Determinants and Correlates of Placebo Response in Children with ADHD

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October, 2014

A thesis submitted to McGill University in partial fulfillment of the requirements of the Degree of Master of Science

Acknowledgements

During my two years at McGill I had the opportunity to work with a number of great people who have contributed, in different ways, to make this dissertation possible. Therefore, I would like to show my gratitude to everyone who assisted and guided me.

First and foremost, I would like to express my sincere gratitude to my supervisor, Dr. Ridha Joober for his endless support, all the encouragement, and persistent guidance. I am so grateful and privileged to work under his supervision. His critical views and continuous motivation have been very valuable and fundamental for the successful completion of my thesis. Thank you so much!

Thanks are also extended to the members of my supervisory committee Drs. Natalie Grizenko, Roberta Palmour, and Sarojini Sengupta for taking the time to give me invaluable feedback on my research.

I am also grateful to ADHD team members for their contribution to this study: Johanne, Marie-Ève, Sherrie, Thao, Jacqueline, Sandra, Mira, Marina, Zia, and Maryam. Marie-Ève many thanks for translating the abstract and for being prepared to help all the time. Mira thank you so much for proofreading the thesis several times. Sincere thanks to Alexandru Traicu, his help, assistance, and efforts were vital to complete the third chapter of this thesis. Thanks to all of you for all the help and support. In addition, warm thanks go to all participants and their families. Their participation was crucial to all the research in the ADHD program and to this thesis.

I am also grateful to the Ministry of Higher Education of Saudi Arabia and the Saudi Cultural Bureau for their financial and academic support and for investing in my education. Both my sponsors and the Department of Human genetics at McGill

University provided me with various awards to share my work at various conferences and different meetings. Your support helped me to explore, learn, and broaden my horizon. Thank you.

Finally, a special word of thanks to my parents: Hanan **&** Yousef for their prayers and endless support through all my decisions. To my siblings Turki, Wesam **&** Tala**,** and the rest of my family for keeping me motivated and believing in me. I would not be the person I am today without you. My deepest gratitude goes to my brother **Wesam** who was standing beside me throughout this journey. Your everlasting support means a lot to me. I will ever remain indebted to you. Thanks are also extended to my friends who have been there in one way or the other. You made me feel surrounded by a loving family.

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Abstract

Over the last several years, scientific interest in the placebo effect has extended significantly in the medical literature. Understanding placebo is important to answer some important conceptual questions (e.g. the mind-body interactions) as well to optimize clinical trials' design and interpretation.

Evidence based medicine relies heavily on clinical trials comparing aiming at showing the superiority of a given treatment over placebo. In fact, having a large placebo response is becoming one of the main reasons behind failed clinical trials. Therefore, understanding the placebo effects by answering "how, where, and when" does placebo work is critical in modern medicine.

This thesis includes two studies aiming at answering some of these questions by identifying psychobiological and biological (genetic) correlates of placebo response in children with Attention-deficit hyperactivity (ADHD). These may in turn serve as biomarkers for predicting placebo responsiveness.

The first study focused on socio-demographic and clinical correlates of placebo response as assessed by parents, teachers and in the laboratory by trained research assistants. Contrary to a strong placebo response in parents and teachers, we identified a reverse placebo response (RPR) in the lab setting. Two specific patterns of placebo response were also identified as function of various socio-demographic and clinical characteristics. The second study demonstrated the contribution of the Catechol-O-methyltransferase (*COMT)* gene to RPR. We also studied the association between MPH response and *COMT* polymorphisms.

Results of these studies could have a significant impact on increasing our knowledge about placebo; hence, they will allow us to improve treatment approaches in clinical practice.

Résumé

Au cours des dernières années, l'intérêt de la communauté scientifique pour l'effet placébo dans diverses conditions médicales s'est accru de manière considérable. Comprendre l'effet placébo est essentiel pour répondre à certaines questions conceptuelles (par exemple les interactions corps-esprit), ainsi que pour optimiser les devis des essais cliniques. L'utilisation actuelle de placébos dans presque tous les protocoles d'essais cliniques illustre l'importance de ce phénomène dans les sciences biomédicales. Compte tenu de l'importance de la réponse au placébo en médecine, l'approche clinique moderne (ou médecine fondée sur les preuves) exige qu'un traitement montre sa supériorité au placébo. Cependant, un effet placébo important peut être la cause principale de l'absence d'un effet thérapeutique observé. Par conséquent, bien comprendre l'effet placébo en répondant au « comment, où et quand » celui-ci fonctionne-t-il est essentiel à la médecine moderne.

Ce mémoire comprend deux études visant à répondre au moins en partie aux questions précédentes en identifiant certaines caractéristiques biologiques (génétiques) et psychobiologiques de la réponse au placébo chez des enfants atteints de trouble déficitaire de l'attention avec hyperactivité (TDAH). Ces caractéristiques pourraient éventuellement servir de biomarqueurs permettant de prédire la réponse au placébo.

La première étude porte sur l'exploration des corrélats sociodémographiques et cliniques de la réponse au placébo. Nous avons observé une réponse hautement significative au placébo selon les parents et le professeur, mais une réponse inverse (RIP) au placébo dans le laboratoire. Deux patron de réponse au placébo on été mis en évidence en fonction des caractéristiques cliniques et démographiques. La seconde étude démontre le rôle du gène de la catéchol-O-méthyltransférase (*COMT*) à la RIP. Nous avons également étudié l'association entre la réponse au méthylphenidate (MPH) et des polymorphismes du gène *COMT*.

Les résultats de ces études pourraient avoir un impact important sur l'approfondissement de nos connaissances sur l'effet placébo; par conséquent, ils pourraient nous permettre d'améliorer les approches du traitement dans la pratique clinique.

Contribution of Authors

CHAPTER 1 - Introduction

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CHAPTER 2 – Study Design

Writing of text = W. Fageera

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Supervision = R. Joober

CHAPTER 5 – Conclusions

Writing of text = W. Fageera

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Symbol	Abbreviations
5- HTTLPR	Serotonin transporter-linked polymorphic region
ADHD	Attention Deficit Hyperactivity Disorder
CCK	Cholecystokinin
CGI	Clinical Global Impression
COMT	Catechol-O-methyltransferase
Conners'-P	Conner's Global Index-Parents
Conners'-T	Conner's Global Index-Teachers
CPE	Convergent placebo effect
DA	Dopamine
DPE	Divergent placebo effect
fMRI	Functional Magnetic Resonance Imaging
IBS	Irritable Bowel Syndrome
MAO	Monoamine Oxidase-A
MDD	Major Depressive Disorder
Met	Methionine
MPH	Methylphenidate
MR	Medication Response
NAc	Nucleus Accumbens
PBO	Placebo
PCR	Polymerase Chain Reaction
PD	Parkinson's Disease
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
PR	Placebo Response
RASS	Restricted Academic Situation Scale

RPR	Reverse Placebo Response
SAD	Social Anxiety Disorder
SNP	Single Nucleotide Polymorphism
Val	Valine

Chapter 1

Literature Review

1. Introduction

It is now well established that the mind, cognition, and emotions of a patient play a critical role in the therapeutic outcome. In other words, taking care of patients is of paramount importance for treating diseases. Plato's dialogue has described this as, "where Socrates points out that the pharmacology is only half of the treatment, and that a spell of the soul —that is a psychotherapeutic or psychosomatic intervention as we would say today—is just as important" (Walach, 2011). Previous studies suggest that in many conditions, one major source of response to treatment is determined by the propensity of a subject to feel better after receiving treatment, regardless of whether the treatment contains a pharmacologically active ingredient or not, a phenomenon that has been known as "***placebo effect***". In other words, what patients do is tricking themselves back into health by using their mind and brain, suggesting that not only physiological effects of medications are important to the healing process, but the subject as an active agent in determining the meaning of a treatment is important as well (Walach, 2011).

While some researchers have questioned whether there is a conclusive evidence of objective neurobiological effects of the placebo effect, it is now very well established that the placebo effects are correlated with neurobiological and physiological changes, including modifications in the electrical (Hunter, Leuchter, Morgan, & Cook, 2006) and functional activity of the brain (Benedetti, 2009; Hunter et al., 2006; Kong et al., 2006; Price, Craggs, Verne, Perlstein, & Robinson, 2007). Neuroimaging studies have provided significant insights to the understanding of this phenomenon. Indeed, they helped to visualize the changes in the brain activity and neurotransmitters after using placebo (Benedetti et al., 2005). Besides, genetic studies have also opened the door for further investigation of biological markers that might account for individual variations

in placebo response.

Contrary to the directly induced effects by the pharmacological proprieties of a substance, placebo effects are much more complicated to study as they may be contingent on psychosocial characteristics of individuals, such as age, suggestibility, personal history, personal beliefs, particularly with regard to the medical model one adheres to (Kleinman, Guess, & JS, 2002). Notwithstanding these individual variations, it is now well accepted that expectations and beliefs are main components of the placebo effect (Watson & Rayner, 1920; Sterzer, Frith, & Petrovic, 2008).

Importantly, placebo effect is not only limited to pathological conditions (both psychological and physical) but it is also observed with regard to normal traits in healthy volunteers (Benedetti, 2009). Over the past 50 years, reduction in symptoms after placebo administration have been reported virtually in every ailment, including "hard" outcomes such as blood pressure (Asmar, Safar, & Queneau, 2001; Brown, 1998; Preston, Materson, Reda, & Williams, 2000) or seizures (Bae et al., 2011; Burneo, Montori, & Faught, 2002), and in countless research trials (Benedetti, 2009; Brody & Brody, 2000; Kienle & Kiene, 1997; Stewart-Williams, 2004).

In this thesis we studied placebo response in children with Attention deficit hyperactivity disorder (ADHD) aged 6 to 12 years. ADHD could be a fertile ground to study placebo response for several reasons. First, ADHD is one of the most frequent neurodevelopmental disorders, affecting 5-10% of school-age children (Faraone, Sergeant, Gillberg, & Biederman, 2003). The treatment of ADHD by various molecules has increased sharply in the last decade. Second, clinical trials of ADHD have shown that there is a moderate to large benefit from placebo. Approximately 30% of ADHD children are placebo responders in double-blind clinical trials (Sandler, Glesne, &

Geller, 2008; Waschbusch, Pelham, Waxmonsky & Johnston, 2009). Third, some studies showed that children are more responsive to placebo than adults (Lewis, Winner, & Wasiewski, 2005; Rheims, Cucherat, Arzimanoglou, & Ryvlin, 2008b). In a meta-analysis on response to anticonvulsive drugs and placebo, it was reported that children were more responsive to placebo compared to older patients (Rheims, Cucherat, Arzimanoglou, & Ryvlin, 2008a). Furthermore, similar age effect was also reported for the treatment of migraine (Fernandes, Ferreira, & Sampajo, 2008). Hence, investigating placebo effects in children with ADHD seems to constitute a good clinical initiative/proposal. Fourth, since response to treatment in children with ADHD is often assessed by various observers (parents, clinicians, and teachers), it is possible to distinguish placebo response according to these various observers, which in turn may shed some light on how placebo response is shaped by psychological and social characteristics of these observers. This also illustrates how placebo effect transmigrate the body limits, such as when placebo is administered to a child, this response will embody to a large extent, the psycho-social dynamic of the parents (or other observers) that is deployed around the problem of ADHD. This thesis aims to answer the following questions: 1- Does placebo response differs according to the observer in ADHD? 2. What are the psychosocial and clinical determinants of placebo response? 3. Does *COMT* gene play a role in this phenomenon?

1.1 History of Placebo

Historically, placebo played an important role in the development of medicine (Wolf, 1959). The term “Placebo” is rooted in the Latin word “placare” meaning, “I

shall please" (Shapiro, 1978). The placebo discovery has a serendipitous history and the idea of "placebo" has existed for centuries.

Distributing sugar pills or inactive substances in order to comfort a patient in order to recognize "real" sick people, was quite customary during the pre-pharmacological era (Walach, 2011). The term "placebo" was first used in the 14th century; however in the late 18th century, it became part of the medical terminology. Importantly, in most cases during that time, physicians did not administer "pure" placebos (i.e. they did not use substances with no pharmacological effect at all). However, they resorted to different kinds of weaker substitute for the real treatment (de Craen, Kaptchuk, Tijssen, & Kleijnen, 1999; Jutte, 2013). Furthermore, in that era physicians tended to prescribe placebo when they thought "nothing was wrong with a patient" (Evans, 2003). In other words, it was used to placate the patient and to satisfy his/her demand rather than exerting a specific effect.

Going back to the nineteen forties, more specifically in the final years of World War II, Henry Beecher, an American anesthetist was using placebo to treat severely wounded soldiers. He used a harmless solution of saline instead of morphine, when he ran out of morphine for surgeries. Most of those treated with the saline settled down and they seemed to have little pain as if they were under the influence of morphine (Evans, 2003). Surprisingly, it did not seem to matter whether the injection had been filled with morphine or saline. Few years later (in 1955), Beecher summarized the history of placebo research and its effect in his article "The powerful placebo". This publication may be considered as one of the most frequently cited placebo reference (it has been cited by over 100 PubMed Central articles to date). The article claimed that 35% of over 1000 patients responded to placebo across 15 clinical trials, covering a wide variety of

areas (Beecher, 1955). Placebo response included both subjective and objective improvements. However, the accuracy of this estimation was later debated (Hrobjartsson & Gotzsche, 2001; Wampold, Minami, Tierney, Baskin, & Bhati, 2005).

By early 1960s, placebo effects were well known and placebo-controlled trials became the standard in order to get a new medication approved (Kaptchuk, 1998). In modern medicine, any treatment is composed of two components: one is related to the drug itself and the other to the perception of administration of drug (i.e. placebo effect or placebo response) (Benedetti, 2012). Because the latter can change as a function of a large number of factors, including psychological, sociological and interpersonal factors, molecule with real physiological and beneficial effects could fail clinical trials because of an inflated placebo effect. In fact it is believed that the last few years, placebo effect has increased and may be the source of many failed clinical trials (Alphs, Benedetti, Fleischhacker, & Kane, 2012; FDA, 2014). Hence, studying the determinants of placebo response in any specific medical condition is of paramount important to improve the effectiveness of clinical trial to detect a specific physiological effect of new pharmaceutical agents.

1.2 Definitions

Several terms have been widely used in placebo research and it is critical to distinguish these terms.

***Placebo*:**

Placebo is a pharmacologically inert substance or any therapeutic procedure designed to simulate medical therapy without being a specific therapy for the target condition (Stewart-Williams, 2004; Turner, Deyo, Loeser, Von Korff, & Fordyce,

1994).

True/Pure Placebo:

A substances with no pharmacological effect, e.g. sugar pills or saline infusions (Fent, Rosemann, Fassler, Senn, & Huber, 2011).

Untrue/Impure Placebo:

A substance with pharmacological effect but not on the targeted condition being treated. In other words, the prescribed substance has not been proven useful or its effect is uncertain for a specific disease, for example using vitamin infusions for the treatment of cancer (Fent et al., 2011).

Placebo Response

It refers to the improvement, regardless of its mechanisms, of an outcome related to a specific condition after administering placebo. Many factors can play a role in observing this improvement including spontaneous remission or regression to the mean, as well as neurobiological and physiological mechanisms associated with placebo (Brown, 2006; Raz, Zigman, & de Jong, 2009; Sonawalla & Rosenbaum, 2002).

Placebo Effect

It is the psychobiological phenomenon attributable to the placebo and treatment context (Raz, Zigman, & de Jong, 2009). Brown (2006) defined it as the placebo response minus the other factors that might have an interaction with it.

Despite the importance of distinguishing between the placebo response and placebo effect, these terms have been widely used interchangeably (Benedetti, 2012; Carlino & Benedetti, 2014).

Nocebo Response

Is derived from a Latin word that means, "I shall harm". It is defined as the adverse outcomes caused because of negative expectations, whereas the placebo effect is associated with positive expectations (Benedetti, 2012; Drici, Raybaud, De Lunardo, Iacono, & Gustovic, 1995; Johansen, Brox, & Flaten, 2003; Sonawalla & Rosenbaum, 2002).

Lessebo Response

This term has two different meanings depends on their context. Postuma & Albin (2014) uses this term to denote the placebo response that can be learned (i.e. lessebo comes from the word lesson). Mestre et al. (2014) defined Lessebo reponse as "patients' expectation of a negative outcome caused by the potential for receiving the placebo" (i.e. Lessebo comes from the word less or reduction).

1.3 How does Placebo Work?

Placebo effect could be considered as one of the least understood phenomenon found in human behavior. In the last two decades, there has been a growing interest in placebo research in various conditions/disorders. The interest in studying the mechanisms underlying placebo response is stimulated by advances in research design and technology and the possibility that this research can shed some light on the mind-brain-body interactions (Colloca & Benedetti, 2005). The changes that follow placebo administration can be elicited by different mechanisms; some of them are psychological (mentalistic) while others are physiological (biological).

1.3.1 Psychological Mechanisms

Placebo response is context sensitive and cannot be attributable to the inert substance alone. Therefore, studying the psychosocial context that surrounds the patients and studying the patient as an agent that can generate meaning from what he experiences in the therapeutic context is important to understand the placebo effects (Benedetti, Mayberg, Wager, Stohler, & Zubieta, 2005). Perceiving these factors is likely to vary among patients; therefore, the qualities and the magnitude of placebo responses could differ from one patient to the other (Price & Barrell, 2012). Different theories have been proposed to explain the psychological mechanisms of placebo response, including: expectations, classical conditioning theory, and emotional change theory (e.g. reward and anxiety reduction). Desire (Vase, Price, Verne, & Robinson, 2004; Vase, Robinson, Verne, & Price, 2003, 2005), motivation (Hyland, 2011b), distortion in memory (Price, Finniss, & Benedetti, 2008a), and patient's feelings, fears and beliefs are important pieces of the placebo puzzle that could modulate it and help to integrate it into the whole picture (Price & Barrell, 2012).

1.3.1.1 Expectation and Anticipation

One of the most prominent theories underlying placebo effect was developed by Kirsch (1985) postulating that placebo response is secondary to the self-fulfilling effects of response expectation. The generation of changes in subjective experience (placebo effect) by corresponding response expectation is suggested to be a basic psychological mechanism and the core of placebo effect in human beings (Geers, Wellman, Fowler, Rasinski, & Helfer, 2011; Haour, 2005; Price et al., 2008a; Laverdure-Dupont D et al., 2010). Expectations refer to "the probabilities associated with a future state of affairs" (Geers, Weiland, Kosbab, Landry, &

Helfer, 2005) based on the patient's beliefs for clinical improvements. Another description of expectation was presented by Vase et al., (2004) as "the experienced likelihood of an outcome or an expected effect". It is mainly generated when patients consciously foresee a positive/negative outcome (Benedetti & Amanzio, 2011) i.e. information about expected treatment results can affect the patients in a positive or a negative manner and lead to a placebo or a nocebo effect. These expectations are primarily contingent on a combination of verbal instructions, environmental clues, emotional arousal, previous experience, trust between the patient and his care providers, and the interaction with care-providers (Moore, 2012). For example, with regard to trust between the patient and the care providers, the belief of patients that the healer "knows his business and is trustworthy" could lead to a drastic improvement (Walach, 2011), in addition to, or sometimes in spite of what is being done. The expectation level could be simply measured; within the context of pain, for example the ideal way to measure it is by asking people directly about the level of pain, or the reduction thereof, they expect to experience (Benedetti, 2009).

