practice.

The dynamic progress in this field is not only reflected in the constantly growing number of publications explicitly focusing on the neurobiology and psychology of the placebo response, but also in the structure and content of scientific meetings on this topic. A 1999 symposium on the Mechanisms of Placebo covered this research area with two presentations on ''expectation/conditioning mechanisms'' and ''opioid mechanisms'' (9th World Congress on Pain, Vienna). In 2000, a NIH-sponsored workshop assembled ten presenters (and more than 500 attendants and discussants), mainly from the US, to cover the field and to assess the state of the art (Guess et al., 2002). A more recent symposium on the Mechanisms of Placebo/Nocebo Response held in Tutzing, Germany, in 2007 and supported by the Volkswagen Foundation, one of the major German research funding agencies, brought together 45 speakers and experts from eight countries with topics like ''general concepts,'' ''learning and memory,'' ''brain-immune interaction,'' ''Parkinson's disease and reward mechanisms,'' ''pain,'' and ''clinical-ethical implications,'' which reflect the steady growth of knowledge in this research field.

This review summarizes (1) current neurobiological models of the placebo response: expectations and reward, Pavlovian conditioning, and anxiety mechanisms of the nocebo response; (2) implications of insights into the placebo mechanisms for clinical trials and testing; and (3) the main research questions currently being discussed.

Comprehensive reviews focusing on the psychological (Price et al., 2008; Klosterhalfen and Enck, 2006), neuropharmacological/neuroanatomical (Colloca and Benedetti, 2005; Benedetti et al., 1995; Pacheco-Lopez et al., 2006; Benedetti, 2008) and methodological aspects of the placebo response (Colloca et al., 2008; Klosterhalfen and Enck, 2008) have been recently published elsewhere.

Current Models of the Placebo Response

A major insight from the recent publications on placebo is that there seems not to be a single neurobiological or psychobiological mechanism which is able to explain placebo and nocebo phenomena in general. Instead, we have learned that different mechanisms exist by which placebo or nocebo responses are steered across diseases and experimental conditions.

Expectation and the Brain Reward Circuitry

It has been proposed that the placebo effect is mediated by the brain reward circuitry (de la Fuente-Fernández et al., 2001; de la Fuente-Fernández and Stoessl, 2002). Based on placebo studies with Parkinson's patients (de la Fuente-Fernández et al., 2004) and in experimental pain (Scott et al., 2007), it has been

hypothesized that reward expectations, such as expectation of clinical improvement, are likely to play an important role in the placebo effect. Thus, expectation may be closely tied to a tonic activation of tegmental or prefrontal dopaminergic neurons, which project to the dorsal and ventral striatum. In the expectation phase, prior to reward, there is uncertainty, and this is reflected in sustained dopaminergic activation, which is maximized when the probability of reward is 0.5. It is known that with a 0.5 probability of reward, 29% of dopaminergic cells are tonically activated (Fiorillo et al., 2003). Conversely, both occurrence and nonoccurrence lead to virtually no tonic activation. There is also phasic dopaminergic activation which takes place after reward, and this is stronger when the reward has come as a surprise. Therefore, uncertainty appears to heighten reward mechanisms in this brain reward circuitry model.

Based on this information, the following neurobiological placebo mechanism has been proposed (de la Fuente-Fernández, 2004; de la Fuente-Fernández et al., 2004). When an interaction (e.g., positive verbal suggestion) creates the possibility of a reward, which in the case of placebo administration is represented by the therapeutic benefit, certain cortical neurons become active in relation to reward probability. These cells send direct excitatory glutamatergic inputs to dopaminergic cell bodies along with indirect inhibitory gamma amino butyric acid inputs (de la Fuente-Fernández et al., 2002a; Fricchione and Stefano, 2005). The combination of these signals arriving at the dopaminergic neurons via direct and indirect pathways contributes to the probability of tonic activation (de la Fuente-Fernández et al., 2002b). Furthermore, it has been reported that neurons in the prefrontal cortex, nucleus accumbens, and the caudate-putamen display tonic activation during expectation of reward (Schultz, 1998).

Compelling evidence of the involvement of reward mechanisms in the placebo effect comes from recent brain imaging studies on placebo analgesia. In fact, in a brain imaging study in which both positron emission tomography and functional magnetic resonance imaging were used, Scott et al. (2007) tested the correlation between the responsiveness to placebo and that to monetary reward. By using a model of experimental pain in healthy subjects, they found that placebo responsiveness was related to the activation of dopamine in the nucleus accumbens, as assessed by using in vivo receptor-binding positron emission tomography with raclopride, a D2-D3 dopamine receptor agonist. The very same subjects were then tested with functional magnetic resonance imaging for activation in the nucleus accumbens to monetary rewards. What these investigators found is a correlation between the placebo responses and the monetary responses: the larger the nucleus accumbens responses to monetary reward, the stronger the nucleus accumbens responses to placebos.

This study strongly suggests that placebo responsiveness depends on the functioning and efficiency of the reward system, and this would explain, at least in part, why some individuals respond to placebos whereas some others do not. Those who have a more efficient dopaminergic reward system would also be good placebo responders. Interestingly, Scott et al. (2007) used an experimental approach that is typical of clinical trials, whereby the subjects know they have a 50% chance to receive either placebo or active treatment, and whereby no prior conditioning was performed.

