Universitat Autònoma de Barcelona

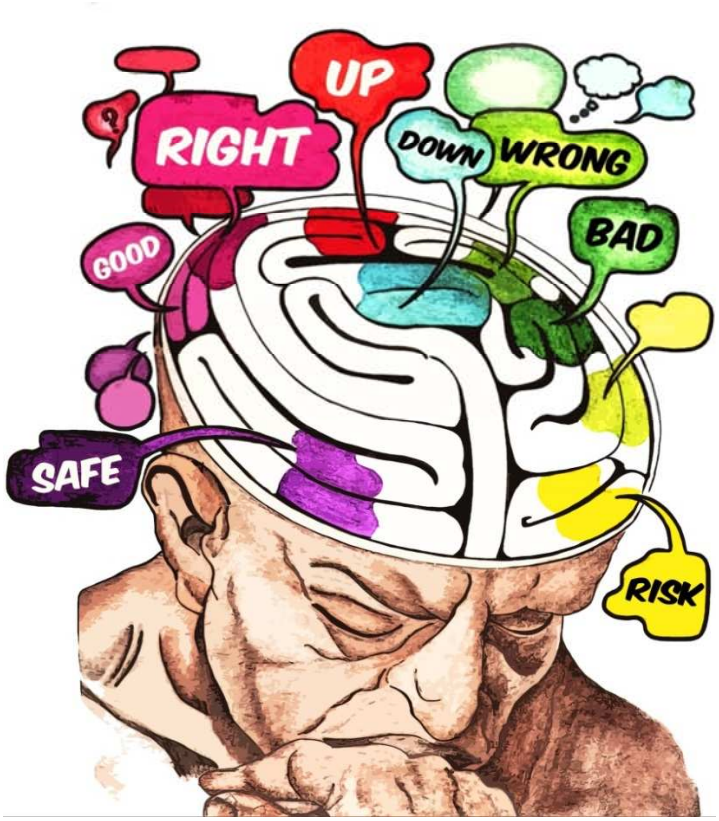
Neuropsychological evaluation of patients with acromegaly and Cushing's syndrome: Long-term effects

Iris Crespo Martín

PhD Thesis

Neuropsychological evaluation of patients with acromegaly and Cushing's syndrome: Long-term effects.

Iris Crespo Martín



Universitat Autònoma de Barcelona

Universitat Autònoma de Barcelona

Doctoral thesis:

Neuropsychological evaluation of patients with acromegaly and Cushing's syndrome: Long-term effects.

Iris Crespo Martín

2015/16

Thesis directors:

Prof. Susan Webb Youdale

Dra. Elena Valassi

Dra. Eugenia Resmini

Programa de Doctorat en Medicina

Departament de Medicina. Facultat de Medicina

Universitat Autònoma de Barcelona

2016

"All truths are easy to understand once they are discovered;
the point is to discover them"
Galileo Galilei

Acknowledgements

Me gustaría aprovechar estas líneas para mostrar mi agradecimiento a todas aquellas personas que directa o indirectamente han hecho posible que esta tesis doctoral se llevara a cabo. Al mismo tiempo, espero que este trabajo les haya reportado, en mayor o menor medida, algo de bueno.

Si tuviese que resumir lo que ha sido para mí realizar esta tesis doctoral expresaría que fundamentalmente ha sido un camino de aprendizaje y que, sin lugar a dudas, me quedo con el crecimiento profesional y personal que ha supuesto. Aunque como todo camino que se precie no ha sido fácil. Nada parecido a dar un paseo por la orilla del mar con el agua fresca mojándote los pies. A mi modo de ver, se acerca más a la subida empinada de una montaña por un caminito estrecho plagado de piedras. Eso sí, cuando por fin llegas al pico más alto la sensación de plenitud es innegable y el aire que te llena los pulmones no puede ser más puro.

Un arduo camino que nadie puede hacer solo, pues resulta abrumador. Siempre es un placer que te acompañen en la caminata gente con experiencia, gente que ya hizo el camino anteriormente. Yo he tenido la suerte de contar con la Dra. Susan Webb, una experta en enfermedades raras hipofisarias y la primera que me dio la oportunidad de formar parte del mundo de la investigación. También he contado con el soporte de la Dra. Eugenia Resmini y la Dra. Alicia Santos. Ellas dos fueron las responsables de que se creara una nueva línea de investigación dentro del grupo dedicada especialmente a los efectos neuropsicológicos y cerebrales que sufren los pacientes con Síndrome de Cushing y acromegalia. Y por último, con la Dra. Elena Valassi que ha sabido contagiarme su pasión por la medicina y la endocrinología.

Tampoco puedo dejar de resaltar la ayuda y colaboración que han aportado otros grandes profesionales como Patricia Pires, Yolanda Vives Gilabert, Esther Granell Moreno, Manel de Juan Delago y Beatriz Gómez Ansón, principalmente en el procesamiento y post-procesamiento de las imágenes cerebrales. Esa parte del camino no hubiese sido posible realizarla sin ellos.

Hay otra parte indispensable para completar este recorrido. Son ellos, los pacientes con síndrome de Cushing y acromegalia, todos esos pacientes que voluntariamente quisieron y pudieron participar, e incluso aquellos que no. Porque si a alguien hay que agradecerle el SENTIDO humano de este camino que inicié hace unos años, es a ellos.

Las personas que han sufrido estas enfermedades y que nos dicen y nos repiten que no se sienten totalmente "curados" independientemente de lo que digan las analíticas.

También le debo mi más profundo agradecimiento a todo ese grupo de personas sanas del banco de sangre que de manera totalmente altruista se ofrecieron voluntarias para participar en estos estudios.

No puedo completar esta cima sin hacer una breve mención a las personas que son una parte íntegra de mí misma y sin las cuales no sería quien soy a día de hoy: mis padres, mi hermano, mi hermana, mis buenas amigas y él. Sin ellos, los grandes momentos sólo son momentos y los grandes retos, abismos imposibles.

Contents

List of publications

This thesis is mainly based on the following two original articles:

- **Paper I:** Crespo, I., Granell-Moreno, E., Santos, A., Valassi, E., Vives-Gilabert, Y., De Juan-Delago, M., Webb, SM., Gómez-Ansón, B., Resmini, E. Impaired decision-making and selective cortical frontal thinning in Cushing's syndrome. Clinical Endocrinology. 2014 Dec; 81(6): 826-833. doi: 10.1111/cen.12564.

- **Paper II:** Crespo, I., Santos, A., Valassi, E., Pires, P., Webb, SM., Resmini, E. Impaired decision making and delayed memory are related with anxiety and depressive symptoms in acromegaly. Endocrine. 2015 Dec; 50(3): 756-763. doi: 10.1007/s12020-015-0634-6.

According to the Postgraduate criteria, the following original article cannot be part of the main body of this thesis because it is not published yet. Nevertheless, the article appears in Annex II, since it reports important evidences in line with this thesis.

- **Paper III:** Crespo, I., Santos, A., Gómez-Ansón, B., López-Mourelo, O., Pires, P., Vives-Gilabert, Y., Webb, SM., Resmini, E. Brain metabolite abnormalities in ventromedial prefrontal cortex are related to duration of hypercortisolism and anxiety in patients with Cushing's syndrome. Endocrine. 2016 *(under review)*

Abbreviations

ACC	Anterior cingulate cortex
ACTH	Adrenocorticotrophic hormone
ANOVA	Analysis of variance
BDI-II	Beck depression inventory II
CS	Cushing's syndrome
DTI	Diffusion tensor imaging
fMRI	Functional magnetic resonance imaging
GC	Glucocorticoid
GH	Growth hormone
Glx	Glutamate+Glutamine
HC	Hydrocortisone
ICD-10	International classification of diseases 10
IGF-1	Insulin-like growth factor 1
IGT	Iowa gambling task
IQ	Intelligence quotient
MRI	Magnetic resonance imaging
MANOVA	Multivariate analysis of variance
NAA	N-acetyl-aspartate
NFPA	Non-functioning pituitary adenomas
NMDA	N-methyl-D-aspartate
QoL	Quality of life
OGTT	Oral glucose tolerance test
RAVLT	Rey auditory verbal learning test
STAI	State-trait anxiety inventory
UFC	Urinary free cortisol
11ß-HSD1	11β-hydroxysteroid dehydrogenase type 1

Introduction and background

Pituitary adenomas are rare, benign tumors, but can cause serious morbidity due to excessive secretion of pituitary hormones. After treatment, multiple physical and psychological complaints may persist, even after long-term remission. The studies described in this thesis focus on the neuropsychological consequences in patients who have suffered two types of endocrine diseases: Cushing's syndrome and acromegaly.

1. Cushing's syndrome

Pituitary and adrenal adenomas cause excessive cortisol production from the adrenal gland and the prolonged exposure to hypercortisolism induces signs and symptoms referred to as Cushing's syndrome (CS). It can also be due to an ectopic secretion of adrenocorticotrophic hormone (ACTH) from a tumor in another location. CS is characterized by high blood pressure, glucose intolerance, abdominal obesity, round red face (moon face), fat lump between the shoulders, muscle weakness, osteopenia, striae, hirsutism, acne, poor wound healing and irregular menstruation (Nieman 2015).

CS can be treated by selective removal of the pituitary adenoma via transsphenoidal surgery, adrenalectomy when the tumor is in the adrenal gland or excision of the tumour that secretes ACTH. Pituitary irradiation is an alternative treatment option when pituitary surgery fails (Biller 2008). Pharmacological treatment can also be used in some patients to reduce cortisol levels. Following successful treatment of hypercortisolism, signs and symptoms of CS patients improve substantially. Nevertheless, prolonged exposure to hypercortisolism may have long-lasting adverse effects. Patients do not completely return to the premorbid level of functioning and quality of life (QoL) is impaired despite long-term cure of CS (Van Aken 2010). In a recent cross-sectional study, only 80% of CS patients felt that they had been cured after surgery (mean time: 7.4 years), even though 92% met biochemical remission criteria (Carluccio 2015).

Residual impairment of QoL may persist after long-term disease remission, in terms of fatigue, physical aspects, anxiety, depression and perception of well-being according to different studies (Lindsay 2006, Wagenmakers 2012). Patients with CS most often

complain of fatigue/weakness (85%), changes in physical appearance (63%), emotional instability (61%), cognitive problems (49%), depression (32%), and sleeping difficulties (12%) (Gotch 1994). These problems in CS patients cause negative effects on family life, partner relations and work/school performance (Gotch 1994). So called “cured” patients still experience a lower general well-being, more anxiety and depression, leading to QoL below that of matched healthy controls (Ragnarsson 2013). The mechanism through which cured CS determines impairment of QoL is probably multifactorial, involving physical and psychological factors.

1.1. Cognitive function

Patients with CS present cognitive alterations, mainly involving memory (Tiemensma 2010a). Impairments in executive functions, attention, visuoconstructive functions, language, information processing speed and motor functions are also present in these patients, although less consistently (Forget 2002, Resmini 2012, Santos 2014, Starkman 1992, Tiemensma 2010a). There are studies that have highlighted the relationship between preoperative urinary free cortisol (UFC) levels and cognitive dysfunction (Milian 2012, Milian 2014).

Cortical thinning and reduced brain volume in general, in the cerebellum, in the anterior cingulate cortex (ACC) and especially in the hippocampus have been described in CS (Andela 2013, Bourdeau 2005, Resmini 2012, Santos 2014). Given the key role of the hippocampus in memory, it was expected that hippocampal atrophy would be related with memory dysfunctions (Resmini 2012, Starkman 1992). Fortunately, the hippocampal atrophy seems to improve one year after surgery and, to a lesser extent, memory function too (Resmini 2012). Using proton magnetic resonance spectroscopy, the hippocampus showed lower levels of N-acetyl-aspartate (NAA) and higher levels of glutamate+glutamine (Glx) in cured CS patients than in matched controls. The authors suggested that decreased NAA concentrations reflected neuronal damage or loss after hypercortisolism, while increased Glx reflected glial proliferantion as a repair mechanism (Resmini 2013).

CS has been associated to aging-like effects on the brain (Michaud 2009). Diffuse white matter alterations using Diffusion Tensor Imaging (DTI) in the brains of CS

patients suggest a widespread loss of integrity and predominant demyelination compared to controls (Pires 2015). Once present, these alterations seem to be independent of concomitant hypercortisolism, persisting after remission (Pires 2015). In fact, a recent systematic review including nineteen MRI studies in a total of 339 patients with CS (active and in remission) has showed smaller hippocampal volumes, enlarged ventricles, cerebral atrophy as well as alterations in neurochemical concentrations and functional activity (Andela 2015). These findings were related to clinical characteristics like cortisol levels, duration of hypercortisolism, age at diagnosis, current age and triglyceride levels, and behavioral outcome comprising cognitive and emotional functioning, mood and QoL (Andela 2015). In the same line, vascular age (calculated according to an algorithm based on the Framingham heart study) appears to negatively influence cognitive function and brain volume in patients in remission (Santos 2015). Only CS patients in remission, but not in the active phase, have more severe white matter lesions than controls, associated to hypertension and hydrocortisone use to treat the postoperative inhibition of the hypothalamic-pituitary-adrenal axis (Santos 2015).

The reversibility of structural, neurochemical and cognitive alterations was incomplete after long-term remission. Recently, an increase in connectivity between the limbic network (like the amygdala and insula) and the ACC along with hypoactivation of the ventromedial prefrontal cortex during emotional processing have been observed in CS patients with long-term remission (Bas-Hoogendam 2015, van der Werff 2015). These results suggest that hypercortisolism may lead to persistent changes in brain functional connectivity involving episodic memories, emotion regulation or inhibitory control. Moreover, mental fatigue, characterized by mental exhaustion and long recovery time following mentally strenuous tasks, is more common in patients with CS in remission compared to healthy education-, age- and gender-matched controls (Papakokkinou 2015). In the last year, an interesting study has shown that the long-term cognitive impairments observed in patients with CS in remission were associated with variants in the glucocorticoid (GC) receptor and the 11β-hydroxysteroid dehydrogenase type 1 (11ß-HSD1) gene, indicating GC sensitivity and pre-receptor regulation of GC action may play a role in the etiology of cognitive dysfunction in these patients (Ragnarsson 2014).

1.2. Psychological status

Psychopathology has been reported in 54-81% in different series of patients with CS (Pereira 2010, Sonino 2001). Major depression and anxiety are the most common affective disorders, but other emotional disabilities like mood lability, mania, paranoia, acute psychosis, and panic attacks may also occur in CS (Pereira 2010, Pivonello 2015, Sonino 2001). Psychopathology (mainly atypical depression) was more prevalent before cure (66.7%) than at 3 months (53.6%), 6 months (36%) and 12 months (24.1%) after successful treatment (Dorn 1997).

Most CS patients have depression or emotional lability especially if they are older, females and have severe hypercortisolism (Dorn 1997). A review from China covering data from the last 20 years on neuropsychiatric disorders confirmed that patients with active CS have more problems, specifically emotional instability, depression, anxiety, and impulsivity (Chen 2013). Regarding gender, both males and females presented similar psychopathological profiles on average 42 months after remission of active hypercortisolism (Milian 2014). Of note that in males prolonged time to diagnosis and in females the presence of comorbidities/stressors were the strongest predictive factors for worse psychopathological status (Milian 2014).

After successful biochemical treatment of CS, psychiatric symptoms decrease, but patients still show decreased QoL and a higher prevalence of affective disorders and apathy compared to healthy controls (Tiemensma 2010b). In fact, CS patients show more emotional problems (depression and anxiety) and slower recovery after surgery than other patients with pituitary adenomas. Higher scores in depression (feeling tired, guilty, hopeless, unworthy, poor appetite and loss of interest) and anxiety (being nervous, tense, panicky, alertness, difficulties falling asleep or early morning awakening) are common in patients with CS (of both adrenal and pituitary origin) in remission (Pivonello 2015).

Personality profile of patients after cure of CS has also been described. Fifty patients with CS (74% biochemically controlled) were compared to 60 patients with non-functioning pituitary adenomas (NFPA) and 100 healthy controls. CS patients had less novelty-seeking behavior, exploratory excitability and extravagance than controls, as

well as more anticipatory worries and pessimism, higher fear of uncertainty, shyness with strangers, fatigability and asthenia. Compared to NFPA, CS patients expressed higher neuroticism. Moreover, persistent hypercortisolism after surgery in CS was associated with higher fear of uncertainty, fatigability and asthenia when compared to CS in remission (Dimopoulou 2013, Tiemensma 2010b).

2. Acromegaly

GH-producing pituitary adenomas cause acromegaly. The biochemical hallmarks are elevated growth hormone (GH) and insulin-like growth factor 1 (IGF-1) concentrations. GH overproduction in adults leads to phenotypical changes like frontal bossing, macroglossia, organomegaly (soft tissue of internal organs), kyphosis, soft tissue swelling visibly resulting in enlargements of hands, feet, nose, lips and ears. Carpal tunnel syndrome, headache, enlarged head, hypertension, diabetes mellitus, compression of the optic chiasm, greasy skin, sleep disturbances, heart failure and malignancies especially colorectal cancer are well known complications of acromegaly patients (Ben-Shlomo 2001).

Acromegaly can be treated by selective removal of pituitary adenoma via transsphenoidal surgery or by medical treatment with somatostatin analogs or pegvisomant. Radiotherapy is a third-line treatment option (Melmed 2009).

The impact of acromegaly and its treatment on the patients' QoL can be great, due to the delay in diagnosis and the specific comorbidities. Impairment of QoL persists in "cured" acromegaly when compared to normal population or patients with non-functioning pituitary adenomas (NFPA) (Biesmasz 2004). Patients with acromegaly showed reduced physical and social functioning, limitations in role functioning due to both emotional and physical problems, increased pain and decreased general well-being, as compared with healthy controls (Biesmasz 2004). Indeed, patients with long-term remission of acromegaly show more negative illness perceptions than patients with acute illness (i.e. acute pain or vestibular Schwannoma) (Tiemensma 2011a).

Among the factors affecting QoL, psychological status is one of the most relevant. In fact, psychopathology seems to predominantly drive reduced QoL in acromegaly (Geraedts 2015). Although patients with acromegaly in remission have a good

understanding of their disease, they experience a lack of personal control and are not likely to seek medical care (Pereira 2012). On the other hand, potential long-lasting effects of GH and IGF-1 on the central nervous system affecting personality, cognition and behaviour have not been considered until recently, but some studies published more than half a century ago reported psychological disturbances after exposure to GH and IGF-1 (Bleuler 1951).

2.1. Cognitive function

Evaluation of cognitive function in patients with acromegaly has revealed some relevant alterations. Brummelman et al. found that a high proportion of patients (72%) scored in the impaired range (<10th percentile) on at least one outcome of memory, independent of both biochemical control and current therapeutic regimen (Brummelman 2012a). However, some differences in memory and cognitive dysfunction between patients with active and cured acromegaly have been shown (Crespo 2014). Self-perception of cognitive function, including ability to learn, concentration and distractibility, mental agility, memory and verbal recall was recently described in acromegaly patients (Yedinak 2014). Patients with active acromegaly expressed decreased ability to learn and more distractibility than patients with NFPA and controlled acromegaly (Yedinak 2014). Specifically, active patients reported difficulties to learn new skills, difficulty concentrating, “head fog”, as well as slow thinking and verbal recall in a conversation (Yedinak 2014). These complaints are consistent with impaired working memory and attenuation of electrophysiological brain activity, which have been previously described in “naïve”/active acromegaly (León-Carrión 2010, Martín-Rodríguez 2013, Tanriverdi 2009).

Regarding cured acromegaly, all the studies seem to indicate impairments of immediate and delayed memory (Martín-Rodríguez 2013, Sievers 2012, Tiemensma 2010c). So, learning and recall difficulties are common after control of hormone excess, as well as in long-term cured acromegalic patients. Nevertheless, there are contradictory findings in others cognitive domains. While Sievers et al. found attention problems in 33.3% of acromegalic patients (in particular, impaired selective attention and alertness), another study reported normal attention function in long-term cured

acromegaly compared to both matched controls and patients treated for NFPA (Sievers 2012, Tiemensma 2010c).

2.2. Psychological status

The psychopathology symptoms of acromegaly include changes of personality traits and coping strategies. Acromegalic patients exhibit higher anxiety-related traits and reduced novelty-seeking behavior and impulsivity, as compared to NFPA, which may affect QoL, treatment adherence and patient-doctor communication (Sievers 2009a). Patients with acromegaly describe themselves as more harm-avoidant and neurotic, and show a high social conformity (Sievers 2009a). Of note, these maladaptive personality traits persist after treatment. Tiemensma et al. have shown that long-term cured acromegaly patients present more symptoms of submissiveness, cognitive distortion, identity problems, affective lability, self-harm, oppositionality and anxiousness than NFPA patients (Tiemensma 2010c).

Interestingly, a recent study showed that reduction of QoL is driven dominantly by psychopathology (anxiety and depression) rather than other biochemical factors in acromegalic patients (Geraedts 2015). Preliminary data on 118 acromegaly patients included in the Moscow Registry of acromegaly found mental disorders in 88 (74,6%) patients, following ICD-10 criteria. Affective disorders were diagnosed in 30% of these patients, accounting for either bipolar disorder II (12.7%) or cyclothymia (5.1%) (Bobrov 2014). Moreover, organic mental disorders, such as mild dementia, mood/anxiety/personality disorders and mild cognitive disorders were detected in 56% of acromegalic patients (Bobrov 2014). These mental disorders are common in chronic and disabling diseases; but an increased rate of lifetime affective disorders, mainly major depression episodes (9.9%) and dysthymia (16.1%), and psychological distress are described in acromegaly more frequently than in patients with chronic somatic disorders (Sievers 2009b). It remains unclear whether the increased risk of mental disorders in acromegaly is due to effects of GH and IGF-1 on brain function, or whether these patients are distressed due to their illness, comorbidities or treatments.

It is known that delay in the diagnosis of acromegaly worsens not only the clinical manifestations of the disease, but also the psychopathological symptoms. Time

elapsed until the diagnosis of acromegaly has been correlated with depressive symptoms, anxiety, reduced psychological QoL, and impaired body image (Siegel 2013). Body image is a key factor in the psychological wellbeing of patients. Since changes in appearance persist even after long-term cure, patients with acromegaly usually report self-consciousness about social-, sexual-, bodily- and facial-appearance and show a negative self-concept which has a greater impact in their psychological status and QoL (Roerink 2014).

3. Decision making

Making a choice is a common behavior in daily life and requires selecting one option among several alternative possibilities. It can seem a simple task but involves a complex procedure. Decision making implies multiples cognitive processes, including information processing, recall of previous experiences and analysis of consequences derived of alternatives describes in terms of different criteria (Martínez-Selva 2006).

The measurement of decision making that currently receives most attention in the literature is the Iowa Gambling Task (IGT). The IGT mimics real-life risk taking situations since it involves uncertainty, reward and punishment (Bechara 1994). The task requires that the user chooses between an immediate reward and a delayed long-term punishment. The IGT involves four decks of cards (A, B, C and D) and subjects should choose from any decks a total of 100 cards in a sequential manner *(Figure 1)*. Virtually, they receive $2000 at the start of the game and are instructed to win as much money as possible. In order to do so, subjects should learn to choose from the most advantageous decks. On every card selection, the subject is informed if have won money or if have won money and also lost it, depending on the deck selection. The payoff of each selection is higher in decks A and B as compared to decks C and D, but losses are also much higher in decks A and B than in decks C and D; therefore, decks A and B are consistently disadvantageous (Bechara 1994). The player is never told the distribution of wins and losses associated with each deck; instead the distribution is learnt from experience, as well as occurs in real life.

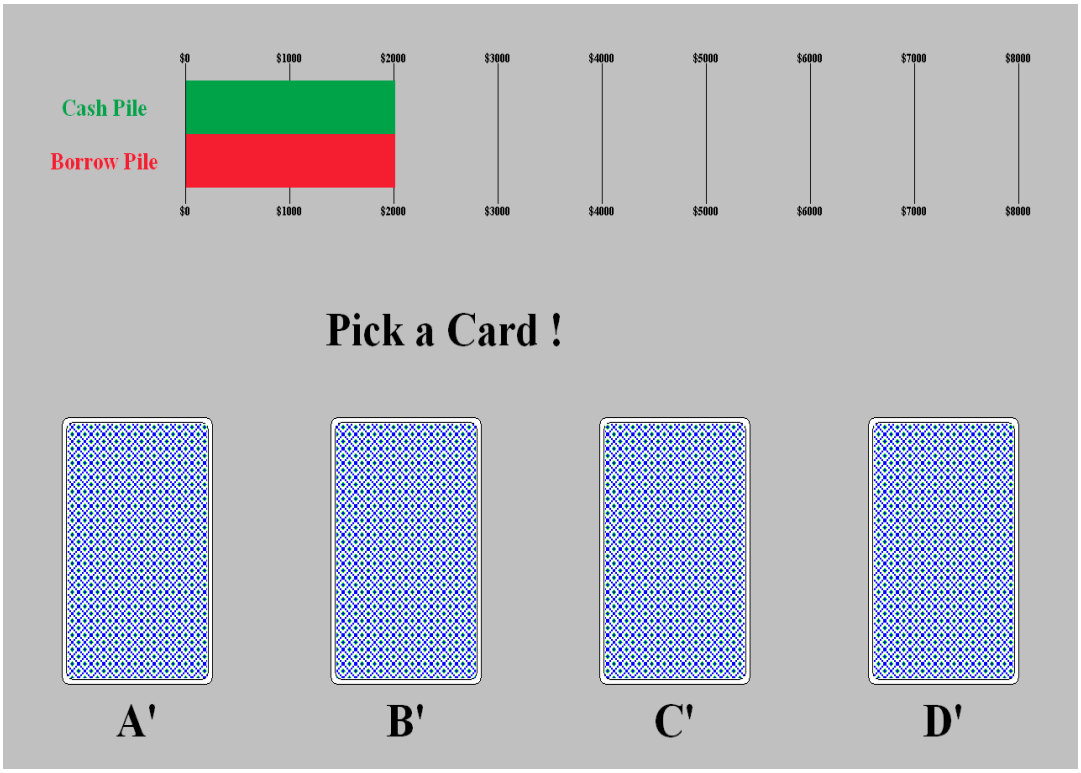


Figure 1. Computer screen of the Iowa Gambling Task.

The main measure is the proportion of selections from the advantageous decks minus the proportion of selections from the disadvantageous decks (Bechara 1994). A typical finding reported with the IGT is that healthy participants gradually learn to favor the advantageous decks and make decisions which help them to win money.

Poor performance on the IGT may occur for multiple reasons, because IGT task depends on multiple processes, including remembering past outcomes, learning long-term contingencies, evaluating immediate wins relative to long-term losses and choices mechanisms (Busemeyer 2002). After a series of experiments, Busemeyer and Stout (2002) concluded that three distinct categories can explain impaired decision making: motivational or emotional reasons, poor memory for past experience and sensitivity of expectancies. Thus, a poor IGT performance could be the result of an over-emphasis on wins or losses, an inability to remember previous negative outcomes or a general erratic strategy of decision making (Busemeyer 2002). It is clear that the use of the IGT to emulate real-world decision making implies examining the complex

interplay between cognitive, motivational and response processes involved with choice behavior.

It is important to highlight that mood can clearly alter performance on decision making as evaluated with the IGT. There is an integral link between affective state and motivations. It suggests that negative emotions are associated with avoidance of stimuli that may have disadvantageous consequences, and positive emotions are associated with a proactive attitude or pleasant consequences (Williams 2007). Moreover, the emotional status influences information processing and may disrupt longer term prioritization of punishment or reward-related information which is memorized (Williams 2007). An association between decision making and psychopathology such as anxiety and depression has been demonstrated (Kirsch 2009, Paulus 2012, Robinson 2015). Patients suffering from affective disorders showed decision-making impairments (Murphy 2001). In fact, even subjects with higher levels of trait anxiety (without psychopathology) can present intrusive memories and impaired decision making (Marzi 2014, Miu 2008). It is therefore important to evaluate affective or anxiety disorders, as well as memory function, in subjects with poor decision making.

4. Frontal cortex

Frontal cortex is one of the major lobes of the cerebral cortex in the human brain and is located at the front of the brain. It is known that frontal cortex is not a homogenous entity and it is divided into three functionally specialized subregions, namely orbitofrontal, dorsolateral and ventromedial frontal cortices. All three regions are present in both the left and the right hemispheres.

The orbitofrontal region is characteristically associated with inhibition of impulses. Patients with damage in this area commonly display poor social judgments, impulsiveness and disinhibition, diminished self-supervision of behavior, tactlessness and limited insight (Cummings 2003). Alternatively, the dorsolateral region mediates working memory and higher executive functions. Lesions of the dorsolateral cortex can result in poor abstraction, reduced mental capacity and control, poor verbal fluency, impaired strategy generation for solving problems and altered strategy in response to

changing stimuli (Cummings 2003). Finally, the ventromedial frontal region is the area associated with motivation and goal direction. Patients with ventromedial frontal dysfunction usually display apathy in affective, emotional and cognitive dimensions, so they show decreased emotional concern, reduced interest and impaired initiation and task maintenance (Cummings 2003).

Moreover, the frontal cortex has interconnections with other brain areas processing external information (with all sensory systems and with cortical and subcortical motor system structures), as well as internal information (limbic and midbrain structures involved in affect and long-term memory) (Miller 2002). Indeed, the anatomy of the frontal cortex suggests that it is well-suited for a role as the brain's executive element (Miller 2002). Thus, the frontal cortex may synthesize information from a wide range of brain systems and exert control over behavior.

4.1. Frontal cortex and decision making

The frontal area is active before and during a variety of actions, like memory for past events, anticipation of expected events and behavioural consequences, and is modulated by internal factors such as emotional and attentional state (Miller 2002). Nevertheless, the frontal cortex is mainly characterized by its implication in executive functions and in decision making.

