Increasing uncertainty in CNS clinical trials: The role of placebo, nocebo, and Hawthorne effects

Placebo, nocebo, Hawthorne effects and the increasing uncertainty in CNS clinical trials

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Search strategy and selection criteria

We searched Pubmed for papers published in English between Jan 1, 2000 and Dec 31, 2015, with the following search terms: “placebo effect”, “placebo response”, “placebo analgesia”, “nocebo effect”, “nocebo response”, “nocebo hyperalgesia”, “Hawthorne effect”. Then we selected those studies aimed at investigating the mechanisms and at discussing some methodological issues of CNS clinical trials. Since this review is aimed at discussing some recent findings on the role of placebo, nocebo and Hawthorne effects in the interpretation of CNS clinical trials, we selected mainly those studies published between 2010 and 2015, but did not exclude frequently referenced and highly regarded older publications, particularly those that help understand the more recent findings. Moreover, we discarded all the studies whose main objective was to merely compare verum to placebo.

Panel 1: definitions

Placebo: A placebo is an inert treatment, be it pharmacological or not, with no specific therapeutic properties for the condition being treated. A placebo effect, or response, may follow the administration of the inert treatment. The main source of confusion about this word depends on its different meaning for the clinical trialist and the neuroscientist/psychologist. Whereas the former considers a placebo effect, or response, any improvement that may take place after placebo administration, including spontaneous remission, regression to the mean, patient's and/or experimenter's biases, the latter considers only those psychobiological factors occurring in the patient's brain, e.g. expectations of therapeutic benefit.[1]

Nocebo: A nocebo is opposite to placebo, that is, it is related to negative outcomes rather than to positive therapeutic outcomes. Therefore, for the neuroscientist it is related to negative expectations of clinical worsening.[1]

Expectation: The patient's expectations play a crucial role in the placebo response,[2] and the term "placebo effect" is often replaced with the word "expectation effect", even though this is not completely correct. In fact, expectation is the most important mechanism that mediates placebo effects, yet not the only one.[1]

Hawthorne effect: This term is used in clinical research to indicate that individuals improve in response to their awareness of being under study. In other words, any improvement that may take place when recruited in a trial might be attributable to several factors, including better attention, better observation, better care, better compliance, better adherence.[3]

Uncertainty in physics and biology: The concept of uncertainty is best explained in physics by the Heisenberg uncertainty principle. This imposes limits on the precision of a measurement, by asserting that a dynamical disturbance is necessarily induced in a system by the measurement *per se*. Whilst this holds true at the subatomic level, i.e. in microsystems, it can also be applied at the level of biological macrosystems, whereby the very act of measuring may change the biological macrosystem itself.[4]

Uncertainty in clinical trials and healthcare: Today experimental and clinical evidence suggests that an important component of the uncertainty in CNS clinical trials derives from expectation-related placebo responses, whereby the effect of a molecule (the drug) upon a biological system (the patient) may be affected by the very act of administering the treatment. In other words, the patient's expectations of clinical improvement may interfere with the specific therapeutic effects of both pharmacological and nonpharmacological treatments.

Abstract

As modern placebo research is digging more and more into the details of the placebo phenomenon in CNS disorders, the uncertainty about therapeutic outcomes in several neurological conditions is increasingly growing. Our current understanding of the mechanisms of different placebo effects emphasizes how the interpretation of CNS clinical trials may be very difficult and challenging. In the past few years, new mechanisms and new concepts have emerged in the study of placebo, nocebo and Hawthorne effects in CNS clinical trials. For example, the mere recruitment or social interaction in a trial may change the baseline conditions, and different genotypes have been found to respond differently to placebos, for example in social anxiety, depression and pain. The very existence of all these factors in the general population raises the question whether it is correct to reduce placebo responses in CNS clinical trials.

Introduction

The very act of measuring something in humans can modulate the expectations of the person who is being measured, which in turn may change behaviors, attitudes and body physiology,[1,2] thus leading to uncertainty (panel 1) in the measurement. This notion of uncertainty is applicable to clinical trials, in which the effects of a treatment (a drug or a non-pharmacological intervention) upon the patient is usually assessed and measured. In this case, the patient's expectations of therapeutic benefit might interfere with the response to drug administration.[4] Indeed, uncertainty pervades clinical trials, particularly CNS trials. As placebo research is digging into the details of the placebo phenomenon more and more, the uncertainty about outcomes in CNS clinical trial is progressively growing. For example, the failure of many recent neurological and psychiatric clinical trials—eg, in pain, Parkinson's disease, and schizophrenia—is related to the very high placebo responses reported across different studies. This finding emphasizes the urgent need to better understand placebo effects within the context of CNS disorders, in order to better design CNS clinical trials. The increasing understanding of placebo and nocebo effects in clinical research and clinical practice will certainly lead to more effective treatments for patients as well as to better medical practice.

This is not a comprehensive review of clinical trial methodology or placebo mechanisms, but rather an overview of recent findings on placebo, nocebo and Hawthorne effects (panel 1) that highlight some new mechanisms and concepts that may contribute to uncertainty in clinical trials.[3,4] In particular, in this review we will outline the problem of uncertainty in CNS clinical trials and suggest possible ways to improve their design, first and foremost by assessing patients' expectations. We will also consider ways in which these issues could be addressed in future studies.

The uncertainty of drug action

The concept of uncertainty in measuring therapeutic outcomes (panel 1) is best evidenced in a clinical trial of postoperative pain that was performed in 1995, in which the cholecystokinin antagonist proglumide was shown to be better than placebo for relieving pain (Fig. 1A).[5] These results seemed to indicate that proglumide is a good painkiller. However, this conclusion proved

to be erroneous, as proglumide was totally ineffective when the very act of proglumide administration was eliminated by means of a hidden injection (unbeknownst to the patient). How is it possible that a drug seems to work as a painkiller when administered overtly accordingly to routine clinical practice, whereas it shows no analgesic action when the patient does not know that he is receiving it? The explanation is that proglumide does not act as a painkiller but, rather, as a placebo (or expectation) enhancer by blocking the cholecystokinin receptors. This in turn potentiates the opioid-mediated placebo analgesic response. In other words, proglumide induces a reduction in pain if, and only if, it is associated with a placebo procedure.[5]

This trial clearly shows how the uncertainty principle can be applied to biological macrosystems, and more specifically to the therapeutic setting and clinical trials. In fact, the very act of administering a drug and measuring its effects can activate the patient's expectations of analgesia, with the drug offering some benefit not by an action on pain pathways, but through the mechanisms of expectation. Of course, this leads to the erroneous interpretation of the outcome, ie the outcome is pain relief, but the interpretation that the drug is an effective analgesic is wrong.