A large number of experiments support the expectation account of placebo effects. For instance, Price et al., (1999) tested the extent to which expectation of pain relief after applying placebo creams (**A**, **B** and **C**) onto three skin areas after heat stimulation. Subjects were given the expectation that the cream **A** was a strong analgesic, cream **B** was a weak analgesic, and cream **C** was a control agent. Immediately after application of the three creams, subjects rated their expected pain levels for the placebo test trials. The actual pain magnitudes were as (**C > B > A**), indicating the role of expectation in placebo outcomes. Another example of the

expectation effect in clinical settings was conducted by Pollo et al., (2011) in post-operative patients. In this study, the authors changed the symbolic meaning of a base IV infusion in three groups of patients for three days by using different verbal instructions. *Group 1* was not told anything about the IV infusion they were given. *Group 2* was told that the basal infusion was either a powerful painkiller or a placebo. And the *last group* was told that they were receiving a real painkiller (deceptive administration). Requesting painkillers decreased significantly among the three groups according to the following order: Group 3 < Group 2 < Group 1. Yet another very interesting study was conducted to illustrate the role of expectations in placebo effect in Alzheimer's patients. In this pathological condition affecting the cognitive capacity of patients, it was shown that the response to placebo and real medicine in reducing pain is lower than what would be expected if compared to the same experiments in non-cognitively impaired subjects. This could be explained, at least in part, by the fact that the capacity of these patients is altered to build expectations, and is based on the clinical context and their previous experiences (Benedetti et al., 2006).

Since placebo response is contingent on expectations, it is highly dependent on several factors that are conceived by the subject as enhancers (or dampeners) of the expected effect. For example, it has been reported that the size (Buckalew & Ross, 1981), color (e.g. blue more effective for sleeping pills; red for pain pills), preparation (pills or capsules) (deCraen, Roos, deVries, & Kleijnen, 1996), branding (Branthwaite & Cooper, 1981), price (Waber, Shiv, Carmon, & Ariely, 2008a) and type of placebo (e.g. pure placebo vs. impure placebo) can influence its effect in a predictable way (Thomson, 1982).

Likewise, other factors such as: verbal or non-verbal information (suggestion), motivation, emotional factors (such as desire and self-efficacy) (Price et al., 2008a), direct personal experience (Colloca & Benedetti, 2006; Price et al., 2008a), social observational learning (Colloca & Benedetti, 2009), memory and perception, can shape expectations. Some of these factors will be highlighted in the next paragraph.

A. Verbal and Nonverbal Suggestions

Verbal and nonverbal, direct and indirect suggestions have been shown to influence placebo and nocebo response in numerous experimental studies (Pollo et al., 2001; Price et al., 1999; Vase, Riley, & Price, 2002). Verbal suggestions that induce certain expectations of analgesia induce larger placebo responses than those inducing uncertain expectations (Price et al., 2008a). Vase et al., (2002) found that placebo analgesia was significantly higher following verbal instructions given to the participants compared to placebo response in the context of double-blind, randomized clinical studies (Enck & Klosterhalfen, 2005; Vase et al., 2002).

B. Motivation

Similarly, motivation is another important modulator of placebo effect (Wager, 2005). Indeed, a motivated subject is more likely to be extra alert to the changes that are compatible with his expectations and to disregard changes that are not well-matched with his/her expectation (Geers, Helfer, Weiland, & Kosbab, 2006). In addition, as motivation is also determined by the meaning individuals attribute to the concepts of illness and health and how they respond to medications and as these concepts are highly determined by social and cultural constructs, placebo response is also a reflection of social and cultural contexts. Parallel to this,

it has also been shown that placebo effect on gastric ulcers is very low in Brazil, high in Northern Europe and very high in Germany; an observation that might be related to cultural factors (Moerman, 2000).

C. Emotional Factors

Several emotional factors have been shown to modulate placebo response:

C.1 Desire

Desire as defined by Price et al., (2008b) is "the experiential dimension of wanting something to happen or wanting to avoid something". This concept has been suggested to be likely involved in placebo response (Vase, Price, Verne, & Robinson, 2003). In pain studies, it is natural that subjects desire to avoid, terminate, or reduce pain (Price et al., 2008a). Vase et al., (2003) reported that the patients' expectations and their desire for relieving pain explain a significant variation in response to placebo in rectal pain. In addition, a subsequent study showed that a decrease in expected pain and negative emotions (such as anxiety) along with increasing desire for pain relief were associated with increased placebo analgesia over time (Vase et al., 2005). Also the desire for approaching or fulfilling goals is consistent with the model described above (Geers et al., 2006). It is also likely that after administration of placebo, the changes of common human emotions, such as sadness and anxiety result from interaction of desire and expectation (Hyland, Whalley, & Geraghty, 2007; Price et al., 2008a).

C.2 Self-efficacy

Self-efficacy is one's belief in his ability to succeed in specific situations to achieve an outcome (Caspi & Bootzin, 2002). This factor can play a major role in how a subject approaches and reaches goals; as such, self-efficacy has been associated with response to placebo (Oken, 2008).

C.3 Stress/Anxiety Reduction

Reducing anxiety was drastically associated with receiving placebo in patients with irritable bowel syndrome (IBS) (Vase et al., 2005). This observation has two possible explanations: 1) either decreasing stress enhances the healing process, as it is well known that chronic anxiety and stress impair function of various systems. 2) Or various other factors such as sleep pattern, level of activity, etc. could improve after reducing stress (that follows placebo administration), and therefore, overall health may improve (Stewart-Williams, 2004).

D) Social Observational Learning

Social observational learning represents another psychological mechanism for modulating subject's expectations and placebo responses (Colloca & Benedetti, 2009). Colloca and Benedetti (2009) showed that observing another person who is undergoing an analgesic treatment improves placebo analgesic responses in the observer. Interestingly, this response was "similar in magnitude to those induced by directly experiencing the benefit through a conditioning procedure".

1.3.1.2 Pavlovian (Classical) Conditioning

Another important approach to the placebo effect is based on classical

conditioning. Classical conditioning may be considered as the first type of learning where organisms respond to an environmental stimulus. The conditioning effect theory has been developed by Ivan Pavlov, a Russian physiologist, in 1903. Pavlov revealed how the classical conditioning could be a reflective type of learning wherein environmental stimulus acquires the capacity to evoke an innate response. He established the laws of classical conditioning by studying dogs' unconditioned response to an unconditioned stimulus (e.g., salivation after noticing food) with a conditioned auditory stimulus (e.g., a bell ringing) that provokes a conditioned response (i.e., salivation which is induced by bell ringing alone) (Figure 1.1) (Benedetti, 2012; Frances K. McSweeney, 2014).

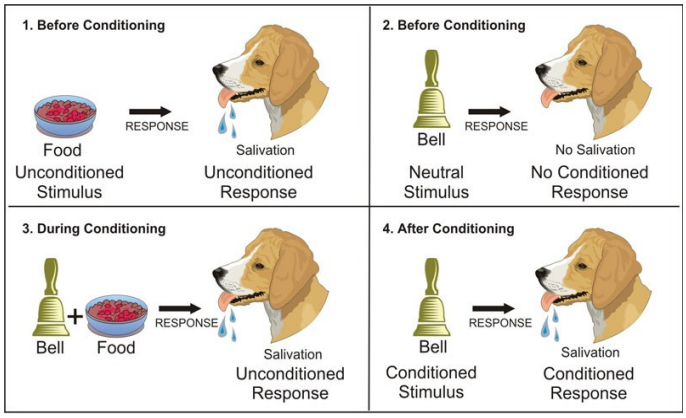

Figure 1.1: Classical conditioning effect (Pavlov's Experiment). Taken from (Sincero, 2011)

John B. Watson (1921) conducted a similar experiment in humans (figure 1.2). Watson conducted his experiment on a 9-month-old child (Albert). He was mainly studying if Albert will become terrified from a white rat, a rabbit, and other things by pairing them with a loud noise (i.e. unconditional stimulus) (Figure 1.2). He observed that the loud noise developed a fear in Albert but no fear signs were detected when he put rat and rabbit alone for the first time. Few weeks later,

Watson repeated the same experiment by using a rat and claimed “little Albert only had to see the rat and he immediately showed every sign of fear”. He would cry (whether or not the hammer was hit against the steel bar) and he would attempt to crawl away” (John B. Watson & Rayner, 1920).

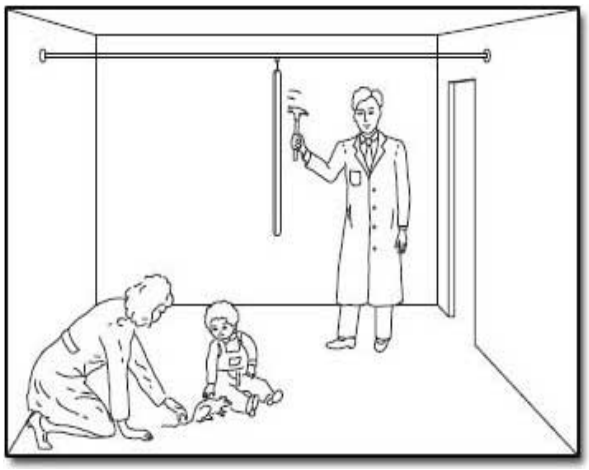

Figure 1.2: Classical conditioning experiment in human, conducted by John B. Watson. Taken from (McLeod, 2008)

Similarly, clinical setting features (such as, syringes, white coats or the peculiar hospital smell) when paired with active medications can act as conditioned stimuli, leading to elicit a therapeutic response even in the absence of an active agent. This could happen mainly by comparing these conditioned stimuli with a previous experience (Voudouris, Peck, & Coleman, 1990; Wickramasekera, 1980). Nowadays, the validity of classical conditioning theory for explaining placebo effects has compelling evidence (Chung, Price, Verne, & Robinson, 2007; Haour, 2005).

Expectations and conditioning are the two main functional approaches to the placebo effect that are involved in eliciting different placebo responses (Benedetti et al., 2003). As whatever is learned in Pavlovian conditioning is expectation (Benedetti,

Carlino, & Pollo, 2011), these mechanisms are compatible and they do not work against each other in order to achieve clinical benefits that are increased by a combination of expectation and conditioning (Amanzio & Benedetti, 1999). For example, in placebo analgesia, researchers have noticed that expectation is not separate from conditioning and suggestion. In fact, Benedetti and colleagues (2003) claim, "conditioning and suggestion induce individual expectations for pain relief" (Figure 1.3). Nevertheless, these findings suggest that the expectations have an effect in conscious functions whereas the conditioning takes place when unconscious physiological functions come into play. For example, expectations are shown to be effective in pain and motor performance (i.e. conscious functions) but not in hormonal secretion (i.e. unconscious functions) (Benedetti et al., 2003). On the other hand, conditioning is shown to mediate placebo responses in hormonal secretion. Therefore, placebo effect could be a conscious or unconscious learned phenomenon, depending on the system that is involved (e.g., pain or hormone secretion) (Benedetti et al., 2005) (Figure 1.4).

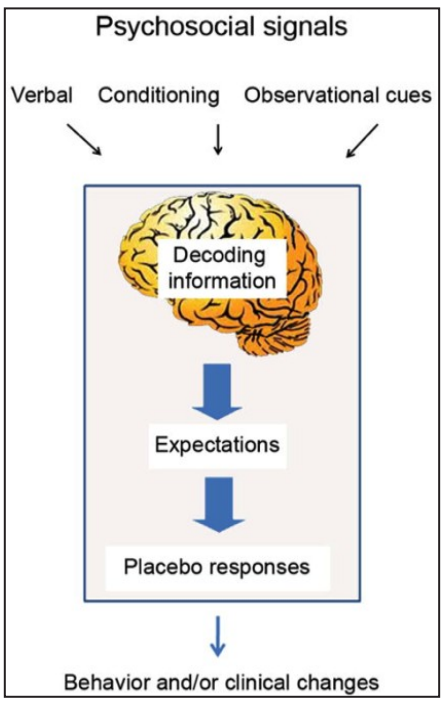

Figure 1.3: Role of conditioning, verbal, observational cues in the brain. Taken from (Meissner, Bingel, et al., 2011).

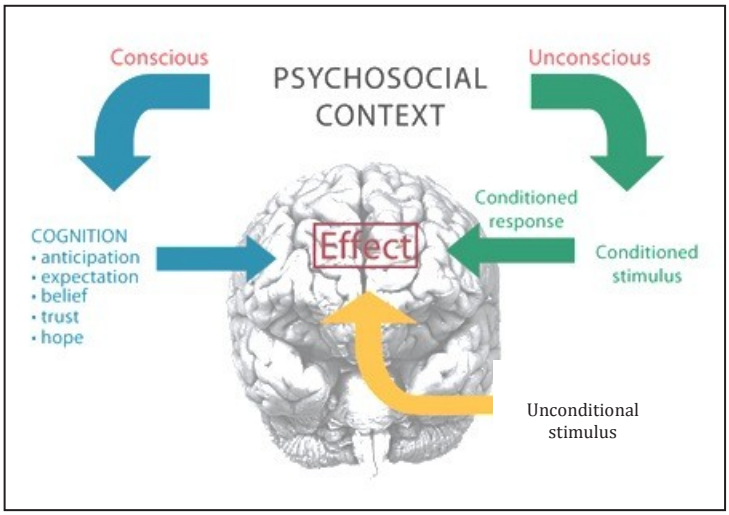

Figure 1.4: The psychosocial context around the patient and the therapy. Taken from (Fabrizio Benedetti, 2009).

Besides all the previous psychological models of placebo response (i.e. expectation and conditioning theory), other mechanisms are also considered when accounting for placebo response. For example, recently, certain personality traits have been identified to be associated with higher placebo effects. These factors include: optimism (Geers, Helfer, Kosbab, Weiland, & Landry, 2005; Geers, Wellman, Fowler, Helfer, & France, 2010; Morton, Watson, El-Deredy, & Jones, 2009), empathy, spirituality (Hyland, 2011a), novelty seeking, and reward responsiveness (Meissner, Kohls, & Colloca, 2011).

1.3.2 Physiological / Neurobiological Mechanisms

Besides or underlying the psychological factors that are related to placebo response, other neurobiological factors may also play a critical role in placebo effects. Use of neuroimaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) facilitated studying the neurobiological mechanisms of placebo response and helped to understand how placebo affects the central nervous system (Benedetti et al., 2005). These techniques allow

observing objective measurements that cannot be readily assessed by direct observations or self-reports. In addition, PET and fMRI facilitate to show that the placebo does not affect behaviors in non-specific way, but affect activity in a disorder-specific neural pathways (Benedetti et al., 2004; de la Fuente-Fernandez et al., 2001; Mayberg et al., 2002), suggesting that the placebo effects are enacted, at least in part, through specific disease related pathways.

Interestingly, placebo and pharmacological treatments yield similar neuronal changes, as supported by studies on pain, motor disorders and depression (Faria, Fredrikson, & Furmark, 2008). Furthermore, placebo effects have shown to be mediated by activating specific areas in the brain that could be dysfunctional in some disorders. It is noteworthy that the neurobiological phenomena induced by placebo, such as activation of some brain regions and releasing endogenous substrates, could be triggered by psychological mechanisms (Benedetti, 2008).

A) Endogenous Opioids System

In 1978, Levine, Gordon and Fields initiated a pioneering study on placebo effect and endogenous opioid release and showed that placebo analgesia could be blocked by the opioid receptor antagonist, naloxone in 39% of the subjects. Recently, Benedetti et al., (2011) provided compelling evidence for the association between modulation of placebo and secretion of endogenous opioids (Benedetti, Carlino, et al., 2011). Zubieta et al., (2005) studied the outcome of using placebo with expectation of analgesia on the endogenous opioid secretion, and confirmed a key role of endogenous opioids in placebo analgesia. They found that the placebo response was significantly associated with higher μ-opioid neurotransmission. A previous comparable finding was reported by another group as well (Wager et al., 2004). Remarkably, activating the

endogenous opioid systems is connected with reducing stress and anxiety and a concomitant increase in positive emotions (Meissner, Kohls, et al., 2011), which have been shown to modulate placebo effects.

B) Endogenous Dopamine System (DA)

Given the role played by dopamine neurotransmission in modulating expectations, it has been suggested that the dopaminergic pathways may play a significant role in modulating placebo response (Faria et al., 2008; Kaasinen, Aalto, Nagren, & Rinne, 2004). In 1999, Altier and Stewart were able to block the analgesic effects by antagonizing nucleus accumbens (NAcc) dopamine receptors (Altier & Stewart, 1999). This observation was not limited to pain, but it has been observed in motor disorders such as Parkinson's disease (PD) (de la Fuente-Fernandez et al., 2001). De la Fuente-Fernandez et al., (2001) observed some changes in release of DA in the striatum of the patients after receiving placebo infusions. More specifically, they noticed an activation of the nigrostriatal dopamine system after placebo administration even though it is in part damaged in this disorder. They found $\geq$ 200% increase in extracellular dopamine concentration in patients who responded to placebo, an equal effect was seen for the active drug (levodopa). Later in 2002, they concluded that release of DA is related to the expectation of reward and not to the reward itself (as the striatum is involved in the circuitry of reward mechanisms) (de la Fuente-Fernandez et al., 2002).

Another study conducted by Benedetti et al., (2004) demonstrated that using placebo leads to a change (reduction) in the activity of single neurons in the subthalamic nucleus, which was correlated with clinical improvement. This region is considered as a

crucial brain area for central motor control. Interestingly, no change in activity was observed when the clinical placebo response was absent (Benedetti et al., 2004).

C) Endorphins

Several studies advocate that endorphins, which are body's natural painkiller, mediate the placebo effect. This association was mainly observed with "analgesia" but it does not explain other symptoms' such as relief of nausea or depression (Thompson, 2000). Placebo responders showed a higher level of β-endorphins in the cerebrospinal fluid (CSF) compared to non-responders. Alphs et al., (2012) also suggested a role of endorphins in placebo effect. From a biological point perspective, some argue that endorphins release is not the cause of placebo analgesia; but is rather itself placebo analgesia (Stewart-Williams, 2004). However, the role of endorphins in relation to placebo effects is still debatable.

D) Endocannabinoids and Cholecystokinin (CCK) Systems

Using naloxone, an opioid receptor antagonist, in a morphine conditioning and/or expectation-inducing protocol led to reverse placebo analgesia and caused arm pain. On the contrary, when ketorolac, a non-opioid analgesic was used in the same protocol, naloxone became ineffective and cannabinoid receptor antagonist rimonabant completely blocked placebo analgesia (Benedetti, Amanzio, Rosato, & Blanchard, 2011). These findings suggest that the placebo effect can still occur after blocking opioid system and may suggest that other systems are also implicated.

Another system that might be implicated in placebo effects is the cholecystokinin (CCK) system, a neuropeptide that has anti-opioid effects. It has been suggested that the CCK system could reduce the effect of opioids. Therefore, the placebo on the contrary may facilitate opioids and inhibit CCK (Benedetti, Carlino, et

al., 2011; Benedetti et al., 2005). However, so far little is known about the non-opioid systems and further research is needed to clarify their role.

It is important to note that biological models are not independent models that compete with psychological models in placebo response (Figure 1.5 and 1.6).

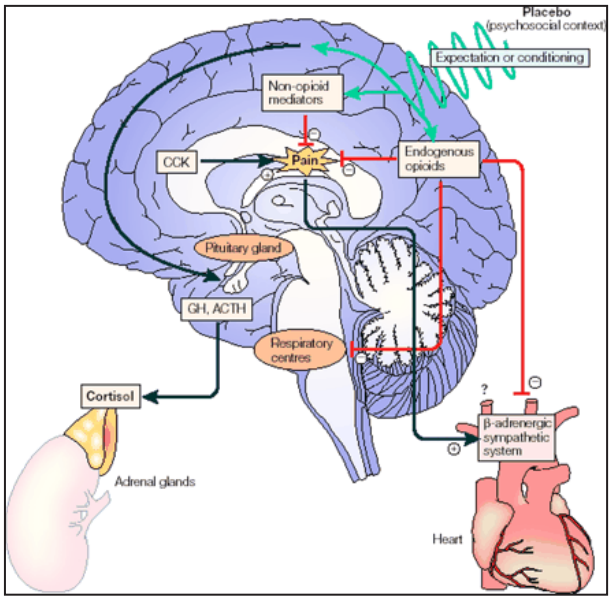

Figure 1.5: Some of the events that might take place in the brain after administering placebo in analgesia. For example, through opioid and/or non-opioid, expectations and /or conditioning mechanisms. Taken from (Colloca & Benedetti, 2005). **Note**: ACTH: adrenocorticotrophic hormone; GH: growth hormone.