Alterations in the decision making measured by the IGT have been associated with damage or injuries to the human frontal cortex (Bechara 2000a y 2000b). In fact, the IGT originally was created to detect poor decision making in patients with lesions in the prefrontal cortex (Bechara 1994). Recently, functional magnetic resonance imaging (fMRI) studies have confirmed that the IGT is related to the different regions of frontal cortex: orbitofrontal, dorsolateral and ventromedial (Li 2010, Lin 2008). Furthermore, other key regions like the insula and the anterior cingulated cortex (ACC, on the medial surface of the frontal lobes) have also shown to be important in decision making *(Figure 2)*. The ACC and the insula are involved in motivation, goal-directed behaviors and adaptative behavior, responding to negative feedback (Devinsky 1995); essential mechanisms to learn an advantageous strategy in the IGT.

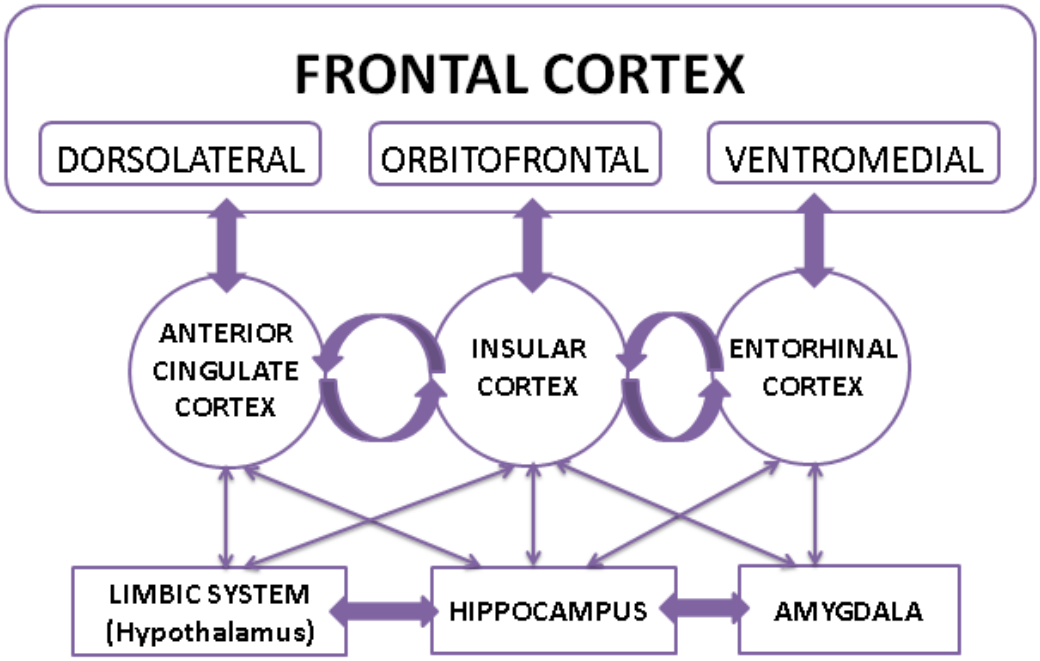


Figure 2. Brain areas related with the frontal cortex and involved in the IGT performance.

Regarding positive feedback in the IGT, the dopamine system seems to have an important role and the frontal cortex contains most of the dopamine-sensitive neurons in the cerebral cortex (Zeeb 2009). Dopamine mechanisms can be essential for implementing decisions because it is associated with rewards, learning and memory, attention, planning and motivation (Ranganath 2015). All these components are involved in the decision-making process and damage to any of these components compromises the ability to make decisions that are advantageous in the long-term.

Frontal neurons seem to have a crucial ability to solve a goal-directed task and excitatory signals seem to enhance activity of neurons representing relevant information which is necessary to make a decision (Miller 2002). Reward-related signals from successful experiences or rewarded decisions foster the formation of associations between prefrontal neurons, especially in the ventromedial frontal region that is sensitive to reward (Miller 2002). As learning proceeds, repeated selection of the rewarded choices reinforces the prefrontal pathway and the behavior becomes habitual (Miller 2002). Therefore, frontal cortex has the ability to coordinate processing among its millions of neurons in order to direct them towards future goals and advantageous decisions.

4.2. Frontal cortex and hormones

The pituitary gland and hypothalamus are relatively small divisions of the vertebrate forebrain that play significant roles in neural mechanisms including homeostasis and neural survival. They also coordinate the somatic, autonomic and neuroendocrine responses. It is known that the hypothalamus connects to the frontal cortex both directly and indirectly (Risold 1997). Thus, impaired hormone secretion, such as hypercortisolism and GH and IGF-1 excess can influence frontal functioning and disrupt or alter bilateral communication between the frontal and the neuroendocrine system.

Glucocorticoid (GC) receptors are abundant in the frontal cortex, an area able to inhibit and regulate hypothalamic-pituitary-adrenal responses to acute stress (Radley 2011). Nevertheless, GC overexposure could have neurotoxic effects and induce neuronal damage. Because the prefrontal cortex is rich in GC receptors, it may be potentially exposed to the neurotoxic effects of hypercortisolism. Simplification of dendrites, reduction of spine synapse density, loss of glutamate receptors and diminished glutamatergic neurotransmission in the prefrontal cortex have been described in rats after chronic stress (Popoli 2013). These changes seem to be a compensatory response to elevated synaptic glutamate activity after initial response to acute stress (which determines high cortisol levels), and have been associated with both decreased transmission efficiency and impaired synaptic plasticity (Popoli 2013). Indeed, when the amount of glutamate exceeds normal limits, it could kill nerve cells, a phenomenon known as “excitotoxicity” (Matute 2006). There is evidence that some brain regions of patients with CS are neuropathologically abnormal due to abnormal metabolite concentrations *(Annex II)*. While low levels of N-Acetyl-aspartate (NAA) have been observed in the hippocampus of cured CS patients, indicating neuronal dysfunction, decreased choline levels have been described in the frontal and thalamic areas of active CS patients as compared with healthy controls (Khiat 1999, Resmini 2013). These brain metabolite alterations could be considered markers of GC neurotoxicity in CS and could imply changes in brain function, and precede volume reduction (Resmini 2013).

Corticosterone-induced changes in neuron morphology of the ventromedial frontal cortex have also been observed (Wellman 2001), suggesting that hypercortisolism could play a direct effect on the ventromedial frontal area by triggering metabolite abnormalities and neuronal damage. In fact, a previous study has observed a selective hypoactivation of the ventromedial frontal area during an emotional task in patients with CS (Bas-Hoogendam 2015). Since the ventromedial area of the prefrontal cortex is directly involved in the representation of elementary positive and negative emotional states, it is congruent to think that neuronal damage or abnormal metabolites in this area can imply affective alterations in CS, as irritability or worrying, which have been frequently reported in CS patients (Davidson 1999) *(Annex II)*. In patients with anxiety and chronic depression, alterations of NAA concentration and glutamate in the ventromedial and orbital prefrontal cortex have been reported (Grachev 2000, Portella 2011).

Structural and functional abnormalities in frontal neurons after GC excess also affect executive functions and behavior. Hypercortisolemia secondary to stress has been related to loss of prefrontal cortex control over subcortical areas, and has been indicated to promote more risk-taking behavior (De Kloet 2005). In fact, Coates and Herbert observed that increased cortisol levels might shift risk preferences and even affect the ability to engage in rational choices (Coates 2008). Moreover, salivary cortisol levels after induced stress and exogenous administration of cortisol have shown to be negatively related to decision making in healthy subjects (Putman 2010 and Santos-Ruiz 2012). However, there are no studies that describe how endogenous hypercortisolism impacts on the frontal cortex and decision making of these patients *(Figure 3)*.

On the other hand, GH and IGF-1 have been related to neuroprotection, regeneration and functional plasticity in the adult brain, and GH/IGF-1 deficiency has been reported to influence cognitive impairments and dementia in elderly people (Aberg 2006, Deak 2012, Fernandez 2007, Messier 2005). Since IGF-1 acts as a neuroprotective factor in the central nervous system, alterations in frontal cortex would not be expected after GH/IGF-1 exposure, but in a condition of chronic hormone excess, its effects could be paradoxically deleterious. Indeed, attenuation of electrophysiological brain activity has been described; Martín-Rodriguez et al. showed that acromegalic patients presented

lower activity in the right dorsolateral prefrontal cortex and left parahippocampal cortex than healthy controls (Martín-Rodríguez 2013). Regarding brain volume, patients with acromegaly present a volumetric expansion of the grey matter and white matter compartments of the brain, leading to the increase in total intracranial volume (Sievers 2009c). However, it is unclear how excessive GH/IGF-1 impacts on the frontal function like decision making in acromegalic patients *(Figure 3)*.

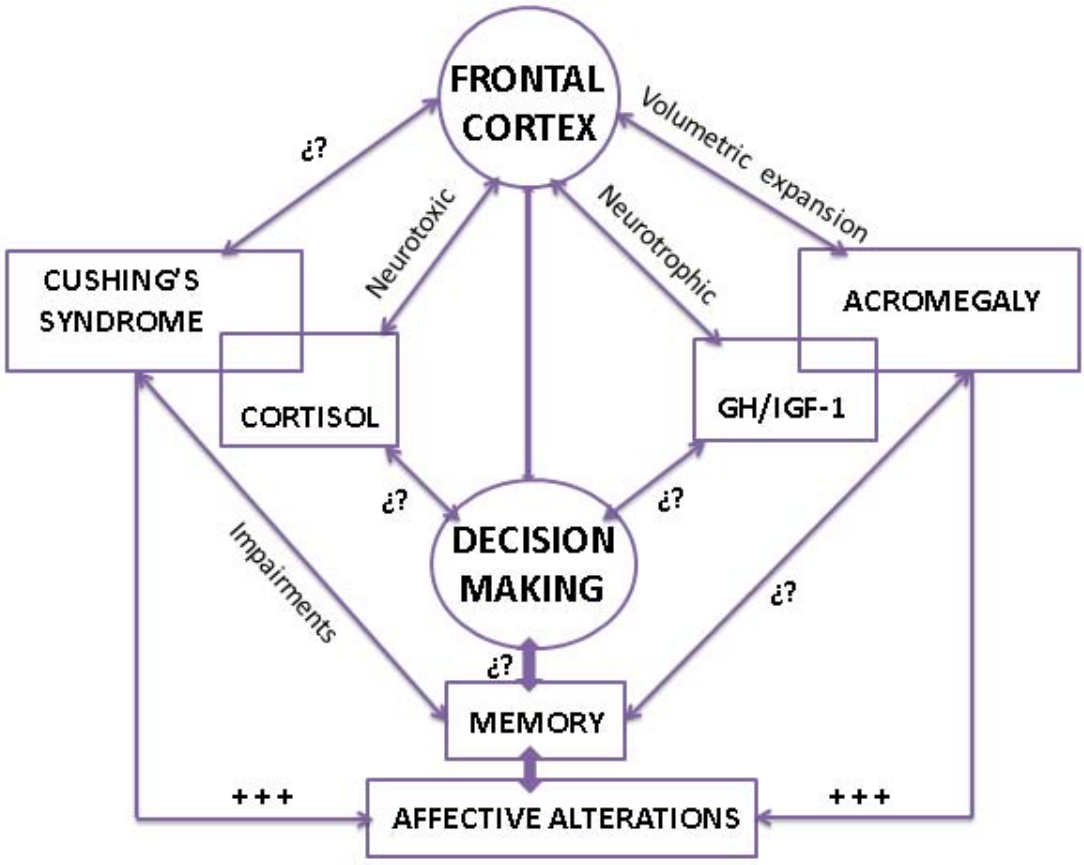


Figure 3. Possible relationships and mechanisms which relate Cushing's syndrome and acromegaly with decision-making impairments.

Hypothesis

Paper I:

Patients with CS (a paradigm of chronic exposure to endogenous hypercortisolism) do not completely return to their premorbid level of functioning after successful treatment, and impaired cognitive function persists despite long-term cure. It is known that GC excess is a potential neurotoxic factor for the brain, and long-term exposure to hypercortisolism can damage neuronal cells and alter cognitive functions. Since frontal cortex is a main target of GC and is involved in decision making, we hypothesize that:

1. Exposure to cortisol excess in CS patients alters the frontal cortex and its functioning.

2. Impaired decision making is related to cortical frontal thinnign and poor memory in CS patients.

Paper II:

Memory dysfunction is frequently described in patients with acromegaly, but GH and IGF-1 have been demonstrated to promote neuronal survival and plasticity. Emotion and cognition are related; in particular, memory and decision making are responsive to emotional alterations. The emotional status influences information processing, and may cause the sustained disruption of the memorized prioritization of punishment or reward-related information. Since affective disorders have been previously documented in acromegaly patients, we hypothesize that:

4. Affective alterations, mainly anxiety and depression, are associated with memory impairments.

5. Affective alterations, mainly anxiety and depression, and memory impairments lead to impaired decision making in patients with acromegaly.

Objectives

Paper I:

1. To compare the decision-making processes, as assessed through the IGT, between CS patients and healthy controls matched by gender-, age-, and years of education.

2. To assess the frontal cortical thickness using a 3Tesla MRI in CS patients and compare the results with those obtained in healthy controls matched by gender-, age-, and years of education.

3. To explore the relationship between decision making and frontal cortical thickness in CS patients.

4. To investigate the relationship between the poor memory function previously described in CS and decision making.

Paper II:

5. To compare memory, as measured through the Rey auditory verbal learning test (RAVLT), between patients with acromegaly and healthy controls matched by gender-, age-, and years of education.

6. To evaluate decision-making processes using the IGT in patients with acromegaly, and compare them with those obtained in healthy controls matched by gender, age, and years of education.

7. To investigate the relationship between anxiety/depressive symptoms and memory in acromegalic patients.

8. To explore the relationship between anxiety and depressive symptoms and decision making in acromegalic patients

9. To investigate the relationship between decision making and memory in acromegalic patients.

Material & methods

1. Design

· **Paper I:** Cross-sectional case-control study.

· **Paper II:** Cross-sectional case-control study.

2. Subjects

· **Paper I:**

Thirty-five CS patients clinically followed in our institution and 35 controls matched for sex, gender and years of education were evaluated with the IGT and a 3Tesla MRI of the brain. Healthy controls were recruited from among blood bank donors at Hospital Sant Pau (Barcelona, Spain). *Table 1 (Annex 1)* shows the baseline clinical characteristics of study participants.

At the time of the study, 27 CS patients were in remission (cured group), 24 with pituitary adenomas and 3 with adrenal adenomas. CS was considered in remission if after surgery patients had achieved adrenal insufficiency or morning cortisol suppression (<50nmol/liter; <1.8µg/dl) after 1mg dexamethasone overnight and repeatedly normal 24h UFC (measured with a commercial RIA after previous urine extraction with an organic solvent) (Arnaldi 2003). Six patients had radiotherapy after surgery. Eight of the patients in remission after successful surgery were adrenal insufficient and required substitution treatment with hydrocortisone (HC) [median HC dose 15mg/d (5-20mg/d)]. Corticotrope insufficiency was defined as an insufficient response to synthetic ACTH (peak <18mcg/dl) or both undetectable serum and urinary cortisol.

Eight CS patients were taking medical therapy with steroid synthesis inhibiting drugs (medically treated group): 4 were taking ketoconazole, 2 were taking metyrapone and 2 were taking both ketoconazole and metyrapone [median ketoconazole dose 400 mg/d (200-400mg/d) and median metyrapone dose 500 mg/d (500-750 mg/d)]. This group included 4 CS patients of pituitary origin, 3 of adrenal origin and 1 ectopic ACTH-secretion of unknown origin. Five were waiting for surgery (2 for neurosurgery and 3

for adrenal surgery) and 2 had previously undergone unsuccessful transsphenoidal neurosurgery and radiotherapy.

All the patients included in this study were eucortisolemic at study evaluation. Eucortisolism was defined as UFC levels within the normal range [50-280 nmol/24h (20-100μg/24h)]. Duration of eucortisolism was calculated from the date of successful surgery or normalization of UFC until the date of the study evaluation. Mean duration of eucortisolism was 41 (6-288) months in the cured and 2 (1-4) months in the medically treated group.

Duration of hypercortisolism was considered as the time from symptom onset until hypercortisolism control (eucortisolism) and was assessed by the endocrinologist in charge. At diagnosis, the duration of hypercortisolism was estimated by personal interview, careful review of medical records and photographs of patients. Mean duration of hypercortisolism was 57.6±34.5 months in the cured group and 46.5±32.5 months in the medically treated group.

The study was approved by the hospital ethics committee, and written informed consent was obtained from all participants.

Exclusion criteria:

CS patients with diabetes, GH deficiency, known intellectual disability, age over 65 years, a history of drug abuse and any current or previous psychopathology were excluded. CS patients with diabetes mellitus and GH deficiency were excluded because cognitive deficits and hippocampal atrophy have been described in these conditions (Bruehl 2009, Popovic 2004). Patients with malignant adrenocortical tumours were also excluded.

Exclusion criteria for controls were prior endocrine disease or exposure to GC, known intellectual disability, a history of drug abuse and any current or previous psychopathology.

· **Paper II:**

Thirty-one patients with acromegaly (mean age 49.5±8.5 years, 14 females and 17 males) and thirty-one age- gender- and years of education- matched controls were included in this study. Healthy controls were recruited from among blood bank donors

of the hospital. The study was approved by the hospital ethics committee, and written informed consent was obtained from all participants.

Twenty-four patients (77.4%) had macroadenomas and 7 (22.6%) had microadenomas. Twenty-nine patients had been treated with transsphenoidal neurosurgery and the remaining two had used somatostatin analogues as primary therapy. Ten patients (32%) had also received postoperative radiotherapy. None were taking antidepressants or anxiolytics at the time of the study. Median duration of disease control was 61(4-300) months. Median of delay to diagnosis after initial symptoms was 49 (1-240) months.

The patients were classified according to clinical control, based on consensus criteria (Melmed 2009). Both GH and IGF-I were measured to assess the biochemical status after treatment (except in patients receiving pegvisomant therapy, in which case only IGF-I was measured). Biochemical control was defined as a normal IGF-I for age and gender and a GH less than 1.0 ng/ml after an oral glucose tolerance test (OGTT).

Serum GH concentration was determined by a chemiluminescent immunometric assay (Immulite 2000®, Siemens Healthcare Diagnostics Products Ltd., Llanberis, UK) which uses the recombinant GH 98/574 calibrator, with an analytical sensitivity of 0.01 μg/L, and with intraassay and total CV of 2.9-4.6 % and 4.2-6.6 %, respectively. Serum IGF-I concentration was determined by a chemiluminescent immunometric assay (Immulite 2000®, Siemens Healthcare Diagnostics Products Ltd., Llanberis, UK) with an analytical sensitivity of 20 μg/L, and with intraassay and total CV of 2.3-3.9 % and 3.7-8.1 %, respectively.

At the time of the current study, 10 patients were biochemically controlled, on medical therapy (8 treated with somatostatin analogues alone and 2 with somatostatin analogues and pegvisomant). The remaining 21 were cured after surgery; of these, 9 patients required treatment for some degree of hypopituitarism, namely with L-thyroxine, hydrocortisone, gonadal steroids, and/or desmopressin.

The baseline clinical characteristics of study participants are showed in *Table 2 (Annex 1)*.

Exclusion criteria:

Acromegalic patients with diabetes, GH deficiency, a history of drug abuse and aged over 65 years were excluded. Patients with diabetes and GH deficiency were excluded

because cognitive deficits and hippocampal atrophy have been described in these conditions (Bruehl 2009, Popovic 2004).

Exclusion criteria for healthy controls were prior endocrine disease (including diabetes), a history of drug abuse and any current or previous psychopathology.

3. Neuropsychological variables (Paper I and II)

3.1. Iowa Gambling Task

The Iowa Gambling Task (IGT) is a test used to assess decision making and its evaluation is focused on 2 components:

I. Evaluation of decision-making strategy

Subjects see four card deck groups (A, B, C and D) on a computer screen. Each card group has different profiles of rewards (money wins) and punishments (money losses). Two card groups (A and B) are considered riskier and two card groups (C and D) are considered safer. Choosing the riskier card groups, subjects win more money in the short-term, but lose more money in the long-term. Choosing the safer card groups, subjects win less money in the short-term, but lose less money in the long run.

Subjects were asked to choose the options which would allow them to "win" more money, at the beginning of the study. A bar on the top of the screen changes according to the amount of money won or lost after each selection. The amount of money accumulated is shown in this bar as a feedback factor used to regulate their choices. An increase in both the amount of lost money and the number of riskier cards reflect a poorer decision-making function.

II. Evaluation of learning during the test

The IGT consists of 100 card choices. These choices are divided into 5 blocks of 20 cards each. The 5 blocks reflect a subject's learning evolution in the decision-making process during the test. The total score for each block is calculated as the difference between advantageous selections (the number of card selections from groups C and D) and disadvantageous selections (the number of selections from groups A and B). This score reflects the ability to learn the correct strategies and the feedback-regulating performance during the IGT.

Studies on decision making indicate that there are two types of decisions in the IGT: decisions under ambiguity (blocks 1 and 2 of the IGT) and decisions under risk (blocks 3-5 of the IGT) (Brand 2006).

3.2. Rey Auditory Verbal Learning Test

Rey Auditory Verbal Learning Test (RAVLT) is one of the most widely used tests to evaluate verbal memory. The RAVLT consist of 1) presentation of a 15-word list (list A) in five learning trials, 2) a single presentation of an interference 15-word list (list B), 3) a postinterference recall trial, and 4) after 30 min subjects are asked to recognize the words of both lists (list A and list B). The score for each trial is the number of words correctly recalled. Its evaluation is focussed on:

I. Immediate memory

It refers to the capacity to retain information for a short period of time. It was evaluated with the scores of "five learning trials" and the score of the "interference trial".

II. Delayed memory

It refers to the capacity to retain information for a long period of time. It was evaluated with the scores of the "postinterference recall trials" and the "recognition trials".

4. Neuroimaging variables (Paper I)

4.1. Magnetic resonance imaging

Acquisition

Magnetic resonance imaging (MRI) scans were acquired using a 3 Tesla Philips-Achieva MRI (software version 2.1.3.2). Dedicated 3D-MPRAGE of the entire brain (Turbo Field Echo, TR=6.7, TE=3.1, voxel size 1.2 × 0.889 × 0.889) was obtained. MRI anonymisation, storage and postprocessing were performed at the Port d'Informació Científica (PIC) in Barcelona through the webportal PICNIC (https://neuroweb.pic.es).

Postprocessing: surface reconstructions and estimation of cortical thickness

MRI data were analyzed and surfaces reconstructed with Freesurfer v4.3.1 (http://surfer.nmr.mgh.harvard.edu), a software tool developed at the Martinos

Center for Biomedical Imaging, according to previously published methodologies (Dale 1999). Images from all participants were registered in the Talairach atlas (Lancaster 2000). Image intensity variations due to magnetic field inhomogeneity were normalized and a skull stripping algorithm was applied (Segonne 2004).

An estimate of the gray/white boundary was constructed by classifying all white matter voxels in an MRI volume. The surface of the white matter voxels was refined to obtain a better accuracy in the gray/white boundary. It was then deformed outward to find the pial surface, according to Fischl (Fischl 2000). The surface deformation was based on a local adaptive estimation of the MRI values at the different surfaces, by minimizing a constrained energy function. Cortical thickness estimates were then obtained with the shortest distance between the white matter and the pial surfaces at each location of the cortex. Subsequently, a blinded investigator carried out a visual check and if necessary, manual correction to refine the segmentation and to correct software delineation errors.

5. Affective variables (Paper II)

5.1. Beck Depression Inventory-II

Beck depression inventory-II (BDI-II) is a self-reported measure of the severity of depressive symptoms. It has 21 items with a four point scale ranging from 0-3. The total score is the sum of each item-rating and can range from 0-63. Higher scores indicate more severe depressive symptoms. Scores 0-13 indicate minimal depression, 14-19 indicate mild depression, 20-28 indicate moderate depression and 29-63 indicate severe depression. The BDI-II can be separated into affective and somatic dimensions.

5.2. State-Trait Anxiety Inventory

State-trait anxiety inventory (STAI) is a self-reported measure that includes two subscales to evaluate two types of anxiety: state anxiety (anxiety related to current events) and trait anxiety (anxiety as a personal characteristic). Each subscale has 20 questions with a four point scale ranging from 0-3. The total score for each subscale is

the sum of each item-rating and can range from 0-60. Higher scores indicate higher levels of anxiety.

6. Statistical analysis

· Paper I:

Analysis was performed using SPSS 17.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA). Data distribution was analysed by the Kolmogorov-Smirnov test. Quantitative data are expressed as mean±SD (Gaussian distribution) or as median (range) (non-Gaussian distribution). Analysis of variance (ANOVA) was used to compare three groups. Comparison between the two groups was performed using Student's t-test, except for the blocks of the IGT that were analyzed using repeated measures ANOVA. Gender, age and duration of hypercortisolism were included as covariates in repeated measures ANOVA. $P<0.05$ was considered significant. Correlations among variables were studied using Pearson's correlation coefficient. Moreover, a multiple linear regression analysis was performed in order to determine if lower performance in verbal memory test could be related to the presence of impaired decision-making. For this, previously published results using the RAVLT in these same patients were used (Resmini 2012).

Statistical maps for cortical thickness were generated using Freesurfer's QDEC 1.4 (Query, Design, Estimate, Contrast), which uses a general linear model (GLM) at each surface vertex. A whole brain analysis between CS patients and controls was performed using a FWHM (full-width/half max) of 10mm. The following thresholds were used: $p<0.05$ for corrected (False Discovery Rate) and $p<0.001$ for uncorrected analysis. The significant clusters obtained were then represented on inflated surfaces in order to allow for better visualization of sulcal regions while maintaining topology. Whole brain correlation analysis between the cortical thickness of each vertex and IGT performance was also obtained with QDEC (QDEC tool). Desikan-Killiany atlas was used to designate the different anatomical areas of the brain.

· **Paper II:**

Data were analysed using SPSS 19.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA) with a level of significance of $p<0.05$. All quantitative data are expressed as mean±SD (Gaussian distribution) or as median (range) (non-Gaussian distribution). Data distribution was analysed by the Kolmogorov-Smirnov test. Comparisons between the two groups were performed using Mann Whitney's U-test for depression score, Student's t-test for anxiety score and card choices of the IGT and repeated measures ANOVA for the blocks of the IGT. Moreover, two multivariate analyses of variances (MANOVA) were performed for RAVLT, one for short-term memory (including scores of trial 1, trial 5 and interference trial) and another for long-term memory (including scores of recall trial, recognition trial A and recognition trial B). Correlations among variables were studied using Pearson's correlation coefficient for parametric measures and Spearman's rho for non-parametric measures. Furthermore, two multiple linear regression analyses were performed in order to investigate the role of anxiety and depression on memory and decision making.

Results

Paper I:

· COMPARISON BETWEEN MEDICALLY TREATED CS PATIENTS, CURED CS PATIENTS AND HEALTHY CONTROLS

No difference in age, gender or years of education was observed between the groups. Regarding ANOVA, no group effect was observed on the cortical thickness and IGT (*Figure 1, Annex 1*). Since this initial approach of analysing 3 groups showed no differences between the groups, a new approach comparing all CS patients (medically treated and cured) with healthy controls was adopted.

· COMPARISON BETWEEN CS PATIENTS AND HEALTHY CONTROLS

Iowa Gambling Task (IGT)

CS patients showed impaired decision making compared to controls as reflected by a smaller number of safe cards (50.4±10.8 vs. 58.3±15.6, $p<0.05$) and a trend towards a higher number of riskier cards (49.6±10.8 vs. 43.9±15.9, $p=0.087$) (*Figure 2, Annex 1*), indicating that CS patients chose short-term rewards and long-term punishments. No significant differences between CS patients and controls were observed in the final amount of money obtained, although there was a trend for CS patients to accumulate a smaller amount (-1188.9±1058.4 vs. -620.4±1470.7, $p=0.066$).

With regards to the blocks, a repeated measures ANOVA (blocks 1-5) was performed, showing a significant effect between the blocks ($F=5.32$, $p<0.01$) and between the groups ($F=3.98$, $p<0.05$). However, the interaction of blocks x groups was not significantly different. See *Table 1* for details.

Table 1. Mean and SD of IGT in CS patients and controls.

	Patients with Cushing's syndrome	**Healthy controls**
Total score of IGT	0.86±21.6	14.56±28.7
Score of block 1	-2.63±6.7	-1.03±8
Score of block 2	0.74±7.2	2.72±7.5
Score of block 3	2.63±7	3.08±8.5
Score of block 4	1.08±8.1	5.06±8.9
Score of block 5	-0.69±8.2	4.72±11.3

We decided to perform a further analysis, excluding block 1 and block 2, consistent with the notion of decisions under risk (final trials of the IGT). The result of repeated measures ANOVA (blocks 3-5) for this new analysis showed a significant effect of the interaction between blocks and groups (blocks x groups $F=5.02$, $p<0.05$) and a significant effect of group ($F=3.67$, $p=0.05$), but no effect of blocks. In addition, a subsequent pairwise comparison (corrected for multiple comparisons) was performed and a difference was found in block 5 between CS patients and healthy controls (-0.69±8.27 vs. 4.72±11.28, $p<0.05$), with a trend in block 4 (1.03±8.10 vs. 5.06±8.89, $p=0.06$). This suggested that the significant differences in decision making appear in the final phase of the test (*Figure 3, Annex 1*). Results did not change when the duration of hypercortisolism, gender and age were included in the IGT analysis as covariates.

Cortical thickness

Whole brain analysis comparison of CS patients with controls showed decreased cortical thickness ($p<0.001$, uncorrected) in certain areas: left superior frontal, left precentral and left insular cortex; bilateral rostral anterior cingulated cortex, and right caudal middle frontal cortex (*Figure 4, Annex 1*). However, no difference was observed at the corrected level (False discovery rate).

· CORRELATIONS BETWEEN IGT, CORTICAL THICKNESS, AND CLINICAL VARIABLES

No correlations were found between the IGT scores and cortical thickness. IGT performance and cortical thickness did not correlate with duration of eucortisolism or with duration of prior hypercortisolism either. There were no correlations between UFC and IGT, or between UFC levels and cortical thickness. This suggests that GC exposure itself, rather than its duration, could be related to altered decision making and reduced cortical thickness.

