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Characterization of the thalamic-subthalamic circuit involved in the placebo response through single-neuron recording in Parkinson patients

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Abstract : The placebo effect, or response, is a complex phenomenon whereby an inert treatment can induce a therapeutic benefit if the subject is made to believe that it is effective. One of the main mechanisms involved is represented by expectations of clinical improvement which, in turn, have been found to either reduce anxiety or activate reward mechanisms. Therefore, the study of the placebo effect allows us to understand how emotions may affect both behavior and therapeutic outcome. The high rate of placebo responders in clinical trials of Parkinson's disease provided the motivation to investigate the biological underpinnings of the placebo response in Parkinsonian patients. The placebo effect in Parkinson's disease is induced through the administration of an inert substance which the patient believes to improve motor performance. By using this approach, different behavioral and neuroimaging studies have documented objective improvements in motor performance and an increase of endogenous dopamine release in both the dorsal and ventral striatum. Recently, single-neuron recording from the subthalamic and thalamic regions during the implantation of electrodes for deep brain stimulation has been used to investigate the firing pattern of different neurons before and after placebo administration. The results show that the subthalamic nucleus, the substantia nigra pars reticulata, and the ventral anterior thalamus are all involved in the placebo response in Parkinson patients, thus making intraoperative recording an excellent model to characterize the neuronal circuit that is involved in the placebo response in Parkinson's disease as well as in other disorders of movement.

Keywords : Placebo response; Parkinson; Single-neuron recording; Basal ganglia

1. Introduction

In recent years, the placebo effect, or response, has evolved from a nuisance in the setting of clinical trials to a topic worthy of scientific inquiry. The placebo response is a change in the body, or the body-mind unit, that occurs as a result of the symbolic significance which one attributes to an event or object in the healing environment (Brody, 2000). Considerable progress has been made in our understanding of its neurobiological mechanisms, particularly in conditions such as pain and Parkinson's disease. Other medical conditions, like depression, anxiety, hormone secretion, immune functions and addiction, are less understood, yet some of their neurobiological mechanisms have been identified as well (Benedetti, 2008). Today we know that the placebo effect is due to the psychosocial context surrounding the patient and the therapy (Benedetti et al., 2011a and Colloca and Benedetti, 2005). In fact, different sensory and social stimuli, which constitute the ritual of the therapeutic act, can induce positive expectations of clinical improvement. The link between expectations and any effect that may take place in the brain and body is represented by at least a twofold mechanism. First, expectation of a positive outcome may reduce anxiety (Petrovic et al.,

2005 and Vase et al., 2005) and second, it can activate reward mechanisms, whereby the reward is the therapeutic benefit itself (de la Fuente-Fernández et al., 2001 and Scott et al., 2007). Therefore, the study of the placebo effect is a window that allows us to understand how emotions may affect both behavior and therapeutic outcome. Indeed, anticipation of either a positive or negative event, which involves its appraisal, has been postulated to take part in emotions (Scherer, Schorr, & Johnstone, 2001).

Most of our knowledge about the neurobiological mechanisms of the placebo effect comes from the field of pain and analgesia (Benedetti, 2008). It is well established that different endogenous neuronal networks are responsible for the modulation of pain by placebos. In particular, the activation of the descending pain-modulating network from the cerebral cortex to the brainstem and spinal cord has been described, with the involvement of opioid, cannabinoid, cholecystokinin and dopamine systems (Benedetti, 2008, Benedetti et al., 2011a and Tracey, 2010). What has emerged over the past few years is that the endogenous opioids and endocannabinoids are involved in placebo analgesia in different circumstances (Benedetti, Amanzio, Rosato, & Blanchard, 2011) whereas cholecystokinin plays a key role in the opposite effect, namely, nocebo hyperalgesia (Benedetti, Amanzio, Vighetti, & Asteggiano, 2006). In this sense, the nocebo response is a phenomenon opposite to the placebo response that is due to the negative symbolic significance which one attributes to an event or object in the healing environment.