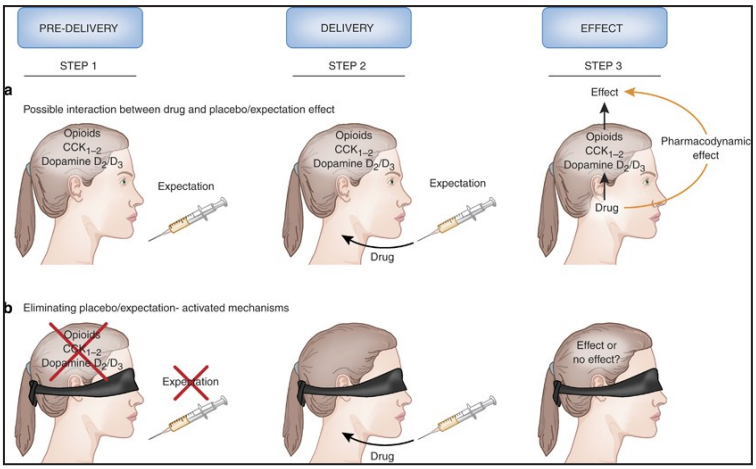

Figure 1.6: Interference between placebo/expectations effects and drug action. (A) Step 1: by presenting a syringe (for example) the placebo/expectation mechanism (such as releasing DA and opioid) will be activated. In step 2, the drug will be injected; however, the effect may be because of the drug action and/or because of an interference with expectation-activated mechanism. (B) By a hidden administration, the placebo/expectations mechanisms could be eliminated; therefore, any observed effect is likely to be because of the drug itself. Taken from (Enck et al., 2013).

1.4 Genetics of Placebo Response

E) Genetic factors

The magnitude of placebo response is variable among individuals. The origin of this variability, as mentioned earlier might depend on many psychosocial factors. However, some of this variability may be explained by biological factors (including genetic factors). Although there are a few preliminary studies investigating genetic association between placebo response and genetic variants in some disorders, our understanding of the genetic modulators of the placebo response is still very limited. To the best of our knowledge, none of these studies were conducted in children in general and children with ADHD in particular.

The genes to study in placebo effects were selected based on their neurobiological effects. Genes coding for brain dopamine related proteins besides other neurotransmitter systems have received some attention. Rausch et al., (2002) found a significant association between serotonin transporter-linked polymorphic region (5-HTTLPR) and placebo response. The homozygous for the long allele of 5-HTTLPR polymorphism showed higher placebo response, as well as more robust response than the short allele group. Few years later, Furmark et al., (2008) found a significant association between 5-HTTLPR, the G-703T polymorphism in the tryptophan hydroxylase-2 (*TPH2*) gene promoter, and placebo response in patients with severe anxiety disorder (SAD). Improvement in the symptoms was mediated by these genes (two serotonin-related enzymes) on amygdala activity, a brain region that is crucial for emotional processing. However, this finding could be linked to placebo-induced anxiety relief and be unique to SAD (Hall et al., 2012).

Other genes that were associated with placebo response in several conditions are the genes coding for Catechol-O-methyltransferase (*COMT*) and Monoamine oxidase-A (*MAO-A*). These two genes are important in the regulation of dopamine and norepinephrine in the brain, particularly in the prefrontal cortex. Since these neurotransmitters work jointly to modulate reward processes (dopamine is involved in encoding reward expectation and norepinephrine is involved in sustaining attention on the possible reward), these associations reinforce the importance of expectation/reward in placebo effects.

**** MAO-A***

Leutcher et al., (2009) found that *MAO-A* variations (rs6323) in patients with Major Depressive Disorder (MDD) are significantly associated with response to placebo and a stronger placebo response in subjects with the lowest enzyme activity (i.e. more norepinephrine was needed in order to translate hope and belief into something physiological). Another SNP in *MAO-A* (rs6609257) also showed a trend of association with placebo response (Tiwari et al., 2013).

****COMT***

COMT gene could be considered as a prime candidate gene in placebo response research for the following reasons:

1. *COMT* has been linked to the susceptibility and treatment of many conditions (Craddock, Owen, & O'Donovan, 2006), including pain (Tammimaki & Mannisto, 2012), Parkinson's disease (Deane, Spieker, & Clarke, 2004), schizophrenia, and ADHD (Fan et al., 2005; Hosak, 2007; Sanchez-Mora et al., 2012; Turic et al., 2005).

2. *COMT* is involved in the physiological pathways in models of reward processing (Schmack et al., 2008).

3. *COMT* val$^{108/158}$met is one of the well-studied SNPs that affect enzyme activity (low activity "met allele" resulting in higher level of dopamine). A significant association between *COMT* and placebo response was reported by Leuchter et al., (2009) in MDD. In contrast to their findings with MAO, they reported that patients with high level of dopamine (i.e. who carry met allele) had a lower placebo response even though; more response was expected with higher DA. However, the authors explained this unexpected observation by stipulating subjects with the met allele may have sustained higher dopamine background level, which makes them less sensitive to reward. This higher tonic dopamine level may blunt the more phasic dopamine signaling and the reward processing. Interestingly, these genes (i.e. *COMT* and *MAO-A*) have no effect on MDD susceptibility, supporting the notion that these genes are involved in placebo effects (Leuchter et al., 2009).

In contrast, more placebo response was found as the copies of met allele of the *COMT* gene increased (i.e. low levels of activity), and therefore high levels of dopamine in Irritable Bowl Syndrome (Hall et al., 2012). Another study (Farrell, Tunbridge, Braeutigam, & Harrison, 2012) in healthy men found that met carriers subjects performed better than Val homozygous subjects on a 2-back task of working memory after administration of placebo. Similar results were found in patients with schizophrenia (Goldberg et al., 2003), suggesting that the *COMT* mediated placebo response pathway might be involved across multiple conditions (Hall et al., 2012).

From these studies, it is possible to conclude that met/met is a genetic marker for the placebo response, followed by heterozygous patients (val/met); whereas,

val/val subjects could be the latest responders to placebo. Although, sample size could be relatively small, these studies show a strong correlation between some gene variants and placebo effects. Importantly, placebo response is a complex phenotype and a single locus like *COMT* val$^{108/158}$met could not be a main factor explaining a large part of the variance in this phenotype (Hall et al., 2012). However, given that it is functional polymorphism it has a greater potential to contribute to behavioral variability than other non-functional or non-coding polymorphisms (Hall et al., 2012).

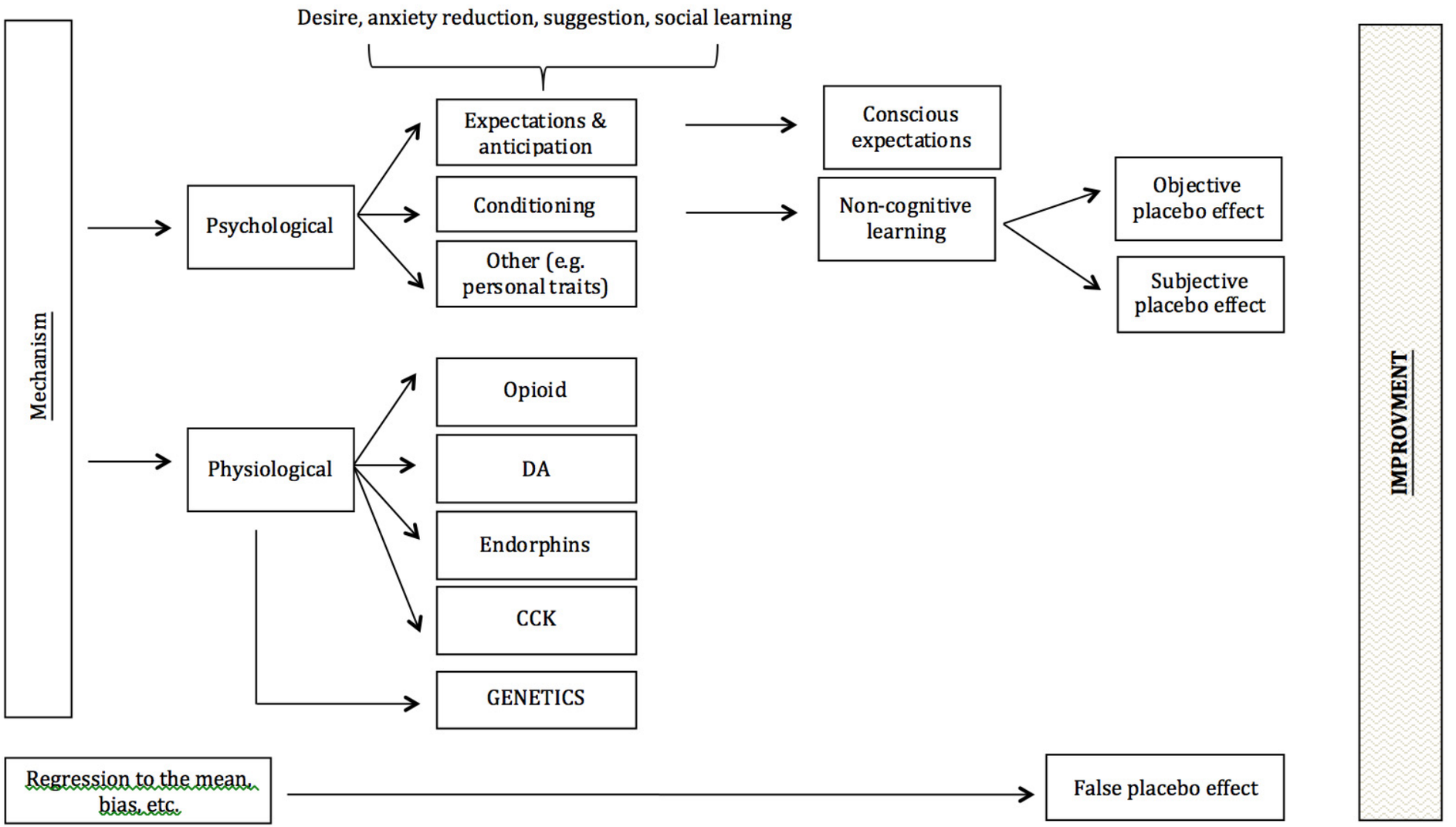

Figure 1.7: Overview on placebo mechanisms; adopted from (Stewart-Williams, 2004)

1.4 Implications of Placebo mechanisms for Clinical Trials

1.4.1 Brief history

The use of placebos in controlled clinical trials developed gradually. It is believed that the first placebo-controlled experiment was conducted in 1784 in Paris to investigate the validity of "mesmerism", a very popular therapeutic technique at that time. "Mesmerism" referrers to Franz Anton Mesmer who believed on the power of planets in human health, and how the universe was connecting people to the planets by an invisible fluid. Any alteration in this fluid affects people's health. He discovered the magnets power in healing open wounds (either by putting magnets over open wounds or by asking subjects to hold magnets sticks). Mesmer was using magnetized water and metallic rods to cure various diseases. People got attracted with this new method for two main reasons: **(1)** patients were successfully cured from different types of diseases, which made Mesmer a miraculous healer. And **(2)** it was preferable to purging and bleeding, the most common medical treatments techniques of that age. In 1784, King Louis XVI appointed the French Academy of Sciences to investigate this technique by conducting an official trial. A patient was blinded to the experiment where a magnetic rod and a dummy one were used. The patient was shown to respond to both rods; and the committee concluded "Mesmerism worked by the action of the imagination" (Finniss, 2013; Walach, 2011).

Moving to the modern medicine era, using placebo in clinical trials became central and mandatory by regulation to establish the therapeutic efficacy and safety of medications. As pain is a subjective experience with important psychological and social modulation, the use of placebo in clinical trials for analgesics has been very important (Benedetti, 2009). That could be true with most studies that looked at the

neurobiological mechanisms of placebo effects. However, several studies have demonstrated both placebo and nocebo effect in many disorders; including: the immune system, motor disorders, hypertension, and mental disorders (Benedetti, 2009; Benedetti et al., 2005; Price, Finniss, & Benedetti, 2008b; Sandler, 2005).

1.4.2 Implication of placebo effect in clinical trial designs

In depth understanding of placebo response is important to improve the quality and validity of clinical trials. Indeed, a clinical trial aims primarily to isolate the effects of the medication that are directly in association with its interaction with the disease mechanisms. Thus, to improve the capacity of a clinical trial to achieve its primary purpose, the knowledge of placebo effect will help to improve the clinical design by:

(1) Minimizing the placebo effects as much as possible so that the effect of the active medication can be identified against a minimum background placebo effect (Enck et al., 2013). It has long been known that there are placebo responders and non-responders, although the reasons behind this are less clear. Different combinations of factors influence the response as implied above. Identifying genetic, neurofunctional, or psychological predictor for placebo responders has important implications in clinical trials. Firstly, treatment approaches can be modified on an individual basis to allow maximum benefit for individual patients. In other words, it will enable physicians to "tailor treatments" based on patients' needs; for example by picking the best drug or adjusting treatments' doses (Hall et al., 2012).

Secondly, it would allow for a further understanding of sources of variance during clinical trials. That is by separating placebo responders from non-responders before the start of a trial, based on specific characteristics will lead to have more efficient trials (Benedetti, 2009). Many other aspects of our knowledge of the

determinants of placebo response (see below) can help to improve the design of clinical trials.

(2) Understanding how beliefs, values and expectation (or other complex mental processes) shape brain functions and in turn affects the human experience and behavior (Benedetti et al., 2005; Finniss & Benedetti, 2005). Indeed, as patients' mind, cognition, and emotions play a central part in therapeutic outcome similar to what the pharmacology, genetics, and physical interventions do, is always important to keep these dimensions in mind when interpreting the "pure" pharmacodynamic effects of medications (Benedetti, 2013).

(3) Helping to generate new study designs and reevaluate clinical trial methodology (Benedetti & Amanzio, 2011). For example, by controlling for patients' expectations as this factor is so critical in responding to a placebo or a real drug (Benedetti & Amanzio, 2011). Previous studies have shown the effect of assigning different groups based on their expectation level (Bausell, Lao, Bergman, Lee, & Berman, 2005; Linde et al., 2007). Bausell et al., (2005) showed how placebo response increases in the group that has high level of expectations/or believes in the treatments compared to the standard group. Another example is the "open/hidden" design that had emerged in the clinical trial field after understanding some of the placebo effect's mechanisms (Benedetti, 2012) (Figure 1.7).

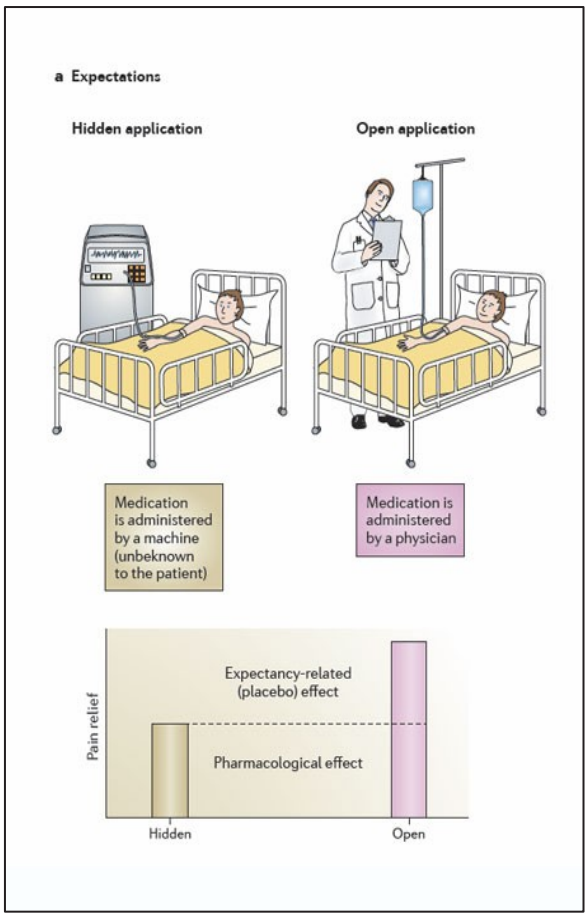

Figure 1.8: The vital role of cognitive factors (e.g. expectations) in mediating placebo responses in open/hidden model. Taken from (Enck, Bingel, Schedlowski, & Rief, 2013)

1.4.3 Important Claims of placebo effect and clinical trials design

1. Secular trend: there is strong evidence in the literature indicating an increase of placebo effect in the last few decades (Andrews, 2001; Walsh, Seidman, Sysko, & Gould, 2002). Understanding the mechanisms behind this trend might be very important to improve the quality of clinical trials.

2. Placebo effects are not restricted to subjective effects, but extend to more objective outcomes (Thompson, 2000). However, placebo response is shown to be greater in continuous subjective measures such as pain (Furukawa, 2002). It was also reported that

placebo effects are greater with self-report outcomes rather than observer measured or objective measures (Hrobjartsson & Gotzsche, 2001).

3. In placebo research both desirable and undesirable effects should be accounted. Both effects can be concurrently monitored in clinical observations. For example, placebo can improve health but can also produce side effects (Shapiro, Chassan, Morris, & Frick, 1974).

4. Non-blind placebos (open–label study design) can be effective. In other words, placebo could work even if the subject is aware of using it (Kaptchuk et al., 2010; Kelley, Kaptchuk, Cusin, Lipkin, & Fava, 2012; Sandler & Bodfish, 2008).

5. Many factors may modulate placebo response and need to be taken into consideration when designing clinical trials:

A. Placebo injections are more powerful and produce stronger effects than capsules and pills (Buckalew & Ross, 1981; Stewart-Williams, 2004).

B. Large pills are more effective than small ones (Stewart-Williams, 2004).

C. Color makes a difference as pink pills improve mood and blue ones could worsen it (Stewart-Williams, 2004).

D. Person who is giving placebo is an important factor as administering placebo by a doctor seems to be more powerful compared to the one given by another medical staff (e.g. by a nurse or a secretary) (Thompson, 2000).

E. Placebo effect could have short (few hours) or long-term (months) effects (Thompson, 2000). Michael Hyland (2011b) explained this observation from a motivational point of view. He claimed that different placebo mechanisms may apply in different contexts. Expectations, conditioning and goal activation may be

responsible for short-term placebo effects whereas satisfaction of higher-level goals, such as a good relationship with the doctor may result in longer-term placebo responses. It has been reported that clinical outcomes are affected by a patient-doctor verbal and non-verbal interaction, when the doctor emphasizes the importance of medication, and when the frequency of medical visits are increased (Vase, Price, Verne, & Robinson, 2004).

F. Medications' price: the more expensive, the longer effect lasts (Waber, Shiv, Carmon, & Ariely, 2008b).

1.4.4 Use of placebo in clinical practices

While using placebo in clinical trials is a major part of experimental medicine, its use in clinical practice is rather limited and often questioned on the basis of ethical grounds. British Parliament's Science and Technology Committee called placebo as a "bad medicine" (Committee, 2010) and The American Medical Association considers using placebos "without patient's consent" is unethical (Kupferschmidt, 2011). The controversy of using placebo in clinical settings raises several ethical issues (Asai & Kadooka, 2013) including: ***(1)*** the use of inactive drugs when effective drugs exist. ***(2)*** Tricking patient by using placebo could violate patient's trust ***(3)*** If the patient is helped by placebo, discontinuing the placebo, in absence of a more effective treatment, would be unethical (Lichtenberg, Heresco-Levy, & Nitzan, 2004).

Chapter 2

Study Design

2.1 Overview

The data collection presented throughout this thesis is based on a two-week double-blind, placebo-controlled, crossover randomized trial of methylphenidate (MPH) conducted at the Douglas Mental Health University Institute in children with ADHD (between 6 - 12 years) under the supervision of Drs. Ridha Joober and Natalie Grizenko.