· CORRELATIONS BETWEEN IGT AND RAVLT

We used previously published data (Resmini 2012) to analyze the relationship between IGT performance and verbal memory performance (using the Rey Auditory Verbal Learning Test, RAVLT). The results showed that Block 4 of the IGT was correlated with recall index ($r=0.277$, $p<0.05$) and total score of the RAVLT ($r=0.297$, $p<0.05$), and Block 5 of the IGT was correlated with the number of words correctly recalled in the last learning trial of the RAVLT ($r=0.235$, $p<0.05$). Moreover, the selection of riskier cards of the IGT was correlated with the number of words correctly recalled in the last learning trial ($r=-0.301$, $p<0.05$), the recall index ($r=-0.246$, $p<0.05$) and the total score of RAVLT ($r=-0.274$, $p<0.05$). The same occurred with the selection of safer cards of IGT, that was correlated with the number of words correctly recalled in the last learning trial ($r=0.298$, $p<0.05$), the recall index ($r=0.243$, $p<0.05$) and the total score of RAVLT ($r=0.272$, $p<0.05$). Including these memory variables in a linear regression model, the main determinant factor for IGT performance was the number of words correctly recalled in the last learning trial of the RAVLT ($r=0.306$, $p<0.05$). These findings support that learning capacity is important to explain our results, namely, lower performance in learning capacity and verbal memory could contribute to impaired decision making.

Paper II:

· COMPARISON BETWEEN ACROMEGALIC PATIENTS AND CONTROLS

Iowa Gambling Task (IGT)

Acromegalic patients showed impaired decision making compared to controls as reflected by a smaller number of safe cards (51.2±15.3 vs. 60.6±13.6, $p<0.05$) and a

higher number of riskier cards (48.8±15.3 vs. 39.3±13.6, p<0.05) were chosen (*Figure 5, Annex 1*).

With regards to the blocks, a repeated measures ANOVA (blocks 1-5) showed a significant effect between the blocks (F=3.512, p<0.05) and between the groups (F=6.493, p<0.05). However, the interaction of blocks x groups was not significantly different, indicating that the significant differences observed between patients and controls in general performance of IGT disappear when the groups are compared in each individual blocks of the test (*Figure 6, Annex 1*). See *Table 3 (Annex 1)* for details.

Rey Auditory Verbal Learning Test (RAVLT)

The MANOVA for long-term memory (including Recall trial, Recognition trial A and Recognition trial B) showed a significant group effect (F=4.22, p<0.01). Specifically, acromegalic patients showed worse scores than controls in the recall trial (F=6.303, p<0.05) and the recognition trial A (F=12.941, p<0.01) of RALVT. No differences were observed between acromegalic patients and controls in the MANOVA for short-term memory (including Trial 1, Trial 5 and Interference trial). See *Table 3 (Annex 1)* for details.

Beck Depression Inventory-II (BDI-II)

Total score of BDI-II was significantly higher in acromegalic patients compared to controls [9(0-27) vs. 1(0-17), p<0.001]. Acromegalic patients showed more affective and somatic symptoms than controls [affective, 3(0-18) vs. 1(0-12); somatic, 5(0-11) vs. 1(0-5); p<0.001 for both comparisons] (*Figure 7, Annex 1*).

Twenty-six acromegalic patients (84%) showed minimal depression, 1 (3%) showed mild depression, 3 (10%) showed moderate depression and 1 (3%) showed severe depression.

State-trait anxiety inventory (STAI)

Acromegalic patients showed both higher state and trait anxiety scores than controls (state, 16.6±11.4 vs. 9.5±6; trait, 20.3±10.2 vs. 11.5±5.6, p<0.01 and p<0.001 respectively). See *Table 3 (Annex 1)* for details.

Four patients (13%) scored above normative cutoff for anxiety trait and three (10%) scored above normative cutoff values for anxiety state indicating severe anxiety problems.

No differences in the tests were found between acromegalic patients with and without hypopituitarism**.**

· RELATIONSHIPS BETWEEN TESTS

Correlations between RAVLT, BDI-II, STAI and IGT

Multiple correlations were found between memory, anxiety and depressive symptoms. The RALVT was negatively correlated with affective dimension, somatic dimension and total score of BDI-II and trait and state anxiety of STAI. Pearson correlations and p values are showed in Table 3. When these variables were included in a linear regression model, trait anxiety still predicted RAVLT score (β=-0.285, R=285; $p<0.05$). These mean that anxiety and depressive symptoms influence memory consolidation (delayed memory).

Regarding decision making, the IGT was negatively correlated with affective dimension of BDI-II and state anxiety of STAI. Pearson correlations and p values are showed in *Table 4 (Annex 1)*.

Moreover, the trial 5 of RAVLT (the last learning trial) was correlated with safer card choices (r=0.268, $p<0.05$), riskier card choices (r=-0.268, $p<0.05$), block 4 (r=0.325, p=0.01) and block 5 (r=-0.245, p=0.05) of IGT. All this indicates that affective alterations and learning capacity influence decision making.

Including depression, anxiety, memory and clinical variables in a linear regression model, the main determinant factor for IGT performance was the score of trial 5 of the RAVLT (β=0.316, R=0.316; $p<0.05$), a measure of learning capacity in a short period of time.

Correlations between evaluated tests and clinical parameters

Delay to diagnosis was inversely correlated with score in Trial 5 (r=-0.447, $p<0.05$) and Recognition trial B (r=-0.491, $p<0.01$) of RAVLT, while duration of control was inversely correlated with score in Trial 1 (r=-0.426, $p<0.05$) and Recall trial (r=-0.363, $p<0.05$). There were no relationships between delay to diagnosis and duration of control and the other evaluated tests. No relationships were found between evaluated tests and hormonal values or other clinical variables, excepting a positive correlation between anxiety state and heart rate at the moment of evaluation (r=0.386, $p<0.01$).

Discussion

These studies show impaired decision making in both CS and acromegalic patients. They selected more disadvantageous choices than advantageous choices. Furthermore, whereas healthy controls gradually learned to favor the advantageous decks, patients with acromegaly and CS continued to experience large punishment throughout training.

We have not found a direct relationship between poor decision making and frontal cortical thinning in CS patients, opposite to previous studies on other clinical conditions. Nevertheless, decision deficits on the IGT were correlated with memory deficits in CS and acromegalic patients. These findings suggest that learning and memory processes were partly responsible for decision-making impairments in these pituitary diseases, in agreement with the findings reported by Busemeyer and Stout (Busemeyer 2002).

Cognitive function is highly responsive to emotional alterations. In our study, acromegalic patients show affective alterations (anxiety and depressive symptoms) that influenced delayed memory and decision making. Therefore, psychological status should be a clinical concern in acromegalic patients, to improve and to address this issue is mandatory in order to better the psychophysical outcomes of the disease (i.e. sleep disorders, pain or energy) and the resulting poorer quality of life (QoL). In fact, a recent study has showed that psychopathology (mainly anxiety and depression) is a strong factor in determining the individual's experience of the diseases and the perceived QoL (Geraedts 2015).

· Decision making and memory in patients with CS (Paper I)

CS patients failed to learn advantageous strategies during the IGT and their behaviour was driven by short-term reward and long-term punishment. This suggests that CS patients may have learning problems because they could not be able to use previous experience as a feedback factor to regulate their choices.

The reported impairments in decision making are in line with previous studies showing a greater degree of maladaptative personality traits and less effective coping strategies in long-term biochemically cured CS patients as compared to matched

controls (Tiemensma 2010c and 2011b). Both learning and feedback-regulating are essential processes for adaptive behaviour in daily life.

It is known that GC allows the brain to deal with stress-provoked responses such as immediate attention and strategy decisions. Since the response to stressors is important for short term survival, motivation and behaviour are usually driven by this need. Interestingly, this pattern of motivation for short- term rewards was also observed in rats undergoing acute stress (Shafiei 2012), or GC excess, further supporting the hypothesis that GC excess may play a pathogenetic role in learning impairments.

On the other hand, complex decision making on the IGT rely on memory (Gupta 2009). Choice-outcome associations in the IGT must be continually formed and updated. A deck that was associated with high rewards in some choices may suddenly yield a severe punishment. In this regard, IGT is similar to real-life situations, in that we often deal with decision-making challenges, implying unpredictable and changeable contingences and magnitudes of both reward and punishment. The memory system, which is critical for relational memory, supports the relational representations of successive events or information required to learn advantageous strategies (Gupta 2009). Therefore, poor memory creates difficulties to learn novel relationship across successive experiences of the individual decks of IGT and their variations in rewards and punishment schedules.

· Frontal cortex in patients with CS (Paper I)

Cognitive alterations were accompanied by decreased cortical thickness, mainly in specific frontal areas. Interestingly, no differences have been found between cured CS and medically treated patients, suggesting an incomplete recovery of the gray matter of the brain after endocrine remission of hypercortisolism.

Gray matter loss in CS patients has been previously described (Resmini 2012). However, to the best of our knowledge, this is the first study to show selective thinning in specific areas of the frontal cortex on MRI in these patients. This cortical thinning was present in CS patients even after normalization of cortisol levels, suggesting that this damage, induced by prior GC excess exposure, is not completely reversible.

Regarding the rational and possible explanations behind this 'residual' morbidity after cortisol normalization, some hypothesis can be formulated. Stress-induced hypercortisolism has a dual mode of action in rodents (De Kloet 2005). The first mode includes rapid changes due to catecholamines rise, which lead to increased excitability and enhanced alertness, vigilance and attention. These changes are fast and of short duration. The second mode involves slow changes as a result of gene transcription activation and consequent increase in corticosterone concentrations. Once this process occurs, the possibility to generate new plasticity is reduced, and cognitive function is impaired (De Kloet 2005). Long-term exposure to stress or cortisol involves irreversible neurobiological changes in gene expression, neuronal structure and neuronal firing patterns throughout the brain (De Kloet 2005, Joëls 2007). Harmful effects of GC on frontal neurons have been widely described in rodents (Radley 2006, Wellman 2001), as well as in humans, in other situations of endogenous GC excess, such as depressive disorders and alcoholism (Le Berre 2014, Mc Ewen 2005). Thus, the decision-making performance in our cured CS patients may reflect a persistent behavioral alteration due to these neuronal changes in the frontal cortex, induced by prior exposure to hypercortisolism.

On the other hand, stress-induced hypercortisolism has been associated with glutamate-induced neurotoxicity and desensitization of NMDA receptors in response to a marked increase in postsynaptic calcium concentration (Diamond 2007). The duration of this neurotoxicity is a crucial for recovering brain functioning. Once neuronal alterations and structural changes are established, recovery is unlikely. Of note, in extreme cases of posttraumatic stress disorder, altered prefrontal function does not return to normal after exposure to stress (Diamond 2007). The same irreversibility could occur in CS patients, explaining why they were unable to recover full prefrontal function after chronic hypercortisolism.

· Interactions between decision-making dysfunctions and frontal cortical thinning in CS patients (Paper I)

Our data did not show a correlation between impaired decision-making and cortical thickness. This probably reflects that other structures or networks are also involved in decision making. It has been proposed that subcortical areas, such as the amygdala or

hippocampus, together with the frontal cortex, interact to facilitate learning and memory during the IGT (Clark 2008, Williams 2007). In fact, the hippocampus may interact with the frontal cortex to maintain the emotional regulation as well as contextual information which are needed to represent and monitor motivationally relevant goals (Williams 2007). The relationship between lower performance in verbal memory and impaired decision making which we have described in our study supports this hypothesis. Therefore, lower performance in learning capacity and verbal memory could contribute to impaired decision making. Interestingly, the significant differences in decision making observed in our patients as compared with controls occur in the final phase of the test, indicating they do not learn the correct strategy during the test.

Decision making requires the interaction between both the hippocampus and the frontal areas, which are deeply interconnected. Indeed, any changes in the hippocampus can indirectly affect the functioning of frontal areas. Our group demonstrated that long-term exposure to GC excess is associated with cortical atrophy and hippocampal atrophy, especially in older subjects (Resmini 2012). Another study described a significant increase in hippocampal volume after thansphenoidal surgery (Toffanin 2011) in patients who were significantly younger than ours. Thus, it may be that age is the main factor regulating the reversibility of cerebral atrophy in CS (Patil 2007). In line with this, Anderson et al. suggested that GC may exacerbate age-related cognitive impairments and predict prefrontal structural plasticity (Anderson 2014). Therefore, duration of GC exposure and age are the main determinants of brain function recovery in CS patients.

In our study, IGT performance and cortical thickness did not correlate with duration of prior hypercortisolism, suggesting that these cellular changes do not depend on the length of GC exposure. For this reason, an earlier diagnosis and prompt treatment may help avoid progression of the frontal damage, with consequent impairment of cortical functioning, affecting coping strategies, executive functions and decision making. Moreover, functional abnormalities could be present and be early markers of GC neurotoxicity, as evidenced in other brain areas (Resmini 2013).

· **Decision making and memory in patients with acromegaly (Paper II)**

Acromegalic patients showed impaired decision making. Their decision-making strategies are driven by short-term reward and long-term punishment. Moreover, poor memory, mainly the delayed component, was observed in these patients.

Multiple processes determine decision-making performance. Learning and memory processes are an intrinsic task which allow to evaluate the risks of a decision (Whitney 2012). Decision making requires the knowledge of the risk/benefit ratio, the ability to retrieve the information from memory, and the ability to hold the data in mind while comparing and contrasting them. The association of riskier profiles with particular card groups is an important component of the IGT, because information on outcomes of the card groups in the IGT must be dynamically updated over time.

The majority of studies reported non-significant associations between IGT performance and intelligence quotient (IQ) (Toplak 2010). Neuropsychological indexes of other executive functions and intelligence leave a large amount of unexplained variance in the IGT performance. Toplak et al. proposed that impaired performance on the IGT may be due to problems in three cognitive processes: implicit and instrumental learning, “overlearned” associations and processes of behavioral regulation by emotions (Toplak 2010). Thus, we could speculate that acromegalic patients failed in some or all these cognitive processes.

Regarding memory, our results show that delay in diagnosis was inversely correlated with RAVLT scores, indicating that a longer delay is associated with worse short-term and long-term memory, whereas duration of control was also inversely correlated with RAVLT scores, indicating that a longer disease control is associated with worse short-term and long-term memory. The first correlation is more obvious than the second, because delay in diagnosis could impair memory processes. This is another finding that underlines the importance of an earlier diagnosis in acromegaly. The second correlation is intriguing, since on the one hand it is known that age worsens memory function, while on the other hand, control of disease improves comorbidities. The message from our data could be that the patients should be controlled as soon as possible, because the positive effect of disease control could be lost due to aging. Further longitudinal studies are needed to clarify the point.

· Interactions between affective alterations and cognitive dysfunction in patients with acromegaly (Paper II)

Consideration of brain connectivity is also important to understand potential cognitive-emotional interactions and integration. Prefrontal cortex has been considered a key region that integrates emotional and cognitive information coming from multiple regions such as the anterior cingulate cortex, amygdala and hippocampus (Pessoa 2008). The hippocampus contributes to the long-term persistence of emotionally significant information, and the accumulated effects of distress may disrupt its networks (Williams 2007). In this condition, prefrontal systems for regulation of punishment-reward cues may not be effectively engaged (Williams 2007). Therefore, convergent evidence points to the role of memory in evaluation of emotionally significant information and decision-making processes.

Our data indicated that acromegalic patients present impairments in decision-making strategies and delayed memory which were related to anxiety and depressive symptoms. As far as we know, these are the first results suggesting that acromegalic patients show affective alterations that influence cognitive function, mainly memory consolidation (delayed memory) and decision making. Several groups of data suggest that mood and affect can influence the decision-making processes because regulation of punishment-reward cues may not be effectively engaged under a state of anxiety or depression (Buelow 2009, Williams 2007). In fact, depressed patients with less severe symptoms and subjects with a higher negative mood, might show impairments of the IGT (Must 2006, Suhr 2007). Along this line, affective state could be a key factor for better decision making and memory in acromegalic patients. Therefore, providing emotional support to the patients, as psychological therapy, could be a way to improve their cognitive function and an important clinical implication for further research.

Affective disorders like anxiety and depression are common in chronic and disabling diseases. Nevertheless, an increased rate of lifetime affective disorders, mainly major depression episodes (9.9%) and dysthymia (16.1%), and psychological distress are described in acromegalic patients more frequently than in patients with chronic somatic disorders (Sievers 2009b). It remains unclear whether the increased risk of affective disorders in acromegaly is due to effects of GH and IGF-1 excess on brain function, or whether these patients are distressed and are more prone to develop

psychopathology, because of their illness, their associated morbidity and/or treatment. Interestingly, a recent study showed that reduction of quality of life is mainly driven by psychopathology (anxiety and depression) rather than other biochemical factors in acromegalic patients (Geraedts 2015). This finding emphasizes our conclusion, adding importance to the measures of affective disorders rather than only biochemical variables, in order to improve patient management and control.

It is known that psychopathological variables are candidate modifiable factors to link pituitary disease, especially acromegaly, to a lower health-related quality of life (Geraedts 2015). A key clinical application of this research would be that depressive symptoms and anxiety, related with decision making, are essentially modifiable factors. A more complex treatment strategy including extensive psychopathological evaluation and therapy may be an attractive possibility to improve patient management. The neuropsychology is being acknowledged as an important clinical tool in patients with hormone excess, due to the frequent occurrence of neuropsychological impairments in them. Therefore, to have a neuropsychologist in the multidisciplinary team dealing with pituitary diseases is highly recommendable. Finally, patient associations can give additional emotional support.

Study limitations

There are several limitations in our studies:

- The **cross-sectional design** limits any causal inference.

- The **small sample size** may have contributed to the loss of significance in some variables, especially in the cortical thickness results when multiple comparison correction (False discovery rate) was applied. However, it is difficult to attain large patient groups because CS and acromegaly are rare diseases.

- **Clinical heterogeneity** should be considered. In the CS patient group, we included patients with tumours of pituitary origin and patients with tumours of adrenal origin, but we hypothesized that an excess of GC may harm the brain whatever the origin. In the acromegalic patient group, we included patients cured after surgery or controlled with therapy as well as patients with treated hypopituitarism, a frequent sequela of pituitary surgery.

- We cannot exclude an **effect of prior radiotherapy**, especially on cortical thinning in patients with CS. However, no differences were observed in this area between CS patients and healthy controls when excluding irradiated patients (n=8). Regarding cognitive function, Brummelman et al. (2012b) found that patients irradiated for a pituitary adenoma showed no differences in memory and executive functions compared to non-irradiated patients (Brummelman 2012b). Moreover, radiotherapy was not a predictor for decision making in the linear regression analysis.

- A possible **effect of current medical treatment** in acromegalic patients could be postulated; however, medical treatment was not a predictor for decision making in the linear regression analysis. Current medical treatment in acromegaly was not found to be related with memory and executive functioning in previous studies (Brummelman 2012a).

Conclusions

1) To our knowledge, these are the first reports describing the presence of altered decision making in patients with both CS and acromegaly.

2) Poor learning and memory consolidation seem to be partly responsible for decision-making impairments.

3) CS patients show a frontal cortical thinning. These morphological frontal alterations described after cortisol normalization provide further support to the concept that chronic hypercortisolism promotes brain changes which are not completely reversible after endocrine remission.

4) Frontal cortical thinning was not directly related to the decision-making deficits observed in CS patients.

5) Acromegalic patients showed impairments of decision making and delayed memory related to anxiety and depressive symptoms.

6) Awareness of these cognitive alterations would appear to justify a routine neuropsychological assessment of these patients.

References

1. Aberg ND, Brywe KG and Isgaard J. (2006) Aspects of growth hormone and insulin-like growth factor-1 related to neuroprotection, regeneration and functional plasticity in the adult brain. *The Scientific World Journal* 6: 53-80

2. Andela CD, van der Werff SJA, Pannekoek JN, et al. (2013) Smaller grey matter volumes in the anterior cingulate cortex and greater cerebellar volumes in patients with long-term remission of Cushing's disease: a case-control study. *European Journal of Endocrinology* 169: 811–819

3. Andela CD, van Haalen FM, Ragnarsson O, et al. (2015) Mechanisms in endocrinology: Cushing's syndrome causes irreversible effects on the human brain: a systematic review of structural and functional magnetic resonance imaging studies. *European Journal of Endocrinology* 173(1):R1–14

4. Anderson RM, Birnie AK, Koblesky NK, et al. (2014) Adrenocortical status predicts the degree of age-related deficits in prefrontal structural plasticity and working memory. *The Journal of Neuroscience* 34, 8387-8397

5. Arnaldi G, Angeli A, Atkinson AB, et al. (2003) Diagnosis and complications of Cushing's syndrome: a consensus statement. *Journal of Clinical Endocrinology & Metabolism* 88: 5593-5602

6. Bas-Hoogendam JM, Andela CD, van der Werff SJA, et al. (2015) Altered neural processing of emotional faces in remitted Cushing's disease. *Psychoneuroendocrinology* 59: 134–146

7. Bechara A, Damasio A, Damasio H et al. (1994) Insensitivity to future consequences following damage to human prefrontal cortex. *Cognition* 50: 7-15

8. Bechara A, Damasio H and Damasio AR. (2000a) Emotion, decision making and the orbitofrontal cortex. *Cerebral Cortex* 10: 295-307

9. Bechara A, Tranel D and Damasio H. (2000b) Characterization of the decision-making deficits of patients with ventromedial prefrontal cortex lesions. *Brain* 123: 2189-2202

10. Ben-Shlomo A and Melmed S. (2001) Acromegaly. *Endocrinology Metabolism Clinics of North America* 30: 565-583

11. Biermasz NR, van Thiel SW, Pereira AM, et al. (2004) Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. *Journal of Clinical Endocrinology and Metabolism* 89(11): 5369-5376

12. Biller BM, Grossman AB, Stewart PM, et al. (2008) Treatment of adrenocorticotropin-dependent Cushing's syndrome: a consensus statement. *Journal of Clinical Endocrinolgy and Metabolism* 93: 2454-2462

13. Bleuler M. (1951) The psychopathology of acromegaly. *The Journal of Nervous and Mental Disease* 113: 497-511

14. Bobrov A, Starostina E, Devral A et al. (2014) Psychopathology in patients with acromegaly. *Abstract of the 22nd European congress of psychiatry* (EPA-1338) 9 (Supplement 1)

15. Bourdeau I, Bard C, Forget H, et al. (2005) Cognitive function and cerebral assessment in patients who have Cushing's syndrome. *Endocrinology and Metabolism Clinics of North America* 34: 357-69

16. Brand M, Labudda K, Markowitsch HJ. (2006) Neuropsychological correlates of decision-making in ambiguous and risky situations. *Neural Networks* 19: 1266-1276

17. Bruehl H, Wolf OT, Sweat V, et al. (2009) Modifiers of cognitive function and brain structure in middle-aged and elderly individuals with type 2 diabetes mellitus. *Brain Research* 1280: 186-194

18. Brummelman P, Koerts J, Dullaart RP et al. (2012a) Effects of previous growth hormone excess and current medical treatment for acromegaly on cognition. *European Journal of Clinical Investigation* 42: 1317-1324

19. Brummelman P, Sattler MGA, Meiners LC, et al. (2012b) Cognitive performance after postoperative pituitary radiotherapy: a dosimetric study of the hippocampus and the prefrontal cortex. *European Journal of Endocrinology* 166, 171-179

20. Buelow MT and Suhr JA. (2009) Construct validity of the Iowa Gambling Task. *Neuropsychology review* 19, 102-114

21. Busemeyer J and Stout J. (2002) A contribution of cognitive decision models to clinical assessment: decomposing of the Bechara Gambling Task. *Psychological assessment* 14(3): 253-262

22. Carluccio A, Sundaram NK, Chablani S, et al. (2015) Predictors of quality of life in 102 patients with treated Cushing's disease. *Clinical Endocrinology* 82: 404–411

23. Chen YF, Li YF, Chen X, et al. (2013) Neuropsychiatric disorders and cognitive dysfunction in patients with Cushing's disease. *Chin Med J* 126: 3156-3160

24. Clark L, Bechara A, Damasio H, et al. (2008) Differential effects of insular and ventromedial prefrontal cortex lesions on risky decision-making. *Brain* 131, 1311-1322

25. Coates JM and Herbert J. (2008) Endogenous steroids and financial risk taking on a London trading floor. *Proceedings of the National Academy of Science of the USA* 105: 6167-6172

26. Crespo I and Webb SM. (2014) Perception of health and cognitive dysfunction in acromegaly patients. *Endocrine* 46: 365-367

27. Cummings J and Mega M. (2003) Neuropsychiatry and behavioral neuroscience. New York: *Oxford University Press*.

28. Dale AM, Fischl B, Sereno MI. (1999) Cortical surface-based analysis: I. Segmentation and surface reconstruction. *Neuroimage* 9: 179–194

29. Davidson RJ and Irwin W. (1999) The functional neuroanatomy of emotion and affective style. *Trends in Cognitive Science* 3, 11-21

30. Deak F and Sonntag WE. (2012) Aging, synaptic dysfunction, and insulin-like growth factor (IGF)-1. *Journals of gerontology series A: Biological Sciences and Medical Sciences* 67A: 611-625

31. De Kloet R, Joëls M and Holsboer F. (2005) Stress and the brain: from adaptation to disease. *Nature Review Neuroscience* 6: 463-475

32. Devinsky O, Morrell MJ and Vogt BA. (1995) Contributions of anterior cingulated cortex to behavior. *Brain* 118: 279-306

33. Diamond DM, Campbell AM, Park CR, et al. (2007) The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-dodson law. *Neural Plasticity*, 0-33

34. Dimopoulou C, Ising M, Pfister H, et al. (2013) Increased Prevalence of Anxiety-Associated Personality Traits in Patients with Cushing's Disease: A Cross-Sectional Study. *Neuroendocrinology* 97: 139-145

35. Dorn LD, Burgess ES, Friedman TC, et al. (1997) The longitudinal course of psychopathology in Cushing's syndrome after correction of hypercortisolism. *Journal of Clinical Endocrinology and Metabolism* 82: 912–919

36. Fernández S, Fernández AM, López-López C, et al. (2007) Emerging roles of insulin-like growth factor-I in the adult brain. *Growth hormone & IGF research* 17: 89-95

37. Fischl B and Dale AM. (2000) Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proceedings of the National Academy of Science of the USA* 97: 11050–11055

38. Forget H, Lacroix A, Cohen H. (2002) Cognitive decline in patients with Cushing's syndrome. *Psychoneuroendocrinology* 6: 20-29

39. Geraedts VJ, Dimopoulou C, Auer M et al. (2015) Health outcomes in acromegaly: depression and anxiety are promising targets for improving reduced quality of life. *Frontiers in Endocrinology* 5: 229 [online]

40. Gotch PM. (1994) Cushing's syndrome from the patient's perspective. *Endocrinology and Metabolism Clinics of North America* 23: 607-617

41. Grachev ID and Apkarian AV. (2000) Anxiety in healthy humans is associated with orbital frontal chemistry. *Molecular Psychiatry* 5, 482-488

42. Gupta, R., Duff, M.C., Denburg, N.L., et al. (2009) Declarative memory is critical for sustained advantageous complex decision-making. *Neuropsychologia*, **47**, 1686-1693

43. Joëls M, Karst H, Krugers HJ et al. (2007) Chronic stress: implications for neuronal morphology, function and neurogenesis. *Frontiers in Neuroendocrinology* 28, 72-96

44. Khiat A, Bard C, Lacroix A, et al. (1999) Brain metabolic alterations in Cushing's syndrome as monitored by proton magnetic resonance spectroscopy. *NRM in Biomedicine* 12, 357-363

45. Kirsch M and Windmann S. (2009) The role of anxiety in decision making. *Review of Psychology* 16: 19-28

46. Lancaster JL, Woldorff MG, Parsons LM, et al. (2000) Automated Talairach atlas labels for functional brain mapping. *Human Brain Mapping* 10: 120–131

47. Le Berre AP, Rauchs G, La Joie R, et al. (2014) Impaired decisión-making and brain shrinkage in alcoholism. *European Psychiatry* 29, 125-133

48. León-Carrión J, Martín-Rodríguez JF, Madrazo-Atutxa A et al. (2010) Evidence of cognitive and neurophysiological impairtment in patients with untreated naïve acromegaly. *Journal of Clinical Endocrinology and Metabolism* 95: 4367-4379

49. Li X, Lu ZL, D'Argembeau A, et al. (2010) The Iowa gambling task in fMRI images. *Human Brain Mapping* 31: 410-423

50. Lin CH, Chiu YC, Cheng CM, et al. (2008) Brain maps of Iowa gambling task. *BMC Neuroscience* 9: 72-87

51. Lindsay JR, Nansel T, Baid S, et al. (2006) Long-term impaired quality of life in Cushing's syndrome despite initial improvement after surgical remission. *Journal of Clinical Endocrinology and Metabolism* 91: 447–453

52. Martín-Rodríguez JF, Madrazo-Atutxa A, Venegas-Moreno E et al. (2013) Neurocognitive function in acromegaly after surgical resection of GH-secreting adenoma versus naïve acromegaly. *PLoS One* 8: e60041 [online]

53. Martínez-Selva JM, Sánchez-Navarro JP, Bechara A et al. (2006) Mecanismos cerebrales de la toma de decisiones. *Revista de Neurología* 42(7): 411-418

54. Matute C, Domercq M, Sánchez-Gómez MV. (2006) Glutamate-mediated glial injury: mechanisms and clinical importance. *Glia* 53, 212-224

55. Marzi T, Regina A and Righi S. (2014) Emotions shape memory suppression in trait anxiety. *Frontiers in Psychology* 4: 1001 [online]

56. Mc Ewen BS. (2005) Glucocorticoids, depression and mood disorders: structural remodelling in the brain. *Metabolism* 54, 20-23

57. Melmed S, Colao A, Barkan A, et al. (2009) Guidelines for acromegaly management: An update. *Journal of Clinical Endocrinolgy and Metabolism* 94: 1509-1517