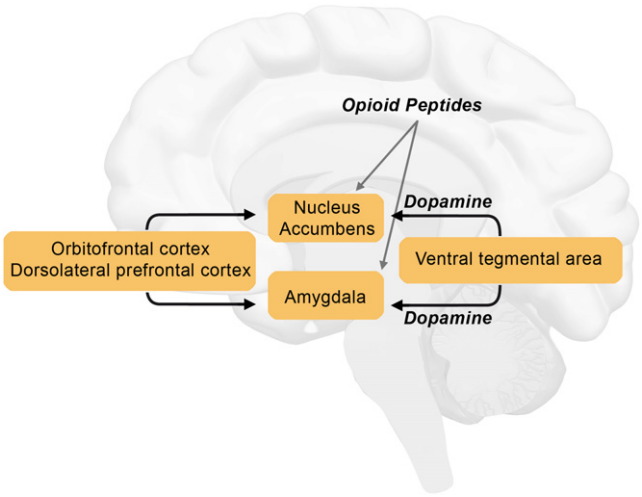

Figure 1. Simplified Scheme of the Reward System
Placebo administration has been found to activate both dopamine and endogenous opioid peptides in the nucleus accumbens, thus suggesting an involvement of reward mechanisms in some types of placebo effects (de la Fuente Fernández et al., 2001; Scott et al., 2008). Note: the main propose of this sketch is to focus on neural substrates of the reward system in the context of the placebo response which, in this case, takes precedence over anatomical accuracy.

In a different study by the same group, Scott et al. (2008) studied the endogenous opioid and the dopaminergic systems in different brain regions, including those involved in reward and motivational behavior. Subjects underwent a pain challenge, in the absence and presence of a placebo with expected analgesic properties. By using positron emission tomography with ^{11}C-labeled raclopride for the analysis of dopamine and ^{11}C-carfentanil for the study of opioids, it was found that placebo induced activation of opioid neurotransmission in the anterior cingulate, orbitofrontal and insular cortices, nucleus accumbens, amygdala, and periaqueductal gray matter. Dopaminergic activation was observed in the ventral basal ganglia, including the nucleus accumbens. Both dopaminergic and opioid activity were associated with both anticipation and perceived effectiveness of the placebo. Large placebo responses were associated with greater dopamine and opioid activity in the nucleus accumbens. Therefore, as shown in the schema of the reward circuitry in Figure 1, both dopamine and endogenous opioids have been found to be activated in the nucleus accumbens after placebo administration, which indicates that these two neurotransmitters play a key role in the modulation of the placebo response.

Pavlovian Conditioning of Placebo Effects: Neuroimmune Responses

The behavioral conditioning of immune responses is based on the intense crosstalk between the CNS and the peripheral immune system (Meisel et al., 2005; Sternberg, 2006; Tracey, 2007). Commonly, in these approaches, experimental animals are presented with a novel taste (e.g., saccharin) as conditioned stimulus (CS) in the drinking water, and subsequently injected with an agent that produces changes in immune status

(unconditioned stimulus, UCS). When the CS (saccharin solution) is re-presented at a subsequent time point, the animals avoid drinking the saccharin, which is termed "conditioned taste aversion" (CTA) (Garcia et al., 1955). Concomitantly, the animals demonstrate a modification of immune parameters that commonly mimics the actual UCS effect (Ader, 2003). Ader and Cohen (1975) demonstrated conditioned suppression of antibody production for the first time. Experimental evidence over the last 25 years has shown behaviorally conditioned effects in rodents, both in humoral and cellular immunity, with behavioral conditioning able to re-enlist changes in lymphocyte circulation and proliferation, cytokine production, natural killer (NK) cell activity, and endotoxin tolerance (reviewed in Exton et al., 2001; Ader, 2003; Pacheco-Lopez et al., 2006; Riether et al., 2008).

Regarding the neurobiological mechanisms, it was demonstrated by employing the immunosuppressant cyclophoshamide as a UCS that the insular cortex and the amygdala are key structures in behaviorally conditioned suppression of antibody production (Ramírez-Amaya et al., 1996, 1998). In parallel, when the calcineurin inhibitor and immunosuppressive agent cyclosporine A was employed as a UCS in a taste aversion paradigm, the behaviorally conditioned suppressive effect on lymphocyte activity in the spleen, as well as cytokine production (interleukin-2, interferon-γ), was affected by brain excitotoxic lesions. This shows that the insular cortex is essential to acquiring and evoking this conditioned response in cellular immune functions. In contrast, the amygdala seems to mediate the input of visceral information necessary at acquisition time, whereas the ventromedial hypothalamic nucleus appears to participate in the output pathway to the immune system, which is needed to evoke the behaviorally conditioned immune response (Pacheco-Lopez et al., 2005). On the peripheral efferent arm, these conditioned effects are mediated via the splenic nerve through noradrenaline and adrenoceptor-dependent mechanisms (Exton et al., 2001, 2002). The neural circuitry is illustrated in Figure 2.

A number of studies have meanwhile demonstrated the clinical relevance of conditioned changes in immune function. Specifically, the morbidity and mortality of animals with autoimmune disease was abated via conditioning using cyclophosphamide (Ader and Cohen, 1982) or with cyclosporine (Klosterhalfen and Klosterhalfen, 1990) as the UCS and, in addition, behavioral conditioning prolonged the survival of heterotopic heart allograft and significantly inhibited the contact hypersensitivity reaction (Exton et al., 1998, 1999, 2000).