Besides pain and analgesia, there is extensive evidence for a prominent placebo effect in Parkinson's disease (see below), which makes this motor disorder an excellent condition to investigate (Benedetti, 2008). There are at least two other reasons why Parkinson's disease is a good model for placebo studies. First, differently from pain, in Parkinson's disease the response to treatment can be assessed objectively by the experimenter, thus providing objective measurements of motor performance. Second, the use of electrodes for deep brain stimulation offers the opportunity to analyze the placebo response at the unprecedented level of single-neuron recording in awake patients, thus allowing us to perform a correlation between the clinical outcome and the activity of single neurons in different brain regions.

2. The placebo response in Parkinson patients

Parkinson's disease is mainly a disorder of movement, although several sensory, cognitive, mood, sleep, autonomic disturbances may be present as well (Blandini et al., 2000 and McRae et al., 2004). There are at least three important motor symptoms: tremor, rigidity, and bradykinesia. Tremor is at rest and involves mainly the upper limbs, although other body parts may be subject to tremor, such as the chin. Rigidity involves all the muscles, with a global impairment of movements and gait. Bradykinesia means that movements slow down, so that any action is performed very slowly and with difficulty.

Substantial improvements in Parkinsonian symptoms are seen in placebo groups of many clinical trials that assess both pharmacological and surgical treatments for Parkinson's disease (Diederich and Goetz, 2008, Galpern et al., 2012, Goetz et al., 2002, Goetz et al., 2008a, Goetz et al., 2000 and Shetty et al., 1999). Interestingly, the side effects of pharmacological therapy, such as dyskinesia, were found to be evoked by placebo treatments as well (Goetz, Laska, et al., 2008).

Substantial improvements in motor symptoms are also present in patients who are assigned to the placebo group in surgical treatments of Parkinson's disease (Gill et al., 2003, Gross et al., 2011, Lang et al., 2006, Marks et al., 2008, Marks et al., 2010, Olanow et al., 2003, Slevin et al., 2005 and Stover et al., 2005). However, it should be noted that these trials are usually aimed at comparing active treatments versus placebos, with no natural history groups. Therefore, the clinical improvements in the placebo groups cannot be attributed to real psychobiological placebo effects only, as spontaneous remission and regression to the mean are taking a part in the reduction of the symptoms in many cases. Nonetheless, the high rate of placebo effects in clinical trials of Parkinson's disease provided the impetus to investigate placebo responses of Parkinson patients in more detail and under strictly controlled conditions.

In order to overcome these limitations in the clinical trials setting, Parkinson patients have been studied under strictly controlled conditions with a rigorous experimental approach, whereby the placebo group is compared to a natural history group, in order to rule out spontaneous remission. In a typical placebo procedure in Parkinson patients, a placebo is administered along with verbal suggestions of motor improvement, so that the patients expect an improvement in their motor symptoms, such as tremor, muscle rigidity, and bradykinesia. For example, the velocity of movements in Parkinson patients who had been implanted with electrodes in the subthalamic nuclei for deep brain stimulation was analyzed in the laboratory setting (Pollo et al., 2002). In order to manipulate the patients' expectations, two opposite conditions were studied. In the first condition, the patients were told that the intensity of stimulation was high, so that they expected good motor performance, whereas in the second they were told that the stimulation intensity was lowered, so as to induce expectations of bad motor performance. The effect of subthalamic stimulation on the velocity of movement of the right hand was analyzed with a movement analyzer, which was characterized by a rectangular surface where the patients performed a visual directional-choice task. To do this, the right index finger was positioned on a central sensor with a green light. After a random interval of a few seconds, a red light turned on randomly in one of the three sensors placed 10 cm away from the green-light sensor. The patients were instructed to move their hand as quickly as possible in order to reach the target red-light sensor. The hand movement was found to be faster when the patients expected a good motor performance than when they expected bad performance. Interestingly, all these effects occurred within minutes, which indicates that expectations of clinical improvement induce neural changes very quickly. Similar findings were confirmed in subsequent studies (Benedetti et al., 2003 and Mercado et al., 2006).