58. Messier C and Teutenberg K. (2005) The role of insulin, insulin growth factor, and insulin-degrading enzyme in brain aging and Alzheimer's disease. *Neural Plasticity* 12: 311-328

59. Michaud K, Forget H, Cohen H. (2009) Chronic glucocorticoid hypersecretion in Cushing's syndrome exacerbates cognitive aging. *Brain and Cognition*, 71: 1-8

60. Milian M, Teufel P, Honegger J, et al. (2012) The development of the Tuebingen Cushing's disease quality of life inventory (Tuebingen CD-25). Part II: normative data from 1784 healthy people. *Clinical Endocrinology* 76: 861–867

61. Milian M, Honegger J, Gerlach C, et al. (2014) Similar psychopathological profiles in female and male Cushing's disease patients after treatment but differences in the pathogenesis of symptoms. *Neuroendocrinology* 100(1): 9-16

62. Miller EK, Freedman DJ and Wallis JD. (2002) The prefrontal cortex: categories, concepts and cognition. *Philosophical Transactions of the Royal Society of London B* 357: 1123-1136

63. Miu AC, Heilman RM and Houser D. (2008) Anxiety impairs decision-making: psychophysiological evidence from an Iowa gambling task. *Biological Psychology* 77: 353-358

64. Murphy F, Rubinsztein J, Michael A, et al. (2001) Decision-making cognition in mania and depression. *Psychological medicine* 31: 679-693

65. Must A, Szabó Z, Bódi N, et al. (2006) Sensitivity to reward and punishment and the prefrontal cortex in major depression. *Journal of Affective Disorders* 90, 209-215

66. Nieman LK. (2015) Cushing's syndrome: update on signs, symptoms and biochemichal screening. *European Journal of Endocrinology* 173: M33-8

67. Papakokkinou E, Johansson B, Berglund P, et al. (2015) Mental Fatigue and Executive Dysfunction in Patients with Cushing's Syndrome in Remission. Behavioural Neurology 1: 1-6 [online]

68. Patil CG, Lad SP, Katznelson L, et al. (2007) Brain atrophy and cognitive deficits in Cushing's disease. *Neurosurgery Focus* 23, E11

69. Paulus MP and Yu AJ. (2012) Emotions and decision-making: affects-driven belief systems in anxiety and depression. *Trends in Cognitive Science* 16: 476-483

70. Pereira AM, Tiemensma J, Romijn JA. (2010) Neuropsychiatric disorders in Cushing's syndrome. *Neuroendocrinology* 92 (Suppl 1): 65–70

71. Pereira AM, Tiemensma J, Romijn JA et al. (2012) Cognitive impairment and psychopathology in patients with pituitary diseases. The *Netherlands Journal of Medicine* 70: 255-260

72. Pessoa L. (2008) On the relationship between emotion and cognition. *Nature Reviews Neuroscience* 9, 148-158

73. Pires P, Santos A, Vives-Gilabert Y et al. (2015) White matter alterations in the brains of patients with active, remitted and cured Cushing syndrome: A DTI Study. *American Journal of Neuroradiology* 36(6): 1043–1048

74. Pivonello R, Simeoli C, De Martino MC et al. (2015) Neuropsychiatric disorders in Cushing's syndrome. *Frontiers in Neuroscience* 9: 129 [online]

75. Popoli M, Yan Z, McEwen B, Sanacora G. (2013) The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nature Reviews Neuroscience* 13, 22-37

76. Popovic V, Pekic S, Pavlovic D, et al. (2004) Hypopituitarism as a consequence of traumatic brain injury (TBI) and its possible relation with cognitive disabilities and mental distress. *Journal of Endocrinological Investigation* 27: 1048-1054

77. Portella MJ, de Diego-Adeliño J, Gómez-Ansón B, et al. (2011) Ventromedial prefrontal spectroscopic abnormalities over the course of depression: A comparison among first episode, remitted recurrent and chronic patients. *Journal of Psychiatric Research* 45, 427-434

78. Putman P, Antypa N, Crysovergi P, et al. (2010) Exogenous cortisol acutely influences motivated decision making in healthy young men. *Psychopharmacology* 208: 257-263

79. Radley JJ, Rocher AB, Miller M, et al. (2006) Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. *Cerebral Cortex* 16, 313-320

80. Radley JJ and Sawchenko PE. (2011) A common substrate for prefrontal and hippocampal inhibition of the neuroendocrine stress response. *The Journal of Neuroscience* 31: 9683-9695

81. Ragnarsson O and Johannsson G. (2013) Cushing's syndrome: a structured short- and long-term management plan for patients in remission. *European Journal of Endocrinology* 169: R139-152

82. Ragnarsson O, Glad CAM, Berglund P, et al. (2014) Common genetic variants in the glucocorticoid receptor and the 11β-hydroxysteroid dehydrogenase type 1 genes influence long-term cognitive impairments in patients with Cushing's syndrome in remission. *Journal of Clinical Endocrinology & Metabolism* 99(9):E1803–1807

83. Ranganath A and Jacob SN. (2015) Doping the mind: Dopaminergic modulation of prefrontal cortical cognition. *The Neuroscientist* (Sep 3) [Epub ahead of print]

84. Resmini E, Santos A, Gómez-Anson B et al. (2012) Verbal and visual memory performance and hippocampal volumes, measured by 3-tesla magnetic resonance imaging, in patients with Cushing's syndrome. *Journal of Clinical Endocrinology & Metabolism* 97: 663-671

85. Resmini E, Santos A, Gómez-Ansón B et al. (2013) Hippocampal dysfunction in cured Cushing's syndrome patients, detected by H-MR-spectroscopy. *Clinical Endocrinology* 79: 700-707

86. Risold PY, Thompson RH, Swanson LW. (1997) The structural organization of connections between hypothalamus and cerebral cortex. *Brain Research Reviews* 24: 197-254

87. Robinson OJ, Bond RL and Roiser JP. (2015) The impact of stress on financial decision-making varies as a function of depression and anxiety symptoms. *PeerJ* 3: e770 [online]

88. Roerink SH, Wagenmakers MA, Wessels JF et al. (2014) Persist self-consciousness about facial appearance, measured with Derriford appearance scale 59, in patients after long-term biochemical remission of acromegaly. *Pituitary* 18(3): 366-375

89. Santos-Ruiz A, García-Rios MC, Fernández-Sánchez JC, et al. (2012) Can decision-making skills affect responses to psychological stress in healthy women? *Psychoneuroendocrinology* 37: 1912-1921

90. Santos A, Resmini E, Crespo I et al. (2014) Small cerebellar cortex volume in patients with active Cushing's syndrome. *European Journal of Endocrinology* 171: 461-469

91. Santos A, Resmini E, Granell E, et al. (2015) Cardiovascular risk and white matter lesions after endocrine control of Cushing's syndrome. *European Journal of Endocrinology* 173: 765-775

92. Segonne F, Dale AM, Busa E, et al. (2004) A hybrid approach to the skull stripping problem in MRI. *Neuroimage* 22: 1060–1075

93. Shafiei N, Gray M, Viau V, et al. (2012) Acute stress induces selective alterations in cost/benefit decision-making. *Neuropsychopharmacology* 37, 2194-2209

94. Siegel S, Streetz-van der Werf C, Schott JS et al. (2013) Diagnostic delay is associated with psychosocial impairments in acromegaly. *Pituitary* 16: 507-514

95. Sievers C, Ising M, Pfister H et al. (2009a) Personality in patients with pituitary adenomas is characterized by increased anxiety related traits: comparison of 70 Acromegalic patients to patients with non-functioning pituitary adenomas and age and gender matched controls. *European Journal of Endocrinology* 160: 367-73

96. Sievers C, Dimopoulou C, Pfister H et al. (2009b) Prevalence of mental disorders in acromegaly: a cross-sectional study in acromegalic patients. *Clinical Endocrinol*ogy 71: 691-701

97. Sievers C, Sämann PG, Dose T, et al. (2009c) Macroscopic brain architecture changes and white matter pathology in acromegaly: a clinicoradiological study. *Pituitary* 12: 177-185

98. Sievers C, Samann PG, Pfister H et al. (2012) Cognitive function in acromegaly: description and brain volumetric correlates. *Pituitary* 15: 350-357

99. Sonino N and Fava GA. (2001) Psychiatric disorders associated with Cushing's syndrome. Epidemiology, pathophysiology and treatment. *CNS Drugs* 15(5): 361–373

100. Starkman MN, Gebarski SS, Berent S, et al. (1992) Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. *Biological Psychiatry* 32: 756-765

101. Suhr JA and Tsanadis J. (2007) Affect and personality correlates of the Iowa Gambling Task. *Personality and individual differences* 43, 27-36

102. Tanriverdi F, Yapislar H, Karaca Z et al. (2009) Evaluation of cognitive performance by using P300 auditory event related potentials (ERPs) in patients with growth hormone (GH) deficiency and acromegaly. *Growth Hormone & IGF-1 Research* 19: 24-30

103. Tiemensma J, Kokshoorn NE, Biermasz NR et al. (2010a) Subtle cognitive impairment in patients with long-term cure of Cushing's disease. *Journal of Clinical Endocrinology & Metabolism,* 95, 1-16

104. Tiemensma J, Biermasz NR, Middelkoop HAM et al. (2010b) Increased prevalence of psychopathology and maladaptive personality traits after long-term cure of Cushing's disease. *Journal of Clinical Endocrinology and Metabolism* 95(10):E129-141

105. Tiemensma J, Biermasz NR, van der Mast RC et al. (2010c) Increased psychopathology and maladaptative personality traits, but normal cognitive functioning, in patients after long-term cure of acromegaly. *Journal of Clinical Endocrinology and Metabolism* 95: E392-E402

106. Tiemensma J, Kaptein AA, Pereira AM et al. (2011a) Affected illness perceptions and the association with impaired quality of life in patients with long-term remission of acromegaly. *Journal of Clinical Endocrinology and Metabolism* 96: 3550-3558

107. Tiemensma J, Kaptein AA, Pereira AM, et al. (2011b) Coping strategies in patients after treatment for functioning or non-functioning pituitary adenomas. *Journal of Clinical Endocrinology & Metabolism* 96, 964-971

108. Toffanin T, Nifosi F, Follador H, et al. (2011) Volumetric MRI analysis of hippocampal subregions in Cushing's disease: A model for glucocorticoids neural modulation. *European Psychiatry* 26, 64-67

109. Toplak ME, Sorge GB, Benoit A, et al. (2010) Decision-making and cognitive abilities: A review of associations between Iowa Gambling Task performance, executive functions and intelligence. *Clinical Psychology Review* 30, 562-581

110. Van Aken MO, Pereira AM, Biermasz NR, et al. (2005) Quality of life in patients after long-term biochemical cure of Cushing's disease. *Journal of Clinical Endocrinolgy and Metabolism* 90: 3279-3286

111. Van der Werff SJA, Pannekoek JN, Andela CD, et al. (2015) Resting-state functional connectivity in patients with long-term remission of Cushing's disease. *Neuropsychopharmacology* 40(8): 1888–1898

112. Wagenmakers MA, Netea-Maier RT, Prins JB, et al. (2012) Impaired quality of life in patients in long-term remission of Cushing's syndrome of both adrenal and pituitary origin: a remaining effect of long-standing hypercortisolism? *European Journal of Endocrinology* 167: 687–695

113. Wellman CL. (2001) Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. *Journal of Neurobiology* 49, 245-253

114. Whitney P and Hinson JM. (2012) The role of source memory in gambling task decision making. *Journal of clinical and experimental neuropsychology* 34, 826-835

115. Williams LM and Gordon E. (2007) Dynamic organization of emotional brain: resposivity, stability and instability. *The Neuroscientist* 13(4): 349-370

116. Yedinak CG and Fleseriu M. (2014) Self-perception of cognitive function among patients with active acromegaly, controlled acromegaly, and non-functional pituitary adenoma: a pilot study. *Endocrine* 46: 585-593

117. Zeeb FD, Robbins TW and Winstanley CA. (2009) Serotonergic and dopaminergic modulation of gambling behaviour as assessed using a novel rat gambling task. *Neuropsychopharmacology* 34: 2329-2343

Annex I

1. Tables

Table 1. Clinical characteristics of CS patients and controls.

	Cushing patients (n=35)		Healthy controls (n=35)
	Cured (n=27)	**Medically treated (n=8)**	
Age	44.5±10	41.4±12.3	44.4±9.5
Gender (female/male)	22/5	8/0	30/5
Years of education	13.2±3.2	13.4±3.1	13.4±3.1
Origin of CS:			_
· Pituitary adenoma	24	4	
· Adrenal adenoma	3	3	
· Ectopic ACTH secretion	0	1	
Duration of prior hypercortisolism (months)	57.6±34.5	46.5±32.5	_
Radiotherapy	6/27	2/8	_
Duration of eucortisolism (months)	41 (6-288)	2 (1-4)	_
Hydrocortisone substitution	8/27	0/8	_

Table 2. Clinical characteristics of acromegalic patients and controls.

	Acromegalic patients (n=31)	**Healthy controls** (n=31)
Gender (male/female)	17/14	17/14
Age (years)	49.5±8.5	49±7.5
Years of education	12.3±3.8	12.84±3.5
Duration of disease control (months)	61(4-300)	-
Medical treatment:	n=10	
- Somatostatin analogue therapy	10/10 (32%)	
- Pegvisomant therapy	2/10 (6.5%)	
Postoperative radiotherapy	10/31 (32%)	
Hypopituitarism:	n=9 (29%)	
- ACTH deficiency	6/9	
- Gonadotrophins deficiency	5/9	
- TSH deficiency	7/9	
- ADH deficiency	1/9	

Table 3. Mean and SD of evaluated tests in acromegalic patients and controls.

	Acromegalic patients	Healthy controls	p-value
Iowa Gambling Task:			
Safer card choices	51.2±15.3	60.6±13.6	<0.05
Riskier card choices	48.8±15.3	39.3±13.6	<0.05
Block 1 score	-3.7±5.8	1.2±7.5	NS
Block 2 score	1.5±6	3.9±8.5	NS
Block 3 score	1.7±9.1	5±8	NS
Block 4 score	1.2±10.9	5.6±8	NS
Block 5 score	1.8±9.7	5.4±10.4	NS
Rey Auditory Learning Verbal Test:			
***Short-term memory**:*			NS
Score of trial 1	5.5±1.7	5.8±1.3	
Score of trial 5	11±2.4	12±2.3	
Score of interference trial	4.7±1.3	5.3±2.2	
Long-term memory:			<0.01
Score of recall trial	8.8±2.4	10.4±2.6	<0.05
Score of recognition trial A	11.7±2.3	13.5±1.5	<0.01
Score of recognition trial B	7.8±3.1	9.1±2.9	0.088
Beck Depression Inventory-II:			
Total score	9(0-27)	1(0-17)	<0.001
Affective dimension score	3(0-18)	1(0-12)	<0.001
Somatic dimension score	5(0-11)	1(0-5)	<0.001
State-Trait Anxiety Inventory:			
State anxiety score	16.6±11.4	9.5±6	<0.01
Trait anxiety score	20.3±10.2	11.5±5.6	<0.001

Table 4. Correlations between tests of cognitive functions (Iowa Gambling Task -IGT- and Rey Auditory Learning Verbal Test -RALVT-) and questionnaires of depression and anxiety (Beck Depression Inventory-II -BDI II- and State Trait Anxiety Inventory -STAI-).

*p<0.05

**p<0.01

	BDI-II **Total**	BDI-II **Affective dimension**	BDI-II **Somatic dimension**	STAI **State anxiety**	STAI **Trait anxiety**
RALVT:					
Short-term memory:					
Trial 1	NS	NS	NS	NS	NS
Trial 5	NS	r=-0.33**	NS	r=-0.26*	r=-0.28*
Interference trial	r=-0.33**	r=-0.34**	r=-0.35**	r=-0.26*	r=-0.34*
Long-term memory:					
Recall trial	r=-0.28*	r=-0.28*	r=-0.28*	r=-0.33*	r=-0.32*
Recognition trial A	r=-0.33**	r=-0.26*	r=-0.36**	r=-0.35**	r=-0.35**
Recognition trial B	NS	NS	NS	NS	NS
IGT:					
Safer card choices	NS	r=-0.28*	NS	NS	NS
Riskier card choices	NS	r=-0.28*	NS	NS	NS
Block 1	NS	NS	NS	NS	NS
Block 2	NS	NS	NS	NS	NS
Block 3	NS	NS	NS	r=-0.33**	NS
Block 4	NS	NS	NS	r=-0.36*	NS
Block 5	NS	NS	NS	NS	NS

2. Figures

Figure 1. Total score of IGT for all single CS patients. No differences were observed between medically treated CS and in remission CS

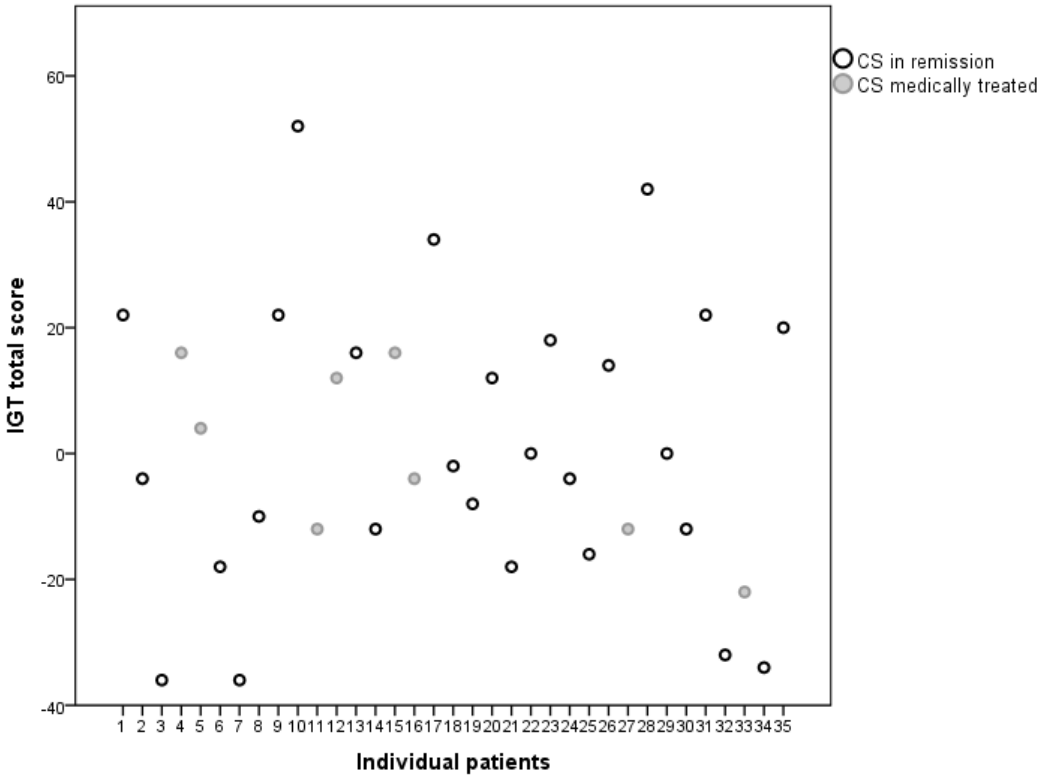


Figure 2. Number of riskier or safer cards chosen in the IGT by CS patients and controls. * = p<0.05

IGT= Iowa gambling task

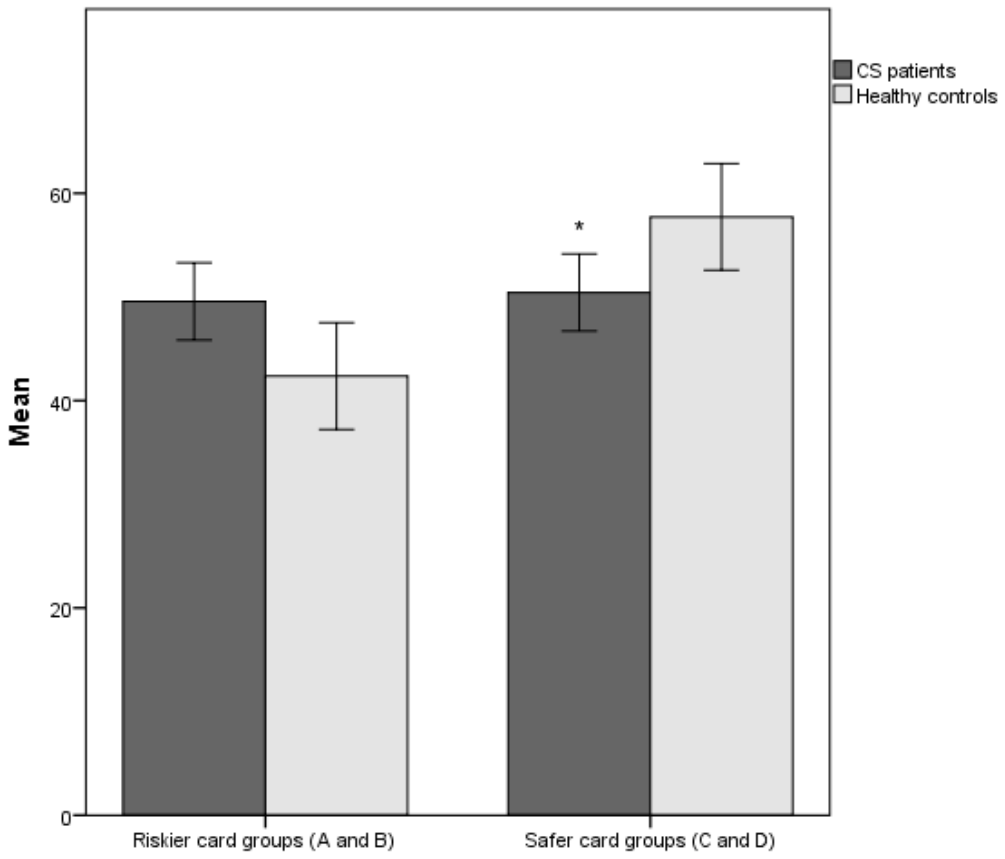


Figure 3. IGT learning curves in CS patients and healthy controls.

The learning curves consist of the score (the number of advantageous selections minus the number of disadvantageous selections) within each block of cards of the IGT (Iowa gambling task)

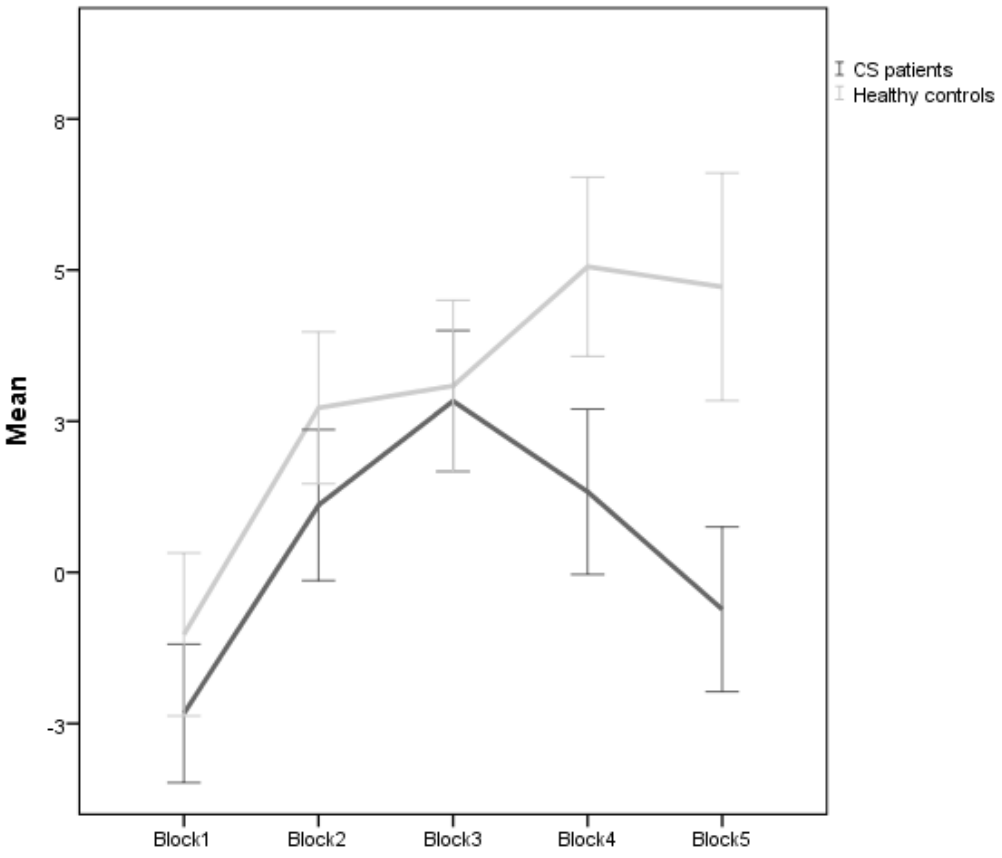


Figure 4. Cortical thinning in CS patients when compared to controls. Areas of significant thinning (whole brain analysis, uncorrected) appear black.

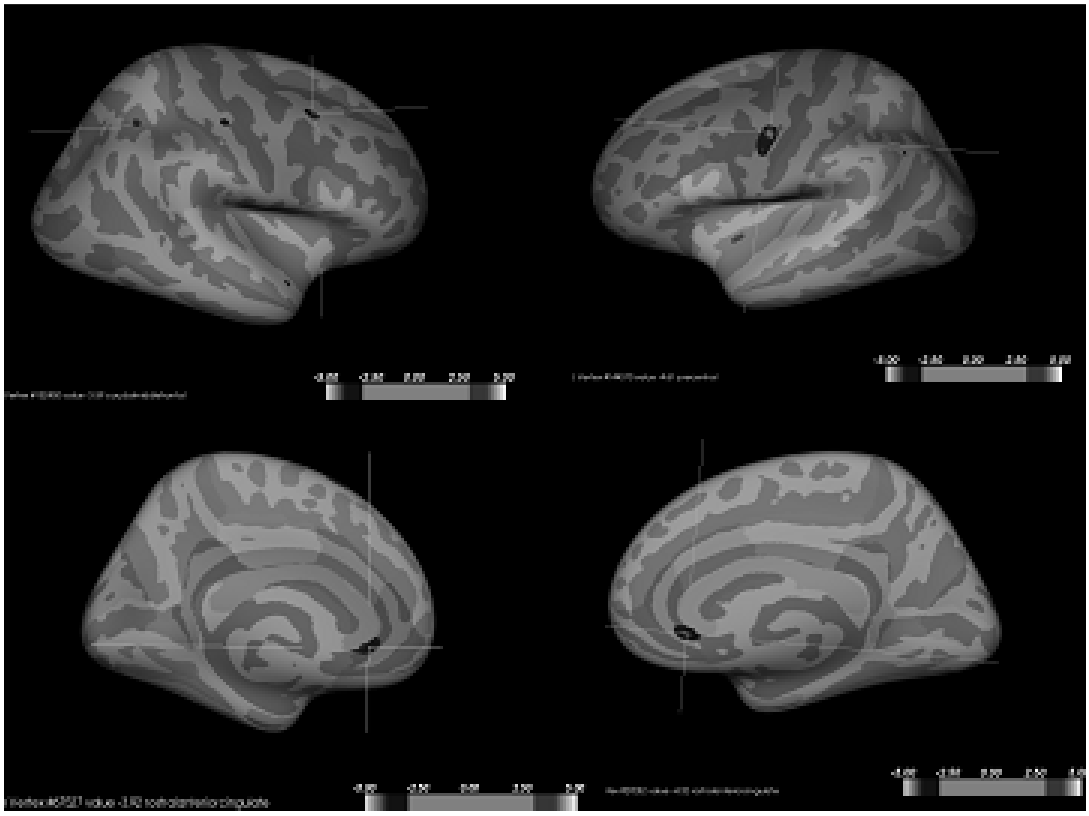


Figure 5. Number of riskier or safer cards chosen in the Iowa Gambling Task (IGT) by acromegalic patients and controls. * = p<0.05

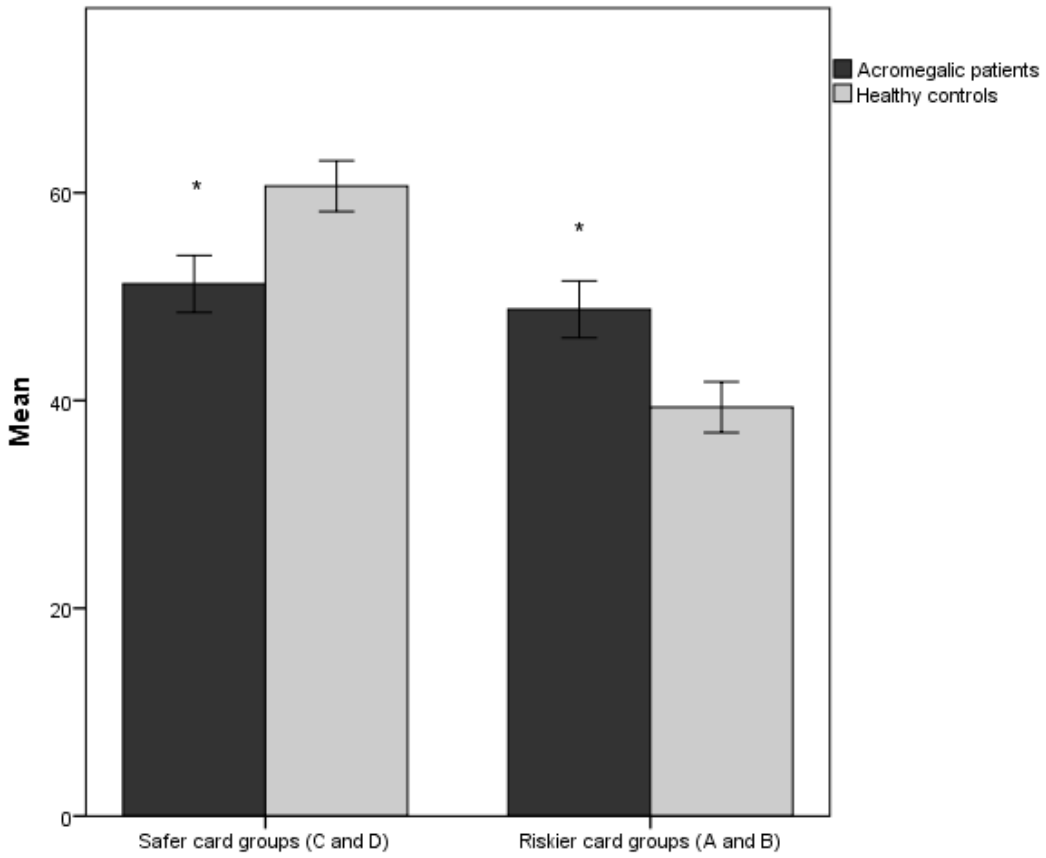


Figure 6. IGT learning curves in acromegalic patients and healthy controls. Even though the performance was different between patients and controls (repeated measures ANOVA, $F=6.493$, $p<0.05$), no differences were observed between any of the 5 blocks of both groups.