Experimental evidence also suggests that behavioral conditioning of immunopharmacological drug effects is possible in humans. Conditioned cyclosphosphamide-induced leucopenia has been reported (Giang et al., 1996), along with a conditioned immune response to the cytokine interferon-γ (Longo et al., 1999), as well as conditioned suppression of the ex vivo production and mRNA expression of interleukin-2 and interferon-γ, and of the proliferation of peripheral lymphocytes (Goebel et al., 2002). Allergic reactions have been shown to be affected by behavioral conditioning and emotional status (Kemeny et al., 2007). However, more recently, it was demonstrated that the antihistaminergic properties of the H_1-receptor antagonist desloratadine can be behaviorally conditioned in patients suffering from allergic house-dust-mite rhinitis, as analyzed by subjective symptom score, skin prick test, and decreased basophile activation (Goebel et al., 2008). Interestingly, subjective symptom score and skin reactivity, but not basophile activation, was reduced in patients who where conditioned but not re-exposed to the novel-tasting drink served as a CS. By contrast, only conditioned patients who were re-exposed to the CS also demonstrated significant inhibition in cellular immune activation. These data support earlier observations indicating that conscious physiological pain and motor mechanisms are mainly affected by patients' conscious expectations, whereas unconscious physiological processes, such as hormone release or immune functions, appear to be mediated by behavioral conditioning (Benedetti et al., 2003).

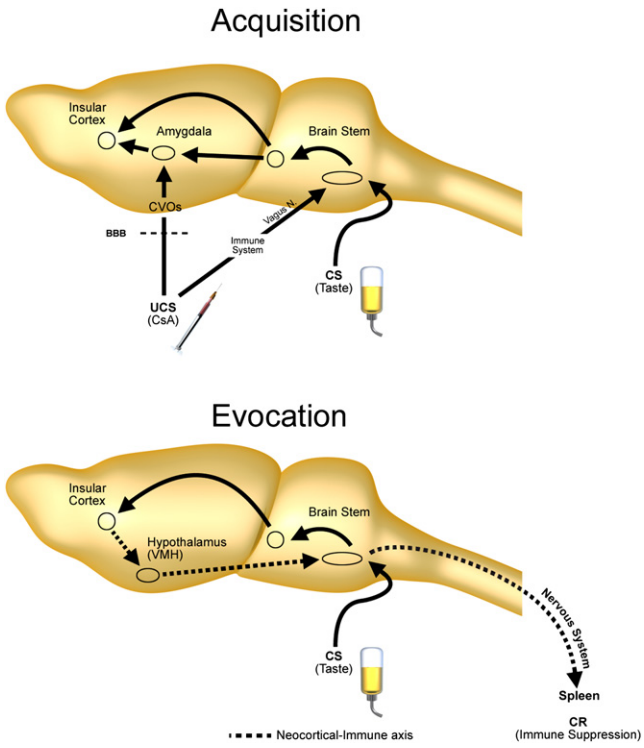

Figure 2. Neural Substrates Involved in Behaviorally Conditioned Immunosuppression in Rats

Brain excitotoxic lesions show that the insular cortex is essential to acquiring and evoking this conditioned immunosuppressive response. In contrast, the amygdala seems to mediate the input of visceral information necessary at acquisition time, whereas the ventromedial hypothalamic nucleus appears to participate in the output pathway to the immune system needed to evoke the behaviorally conditioned immune response (CS, conditioned stimulus, saccharin taste; UCS, unconditioned stimulus; CsA, cyclosporine A; BBB, blood-brain barrier; CVOs, circumventricular organs; VMH, ventromedial hypothalamic nucleus) (Pacheco-Lopez et al., 2005).

Similar conditioning mechanisms have been found in the endocrine system. In one study aimed at differentiating the effects of conditioning and expectation, plasma levels of both growth hormone and cortisol were measured in different conditions (Benedetti et al., 2003). In the first experimental condition, verbal suggestions of growth hormone increase and cortisol decrease were delivered to healthy volunteers, so as to make them expect hormonal changes. These verbal instructions did not have any effect on both hormones, and in fact no plasma concentration

change was detected. In the second experimental condition, sumatriptan, a serotonin 5-$HT_{1B/1D}$ receptor agonist that stimulates growth hormone and inhibits cortisol secretion, was administered for 2 days in a row and then replaced with a placebo on the third day. A significant increase of growth hormone and decrease of cortisol plasma concentrations were found after placebo administration. These conditioned effects occurred regardless of the verbal suggestions the subjects received. In other words, the placebo mimicked the sumatriptan-induced growth hormone increase, even though the subjects expected a growth hormone decrease. Likewise, the placebo mimicked the sumatriptan-induced cortisol decrease, even though the subjects expected a cortisol increase. It can be assumed that in this case the conditioned stimulus was represented by the act of injecting the pharmacological agent (i.e., the context around the treatment).

This experimental evidence demonstrates the potential applicability of such behavioral conditioning protocols in clinical practice. However, in future studies it will be necessary to analyze the kinetics of the behaviorally conditioned immunopharmacological and endocrine response and to elucidate whether and to what extent these conditioned responses can be reconditioned on multiple occasions. Only with this information and more detailed knowledge of the mechanisms behind the CNS-immune system and CNS-endocrine system interaction will it be possible to design conditioning protocols which can be employed in clinical situations to the patients' advantage.

Mechanisms of the Nocebo Effect

Compared to the placebo effect, much less is known about the nocebo effect, since the induction of a nocebo response represents a stressful and anxiogenic procedure, thus limiting its ethical investigation. The term nocebo ("I shall harm") was introduced in contraposition to the term placebo ("I shall please") by a number authors in order to distinguish the pleasing from the noxious effects of placebo (Kennedy, 1961; Kissel and Barrucand, 1964; Hahn, 1985, 1997). If the positive psychosocial context, which is typical of the placebo effect, is reversed, the nocebo effect can be studied. Therefore, it is important to stress that the study of the nocebo effect relates to the negative psychosocial context surrounding the treatment, and its neurobiological investigation is the analysis of the effects of this negative context on the patient's brain and body. As for the placebo effect, the nocebo effect follows the administration of an inert substance, along with the suggestion that the subject will get worse. However, the term nocebo-related effect can also be used whenever symptom worsening follows negative expectations without the administration of any inert substance (Benedetti et al., 2007b; Benedetti, 2008).