This chapter will present an overview of the methods used in this thesis (chapters 3 and 4).

2.2 Study Design

Following baseline evaluations (which served as a wash-out period for previously medicated children), children with ADHD received for a week either placebo or 0.5mg/kg of MPH in a b.i.d dose, and were then crossed over during the second week. Treatment response (i.e. to MPH and placebo) was assessed by examining the change in scores obtained from various lab assessments using Conners' scales as evaluated by parents at home and teachers at school. A more "objective response to treatment was assessed by a system of behavioral observation in the laboratory, where a set of behaviors pertinent to ADHD are evaluated by the research staff.

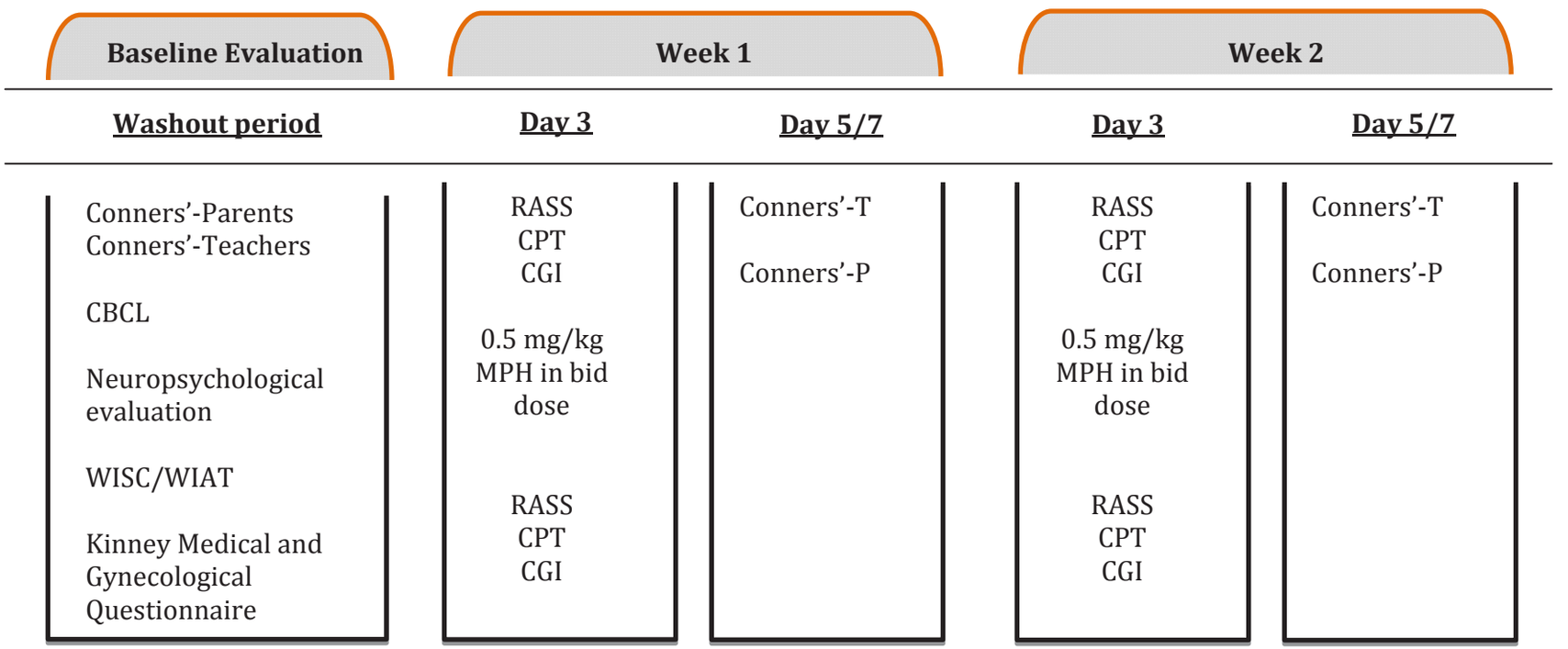

Figure 2.1: Timeline of the two-week double-blind, placebo-controlled crossover trial of methylphenidate

2.3 Recruitment of Children with ADHD

Children with ADHD (aged 6-12years) were referred to the ADHD clinic at the Douglas Mental Health University Institute, Montreal, by schools, community social workers, family doctors, pediatricians, and child psychiatry outpatient clinics. The research protocol for the study was approved by the Research Ethics Board of the Douglas Institute. During the recruitment process, all details related to the study were explained to the parents who provided written consent on behalf of their children. Additionally, children with ADHD gave their verbal assent to participate in the project. Recruitment into the study was based on the following inclusion and exclusion criteria:

Table 2.1: Inclusion and exclusion criteria for study participants

Inclusion Criteria	Exclusion Criteria
✓ Age: 6-12 years ✓ Diagnosis of ADHD based on: • Clinical interview of the child and at least one parent • Structured interview with parents using DISC-IV, parental report • Evaluation of behavior in different settings: 1) In school by teacher (Conners' Global Index (CGI) –Teacher 2) At home by parents (CGI-Parents). N.B. at least one CGI score either Parents or Teachers should be 65 or above.	✓ Previous history of mental retardation with an IQ less than or equal to 70 ✓ Previous history of autism, Tourette's syndrome, pervasive developmental disorder or psychosis ✓ Any major medical condition or impairment that would prevent the child to complete testing during the study. ✓ Synchronous treatment with any other medication except MPH.

2.4 Evaluation of Behavioral and Therapeutic Response to Methylphenidate

Following baseline evaluations, all children with ADHD received either MPH or placebo (for 7 days) in a randomized, double blind sequence. Both MPH and placebo are packed in colored gelatin capsules. All capsules were sealed in a daily basis and prepared by a clinical pharmacist who was not involved in the current study. Their order of administration was determined by counterbalanced random assignment (using computer-generated random numbers). MPH was prescribed in a b.i.d. dose (0.5 mg/kg/day; in the morning before school and at noon). Medication administration for each child was at the same dose and at the same time every day. Furthermore, in each treatment week, on the 3rd day, the children were evaluated in the clinic (RASS, CPT and SOPT) before taking the medication and 60 minutes after the medication. In addition, the Clinical Global Impression (CGI) for illness severity and improvement were completed by the clinical staff based on their observation during the testing. On the 5th and the 7th day, information on therapeutic response was collected from the teachers (Conners'-T) and the parents (Conners'-P), respectively, by a research assistant. Figure 2.2 is an illustration of a child who was administered MPH in the first week followed by placebo in the second week:

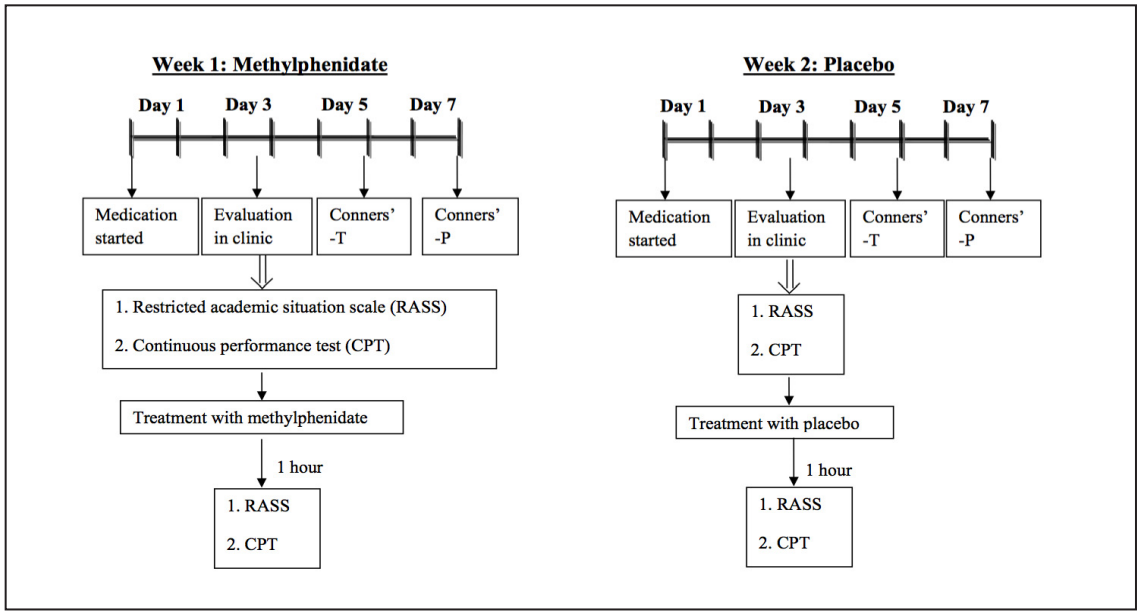

Figure 2.2: Description of behavioral measures and neurocognitive assessments administered during the two-week trial.

2.5 Baseline Evaluation

During this evaluation, participants were off any medication. Baseline evaluations included: (1) diagnosis of ADHD based on DSM-IV criteria, and its associated comorbid disorders; (2) collection of demographic data; (3) Full scale, verbal and performance IQ were measured by the Wechsler Intelligence Scale for Children-III (WISC-III). (4) Behavioral profiles of children were assessed by a psychiatrist and by research assistants using the Clinical Global Impression for severity (CGI-severity), by parents (CBCL, Conners'-P), and by teachers (Conners'-T).

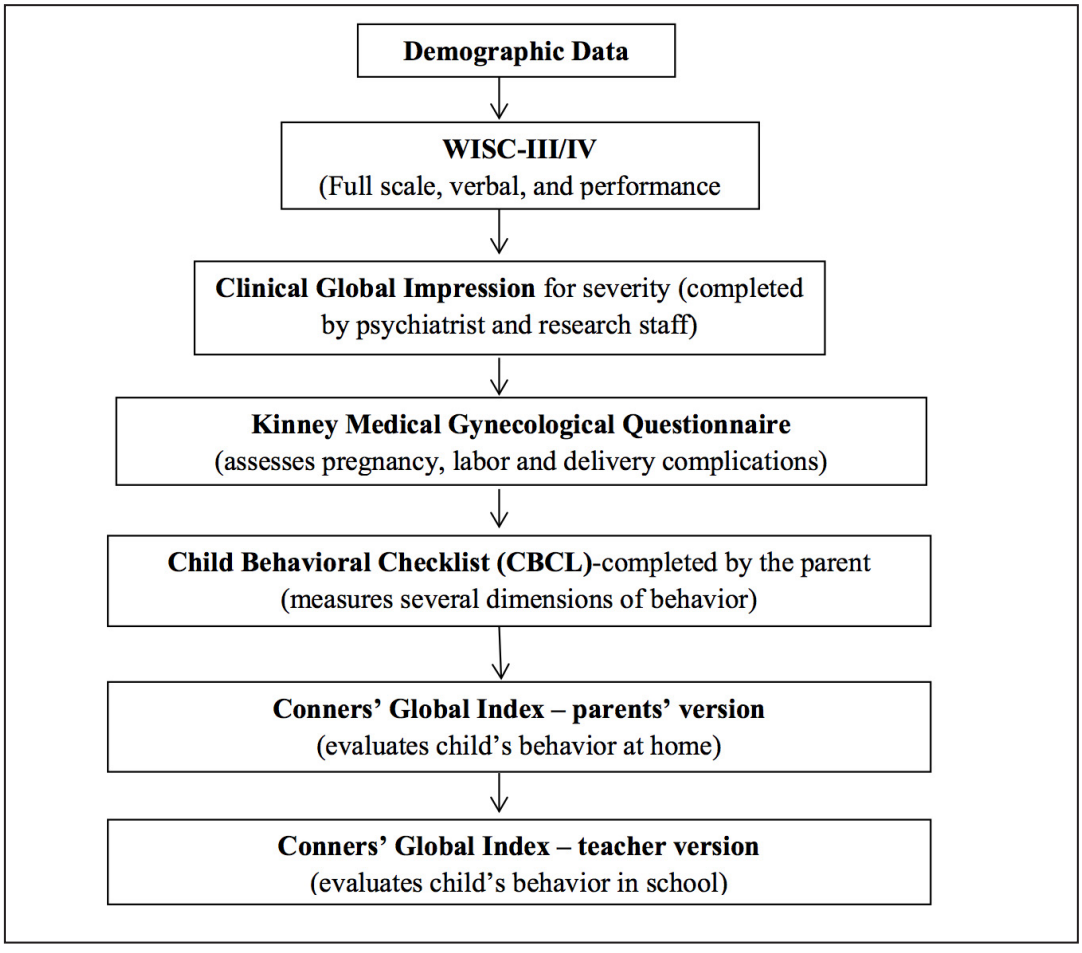

Figure 2.3: Outline of baseline evaluations conducted in study participants

2.5.1 Conners' Global Index (CGI)

Conners' Global Index is an assessment tool used to obtain information about the child's behavior from those who interact closely with the child on a regular basis (i.e. the parents at home (Conners'-P) and the teachers in the classroom (Conners'-T)) by using series of questions about the behavior of child. It is a reliable scale used to discriminate between children with ADHD and normal children. Conners'-P and Conners'-T scales assess the frequency of occurrence of 10 types of ecologically relevant behaviors. The Conners' scales are composed of two factors: Restless/Impulsive and Emotional Lability. Three of the ten items belong to the emotional factors: temper outbursts, frequent crying, and mood changes. The other items belong to Restless/Impulsive factor: disturbs other children, restless, excitable, fails to finish things, inattentive, fidgeting, and child's demands must be met immediately (Conners, 1999). Conners' scales are T standardized, with scores ranging from 0 to 100. A score of 50 is the average score and one standard deviation is represented by 15 points, thus scores of 65 or 70 are considered problematic.

2.5.2 Restricted Academic Situation Scale (RASS)

The Restricted Academic Situation Scale (RASS) (Barkley, 1990) is a coding system that is used to observe and record the child's ability to sustain attention during an assigned independent academic task (a set of math problems) with the presence of potential distractions, without any kind of direct observation (Barkley, 1990). RASS has been used to distinguish between normal children and children with ADHD (Milich, Loney, & Landau, 1982). RASS coding system has two factors: engagement/disengagement and motor activation (Gupta & Kar, 2009; Karama et al., 2009). In the current study, this assessment was conducted in a room equipped with a

worktable, a chair, an intercom, and some toys. Participants were given a set of math problems (based on their age and academic achievements) and instructed to complete as many problems as possible in 15 minutes without leaving the seat or playing with the toys. During these 15 minutes, a research assistant assessed the child's behavior from behind a one-way mirror. This assessment targets five behaviors: “off-task”, “vocalizing”, “playing with objects”, “fidgeting”, and “out of seat”. All behavioral events were recorded at 30-second intervals. In addition, this task is conducted twice on each testing day (before and after the treatment), it may be considered a good predictor of the child's motivation during a monotonous and repetitive task.

N.B. for both Conners’ and RASS assessments; the higher score is an indicative of worse behavior and/or performance

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Chapter 3

Placebo Response in Children with ADHD: Multidimensional evaluation and exploration of its determinants

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Manuscript to be submitted!

Preface

The previous chapters reviewed general literature on placebo response in a wide variety of clinical disorders, showing that the magnitude of placebo responses is favorably inconsistence among individuals (depending on many factors). This chapter presents the findings of studying the correlates of placebo response in children with ADHD. Although, an emerging literature has studied the predictors of placebo response in ADHD, we tried to fill the gaps of the previous studies. In the current study, we aim to understand this phenomenon more deeply by studying its predictors as assessed by different raters/ observers (parents, teachers and research team) by using a quantitative approach. Identifying predictors is critical for measuring and controlling placebo effects in all kinds of studies.

Abstract

Background: Placebo (PBO) effect is a change in clinical outcome while the patient is taking a sham treatment. It is a complex psychological phenotype reflecting positive (placebo) or negative (nocebo) expectation and/or behavioral conditioning. While progress has been made to better understand the neurobiological mechanisms underlying placebo effect, its psychosocial determinants have rarely been studied, even though they might be very important in modulating expectations.

Objectives: To evaluate placebo response (PR) and its psychosocial determinants in children with ADHD.

Method: As part of a large pharmacogenetic study, 614 children with ADHD aged 6 to 12 years were recruited to a randomized, double-blind placebo-controlled crossover trial with a fixed dose of methylphenidate or placebo (1 week for each arm). Placebo response was calculated as the difference in Conners scores (parents and teachers) at baseline and during PBO weeks. PR in children was calculated as a difference in the RASS scores (classroom simulated behavioral assessment as assessed by direct observation of the child behavior by trained research assistant) before and 1 hour after placebo administration.

Results: A highly significant PR was identified according to parents and teachers assessments ($p < 0.001$). In sharp contrast to these expected results, behavior of the children, as assessed by the RASS scores, worsened after taking PBO ($p < 0.001$). Several psychosocial factors such as parental income, mothers' marital status, mother's level of education, maternal smoking during pregnancy (MSDP), ethnicity of the child and prior exposure to psychostimulant showed a significant and specific effect on PR as assessed by parents and teachers. Furthermore, the interaction between these different factors and treatment with placebo revealed two distinct pattern of PR.

Conclusion: Psychosocial factors are important in determining the magnitude and the profile of placebo response. Given the centrality of placebo to clinical trials, these psychosocial determinants may be important to take into consideration when evaluating the results of such trials.

1. INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, affecting 5-10% of school-age children (Faraone, Sergeant, Gillberg, & Biederman, 2003). Psychostimulants, such as methylphenidate (MPH), are most commonly used to treat ADHD and they act primarily through enhancing the dopamine (DA) and norepinephrine (NE) transmission in the synaptic cleft of neurons (Rowland et al., 2002; Russell, de Villiers, Sagvolden, Lamm, & Taljaard, 1998). Although majority of the ADHD patients respond to psychostimulant medications, there is a substantial level of variance in therapeutic response (TR) from one patient to the other (McGough, 2005) but the determinants of this variability are not very well established (Froehlich, McGough, & Stein, 2010). However, previous studies suggest that in many psychiatric conditions, one major source of response to treatment is determined by the propensity of subject to feel better after receiving treatment, regardless of whether it contains a pharmacologically active ingredient or not, which is known as "*placebo effect*".

Placebo effect can be defined as "a genuine psychological or physiological effect attributable to receiving a substance or undergoing a procedure, which is not due to the inherent powers of that substance" (Stewart-Williams & Podd, 2004). In clinical trials, placebo (PBO) is a critical component for the evaluation of efficacy of the medication. In order to better understand placebo effect, several researchers have proposed various theories and psychological mechanisms that could explain this effect (Benedetti, 2008; Benedetti, Mayberg, Wager, Stohler, & Zubieta, 2005; Price, Finniss, & Benedetti, 2008). Patients' expectations, suggestions, and motivations are considered as core mechanism of placebo response (Geers, Helfer, Kosbab, Weiland, & Landry, 2005; Kirsch, 1985). Recently, several neuroimaging studies have shown the effect of placebo

on brain activation and neurochemical changes. These changes have been observed in different brain regions; such as the prefrontal area (Craggs, Price, Verne, Perlstein, & Robinson, 2007), a brain region that is postulated to be involved in ADHD. It is also the region that could be related to recalling the expected effects of the treatment. Other brain regions such as the right ventrolateral prefrontal cortex may be associated with the emotional connectivity of treatment expectations (Petrovic et al., 2005).

In a large meta-analysis, it has been reported that the placebo effect is almost trivial in long-term studies when placebo treatment is compared to no treatment, suggesting that placebo response is more prominent in short-term treatment and tends to vanish with time (Hrobjartsson & Gotzsche, 2001, 2004). In recent years, the placebo effect has received increasing attention in the treatment. Approximately 30% of ADHD children are placebo responders in double-blind clinical trials (Sandler, Glesne, & Geller, 2008). Previous studies investigated factors that influenced this response. However, these studies have mainly relied on parental evaluation of response to treatments and neglected how other observers (i.e. teachers) perceived response to treatment (Newcorn et, al. 2009; Waxmonsky et, al. 2011). Teachers' evaluation is vital in assessing the improvement in behavior of child as they might have different perception from parents of same child. In addition, most of the previous studies relied on arbitrary categorical definition of treatment response, which might introduce some bias in research results.