The learning curves consist of the score (the number of advantageous selections minus the number of disadvantageous selections) within each block of cards of the IGT (Iowa gambling task)

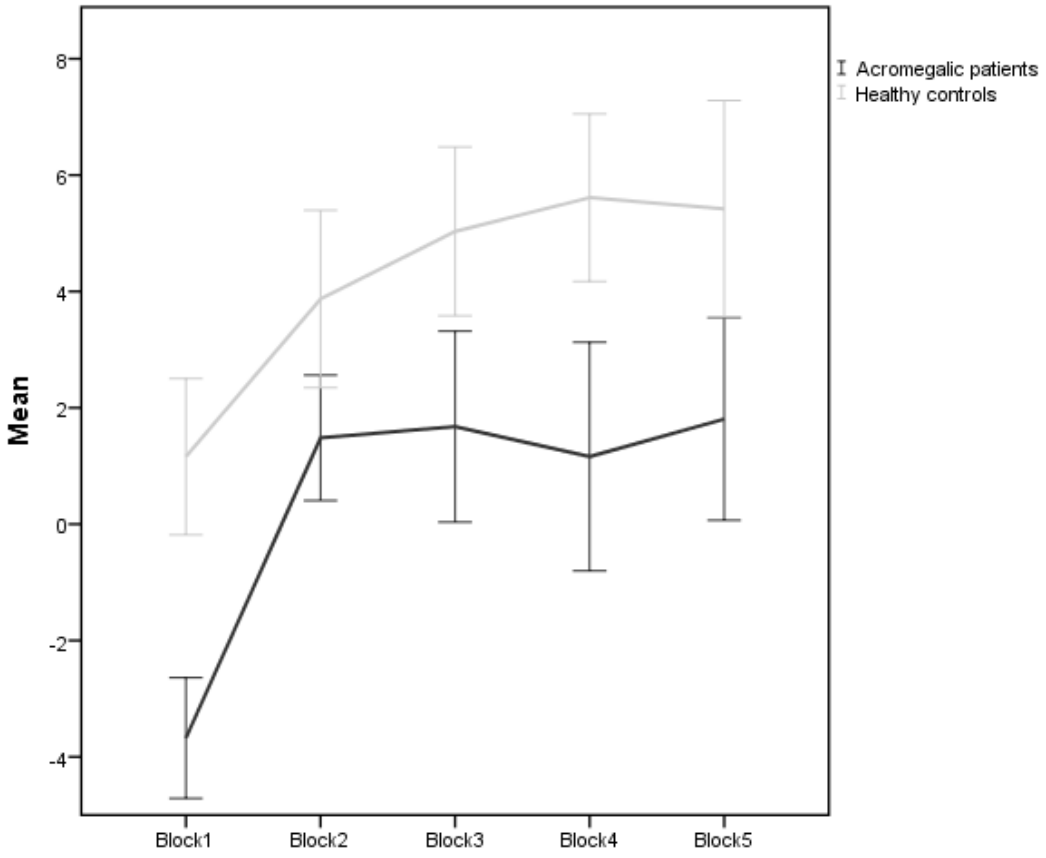
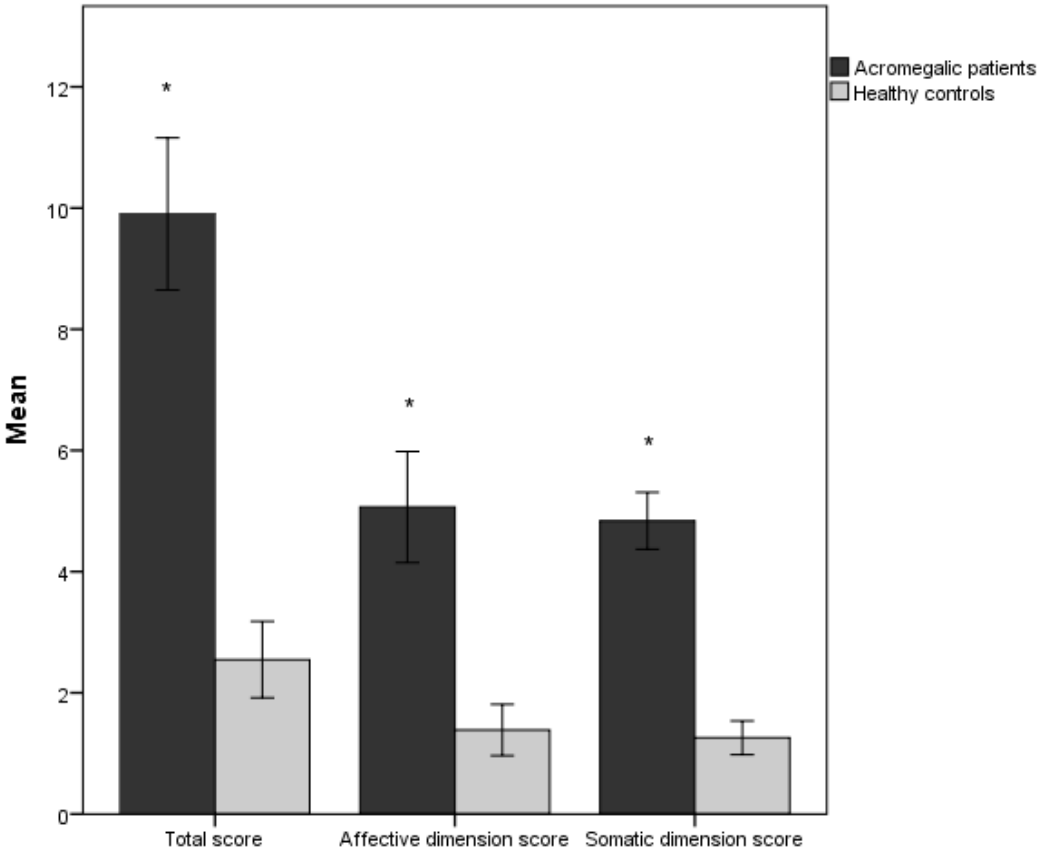


Figure 7. Total score, affective dimension score and somatic dimension score in the Beck Depression Inventory-II (BDI-II) in acromegalic patients and controls. * = p<0.05



Annex II

BRAIN METABOLITE ABNORMALITIES IN VENTROMEDIAL PREFRONTAL CORTEX ARE RELATED TO DURATION OF HYPERCORTISOLISM AND ANXIETY IN PATIENTS WITH CUSHING'S SYNDROME

Iris Crespo[1], Alicia Santos[1], Beatriz Gómez-Ansón[2], Olga López-Mourelo[3], Patricia Pires[4], Yolanda Vives-Gilabert[4], Susan M. Webb[1], Eugenia Resmini[1]

[1]Endocrinology/Medicine Departments, Hospital Sant Pau, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER, Unidad 747), IIB-Sant Pau, ISCIII and Universitat Autònoma de Barcelona (UAB), Spain

[2]Neuroradiology Unit, Hospital de Sant Pau, and IIB-Sant Pau, UAB, Barcelona, Spain

[3]Parc de Recerca-UAB, Barcelona, Spain

[4]INNDACYT, Avda. Europa, 20, Planta Baja Puerta D 08907, Hospitalet de Llobregat, Spain

Short title: Abnormal prefrontal metabolites in Cushing's syndrome

Word count: Abstract= 235; Text= 3222

Number of references= 46; Table= 2; Figures= 3

Corresponding author:

Eugenia Resmini, MD, PhD

Endocrinology Department

Hospital de Sant Pau,

C/Sant Antoni Maria Claret n.167

08025 Barcelona, Spain

E-mail: EResmini@santpau.cat

Abstract

Purpose: Chronic exposure to excessive glucocorticoid (GC) concentration in Cushing's syndrome (CS) can affect the brain structurally and functionally; ventromedial prefrontal cortex (vmPFC) is rich in GC receptors and therefore particularly vulnerable to excessive GC concentration. Proton magnetic resonance spectroscopy (^{1}H-MRS) is a sensitive, non-invasive imaging technique that provides information on brain metabolites *in vivo.* Our aim was to investigate metabolite concentrations in vmPFC of CS patients and their relationship with clinical outcome.

Methods: Twenty-two right-handed CS patients (7 active/15 in remission, 19 females, 41.6±12.3 years) and 22 right-handed healthy controls (14 females, 41.7±11 years) underwent brain MRI and ^{1}H-MRS exams at 3 Tesla. Concentrations of glutamate (Glu), glutamate + glutamine (Glx), creatine (Cr), N-Acetyl-aspartate (NAA), N-Acetyl-aspartate + N-acetylaspartylglutamate (total NAA), choline-containing compounds (Cho) and myoinositol (MI) were determined. Moreover, anxiety and depressive symptoms were evaluated with the State-Trait Anxiety Inventory (STAI) and the Beck Depression Inventory-II (BDI-II) test, respectively.

Results: CS patients had lower concentrations of glutamate and total NAA in the vmPFC than healthy controls (8.6±1.2 vs. 9.3±0.7 mmol/L, and 6.4±0.8 vs. 6.8±0.4 mmol/L, respectively; $p<0.05$). Duration of hypercortisolism was negatively correlated with total NAA ($r=-0.488$, $p<0.05$). Moreover, the concentration of total NAA was negatively correlated with anxiety state ($r=-0.359$, $p<0.05$).

Conclusions: Brain metabolites are abnormal in the vmPFC of patients with CS. Decreased total NAA and glutamate concentrations indicate neuronal dysfunction that appear to be related with duration of hypercortisolism and anxiety.

Key words: Cushing's syndrome, brain metabolites, anxiety, spectroscopy, ventromedial prefrontal cortex.

Introduction

Exposure to excessive glucocorticoid (GC) concentration in Cushing's syndrome (CS) can have deleterious effects on the brain. Several studies had described cognitive impairments and reduced brain volume in CS patients even after biochemical cure [1-5]. Thus, excessive GC concentration can provoke neuronal changes that persist (at least partially) following correction of hypercortisolism.

Excessive GC concentration can affect the brain at the cellular, structural and functional level [4]. For example, studies focusing on the hippocampus (a major target region for GC action) have described abnormal brain metabolite concentrations, volume reduction and memory impairments in CS patients [2,7-9]. Other structures strongly connected to the hippocampus, as the ventromedial prefrontal cortex (vmPFC) may be also affected by excessive GC concentration. The vmPFC is rich in GC receptors, thus, being potentially exposed to the neurotoxic effects of hypercortisolism. Previous studies have observed a selective cortical thinning in frontal subregions close to the vmPFC, as well as hypoactivation of this area during an emotional task in patients with CS [10-11]. These persistent structural and functional abnormalities after control of hypercortisolism suggest that excessive GC concentration can impair the vmPFC.

These impairments in the vmPFC could be related to altered metabolite concentrations. There is evidence that some brain regions of CS patients are neuropathologically abnormal related to altered metabolite concentrations. Low levels of N-Acetyl-aspartate (NAA) had been observed in the hippocampus of cured CS patients, indicating neuronal dysfunction, and decreased choline levels had been described in the frontal and thalamic areas in active CS patients as compared with healthy controls [7,12]. These brain metabolite alterations could be considered markers of GC neurotoxicity in CS and could imply changes in brain function and precede volume reduction [7].

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive spectroscopic technique that provides information on brain tissue in vivo by measuring the concentration of several metabolites in specific brain regions (voxels).

The vmPFC plays a critical role in emotional processing and it is known that CS patients show anxiety, depression, affective lability, apathy and irritability [13]. It could be

dysfunction as reflected in abnormal emotional symptoms is related to vmPFC dysfunction and abnormal brain metabolites in this area. In fact, abnormalities of glutamate, choline and NAA levels in the vmPFC have been observed in patients with chronic depression [14].

Studies converge to suggest that glutamate and NAA are the most common metabolites to show abnormalities in prefrontal areas. Glutamate is the most abundant metabolite in the human brain [15-16] and an important excitatory neurotransmitter that can also be a potent neurotoxin if it exceeds normal limits [17]. NAA is a marker of neuronal integrity [18]. No data are available regarding these brain metabolites in the vmPFC of CS patients.

The aim of this study was to investigate whether brain metabolites in the vmPFC differed between CS patients and healthy controls. We hypothesized that exposure to excessive GC concentration would cause metabolite abnormalities in the vmPFC, which could be correlated with emotional symptoms previously observed in CS patients.

Methods

Subjects

This cross-sectional study included 22 right-handed CS patients (7 active and 15 in remission) clinically followed at our institution and 22 right-handed healthy controls. Control subjects were recruited among healthy volunteers who had previously participated in other clinical studies [5, 7, 10]. All subjects gave full informed consent and underwent brain examination by MRI and proton magnetic resonance spectroscopy (1H-MRS) at 3 Tesla.

We studied 19 females and 3 males with CS, with a mean age of 41.6±12.3 years and 13.7±3.2 years of education. In 17 patients, CS was of pituitary origin, and in the remaining 5 it was adrenal.

Fifteen patients were considered in remission after surgery, since adrenal insufficiency was demonstrated or morning cortisol suppression (<50 nmol/l) was observed after 1 mg dexamethasone overnight and repeated 24h urinary free cortisol measures were normal (<280 nmol/L), according to the consensus criteria [19]. Seven patients who did not fulfil these criteria were considered to have active CS.

In the remission group, 3 CS patients were taking hydrocortisone replacement and 4 had received postoperative pituitary radiotherapy (Table 1). The median of duration of remission was 42 (range 6-216) months.

In the active group, 4 patients were on medical therapy with steroid synthesis inhibiting drugs: 2 were taking ketoconazole, 1 was taking metyrapone and 1 was taking both ketoconazole and metyrapone. The remaining 3 were naïve to therapy.

Duration of hypercortisolism was considered as the time from symptom onset until cortisol normalization (remission group) or until the date of enrollment in this study (active group) and was assessed by the endocrinologist in charge. At diagnosis, the duration of hypercortisolism was estimated by personal interview, careful review of medical records and photographs of patients. Mean duration of hypercortisolism was 87±75 months in the active group and 58±27 months in the remission group (Table 1).

In the control group, 14 females and 8 males were studied, their mean age was 41.7±11 years and educational level was 13.8±4 years of education. All healthy controls were free of comorbid disease and of previous or current endocrine diseases.

Exclusion criteria in patients and controls were diabetes mellitus, taking exogenous glucocorticoid medication, growth hormone deficiency, past medical history of head injury, cerebrovascular disease, neurological disorders (like Parkinson's disease or dementia), severe mental illness and a history of drug or alcohol abuse.

MRI scanning technique

Magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (1H-MRS) were performed using a 3 Tesla Philips Achieva instrument (software version 2.1.3.2) and a dedicated acquisition protocol which included a 3D-MPRAGE whole brain MRI sequence (Turbo Field Echo, TR=6.7 ms, TE = 3.1 ms) followed by 1H-MRS (SVS-PRESS, TR = 2000 ms, TE = 38 ms, NSA = 128, VOI = 2 x 2 x 2 cm3, AutoWS-Prescan) from the ventromedial prefrontal region (Figure 1) including the rostral cingulated gyrus and Broadmann's areas 10, 24 and 32 [20].

^{1}H-MRS raw data were exported and postprocessed, a posteriori, using the LC Model software which provides concentrations (mmol/L) of the single metabolite peaks [21]. For the purpose of the current study, we determined and quantified the concentrations of glutamate (Glu), glutamate + glutamine (Glx), creatine (Cr), N-Acetyl-

aspartate (NAA), N-Acetyl-aspartate+N-acetylaspartylglutamate (total NAA), choline-containing compounds (Cho) and Myoinositol (MI).

Glu is the principal excitatory neurotransmitter. It is also a potent neurotoxin that can kill nerve cells when its amount exceeds normal limits; this phenomenon is known as excitotoxicity [17]. The glutamate–glutamine cycle between neuron and glia avoid the glutamate excitotoxicity [17]. NAA is the second most abundant metabolite in the CNS and the most prominent peak of a spectrum obtained with ^{1}H-MRS of the normal brain [22]. The Cho signal corresponds mostly to unbound phosphocholine and glycerophosphocholine, indicators of membrane metabolism. Accordingly, elevated Cho is interpreted as evidence for increased membrane turnover [23]. Cr is a marker of energetic systems and intracellular metabolism being a reservoir for the generation of adenosine triphosphate (ATP). Concentration of Cr is relatively constant and is considered a most stable cerebral metabolite. The peak represents a combination of molecules containing Cr and phosphocreatine [24]. MI is highly concentrated in astrocytes and is considered a glial marker [25]. Elevated MI occurs with increased glial-cell size as found in inflammation, as well as in gliosis, astrocytosis and in Alzheimer's disease [26].

All spectra were evaluated by an experienced observer (BGA), and only good quality spectra were included. Good quality was defined as metabolite concentrations with standard deviations below 10% and when the major metabolite peaks were identifiable.

Questionnaires

State-Trait Anxiety Inventory (STAI) and Beck Depression Inventory-II (BDI-II) tests were used to measure anxiety and depressive symptoms, respectively.

BDI-II has 21 items with a four point scale ranging from 0-3 and total score is the sum of each item-rating (range 0-63). Higher scores indicate more severe depressive symptoms [27].

STAI evaluates two types of anxiety: state anxiety (anxiety related to current events) and trait anxiety (anxiety as a personal characteristic). Each subscale has 20 questions with a four point scale ranging from 0-3 and the total score for each subscale is the

sum of each item-rating (range 0-60). Higher scores indicate higher levels of anxiety [28].

Statistical analysis

Statistical analysis was performed using SPSS 21 software (IBM-SPSS Inc., Chicago, IL, USA). Data distribution was analysed with the Kolmorov-Smirnov test. Quantitative data were expressed as mean±SD and non-quantitative data as median (range). Chi-square was used to compare categorical variables. ANOVA followed by Bonferroni test as a *post hoc* was used for comparisons between three groups and Students t-test when comparing two groups. Adjustment for multiple comparisons was not performed. Bivariate correlations using Pearson coefficient were performed between metabolite concentrations and clinical variables (i.e. duration of hypercortisolism and anxiety). Multiple linear stepwise regression analysis was used to evaluate the role of clinical variables on metabolites concentrations.

Results

Differences of metabolite concentrations between the groups

ANOVA followed by a Bonferroni test showed no group effect on metabolites between active CS patients, CS patients in remission and healthy controls. Since this initial approach showed no differences, a new approach comparing all CS patients with healthy controls was adopted.

CS patients had lower concentrations of glutamate and total NAA in the ventromedial prefrontal cortex than healthy controls (8.6±1.2 vs. 9.3±0.7 mmol/L, and 6.4±0.8 vs. 6.8±0.4 mmol/L, respectively; $p<0.05$). There was also a trend towards lower levels of creatine (Cr) in CS patients, compared to healthy controls (5.6±0.4 vs. 5.9±0.4 mmol/L, $p=0.07$). For all other metabolites, no differences were found between CS patients and healthy controls (Table 2).

Difference of emotional symptoms between the groups

CS patients showed more depressive symptoms than healthy controls (BDI-II: 10.4±6.1 vs. 1.8±1.8, $p<0.001$). Furthermore, CS patients had higher scores of state and trait anxiety compared to controls (20±11 vs. 11.4±7.7; $p<0.05$ and 24.9±11.6 vs. 11.8±7.7;

p<0.01). No differences were observed between active CS patients and CS patients in remission.

Relationship between metabolites and clinical parameters

Duration of hypercortisolism was negatively correlated with total NAA and Cr (r=-0.488 and r=-0.463, respectively; p<0.05 for both) (Figure 2). To avoid a potential confounding effect, gender, radiotherapy and illness state (active or cured) were included in a multiple linear stepwise regression analysis together with duration of hypercortisolism. Only duration of hypercortisolism predicted levels of total NAA (β=-0.488, R^2=0.239, p<0.05) and Cr (β=-0.463, R^2=0.214, p<0.05).

Correlations between age and both glutamate and total NAA were found in healthy controls (r=-0.466 and r=-0.472, respectively; p<0.05), but these correlations were lost in CS patients.

A strongly positive correlation was found between total NAA and both Cr and glutamate (r=0.91 and r=0.617, respectively; p<0.001) in the vmPFC. When total NAA was entered as a dependent variable and Cr, glutamate and duration of hypercortisolism as independent factors in the regression analysis, glutamate and duration of hypercortisolism were still independent predictors of total NAA (β=0.540, R^2=0.527, p<0.01).

Moreover, total NAA was negatively correlated with anxiety state (r=-0.359, p<0.05) (Figure 3), and Cr was negatively correlated with anxiety state, anxiety trait and depressive symptoms (r=-0.443, r=-0.413 and r=-0.358, respectively; p<0.05 for all correlations).

Discussion

Our study has demonstrated for the first time that glutamate and NAA concentration in the vmPFC, measured by proton magnetic resonance spectroscopy (^{1}H-MRS), are significantly reduced in CS patients. Our data also shown that total NAA is negatively correlated with duration of hypercortisolism and anxiety, suggesting that long-term exposure to glucocorticoid (GC) excess may induce metabolite alterations in the vmPFC, related with anxiety states.

The alteration of these metabolite concentrations could be considered markers of neuronal dysfunction. Total NAA is a marker of neuronal density, integrity and viability

[16]; therefore, a decrease of total NAA may reflect diminished neuronal integrity and/or neuronal loss in CS patients. Reduction of NAA levels were observed in both hippocampi of patients with CS despite endocrine cure and the current data confirm the same reduction of total NAA in the vmPFC, indicating impaired neuronal integrity in CS patients [7].

On the other hand, glutamate is an important excitatory neurotransmitter that contributes to brain functions and neuronal plasticity [15]. Our data revealed lower levels of glutamate in CS patients as compared to healthy controls, indicating poorer excitatory neurotransmission in the vmPFC of these patients. These findings are in line with recent published data using functional MRI in CS patients that showed hypoactivation of vmPFC in response to emotional stimuli [11]. Therefore, we suggest that hypoactivation of vmPFC in CS patients could be related to down-regulation of the glutamatergic system and reduced neuronal integrity.

It is known that the prefrontal cortex is sensitive to excessive GC concentration [29]. Simplification of dendrites, reduction of spine synapse density, loss of glutamate receptors and diminished glutamatergic neurotransmission in vmPFC has been described in rats after chronic stress [30]. These changes seem to be a compensatory response to elevated synaptic glutamate activity after initial response to acute stress and have been associated with decreased transmission efficiency and impaired synaptic plasticity [30]. Indeed, if the amount of glutamate exceeds normal limits it can kill nerve cells, a phenomenon known as excitotoxicity [17]. For this reason there is a glutamate–glutamine cycle, where glutamate released at nerve terminals is taken up in white matter by astrocytes and converted into glutamine; the glutamine is shuttled back to neurons and converted into glutamate. All these physiological mechanisms to avoid the excessive glutamate signaling after GC overexposure could explain the reduced levels of glutamate and total NAA observed in CS patients.

Regarding creatine (Cr), it is a marker of energetic systems and intracellular metabolism. Concentration of Cr is relatively constant; in fact, it is considered a most stable brain metabolite [24]. We have found a non-significant trend in Cr levels to be lower in CS patients than controls. Reductions in the Cr concentrations are associated with a disruption of brain energy production and dearth of ATP and are common after periods of intense neuronal activity [31]. We speculate that long-term exposure to

excessive GC concentration could cause overactivity and hypermetabolism of the vmPFC and consequently overconsumption of Cr; thereafter, a decrease of Cr may render CS patients susceptible to emotional alterations. This idea is congruent with the inverse relationship observed between Cr concentration and emotional variables, both anxiety and depressive symptoms. Thus, a subtly decreased creatine concentration, which suggests low energy metabolism in vmPFC neurons, could contribute to the emotional state in CS patients, as occurs in patients with other affective disorders (i.e. social anxiety and bipolar disorder) [31-32]. In fact, a previous study had reported that creatine supplementation can have a beneficial effect in the treatment of emotional symptoms of patients with resistant depression [33].

Alterations in brain metabolite concentrations due to exposure to elevated levels of GC have previously been observed [12, 34]. Khiat et al. (2001) showed that patients treated with exogenous GC therapy for many years (range of 2-22 years) had decreased choline level in the thalamic area [34], analogous to that observed in patients with active CS [12]. However, the reduction of choline levels in thalamic and frontal regions was recovered after correction of hypercortisolism [35]. No differences were found between CS patients and controls in the choline concentrations of regions in the vmPFC neither of the hippocampus [7]. However, comparisons between the present study and the previous studies are difficult. The brain areas included were different and they reported metabolite ratios instead of concentrations, using a 1.5-Tesla MRI.

Direct effects of age on different metabolites in healthy human brain have been demonstrated [36-37]. In this line, correlations between age and both glutamate and total NAA were found in healthy controls but not in CS patients. Exposure to GC hypersecretion has been shown to have “ageing-like” effects on brain function [37-40], and we hypothesize that the relationship between metabolite concentration and age is lost in CS patients due to premature ageing of the brain. It is known that long-term exposure to GC hypersecretion stimulates oxidative stress (an important ageing mechanism), mainly in the neurons, and can increase the vulnerability of the brain to other insults [38].

Interestingly, duration of hypercortisolism correlated with concentrations of creatine and total NAA. Indeed, after adjusting for gender, radiotherapy and illness state,

duration of hypercortisolism was the strongest negative predictor of creatine and total NAA, suggesting that long-term excessive GC concentration determines brain metabolite impairments in the vmPFC area. Published data have indicated that, contrary to long-term effects, short-term GC exposure does not lead to changes in cerebral metabolites [41]. Therefore, the duration of exposure to GC hypersecretion is the key to recover neuronal and brain functioning, especially in the PFC cortex, because once neuronal alterations and structural changes are established, recovery is unlikely [42]. In extreme cases of posttraumatic stress disorder, for example, altered PFC function did not return to normal after long-term exposure to stress [43]. This can explain why there are no differences between patients with active CS and patients in remission; the previous long-term exposure to hypercortisolism determines altered metabolite concentration independently of the current clinical status. Thus, an earlier diagnosis and prompt treatment are essential to avoid progression of the prefrontal damage.

The ventromedial area of the prefrontal cortex (vmPFC) is directly involved in the representation of elementary positive and negative emotional states [44]. Therefore, it is congruent to think that abnormal metabolites in this area can imply affective alterations, as irritability or worrying. Alterations of NAA concentration and glutamate in the vmPFC and orbital prefrontal cortex have been described in patients with anxiety disorders and chronic depression [14,45]. Our findings showed that total NAA was negatively correlated with anxiety state, and Cr was negatively correlated with both anxiety and depressive symptoms. Although the correlation between total NAA and BDI-II was not significant, the Pearson coefficient was -0.277, and p-value 0.079. Thus, with a longer sample, may have been significant. Moreover, it is known that GC receptors are plentiful in vmPFC and prolonged occupation of these receptors due to excessive GC concentration produces toxic effects on brain cells. Corticosterone-induced changes in neuron morphology of the vmPFC had been observed [46], suggesting that hypercortisolism could play a direct effect on the vmPFC and consequently could trigger metabolite abnormalities and affective alterations. In our study, CS patients showed subclinical anxiety and depressive symptoms, an emotional state not completely comparable with affective disorders such as major depression or

generalized anxiety disorder. Even so, it cannot be excluded that these affective alterations also contribute to maintain metabolite abnormalities in CS.

This study has several limitations. First, its cross-sectional design prevents from inferring causation based on our data. Second, only very high quality spectra were chosen as suitable for analysis (representing only 40% of all ^{1}H-MRS performed), resulting in a limited sample size and unequal number of female/male between the groups. Therefore, the healthy controls were not perfectly matched with the patients. No significant differences were found between CS patients and controls for age, gender and educational levels (results shown in Table 1). Even so, we included these characteristics in the linear regression analysis. Age, gender and educational level were not predictors of total NAA, glutamate and Cr concentrations. Finally, the sample is clinically heterogeneous; however, this is practically unavoidable in a rare disease like CS.

In conclusion, decreased total NAA and glutamate concentrations have been found in CS patients, indicating neuronal dysfunction. These brain metabolite abnormalities were consistently related to duration of hypercortisolism, highlighting the role of GC neurotoxicity on functional alterations of the vmPFC, including affective alterations, mainly anxiety.

Acknowledgements

The authors thank all the patients and controls that voluntarily participated in the study. This work was supported by Instituto de Salud Carlos III, Spanish Ministry of Science and Innovation (MICINN, FIS080302) and the European Commission (ERCUSYN PHP800200). We thank Dr. Ignasi Gich, from the Department of Epidemiology of the Hospital Sant Pau, for the critical review of the statistical analysis.

Conflict of interest: Nothing to declare.