Brain imaging techniques have been crucial to understanding the neurobiology of negative expectations, and most of this research has been performed in the field of pain. Overall, negative expectations may result in the amplification of pain (Koyama et al., 1998; Price, 2000; Dannecker et al., 2003) and several brain regions, like the anterior cingulate cortex (ACC), the prefrontal cortex (PFC), and the insula, have been found to be activated during the anticipation of pain (Chua et al., 1999; Hsieh et al., 1999; Ploghaus et al., 1999; Porro et al., 2002, 2003; Koyama et al., 2005; Lorenz et al., 2005; Keltner et al., 2006). For example, Sawamoto et al. (2000) found that expectation of a painful stimulus amplified the perceived unpleasantness of innocuous thermal stimulation, and that these subjective hyperalgesic reports were accompanied by increased brain activations in the anterior cingulate cortex (ACC), the parietal operculum (PO), and posterior insula (PI). In another study by Koyama et al. (2005), as the magnitude of expected pain grew, activation increased in the thalamus, insula, PFC, and ACC. By contrast, expectations of decreased pain reduced activation of pain-related brain regions, like the primary somatosensory cortex, the insular cortex, and ACC. Likewise, Keltner et al. (2006) found that the level of expected pain intensity altered the perceived intensity of pain along with the activation of different brain regions, like the ipsilateral caudal ACC, the head of the caudate, the cerebellum, and the contralateral nucleus cuneiformis (nCF).

Besides neuroimaging, pharmacological studies give us insights into the biochemistry of the nocebo effect and of negative expectations. For example, the antagonist action of CCK on endogenous opioids (Benedetti, 1997) is particularly interesting in the light of the opposing effects of placebos and nocebos. A model has recently been proposed whereby the opioidergic and the CCK-ergic systems may be activated by opposite expectations of either analgesia or hyperalgesia, respectively. In other words, verbal suggestions of a positive outcome (pain decrease) activate endogenous μ-opioid neurotransmission, while suggestions of a negative outcome (pain increase) activate CCK-A and/or CCK-B receptors. This neurochemical view of the placebo-nocebo phenomenon, in which two opposite systems are activated by opposite expectations about pain, is in keeping with the opposite action of opioids and CCK in other studies (Benedetti et al., 2007a). Interestingly, the CCK-antagonist proglumide has been found to potentiate placebo-induced analgesia, an effect that is probably due to the blockade of the anti-opioid action of CCK (Benedetti et al., 1995; Benedetti, 1996). Therefore, CCK appears to play a pivotal role in the psychological modulation of pain, antagonizing placebo-induced opioid release on the one hand and mediating nocebo-induced facilitation of pain on the other hand.

The involvement of CCK in nocebo hyperalgesia is likely to be mediated by anxiety, as benzodiazepines have been found to block both nocebo-induced hyperalgesia and the typical anxiety-induced hypothalamus-pituitary-adrenal hyperactivity. Conversely, the CCK antagonist, proglumide, has been found to prevent nocebo hyperalgesia but not the hypothalamus-pituitary-adrenal hyperactivity, which suggests two independent biochemical pathways activated by nocebo suggestions and anxiety (Figure 3).

More recent studies have found that nocebo effects are also associated to a decrease in dopamine and opioid activity in the nucleus accumbens, thus underscoring the role of the reward and motivational circuits in nocebo effects as well (Scott et al., 2008). In other words, the activation/deactivation balance of both dopamine and opioids in the nucleus accumbens would account for the modulation of placebo and nocebo responses. Therefore, a complex interaction among different neurotransmitters, such as CCK, dopamine, and opioids, occurs when either placebos or nocebos are administered.

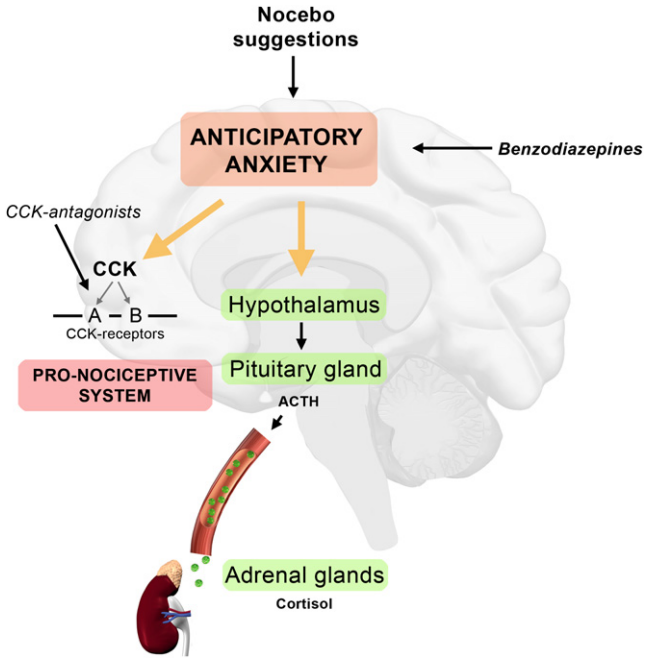

Figure 3. Mechanisms of the Hyperalgesic Nocebo Effect
Nocebo suggestions induce anticipatory anxiety, which activates two independent pathways, the hypothalamus-pituitary-adrenal (HPA) axis on the one hand and a CCK-ergic pronociceptive system on the other hand. Benzodiazepines act on anxiety, thus blocking both the HPA hyperactivity and the CCK pronociceptive system. In contrast, CCK antagonists act on the pronociceptive system only, thus preventing nocebo hyperalgesia but not HPA-hyperactivity (Benedetti et al., 2006). Note: the main propose of this sketch is to focus on neural substrates of the hyperalgesic nocebo effect which, in this case, takes precedence over anatomical accuracy.