This effect of expectation on drug action has been confirmed recently for narcotic drugs.[6,7] Expectation of remifentanil (the patient gets remifentanil and is told it is remifentanil) produces more pronounced analgesic effects compared to no-expectation (gets remifentanil, told saline) (Fig. 1B). Moreover, expectation of interruption (gets remifentanil, told its administration has been interrupted) , abolishes the overall analgesic effect. These effects are associated with activity in the dorsolateral prefrontal cortex and pregenual anterior cingulate cortex in the positive expectation condition, and with activity in the hippocampus in the negative expectation of interruption.

In light of the recent findings on the neurobiology of placebo and expectation, which represents one of the main mechanisms of placebo responses (panel 1) and whereby different neurotransmitters have been found to be involved,[8-13] the interference between pharmacodynamic and psychological (placebo) effects could take place in the following way. A drug, e.g. a narcotic, binds to the opioid receptors, but the act of its administration induces expectations of benefit that activate endogenous ligands, which in turn may compete with the narcotic for the same receptors, thereby interfering with its action. The only way to eliminate this

interference is the administration of the narcotic unbeknownst to the patient, which eliminates the ritual of the therapeutic act and thus the positive expectations.[14,15]

This pharmacodynamics/placebo interference can envisage at least two possible mechanisms: drugs and placebos can act either on the same receptors or, otherwise, on the same type of receptor but in different regions of the brain.[16] There is some experimental evidence that the second mechanism is more likely. For example, narcotics bind to the mu opioid receptors in one region of the brain, whereas placebos act, through the activation of endogenous ligands, on mu opioid receptors in a different region, with an overall additive effect.[17] None the less, as far as we know today, an interactive and synergic relation between endogenous ligands and narcotics cannot be ruled out completely.[18] Whatever the case, this points raises several ethical and methodological questions that requires further research and discussion. For example, what about using a drug A in clinical practice that amplifies placebo effects and results in better outcome than a drug B whose action is purely based on disease-specific influences? Although to date we have no idea of which drugs can modulate placebo effects, virtually all drugs are potentially capable of such an action, and this possibility should stimulate further pharmacological investigation in this direction.

The uncertainty of drug action embraces adverse events as well, whereby the patients who take placebos often show side effects (nocebo effects). For example, in clinical trials of three classes of antimigraine drugs—nonsteroidal anti-inflammatory drugs, triptans and anticonvulsants—the adverse events reported in the placebo arms of these trials corresponded to those of the antimigraine medication against which the placebo was compared.[19] In fact, anorexia and memory difficulties, which are typical adverse events of anticonvulsants, were present only in the placebo arm of these trials.[19] These results suggest that the adverse events in placebo arms of clinical trials of antimigraine medications depend on the adverse events of the active medication against which the placebo is compared. These findings are in keeping with the expectation mechanism of placebo and nocebo effects. Similar findings have been found for depression,[20,21] headache,[22] fibromyalgia,[23] diabetic peripheral neuropathy,[24] neuropathic pain.[25] This is a major drawback, because drop outs due to nocebo effects may confound the interpretation of many clinical trials.

It should be noted that the clinical trial setting is not appropriate to understand the mechanisms of placebo and nocebo effects, as many factors may contribute to the improvement that may take

place after placebo administration, such as spontaneous remission, regression to the mean, selection biases, experimenter's measurement biases, patient's report biases.[1] By contrast, these elements can be ruled out in the experimental setting, in which genuine psychobiological placebo effects can be identified. This typical experimental approach has been translated to the clinical arena for some medical conditions, such that patients have been studied under strictly controlled laboratory conditions. To date, we know some neurobiological mechanisms of genuine placebo responses only in a few CNS disorders and therapeutic interventions, such as pain, Parkinson's disease, depression, anxiety, addiction, deep brain stimulation, Alzheimer's disease, headache (panel 2).[1,26]

Failed trials and the progressive increase of uncertainty

Many failures to detect a significant difference between the effects of medication and placebo are due to very high placebo responses, although the mechanism of high placebo responsiveness is unknown in most of the cases. Failure to demonstrate benefit over placebo in CNS clinical trials has become the rule over the past years, and pain is certainly one of the conditions in which the rate of failed trials is very high. Failed analgesic trials, in which the outcome does not prove the efficacy of a treatment, involve drugs like lamotrigine for the treatment of mixed neuropathic pain conditions,[27] levetiracetam for post-herpetic neuralgia,[28] lacosamide for painful peripheral diabetic neuropathy,[29] pregabalin for painful HIV neuropathy,[30] just to mention a few.

The number of drugs that are axed after Phase II/III clinical trials because they are not better than placebos is huge. For example, the drugs for the treatment of neuropathic and cancer pain that were dropped because of the failure rate of putative new painkiller has been over 90% in the past ten years.[31,32] In 2011, Clinicaltrials.gov listed 4152 pain trials, yet in a time window of 3 years, the only new approvals were for already existing drugs, e.g. duloxetine, oxycodone, and fentanyl in new formulations or dosage forms.[32] In neuropathic pain, the medication-placebo difference is greater when studies were published earlier, and this is attributable to larger placebo responses in more recent studies and to longer and larger trials.[33,34] This lack of benefit of painkillers over placebo echoes the findings of clinical trials in depression,[35] suggesting similarities in patients' placebo responses between neuropathic pain and neuropsychiatric disorders.[36] For example, evidence of significant and increasing rates of placebo responses in antidepressant trials has been

documented in several studies,[35,37,38] although the reason of this increase is not clear: one explanation could be the greater participant expectation from clinical trials, but this surely requires further research. In this regard, it is worth mentioning a less recent study by Kirsch and Sapirstein,[39] in which the response to placebo was always found to be 75% of the response to verum antidepressant therapy, regardless of antidepressant class/mechanism, thus indicating that the effects of all antidepressants are much smaller than the effects of placebo.