Given these gaps in the literature, the present study is a quantitative rather than qualitative approach to Placebo response. Placebo response analysis was derived from the assessment of parents, teachers and the direct observation of children's behavior in our laboratory. This study aims to examine the magnitude and the direction of placebo response in children with ADHD from different perspective and to explore the factors,

particularly the psychosocial factors that may influence this response.

2. MATERIALS AND METHODS

2.1 Participants and Study Design

For detailed description of the sample characteristics and study procedures, see section 2. In this study a total of 628 ADHD children (492 boys and 136 girls), between the age of 6 and 12 years (mean age = 9.07; SD = 1.83) were recruited from the child psychiatry outpatient clinic at the DMHUI. In total 539 children completed the Conners' parents and 528 completed the Conners' teacher after receiving MPH and placebo.

2.2 Assessments

2.2.1 *Baseline assessment*

- **Conners Global Index-Parents (Conners-P) and Conners Global Index-Teachers (Conners-T)**

These scales were used to evaluate behavior of child at home and in the classroom. Parents were asked to complete the Conners-P on weekdays and during the weekend. Whereas, teachers were asked to complete the Conners-T after 5 days of observing the children at school. For more details, see section 2.

- **Task-oriented behavior assessment within a Restricted Academic Situation Scale (RASS)**

Please refer to chapter 2 for more details about RASS.

2.3 Predictors of placebo response

We explored the following predictors of placebo response: **(1)** Sociodemographic variables including: socioeconomic status, sex, ethnicity, marital status of parents. **(2)** Psychopathological characteristics (i.e. other comorbid disorders) include: conduct disorder (CD), oppositional defiant disorder (ODD), mood disorders, and anxiety

disorders. **(3)** Other factors include: previous psychostimulant exposure, maternal smoking during pregnancy (MSDP), mothers' level of education, and order of administrating placebo (i.e. before or after MPH). Socioeconomic status (SES) was computed based on annual family income. Low SES was defined as having a family income of less than CAD $30,000/year. Marital status categorized as one parent (for separated, divorced, not married, and widow or widower) and two-parents (for married or living together). Mother's education level of less than 11 years was considered low.

2.4 Statistical Analysis

All the statistical analyses were conducted using the SPSS software, version 20. A series of univariate repeated measure analysis of variance was performed to explore different socioeconomic-demographic and clinical factors that may modulate placebo response of teachers, parents and children. Level of psychopathology during the baseline and the placebo treatment was entered as the within-subject measure while relevant characteristics that may influence response to placebo were entered as the between-subject measure. Subsequently all factors that were found to influence placebo response in the univariate approach were included in a linear regression analysis to understand the effect of the combined factors on placebo response while taking into account their correlations.

Placebo response in this study was defined as the total difference in Conners' scores between the baseline and the week of placebo for parents and teachers. Whereas placebo response in children was derived from the RASS and calculated as the difference in RASS score before and after taking placebo.

3. RESULTS

We identified a very significant placebo response as assessed by parents [F(1,

539) = 380.175, $p < 0.001$]. The mean for Conners-P was 72.2 (SD = 11.1) and 61.62 (SD = 13.9) during baseline and placebo week assessments respectively. For teachers the placebo response was also highly significant [$F(1, 527) = 75.761, p < 0.001$], although its magnitude was lower compared to parents' assessment. Indeed the Conners-T average score during the baseline evaluation was 69.3 (SD = 12.3) and 65.3 (SD = 13.5) during the placebo week. When we compared the mean score of response to placebo as assessed by parents [M = 10.57, SD = 10.36] and teachers [M = 3.92, SD = 12.83], we identified a highly significant difference [$t(539) = 19.49$, $p < 0.001$]. Surprisingly, in sharp contrast to what was observed in parents and teachers, children's behavior as assessed by RASS, deteriorated significantly [$F(1, 613) = 97.1$, $p < 0.001$] after placebo suggesting a reverse placebo response (RPR) (Figure 3.1).

No significant difference was observed with the order of treatment. The results profile was the same regardless of whether the children received placebo the first week or the second week. In order to determine the interaction between several factors that may modulate response to placebo, we applied a series of repeated measure ANOVA where the patients are subdivided into two independent groups (e.g. previously exposed to medication or not) and the response to placebo is taken as the within subject repeated measure.

- **Parents**

Significant interactions between response to placebo and parental income [$F(1, 511) = 10.3, p = 0.001$], marital status, [$F(1, 485) = 4.3, p = 0.039$], mother's level of education, [$F(1, 481) = 7.158, p = 0.008$], maternal smoking during Pregnancy (MSDP) [$F(1, 494) = 4.822, p = 0.029$], and prior psychostimulant exposure [$F(1, 517) = 10.808, p = 0.001$] were found. Low family income, single parental status, low education level of mothers, MSDP and medication naïvety were associated with

significantly higher placebo response. Linear regression analysis including all factors that were significantly associated with placebo response revealed that prior medication [β = -.144, p = 0.003] significantly contributed to predict placebo response as assessed by parents (Table 3.1).

Significant main effects were found with gender [F(1, 538) = 23.51, p < 0.001], conduct disorder (CD) [F(1, 532) = 36.85, p < 0.001], oppositional defiant disorder (ODD) [F(1, 533) = 34.98, p < 0.001], anxiety disorder [F(1, 502) = 1.96, p = 0.003], and ADHD subtype [F(2, 536) = 39.99, p < 0.001]. Girls, and children with CD, ODD, and anxiety disorders had more behavioral events compared to the other groups (i.e. boys and children without any psychopathological characteristics). Post-hoc analysis using Tukey HSD for the ADHD subtype indicated that the mean score for the combined type (with more behavioral events) was significantly different than the inattentive (p <.001) and the hyperactive (p=0.008) subtype. However, the inattentive subtype did not differ significantly from the hyperactive type, even though a marginal p-value was observed (p=0.057) (Table 3.1).

- **Teachers**

Placebo response as assessed by teachers showed significant interaction with ethnicity [F(1, 525) = 5.387, p = 0.021] and prior medication exposure [F(1, 506) = 15.503, p = 0.000]. Caucasians and medication naïvety were associated with significantly higher placebo response. Linear regression analysis revealed that both of these factors contributed independently to placebo response [β = -.171, p = 0.000; β = -.084, p = 0.055 respectively].

Significant main effects were found with income [F(1, 949) = 15.6, p < 0.001], marital status [F(1, 471) = 8.01, p = 0.005], oppositional defiant disorder (ODD) [F(1, 520) = 10.97, p < 0.001], ADHD subtype [F(2, 525) = 16.15, p < 0.001], and mother's

level of education [$F(1, 470) = 18.47$, $p < 0.001$]. Low family income, children who live with one parent, children with CD, ODD, hyperactive children, and whose mothers are less educated had more behavioral events had more behavioral events compared to the other groups (Table 3.2).

Gender, CD, anxiety and mood disorders did not show any association with PR [all p values > 0.05] in ADHD children as assessed by teachers.

- **Children:**

Contrary to parents and teachers, children's behavior as assessed by RASS deteriorated significantly suggesting a reverse placebo response (RPR). Significant interactions were identified with parental income [$F(1, 577) = 5.53$, $p = 0.019$] and mother's level of education, [$F(1, 543) = 5.16$, $p = 0.023$]. Low family income and low maternal education tend to show more RPR.

Significant main effects were found with gender, ethnicity, ADHD subtype, and prior medication exposure. Interestingly, in contrast to what we observed with parents regarding the sex, we found that boys had higher scores on the RASS both before and after the administration of placebo (i.e. more behavioral events) compared to girls. Also, in contrast to teachers and ethnicity, we found that Caucasians had more behavioral events and were less engaged in the math task. Regarding the prior medication exposure factor, children who were not medicated previously were performing better in RASS and they were more engaged in the math task. Post-hoc analysis for the ADHD subtypes indicated that the mean score for the combined subtype (less engaged) was significantly different than the inattentive ($p<.001$) and slightly different from the hyperactive ($p =.096$) subtype. However, the inattentive subtype did not significantly differ from the hyperactive subtype ($p =0.798$) (Table 3.3).

- **Placebo Response Patterns**

A closer examination of placebo response in patients stratified into two groups according to the factors that were found to interact with placebo (e.g. exposed to MSDP or not) suggests that there are two different patterns of placebo response (Figure 3.2). The first pattern, which we can call *convergent placebo effect* (CPE), is characterized by the fact that the *baseline evaluation* drove the interaction between the group membership (Figure 3.2.A). Patients with different group membership had significantly different levels of psychopathology during baseline evaluation and tended to converge to be more similar when they were treated with placebo. This was the case for income, mothers' level of education, marital status, and MSDP (Figure 3.3 D-G). The reverse pattern, that is similar levels of psychopathology during the baseline evaluation and *more dissimilar levels of psychopathology after treatment with placebo* (Figure 3.2.B) was observed for ethnicity in the teachers assessments [$F(1, 524) = 4.93$, $p = 0.027$], and prior history of medication exposure [$F(1, 516) = 16.84$, $p = 0.000$], [$F(1, 505) = 19.43$, $p = 0.000$] in both parents and teachers respectively (Figure 3.3 A-C). This pattern is called *divergent placebo effect* (DPE).

4. DISCUSSION

A robust overall improvement in children's behaviors after receiving placebo was perceived by both parents and teachers, suggesting a significant placebo response. This observation confirms previous studies showing placebo response in ADHD participants (Swanson et al., 1995; Newcorn et al., 2009; Waxmonsky et al., 2011). On the contrary, a novel finding in the ADHD placebo literature was identified. A significant deterioration during performing a goal-oriented task (RASS) was observed in the laboratory after receiving placebo, suggesting a ***reverse placebo response*** (RPR). This result validates earlier findings from our group (Sengupta et al., 2008). Because the deterioration happens while suggesting and expecting improvement under PBO we

preferred to use the RPR term and not the nocebo effect term, which refers to the deterioration observed under negative suggestion and expectation. Theses contrasting responses (between parents, teachers, and children) indicate that the overall improvement observed by parents or teachers does not essentially match the children's behaviour.

Several mechanisms may explain the opposing effects of placebo between trained raters, parents, and teachers. Firstly, it is possible that the parents and teachers might change their perception of behaviors when they believe in the efficacy of the treatment, which could in turn influence the child's behavior (Waschbusch et al., 2009). Secondly, the emotional context plays an effective role in response to placebo (Moore, 2012), therefore, response to placebo might differ when it is given by parents, teachers (in a caring setting), or by research personnel (in a more objective laboratory setting). Thirdly, the RPR may be due to the fact that ADHD children performed the RASS before and after taking placebo on the same day. Thus, the repetition of monotonous activities associated with the RASS may result in a lack of interest in completing the RASS on second time (i.e. after taking the placebo). Supporting this idea, previous studies have suggested that children with ADHD abhor repetitive monotonous tasks (Biederman et al., 2007; Sengupta et al., 2008), given their executive function deficit and lack of motivation (Sonuga-Barke, 2003). Finally, the placebo response may be higher in the passive perception of inner states (e.g., pain, sadness, and anxiety), compared to active engagement in a goal-oriented task. Similarly, in ADHD, higher placebo responses have been observed in subjective compared to objective assessments. Both observations may help explain the improvement in passively perceived, subjective ratings of ADHD from parents and teachers, compared to the objective, task-oriented RASS.

These results reveal the association between children's behavior and how the observers see and report these behaviors. Therefore, studying placebo response by specifying the observers and combining ratings from several sources into a single outcome variable would be critical.

Furthermore, this study shows the complex construct of placebo response, as various components were involved. In parents, family income, marital status, mothers' level of education, MSDP, and prior psychostimulant exposure were shown to significantly influence placebo response. In teachers, response to placebo was significantly associated with ethnicity and previous medication treatment. Finally, the RPR in children was associated with maternal education and income. By stratifying children according to several clinical and demographic strata, two patterns of response to placebo were emerged. The first pattern, which we called ***convergent placebo effect*** (CPE), was characterized by sever rating at baseline (i.e. higher scores at baseline) but no/or trivial differences after administration of placebo. In other words, the higher scores of behavioral disturbances at baseline tend to disappear upon treatment with placebo, suggesting a higher placebo response as the higher groups try to 'catch up' with the rest of the sample during the placebo phase. This pattern was observed with parents' assessments in children with the following characteristics: exposed to MSDP, lower maternal education, single parent status, and low income. "Overestimation" theory may explain this pattern. Alternatively, raters tend to unconsciously overestimate the psychopathology of a child to secure treatment and services because of **(1)** difficulty in accessing care when parents might expect that more severe the behavior, more likely is the child to get a treatment. **(2)** Also it is possible that in clinical trials where patients are recruited through advertisement and are encouraged to participate for free access to treatment or other financial compensations, patients may overestimate the level of their

symptoms at baseline evaluations to better justify treatment. This is often the case in clinical trial recruitment, where some patients may exaggerate their symptoms to be included in a trial (Benedetti, 2009; Kleinman et al., 2002). The fact that this pattern is also observed among parents with low SES may also support this hypothesis.

Prior stimulant treatment and ethnicity drove the second pattern of the placebo response. ***Divergent placebo effect*** (DPE), which is in line with the common and the classical conception of placebo response. This pattern is characterized by no/or trivial differences at baseline but significant differences after administration of placebo. DPR is predictable because it may be explained by the "expectation effect" theory. It is assumed that the parents and teachers might have less expectation of response in children who have already been taking medication and for whom response is already "calibrated" by the observer's experience. Lower expectation of response for these children could be explained by the fact that these children might be referred to our clinic because of poor response to treatment. It could also be explained by the fact that the placebo effect can be a learned response, meaning that the parents and teachers who observed the real medication effect will be able to easily detect the difference between real medication and sham treatments (placebo). This finding replicates previous studies showing lower placebo response after exposure to prior treatment (Sandler & Bodfish, 2008; Newcorn et al., 2009). A clinically relevant observation that follows this pattern is that the teachers showed an ethnic bias and tended to report more improvement with placebo in Caucasian children compared to non-Caucasians. This is particularly interesting in view of the fact that non-Caucasian children have in fact less task disengagement in a school like test (the RASS), after the treatment with placebo. No association between ethnicity and placebo response from parents' perspective was found which is in contrast to Newcorn et al., (2009), as they reported that non-

Caucasian parents noted more placebo response.

Other variables including psychopathological characteristics, such as participants' gender, ADHD subtype, and time of administering placebo were not associated with placebo response. These findings join an array of prior inconsistent results regarding these variables (Sandler & Bodfish 2008; Benedetti 2009; Newcorn et al., 2009).

Study's strengths and weaknesses

The current study is one of the largest studies that examined placebo response in children with ADHD. The major strength of this study is the comparison of parents and teachers' placebo response with that of trained raters' assessments by using RASS (no prior study has used this scale). Also, within-subject crossover design of our study is a strong point compared to more commonly applied parallel designs. Use of this within-subject crossover design allowed increasing precision in evaluation of the placebo response. As it compares the effect of placebo to the baseline level of psychopathology in each subject whereas in parallel designs, only the average effect of placebo can be used. We also relied on quantitative measures to avoid the cut off points used in previous studies, which may bias results. In addition, it is an extremely comprehensive study and examined in depth a large number of predictors; some of them have not been previously discussed in the literature, e.g. marital status, MSDP, and mothers' level of education.

Including more boys in the study may limit the generalizability of our findings. However, this could be expected since boys are more frequently diagnosed with ADHD.

Conclusion

Since last decade, there has been increasing evidence related to placebo effects in the literature (Walsh et al., 2002). The exact origin of this phenomenon is not known,

although some explanations have been proposed. Our observations might provide some insight into this phenomenon especially with the CPE pattern where subjects might over-evaluate their psychopathology in the hope of having access to care through clinical trials. Current findings improve our understanding of placebo response in children with ADHD and its determinants. This in turn may inform us about the expectations of parents, teachers and children about medication effects, and thus better adjust to their expectations. It can also influence future psychopharmacological research that focuses on studying "true" medications effect. This could be done by improving subjects' selection criteria e.g. excluding the subjects who would not "truly" benefit from medication. Finally, paying more attention to placebo effects, and adjusting for their effects in clinical trials may improve the quality of psychopharmacological research and clinical trials studies.

Table 3.1: Summary of the factors examined to understand placebo response from the parents' perspective

	Baseline Week Mean (SD)	***Placebo Week Mean (SD)***	***Interaction Statistic & p-value, Partial Eta Squared***	***Main Effect Statistic & p-value, Partial Eta Squared***
A) Sociodemopgraphic Factors				
SEX			$F_{1,538}$=1.26, p=0.261; 0.002	$F_{1,538}$=23.51, **p=0.000***; 0.042
Boys (N=423)	70.86 (10.54)	60.61 (13.55)		
Girls (N=117)	77.01 (11.72)	65.27 (14.62)		
Ethnicity			$F_{1,537}$=.677, p=.411; 0.001	$F_{1,537}$=1.17, p=.280; 0.002
Caucasian (N=474)	72.46 (10.98)	61.72 (13.91)		
Others (N=65)	70.22 (11.77)	60.85 (14.13)		
[A] Income			$F_{1,511}$=10.28, **p=0.001***; 0.020	$F_{1,511}$=6.15, **p=0.013***; 0.012
Low (N=195)	74.93 (9.97)	61.91 (14.18)		
High (N=318)	70.68 (11.19)	61.34 (13.65)		
[B] Marital status			$F_{1,485}$=4.28, p=0.039*; 0.009	$F_{1,485}$=4.67, p=0.031*; 0.010
Single (N=200)	74.34 (10.39)	62.12 (14.13)		
Couples (N=287)	70.01 (11.04)	61.17 (13.73)		
B) Psychopathological characteristics*				
CD			$F_{1,532}$=.88, p=0.348; 0.002	$F_{1,532}$= 36.85, p=0.000*; 0.065
No (N=443)	71.07 (10.98)	60.26 (13.55)		
Yes (N=91)	77.73 (9.77)	68.27 (13.43)		
ODD			$F_{1,533}$=.64, p=0. 43; 0.001	$F_{1,533}$= 34.98, p=0.000*; 0.062
No (N=311)	69.77 (11.48)	59.58 (13.13)		
Yes (N=224)	75.67 (9.50)	64.60 (14.3)		
Anxiety disorders			$F_{1,504}$=2.09, p=.149; 0.004	$F_{1,504}$=9.874, p=.002*; 0.019
No (N=293)	70.48 (11.26)	60.56 (13.71)		
Yes (N=213)	74.32 (10.39)	62.76 (13.76)		
Mood disorders			$F_{1,504}$=1.75, p=.187; 0.003	$F_{1,504}$=1.05, p=.305; 0.002
No (N=470)	71.89 (11.10)	61.46 (13.66)		
Yes (N=36)	75.25 (10.33)	61.92 (15.47)		
C) Other factors				
ADHD subtype			$F_{2,536}$=1.70, p=.184; 0.006	$F_{2,536}$=39.99, p=.000*; 0.130
Inattentive (N=209)	67.85 (11.04)	56.72 (12.19)		
Hyperactive (N=51)	72.37 (10.31)	59.49 (13.28)		
Combined (N=279)	75.44 (10.15)	65.72 (14.01)		
[C] Mothers' level of education			$F_{1,481}$=7.15, p=.008*; 0.015	$F_{1,481}$=2.83, p=.093; 0.006
Low (N=166)	74.36 (10.65)	61.31 (14.77)		
High (N=317)	71.03 (10.71)	61.20 (13.29)		
Previous Medications			$F_{1,517}$=10.80, p=.001*; 0.020	$F_{1,517}$=14.48, p=.000*; 0.027
No (N=357)	71.75 (10.92)	59.9 (13.57)		
Yes (N=162)	73.66 (11.14)	65.71 (13.90)		
Smoking during pregnancy			$F_{1,494}$=4.82, p=.029*; 0.010	$F_{1,494}$=3.68, p=.056*; 0.007
No (N=317)	71.06 (10.79)	61.35 (13.67)		
Yes (N=179)	74.27 (11.07)	62.00 (14.15)		
Duration of placebo taking			$F_{1,537}$=.77, p=.380; 0.001	$F_{1,537}$=.044, p=.835; 0.000
1st week (N=291)	72.33 (11.19)	61.32 (13.40)		
2ed week (N=248)	72.04 (11.02)	61.99 (14.54)		

[A] Income was grouped into 2 categories: (1) Low < $30,000 CAD and (2) High > $30,000 CAD.