Placebo Responses in Clinical Trials

Ever since the dawn of the first randomized placebo-controlled trials testing new drugs and treatments in the middle of the last century, and even before (Hill, 1990), placebo responses in clinical trials have given rise to discussion and concern regarding their mechanisms and have usually been regarded as a nuisance or a barrier to a rational approach in modern drug development. High placebo responses have induced false expectations regarding drug efficacy and resulted in the refusal of drug approval in some cases, e.g., neurokinins in the treatment of depression (Kramer et al., 1998; Enserink, 1999).

Not only do placebo responses in clinical trials impose significant limits to the testing of new compounds, but they are also linked to the drug adherence and compliance of patients in such trials in a paradoxical way. Patients that adhered to medication instructions by more than 80% showed better survival in a coronary disease study (Coronary Drug Project Research Group, 1980), and poor drug adherence in a myocardial infarction survivor study was associated with a higher risk of mortality (Beta-Blocker Heart Attack Trial, Horwitz et al., 1990), irrespective of whether the active compound or a placebo was taken, and regardless of other potential risk factors. This has been attributed to the greater expectancies or beliefs, both in drug and placebo responders that the medication may be of help, although other factors, such as health behaviors, cannot be ruled out completely. These findings have certainly fostered the development of further experimental approaches to the placebo phenomenon.

Attempts to unravel the mechanisms of the placebo response in clinical trials have used meta-analytic approaches of the placebo arm of trials—with mixed results. The placebo effect in randomized controlled trials has been reported to be around 40% in functional disorders (Enck and Klosterhalfen, 2005) but lower in depression (29%), bipolar mania (31%) (Sysko and Walsh, 2007), and migraine (21%) (Macedo et al., 2008). The reasons for these variable placebo response rates are unknown but may include the sample size (Enck and Klosterhalfen, 2005), the year of study (Walsh et al., 2002), design characteristics (Macedo et al., 2006), and recruitment pattern (Kobak et al., 2007). Meta-analyses can come to opposite conclusions on the same data set, e.g., with respect to the direction of the effects of the number of study visits on the placebo effect size (e.g., Pitz et al., 2005; Patel et al., 2005), but this may be due to data extraction errors that lead to false findings and conclusions (Gøtzsche et al., 2007). Hróbjartsson and Gøtzsche (2001, 2004) came to conclude that the placebo response appears to be powerful only because of a lack of "no treatment" control groups in most studies. However, their argument has been challenged by data indicating that among the trials they included into their meta-analyses, those with endpoints regulated directly by the autonomic nervous system do report stronger response to placebo treatment, while endocrine and other endpoints are less responsive (Meissner et al., 2007).

Other contributing factors to the placebo response rate in clinical trials were: the origin of patients—response rates in migraine prophylaxis were higher in Europeans than in North Americans (Macedo et al., 2008), personal expectations (Linde et al., 2007) and the loss thereof, e.g., in Alzheimer's disease (Benedetti et al., 2006), the study center (Ondo, 2007), and patient recruitment and physician training (Kobak et al., 2007). A genetic contribution to placebo responsiveness has been proposed (Bendesky and Sonabend, 2005; Raz, 2008) but empirical evidence is still lacking.

Because of the difficulties to reliably identify placebo responders and predicting placebo response rates in clinical trials, different methodological attempts have been made to the way (novel) drugs are tested against placebo.

The most traditional way to attempt to control for placebo response in clinical trials was the use of a crossover design, in which an individual patient serves as her/his own control, reducing the between-subject variability and the number of patients studied. This model was almost completely abolished due to the fact that blinding may be rather difficult in such studies (Boutron et al., 2006), unless one is able to implement "active placebos" that mimic the side-effects of a compound without inducing its main effects (Edward et al., 2005). Another conventional model to control for placebo effects is the use a placebo run-in phase prior to drug and placebo dispensing to identify and exclude placebo responders: placebo responders tend to exhibit less severe symptoms during run-in (Evans et al., 2004) and to respond faster to treatment with symptom improvement (Gomeni and Merlo-Pich, 2007) than patients in the drug arm. Drug-free run-in periods have also been used to identify

individual and group characteristics of placebo responders. However, these results are not generalizable across medical conditions, (Talleyn et al., 2006) since most of the variables that are regularly documented at study initiation are related to symptoms and disease characteristics rather than to individual personality traits or states (Hyland et al., 2007). An extension of placebo run-in periods are studies with multiple drug/placebo phases that alternate, with or without washout periods in between (Kleveland et al., 1985). These models were more recently requested again by drug approval authorities to account for variable symptom courses and the alternation of symptom-free with relapse periods in many chronic diseases. It has, however, been shown that the placebo response in a first medication period does not reliably predict the response (to drug or placebo) in a second phase (Tack et al., 2005). If being a placebo responder is a characteristic of an individual patient, study designs should take this into account by employing a design with multiple (>2) crossovers between placebo and drug and to randomize and individualize in a ''single-subject trials'' (SST) the timing for run-in and run-out for each phase (Madsen and Bytzer, 2002). In theory, this should allow us to reliably distinguish placebo responders from nonresponders. However, multiple crossovers with randomly assigned treatment periods, with a complete random order or a random starting day generate specific methodological problems and need new statistical models before being applicable in clinical drug testing.