References

1. Bourdeau, I., Bard, C., Forget, H., Boulanger, Y., Cohen, H., Lacroix, A.: Cognitive function and cerebral assessment in patients who have Cushing's syndrome. Endocrinology Metabolism Clinics of North America. 34, 357-369 (2005)
2. Resmini, E., Santos, A., Gómez-Anson, B., Vives, Y., Pires, P., Crespo, I., Portella, MJ., Juan-Delago, M., Barahona, MJ., Webb, SM.: Verbal and visual memory performance and hippocampal volumes, measured by 3-tesla magnetic resonance imaging, in patients with Cushing's syndrome. Journal of Clinical Endocrinology & Metabolism. 97, 663-671 (2012)
3. Tiemensma, J., Kokshoorn, NE., Biermasz, NR., Keijser, BSA., Wassenaar, MJE., Middelkoop, HAM., Pereira, AM., Romijn, JA.: Subtle cognitive impairment in patients with long-term cure of Cushing's disease. Journal of Clinical Endocrinology & Metabolism. 95, 1-16 (2010)
4. Andela, CD., van Haalen, FM., Ragnarsson, O., Papakokkinou, E., Johannsson, G., Santos, A., Webb, SM., Biermasz, NR., van der Wee, NJ., Pereira, AM.: Mechanisms in endocrinology: Cushing's syndrome causes irreversible effects on the human brain: a systematic review of structural and functional magnetic resonance imaging studies. European Journal of Endocrinology. 173, R1–14 (2015)
5. Santos, A., Resmini, E., Gómez-Ansón, B., Crespo, I., Granell, E., Valassi, E., Pires, P., Vives-Gilabert, Y., Martínez-Momblán, MA., de Juan, M., Mataró, M., Webb, SM.: Cardiovascular risk and white matter lesions after endocrine control of Cushing's syndrome. European Journal of Endocrinology. 173, 765-775 (2015)
6. Joëls, M., Karst, H., Krugers, HJ., Lucassen, PJ.: Chronic stress: implications for neuronal morphology, function and neurogenesis. Frontiers of Neuroendocrinology. 28, 72-96 (2007)
7. Resmini, E., Santos, A., Gómez-Ansón, B., López-Mourelo, O., Pires, P., Vives-Gilabert, Y., Crespo, I., Portella, MJ., de Juan-Delago, M., Webb, SM.: Hippocampal dysfunction in cured Cushing's syndrome patients, detected by ^{1}H-MR-spectroscopy. Clinical Endocrinology. 79, 700-707 (2013)
8. Starkman, MN., Gebarski, SS., Berent, S., Schteingart, DE.: Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. Biological Psychiatry. 32, 756-765 (1992)

9. Forget, H., Lacroix, A., Somma, M., Cohen, H.: Cognitive decline in patients with Cushing's syndrome. Journal of International Neuropsychological Society. 6, 20-29 (2002)
10. Crespo, I., Granell-Moreno, E., Santos, A., Valassi, E., Vives-Gilabert, Y., de Juan-Delago, M., Webb, SM., Resmini, E.: Impaired decision making and selective cortical frontal thinning in Cushing's syndrome. Clinical Endocrinology. 81, 826-833 (2014)
11. Bas-Hoogendam, JM., Andela, CD., van der Werff, SJA., Pannekoek, JN., van Steenbergen, H., Meijer, OC., van Buchem, MA., Rombouts, SARB., van der Mast, RC., Biermasz, NR., van der Wee, NJA., Pereira, AM.: Altered neural processing of emocional faces in remitted Cushing's disease. Psychoneuroendocrinology. 59, 134-146 (2015)
12. Khiat, A., Bard, C., Lacroix, A., Rousseau, J., Boulanger, Y.: Brain metabolic alterations in Cushing's syndrome as monitored by proton magnetic resonance spectroscopy. NMR in Biomedicine. 12, 357-363 (1999)
13. Tiemensma, J., Biermasz, NR., Middlekoop, HAM., van der Mast, RC., Romijn, JA., Pereira, AM.: Increased prevalence of psychopathology and maladaptive personality traits after long-term cure of Cushing's disease. Journal of Clinical Endocrinology & Metabolism. 95, 129-141 (2010)
14. Portella, MJ., de Diego-Adeliño, J., Gómez-Ansón, B., Morgan-Ferrando, R., Vives, Y., Puigdemont, D., Pérez-Egea, R., Ruscalleda, J., Álvarez, E., Pérez, V.: Ventromedial prefrontal spectroscopic abnormalities over the course of depression: A comparison among first episode, remitted recurrent and chronic patients. Journal of Psychiatric Research. 45, 427-434 (2011)
15. Soares, DP. and Law, M.: Magnetic resonance spectroscopy of the brain: review of metabolites and clinical applications. Clinical Radiology. 64, 12-20 (2009)
16. Yüksel, C. and Öngür, D.: Magnetic resonance spectroscopy studies of glutamate-related abnormalities in mood disorders. Biological Psychiatry. 68, 785-794 (2010)
17. Matute, C., Domercq, M., Sánchez-Gómez, MV.: Glutamate-mediated glial injury: mechanisms and clinical importance. Glia. 53, 212-224 (2006)
18. Arnold, DL., de Stefano, N., Matthews, PM., Trapp, BD.: N-acetylaspartate: usefulness as an indicator of viable neuronal tissue. Annals of neurology. 50, 824-825 (2011)

19. Arnaldi, G., Angeli, A., Atkinson, AB., Bertagna, X., Cavagnini, F., Chrousos, GP., Fava, GA., Findling, JW., Gaillard, RC., Grossman, AB., Kola, B., Lacroix, A., Mancini, T., Mantero, F., Newell-Price, J., Nieman, LK., Sonino, N., Vance, ML., Giustina, A., Boscaro, M.: Diagnosis and complications of Cushing's syndrome: a consensus statement. Journal of Clinical Endocrinology & Metabolism. 88, 593-602 (2003)
20. Talairach, J. and Tournoux, P.: Co-planar stereotaxic atlas of the human brain. New York: Thieme (1988)
21. Provencher, SW.: Automatic quantitation of localized in vivo 1H spectra with LC Model. NMR in Biomedicine. 14, 260-264 (2001)
22. Baslow, MH.: Evidence supporting a role for N-acetyl-L-aspartate as a molecular water pump in myelinated neurons in the central nervous system. An analytical review. Neurochemistry International. 40, 295–300 (2002)
23. Malhi, GS., Valenzuela, M., Wen, W., Sachdev, P.: Magnetic resonance spectroscopy and its applications in psychiatry. Australian & New Zealand Journal of Psychiatry. 36, 31-43 (2002)
24. Hajek, M. and Dezortova, M.: Introduction to clinical in vivo MR spectroscopy. European Journal of Radiology. 67, 185-190 (2008)
25. Schirmer, T. and Auer, DP.: On the reliability of quantitative clinical magnetic resonance spectroscopy of the human brain. NMR in Biomedicine. 13, 28–36 (2000)
26. Soares, DP. and Law, M.: Magnetic resonance spectroscopy of the brain: review of metabolites and clinical applications. Clinical Radiology. 64, 12-20 (2009)
27. Whisman, MA., Perez, JE., Ramel, W. Factor structure of the Beck Depression Inventory-Second Edition (BDI-II) in a student sample. Journal of Clinical Psychology. 56, 545-551. (2000).
28. Spielberger, C. Assessment of state and trait anxiety: Conceptual and methodological issues. Southern Psychologist. 2, 6-16 (1985)
29. Diorio, D., Viau, V., Meaney, MJ.: The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. The Journal of Neuroscience. 13, 3839-3847 (1993)
30. Popoli, M., Yan, Z., McEwen, B., Sanacora, G.: The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nature Reviews Neuroscience. 13, 22-37 (2013)

31. Öngur, D., Prescot, AP., Jensen, JE., Cohen, BM., Renshaw, PF.: Creatine abnormalities in schizophrenia and bipolar disorder. Psychiatry Research. 172, 44-48 (2009)
32. Yue, Q., Liu, M., Nie, X., Wu, Q., Li, J., Zhang, W., Huang, X., Gong, Q.: Quantitative 3.0T MR Spectroscopy reveals decreased creatine concentration in the dorsolateral prefrontal cortex of patients with social anxiety disorder. Plos One. 7, e48105 (2012)
33. Roitman, S., Green, T., Osher, Y., Karni, N., Levine, J.: Creatine monohydrate in resistant depression: a preliminary study. Bipolar Disorders. 9, 754-758 (2007)
34. Khiat, A., Yared, Z., Bard, C., Lacroix, A., Boulanger, Y.: Long-term brain metabolic alterations in exogenous Cushing's syndrome as monitord by proton magnetic resonance spectroscopy. Brain Reserch. 134-140 (2001)
35. Khiat, A., Bard, C., Lacroix, A., Boulanger, Y.: Recovery of the brain choline level in treated Cushing's patients as monitored by proton magnetic resonance spectroscopy. Brain Research. 301-307 (2000)
36. Hädel, S., Wirth, C., Rapp, M., Gallinat, J., Schubert F. Effects of age and sex on the concentrations of glutamate and glutamine in the human brain. Journal of magnetic resonance imaging. 38, 1480-1487 (2013)
37. Chiu, PW., Mak, HK., Yau, KK., Chan, Q., Chang, RC., Chu, LW.: Metabolic changes in the anterior and posterior cingulate cortices of the normal aging brain: proton magnetic resonance spectroscopi study at 3T. Age. 36, 251-264 (2014)
38. Vitale, G., Salvioli, S., Franceschi, C.: Oxidative stress and the ageing endocrine system. Nature Reviews of Endocrinology. 9, 228-240 (2013)
39. Tirabassi, G., Boscaro, M., Arnaldi, G.: Harmful effects of functional hypercortisolism: a working hypothesis. Endocrine. 46, 370-386 (2014)
40. Michaud, K., Forget, H., Cohen, H.: Chronic glucocorticoid hypersecretion in Cushing's syndrome exacerbates cognitive aging. Brain Cognition. 71, 1-8 (2009)
41. Scheel, M., Ströhle, A., Bruhn, H.: Effects of short-term stress-like cortisol on cerebral metabolism: A proton magnetic resonance spectroscopy study at 3.0T. Journal of Psychiatric Research. 44, 521-526 (2010)
42. Joëls, M., Karst, H., Krugers, HJ., Lucassen, PJ.: Chronic stress: implications for neuronal morphology, function and neurogenesis. *Frontiers in Neuroendocrinology*. 28, 72-96. (2007)

43. Diamond, DM., Campbell, AM., Park, CR., Halonen, J., Zoladz, PR.: The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-dodson law. *Neural Plasticity*. 0-33 (2007)

44. Davidson, RJ. and Irwin, W.: The functional neuroanatomy of emotion and affective style. Trends in Cognitive Science. 3, 11-21 (1999)

45. Grachev, ID. and Apkarian, AV.: Anxiety in healthy humans is associated with orbital frontal chemistry. Molecular Psychiatry. 5, 482-488 (2000)

46. Wellman, CL.: Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. Journal of Neurobiology. 49, 245-253 (2001)

Figures and tables

Figure 1. 3 T MR images of the brain showing the volume of interest (VOI, 2 x 2 x 2 cm^3) in the axial (a), sagittal (b) and coronal (c) sections used for ^{1}H-MRS data acquisition in the ventromedial prefrontal area. A representative spectrum with identification of the different metabolic peaks after processing with LCModel software is presented (d).

*MI: Myoinositol; Cho: Choline-containing; Cr: Creatine; Glu: Glutamate; Glx: Glutamate + glutamine; NAA: N-Acetyl-aspartate.

* X axis indicates the frequency chemical shift in parts per million (ppm) of the various metabolites in a given tissue sample.

Y axis indicates the intensity signal of the metabolites.

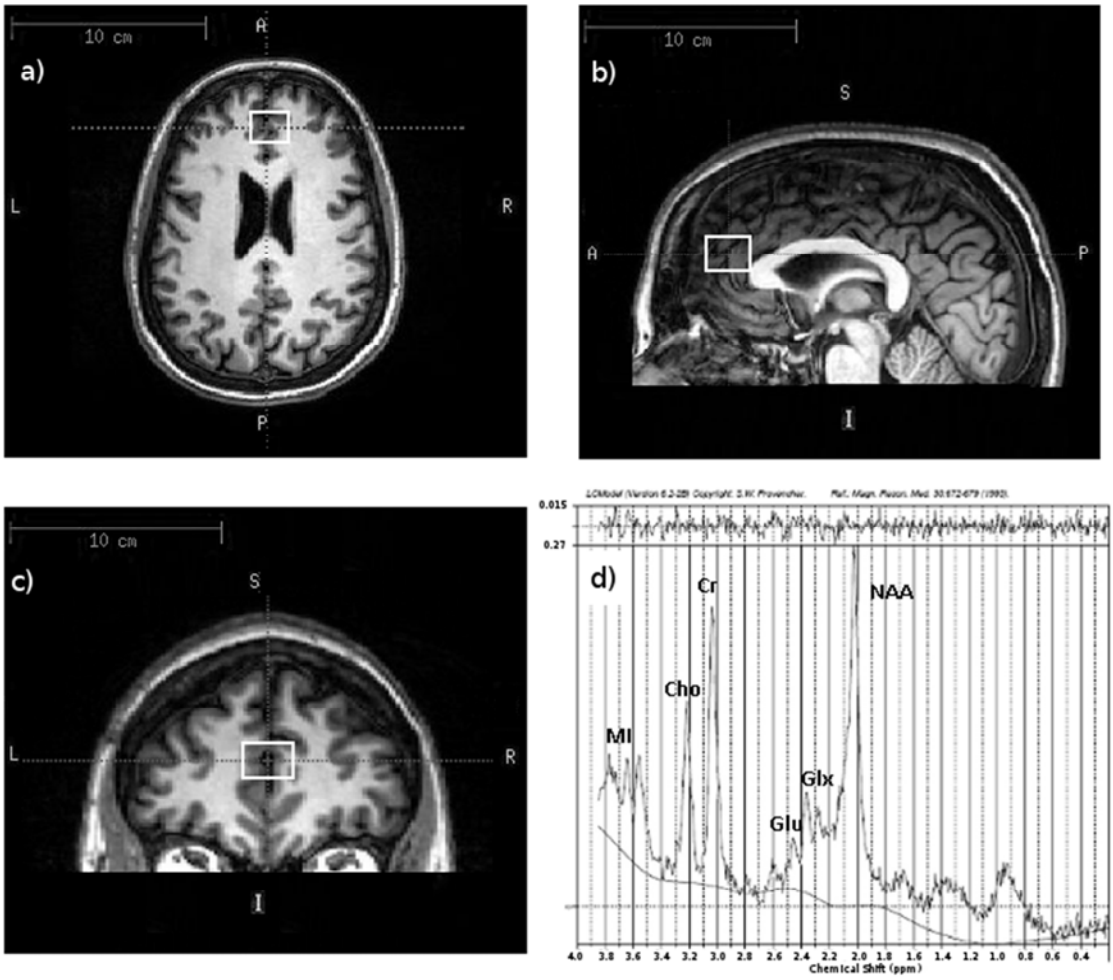


Figure 2. Graph showing the negative correlation between total N-Acetyl-aspartate (NAA) concentration and duration of hypercortisolism in 22 patients with Cushing's syndrome ($r = -0.488$. $p < 0.05$).

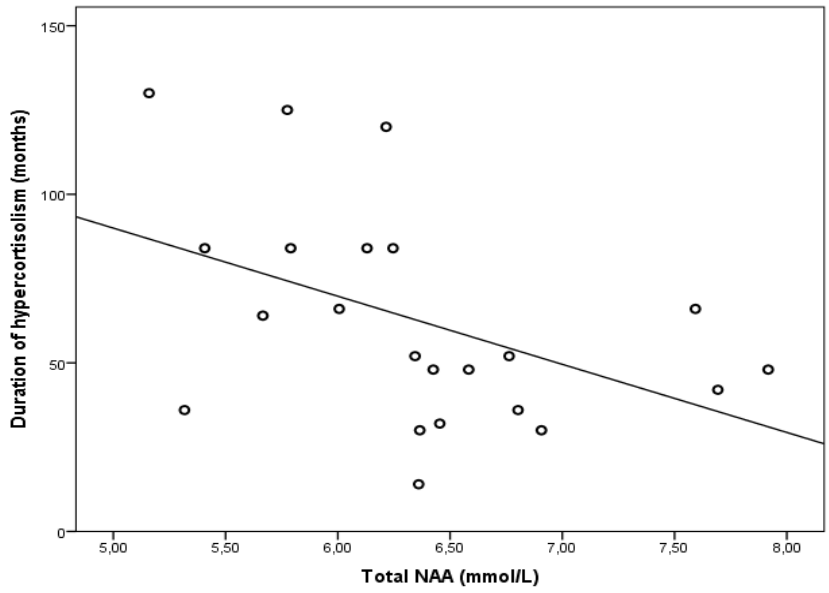


Figure 3. Graph showing the negative correlation between total N-Acetyl-aspartate (NAA) concentration and anxiety state (measured using STAI test) in 22 patients with Cushing's syndrome ($r = -0.359$. $p < 0.05$).

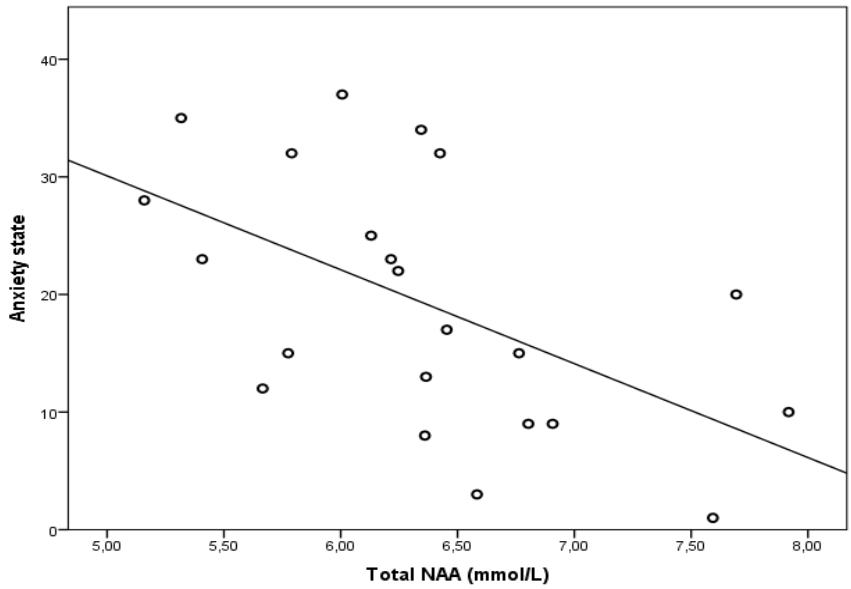


Table 1. Clinical characteristics of patients with Cushing's syndrome (CS) and healthy controls.

	CS patients (n=22)		**Healthy controls (n=22)**	**p value**
	Active (n=7)	***In remission (n=15)***		
Age (years)	41.8±10	41.3±12	41.7±11	0.993
Gender ratio (female/male)	6/1	13/2	14/8	0.114
Education level (years)	13.7±2	13.7±3	13.8±4	0.996
Origin of CS:				
- Pituitary adenoma	4	13		
- Adrenal adenoma	3	2		
Mean urinary free cortisol (UFC) (nmol/24h)	422±175	141±84		<0.001
Mean duration of hypercortisolism (months)	87.3±75	58.3±27		0.192
Radiotherapy	2	4		
Median duration of remission (months)		42 (6-216)		
Hydrocortisone substitution		3		
Medical therapy:				
- Ketokonazole (KTZ)	2			
- Metyrapone (MTP)	1			
- KTZ + MTP	1			

Table 2. Metabolite concentrations in CS patients and healthy controls.

Metabolite concentrations (mmol/L)	CS patients	Healthy controls	p value
· **Glutamate (Glu)**	8.6±1.2	9.3±0.7	0.044
· **Glutamate + glutamine (Glx)**	10.5±1.2	11±1	0.247
· **Creatine (Cr)**	5.6±0.4	5.9±0.4	0.07
· **N-Acetyl-aspartate (NAA)**	6.1±0.9	6.5±0.4	0.082
· **N-Acetyl-aspartate+N-acetylaspartylglutamate (total NAA)**	6.4±0.8	6.8±0.4	0.045
· **Choline-containing compounds (Cho)**	1.4±0.2	1.4±0.2	0.441
· **Myoinositol (MI)**	4.5±0.7	4.7±0.7	0.253

Publications

· **Paper I:**

Crespo, I., Granell-Moreno, E., Santos, A., Valassi, E., Vives-Gilabert, Y., De Juan-Delago, M., Webb, SM., Gómez-Ansón, B., Resmini, E. Impaired decision-making and selective cortical frontal thinning in Cushing's syndrome. Clinical Endocrinology. 2014 Dec; 81(6): 826-833. doi: 10.1111/cen.12564.

· **Paper II:**

Crespo, I., Santos, A., Valassi, E., Pires, P., Webb, SM., Resmini, E. Impaired decision making and delayed memory are related with anxiety and depressive symptoms in acromegaly. Endocrine. 2015 Dec; 50(3): 756-763. doi: 10.1007/s12020-015-0634-6.

Clinical Endocrinology (2014) **81**, 826–833 doi: 10.1111/cen.12564

ORIGINAL ARTICLE

Impaired decision-making and selective cortical frontal thinning in Cushing's syndrome

Iris Crespo*, Granell-Moreno Esther†, Alicia Santos*, Elena Valassi*, Vives-Gilabert Yolanda‡, Manel De Juan-Delago†, Susan M. Webb*, Beatriz Gómez-Ansón† and Eugenia Resmini*

**Endocrinology/Medicine Department, Hospital Sant Pau, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBER-ER, Unidad 747), ISCIII and Universitat Autònoma de Barcelona (UAB), †Neuroradiology Unit, Hospital Sant Pau, and ‡INNDACYT, Barcelona, Spain*

Summary

Context and objective Cushing's syndrome (CS) is caused by a glucocorticoid excess. This hypercortisolism can damage the prefrontal cortex, known to be important in decision-making. Our aim was to evaluate decision-making in CS and to explore cortical thickness.

Subjects and methods Thirty-five patients with CS (27 cured, eight medically treated) and thirty-five matched controls were evaluated using Iowa gambling task (IGT) and 3 Tesla magnetic resonance imaging (MRI) to assess cortical thickness. The IGT evaluates decision-making, including strategy and learning during the test. Cortical thickness was determined on MRI using FREESURFER software tools, including a whole-brain analysis.

Results There were no differences between medically treated and cured CS patients. They presented an altered decision-making strategy compared to controls, choosing a lower number of the safer cards ($P < 0{\cdot}05$). They showed more difficulties than controls to learn the correct profiles of wins and losses for each card group ($P < 0{\cdot}05$). In whole-brain analysis, patients with CS showed decreased cortical thickness in the left superior frontal cortex, left precentral cortex, left insular cortex, left and right rostral anterior cingulate cortex, and right caudal middle frontal cortex compared to controls ($P < 0{\cdot}001$).

Conclusions Patients with CS failed to learn advantageous strategies and their behaviour was driven by short-term reward and long-term punishment, indicating learning problems because they did not use previous experience as a feedback factor to regulate their choices. These alterations in decision-making and the decreased cortical thickness in frontal areas suggest that chronic hypercortisolism promotes brain changes which are not completely reversible after endocrine remission.

(Received 23 April 2014; returned for revision 16 May 2014; finally revised 30 June 2014; accepted 17 July 2014)

Correspondence: Iris Crespo, Department of Endocrinology, Hospital de Sant Pau, C/Sant Antoni Maria Claret n. 167, 08025 Barcelona, Spain. Tel.: 0034 935537917; E-mail: icrespo@santpau.cat

Introduction

Cushing's syndrome (CS) is a rare disease, caused by an excessive exposure to endogenous glucocorticoids (GC). It has been associated with deficits in cognitive function, especially memory and attention.[1] GC excess is a potential neurotoxic factor for the brain, and long-term exposure to hypercortisolism damages neuronal cells.[2]

Frontal cortex is involved in executive functions, in decision-making and in emotional stress responses.[3] Glucocorticoid (GC) receptors are abundant in the frontal cortex, an area able to inhibit and regulate hypothalamic–pituitary–adrenal responses to acute stress.[4] Hypercortisolemia secondary to stress has been related to loss of prefrontal cortex control over subcortical areas and to promote more risk-taking behaviour.[5] In fact, Coates and Herbert observed that increased cortisol levels may shift risk preferences and even affect ability to engage in rational choices.[6] Moreover, salivary cortisol levels after induced stress and exogenous administration of cortisol have shown to be negatively related to decision-making in healthy subjects.[7,8]

Several studies have shown that patients with CS do not completely return to their premorbid level of functioning after successful treatment, and impaired cognitive function persists despite long-term cure. Cured CS patients performed significantly worse on memory and executive functions compared to matched controls and patients treated for nonfunctioning pituitary macroadenomas, not exposed to hypercortisolism.[9,10] Moreover, long-term cured CS patients showed high prevalence of psychopathology (mainly depressive disorders), maladaptive personality and worse coping strategies compared to healthy controls and to patients with nonfunctioning pituitary adenomas.[11,12]

Advances in neuroimaging have allowed accurate quantification of cortical thickness. Specific MRI postprocessing software to assess grey matter alterations could be useful to detect their relationship with cognitive function. In particular, analysis of cortical thickness has shown to be more accurate in depicting

grey matter loss than other more established tools such as Voxel-based morphometry.[13] Frontal grey matter volume loss has been associated with impairments in the decision-making in alcoholism, and chronic alcohol consumption determines high circulating cortisol levels.[14]

Iowa gambling task (IGT) is one of the most widely used tests to evaluate decision-making. In functional magnetic resonance imaging (fMRI) studies, the IGT has been related to the frontal cortex.[15] Alterations in the IGT have been also associated with damage or injuries to the human prefrontal cortex.[16,17] Furthermore, the insula and the anterior cingulated cortex (ACC, on the medial surface of the frontal lobes) have also shown to be important in motivation, goal-directed behaviours and adaptative behaviour, responding to negative feedback,[18] essential mechanisms to learn an advantageous strategy in the IGT.

The aim of our study was to evaluate the decision-making in patients with CS using the IGT, and to explore its relationship with cortical thickness using a 3 Tesla MRI. Our main hypothesis was that exposure to cortisol excess in patients with CS alters the decision-making process and may be related to frontal thickness.

Methods

Subjects

In this cross-sectional study, thirty-five patients with CS clinically followed in our institution and 35 controls matched for sex, gender and years of education were evaluated with the IGT and a 3 Tesla MRI of the brain. Table 1 shows the baseline clinical characteristics of study participants.

CS patients with diabetes, GH deficiency, known intellectual disability, age over 65 years, a history of drug abuse and any current or previous psychopathology were excluded. CS patients with diabetes mellitus[19] and GH deficiency[20] were excluded because cognitive deficits and hippocampal atrophy have been described in these conditions.[21,22] Patients with malignant adrenocortical tumours were also excluded.

Table 1. Clinical characteristics of patients with Cushing's syndrome and controls

	Patients with Cushing's syndrome (n = 35)		
	Cured (n = 27)	Medically treated (n = 8)	Healthy controls (n = 35)
Age	44·5 ± 10	41·4 ± 12·3	44·4 ± 9·5
Gender (female/male)	22/5	8/0	30/5
Years of education	13·2 ± 3·2	13·4 ± 3·1	13·4 ± 3·1
Origin of CS			
Pituitary adenoma	24	4	–
Adrenal adenoma	3	3	
Ectopic ACTH secretion	0	1	
Duration of prior hypercortisolism (months)	57·6 ± 34·5	46·5 ± 32·5	–
Radiotherapy	6/27	2/8	–
Duration of eucortisolism (months)	41 (6–288)	2 (1–4)	–
Hydrocortisone substitution	8/27	0/8	–

Healthy controls were recruited from among blood bank donors at Hospital Sant Pau (Barcelona, Spain). Exclusion criteria for controls were prior endocrine disease or exposure to GC, known intellectual disability, a history of drug abuse and any current or previous psychopathology.

At the time of the study, 27 patients with CS were in remission (cured group), 24 with pituitary adenomas and three with adrenal adenomas. CS was considered in remission if after surgery patients had achieved adrenal insufficiency or morning cortisol suppression (<50 nmol/l; <1·8 μg/dl) after 1 mg dexamethasone overnight and repeatedly normal 24 h UFC (measured with a commercial RIA after previous urine extraction with an organic solvent).[23] Six patients had radiotherapy after surgery. Eight of the patients in remission after successful surgery were adrenal insufficient and required substitution treatment with hydrocortisone (HC) [median HC dose 15 mg/d (5–20 mg/d)]. Corticotrope insufficiency was defined as an insufficient response to synthetic ACTH (peak <18 mcg/dl) or both undetectable serum and urinary cortisol.

Eight patients with CS were taking medical therapy with steroid synthesis inhibiting drugs (medically treated group): four were taking ketoconazole, two were taking metyrapone, and two were taking both ketoconazole and metyrapone [median ketoconazole dose 400 mg/d (200–400 mg/d) and median metyrapone dose 500 mg/d (500–750 mg/d)]. This group included four patients with CS of pituitary origin, three of adrenal origin and one ectopic ACTH secretion of unknown origin. Five were waiting for surgery (two for neurosurgery and three for adrenal surgery), and two had previously undergone unsuccessful transsphenoidal neurosurgery and radiotherapy.

All the patients included in this study were eucortisolemic at study evaluation. Eucortisolism was defined as urinary free cortisol (UFC) levels within the normal range [50–280 nmol/24 h (20–100 μg/24 h)]. Duration of eucortisolism was calculated from the date of successful surgery or normalization of UFC until the date of the study evaluation. Mean duration of eucortisolism was 41 (6–288) months in the cured and 2 (1–4) months in the medically treated group.

Duration of hypercortisolism was considered as the time from symptom onset until hypercortisolism control (eucortisolism) and was assessed by the endocrinologist in charge. At diagnosis, the duration of hypercortisolism was estimated by personal interview, careful review of medical records and photographs of patients. Mean duration of hypercortisolism was 57·6 ± 34·5 months in the cured group and 46·5 ± 32·5 months in the medically treated group.

The study was approved by the hospital ethics committee, and written informed consent was obtained from all participants.

Iowa gambling task

The Iowa Gambling Task (IGT) is a test used to assess decision-making and its evaluation and it is focused on 3 components:

Evaluation of decision-making strategy. Subjects see four card deck groups (A, B, C and D) on a computer screen. Each card group has different profiles of rewards (money wins) and punishments (money losses). Two card groups (A and B) are considered riskier, and two card groups (C and D) are considered safer. Choosing the riskier card groups, subjects win more money in the short term, but lose more money in the long term. Choosing the safer card groups, subjects win less money in the short term, but lose less money in the long run.