The important role of expectations is further supported by a clinical trial of human fetal mesencephalic transplantation (McRae et al., 2004). Besides the classical clinical trial approach, these investigators 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 at twelve months after surgery. Patients who believed they received transplanted tissue had significant improvements in both quality of life and motor outcomes, regardless of whether they received sham surgery or fetal tissue implantations. Therefore, the expectation of therapeutic benefit represents one of the main factors that mediates the placebo response in Parkinson's disease (Keitel et al., 2013).

3. Dopamine and the placebo response

The disruption of dopamine function in the neural pathway from the substantia nigra pars compacta to the striatum (putamen and caudate nucleus) represents the pathophysiological substrate of Parkinson's disease. The primary deficit involves the selective degeneration of the nigrostriatal dopamine-producing neurons, although at later stages of the disease, other dopamine projections and other neurotransmitters may also be affected. There are in fact different dopaminergic pathways in the brain, such as the mesocortical, the mesolimbic, and the tuberoinfundibular. These pathways are associated with volition and emotional responsiveness, desire and reward, and sensory processes and maternal behavior. The neuropsychiatric pathology associated with Parkinson's disease is attributable to the loss of dopamine along the non-striatal pathways. As far as the motor system is concerned, dopamine has a critical role in the modulation of the basal ganglia functioning (Alexander et al., 1986 and Haber, 2003), and its depletion results in difficulties initiating movement (akinesia), bradykinesia (slowness of movement), rigidity, tremor at rest, and postural instability.

By using positron emission tomography, de la Fuente-Fernández et al. (2001), de la Fuente-Fernández, Schulzer, and Stoessl (2002) and de la Fuente-Fernández, Phillips, et al. (2002) found that placebo administration induces the activation of the dopaminergic system in Parkinson patients (Fig. 1). The release of dopamine in the motor striatum (putamen and dorsal caudate) was greater in those patients who reported clinical improvement. Although all patients showed dopamine placebo responses, only half of the patients reported motor improvement. These patients also released larger amounts of dopamine in the dorsal motor striatum, suggesting a relationship between the amount of dorsal striatal dopamine release and clinical benefit. This relationship was not present in the ventral striatum, in which all patients showed increased dopamine release, irrespective of whether they perceived any improvement. Compared to the dorsal motor striatum, the ventral striatum (nucleus accumbens) is involved in motivation and reward anticipation (Ikemoto and Panksepp, 1999, Knutson and Cooper, 2005, Schultz, 2002 and Schultz et al., 2000). Accordingly, de la Fuente-Fernández et al., 2001 and de la Fuente-Fernández et al., 2002a and de la Fuente-Fernández, Phillips, et al. (2002) proposed that the dopamine released in the ventral striatum was associated with the patients' expectation of improvement in their symptoms, which could in turn be considered a form of reward. This finding was confirmed by Strafella, Ko, and Monchi (2006) in Parkinson patients and by Scott et al. (2007) in pain.
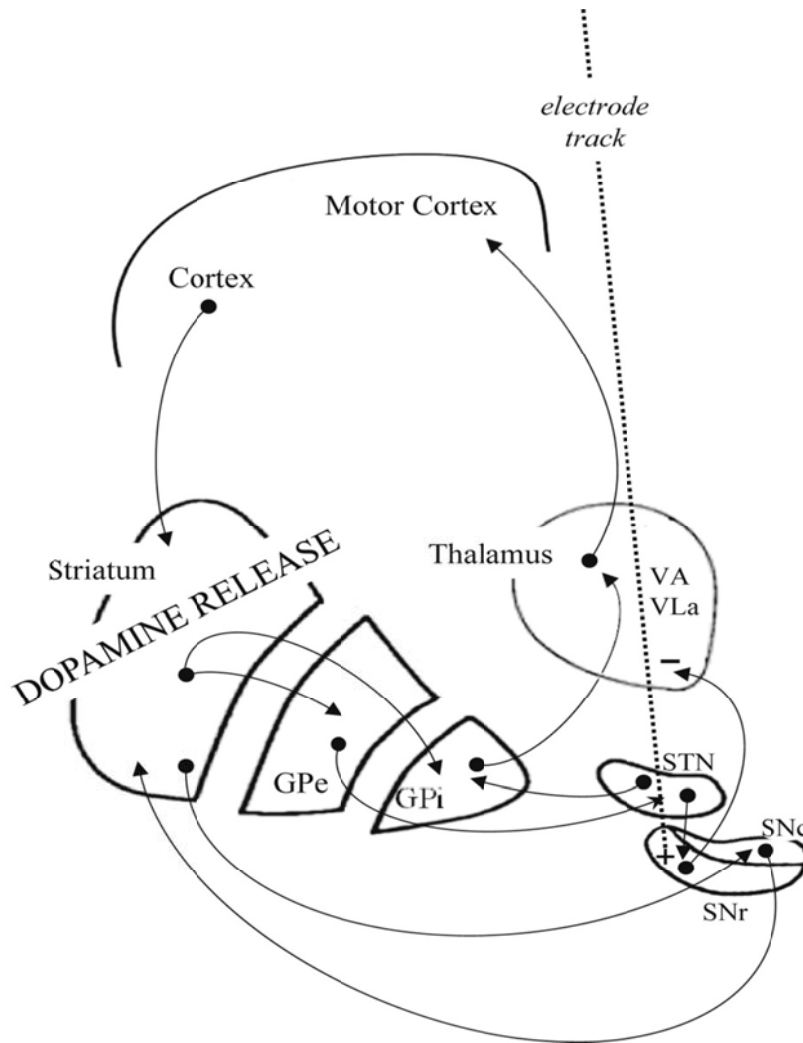
Fig 1: Schematic representation of the principal connections in the basal ganglia circuitry. During the placebo response in Parkinson patients, there is a release of dopamine in both the dorsal and ventral striatum. By using intraoperative recording in awake patients, it is possible to characterize both the neuronal network and the pattern of neuronal activity that is involved in the placebo response. The dotted line represents the intraoperative electrode track which allowed us to record from the thalamus, the subthalamic nucleus (STN) and the substantia nigra pars reticulata (SNr). Note the excitatory pathway from STN to SNr, and the inhibitory pathway from SNr to VA/VLa. GPe = external globus pallidus, SNc = substantia nigra pars compacta.