The increase in placebo responses over time has also occurred in other brain disorders, such as schizophrenia.[40] Supportive evidence for this was found in a comparison of placebo effect observed in studies from two different phase III clinical development programs that were used to support registration of two antipsychotic medications. These programs were completed about ten years apart and had similar designs. In these trials, placebo effects measured by amount of reduction in the Positive and Negative Syndrome Scale (PANSS) total score increased over time.[41] In a different study, ten schizophrenia drug programs that were submitted to the US Food and Drug Administration between December 1993 and December 2005 were identified.[42] The investigators identified 31 trials (22 positive and 9 negative) that included 12,585 patients from 37 countries (64% from North America). In the US trials, placebo effects as measured by reduction in the PANSS total score increased over time, with no apparent trend over time observed in the non-US or 'mixed' trials. Unfortunately, nothing is known about this difference, and a future challenge will be to understand it. In addition, it would be interesting to assess expectations in schizophrenic patients in the context of drug trials, although this certainly represents a difficult task.

Interestingly, an increase in nocebo responses (adverse events in placebo-treated patients) over the past years has been found in multiple sclerosis. Papadopoulos and Mitsikostas[43] conducted a meta-analysis of multiple sclerosis trials published between 1989 and 2009, and the incidence of nocebo responses was performed by pooling the percentage of placebo-treated patients that exhibited adverse events. A total of 56 disease-modifying treatment trials (i.e., affecting the time course of the disease) and 44 symptomatic treatment trials (i.e., affecting only the symptoms and not the disease itself) were analysed. The incidence of nocebo responses was 74·4% in disease-modifying treatment trials and 25·3% in symptomatic treatment trials, and nocebo severity in disease-modifying treatment trials was correlated to the year of publication, with stronger nocebo responses in more recent clinical trials. Again, the nature of such an increase is not known, although it is tempting to speculate that the high rate of nocebo responses in disease-modifying

treatment trials occurs because the active treatment is often toxic and the consent form is worded to reflect this.

Substantial improvements in placebo groups of many clinical trials of Parkinson's disease have been observed,[44,45] and many recent active treatments have failed beating placebos. For example, placebo data from two studies comparing sarizotan to placebo for the management of dyskinesia showed that whereas sarizotan did not show any difference compared to placebo, both sarizotan and placebo ameliorated dyskinesia compared to baseline. Older age, lower baseline parkinsonism score, and lower total daily levodopa doses were related to placebo improvement, whereas lower baseline dyskinesia was associated with worsening of symptoms for those taking placebo.[46] In addition, Goetz and colleagues examined rates and timing of placebo responses to identify patient- and study-based characteristics, ie factors that affect placebo, predicting positive placebo response in several clinical trials.[47] The authors collected individual patient data from the placebo groups of 11 medical and surgical treatment trials involving Parkinson patients with differing disease severities and placebo-assignment likelihoods. A total of 858 patients on placebo met inclusion criteria for analysis. The overall placebo response rate was 16% (range: 0–55%). Placebo responses were temporally distributed similarly during early, mid, and late phases of follow-up. Substantial improvements in patients who receive placebo are also present in surgical treatments of Parkinson's disease. For example, in a study on the effect of intrastriatal implantation of fetal porcine ventral mesencephalic tissue to treat Parkinson's disease, the degree of motor performance improvement at 18 months was substantial in both the real surgery group and the sham surgery group.[48] In one multicenter, randomized, double-blind, sham-surgery-controlled study of human fetal transplantation there was no difference between the transplant and the sham surgery group.[49]

Another example of failure in CNS clinical trials is represented by the more recent gene therapies. Although nine gene therapy clinical trials for Parkinson's disease have been initiated and completed over the past decade, either placebo-controlled or uncontrolled, none has yet demonstrated sufficiently robust clinical efficacy.[50] These include studies in which the targets were the subthalamic nucleus,[51] putamen,[52,53] putamen and substantia nigra.[54] Although the gene therapy approach is different from the traditional drug development, for example in establishing initial dosing and in quantifying targeting success, it certainly requires improvement in clinical trial design and a better selection of outcome measures.

These are just a few examples of CNS clinical trial failures, whereby a substantial increase of placebo responses has occurred over the past years or the active treatment did not beat the placebo. Although the nature of all these failures encompasses many factors, the magnitude of the placebo responses plays a crucial role in many cases. Placebo responses are constituted of many elements, which are not easy to identify across different studies. However, it is interesting to note that, at least in some circumstances, when patients' expectations are used to dichotomize the results, those patients who have high expectations perceive the treatment as efficacious, even if it lacks of specific effects. The following are two examples, the first from a trial for Parkinson's disease, the second from a trial on pain.

In a clinical trial of human fetal mesencephalic transplantation for Parkinson's disease, McRae and colleagues studied the effect of this treatment compared with placebo treatment for 12 months. They also assessed the patient's perceived assignment to either the active (fetal tissue implant) or placebo treatment (sham surgery). There were no differences between the transplant and sham surgery groups on several outcome measures, such as physical and quality of life scores. However, the perceived assignment of treatment group had a beneficial impact on the overall outcome and this difference was still present 12 months after surgery. Patients who believed they received transplanted tissue had significant improvements in both their quality of life and motor outcomes, regardless of whether they received sham surgery or fetal tissue implantations.[55] It is worth noting that the patients' perceived treatment assignment had an impact on objective measures of motor function, and not only on subjective outcome measures.

The effects of the perceived assignment, and hence of the expectation of therapeutic benefit, hold true for pain as well. In one clinical trial, real acupuncture was compared to sham acupuncture and no difference was found between the two groups. However, when patients were asked which group they believed they belonged to (either placebo or real treatment), patients who believed they belonged to the real treatment group experienced larger clinical improvement than those who believed they belonged to the placebo group.[56] In another clinical trial, patients were asked whether they considered acupuncture to be an effective therapy in general and what they personally expected from the treatment. Patients with higher expectations about acupuncture experienced larger clinical benefits than those with lower expectations, regardless of their allocation to real or sham groups.[57] It did not really matter whether the patients actually received the real or the sham procedure–what mattered was whether they believed in acupuncture and expected a benefit from it.