In experimental laboratory research, a number of experimental designs have been employed that may help to identify predictors of the placebo response in the future. The so-called ''balanced placebo design'' (BPD) was traditionally used in the testing for placebo effects of frequently consumed everyday drugs such as caffeine, nicotine, and alcohol (e.g., Dagan and Doljansky, 2006; Kelemen and Kaighobadi, 2007; Cole-Harding and Michels, 2007). While one-half of the study sample receives placebo and the other half the drug, half of each group is receiving correct information while the other half is receiving false information on the nature of their study condition (drug or placebo) immediately prior to drug testing, thus allowing to differentiate between the ''true'' drug effect (those receiving the drug but are told they received placebo) and the true placebo effect (those receiving placebo but are told they received the drug). As is evident, the BPD implies ''deception'' of the subjects (Miller et al., 2005), which limits its suitability and acceptance outside the laboratory and in patients for ethical reasons (Ehni and Wiesing, 2008).

Hidden treatment (HT) or covert treatment is another option that may be specifically useful for the test of drug effects in acute and highly symptomatic conditions such as with postoperative pain (Levine et al., 1981), anxiety, and motor dysfunction in Parkinson's disease (Benedetti et al., 2004b; Lanotte et al., 2005). It resembles some of the features of the SSTs (Madsen and Bytzer, 2002). In case of HT, the patient may receive a drug unnoticed in terms of timing and dosage, and the drug effect (or its missing action) can be determined independent of the patient's expectations. Benedetti and colleagues demonstrated that under these circumstances drugs commonly believed to have analgesic properties such as CCK-antagonists failed to show any antinociceptive effects (Colloca et al., 2004). Evidently, HT can only be applied with the patient agreeing prior to the test that she/he may or may not receive a drug at all, which may raise other ethical concerns (Machado, 2005), especially with the test of novel compounds of unknown properties.

Finally, a free-choice paradigm (FCP), which maybe regarded as a modification of the adaptive response design (Rosenberger and Lachin, 1993) or the early-escape design (Vray et al., 2004) may offer an alternative approach to common drug test procedures. FCP allows the patient to choose between two pills, of which one is the drug and one the placebo, at medication-dispensing time; it is, however, essential that the patient does not take both pills at the same time (hence, a technical or administrative modus has to be implemented to prevent this and to prevent over-dosage etc.), and that he/she may switch to the other condition at any time (hence, the pharmacodynamics of the compound under investigation have to be appropriate, e.g., the speed of action, the feasibility of on-demand medication, etc.). It would, on the other hand, allow assessment of drug efficacy via the choice behavior rather than with symptomatic endpoints. The FCP has been used occasionally in optimizing dosage of drugs (Perkins et al., 1997; Pinsger et al., 2006) in clinical trials. It bypasses many of the ethical concerns against the use of placebos (Ehni and Wiesing, 2008), but its methodology and statistics in assessing drug superiority over placebo have not been validated (Zhang and Rosenberger, 2006).

Research Questions for Future Research

The experimental work on the neurobiological and neuropsychological mechanisms of the placebo/nocebo response from the last decade has impressively increased our knowledge of this long-known phenomenon. It became clear that these approaches will not only help us to better understand human physiology but might have many practical consequences such as on the design of clinical studies, our health care systems, in particular the doctor-patient relationship as well as the education of medical care professionals. However, there are still numerous open questions which urgently need to be addressed in future studies.

The Relationship between Suggested and Conditioned Placebo Effects

It has been postulated that the placebo response is generated by two distinct mechanisms across clinical conditions, one of which concerns suggestion and expectation, and one learning via Pavlovian conditioning (Benedetti et al., 2003; Klosterhalfen and Enck, 2006). The relationship between these two is still unclear, but it has been the subject of experimental research in recent years. Benedetti et al. (2003) were able to demonstrate in experimental pain and in Parkinson's disease that conditioning is actually mediated by expectations and that expectations do not affect conditioned responses. Similar explanations have been put forward, for example, that expectancies acquired through verbal instructions might also be seen as conditioning stimuli that reactivate earlier stimulus association (Klinger et al., 2007).

In a set of experiments, it has recently been demonstrated that prior experience is able to shape placebo analgesia (Colloca and Benedetti, 2006). Subjects that were conditioned to experience placebo analgesia in an acute paradigm showed reduced pain experiences for up to seven days and exhibited no extinction

of responses in the range of minutes. However, placebo analgesia was reduced by prior exposure to negative painful experience. These data emphasize that previous experience with the treatment of pain, both successful and unsuccessful, will have lasting effects on how the second and subsequent treatments of the same conditions are perceived. The analogy to clinical conditions is evident, but relative. While experimental pain is phasic and acute, clinical pain is usually chronic, long-lasting. Whether and to what degree previous pain treatment contributes to the experience of placebo analgesia in a clinical trial—usually 15%–20% of the effect size achieved under experimental pain conditions (Vase et al., 2002)—probably needs to be tested with a different experimental or clinical design. When experimental placebo analgesia was directly compared to pain relief in pain patients, the data suggested that mechanisms counteracting the proanalgesic effects of placebo suggestions are involved (Charron et al., 2006).