It is interesting to note that the strength of expectation of clinical improvement influences the degree of striatal dopamine release in response to placebo. Parkinson patients who are told that they have a specific probability (25%, 50%, 75%, or 100%) of receiving active medication, when in fact they always received a placebo, show different degrees of dopamine activation. Only when patients are informed that they have a 75% probability of receiving active medication, a significant dopamine release occurs (Lidstone et al., 2010). Besides the understanding of the neurobiological underpinnings of the placebo response, this finding may have important implications in the design of clinical trials.

4. Characterization of the neuronal circuit through intraoperative recording

Following the finding of a placebo-induced dopamine release, Benedetti et al. (2004) started looking for possible neuronal changes in different regions of the basal ganglia by using single-neuron recording in awake patients. Indeed, the subthalamic nucleus is now the major target in the surgical therapy of Parkinson's disease and its identification can require the recording of electrical activity in the subthalamic nucleus (Hutchison et al., 1998). Therefore, the activity of single neurons in the subthalamic nucleus as well as in the surrounding regions can be recorded in different conditions, for example after the administration of a pharmacological agent. Several studies have reported that the anti-Parkinson agent, apomorphine, induced changes in the subthalamic nucleus firing pattern of patients with Parkinson's disease (Levy et al., 2001, Lozano et al., 2000 and Stefani et al., 2002). Although Levy et al. (2001) found a certain variability in the firing rates of single neurons under the effect of apomorphine, Stefani et al. (2002) reported that the administration of apomorphine is invariably followed by a reduction of firing rate from about 40 to about 27 Hz.