These findings clearly show that a major confounding element in clinical trials is the patient's expectation, which represents one of the main mechanisms of the placebo response (panel 1). The therapeutic outcome can go, at least in part and in some circumstances, in the same direction of the patient's expectations. In other words, in some clinical trials patients get what they expect. If expectations are not assessed, uncertainty about the outcome may be high. Younger and colleagues created a six items scale for measuring positive and negative treatment expectancies, and found that this assessment was capable of predicting between 12% and 18% outcome variance in pain patients.[58] Therefore, patients' expectations can *per se* produce an improvement, and this indicates that patients' attitude and satisfaction matter in clinical practice and need to be carefully valued. In the arena of clinical trials, it can be helpful to strategize ways to randomize patients based on their baseline attitudes, and to use patients' expectations as co-variables. As a consequence, a crucial question in any clinical trial should be: Which group do patients believe they belong to? Needless to say, blinding is crucial within this context. In the next sections we will show that expectations can be even more subtle and treacherous than previously supposed.

Changes in baseline clinical/biological parameters in the absence of any treatment

The mere recruitment in a clinical trial may trigger clinical and biological effects that are much stronger than so far supposed. Behavioral, clinical and biological parameters can change even before receiving a treatment, thus altering baseline parameters (baseline drift) of the population under study. Concerns about biases being introduced by having research participants complete questionnaires and receive tests have long been known by clinicians, psychologists and scientists.[59] Today clinical trialists know this phenomenon as the Hawthorne effect.[3] This term derives from Hawthorne Works, a factory outside Chicago, where a study was commissioned to see whether workers would become more productive in higher or lower levels of light. Although productivity improved when changes were made, it was suggested that the improvement occurred because the workers were under observation. Thereafter, the term was used in clinical research to indicate that individuals improve in response to their awareness of being under study. Indeed, any improvement that may take place when recruited in a trial might be attributable to several factors, including better attention, better observation, better care, better compliance, better adherence. Unfortunately, the concept of the Hawthorne effect has often been badly

interpreted and/or misunderstood in important issues of clinical trial methodology, for example in studies on surgical safety checklist to reduce morbidity and mortality, in which surgeons under observation may change their behavior and performance. Indeed, sophisticated studies of the Hawthorne effect are long overdue.[60]

Recent studies have found significant changes in baseline levels of several biological/physiological parameters between recruitment and the beginning of treatment, namely, much before any therapy had been started. For example, Cizza and colleagues evaluated the effects of study participation per se at the beginning of a sleep extension trial between screening and randomization in obese patients.[61] The participants were screened and returned for randomization after 81 days. Sleep duration, sleep quality, daily sleepiness, fasting glucose, insulin and lipids were measured. The authors found significant improvements between screening and randomization, well before any intervention. Sleep duration increased, sleep quality improved, insulin resistance decreased and lipids improved. Moreover, both abnormal fasting glucose and metabolic syndrome decreased. Therefore, improvements in biochemical and behavioral parameters between screening and randomization, before the administration of any treatment, changed the true study baseline, thereby potentially affecting outcome. Although the study by Cizza and colleagues[61] is interesting, only a few studies of this kind have been performed so far, thus further research is in order to confirm these findings.

The Hawthorne effect is a nice example of how a measurement may interfere with a biological system, thereby changing the baseline parameters. In other words, the very act of recruiting and measuring in a clinical trial leads to uncertainty in the therapeutic outcome (Fig. 2A). The awareness of being under study and observation may change behavioral, clinical and physiological parameters in many ways, such as better compliance and adherence as well as increased expectations of better improvement and better health. It is also worth noting that in clinical trials Hawthorne effect and regression to the mean effect are often intertwined. In fact, clinical trials are characterized by the fact that only people during a current state of suffering are included, so that the most likely progress for many clinical conditions is the reduction of symptoms. In other words, in the same way that the Hawthorne effect is related to participation biases, regression to the mean is related to patients selection biases. Although many factors are surely at work here, recent research suggests that social interaction among participants in a clinical trial may play a role, as shown by the following studies.

There is now compelling evidence that both placebo and nocebo responses can also be learned through social learning and that manipulation of expectations may induce mass psychogenic illness.[62] The observation of the beneficial effects in other people, for instance through the observation of pre-recorded or face-to-face facial expressions with different emotional content,[63,64] induces substantial placebo analgesic responses that are positively correlated with empathy scores.[65] The same holds true for nocebo effects.[66,67] After the observation phase, the experimental subjects who had been exposed to other subjects in pain show robust nocebo responses, i.e., hyperalgesic responses, and this can be correlated to either empathy scores[66] or pain catastrophizing,[67] thus suggesting that both social and psychological factors are crucial in nocebo hyperalgesia. These findings have implications for clinical trials, because the observation of others must be taken into consideration whenever a clinical trial is performed. Participants in a trial might be influenced by observing other patients belonging to the same trial, for example, by communicating with each other.

A recent study tested the possible propagation of negative information, and hence of negative expectations, across individuals, and the possible consequences in a clinical trial. High-altitude headache has been studied as a model (Fig. 2B).[68] In this experimental model, a subject (the trigger) received negative information about the risk of headache at high altitude and disseminated this negative information across a number of other subjects. After the retrospective assessment of the negative information received, the researchers found that in one week this negative information propagated across 36 subjects. This (nocebo) group showed a significant increase in headache and salivary prostaglandins, particularly PGE2, and thromboxane A2 when at high altitude compared to the control group. More interesting, this interindividual communication had a crucial role in the outcome of a clinical trial. In fact, the same authors ran two aspirin versus placebo clinical trials at high altitude for the control of high-altitude headache (Fig. 2B).[68] The first trial was performed in the control subjects, whereas the second trial was performed in the "socially infected" individuals. No placebo response was found in the first trial: aspirin was effective in reducing both pain and prostaglandins synthesis in the control subjects, whereas placebo was totally ineffective. By contrast, high placebo responses were found in the second trial: both aspirin and placebo reduced pain and prostaglandins in the socially infected individuals. The difference in placebo response between the two groups is attributable to the different baseline levels of headache pain and prostaglandins, e.g. PGE2, induced by the spread of negative information (Fig. 2B). A placebo effect was present only in the socially infected

individuals because the placebo acted only on the nocebo component of the prostaglandin and pain increase.[26,68]

Therefore, both the Hawthorne effect[61] (Fig. 2A) and the social propagation of expectations[68] (Fig. 2B) may influence the baseline of biochemical and behavioral parameters before patients are randomized to either placebo or active treatment, thereby potentially affecting the outcome of a study. Again, the very act of recruiting, measuring, and putting a person under study may trigger changes in his behavior and physiology.

