Studying placebo effects in model organisms will help us understand them in humans

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Abstract

The placebo effect is widely recognized, but important questions remain, for example whether the capacity to respond to a placebo is an evolved, and potentially ubiquitous trait, or an unpredictable side-effect of another evolved process. Understanding this will determine the degree to which the physiology underlying placebo effects might be manipulated or harnessed to optimize medical treatments. We argue that placebo effects are cases of phenotypic plasticity where once predictable cues are now unpredictable. Importantly, this explains why placebo-like effects are observed in less complex organisms such as worms and flies. Further, this indicates that such species present significant opportunities to test hypotheses that would be ethically or pragmatically impossible in humans. This paradigm also suggests that data informative of human placebo effects pre-exists in studies of model organisms.

Keywords: *Caenorhabditis*; *Drosophila*; nocebo effect; phenotypic plasticity, placebo effect.

The non-living environment does not lie. What you see, feel or smell is what you get. It therefore pays to behave, develop and respond appropriately. If it's raining then use an umbrella, and if you know what the future has in store – perhaps because you can see nothing but black clouds – then you should prepare accordingly and take your umbrella. The key is the predictability; if a cue can be used to accurately assess what the future holds then responding appropriately is the best strategy. Predictable cues make it possible for organisms to evolve ways to modify their biology to maximize fitness. We see this in various types of phenotypic plasticity where single genotypes produce, via changes in development or physiology, different phenotypes in response to the environment (1). Such responses range from short-term modifications of physiology to trans-generational effects that persist for many generations.

The key difference between a direct response to the environment and phenotypic plasticity is that the latter relies on the detection of a cue. Hence, an organism's response to food might be a direct response to glucose in the bloodstream or an indirect, and phenotypically plastic, response arising from the smell of food. Phenotypic plasticity is ubiquitous. Organisms that are not responsive to environmental conditions were long ago out-competed by mutants that can accurately tailor their biology to the conditions they will experience. This ubiquity also suggests that phenotypic plasticity is evolutionarily ancient, and hence for metazoans will be grounded in the basics of the nervous and endocrine systems that control and regulate life.

Placebo effects, real responses to false cues, potentially stand at odds with the view that, for adaptive reasons, organisms respond only to true environmental cues. In placebo effects, phenotypic responses are seen in the absence of a biologically active agent. Most frequently associated with placebo control conditions in clinical trials of drugs, placebo effects actually represent a broader class of responses. Hence, 'placebo', 'placebo control' and 'placebo effect' refer respectively to sham/dummy treatments, a control process for a set of experimental artifacts, and legitimate neurophysiological events. Here we argue that what we currently recognize as the placebo effect is the result of humans as a species changing our environment such that cues that once accurately predicted the future environment no longer do so, *i.e.* we have made the environment lie. Placebo effects are not therefore artifacts or anomalies, but rather are examples of phenotypic plasticity that evolved to respond to what were once predictable cues in our environment.

Placebo effects result from a broad range of environmental stimuli that goes beyond traditional ideas of sham tablets only. In fact, the range of information that can influence the response to a sham treatment is broad and includes many environmental and psychosocial variables, such as verbally communicated expectations of an effect, previous experience of an effect (learning), manipulated learning (conditioning), context such as the 'white coat effect', and emotional responses such as hope and anxiety. All are manifest in responses via a number of discrete biological processes, including dopamine (2), opioid (3), and cannabinoid (4) pathways, with similar pathways often observed for both placebo effects and the drug they are mimicking (2, 5). This has collectively been described as "the new

physiology of the doctor-patient relationship" (6). This emergent viewpoint is shifting thinking around the placebo effect away from vague self-reported and subjective responses towards robust and directly measured biological events.

Arguably, if the placebo effect is *biologically real* in humans, it evolved for a purpose. An evolutionary case can be made for a response that can reduce the severity of subjective symptoms, thereby allowing the organism to better cope with survival-relevant situations. For example, if pain is tolerated for longer it might allow an individual to produce greater muscle force and therefore running speed and/or physical strength to capture prey or avoid predation, both potentially life-critical contexts.