According to the classic pathophysiological view of Parkinson's disease, the dopamine depletion in the striatum induces both hyperactivity (high firing rate) (Blandini et al., 2000) and bursting activity (Bergman et al., 1994 and Levy et al., 2001) of subthalamic nucleus neurons. This might be due to a lower activity of the external globus pallidus which sends inhibitory projections to the subthalamic nucleus (see Fig. 1 for the main basal ganglia connections). Therefore, the external globus pallidus hypoactivity would result in decreased inhibition upon the neurons of the subthalamic nucleus. The high-frequency therapeutic stimulation of the subthalamic nucleus would modify this abnormal activity (Limousin et al., 1997), and this might be achieved through the stimulation of the inhibitory afferents from the external globus pallidus to the subthalamic nucleus. Other mechanisms have also been hypothesized (Benedetti, 2008).

On the basis of these pathophysiological considerations, in 2004, the first study of the placebo effect at the single-neuron level was performed by Benedetti et al. (2004). Since the subthalamic nucleus plays an essential role in basal ganglia functioning and is a major target in the surgical therapy of Parkinson's disease (Limousin et al., 1997) and, in addition, its identification requires the recording of intranuclear electrical activity in awake Parkinson patients, these authors performed a double-blind study in which the activity from single neurons in the subthalamic nucleus before and after placebo administration was recorded to see whether neuronal changes were associated to the clinical placebo response. In order to make the placebo response stronger, the placebo was administered in the operating room after several pre-operative administrations of the anti-Parkinsonian drug apomorphine (pharmacological pre-conditioning procedure).

Before placebo administration, the activity of neurons was recorded from one subthalamic nucleus prior to implantation of the first electrode and used as a control. After the placebo, which consisted of a subcutaneous injection of saline solution along with the verbal suggestion of motor improvement, neuronal activity was recorded from neurons prior to implantation of the second electrode into the other subthalamic nucleus. A placebo response was defined as the decrease of arm rigidity of at least 1 point on the clinical evaluation scale. Those patients who showed a straightforward clinical placebo response, assessed by means of arm rigidity and subjective report of well-being, also showed a significant decrease of firing rate compared to the pre-placebo subthalamic nucleus. In order to rule out the possibility that the difference in firing rate between the

pre- and post-placebo subthalamic nucleus was independent of the placebo treatment itself, a no-treatment group (natural history) was studied. The patients of this no-treatment group did not undergo any placebo treatment between the implantation of the first and second electrode. All these patients showed no significant differences between the neuronal firing rates of the two subthalamic nuclei, which indicates that the difference between the first and the second side of implantation in the placebo group was due to the placebo intervention per se. It is also important to point out that the clinical placebo response was assessed before lowering the electrode tip into the subthalamic nucleus, in order to avoid the possible microlesional effects of the electrode, which might represent a confounding factor.

Although the mean firing rate is a good parameter to assess the activity of the subthalamic nucleus, bursting and oscillatory patterns have also been described in Parkinson's disease and related to motor symptoms and to apomorphine effects (Bergman et al., 1994 and Levy et al., 2001). Therefore, in the single-neuron analysis by Benedetti et al. (2004), the bursting activity of the subthalamic nucleus neurons before and after placebo administration was also investigated, in order to see whether, beside the frequency decrease, there was also a change in the pattern of discharge. It was found that the subthalamic nucleus neurons of all the placebo responders shifted significantly from a pattern of bursting activity to a pattern of non-bursting discharge. All the placebo non-responders did not show any difference in the number of bursting neurons before and after placebo administration. Likewise, the no-treatment group did not show any significant difference in bursting activity between the first-side and second-side subthalamic nucleus.