Should we really reduce placebo responses in clinical trials?

By considering the large placebo effects in many CNS clinical trials and their increase over the past few years, as described above, today one of the priorities in clinical research is the development of designs that are aimed at reducing the placebo effect. There are many approaches of this kind.[69–71] For example, a placebo run-in phase is often used to identify placebo responders and to discard them from further randomization. In the placebo run-in design, patients are given a placebo treatment for several days, then those who respond to the placebo and those who show poor adherence are discarded. The remaining patients, who are mainly represented by placebo nonresponders and good adherers, are randomized to verum or placebo. In this way, the specific effect of the treatment under test is supposed to be better evidenced.

A variant of the placebo run-in design is represented by the sequential parallel comparative design (SPCD).[72] In the first part of the trial, a randomization is performed across patients to either verum or placebo. However, differently from other designs, more than half of patients are assigned to the placebo group. After the first part is over, placebo responders and nonresponders are identified. The patients who did not respond to placebo are re-randomized to another trial with half receiving verum and half placebo. The advantage of this design is that a large amount of patients are maintained in the second part of the trial, thus the statistical power is not lost. SPCD is particularly advantageous in trials that involve subjective outcome measures, such as those of antidepressants and analgesics. By using this design in a trial of major depressive

disorder, Fava and colleagues were able to reduce the placebo effect from 17% in the first part of the trial to 8% in the second part.[73]

Recent research on the genetics of the placebo responses also suggests a possible genetic screening of placebo responders and nonresponders, aimed at enrolling only placebo nonresponders in a clinical trial.[74,75] For example, in one study,[76] patients with social anxiety disorder were genotyped with respect to the serotonin transporter-linked polymorphic region (5-HTTLPR) and the G-703T polymorphism in the tryptophan hydroxylase-2 (TPH2) gene promoter. It was found that only those patients who were homozygous for the long allele of the 5-HTTLPR or the G variant of the TPH2 G-703T polymorphism showed robust placebo responses and reduced activity in the amygdala, as assessed by functional magnetic resonance imaging. Conversely, carriers of short or T alleles were identified as placebo nonresponders. In patients with major depressive disorder,[77] polymorphismsin genes encoding the catabolic enzymes catechol-O-methyltransferase (COMT) and monoamine oxidase A were examined. Small placebo responses were found in those patients with monoamine oxidase A G/T polymorphisms (rs6323) coding for the highest activity form of the enzyme (G or G/G). Similarly, lower placebo responses were found in those patients with ValMet catechol-O-methyltransferase polymorphisms coding for a lower-activity form of the enzyme (2 Met alleles). In addition, the COMT functional val158met polymorphism was found to be associated to the placebo effect in irritable bowel syndrome. The strongest placebo response occurred in met/met homozygotes.[78] More recently, the functional missense variant Pro129Thr of the gene coding fatty acid amide hydrolase (FAAH), the major degrading enzyme of endocannabinoids, has been found to affect the analgesic responses to placebo as well as placebo-induced mu opioid neurotransmission.[79]

What are the pros and cons of controlling and minimizing placebo responses in CNS clinical trials? This is a highly controversial issue in modern clinical trials. First of all, there are at least four reasons why we should be very cautious about the interpretation of the placebo run-in and SPCD trials, as well as about eliminating placebo responders on the basis of a genetic screening.[80] The first reason is that the degree of placebo responsiveness may vary from one time to another within the same individual.[1]

The second reason is that reducing placebo responses may not necessarily increase assay sensitivity. In fact, a randomization of placebo nonresponders to placebo and verum may lead to

low placebo responses in both groups, with no real advantage (Fig. 3A). In other words, the response may change in both groups, with no change in the difference between verum and placebo group. Indeed, the use of a single-blind placebo run-in period has been found to have limited utility, since it does not appreciably differ in terms of placebo response or in detecting treatment differences, compared to trials that do not use such an approach.[81] However, it should be noted that the use of a double-blind, variable duration, placebo run-in period, whereby both patients and experimenters are blinded to the length of the placebo run-in period and start of active treatment, has shown better sensitivity in detecting placebo responses.[81]

The third reason is that some drugs need the interaction with placebo mechanisms to show their full potential, as shown by the open-hidden administration paradigm,[14,15] in which excluding the placebo/psychological influence leads to a reduction of the drug effect. In other words, the global action of any treatment, be it pharmacological or not, is a mix of psychological and specific pharmacodynamic/physical factors. Thus, excluding placebo responders from a clinical trial could leads to artificial situations and could work substantially against the interest of the scientist to detect the full potential of a therapy.

The fourth reason why we should be careful when interpreting clinical trials in which only placebo nonresponders have been included is that they do not represent the real world. In this regard, the classical distinction between efficacy and effectiveness trials is crucial.[82–84] Whereas efficacy studies are carried out under ideal and strictly controlled conditions, effectiveness studies are more similar to the real world, namely, to routine medical practice (Fig. 3B). By considering the many factors involved in uncertainty, including placebo effects, Hawthorne effects as well as the more recently described social effects, it appears clear how the more we select patients and circumstances, the farther we go from the real world. For example, efficacy trials use strict inclusion and exclusion criteria, whereas effectiveness trials have limited exclusion criteria and, in addition, may include patients with comorbid conditions and poor compliance. Moreover, whereas the intervention is standardized in efficacy studies regarding timing, dosage, clinical setting and trained health professionals, effectiveness studies do not require this standardization, particularly regarding the medical personnel and equipment/setting. As to analysis and data reporting, effectiveness studies have higher rates of missing data compared to efficacy trials.