[B] Mothers' marital status was grouped into 2 categories: (1) one parent (includes separated/divorced, single or widow/windower), and (2) two parents (includes married or living together).

[C] Mother's education level was divided into 2 groups: (1) Low education ≤ 11 years and (2) High education > 11 years.

* ODD = oppositional defiant disorder; CD = conduct disorder

* Anxiety disorder means having at least one of these disorders: social phobia, separation anxiety disorder, specific phobia, panic disorder, agoraphobia, generalized anxiety disorder, and selective mutism, post traumatic stress disorder.

* Mood disorder means having at least one of these disorders: major depressive episode, dysthymic disorder, manic episode, and hypomanic episode.

Table 3.2. Summary of the factors examined to understand placebo response from the teachers' perspective.

	Baseline Week Mean (SD)	***Placebo Week Mean (SD)***	***Interaction* Statistic & p-value, Partial Eta Squared**	***Main Effect* Statistic & p-value, Partial Eta Squared**
A) Sociodemopgraphic Factors				
SEX			$F_{1,526}$=1.52, p=0.217; 0.003	$F_{1,526}$=2.79, **p=0.095**; 0.005
Boys (N=420)	68.72 (11.40)	65.08 (12.88)		
Girls (N=108)	71.56 (15.52)	66.53 (15.85)		
Ethnicity			$F_{1,525}$=5.397, p=.021*; 0.010	$F_{1,525}$=0.10, p=.752; 0.000
Caucasian (N=459)	69.48 (12.27)	65.14 (13.40)		
Others (N=68)	68.41 (13.07)	67.19 (14.33)		
[A] Income			$F_{1,494}$=0.059, p=.808	$F_{1,949}$=15.6, **p=0.000***; 0.031
Low (N=197)	71.91 (11.90)	67.80 (13.26)		
High (N=299)	67.57 (12.17)	63.68 (13.44)		
[B] Marital status			$F_{1,471}$=.36, p=0.55; 0.001;	$F_{1,471}$=8.01, p=0.005*; 0.017
Single (N=208)	71.39 (12.01)	66.85 (13.93)		
Couples (N=265)	68.02 (12.30)	64.06 (13.25)		
B) Psychopathological characteristics				
CD			$F_{1,519}$=.41, p=0.523; 0.001	$F_{1,519}$= .029, p=0.86; 0.00
No (N=425)	69.34 (12.56)	65.18 (13.53)		
Yes (N=96)	69.20 (11.43)	65.78 (13.38)		
ODD			$F_{1,520}$=.298, p=0.586; 0.001	$F_{1,520}$= 10.97, p=0.001*; 0.021
No (N=318)	68.08 (12.11)	63.86 (12.93)		
Yes (N=204)	71.31 (12.49)	67.60 (14.07)		
Anxiety disorders			$F_{1,492}$=.306, p=.580; 0.001	$F_{1,492}$=.437, p=.509; 0.001
No (N=283)	69.37 (12.24)	65.6 (13.43)		
Yes (N=211)	68.91 (12.48)	64.62 (13.62)		
Mood disorders			$F_{1,491}$=.010, p=.920; 0.000	$F_{1,491}$=.180, p=.672; 0.000
No (N=456)	69.11 (12.31)	65.07 (13.43)		
Yes (N=37)	70.05 (13.19)	65.84 (14.36)		
C) Other factors				
ADHD subtype			$F_{2,525}$=1.95, p=.143; 0.007	$F_{2,525}$=16.15, p=.000*; 0.058
Inattentive (N=196)	65.68 (13.11)	61.55 (12.87)		
Hyperactive (N=48)	73.13 (10.69)	66.63 (13.77)		

Combined (N=284)	71.15 (11. 56)	67.80 (12.69)		
[C] Mothers' level of education			$F_{1,470}$=2.04, p=.154; 0.004	$F_{1,470}$=18.47, p=.000*; 0.006
Low (N=170)	72.06 (11.64)	68.59 (13.15)		
High (N=302)	68.03 (12.24)	63.14 (13.23)		
Previous Medications			$F_{1,506}$=15.50, p=.000*; 0.030	$F_{1,506}$=6.89, p=.009*; 0.013
No (N=324)	69.05 (12.23)	63.78 (13.69)		
Yes (N=184)	70.05 (12.42)	68.49 (12.81)		
Duration of placebo taking			$F_{1,524}$=.170, p=.680; 0.000	$F_{1,524}$=.714, p=.398; 0.001
1st week (N=279)	68.94 (12.27)	64.86 (13.43)		
2ed week (N=247)	69.64 (12.57)	65.93 (13.72)		

[A] Income was grouped into 2 categories: (1) Low < $30,000 CAD and (2) High > $30,000 CAD.

[B] Mothers' marital status was grouped into 2 categories: (1) one parent (includes separated/divorced, single or widows/widower, and (2) two parents (includes married or living together).

[C] Mother's education level was divided into 2 groups: (1) Low education ≤ 11 years and (2) High education > 11 years.

* ODD = oppositional defiant disorder; CD = conduct disorder

* Anxiety disorder means having at least one of these disorders: social phobia, separation anxiety disorder, specific phobia, panic disorder, agoraphobia, generalized anxiety disorder, and selective mutism, post traumatic stress disorder.

* Mood disorder means having at least one of these disorders: major depressive episode, dysthymic disorder, manic episode, and hypomanic episode.

Table 3.3. Summary of the factors examined to understand reverse placebo response in children with ADHD

	Baseline Week Mean(SD)	***Placebo Week Mean (SD)***	***Interaction* Statistic & p-value, Partial Eta Squared**	***Main Effect* Statistic & p-value, Partial Eta Squared**
A) Sociodemopgraphic Factors				
SEX			$F_{1, 612}$=0.032, p=0.86; 0.000	$F_{1,612}$=20.12, **p=0.000**; 0.032
Boys (N=481)	51.31 (27.65)	58.81 (29.58)		
Girls (N=133)	39.47 (24.87)	47.31 (28.32)		
Ethnicity			$F_{1,611}$=1.11, p=.292; 0.002	$F_{1,611}$=5.11, p=.024*; 0.008
Caucasian (N=532)	49.58 (27.40)	57.48 (29.81)		
Others (N=81)	43.53 (27.74)	49.03 (27.85)		
Income			$F_{1,577}$=5.53, p=.019, 0.009	$F_{1,577}$=.77, p=.38, 0.001
Low (N=232)	51.56 (28.37)	56.66 (30.49)		
High (N=347)	47.64 (26.98)	56.54 (29.41)		
Marital status			$F_{1,551}$=1.41, p=0.24; 0.003	$F_{1,551}$=0.054, p=0.816; 0.000
Single (N=239)	50.44 (29.27)	56.97 (31.30)		
Couples (N=314)	48.94 (26.27)	57.39 (28.53)		
B) Psychopathological characteristics				
-CD			$F_{1,603}$=.260, p=0.61; 0.000	$F_{1,603}$= 2.38, p=0.123; 0.004
No (N=493)	47.87 (27.39)	55.56 (29.39)		
Yes (N=112)	51.73 (27.73)	60.42 (30.95)		
ODD			$F_{1,605}$=.285,	$F_{1,605}$= .103, p=.75;

No (N=356)	48.15 (26.99)	56.25 (29.65)	p=0.59; 0.000	0.000
Yes (N=251)	49.29 (28.39)	56.55 (29.96)		
Anxiety disorders			$F_{1,568}$=1.96, p=.304; 0.002	$F_{1,568}$=.091, p=.763; 0.000
No (N=228)	48.41 (27.51)	57.06 (29.62)		
Yes (N=242)	48.54 (26.85)	55.57 (29.39)		
Mood disorders			$F_{1,569}$=.190, p=.663; 0.000	$F_{1,569}$=.44, p=.507; 0.001
No (N=528)	48.66 (27.43)	56.76 (29.73)		
Yes (N=43)	46.49 (23.79)	53.30 (26.73)		
C) Other factors				
ADHD subtype			$F_{2,610}$=.132, p=.877; 0.000	$F_{2,610}$=10.51, p=.000*; 0.033
Inattentive (N=227)	43.26 (25.35)	50.40 (28.07)		
Hyperactive (N=59)	45.14 (29.47)	53.51 (30.37)		
Combined (N=327)	53.22 (27.88)	61.01 (29.92)		
Mothers' level of education			$F_{1,543}$=5.16, p=.023*; 0.009	$F_{1,543}$=1.03, p=.309; 0.002
Low (N=200)	51.82 (29.13)	56.89 (30.43)		
High (N=345)	47.44 (26.71)	56.38 (29.25)		
Previous Medications			$F_{1,588}$=0.019, p=.891; 0.000	$F_{1,588}$=13.43, p=.000*; 0.022
No (N=317)	46.19 (26.38)	53.72 (29.65)		
Yes (N=219)	45.45 (28.90)	62.19 (29.36)		
Time of taking placebo			$F_{1,611}$=1.50, p=.22; 0.002	$F_{1,611}$=3.02, p=.083; 0.005
1st week (N=323)	46.51 (24.95)	54.95 (28.31)		
2ed week (N=290)	51.24 (29.96)	57.79 (31.12)		

[A] Income was grouped into 2 categories: (1) Low < $30,000 CAD and (2) High > $30,000 CAD.

[B] Mother's marital status was grouped into 2 categories: (1) one parent (includes separated/divorced, single or widow/widower), and (2) two parents (includes married or living together).

[C] Mother's education level was divided into 2 groups: (1) Low education ≤ 11 years and (2) High education > 11 years.

* ODD = oppositional defiant disorder; CD = conduct disorder

* Anxiety disorder means having at least one of these disorders: social phobia, separation anxiety disorder, specific phobia, panic disorder, agoraphobia, generalized anxiety disorder, and selective mutism, post traumatic stress disorder.

* Mood disorder means having at least one of these disorders: major depressive episode, dysthymic disorder, manic episode, and hypomanic episode.

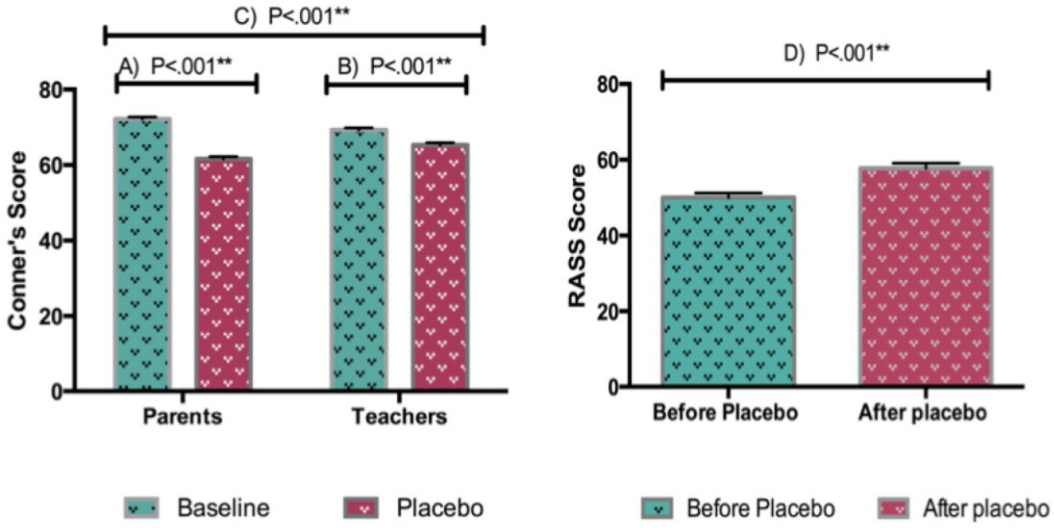

Figure 3.1: Changes in ADHD symptoms between baseline and placebo according to different observers. A) and B) Show a significant difference between baseline week score and placebo week score as assessed by Conners parents and Conners teachers respectively (p<0.001). Although, parents perceive a significantly higher placebo response compared to teachers (c). D) Shows a significant difference in an opposite way between baseline and placebo week score in children as assessed by RASS. * Error bars represent SEM.

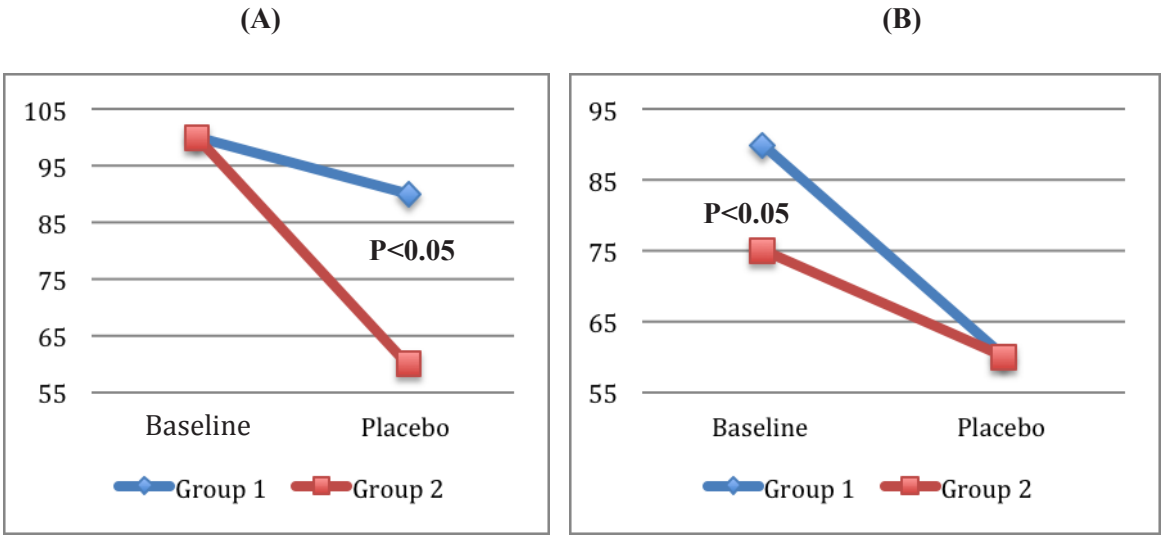

Figure 3.2: Illustrates two patterns of placebo response observed (A) shows that the means of both groups are significantly different in the baseline week and placebo week. (B) Shows significant differences in symptoms severity among the groups in the baseline week, while no difference was observed after placebo.

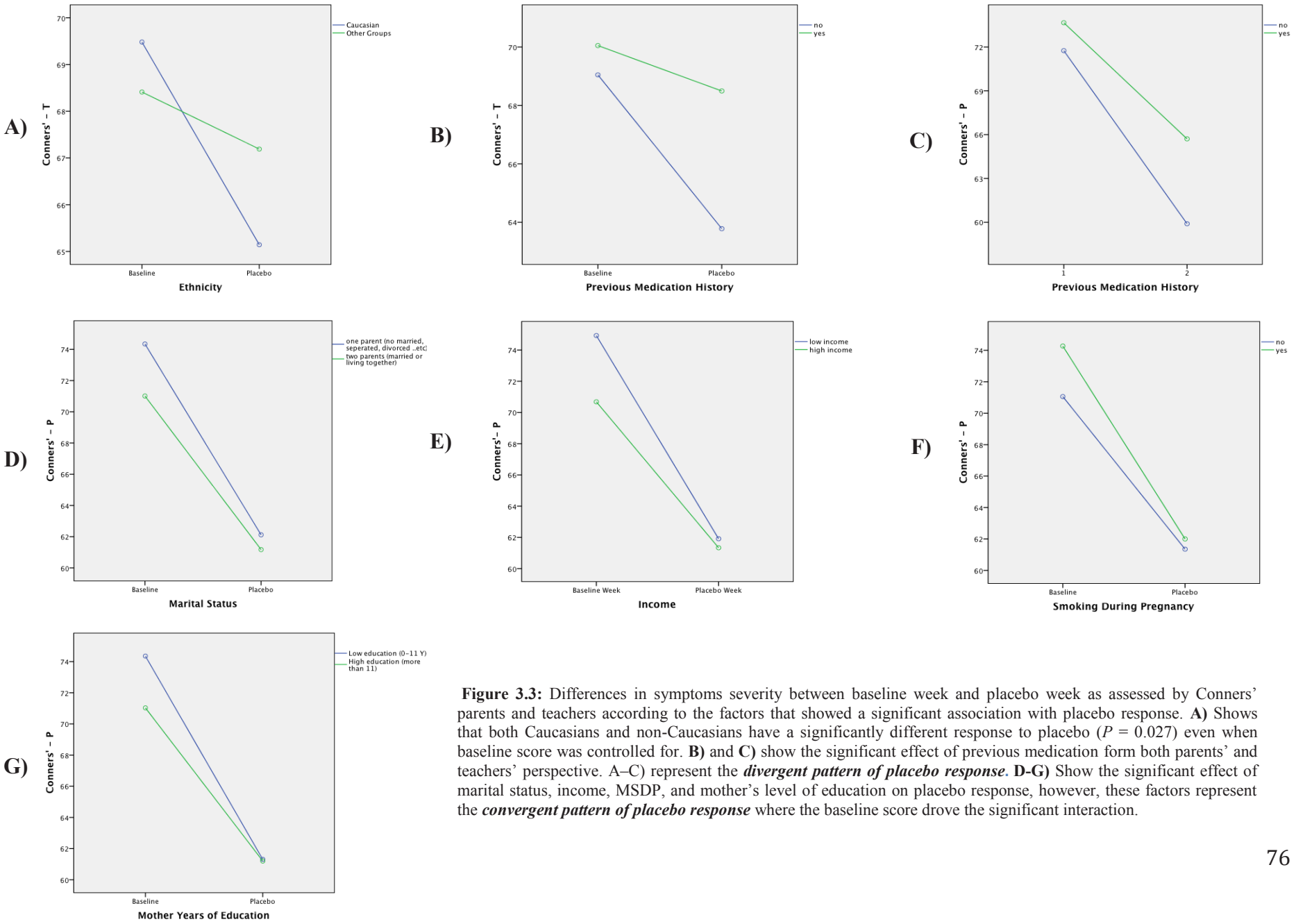

Figure 3.3: Differences in symptoms severity between baseline week and placebo week as assessed by Conners' parents and teachers according to the factors that showed a significant association with placebo response. **A)** Shows that both Caucasians and non-Caucasians have a significantly different response to placebo (P = 0.027) even when baseline score was controlled for. **B)** and **C)** show the significant effect of previous medication form both parents' and teachers' perspective. A–C) represent the ***divergent pattern of placebo response***. **D-G)** Show the significant effect of marital status, income, MSDP, and mother's level of education on placebo response, however, these factors represent the ***convergent pattern of placebo response*** where the baseline score drove the significant interaction.

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Chapter 4

Association of catechol-O-methyltransferase (COMT) gene with the reverse placebo response and medication response in children with ADHD

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Manuscript to be submitted!

Preface

In the previous chapter, we described placebo response and its correlates in children with ADHD as assessed by parents, teachers and research team. We identified a significant placebo response (PR) as assessed by parents and teachers. In sharp contrast to these observers' outcomes, children's behavior significantly deteriorated after placebo as assessed by research team using the RASS, a fine-grained observational tool used in clinical laboratory setting, thus suggesting a reverse placebo response (RPR). In this chapter, we will focus more on this latter observation (i.e. the RPR). After studying the influence of many environmental factors in the previous chapter, we aimed to investigate the role of genetics as well. Given the importance of dopamine (DA) in modulation of placebo/nocebo effects and the fact that the DA deregulation is implicated in ADHD, we decided to investigate the potential role of a candidate gene that has been previously associated with ADHD: the Catechol-O-methyltransferase gene (*COMT*) and in the dopamine metabolism.