However, whilst anecdote abounds, it is rare that placebo effects are reliably observed in any contemporary life-critical context in humans (although the ethical constraints of administering experimental placebo treatments in such contexts must be acknowledged). This appears slightly at odds with the idea that traits closely linked to fitness are likely to be the most responsive to environmental variation. This presents a paradox; if placebo effects occurred only in relation to subjective symptoms, no matter how unpleasant to the individual (e.g. pain, fatigue and depression), it is doubtful they would have been sufficiently responsive to natural selection for the placebo effect to earn its place in contemporary human biology.

There are exceptions. For example, Benedetti and co-workers (7) reported the effects of sham oxygen on pain, fatigue and performance among subjects

experiencing chronic exposure to severe hypoxia at 3,500m of altitude. Whilst the main outcome variable of the study was hypoxia induced pain, the ability of subjects to produce physical work at reduced perception of fatigue suggests that even life-critical functions such as oxygen supply are placebo responsive.

Another interesting exception is Parkinson's disease (PD), which although not life critical *per se*, reduces lifespan in the majority of patients. In fact, examining the table of contents of books on placebo effects, PD stands out from the list of otherwise ostensibly subjective conditions like the proverbial sore thumb.

The role of dopamine in PD has long been recognized. Increasingly the role of the same neurotransmitter is recognized in placebo responses in many scenarios. Benedetti and co-workers analysed the effects of a placebo in PD patients, specifically the effects on dopamine release in the striatum and the modification of neuronal activity in both the thalamus and subthalamic nuclei. In naïve patients, a first placebo administration resulted in no change in neural or clinical measures. However, in patients previously administered the anti-Parkinson's drug apomorphine, the number of repeated exposures to apomorphine predicted both the neural and clinical responses of patients to placebos. Critically, these effects were of the same magnitude as those elicited by the drug itself, suggesting a significant role for learning in the placebo response observed (2).

Placebo responses have also been observed in response to nutritional treatment that were presented, but not actually ingested (8). For example, athletes who were

not glucose deficient show performance increases when glucose is introduced to the mouth and then withdrawn without being ingested, this 'glucose rinsing' also resulting in clear neurophysiological responses. An analogous effect has been observed with caffeine rinsing (9) These placebo effects can be understood in terms of the body responding to a predictable cue – detecting glucose or caffeine in the mouth normally indicates that it will soon be available in the intestine – by altering resource allocation.

If placebo responses seen in humans are the result of phenotypic plasticity, then placebo-like effects should be observable in other species – particularly in cases where the environment has been altered to disrupt its reliability. This is the case, and placebo-like effects are seen in a variety of model systems. Importantly, these examples are directly linked to fitness and rely on widely conserved signaling pathways.

Although addressing placebo effects *per se*, Ader & Cohen (10) reported the conditioned immunosuppression of rats, a study that whilst Sokolowsa et al (11) demonstrated how a 'probe dose' of 10% of the normal dose of morphine triggered morphine like effects, again in rates. However, placebo effects have to date been largely studies in humans, and when not in humans, in vertebrates. Little or no work to date has examined the possibility of placebo effects in simple invertebrate model organisms. However, it is plausible that such species experience similar responses.

In situations analogous to those seen in human experiments above, the perception of food matters in model organisms. A widespread means of extending lifespan is to reduce the overall calorific intake whilst preserving vitamin/mineral needs and avoiding starvation (12). This lifespan extending response to calorific or dietary restriction (DR) is seen widely in eukaryotes, and represents potentially the most viable non-pharmacological means of extending human life and health-span (13). In the nematode worm *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, DR via a range of methods extends lifespan, with lifespan extensions of up to 50% observed in *C. elegans*. The regulation of the DR response is complex, but work in many systems demonstrates the involvement of insulin-like signaling and the highly-conserved mTOR (mechanistic target of rapamycin) pathway (14). The life-extending effects of DR can however be blocked in both worms and flies by the smell of food alone (15, 16). Hence in situations where there is a mismatch between perception and reality, the standard DR response is not seen. In these cases, the smell of food is therefore acting as a placebo, or more correctly what is termed a 'nocebo', a negative placebo response resulting in no lifespan extension.