Therefore, the objectives of efficacy studies and real-world effectiveness studies are different and need to be weighted carefully in order to answer the proper question. Since all the factors responsible for the uncertainty of measuring therapeutic outcomes, including placebo effects, social effects, Hawthorne effects and nocebo effects, are an integral part of the general population, one wonders if it is realistic and it really makes sense to reduce placebo effects when running a CNS clinical trial. If we really want to identify placebo responders and nonresponders by controlling all these factors, we also need a better discussion on what use we make of this. As far as we know today from placebo research, as described throughout this review, the banes of excluding placebo responders from clinical trials are more than the boons.

From mechanisms to practice

There are many reasons why the study and understanding of placebo/nocebo mechanisms should represent a priority in biomedical research, clinical trials and medical practice. One of the most important derives directly from the crucial role of patients' expectations about the therapeutic benefit and their biological underpinnings, as described throughout this review. In CNS clinical trials, the assessment of expectations should represent the rule and a priority before, during and after the course of a trial.

That the investigation of placebo should be a priority in modern medicine is also shown by more recent findings that found that all placebos are not equal. Indeed, clinical trials use placebos with the assumption that they are inert, thus all placebos are considered to be equal. By contrast, there is now compelling evidence that different placebos may use different mechanisms that, in turn, may influence different outcome measures. Therefore, in CNS trials placebos and outcome measures should be selected very carefully in order not to incur in wrong interpretations. For example, Kong et al[85] found that individuals may show analgesic responses to unique healing rituals through different mechanisms in different situations, e.g. to pills or acupuncture needles. Likewise, Benedetti and Dogue[86] found that placebo oxygen inhaled through a mask induces a reduction of high altitude headache with a mechanism that is different from placebo aspirin swallowed with a pill. In addition, and probably more important, these two different placebos

(oxygen mask and pill) affect different outcome measures, such as ventilation, blood pH, and prostaglandins, thus emphasizing that the choice of a placebo may be crucial in the setting of a clinical trial.

The study of placebo, nocebo and Hawthorne effects within the setting of clinical trials also provides important information for clinicians and their routine clinical practice. For example, clinicians should bear in mind that patients' expectation often make the difference in the therapeutic outcome. Therefore, they do not need to give placebos to treat their patients, but rather they could enhance the specific effects of a therapy by adding those positive psychosocial elements that boost the expectations of their patients. Furthermore, it would be interesting to test patients' expectations when the drug, or any other treatment, is on the market, often referred to as phase IV trial. Both medical practice and drug companies would benefit from this approach. In fact, the increase in placebo responsiveness over the past years, described throughout this review, could find an explanation by assessing expectations after the advertisements of new drugs.

Conclusions and future directions

Placebo, nocebo, Hawthorne effects pervade routine clinical practice and produce uncertainty in measuring therapeutic outcomes. The more we know about the underlying mechanisms, the more we need to reframe our approach to clinical trial methodology. Although we believe that placebo responses should not be reduced artificially in clinical trials, some considerations for future trial design have to be taken into account (panel 3). For example, the recently described social interaction among the participants of a trial should be taken into account as an important factor in changing the baseline parameters. Since all these elements are at work across a variety of patient populations and CNS disorders, a crucial question is whether it is correct to reduce them in order to demonstrate efficacy of a new treatment. If, on the one hand, they can be minimized in efficacy studies as a starting point, on the other hand we need to take real-world effectiveness as the main objective. To do this, placebo and nocebo effects should be considered as an integral and essential part of the real-world routine medical practice. Therefore, a better approach in the setting of clinical trials should view patients' expectations as crucial determinants of the

therapeutic outcome, and should consider the assessment of patients' expectations as routine practice.

Future research should be aimed at clarifying at least three crucial points. First, we need to understand the very nature and the neurobiological mechanisms of placebo, nocebo and Hawthorne effects across a variety of CNS disorders. Second, it is crucial to understand in which CNS diseases these effects are large, small or totally lacking. Third, once we have identified all the elements responsible for these effects, we will need to carefully compare efficacy and effectiveness trials in order to verify if it is really correct to control placebo, nocebo and Hawthorne effects to validate a new therapy. Therefore, at least three questions should be addressed in future studies: where, when and how placebos and nocebos work. This is certainly a challenging agenda for future research, because it entails mapping placebo/nocebo responses across a large number of medical conditions, from neurology to psychiatry and many other medical disciplines. A starting point for this exciting enterprise is certainly represented by the understanding of the role of expectations across diseases. Both clinical trial design and medical practice will benefit from the investigation of expectations across neuropsychiatric disorders and other pathologies. We also believe that a fourth question that needs to be addressed is why placebo and nocebo effects exist at all. This is not only a matter of better evolutionary understanding of human biology, but also a way to improve the doctor-patient relationship.[87-89]

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AUTHORS CONTRIBUTION

All the authors searched and selected the literature, critically discussed the paper, and wrote the review.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Panel 2: **Mechanisms of placebo/nocebo responses identified in CNS disorders and therapeutic interventions. See [1,25] for recent reviews of all these mechanisms.**

Pain	Activation of endogenous opioids, endocannabinoids, and dopamine (placebo); activation of a descending inhibitory network from the dorsolateral prefrontal cortex to the periaqueductal grey and to the spinal cord (placebo); activation of cholecystokinin and deactivation of dopamine (nocebo); activation of the spinal dorsal horns (nocebo)
Parkinson's disease	Activation of dopamine in the striatum and changes in activity of neurons in basal ganglia and thalamus
Depression	Changes of electrical and metabolic activity in different brain regions (e.g., ventral striatum)
Anxiety	Changes in activity of the anterior cingulate and orbitofrontal cortices; modulation of amygdala activity; involvement of genetic variants of serotonin transporter and tryptophan hydroxylase
Addiction	Changes of metabolic activity in different brain regions, e.g. thalamus and cerebellum
Autonomic responses to deep brain stimulation	Change of neuronal excitability in limbic regions, as assessed by means of autonomic responses (e.g. heart rate) to brain stimulation
Alzheimer's disease	Involvement of prefrontal executive control and functional connectivity of prefrontal areas
Headache	Modulation of cyclooxygenase products (prostaglandins and thromboxane) in hypobaric hypoxia headache

Panel 3: **Ten tips for better future CNS clinical trials**

1. The more placebo nonresponders we include in clinical trials, the farther we go from the real world, thus any design aimed at eliminating placebo responders from a trial should be considered very carefully.