COMT plays a pivotal role in the regulation of catecholamines and is involved in the degradation/ catabolization of neurotransmitter DA in the prefrontal cortex (PFC), a brain region that is postulated to be involved in ADHD. *A priori* evidence suggests that the *COMT* gene, more specifically the val$^{108/158}$met polymorphism, is an interesting candidate for genetic studies of PR. Furthermore, since ADHD medications such as methylphenidate (MPH) specifically target the DA system, we also studied the association between medication response (MR) and specific *COMT* gene polymorphisms in the same group of children with ADHD.

Abstract:

Introduction: Earlier literature suggests that placebo response (PR) is modulated by specific brain circuits, especially the brain dopamine (DA) system. Thus, genetic factors coding for proteins involved in DA neurotransmission may modulate PR. In addition, methylphenidate (MPH), that has been widely used to treat ADHD, targets DA system in the brain as well. Therefore, variations within the *Catechol-O-methyltransferase* (*COMT*) gene, a major catabolizing enzyme for DA, may be implicated in modulation of both PR and medication response (MR).

Methods: Four *SNPs* (rs6269, rs4633, rs4818, and rs4680) in the *COMT* gene were genotyped in 371 Caucasian children with ADHD (6-12 years). *COMT* genotypes and diplotypes were tested for association with PR and MR using repeated measures analysis. PR and MR were calculated as the difference in Restricted Academic Situation Scale (RASS) score before and after placebo (PBO) and MPH, respectively, in a two-week double-blind, PBO-controlled MPH trial.

Results: Children performance on the RASS deteriorated after PBO administration, suggesting a reverse placebo response (RPR). This RPR was completely reversed by methylphenidate. Diplotypes [P = 0.046] and two SNPs (rs6269 [P = 0.011] and rs4818 [P = 0.008]) were significantly associated with the RPR in children with ADHD. No significant interaction was detected with MR. However, significant main effects of *COMT* genotypes on RASS were observed.

Conclusion: These results suggest that DA system and *COMT* gene variations are involved in RPR as well as in modulation of task-oriented behavior but not in the response of this behavior to MPH treatment in children with ADHD.

Introduction

Placebo (PBO) treatment shows beneficial effects in diverse medical conditions that are indistinguishable from those of active medication (Finniss, Kaptchuk, Miller, & Benedetti, 2010). Placebo effect is so ubiquitous that no molecule can be marketed as a therapeutic agent without showing its superiority to PBO. Suggestions of positive therapeutic effects where non-active agent exists and expectations of these effects are considered to be the key psychological mechanisms underlying the placebo effect. In contrast, the same psychological mechanisms could produce deterioration in a subject's condition when the content of the suggestions and their co-extensive expectations are negative, a phenomenon known as a nocebo effect. The factors underlying the neurobiology of placebo response (PR) are not very well understood (Zubieta & Stohler, 2009). It is currently believed that genetic factors may be implicated in modulating PR in patients (Raz, 2008). Very few studies to date have provided empirical evidence linking genetic variations with PR (Furmark et al., 2008; Hall et al., 2012; Leuchter, McCracken, Hunter, Cook, & Alpert, 2009; Tiwari et al., 2013).

A convergent body of data correlates brain systems with PR in a broad spectrum of disorders including pain, Parkinson's disease, depression, irritable bowel syndrome, anxiety, etc. These studies suggest that PR is modulated by specific brain circuits, especially the dopamine (DA) system (de la Fuente-Fernandez, Lidstone, & Stoessl, 2006; de la Fuente-Fernandez et al., 2001; Hall et al., 2012). In Parkinson's disease and pain, PR has shown to be associated with higher dopaminergic and endogenous opioid activity in the nucleus accumbens. Conversely, nocebo effect is associated with "deactivation" of DA and opioid release (Scott et al., 2008). In other words, opposite responses of DA and opioid neurotransmission are associated with the placebo and nocebo effect.

Dopamine levels at the synapse are controlled either by re-uptake mechanism involving the dopamine transporter (DAT) or by catabolism using the *Catechol-O-methyltransferase* (COMT) enzyme. The DA clearance mechanisms differ in various brain regions; for instance, DAT is responsible for regulating DA levels within the striatum, whereas in prefrontal cortex (PFC), a brain region postulated to be involved in ADHD and in PR, the *COMT* is critical for DA regulation. This observation is of particular interest given the possibility that the genetic factors coding for proteins involved in DA neurotransmission may modulate both PR and medication response (MR).

The *COMT* gene may be considered a prime candidate gene for modulating PR and MR. Variations in the *COMT* gene have been often associated with the DA level in the brain. Although the best studied (Or the most well studied) SNP about the *COMT's* role in the neurobiological phenotypes in humans have focused on the $Val^{108/158}$Met polymorphism (rs4680) (Diamond, 2007; Solis-Ortiz, Perez-Luque, Morado-Crespo, & Gutierrez-Munoz, 2010; Truong et al., 2009; Winterer & Goldman, 2003), it is likely that many other polymorphisms (such as rs6269 in the P1 promoter, rs4633 in Exon 3, rs4818 in Exon 4) within the *COMT* gene could also affect DA levels in the brain by modulating the enzyme's activity (Diatchenko et al., 2005; Benedetti, 2009; Nackley et al., 2006). In addition, a number of *COMT* haplotypes formed by the combination of the previous SNPs (rs6269, rs4633, rs4818 and rs4680) have been found to alter mRNAs secondary structures, leading to the different levels of protein expression (Diatchenko et al., 2005; Nackley et al., 2006). According to Nackley et al., (2006), the $Val^{108/158}$Met (rs4680) polymorphism "interacts with other SNPs and this determines the functional expression of the gene". Given the role of the variations in *COMT* on regulation of DA levels and its role in PFC functioning, we hypothesized that the $Val^{108/158}$Met, other

SNPs, and the haplotypes might alter both response to placebo and medication in normal as well as psychiatric population by affecting synaptic DA levels. Therefore, we examined their role in modulating PR and MR in children with ADHD.

In a previous study, we have investigated PR in children with ADHD. We found that while parents and teachers tend to show a very robust PR, children with ADHD showed a significant deterioration in their behavior after the administration of PBO in a task that requires goal directed behavior (i.e. RASS). Because this deterioration happens instead of the suggested and expected improvement under PBO, we called it a ***reverse placebo response*** (RPR). We preferred not to use the term nocebo effect, which refers to the deterioration observed under negative suggestion and expectation. Remarkably, this RPR is observed in spite of the putative dopamine enhancement postulated to be associated with the administration of PBO. To further test the hypothesis of the involvement of dopamine in the RPR, we decided to investigate the association between *COMT* gene polymorphisms and RPR. We also aimed to study the link between *COMT* and MR in children with ADHD. To our knowledge, no previous studies have investigated RPR in children with ADHD in relation to genetic variability within the *COMT* gene or any other functional polymorphisms in other genes.

Subjects and Methods

Participants

Three hundred seventy one Caucasian children with ADHD between the age of 6-12 years (mean age = 9.05; SD = 1.80) were enrolled in this study. These children participated as part of an ongoing pharmacogenetic study of children with ADHD conducted at the Douglas Mental Health University Institute (DMHUI) in Montreal. Children with low IQ, with a history of Tourette's syndrome, and psychosis were

excluded from the study.

Study Design and RASS assessments

For detailed description of the study procedures, see chapter 2.

Genotyping

Blood or saliva samples were collected from affected child, parents and unaffected siblings (whenever possible) and genomic DNA was extracted from the lymphocytes. The samples were restricted to Caucasians to limit the effects of population stratification (n= 371). Four *COMT* SNPs (rs6269, rs4633, rs4818, and $rs4680_{val/met}$) were genotyped.

Three SNPs: rs6269, rs4633, rs4818 were genotyped by using Sequenom iPlex Gold technology at Genome Quebec, Montreal, Canada. The $Val^{108/158}Met$ polymorphism of the *COMT* gene was amplified using polymerase chain reaction (PCR)–based method. Genomic DNA (100 ng) fragment was amplified in a 20 ul PCR reaction mix containing the following: 10 µM forward 5'ACTGTGGCTACTCAGCTGTG3', and 10 µM reverse 5'CCTTTTTCCAGGTCTGACAA3' primers, 25 µM dNTPs, 1 unit of Taq DNA polymerase (Qiagen, Canada) and lx PCR buffer & 1.5 µM $MgCl_2$ (Qiagen, Canada). Reaction cycle conditions were as follow: 2 min denaturation at 95^0C, followed by 35 cycles of 30s denaturation at 94°C for denaturation, 20 s annealing at 57°C, one final extension of 5 minutes at 72°C, and cooling to 4°C. Success of the PCR was evaluated on a 2% agarose gel to which a current of 130 volt was applied for 45 min. Next, the 169bp *COMT* fragment was digested with 5U of NlaIII enzyme (New England Biolabs, Canada) overnight at 65^0C. PCR products were electrophoresed on 3% agarose-TAE gel with 100 bp DNA ladders, visualized under UV-light and coded according to the length of the PCR product.

Ethics

The Research Ethics Board of the Douglas Mental Health University Institute approved the research protocol. A written consent was obtained from parents and a verbal assent was given by participants after explaining the study.

Statistical Analysis

Hardy–Weinberg Equilibrium and Linkage Disequilibrium were computed using Haploview (Choudhry et al., 2014). A series of univariate repeated measure analysis of variance was used to test the effects of the four *COMT* SNPs, reverse placebo response, MPH response, and SNPs by response interaction on the main outcome variables (i.e. RASS score). Analyses were carried out in SPSS (version 20). RASS score before and after administration of placebo and MPH was entered as the within-subject factor while the genotypes and the haplotypes in the various SNPs were entered as the between-subject factor. Main gene effects were further explored by post-hoc pairwise comparisons using the Tukey HSD method.

COMT haplotypes' effect was examined in 335 children with ADHD by comparing six diplotype groups: three of them are homozygotes: GCGGval/GCGGval (n = 57), ACCGval/ACCGval (n = 5), ATCAmet/ATCAmet (n = 75) and three are heterozygotes: GCGGval/ATCAmet (n = 144), ACCGval/ATCAmet (n = 30), and GCGGval/ACCGval (n = 24).

Chi-square statistics and ANOVA were employed to evaluate the differences in clinical characteristics between *COMT* groups in the current sample. Partial eta-squared effect sizes were calculated and the statistical significance for all analyses was set at $p < .05$.

Results

For all SNPs, the three-genotype groups did not differ with respect to their demographic characteristics or to overall number of behavioral symptoms on the child behavioral check list (CBCL) and incidence of comorbid disorders (Table 4.1). None of these SNPs departed from Hardy-Weinberg equilibrium and they all formed a single haplotype block (Figures 4.1 and 4.2).

Association of COMT polymorphism and the reverse placebo response

Two SNPs, rs6269 (p =0.011) and rs4818 (p =0.008) interacted significantly with the RPR in children with ADHD during the RASS. An over-dominance model fitted best the data for these SNPs. Both homozygous groups presumably associated with extreme DA levels (either low or high) in the PFC, are correlated with higher RPR compared to heterozygous group (Figure 4.3). Post-hoc analysis showed that **(a)** for rs6269, the AA (p= 0.041) and GG (p=0.035) were significantly different from AG. **(b)** The same pattern was observed with rs4818; with CC and GG groups both acted similarly: a significant difference was observed between CC and CG (p= 0.012) and a significant trend between GG and CG (p= 0.073). These results suggest that the genetic variation of the *COMT* may be associated with the RPR in ADHD. In addition, by using the over-dominance model (i.e. comparing heterozygous vs. homozygous groups) we observed a clear tendency of the homozygous group to have higher level of tasks disengagement with the following SNPs: rs6269 (p= 0.003), rs4818 (p=0.002), and a marginal association with Val$^{108/158}$Met (rs4680) (p=0.061). Furthermore, it has been suggested by Nackley et al., (2006) that *COMT* haplotypes may have more impact on *COMT* activity than single SNPs. We therefore tested for the effect of the six-diplotype groups on RPR by using total RASS score, and a significant interaction with RPR was observed (p =0.045). Even though no significant differences were observed among the

diplotype groups (by using Tukey HSD analysis), the homozygous tend to have higher mean scores (indicating worse behavior).

Association of COMT polymorphism and the medication response

Interestingly, the RPR was completely reversed by MPH [$F(1, 518) = 386.25$, $p = 0.000$] (Figure 4.4). A significant main effect was observed with the haplotypes (P=0.030) and all four our SNPs, rs4633 [$F(2, 356) = 4.24$, $p = 0.015$], rs4818 [$F(2, 352) = 3.42$, $p = 0.034$], rs4680 [$F(2, 368) = 3.37$, $p = 0.035$], and rs6269 [$F(2, 360) = 2.93$, $p = 0.055$]. Post-hoc analysis did not show any significant differences between the homozygous groups (i.e. between AA and GG in rs6269, TT and CC in rs4633, CC and GG in rs4818, val/val and met/met in rs4680). However, heterozygous children were more engaged in the task (P values for all > 0.05) (Figure 4.4).

Discussion

Previous studies have shown diverse effects of *COMT* polymorphisms on behaviors that are associated with the functions of prefrontal cortex (Bilder, Volavka, Lachman, & Grace, 2004; Craddock, Owen, & O'Donovan, 2006; Egan et al., 2001). These effects might be due to an expected role of this gene in metabolizing the catecholamine neurotransmitters in the brain. In the current study we attained two main findings:

First on ***placebo***: we identified a significant association between RPR and *COMT* diplotypes and two polymorphisms (rs6269 and rs4818). This is a novel finding in the ADHD and placebo response literature. To the best of our knowledge, this is the first work that studies the association between RPR and COMT in children with ADHD. The association pattern between *COMT* and RPR clearly support an over-dominance model. Both homozygous groups of children experienced more deterioration of

behavior (i.e. more RPR) and were more disengaged while doing RASS compared to the heterozygous group when evaluated with placebo. In addition, using an over-dominance model (heterozygous vs. both homozygous groups) revealed a clear tendency of the heterozygous group to have higher level of tasks engagement. This observation could be explained by the fact that while doing the RASS and by repeating the task, it is possible that the DA system will be under high demand; thus children with suboptimal levels of synaptic DA (homozygous) will perform worse compared to children with the optimal level of DA (heterozygous). These results are consistent with the inverted "U" model of dopamine effect on the prefrontal cortical efficiency, where the cortex functions reach their optimum (i.e. the top of the curve) when the dopamine level is neither too low nor too high (Cools & D'Esposito, 2011; Robbins, 2005; Williams & Goldman-Rakic, 1995) (Figure 4.5).

Second on ***MPH***: As expected MPH significantly improved RASS scores. Earlier studies have shown that the MPH enhances the saliency of a mathematical task in healthy adult subjects by increasing DA (Volkow et al., 2004). *COMT* polymorphisms do not modulate response of task-oriented behavior hence suggesting that this response is independent of *COMT* genotypes (Volkow et al., 2004). This negative result is an addition to an inconclusive outcomes array regarding the association between *COMT* (more specifically, $Val^{108/158}Met$) and medication response (Cheon, Jun, & Cho, 2008; Froehlich et al., 2011; Kereszturi et al., 2008; McGough et al., 2009; Park et al., 2014; Sengupta et al., 2008; Tahir et al., 2000). These variations could be due to the use of different doses of MPH, samples size, as well as use of different scales to define medication response.

However, we observed a main effect of the four SNPs of *COMT* gene and performance of goal-oriented tasks. Again, the same over-dominance model was

observed with all SNPs, which is in line with the inverted U-model of dopamine theory. This finding confirms previous studies showing an association between *COMT* and the ADHD endophenotype (Bilder, Volavka, Lachman, & Grace, 2004; Craddock, Owen, & O'Donovan, 2006). Therefore, using *COMT* inhibitor to regulate *COMT* activity could be useful for children with ADHD, particularly those who are homozygous.

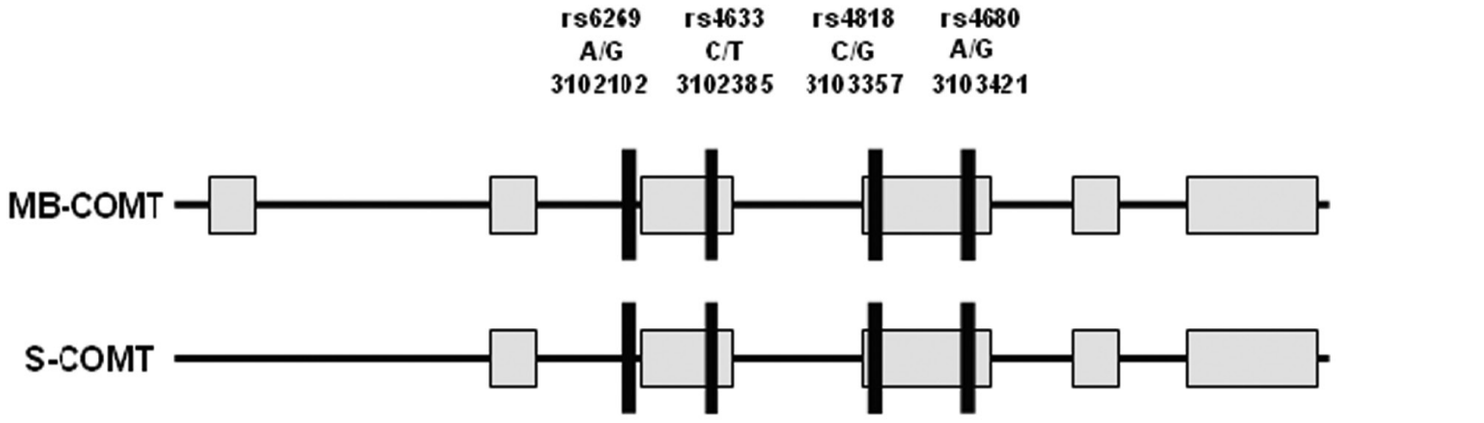
Previously our group observed no significant association between $Val^{108/158}Met$ and MR, and our new findings with the same SNP in larger group of patients confirms similar result. Contrary to the over-dominance model we currently observed, Sengupta et al. reported "ADHD children with the Val/Val genotype showed better-sustained attention than the Met carriers". But increasing the sample size and limiting the current analysis for Caucasian only to avoid population stratification effect might be the reason behind this difference since Sengupta et al., looked at the whole population with almost half of the sample size.

Importantly, we did not correct for multiple tests in the current study, as our main hypothesis was to test the haplotypes, as "they are better determinants for the final *COMT* activity than single SNPs" (Nackley et al., 2006). Therefore, we tried to minimize the number of statistical testing.

This study is one of the largest studies using the double-blind, placebo-controlled, crossover design for the evaluation of behavioral response to both MPH and placebo in ADHD. A major strength of the current study was to analyze the behavior dynamically by using active medication or placebo that can increase the variance between subjects, thus improve our capacity to identify gene effects. In addition, using the RASS as an assessment tool offers a multi-dimensional, ecologically-relevant evaluation of the child's goal-oriented behavior. It also helps to assess the child's behavior objectively while simulating academic situation.

In conclusion, results of this study strongly suggest that the children with homozygous genotype demonstrate poor task oriented behavior and less response to placebo. It is interesting to note that the statistically significant interaction observed between the groups was seen **only** after administration of placebo suggesting that *COMT* polymorphisms modulate behavior relevant to ADHD and RPR but not the response of this behavior to methylphenidate. In addition, the fact that RPR is prevented and even reversed with MPH, suggests that the dopamine mechanisms are involved in this effect. Although the present study demonstrates that *COMT* polymorphisms influence PFC activity and the propensity to response to placebo in children with ADHD, further research with non-ADHD children is warranted in order to elucidate the generalizability of these findings.