It is puzzling to realize that, beyond the laws of Pavlovian learning studied for almost a century now, there is basically no model available that allows us to predict the maintenance of a strong placebo response in a clinical trial that may last for a year or longer (e.g., Chey et al., 2004). According to these laws (Zimmer-Hart and Rescorla, 1974), any conditioned response should diminish over time if no further pairing of the UCS (e.g., an effective drug) and the CS (a pill or injection) occurs but the CS is presented alone. In such trials, extinction does not seem to occur at all. Hence, one may speculate that if conditioning (learning) is part of this placebo response, it cannot be of a Pavlovian nature. Alternatively, in the case of newly developed compound, previous experience with a drug, or a similar compound, that might shape the response can have been gained only by generalization.

The other issue that requires attention is the clinical applicability of conditioned and suggested placebo responses in daily medicine, as many of the studies have so far been conducted in the laboratory and with healthy subjects. One example of a successful transfer from bench to bedside, however, has been documented by studies demonstrating behaviorally conditioned effects in peripheral immune responses (see above).

Is There a Consistent Brain Network for All Placebo Effects?

The number of brain imaging studies on the placebo response has increased greatly over the past few years, in particular in the area of pain and placebo analgesia (Petrovic et al., 2002; Wager et al., 2004; Bingel et al., 2006; Kong et al., 2006; Price et al., 2006), but also to a lesser degree with regard to neurological and psychiatric diseases, such as Parkinson's disease, depression, or irritable bowel disorder (reviewed, e.g., by Benedetti et al., 1995; Colloca and Benedetti, 2005; Beauregard, 2007; Lidstone and Stoessl, 2007, Enck and Klosterhalfen, 2005).

As to experimental pain, different cortical (prefrontal cortex, anterior cingulate gyrus, insula, supplementary motor area), and subcortical structures (amygdala, periacqueductal gray, thalamus) have been found to be involved in the placebo response, and they seem to differentiate between the sensory and the emotional/affective components of pain signals. PET receptor-binding studies have provided direct evidence that the μ-opioid system involving the brain stem and elaborated cortical networks mediates placebo analgesia (Zubieta et al., 2005; Wager et al., 2007), thus confirming previous studies on the blockade of placebo analgesia by the opioid antagonist naloxone (Levine et al., 1978; Amanzio and Benedetti, 1999). It should be noted that other neurochemical systems have been found to contribute to the placebo effect, e.g., the dopaminergic system (Scott et al., 2007, 2008) and CCK (Benedetti et al., 1995; Benedetti, 1996). It remains unclear, however, whether each of these systems contributes to all placebo responses or only to those under specific clinical and experimental conditions. Placebo responses in Parkinson's disease and pain have been linked to a subcortical dopaminergic "reward" in the ventral striatum (de la Fuente-Fernández et al., 2001; Scott et al., 2007); however, the involvement of dopamine was recently questioned with regard to the placebo response in experimental pain (Martikainen et al., 2005). Nevertheless, it is worth mentioning that a possible downstream effect of dopamine activation after placebo administration was found in the subthalamic nucleus, in which single neurons changed their firing pattern (Benedetti et al., 2004a).

It is one of the drawbacks of imaging studies that they rely on a stable and dominant activation pattern across all subjects, since group means are necessary for adequate data analysis. Therefore, placebo nonresponders in small samples of subjects are frequently excluded or used as a type of control (Petrovic et al., 2002; Leuchter et al., 2002; Nemoto et al., 2007). Assessment of individual responsiveness to placebo (Chung et al., 2007) is, however, necessary to advance the field.

Other neurophysiological and psychobiological mechanisms of placebo analgesia and placebo response are currently being discussed. Placebo analgesia following heat pain application may change spinal cord pain processing via descending pathways (Matre et al., 2006), and expectations have been found to alter spinal reflexes and the descending noxious inhibitory control (Goffaux et al., 2007). This raises an important issue that needs to be addressed in future research: While for expectation-induced placebo responses, higher centers of the CNS are needed, Pavlovian conditioning may also occur within the peripheral neural circuitry, e.g., within the enteric nervous system (Drucker and Sclafani, 1997). Whether this also relates to conditioned placebo responses warrants further research.

The Role of Gender in Placebo Effects

Gender effects of the placebo response have rarely been documented in clinical trials but have occasionally been noted in experimental settings (Flaten et al., 2006). However, whether and to what extent gender differences may account for some of the variance in the placebo imaging studies is unknown so far. Cortical processing, independent of the placebo response, has shown significant gender variation both in volunteers and in patients with somatic and visceral pain (Paulson et al., 1998; Berman et al., 2000) and with nonpainful stimuli (Sabatinelli et al., 2004; Gizewski et al., 2006). Unfortunately, most imaging studies on the placebo response have ignored the potential role of gender (Klosterhalfen and Enck, 2008).

Gender effects in the placebo response were reported in an experimental setting with placebo analgesia during ischemic pain, whereby males responded to the manipulation of expectancies through pain information, while women did not (Flaten et al., 2006). However, an experimenter effect could not be

excluded, as all the experimenters were female nurses, which could have induced a reporting bias (Kallai et al., 2004). Gender effects were also noted in an acupuncture trial with male and female acupuncturists, with females inducing greater trust than male experimenters (White et al., 2003). Employing a motion-sickness paradigm, conditioning was effective predominantly in women, while in the suggestion experiment, men exhibited a significantly greater reduction in rotation tolerance and responded more strongly to rotation and to suggestions than women (Klosterhalfen et al., 2007). However, other data from this group pointed toward the role of biological factors (e.g., the menstrual cycle) on processing of visceral and vestibular sensations (Klosterhalfen et al., 2008b) and on differential effects of stress hormone release on nausea and motion sickness (Rohleder et al., 2006). These observations clearly show the necessity to investigate gender effects in the placebo and nocebo responses.