Subjects were asked to choose the options which would allow them to 'win' more money, at the beginning of the study. A bar on the top of the screen changes according to the amount of money won or lost after each selection. The amount of money accumulated is shown in this bar as a feedback factor used to regulate their choices. An increase in both the amount of lost money and the number of riskier cards reflect a poorer decision-making function.

Evaluation of learning during the test. The IGT consists of 100 card choices. These choices are divided into five blocks of 20 cards each. The five blocks reflect a subject's learning evolution in the decision-making process during the test. The total score for each block (TSB) is calculated as the difference between advantageous selection (the number of card selections from C and D) and disadvantageous selection (the number of selections from A and B). This score reflects the ability to learn the correct strategies and the feedback-regulating performance during the IGT. [TSB = (number of C card selections + number of D card selections) - (number of A card selections + number of B card selections)].

Studies on decision-making indicate that there are two types of decisions in the IGT: decisions under ambiguity (blocks 1 and 2 of the IGT) and decisions under risk (blocks 3–5 of the IGT).[24]

Evaluation of relationship between IGT and memory. Memory is one of the main cognitive processes involved in decision-making. The IGT requires learning novel relationships across successive experiences of the individual card groups and their reward schedules.[25] To evaluate whether lower memory performance influence decision-making, we used Rey Auditory Verbal Learning Test (RAVLT) as previously described.[9] The RAVLT was administered in a standard manner consisting of (i) presentation of a 15-word list in five learning trials, the score for each trial being the number of words correctly recalled, (ii) a single presentation of an interference 15-word list, (iii) two postinterference recall trials, one immediate and the other delayed after 15 min, (iv) after 30 min, subjects are asked to recognize the words of both lists, and (v) total score was calculated (the sum of trials 1–5).

Magnetic resonance imaging

Acquisition. Magnetic resonance imaging (MRI) scans were acquired using a 3 Tesla Philips-Achieva MRI (software version 2.1.3.2). Dedicated 3D-MPRAGE of the entire brain (turbo field echo, TR = 6·7, TE = 3·1, voxel size 1·2 × 0·889 × 0·889) was obtained. MRI anonymization, storage and postprocessing were performed at the Port d'Informació Científica (PIC) in Barcelona through the web portal PICNIC (https://neuroweb.pic.es).

Postprocessing: surface reconstructions and estimation of cortical thickness. MRI data were analysed and surfaces reconstructed with FREESURFER v4.3.1 (http://surfer.nmr.mgh.harvard.edu), a software tool developed at the Martinos Center for Biomedical Imaging, according to previously published methodologies.[26] Images from all participants were registered in the Talairach atlas.[27] Image intensity variations due to magnetic field inhomogeneities were normalized, and a skull stripping algorithm was applied.[28]

An estimate of the grey/white boundary was constructed by classifying all white matter voxels in an MRI volume. The surface of the white matter voxels was refined to obtain a better accuracy in the grey/white boundary. It was then deformed outward to find the pial surface, according to Fischl.[29] The surface deformation was based on a local adaptive estimation of the MRI values at the different surfaces, by minimizing a constrained energy function. Cortical thickness estimates were then obtained with the shortest distance between the white matter and the pial surfaces at each location of the cortex. Subsequently, a blinded investigator carried out a visual check and if necessary, manual correction to refine the segmentation and to correct software delineation errors.

Statistical analysis

Analysis was performed using SPSS 17·0 statistical package for WINDOWS (SPSS Inc., Chicago, IL, USA). Data distribution was analysed by the Kolmogorov–Smirnov test. Quantitative data are expressed as mean±SD (Gaussian distribution) or as median (range) (non-Gaussian distribution). ANOVA was used to compare three groups. Comparison between the two groups was performed using Student's *t*-test, except for the blocks of the IGT that were analysed using repeated measures ANOVA. Gender, age and duration of hypercortisolism were included as covariates in repeated measures ANOVA. $P < 0{\cdot}05$ was considered significant. Correlations among variables were studied using Pearson's correlation coefficient. Moreover, a multiple linear regression analysis was performed in order to determine whether lower performance in verbal memory test could be related to the presence of impaired decision-making. For this, previously published results using the RAVLT in these same patients were used.[9]

Statistical maps for cortical thickness were generated using FREESURFER's QDEC 1·4 (Query, Design, Estimate, Contrast), which uses a general linear model (GLM) at each surface vertex. A whole-brain analysis between patients with CS and controls was performed using a FWHM (full-width/half max) of 10 mm. The following thresholds were used: $P < 0{\cdot}05$ for corrected (false discovery rate) and $P < 0{\cdot}001$ for uncorrected analysis. The significant clusters obtained were then represented

on inflated surfaces to allow for better visualization of sulcal regions while maintaining topology. Whole-brain correlation analysis between the cortical thickness of each vertex and IGT performance was also obtained with QDEC (QDEC tool). Desikan-Killiany atlas was used to designate the different anatomical areas of the brain.

Results

Comparison between medically treated patients with CS, cured CS patients and healthy controls

No difference in age, gender or years of education was observed between the groups. Regarding ANOVA, no group effect was observed on the cortical thickness and IGT (Fig. 1). As this initial approach of analysing three groups showed no differences between the groups, a new approach comparing all patients with CS (medically treated and cured) with healthy controls was adopted.

Comparison between patients with CS and healthy controls

Iowa gambling task (IGT). Patients with CS showed impaired decision-making compared to controls as reflected by a smaller number of safe cards (50·4 ± 10·8 *vs* 58·3 ± 15·6, $P < 0·05$) and a trend towards a higher number of riskier cards (49·6 ± 10·8 *vs* 43·9 ± 15·9, $P = 0·087$) (Fig. 2), indicating that patients with CS chose short-term rewards and long-term punishments. No significant differences between patients with CS and controls were observed in the final amount of money obtained, although there was a trend for patients with CS to accumulate a smaller amount (−1188·9 ± 1058·4 *vs* −620·4 ± 1470·7, $P = 0·066$).

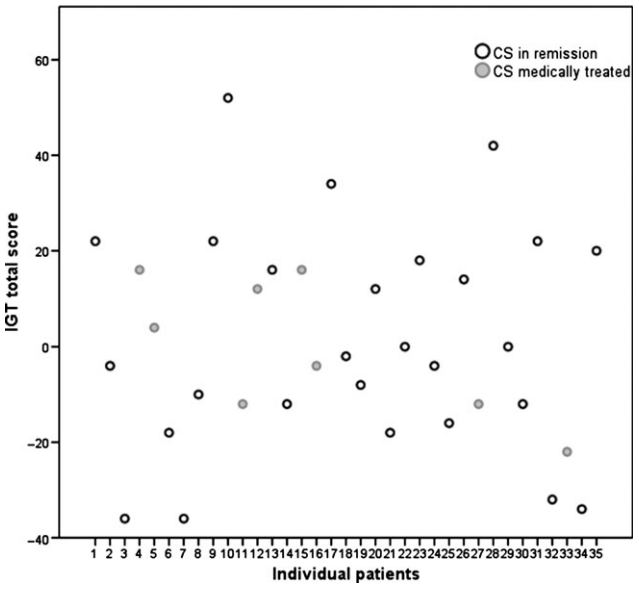


Fig. 1 Total score of Iowa gambling task for all single patients with Cushing's syndrome (CS). No differences were observed between medically treated CS and in remission CS.

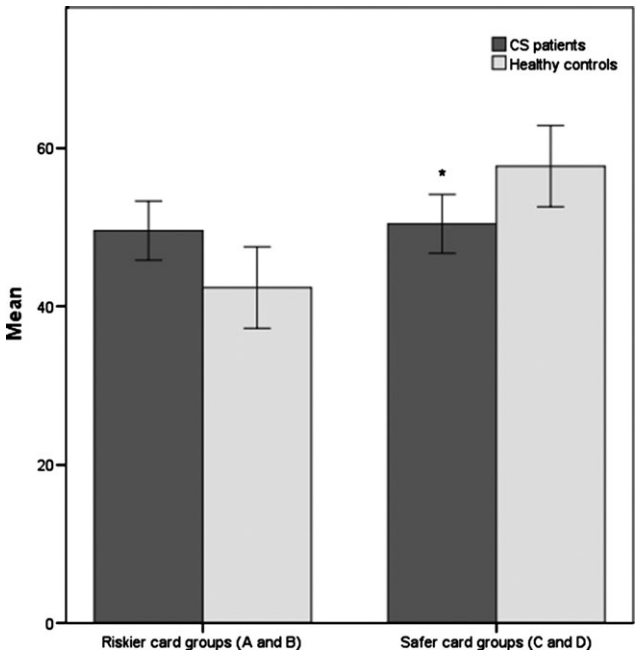


Fig. 2 Number of riskier or safer cards chosen in the IGT by patients with Cushing's syndrome and controls. *$P < 0·05$. IGT = Iowa gambling task.

With regard to the blocks, a repeated-measures ANOVA (blocks 1–5) was performed, showing a significant effect between the blocks ($F = 5·32$, $P < 0·01$) and between the groups ($F = 3·98$, $P < 0·05$). However, the interaction of blocks × groups was not significantly different.

We decided to perform a further analysis, excluding block 1 and block 2, consistent with the notion of decisions under risk (final trials of the IGT). The result of repeated measures ANOVA (blocks 3–5) for this new analysis showed a significant effect of the interaction between blocks and groups (blocks × groups $F = 5·02$, $P < 0·05$) and a significant effect of group ($F = 3·67$, $P = 0·05$), but no effect of blocks. In addition, a subsequent pairwise comparison (corrected for multiple comparisons) was performed, and a difference was found in block 5 between patients with CS and healthy controls (−0·69 ± 8·27 *vs* 4·72 ± 11·28, $P < 0·05$), with a trend in block 4 (1·03 ± 8·10 *vs* 5·06 ± 8·89, $P = 0·06$). This suggested that the significant differences in decision-making appear in the final phase of the test (Fig. 3). Results did not change when the duration of hypercortisolism, gender and age were included in the IGT analysis as covariates.

Cortical thickness. Whole-brain analysis comparison of patients with CS with controls showed decreased cortical thickness ($P < 0·001$, uncorrected) in certain areas: left superior frontal, left precentral and left insular cortex; bilateral rostral anterior cingulated cortex and right caudal middle frontal cortex (Fig. 4). However, no difference was observed at the corrected level (false discovery rate).

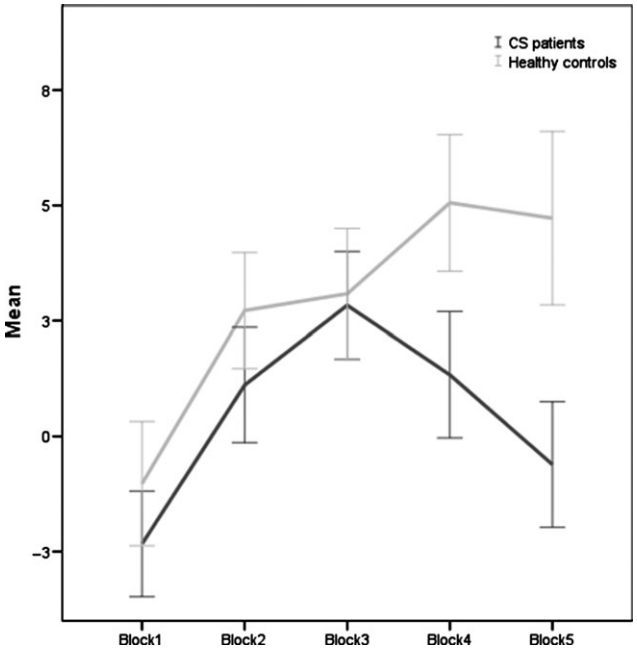


Fig. 3 IGT learning curves in patients with Cushing's syndrome and healthy controls. The learning curves consist of the score (the number of advantageous selections minus the number of disadvantageous selections) within each block of cards of the IGT (Iowa gambling task).

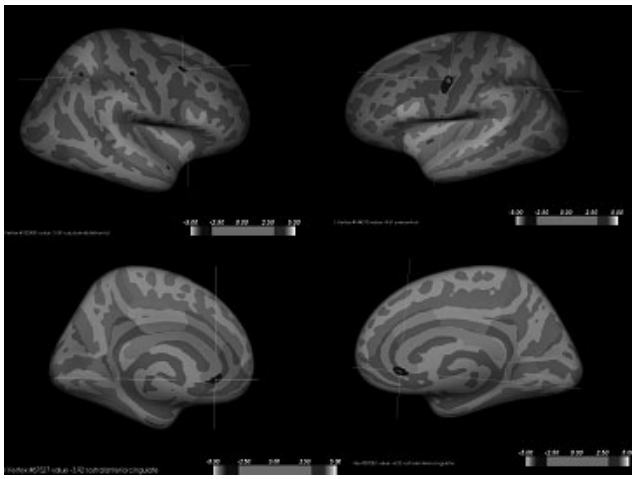


Fig. 4 Cortical thinning in patients with Cushing's syndrome when compared to controls. Areas of significant thinning (whole-brain analysis, uncorrected) appear black.

Correlations between IGT, cortical thickness and clinical variables

No correlations were found between the IGT scores and cortical thickness. IGT performance and cortical thickness did not correlate with duration of eucortisolism or with duration of prior hypercortisolism either. There were no correlations between UFC and IGT, or between UFC levels and cortical thickness. This suggests that GC exposure itself, rather than its duration, could be related to altered decision-making and reduced cortical thickness.

Relationship between IGT and verbal memory

We used previously published data (Resmini *et al.*, 2012) to analyse the relationship between IGT performance and verbal memory performance (using the Rey Auditory Verbal Learning Test, RAVLT). The results showed that block 4 of the IGT was correlated with recall index ($r = 0{\cdot}277$, $P < 0{\cdot}05$) and total score of the RAVLT ($r = 0{\cdot}297$, $P < 0{\cdot}05$), and block 5 of the IGT was correlated with the number of words correctly recalled in the last learning trial of the RAVLT ($r = 0{\cdot}235$, $P < 0{\cdot}05$). Moreover, the selection of riskier cards of the IGT was correlated with the number of words correctly recalled in the last learning trial ($r = -0{\cdot}301$, $P < 0{\cdot}05$), the recall index ($r = -0{\cdot}246$, $P < 0{\cdot}05$) and the total score of RAVLT ($r = -274$, $P < 0{\cdot}05$). The same occurred with the selection of safer cards of IGT, that was correlated with the number of words correctly recalled in the last learning trial ($r = 0{\cdot}298$, $P < 0{\cdot}05$), the recall index ($r = 0{\cdot}243$, $P < 0{\cdot}05$) and the total score of RAVLT ($r = 0{\cdot}272$, $P < 0{\cdot}05$). Including these memory variables in a linear regression model, the main determinant factor for IGT performance was the number of words correctly recalled in the last learning trial of the RAVLT ($r = 0{\cdot}306$, $P < 0{\cdot}05$). These findings support that learning capacity is important to explain our results, namely, lower performance in learning capacity and verbal memory could contribute to impaired decision-making.

Discussion

In this study, patients with CS failed to learn advantageous strategies during the IGT and their behaviour was driven by short-term reward and long-term punishment. This suggests that patients with CS had learning problems because they did not use previous experience as a feedback factor to regulate their choices. Moreover, these cognitive alterations were accompanied by decreased cortical thickness, mainly in specific frontal areas. Interestingly, no differences have been evidences between cured and medically treated group, suggesting an incomplete recovery of the grey matter of the brain after endocrine remission of hypercortisolism.

The impairments observed in decision-making are congruent with previous studies that reported a greater degree of maladaptative personality traits and less effective coping strategies in long-term biochemically cured CS patients compared to matched controls.[11,12] Both learning and feedback-regulating are essential processes for adaptative behaviour in daily life.

It is known that GC function allows the brain to deal with stress-provoked responses (as immediate attention or strategy decisions). As the response to stressors is important for survival in the short term, motivation and behaviour are usually driven by this need. This pattern of motivation for shorter rewards was observed in rats undergoing acute stress,[30] or GC excess, supporting the hypothesis that the shorter-reward motivation could be the result of brain alterations after exposure to an excess of GC.

We observed also a frontal cortical thinning in patients with CS. Grey matter loss in patients with CS has been previously

described.[9] However, to the best of our knowledge, this is the first study to show selective thinning in specific areas of the frontal cortex on MRI in these patients. This cortical thinning was present in patients with CS even after normalization of cortisol levels, suggesting that this damage, induced by prior GC excess exposure, is not completely reversible.

Regarding the rational and possible explanations behind this 'residual' morbidity after cortisol normalization, some hypothesis could be evaluated.

On the one hand, hypercortisolism due to stress response has a dual mode of action in rodents.[5] The first mode includes rapid changes through catecholamines, which lead to increased excitability and enhanced alertness, vigilance and attention. These changes are fast and of short duration. The second mode involves slow changes that are mediated by corticosterone through gene transcription. At this level, the possibility to generate new plasticity is reduced and cognitive function is impaired.[5] Long-term exposure to stress or cortisol involves irreversible neurobiological changes in gene expression, in neuronal structure and in neuronal firing patterns throughout the brain.[5,31] Harmful effects of GC on frontal neurons have been widely described in rodents,[32,33] as well as in humans, in other situations of endogenous GC excess, such as depressive disorders and alcoholism.[14,34] The decision-making performance in our patients with CS may therefore be a behavioural alteration due to these neuronal changes in the frontal cortex, induced during the presence of hypercortisolism, still present after the remission.

On the other hand, hypercortisolism due to stress in experimental studies has been associated with glutamate-induced neurotoxicity and desensitization of NMDA receptors in response to a marked increase in postsynaptic calcium concentration.[35] The duration of this neurotoxicity is a key for recovering brain functioning. Once neuronal alterations and structural changes are established, recovery is unlikely. In extreme cases of post-traumatic stress disorder, for example, altered prefrontal function did not return to normal after exposure to stress.[35] The same could occur in patients with CS, explaining why they were unable to recover full original prefrontal function after chronic hypercortisolism.

Another finding of interest in our study is that IGT performance and cortical thickness did not correlate with duration of prior hypercortisolism, suggesting that these cellular changes do not depend on the length of GC exposure. For this reason, an earlier diagnosis and prompt treatment may help avoid progression of the frontal damage, with consequent impairment of cortical functioning, affecting coping strategies, executive functions and decision-making. Moreover, functional abnormalities could be present and be early markers of GC neurotoxicity, as evidenced in other brain areas.[36]

Our data do not show a correlation between impaired decision-making and cortical thickness. This probably reflects that other structures or networks are also involved in decision-making. It has been proposed that subcortical areas, such as the amygdala or hippocampus, together with the frontal cortex interact to facilitate learning and memory during the IGT.[37,38] In fact, the hippocampus may interact with the frontal cortex to sustain emotional regulation and to maintain contextual information needed to represent and monitor motivationally relevant goals.[38] The relationship between lower performance in verbal memory and impaired decision-making showed in this study supports this proposal. Therefore, lower performance in learning capacity and verbal memory could contribute to impaired decision-making. Interestingly, the significant differences in decision-making observed in our patients appear in the final phase of the test, indicating they do not learn the correct strategy during the test.

Decision-making requires the interaction between both hippocampus and frontal areas. There are important connections between both, indeed any changes in the hippocampus can indirectly affect the functions of frontal areas. Our group demonstrated that GC excess is associated with cortical atrophy and hippocampal atrophy after long-term exposure, especially in older subjects.[9] Another study described a significant increase in hippocampal volume after transsphenoidal surgery.[39] Interestingly, these were younger patients; thus, age seems to be the main factor regulating the reversibility of cerebral atrophy in CS.[40] In this line, Anderson *et al.*[41] suggested that GC may exacerbate age-related cognitive impairments and predict prefrontal structural plasticity. Therefore, duration of GC exposure and age are main determinants of brain function recovery in patients with CS.

Our study has several limitations. First, the small sample size may have contributed to the lost of significance in the cortical thickness results when multiple comparison correction (false discovery rate) was applied. However, it is difficult to attain large patient groups because CS is a rare disease. Second, clinical heterogeneity should be considered as we included patients with tumours of pituitary origin and patients with tumours of adrenal origin, but we hypothesized that an excess of GC may harm the brain whatever the origin. And third, we cannot exclude an effect of prior radiotherapy, especially on cortical thinning. However, no differences were observed in this area between patients with CS and healthy controls when excluding irradiated patients ($n = 8$). Regarding cognitive function, Brummelman *et al.* (2012)[42] found that patients irradiated for a pituitary adenoma showed no differences in memory and executive functions compared to nonirradiated patients.

Conclusions

In conclusion, to our knowledge, this is the first report describing the presence of altered decision-making and cortical thickness in patients with CS. These neuropsychological and morphological frontal alterations described after cortisol normalization provide further support to the concept that chronic hypercortisolism promotes brain changes which are not completely reversible after endocrine remission. Awareness of these alterations would appear to justify a neuropsychological assessment of patients exposed to high cortisol levels.

Acknowledgements

We thank Dr Ignasi Gich, from the Department of Epidemiology of the Hospital Sant Pau, for the critical review of the

statistical analyses. The collaboration of the patients and the controls who participated in this study is also acknowledged.

This work was supported by Instituto de Salud Carlos III, Spanish Ministry of Science and Innovation (MICINN, FIS080302) and the European Commission (ERCUSYN PHP800200).

References

1 León-Carrión, J., Atutxa, A.M., Mangas, M.A. *et al.* (2009) A clinical profile of memory impairment in humans due to endogenous glucocorticoid excess. *Clinical Endocrinology*, **70**, 192–200.
2 Uno, H., Eisele, S., Sakai, A. *et al.* (1994) Neurotoxicity of glucocorticoids in the primate brain. *Hormones and Behavior*, **28**, 336–348.
3 Miller, E.K., Freedman, D.J. & Wallis, J.D. (2002) The prefrontal cortex: categories, concepts and cognition. *Philosophical Transactions of the Royal Society of London B*, **357**, 1123–1136.
4 Radley, J.J. & Sawchenko, P.E. (2011) A common substrate for prefrontal and hippocampal inhibition of the neuroendocrine stress response. *The Journal of Neuroscience*, **31**, 9683–9695.
5 De Kloet, R., Joëls, M. & Holsboer, F. (2005) Stress and the brain: from adaptation to disease. *Nature Reviews Neuroscience*, **6**, 463–475.
6 Coates, J.M. & Herbert, J. (2008) Endogenous steroids and financial risk taking on a London trading floor. *Proceedings of the National Academy of Science of the United States of America*, **105**, 6167–6172.
7 Putman, P., Antypa, N., Crysovergi, P. *et al.* (2010) Exogenous cortisol acutely influences motivated decision making in healthy young men. *Psychopharmacology (Berl)*, **208**, 257–263.
8 Santos-Ruiz, A., García-Rios, M.C., Fernández-Sánchez, J.C. *et al.* (2012) Can decision-making skills affect responses to psychological stress in healthy women? *Psychoneuroendocrinology*, **37**, 1912–1921.
9 Resmini, E., Santos, A., Gómez-Anson, B. *et al.* (2012) Verbal and visual memory performance and hippocampal volumes, measured by 3-tesla magnetic resonance imaging, in patients with Cushing's syndrome. *Journal of Clinical Endocrinology & Metabolism*, **97**, 663–671.
10 Tiemensma, J., Kokshoorn, N.E., Biermasz, N.R. *et al.* (2010a) Subtle cognitive impairment in patients with long-term cure of Cushing's disease. *Journal of Clinical Endocrinology & Metabolism*, **95**, 1–16.
11 Tiemensma, J., Biermasz, N.R., Middlekoop, H.A.M. *et al.* (2010b) Increased prevalence of psychopathology and maladaptive personality traits after long-term cure of Cushing's disease. *Journal of Clinical Endocrinology & Metabolism*, **95**, 129–141.
12 Tiemensma, J., Kaptein, A.A., Pereira, A.M. *et al.* (2011) Coping strategies in patients after treatment for functioning or nonfunctioning pituitary adenomas. *Journal of Clinical Endocrinology & Metabolism*, **96**, 964–971.
13 Pereira, J.B., Ibarretxe-Bilbao, N., Marti, M.J. *et al.* (2012) Assessment of cortical degeneration in patients with Parkinson's disease by voxel-based morphometry, cortical holding, and cortical thickness. *Human Brain Mapping*, **33**, 2521–2534.
14 Le Berre, A.P., Rauchs, G., La Joie, R. *et al.* (2014) Impaired decisión-making and brain shrinkage in alcoholism. *European Psychiatry*, **29**, 125–133.
15 Li, X., Lu, Z.L., D'Argembeau, A. *et al.* (2010) The Iowa gambling task in fMRI images. *Human Brain Mapping*, **31**, 410–423.
16 Bechara, A., Damasio, H. & Damasio, A.R. (2000a) Emotion, decision making and the orbitofrontal cortex. *Cerebral Cortex*, **10**, 295–307.
17 Bechara, A., Tranel, D. & Damasio, H. (2000b) Characterization of the decision-making deficits of patients with ventromedial prefrontal cortex lesions. *Brain*, **123**, 2189–2202.
18 Devinsky, O., Morrell, M.J. & Vogt, B.A. (1995) Contributions of anterior cingulated cortex to behavior. *Brain*, **118**, 279–306.
19 American Diabetes Association (2013) Standards of medical care in diabetes. *Diabetes Care*, **36**(Suppl 1), S1–S2.
20 Ho, K.K. and on behalf of the 2007 GH deficiency consensus workshop participants. (2007) Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: a statement of the GH Research Society in association with the European Society for Pediatric Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society, and Endocrine Society of Australia. *European Journal of Endocrinology*, **157**, 695–700.
21 Bruehl, H., Wolf, O.T., Sweat, V. *et al.* (2009) Modifiers of cognitive function and brain structure in middle-aged and elderly individuals with type 2 diabetes mellitus. *Brain Research*, **1280**, 186–194.
22 Popovic, V., Pekic, S., Pavlovic, D. *et al.* (2004) Hypopituitarism as a consequence of traumatic brain injury (TBI) and its possible relation with cognitive disabilities and mental distress. *Journal of Endocrinological Investigation*, **27**, 1048–1054.
23 Arnaldi, G., Angeli, A., Atkinson, A.B. *et al.* (2003) Diagnosis and complications of Cushing's syndrome: a consensus statement. *Journal of Clinical Endocrinology & Metabolism*, **88**, 5593–5602.
24 Brand, M., Labudda, K. & Markowitsch, H.J. (2006) Neuropsychological correlates of decision-making in ambiguous and risky situations. *Neural Networks*, **19**, 1266–1276.
25 Gupta, R., Duff, M.C., Denburg, N.L. *et al.* (2009) Declarative memory is critical for sustained advantageous complex decision-making. *Neuropsychologia*, **47**, 1686–1693.
26 Dale, A.M., Fischl, B. & Sereno, M.I. (1999) Cortical surface-based analysis: I. Segmentation and surface reconstruction. *Neuroimage*, **9**, 179–194.
27 Lancaster, J.L., Woldorff, M.G., Parsons, L.M. *et al.* (2000) Automated Talairach atlas labels for functional brain mapping. *Human Brain Mapping*, **10**, 120–131.
28 Segonne, F., Dale, A.M., Busa, E. *et al.* (2004) A hybrid approach to the skull stripping problem in MRI. *Neuroimage*, **22**, 1060–1075.
29 Fischl, B. & Dale, A.M. (2000) Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proceedings of the National Academy of Science of the United States of America*, **97**, 11050–11055.
30 Shafiei, N., Gray, M., Viau, V. *et al.* (2012) Acute stress induces selective alterations in cost/benefit decision-making. *Neuropsychopharmacology*, **37**, 2194–2209.
31 Joëls, M., Karst, H., Krugers, H.J. *et al.* (2007) Chronic stress: implications for neuronal morphology, function and neurogenesis. *Frontiers in Neuroendocrinology*, **28**, 72–96.
32 Wellman, C.L. (2001) Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. *Journal of Neurobiology*, **49**, 245–253.
33 Radley, J.J., Rocher, A.B., Miller, M. *et al.* (2006) Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. *Cerebral Cortex*, **16**, 313–320.

34 Mc Ewen, B.S. (2005) Glucocorticoids, depression and mood disorders: structural remodelling in the brain. *Metabolism*, **54**, 20–23.
35 Diamond, D.M., Campbell, A.M., Park, C.R. *et al.* (2007) The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-dodson law. *Neural Plasticity*, 2007, 0–33.
36 Resmini, E., Santos, A., Gómez-Anson, B. *et al.* (2013) Hippocampal dysfunction in cured Cushing's syndrome patients, detected by ^{1}H-MR-spectroscopy. *Clinical Endocrinology*, **97**, 663–671.
37 Clark, L., Bechara, A., Damasio, H. *et al.* (2008) Differential effects of insular and ventromedial prefrontal cortex lesions on risky decision-making. *Brain*, **131**, 1311–1322.
38 Williams, L.M. & Gordon, E. (2007) Dynamic organization of the emotional brain: responsivity, stability and instability. *The Neuroscientist*, **13**, 349–370.
39 Toffanin, T., Nifosi, F., Follador, H. *et al.* (2011) Volumetric MRI analysis of hippocampal subregions in Cushing's disease: a model for glucocorticoids neural modulation. *European Psychiatry*, **26**, 64–67.
40 Patil, C.G., Lad, S.P., Katznelson, L. *et al.* (2007) Brain atrophy and cognitive deficits in Cushing's disease. *Neurosurgery Focus*, **23**, E11.
41 Anderson, R.M., Birnie, A.K., Koblesky, N.K. *et al.* (2014) Adrenocortical status predicts the degree of age-related deficits in prefrontal structural plasticity and working memory. *The Journal of Neuroscience*, **34**, 8387–8397.
42 Brummelman, P., Sattler, M.G., Meiners, L.C. *et al.* (2012) Cognitive performance after postoperative pituitary radiotherapy: a dosimetric study of the hippocampus and the prefrontal cortex. *European Journal of Endocrinology*, **166**, 171–179.