Likewise, work on *C. elegans* shows that the neuronal perception of cold, rather than the system-wide effects of temperature on cellular function *per se*, are critical for cold stress survival. Low temperatures damage *C. elegans* and can kill them, with adult worms dying if they are exposed to temperatures lower than 5°C for prolonged periods (17, 18). This mortality can be greatly reduced, or even blocked, by habituation – worms exposed to low, but non-stressful, temperatures are then highly resistant to subsequent acute cold stress (17, 18). Critically, this habituation is

a result of worm's perception of temperature as opposed to the environmental reality, as disruption of specific neurons can replicate the habituation response in the absence of any temperature change (18, 19). The response, in this case the survival or not of a subsequent stress, is therefore based not on the temperature itself, but on the *perception of temperature*. This perception of temperature then feeds into the insulin-like signaling pathway (18, 20), one of the core highly-conserved pathways that regulates nutrient allocation and lifespan in eukaryotes.

Hence, in both worms and flies we can observe placebo effects; there is an expectation of something and a subsequent set of changes that (should) optimize fitness under the expected condition. Critically though, the trigger is neuronal and hence can be separated from the actual environment that is being perceived or expected. The reality of environmental temperature is less positive than the signal, and it is the signal to which the organism responds; temperature sensation in a light and pheromone-sensing neuron produces a robust effect on insulin signalling that controls experience-dependent temperature habituation. Likewise, the reality of reduced caloric intake is less critical to survival than the perception, suggesting a calorie-independent mechanism for life span extension by caloric restriction.

It is increasingly clear that the biology underpinning the placebo effect in humans could have significant clinical and societal impacts. One reasons that we know so much about the neurophysiological response to placebo treatments among PD patients is that we are able to conduct research on real-time neuronal activity by using the electrodes implanted for the treatment of the disease itself, deep brain

193 stimulation. PD might not be, as is suggested above, an exception, it might simply be
194 the medical condition in humans in which it has been easiest to secure relevant data.
195 In PD therefore, the response to placebos is less a problem for clinical trials and/or a
196 unique opportunity for placebo effect researchers, but a potential clue to future
197 treatments. Far from controlling for the placebo effect in clinical trials, scientists
198 should in fact be seeking to understand and harness the biological processes that in
199 many cases constitute to a substantial percentage of the overall effectiveness of the
200 drug when compared to no-treatment. In short, this field of study could become
201 important and impactful.
202
203 However, the study of placebo responding in humans is plagued by ethical, logistical
204 and cost issues. Scenarios such as PD in which conventional medical treatment
205 facilitates easy and reliable access to brain activity are rare. There are many
206 questions that might be asked in humans with relative ease, for example we should
207 be able to answer the question of whether all individuals respond similarly to non-
208 ingested glucose mouthwash, arguably a direct neuronal prediction. But these are
209 not the critical questions; placebo responses that require a cognitive prediction are
210 more problematic to study. For example, those effects that result from anticipation
211 or expectation of an effect, that might be moderated by a number of affective and
212 cognitive factors such as environmental cues, emotion, memory, sensation and
213 perception.
214
215 That placebo responses might be open to study and to systematic manipulation in
216 simple model organisms could significantly enhance our understanding of the

response in humans. We therefore propose five hypotheses that could be tested in animal models: 1) Placebo-like effects are ubiquitous responses to a set of standardized environmental cues across organisms (*i.e.*, we would expect to see placebo effects to a similar type of cue across numerous organisms); 2) Placebo-like responses will be limited to certain types of species-relevant environmental information such as temperature and energy availability (*i.e.*, we would expect to see evolved responses in life-critical contexts); 3) The capacity to respond to a novel placebo-like cue can be acquired through evolution within a species and/or learning within an organism; 4) A threshold magnitude of information (*e.g.*, temperature, energy availability) is required to elicit a placebo-like effect (this threshold likely related to the significance of the information in critical survival terms); and 5) The threshold magnitude for a placebo-like cue can be experimentally modified to enhance the dose-response relationship. Several of the above questions might be addressed by experimental evolution in model systems which should be able to test the levels of predictability required to maintain and modify responses. Critically, this paradigm also suggests that a wealth of data informative of human placebo effects already exists in studies of model organisms, and several of the hypotheses might be amenable to secondary analysis.

Competing interests

We have no competing interests.

Authors' contributions

SH and CB drafted the manuscript and gave final approval for publication.

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Data, code and materials

No primary data are reported here.

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