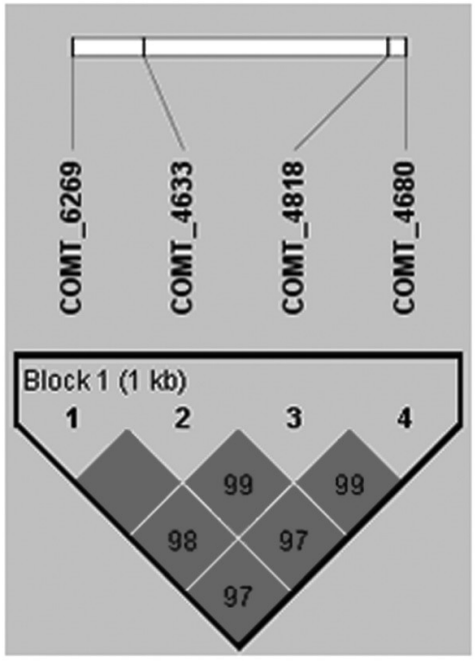
Figure 4.1: **Haplotypes of the *COMT* gene in children with ADHD and their families**



Haplotype	COMT SNPs				% Freq.	T:U	χ^2	*P*
LPS	G	C	G	G	0.391	71.0 : 64.0	0.37	0.54
APS	A	T	C	A	0.457	70.0 : 67.0	0.06	0.79
HPS	A	C	C	G	0.113	21.0 : 26.0	0.52	0.46

Schematic representation of *COMT* polymorphisms (rs6269, rs4633, rs4818, and rs4680) in MB-*COMT* and S-*COMT* illustrating the three *COMT* haplotypes (Nackley et al., 2006). **Note:** S-*COMT* = soluble *catechol-o-methyltransferase*; MB-*COMT* = membrane-bound *catechol-o-methyltransferase*; Haplotype = *COMT* haplotypes, **%** Freq. = haplotype frequency in the data sample, T:U = transmitted vs. untransmitted ratio for haplotypes, $\chi 2$= chi-square statistic for association with ADHD diagnosis. Taken from (Choudhry et al., 2014).

Figure 4.2. **Haplotype block structure of the *COMT* gene in children with ADHD and their families**



LD Plot statistics

L1	L2	D'	LOD	r2
rs4633	rs4818	0.99	129.58	0.59
rs6269	rs4818	0.98	213.07	0.90
rs6269	rs4680	0.97	132.15	0.60
rs4633	rs4680	0.97	224.68	0.94
rs4818	rs4680	0.99	129.34	0.59

Haplotype block structure (depicted by Haploview). **Note:** LD = linkage disequilibrium; LD plot statistics of rs6269, rs4633, rs4818, and rs4680; L1 = Locus 1, L2 = Locus 2, D'= D prime, a measure of pair-wise LD; logarithm of odds (LOD) = LOD score, *r*2 = goodness of fit. Taken from (Choudhry et al., 2014).

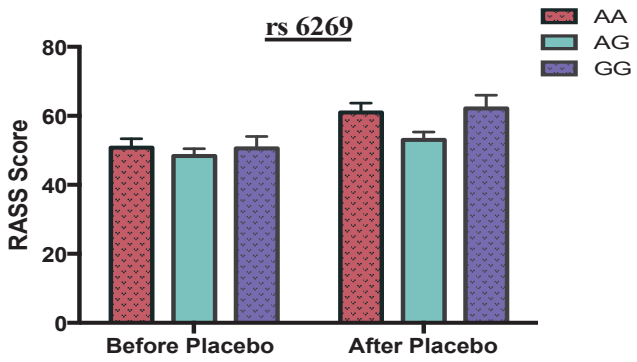


Figure 4.3: Association between *COMT* polymorphisms and the reverse placebo effect. Note. This pattern represents the over-dominance model we have observed with *COMT* SNPs. Both homozygous groups, presumably associated with extreme DA levels (either low or high) in the PFC, are correlated with higher RPR compared to heterozygous group. Error bars represent SEM.

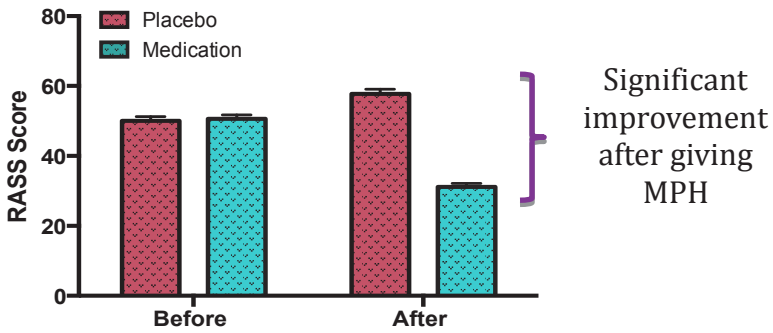


Figure 4.4: Treatment effect (after giving MPH) on the Reverse placebo response in children with ADHD. Note. The lower score means better behavior. Error bars represent SEM.

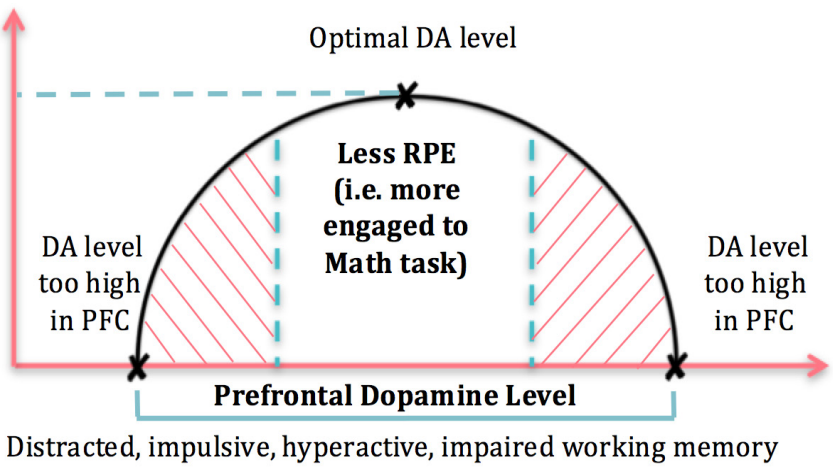


Figure 4.5: Inverted U curve of Dopamine; adopted from (Urban & Gao, 2014)

Table. 4.1 Demographic and clinical characteristics of Caucasian children with ADHD separated according to their genotypes:

rs6269				
	AA (n=121)	**AG (n=181)**	**GG (n=65)**	**Statistic and p-value**
M/F (% males)	96/25 (79.3%)	142/39 (78.5%)	49/16 (75. 5%)	χ^2= .401, df=2, p=.82
Age, yrs	9.02 (1.82)	9.05 (1.75)	9.11 (1.79)	$F_{2,366}$= .055, p=.95
Income (%<$20,000 per yr)	28.1%	29.9%	35.5%	χ^2= 1.1, df=2, p=.59
WISQ full scale IQ	97. 30 (14.13)	96.71 (13.66)	99.24 (12.28)	$F_{2,337}$= .75, p=.47
Comorbidity (%) with:				
- CD	42.5%	40.8%	50.8%	χ^2= 1.93, df=2, p=.38
- ODD	18.3%	26.6%	20.6%	χ^2= 2.97, df=2, p=.23
- MD	5.2%	7. 6%	3.3%	χ^2= 1.59, df=2, p=.45
CBLC total T-score	68.80 (9.09)	69.41 (8.74)	70.45 (7.96)	$F_{2,360}$= .752, p=.672

rs4633				
	CC (n=101)	**CT (n=182)**	**TT (n=80)**	**Statistic and p-value**
M/F (% males)	77/24 (76.2%)	139/43 (76.4%)	67/13 (83. 8%)	χ^2= 2.0, df=2, p=.37
Age, yrs	8.95 (1.69)	8.99 (1.79)	9.26 (1.85)	$F_{2,362}$= .81, p=.45
Income (%<$20,000 per yr)	28.6%	30.6%	32.9%	χ^2= .38, df=2, p=.83
WISQ full scale IQ	96. 84 (12.72)	98.35 (14.18)	96.04 (13.62)	$F_{2,333}$= .85, p=.43
Comorbidity (%) with:				
- **CD**	16.5%	27.4%	21.2 %	χ^2= 4.39, df=2, p=.11
- **ODD**	45.4%	41.4%	45 %	χ^2= .52, df=2, p=.77
- **MD**	2.2%	7. 4%	5.2%	χ^2= 3.02, df=2, p=.22
CBLC total T-score	96.67 (7.87)	69.29 (9.41)	69.48 (8.26)	$F_{2,356}$= .063, p=.939

rs4818				
	CC (n=128)	**CG (n=170)**	**GG (n=61)**	**Statistic and p-value**
M/F (% males)	104/24 (81.2%)	131/39 (77.1%)	45/16 (73. 8%)	χ^2= 1.51, df=2, p=.47
Age, yrs	9.01 (1.81)	9.12 (1.79)	9.1 (1.77)	$F_{2,358}$= .118, p=.89
Income (%<$20,000 per yr)	26.4%	31.9%	34.5%	χ^2= 1.5, df=2, p=.47
WISQ full scale IQ	97. 79 (13.97)	96.22 (13.75)	98.78 (11.80)	$F_{2,329}$= .88, p=.41
Comorbidity (%) with:				

- **CD**	19.7%	26.5%	18.6%	χ^2= 2.58, df=2, p=.28
- **ODD**	39.4%	42.3%	54.2%	χ^2= 3.75, df=2, p=.153
- **MD**	5%	8.1%	3.6%	χ^2= 1.88, df=2, p=.39
CBLC total T-score	68.72 (8.98)	69.48 (8.88)	71.03 (7.09)	$F_{2,352}$= 1.47, p=.231

COMT Val$^{108/158}$Met

	Met/Met (n=81)	**Val/Met (n=192)**	**Val/Val (n=102)**	**Statistic and p-value**
M/F (% males)	68/13 (83.95%)	146/46 (76.04%)	79/23 (77.45%)	χ^2= 2.12, df=2, p=.35
Age, yrs	9.3 (1.81)	8.98 (1.79)	8.89 (1.70)	$F_{2,347}$= 1.41, p=.25
Income (%<$20,000 per yr)	30.3%	31.15%	27.27%	χ^2= .467, df=2, p=.79
WISQ full scale IQ	95.93 (12.98)	98.12 (14.04)	96.74 (12.89)	$F_{2,345}$= .79, p=.45
Comorbidity (%) with:				
- **CD**	18.5%	27.5%	17.35%	χ^2= 4.88, df=2, p=.087
- **ODD**	43.21%	41.36%	43%	χ^2= .194, df=2, p=.91
- **MD**	6.41%	7.56%	2.2%	χ^2= 3.15, df=2, p=.21
CBLC total T-score	68.85 (8.05)	69.46 (9.34)	69.53 (7.8)	$F_{2,367}$= .171, p=.84

Note: M = Male, F = Female. Income <$20,000 = low income family. WISC-full scale IQ = Wechsler Intelligence Scale for Children–III; CD = conduct disorder; ODD = oppositional defiant disorder; MD = major depression disorder. CBCL = Child Behavioral Checklist. Values are mean (SD), counts, proportions unless otherwise indicated. Demographic, clinical, and comorbid characteristics were compared between these groups using the appropriate statistic depending on the nature of the data. Number of observations varied sometimes with regard to variables.

Table 4.2 *COMT* Alleles of Caucasian Children with ADHD and Reverse placebo response by using total RASS score

SNPs	Mean (SD)						***Statistic & p-value, Partial Eta Squared***
rs6269	***AA (N=120)***	***AG (N=178)***		***GG (N=65)***			$F_{2,360}$=4.55, **p=0.011****; 0.025
Before PBO	50.79 (28.21)	48.38 (28.65)		50.56 (27.67)			
After PBO	60.95 (29.91)	53.02 (30.53)		62.16 (30.49)			
rs4633	***TT (N=79)***	***CT (N=181)***		***CC (N=99)***			$F_{2,360}$=.835, p=.435; 0.005
Before PBO	51.5 (29.41)	47.9 (27.97)		50.9 (28.41)			
After PBO	61.08 (30.39)	54.40 (31.12)		59.5 (29.59)			
rs4818	***CC (N=127)***	***CG (N=167)***		***GG (N=61)***			$F_{2,352}$=4.95, **p=0.008****; 0.027
Before PBO	50.52 (28.6)	48.13 (28.42)		50.40 (28.10)			
After PBO	61.36 (30.73)	52.48 (30.20)		61.08 (30.36)			
rs4680 (Val$^{108/158}$Met)	***AA met/met (N=80)***	***AG val/met (N=191)***		***GGval/val (N=100)***			$F_{1,285}$=5.15, p=0.169; 0.10
Before PBO	51.48 (29.11)	48.33 (27.97)		49.58 (27.43)			
After PBO	61.67 (30.2)	54.33 (31.19)		59.04 (29.58)			
Diplotypes:	**ACCG/ACCG (N=5)**	**ACCG/GCGG (N=24)**	**ATCA/GCGG (N=144)**	**ATCA/ACCG (N=30)**	**ATCA/ ATCA (N=75)**	**GCGG/GCGG (N=57)**	$F_{5,329}$=2.28, **p=0.046****; 0.034
Before PBO	56.20 (23.66)	52.33 (29.75)	47.59 (28.13)	49.26 (28.09)	52.10 (29.35)	50.26 (27.01)	
After PBO	65.20 (27.86)	53.37 (27.60)	51.72 (31.09)	59.80 (31.88)	62.08 (30.05)	61.82 (29.55)	

Table 4.3 *COMT* Alleles of Caucasian Children with ADHD and Medication Response by using total RASS score

SNPs	Mean (SD)						***Statistic & p-value, Partial Eta Squared***
rs6269	***AA (N=121)***	***AG (N=177)***		***GG (N=65)***			$F_{2,360}$=2.93, **p=0.055****; 0.016
Before PBO	50.52 (28.26)	47.09 (26.33)		56.93 (27.84)			
After PBO	34.23 (25.31)	29.68 (22.78)		34.69 (27.11)			
rs4633	***TT (N=80)***	***CT (N=180)***		***CC (N=99)***			$F_{2,356}$=4.24, **p=.015**;** 0.023
Before PBO	51.28 (28.79)	46.62 (26.62)		55.50 (27.44)			
After PBO	34.86 (25.28)	28.98 (22.99)		35.65 (26.15)			
rs4818	***CC (N=128)***	***CG (N=166)***		***GG (N=61)***			$F_{2,352}$=3.42, **p=0.034****; 0.019
Before PBO	51.50 (29.06)	46.35 (26.02)		55.37 (26.68)			
After PBO	34.21 (25.44)	29.35 (22.83)		36.19 (27.27)			
rs4680 (Val$^{108/158}$Met)	***AA met/met (N=81)***	***AG val/met (N=190)***		***GGval/val (N=100)***			$F_{2,368}$=3.37, **p=0.035****; 0.018
Before PBO	51.32 (28.82)	47.70 (27.04)		54.56 (26.82)			
After PBO	34.19 (24.22)	28.87 (23.15)		35.60 (25.49)			
Diplotypes:	**ACCG/ACCG (N=5)**	**ACCG/GCGG (N=24)**	**ATCA/GCGG (N=144)**	**ATCA/ACCG (N=30)**	**ATCA/ ATCA (N=75)**	**GCGG/GCGG (N=57)**	$F_{5,329}$=2.50, **p=0.030****; 0.037
Before PBO	57.20 (22.90)	51.62 (27.19)	45.56 (25.97)	50.26 (29.89)	51.51(28.66)	55.91(25.21)	
After PBO	55.00 (31.33)	53.16 (23.56)	28.06 (22.47)	29.69 (25.15)	34.76 (24.57)	38.10 (27.20)	

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Chapter 5

Conclusions

Interest in the area of placebo response has grown drastically since last few decades. This interest resulted in increasing our knowledge about both the psychological and the physiological mechanisms of placebo. It also helped us to understand the power of human mind in healing the body. In fact, the sophisticated brain imaging techniques available today play a vital role in understanding the complex interaction of mind and body in healing processes. Currently, for clinical trials, medication has to show its superiority over placebo to judge its efficacy and to get its approval. As people respond differently to placebo, studying the determinants of this phenomenon became of a high interest.

This thesis was set out to explore the concept of placebo response determinants in children with ADHD as they have shown to respond to placebo. The studies sought to answer the following questions:

1. Does placebo response differ according to the observer in ADHD?
2. What are determinates and patterns of placebo response?
3. Does *COMT* gene play a role in this phenomenon?

This section will synthesize the empirical findings to answer these above mentioned questions. The main empirical findings of chapter 2 of this thesis were to answer the first two questions:

1. Does placebo response differ according to the observer in ADHD?

Different observers responded differently to placebo. While parents and teachers perceive an overall improvement in children's behavior on placebo, a fine grained observation of the children behavior in the laboratory indicated that their condition deteriorates for the capacity to orient to a task an hour after the administration of placebo (i.e. reverse placebo response "RPR"). Importantly, within the overall

improvement in children's behavior from parents' and teachers' perspectives, parents tend to report higher improvement compared to teachers. This observation suggests that the improvement observed by parents or teachers is not in parallel to the behavior of the child. Therefore, studying placebo response by specifying the observers would be critical in future studies. Combining ratings from several sources into a single outcome variable, may in fact obscure opposing trends and confuse the interpretation of clinical trials.

2. What are determinates and patterns of placebo response?

In light of the previous paragraph, finding different factors associated with the placebo response across observers would not be surprising. In parents, family income, marital status, mothers' level of education, MSDP, and prior psychostimulant exposure were shown to significantly influence placebo response. In teachers, response to placebo was significantly associated with ethnicity and previous medication treatment. Finally, in children, the RPR was associated with maternal education and income.

Two main patterns of placebo response emerged after stratifying the data. (1) ***Convergent placebo effect*** where parents tend to exaggerate the level of children's symptoms at baseline evaluations but they reach same level psychopathology as the rest of the sample during the placebo phase indicating higher placebo response. This pattern was driven by MSDP and low SES (i.e. low income, single parent status, and lower maternal education). Parents' beliefs or expectations about the link between severe behavior and securing treatment may explain this pattern. (2) ***Divergent placebo effect,*** this pattern is consistent with the classical conception of placebo response where groups of children start at the same point for baseline but they get different during the placebo phase. It was particularly observed with prior stimulant treatment (for both parents and

trachers) and ethnicity (for teachers), which can be explained by the "expectation effect" theory.

Chapters 4 of this thesis focused more on the biological role of the *COMT* gene polymorphisms on both RPR and medication response in children with ADHD.

3. Does COMT gene play a role in this phenomenon?

A significant association between *COMT* gene polymorphisms and RPR was observed in children with ADHD, thus supporting the DA role in RPR. However, even though no such association was observed with the medication response, *COMT* polymorphisms showed to affect children's behavior while performing a goal-oriented task. Interestingly, both results (i.e. RPR and children's behavior) best fit an over-dominance model, a model that successfully reflects the inverted U-curve of dopamine effect in the brain. These empirical findings may help to understand the mechanisms involved in the role of *COMT* in response to treatment, either to placebo or to active medication, in children with ADHD.

Studying placebo response by using two different scales (objective and subjective), different observers (parents, teachers, research personnel), in different settings (i.e. at home, in school, in clinic), and in a large sample size helped us to understand the complexity of placebo response in an extremely comprehensive way. However, in order to fill in the gaps of the current knowledge, there is a need to study more control cases to allow further understanding of placebo response dimensions.

From a genetic perspective, exploring more genes such as *MAO-A* and serotonin related genes, would help to better manage placebogenic effects. In addition, the general theoretical literature on the subject of RPR specifically in the context of ADHD is still

inconclusive, and further work is needed to answer vital questions about this phenomenon.

In conclusion, beyond the scientific interest in exploring the placebo response determinants, findings reported in this thesis could help to expand our understanding of this phenomenon that can be translated into an improved patient care and the clinical trials design.