2. Any trial should include the assessement of patients' expectations.

3. Any trial should assess the patients' perceived assignment by asking participants which group they believe they belong to.

4. Adverse events in placebo arms (nocebo effects) may depend on the adverse events of the active medication against which the placebo is compared, thus such comparison could provide important information on the role of patients' expectations.

5. The Hawthorne effect, or being-under-study effect, should be considered in any trial and investigated in detail.

6. Social interactions among trial participant should be avoided in order to prevent possible effects on baseline clinical and biological parameters.

7. Experimenter-patient interaction must be evaluated carefully, eg number of visits, because this may influence patients' expectations.

8. Different placebos use different mechanisms which in turn may lead to different outcomes, thus a careful selection of placebos (pills, injections, delivery systems, etc) and outcome measures is crucial.

9. Longer and larger trials may produce large placebo responses, thus short and small trials are preferable to long, large, multicentric trials.

10. A priority in biomedical research, both at the academic and industry level, should be to understand where (which medical condition), when (which circumstance), how (which mechanism) placebos and nocebos work.

Figure legends

Figure 1

Uncertainty in drug action and the ambiguity in defining a painkiller. A) The cholecystokinin-antagonist proglumide (solid line) is better than placebo (dotted line) in relieving pain, thus suggesting that it is a painkiller. However, its hidden administration (solid line), unbeknownst to the patients, is totally ineffective and not different from a hidden injection of saline solution (dotted line). B) The analgesic action of the opioid agonist remifentanil is not always the same, depending on the verbal suggestions given to patients. If they are told that the drug is being delivered, the analgesic effect is larger than when told saline is being given. If patients are told that the drug has been interrupted, though it is still being delivered, pain can even increase.

Figure 2

Changes in baseline clinical and/or biological parameters before treatment. A) In the typical Hawthorne effect, the mere recruitment in a clinical trial may change baseline, thus potentially affecting therapeutic outcomes after randomization to either placebo or verum. B) Some subjects are informed about the risk of high altitude headache through interindividual social spread of negative information (dotted line) whereas other subjects receive no such information (solid line). Then headache is induced by going up to high altitude. It can be seen that the socially infected subjects show more severe pain and larger increase in PGE2 than those who received no information. This change in baseline produces large placebo responses in the first group (dotted line) and no placebo response at all in the second group (solid line).

Figure 3

Methodological and ethical problems when reducing placebo responses in clinical trials. A) Including only placebo nonresponders in a clinical trial may not necessarily increase assay sensitivity, because the placebo effect will be smaller in both groups but the differences Δx and Δy will not change. B) The more we select, the farther we go from the real world. In efficacy studies (highly selected patients), therapeutic efficacy is studied in a highly restricted sub-population of patients. In effectiveness studies (less selected patients), less restrictive inclusion and exclusion criteria are adopted, thus the situation is more similar to the real world.

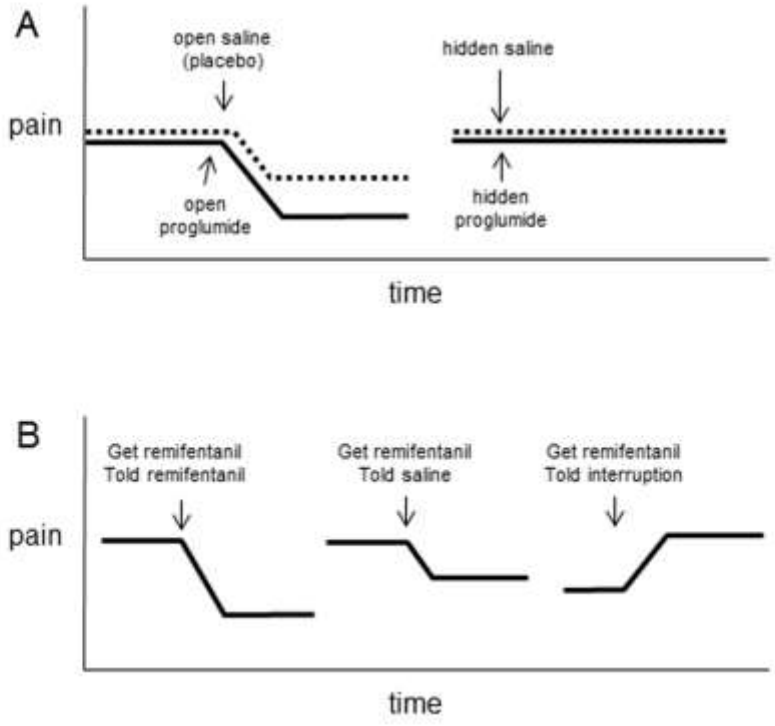
A
open saline
(placebo)
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Fig. 1