In the study by Benedetti et al. (2004) there was a correlation between subjective reports of the patients, clinical responses and neurophysiological responses. In fact, a decrease in firing rate as well as a change from bursting to non-bursting activity of subthalamic nucleus neurons was correlated with both the patients' subjective reports of well-being and the muscle rigidity reduction at the wrist, as assessed by a blind neurologist. Although it is tempting to speculate that these neuronal changes represent a downstream effect of dopamine release in the striatum, the dopamine release in the striatum (de la Fuente-Fernández et al., 2001) and the single-neuron changes (Benedetti et al., 2004) were observed in two different studies, thus no definitive conclusion can be drawn. Nonetheless, on the basis of our knowledge about the basal ganglia circuitry, it is plausible that a placebo-induced release of dopamine acting on the inhibitory D2 receptors disinhibits the gamma-aminobutyric acid (GABA) neurons of the external globus pallidus which, in turn, increase their inhibition onto the subthalamic nucleus (see Fig. 1 for a hypothesized model of the basal ganglia circuitry).

In a more recent study, the same group extended the previous results on single-neuron recording in the subthalamic nucleus to two thalamic nuclei (ventral anterior - VA and anterior ventral lateral - VLa) and the substantia nigra pars reticulata (Benedetti et al., 2009). The subthalamic nucleus receives inputs from both the cortex and the external globus pallidus and sends excitatory output pathways to both internal globus pallidus (GPi) and substantia nigra pars reticulata (Fig. 1). Considering the effect of placebo administration on this nucleus (Benedetti et al., 2004), a significant placebo effect should also be expected in the subthalamic nucleus output regions. Indeed, in Parkinson patients who exhibited a clinical placebo response, the decrease in firing rate in the subthalamic nucleus was associated with a decrease in the substantia nigra pars reticulata and

an increase in the thalamic nuclei (Fig. 2a). Conversel
changes or partial changes in the subthalamic nucle
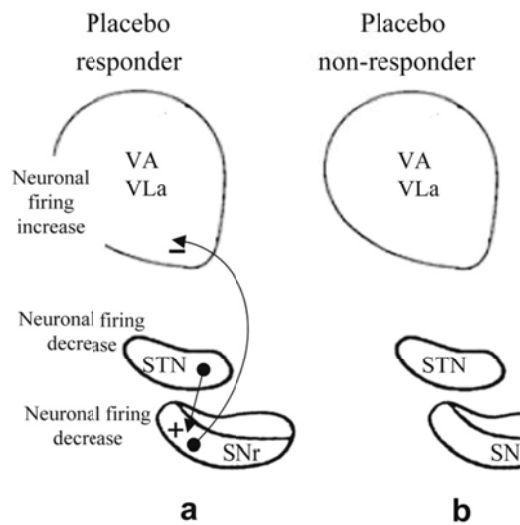subthalamic-nigral-thalamic circuit appears to be impor

Placebo
responder

Placebo
non-responder

Neuronal
firing
increase

VA
VLa

VA
VLa

Neuronal firing
decrease

STN

STN

Neuronal firing
decrease

SNr

SNr

a

b

*Fig 2: Electrophysiological differences between pla
Clinical placebo responders show neuronal activity
substantia nigra pars reticulata (SNr) and thalamus.
responders in the study by Benedetti et al. (2009). (b)
in neuronal activity. This pattern was present in four o
Benedetti et al. (2009). (c) Another placebo non-respo
which are not sufficient to induce changes in SNr and
placebo non-responders in Benedetti et al. (2009).*

Besides these subcortical changes in neuronal ac
change as well (Lou et al., 2013). In fact, a decrease i
elicited by transcranial magnetic stimulation, was four
either a 50% or 100% probability of receiving an anti-
with 0% probability (i.e., no expectation) showed
Therefore, the degree of expectancy has a significant
agreement with the subcortical neuronal changes at the

It should be noted that many questions still re
ethical limitations of intraoperative recording, we have
and duration of the neuronal changes associated to the
these changes seems to be shorter than 30 min (Bened
from single neurons during surgery is too short to draw
nuclei, such as the striatum and the GPi, may be involv
limitations of the electrode trajectory do not allow to
An additional important point is represented by the po
example those related to sensory feedback arising from
response to placebos. Finally, the functional role of tl
placebo response needs further exploration. For exam