The Impact of Obtaining Drug-like Effects without Drugs

One of the most practical implications of the recent neurobiological advances in placebo research is the possibility to induce, at least in some circumstances, drug-like effects without the administration of drugs. Throughout this review we have seen that placebos can induce the activation of endogenous opioids and dopamine, that placebo-conditioned responses of several immune mediators can be obtained through behavioral conditioning, and that nocebos activate the endogenous CCK-ergic systems. The obvious consequence of these findings is their exploitation both in the clinic and in other areas of society, although important ethical constraints have so far limited the development of therapeutic paradigms with placebos.

As far as the clinic is concerned, it would be conceivable today to use a translational approach whereby many experimental protocols, so far carried out in animals and healthy volunteers, could be applied to real medical conditions. For example, there is compelling evidence that pharmacological conditioning can induce powerful placebo responses when the real drug is replaced with a placebo. This phenomenon is well documented in humans, for example in pain (Amanzio and Benedetti, 1999), the immune system (Goebel et al., 2002), and the endocrine and motor systems (Benedetti et al., 2003), although unfortunately no systematic investigation has been done in a real clinical setting. There are, however, some indications that the application of placebo-induced drug-like effects without drugs is possible in the clinic. For example, Benedetti et al. (2004a) conditioned Parkinson's patients with repeated administrations of the anti-Parkinson's drug apomorphin before the surgical implantation of electrodes for deep brain stimulation. Then, the investigators replaced apomorphin with a placebo in the operating room and obtained a powerful placebo reduction of muscle rigidity that mimicked the effects of apomorphin during the previous days. Although the effect was short-lasting (no longer than 20–30 min), it was useful from a clinical point of view because the patient improved and felt better for a while, thus making some surgical procedures easier and faster. These drug-mimicking effects could be particularly useful whenever the drug has important side effects. For example, in the study by Benedetti et al. (2004a), the presurgical apomorphin resulted in both clinical improvement and some side effects, like dyskinesia, whereas the placebo in the operating room induced improvement but not dyskinesia.

Besides the clinic, there are also some other areas of society in which the drug-like effects of placebos may have a strong impact. In a very recent study, Benedetti et al. (2007b) used placebos in an experimental simulation of a sporting event, whereby a placebo was given on the competition day after preconditioning with a narcotic in the training phase. In fact, after repeated administrations of morphine in the training phase, its replacement with a placebo on the day of the competition induced an opioid-mediated increase in pain endurance and physical performance, even though no illegal drug was administered. This shows that athletes can be preconditioned with narcotics and then a placebo given just before the competition, thus avoiding the administration of illegal drugs on the competition day. These narcotic-like effects of placebos raise the important question of whether opioid-mediated placebo responses are ethically acceptable in sport or whether they should rather be considered as a doping procedure in all respects. In the light of the distinction between drugs that are prohibited during and/or out of competition, the preconditioning procedure may be deemed ethical and legal for drugs that are prohibited only during competition, like narcotics (World Anti-Doping Agency 2007, www.wada.ama.org). However, it may also be considered illegal because morphine administration is aimed at conditioning the subjects for subsequent replacement with a placebo, which is supposed to show morphine-like effects during the competition. This issue is not easy to be resolved and needs both an ethical and a legal discussion. In fact, doping is a matter of great public concern today, and we should be aware that if a procedure like the one described by Benedetti et al. (2007b) is performed, illegal drugs in sport would no longer be discoverable, nor would they violate the current antidoping rules.

Where Does Placebo Research Go from Here?

Despite the recent explosion of neurobiological placebo research using sophisticated tools, such as neuroimaging, in vivo receptor binding, and single-neuron recording in awake subjects, our knowledge of the mechanisms underlying the placebo effect is still in its infancy, and several issues need to be addressed in future research. The major questions to be answered are where, when, how, and why placebo effects occur. In fact, we need to know where they work exactly, that is, in which medical conditions. For example, are all diseases and symptoms subject to placebo effects? We also need to know when they work, that is, whether there are special circumstances that are particularly amenable to placebo effects. How they work is also a major question, as we need to understand the brain mechanisms at both the macroscopic (brain regions and their interactions with body functions) and microscopic (cellular and molecular) level. Finally, determining why placebo effects exist at all represents a major scientific challenge, and meeting that challenge will give us insights into the possible evolution of endogenous healthcare systems.

Besides the profound implications of placebo research for a better understanding of human biology, some practical aspects should not be forgotten. For example, placebo and nocebo

phenomena are a major hurdle in the development and validation of new treatments, as high placebo responses sometimes distort the effects of a therapy. If we can identify in more detail the major mechanisms involved in placebo responsiveness, we could also develop strategies aimed at minimizing placebo effects, thereby uncovering the real effect of a therapy. Likewise, nocebo effects can be a serious drawback, as negative reactions to drugs are sometimes due to psychological effects rather than to specific negative effects of the drug itself. Therefore, research aimed at investigating nocebo mechanisms would enable us to disentangle the negative effects of the drug from those of the psychological state of the patient. In addition, a better understanding of the neurobiology of the placebo and nocebo responses will form the basis for designing behavioral protocols that can be employed as supportive therapy together with standard pharmacological regimen, the aim being to maximize the therapeutic outcome for the patient's benefit.

We believe that the future years will be characterized by a deeper understanding of both the placebo and nocebo phenomena, which in turn will give us profound insights into many aspects of human biology.

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