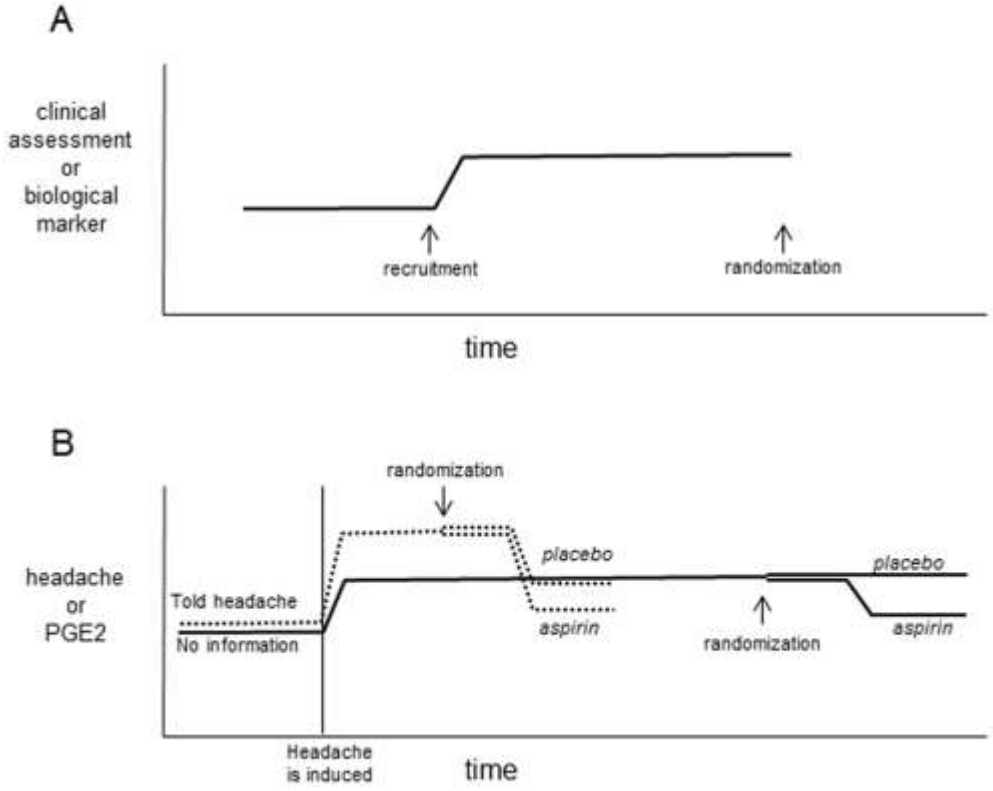
A
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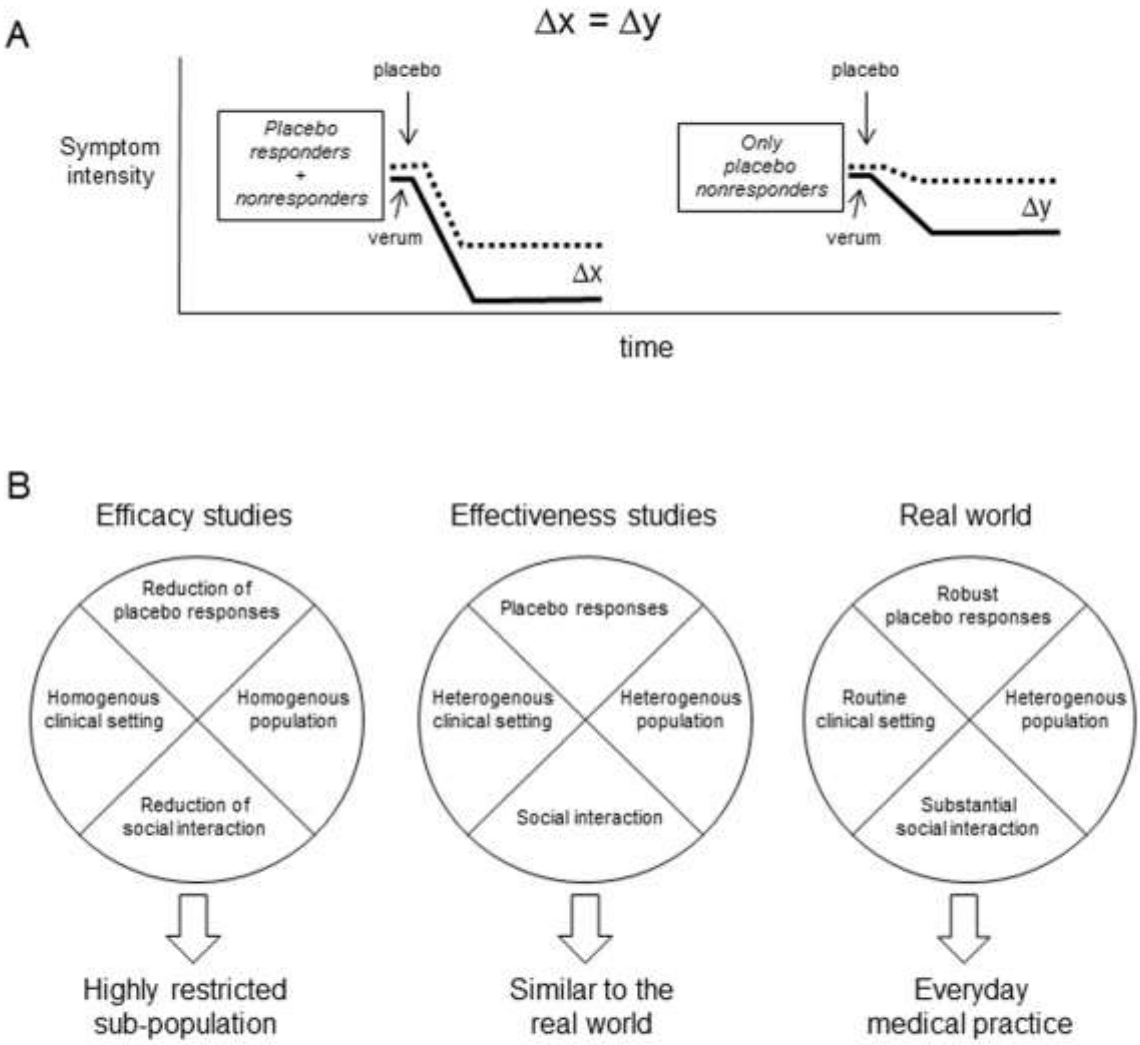
Fig. 2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Symptom intensity
Placebo responders + nonresponders
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verum
Δx
Δy
time
B
Efficacy studies
Effectiveness studies
Real world
Reduction of placebo responses
Homogenous clinical setting
Homogenous population
Reduction of social interaction
Placebo responses
Heterogenous clinical setting
Heterogenous population
Social interaction
Robust placebo responses
Routine clinical setting
Heterogenous population
Substantial social interaction
Highly restricted sub-population
Similar to the real world
Everyday medical practice

Fig. 3