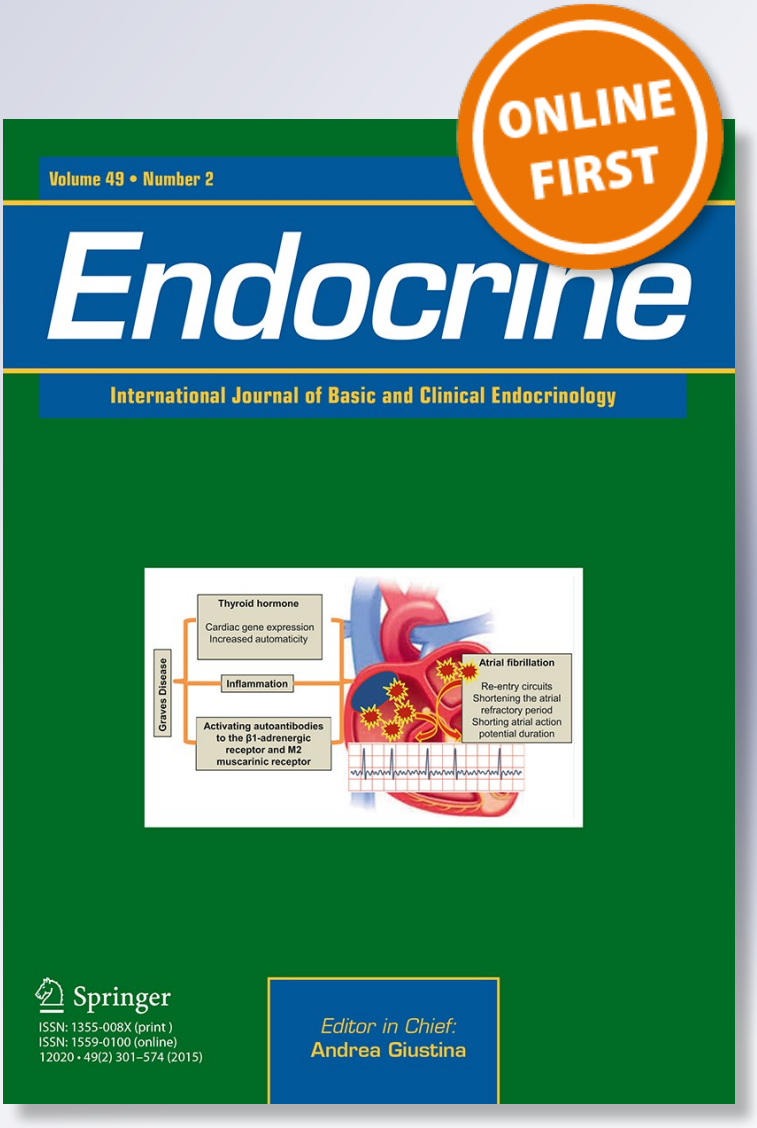
Impaired decision making and delayed memory are related with anxiety and depressive symptoms in acromegaly

Iris Crespo, Alicia Santos, Elena Valassi, Patricia Pires, Susan M. Webb & Eugenia Resmini

Endocrine
International Journal of Basic and Clinical Endocrinology

ISSN 1355-008X

Endocrine
DOI 10.1007/s12020-015-0634-6



Springer

Endocrine
DOI 10.1007/s12020-015-0634-6

ORIGINAL ARTICLE

Impaired decision making and delayed memory are related with anxiety and depressive symptoms in acromegaly

Iris Crespo[1] · Alicia Santos[1] · Elena Valassi[1] · Patricia Pires[2] · Susan M. Webb[1] · Eugenia Resmini[1]

Received: 11 March 2015 / Accepted: 14 May 2015

Abstract Evaluation of cognitive function in acromegaly has revealed contradictory findings; some studies report normal cognition in patients with long-term cured acromegaly, while others show attention and memory deficits. Moreover, the presence of affective disorders in these patients is common. Our aim was to evaluate memory and decision making in acromegalic patients and explore their relationship with affective disorders like anxiety and depressive symptoms. Thirty-one patients with acromegaly (mean age 49.5 ± 8.5 years, 14 females and 17 males) and thirty-one healthy controls participated in this study. The Iowa Gambling Task (IGT), Rey Auditory Verbal Learning Test, State-Trait Anxiety Inventory, and Beck Depression Inventory-II (BDI-II) were used to evaluate decision making, verbal memory, anxiety, and depressive symptoms, respectively. Acromegalic patients showed impairments in delayed verbal memory ($p < 0.05$) and more anxiety and depressive symptoms ($p < 0.05$) than controls. In the IGT, acromegalic patients presented an altered decision-making strategy compared to controls, choosing a lower number of the safer cards ($p < 0.05$) and higher number of the riskier cards ($p < 0.05$). Moreover, multiple correlations between anxiety and depressive symptoms and performance in memory and decision making were found. Impaired delayed memory and decision making observed in acromegalic patients are related to anxiety and depressive symptoms. Providing emotional support to the patients could improve their cognitive function. A key clinical application of this research is the finding that depressive symptoms and anxiety are essentially modifiable factors.

Keywords Acromegaly · Memory · Decision making · Depression · Anxiety · Cognitive function

Introduction

Acromegaly is a rare disease mainly caused by a growth hormone-producing pituitary adenoma. It is characterized by elevated plasma growth hormone (GH) and insulin-like growth factor I (IGF-1), which generate morphological changes, and physical and psychological limitations. The presence of affective disorders is also frequent in these patients [1–3]. The impact of the disease and its treatment on the patient's quality of life are great [4].

Under normal conditions, IGF-1 acts as a neuroprotective factor in the central nervous system [5]. Therefore, alterations in neurological function would not be expected after GH/IGF-1 exposure, but in a condition of chronic hormone excess, its effects could be paradoxically deleterious. Indeed, attenuation of electrophysiological brain activity has been described; Martín-Rodriguez et al. showed that acromegalic patients presented lower activity in the right dorsolateral prefrontal cortex and left parahippocampal cortex than healthy controls [6]. It is known that these areas are key in several cognitive functions, like memory and decision making.

✉ Eugenia Resmini
resminieugenia@libero.it

[1] Endocrinology/Medicine Department, Hospital Sant Pau, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBER-ER, Unidad 747), IIB-Sant Pau, ISCIII and Universitat Autònoma de Barcelona (UAB), Barcelona, Spain

[2] INNDACYT, CR Laureà Miró, 107, Sant Feliu de Llobregat, Barcelona, Spain

Published online: 28 May 2015

Evaluation of cognitive dysfunction in acromegalic patients yielded different findings. Tiemensma et al. reported normal cognitive function in patients with long-term cured acromegaly [3], but a worse delayed memory than in healthy controls was observed, with a no clear explanation. This last finding has been confirmed by several other studies [6–9]; moreover, deficits in attention and executive functions have also been reported [7–10].

Emotion and cognition are known to be related; especially memory and executive functions are very sensitive to emotional alterations [11]. An association between decision making and psychopathology such as anxiety and depression has been demonstrated [12–14]. The emotional status influences information processing and may disrupt longer term prioritization of punishment or reward-related information which is memorized [15]. In fact, even subjects with higher levels of trait anxiety (without psychopathology) can present intrusive memories and impaired decision making [16, 17]. It is therefore important to evaluate cognitive functions, like decision making, in patients with affective or anxiety disorders.

Affective disorders, psychological distress, maladaptive personality traits, anxiety-related traits, anxiety disorders, and major depression have been described in acromegalic patients [1–3, 18]. Specifically, after treatment, acromegalic patients showed more symptoms of affective lability, self-harm, oppositionality, anxiousness, apathy, and irritability than matched controls [3].

The aim of our study was to evaluate memory and decision making in patients with controlled acromegaly, and to explore their relationship with anxiety and depressive symptoms. Our main hypothesis was that alterations in affective processing, mainly anxiety and depression, would lead to impairments in memory and decision-making processes.

Materials and methods

Subjects

Thirty-one patients with acromegaly (mean age 49.5 ± 8.5 years, 14 females and 17 males) and thirty-one age-, gender-, and years of education-matched controls were included in this study. Patients with diabetes, GH deficiency, a history of drug abuse, and aged over 65 years were excluded.

Healthy controls were recruited from among blood bank donors of the hospital. Exclusion criteria for healthy controls were prior endocrine disease (including diabetes), a history of drug abuse, and any current or previous psychopathology. The study was approved by the hospital ethics committee, and written informed consent was obtained from all participants.

Twenty-four patients (77.4 %) had macroadenomas and 7 (22.6 %) had microadenomas. Twenty-nine patients had been treated with transsphenoidal neurosurgery and the remaining two had used somatostatin analogs as primary therapy. Ten patients (32 %) had also received postoperative radiotherapy. None were taking antidepressants or anxiolytics at the time of the study. Median duration of disease control was 61 (4–300) months. Median of delay to diagnosis after initial symptoms was 49 (1–240) months.

The patients were classified according to clinical control, based on consensus criteria [19]. Both GH and IGF-I were measured to assess the biochemical status after treatment (except in patients receiving pegvisomant therapy, in which case only IGF-I was measured). Biochemical control was defined as a normal IGF-I for age and gender and a GH <1.0 ng/ml after an OGTT.

Serum GH concentration was determined by a chemiluminescent immunometric assay (Immulite 2000®, Siemens Healthcare Diagnostics Products Ltd., Llanberis, UK) which uses the recombinant GH 98/574 calibrator, with an analytical sensitivity of 0.01 μg/L, and with intraassay and total CV of 2.9–4.6 % and 4.2–6.6 %, respectively. Serum IGF-I concentration was determined by a chemiluminescent immunometric assay (Immulite 2000®, Siemens Healthcare Diagnostics Products Ltd., Llanberis, UK) with an analytical sensitivity of 20 μg/L, and with intraassay and total CV of 2.3–3.9 and 3.7–8.1 %, respectively.

At the time of the current study, ten patients were biochemically controlled, on medical therapy (8 treated with somatostatin analogs alone and 2 with somatostatin analogs and pegvisomant). The remaining 21 were cured after surgery; of these, 9 patients required treatment for some degree of hypopituitarism, namely with L-thyroxine, hydrocortisone, gonadal steroids, and/or desmopressin.

The baseline clinical characteristics of study participants are shown in Table 1.

Decision making

Decision making was evaluated using the Iowa Gambling Task (IGT), focused on two components:

Evaluation of decision-making strategy

Subjects see four card deck groups (A, B, C, and D) on a computer screen. Each card group has different profiles of rewards (money wins) and punishments (money losses). Two card groups (A and B) are considered riskier and two card groups (C and D) are considered safer. Choosing the riskier card groups, subjects win more money in the short-term, but lose more money in the long-term. Choosing the safer card groups, subjects win less money in the short-term, but lose less money in the long run.

Table 1 Clinical characteristics of acromegalic patients and controls

	Acromegalic patients ($n = 31$)	Healthy controls ($n = 31$)
Gender (male/female)	17/14	17/14
Age (years)	49.5 ± 8.5	49 ± 7.5
Years of education	12.3 ± 3.8	12.84 ± 3.5
Duration of disease control (months)	61 (4–300)	
Medical treatment	$n = 10$	
Somatostatin analog therapy	10/10 (32 %)	
Pegvisomant therapy	2/10 (6.5 %)	
Postoperative radiotherapy	10/31 (32 %)	
Hypopituitarism	$n = 9$	
ACTH deficiency	6/9	
Gonadotrophins deficiency	5/9	
TSH deficiency	7/9	
ADH deficiency	1/9	

Subjects were asked to choose the options which would allow them to "win" more money, at the beginning of the study. A bar at the top of the screen changes according to the amount of money won or lost after each selection. The amount of money accumulated is shown in this bar as a feedback factor used to regulate their choices. An increase in both the amount of lost money and the number of riskier cards reflects a poorer decision-making function.

Evaluation of learning during the test

The IGT consists of 100 card choices. These choices are divided into 5 blocks of 20 cards each. The 5 blocks reflect a subject's learning evolution in the decision-making process during the test. The total score for each block is calculated as the difference between advantageous selections (the number of card selections from groups C and D) and disadvantageous selections (the number of selections from groups A and B). This score reflects the ability to learn the correct strategies and the feedback-regulating performance during the IGT.

Verbal memory

Rey Auditory Verbal Learning Test (RAVLT) is one of the most widely used tests to evaluate verbal memory. The RAVLT consists of (1) presentation of a 15-word list (list A) in five learning trials, (2) a single presentation of an interference 15-word list (list B), (3) a postinterference recall trial, and (4) after 30 min, subjects are asked to recognize the words of both lists (list A and list B). The score for each trial is the number of words correctly recalled. Its evaluation is focussed on the following:

Immediate memory

It refers to the capacity to retain information for a short period of time. It was evaluated with the scores of "five learning trials" and the score of the "interference trial."

Delayed memory

It refers to the capacity to retain information for a long period of time. It was evaluated with the scores of the "postinterference recall trials" and the "recognition trials."

Anxiety and depressive symptoms

Beck depression inventory-II

Beck Depression Inventory-II (BDI-II) is a self-reported measure of the severity of depressive symptoms. It has 21 items with a four-point scale ranging from 0 to 3. The total score is the sum of each item-rating and can range from 0 to 63. Higher scores indicate more severe depressive symptoms. Scores 0–13 indicate minimal depression, 14–19 indicate mild depression, 20–28 indicate moderate depression, and 29–63 indicate severe depression. The BDI-II can be separated into affective and somatic dimensions.

State-trait anxiety inventory

State-Trait Anxiety Inventory (STAI) is a self-reported measure that includes two subscales to evaluate two types of anxiety: state anxiety (anxiety related to current events) and trait anxiety (anxiety as a personal characteristic). Each subscale has 20 questions with a four-point scale ranging from 0 to 3. The total score for each subscale is the sum of

each item-rating and can range from 0 to 60. Higher scores indicate higher levels of anxiety.

Statistical analysis

Data were analyzed using SPSS 19.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA) with a level of significance of $p < 0.05$. All quantitative data are expressed as mean ± SD (Gaussian distribution) or as median (range) (non-Gaussian distribution). Data distribution was analyzed by the Kolmogorov–Smirnov test. Comparisons between the two groups were performed using Mann–Whitney's U test for depression score, Student's t test for anxiety score, and card choices of the IGT and repeated measures ANOVA for the blocks of the IGT. Moreover, two MANOVA analyses were performed for RAVLT, one for short-term memory (including scores of trial 1, trial 5, and interference trial) and another for long-term memory (including scores of recall trial, recognition trial A and recognition trial B). Correlations among variables were studied using Pearson's correlation coefficient for parametric measures and Spearman's rho for non-parametric measures. Furthermore, two multiple linear regression analyses were performed in order to investigate the role of anxiety and depression on memory and decision making.

Results

Comparison between acromegalic patients and controls

Iowa gambling task (IGT)

Acromegalic patients showed impaired decision making compared to controls as reflected by a smaller number of safe cards (51.2 ± 15.3 vs. 60.6 ± 13.6, $p < 0.05$), and a higher number of riskier cards (48.8 ± 15.3 vs. 39.3 ± 13.6, $p < 0.05$) were chosen (Fig. 1).

With regard to the blocks, a repeated measures ANOVA (blocks 1–5) showed a significant effect between the blocks ($F = 3.512$, $p < 0.05$) and between the groups ($F = 6.493$, $p < 0.05$). However, the interaction of blocks × groups was not significantly different, indicating that the significant differences observed between patients and controls in general performance of IGT disappear when the groups are compared in each individual blocks of the test (Fig. 2).

Rey Auditory Verbal Learning Test (RAVLT)

The MANOVA for long-term memory (including Recall trial, Recognition trial A and Recognition trial B) showed a significant group effect ($F = 4.22$, $p < 0.01$). Specifically, acromegalic patients showed worse scores than controls in the recall trial ($F = 6.303$, $p < 0.05$) and the recognition trial A ($F = 12.941$, $p < 0.01$) of RALVT. No differences

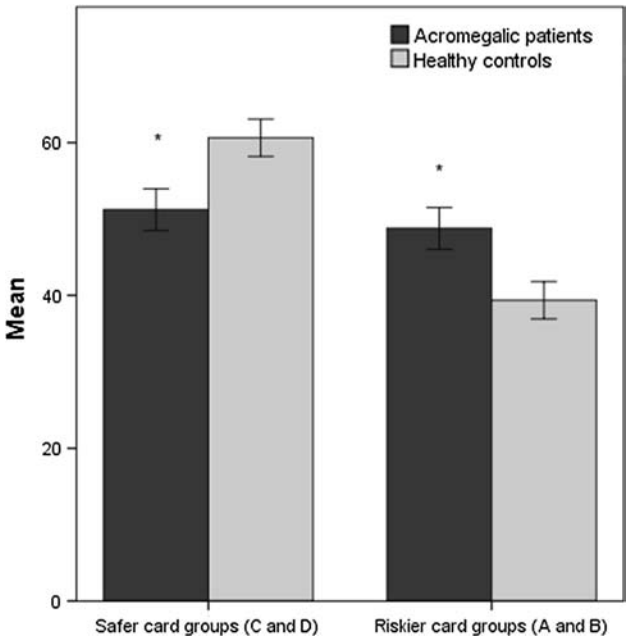


Fig. 1 Number of riskier or safer cards chosen in the Iowa Gambling Task (IGT) by acromegalic patients and controls. *= $p < 0.05$

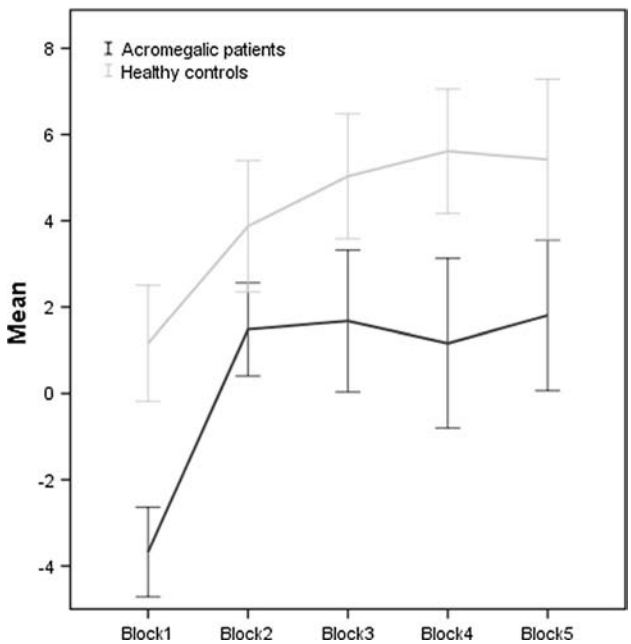


Fig. 2 IGT learning curves in acromegalic patients and healthy controls. Even though the performance was different between patients and controls (repeated measures ANOVA, $F = 6.493$, $p < 0.05$), no differences were observed between any of the five blocks of both groups. The learning curves consist of the score (the number of advantageous selections minus the number of disadvantageous selections) within each block of cards of the IGT (Iowa gambling task)

were observed between acromegalic patients and controls in the MANOVA for short-term memory (including Trial 1, Trial 5, and Interference trial). See Table 2 for details.

Beck depression inventory-II (BDI-II)

Total score of BDI-II was significantly higher in acromegalic patients compared to controls [9 (0–27) vs. 1 (0–17), $p < 0.001$]. Acromegalic patients showed more affective and somatic symptoms than controls [affective, 3(0–18) vs. 1(0–12); somatic, 5(0–11) vs. 1(0–5); $p < 0.001$ for both comparisons] (Fig. 3).

Twenty-six acromegalic patients (84 %) showed minimal depression, 1 (3 %) showed mild depression, 3 (10 %) showed moderate depression, and 1 (3 %) showed severe depression.

State-trait anxiety inventory (STAI)

Acromegalic patients showed both higher state and trait anxiety scores than controls (state, 16.6 ± 11.4 vs. 9.5 ± 6; trait, 20.3 ± 10.2 vs. 11.5 ± 5.6, $p < 0.01$ and $p < 0.001$, respectively).

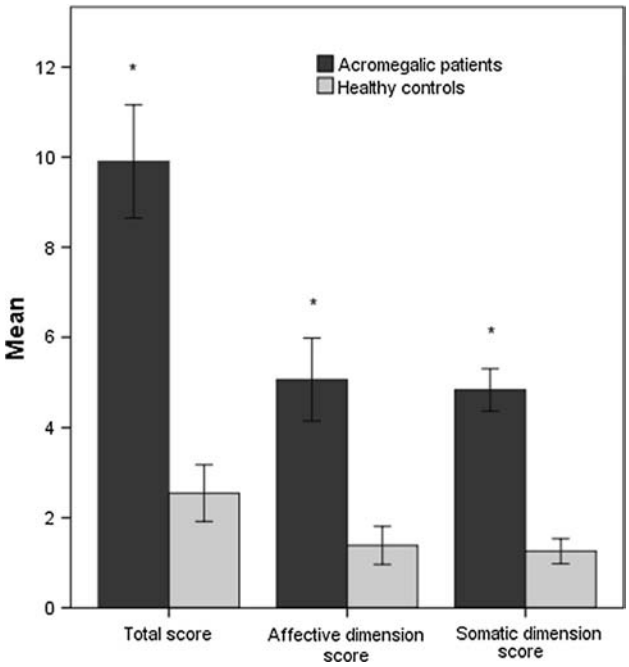


Fig. 3 Total score, affective dimension score, and somatic dimension score in the Beck Depression Inventory-II (BDI-II) in acromegalic patients and controls. *= $p < 0.05$

Table 2 Mean and SD of evaluated tests in acromegalic patients and controls

	Acromegalic patients	Healthy controls	*p* value
Iowa gambling task			
Safer card choices	51.2 ± 15.3	60.6 ± 13.6	<0.05
Riskier card choices	48.8 ± 15.3	39.3 ± 13.6	<0.05
Block 1 score	−3.7 ± 5.8	1.2 ± 7.5	NS
Block 2 score	1.5 ± 6	3.9 ± 8.5	NS
Block 3 score	1.7 ± 9.1	5 ± 8	NS
Block 4 score	1.2 ± 10.9	5.6 ± 8	NS
Block 5 score	1.8 ± 9.7	5.4 ± 10.4	NS
Rey auditory learning verbal test			
Short-term memory			
Score of trial 1	5.5 ± 1.7	5.8 ± 1.3	NS
Score of trial 5	11 ± 2.4	12 ± 2.3	NS
Score of interference trial	4.7 ± 1.3	5.3 ± 2.2	NS
Long-term memory			
Score of recall trial	8.8 ± 2.4	10.4 ± 2.6	<0.05
Score of recognition trial A	11.7 ± 2.3	13.5 ± 1.5	<0.01
Score of recognition trial B	7.8 ± 3.1	9.1 ± 2.9	0.08
Beck depression inventory-II			
Total score	9 (0–27)	1(0–17)	<0.001
Affective dimension score	3 (0–18)	1(0–12)	<0.001
Somatic dimension score	5 (0–11)	1(0–5)	<0.001
State-trait anxiety inventory			
State anxiety score	16.6 ± 11.4	9.5 ± 6	<0.01
Trait anxiety score	20.3 ± 10.2	11.5 ± 5.6	<0.001

Four patients (13 %) scored above normative cutoff for anxiety trait and three (10 %) scored above normative cutoff values for anxiety state, indicating severe anxiety problems.

No differences in the tests were found between acromegalic patients with and without hypopituitarism.

Relationships between tests

Correlations between RAVLT, BDI-II, STAI, and IGT

Multiple correlations were found between memory, anxiety, and depressive symptoms. The RALVT was negatively correlated with affective dimension, somatic dimension, and total score of BDI-II and trait and state anxiety of STAI. Pearson correlations and p values are shown in Table 3. When these variables were included in a linear regression model, trait anxiety still predicted RAVLT score ($\beta = -0.285$, $R = 0.285$; $p < 0.05$). All this means that anxiety and depressive symptoms influence memory consolidation (delayed memory).

Regarding decision making, the IGT was negatively correlated with affective dimension of BDI-II and state anxiety of STAI. Pearson correlations and p values are shown in Table 3.

Moreover, the trial 5 of RAVLT (the last learning trial) was correlated with safer card choices ($r = 0.268$, $p < 0.05$), riskier card choices ($r = -0.268$, $p < 0.05$), block 4 ($r = 0.325$, $p = 0.01$), and block 5 ($r = -0.245$, $p = 0.05$) of IGT. All this indicates that affective alterations and learning capacity influence decision making.

Including depression, anxiety, memory, and clinical variables in a linear regression model, the main determinant factor for IGT performance was the score of trial 5 of the RAVLT ($\beta = 0.316$, $R = 0.316$; $p < 0.05$), a measure of learning capacity in a short period of time.

Correlations between evaluated tests and clinical parameters

Delay to diagnosis was inversely correlated with score in Trial 5 ($r = -0.447$, $p < 0.05$) and Recognition trial B (r = −0.491, $p < 0.01$) of RAVLT, while duration of control was inversely correlated with score in Trial 1 ($r = -0.426$, $p < 0.05$) and Recall trial ($r = -0.363$, $p < 0.05$). There were no relationships between delay to diagnosis and duration of control and the other evaluated tests. No relationships were found between evaluated tests and hormonal values or other clinical variables, excepting a positive correlation between anxiety state and heart rate at the moment of evaluation ($r = 0.386$, $p < 0.01$).

Discussion

Our study indicates that acromegalic patients present impairments in decision-making strategies and delayed memory, which are related to anxiety and depressive

Table 3 Correlations between tests of cognitive functions (Iowa Gambling Task—IGT, and Rey Auditory Learning Verbal Test—RAVLT) and questionnaires of depression and anxiety (Beck Depression Inventory-II—BDI II, and State-Trait Anxiety Inventory—STAI)

	BDI-II total	BDI-II affective dimension	BDI-II somatic dimension	STAI state anxiety	STAI trait anxiety
RAVLT					
Short-term memory					
Trial 1	NS	NS	NS	NS	NS
Trial 5	NS	$r = -0.33$**	NS	$r = -0.26$*	$r = -0.28$*
Interference trial	$r = -0.33$**	$r = -0.34$**	$r = -0.35$**	$r = -0.26$*	$r = -0.34$*
Long-term memory					
Recall trial	$r = -0.28$*	$r = -0.28$*	$r = -0.28$*	$r = -0.33$*	$r = -0.32$*
Recognition trial A	$r = -0.33$**	$r = -0.26$*	$r = -0.36$**	$r = -0.35$**	$r = -0.35$**
Recognition trial B	NS	NS	NS	NS	NS
IGT					
Safer card choices	NS	$r = -0.28$*	NS	NS	NS
Riskier card choices	NS	$r = -0.28$*	NS	NS	NS
Block 1	NS	NS	NS	NS	NS
Block 2	NS	NS	NS	NS	NS
Block 3	NS	NS	NS	$r = -0.33$**	NS
Block 4	NS	NS	NS	$r = -0.36$*	NS
Block 5	NS	NS	NS	NS	NS

* $p < 0.05$; ** $p < 0.01$

symptoms. As far as we know, these are the first results suggesting that acromegalic patients showed affective alterations that influence cognitive function, mainly memory consolidation (delayed memory) and decision making.

Multiple processes determine decision-making performance. Learning and memory processes are an intrinsic task to evaluate the risks of a decision [20]. Decision making requires the knowledge of the risk/benefit ratio, the ability to retrieve the information from memory, and the ability to hold the data in mind while comparing and contrasting them. The association of riskier profiles with particular card groups is an important component of the IGT, because information on outcomes of the card groups in the IGT must be dynamically updated over time. Moreover, several data suggest that mood and affect can influence the decision-making processes because regulation of punishment–reward cues may not be effectively engaged under state of anxiety or depression [15, 21]. In fact, depressed patients with less severe symptoms and persons with a higher negative mood may show impairments of the IGT [22, 23]. Along this line, affective state could be a key factor for better decision making and memory in acromegalic patients. Therefore, providing emotional support to the patients, as psychological therapy, could be a way to improve their cognitive function and an important clinical implication for further research.

The majority of studies reported non-significant associations between IGT performance and intelligence quotient (IQ) [24]. Neuropsychological indexes of other executive functions and intelligence leave a large amount of unexplained variance in the IGT performance. Toplak et al. proposed that impaired performance on the IGT may be due to problems in three cognitive processes: implicit and instrumental learning, "overlearned" associations, and processes of behavioral regulation by emotions [24]. Thus, we could speculate that acromegalic patients failed in some or all these cognitive processes.

Consideration of brain connectivity is also important to understand potential cognitive–emotional interactions and integration. Prefrontal cortex has been considered a key region that integrates emotional and cognitive information coming from multiple regions such as the anterior cingulate cortex, amygdala, and hippocampus [11]. The hippocampus contributes to the long-term persistence of emotionally significant information, and the accumulated effects of distress may disrupt its networks [15]. In this condition, prefrontal systems for regulation of punishment–reward cues may not be effectively engaged [15]. Therefore, convergent evidence points to the role of memory in evaluation of emotionally significant information and decision-making processes.

Our results showed that delay in diagnosis was inversely correlated with RAVLT scores, indicating that a longer delay is associated with worse short-term and long-term memory, whereas duration of control was also inversely correlated with RAVLT scores, indicating that a longer disease control is associated with worse short-term and long-term memory. The first correlation is more obvious than the second, because delay in diagnosis can impair memory processes. This is another finding that underlines the importance of an earlier diagnosis in acromegaly. The second correlation is intriguing, since on the one hand, it is known that age worsens memory function, while on the other hand, control of disease improves comorbidities. The message from our data could be that the patients should be controlled as soon as possible, because the positive effect of the control could be lost due to aging. Further longitudinal studies are needed to clarify the point.

Affective disorders like anxiety and depression are common in chronic and disabling diseases. Nevertheless, an increased rate of lifetime affective disorders, mainly major depression episodes (9.9 %) and dysthymia (16.1 %), and psychological distress are described in acromegalic patients more frequently than in patients with chronic somatic disorders [2]. It remains unclear whether the increased risk of affective disorders in acromegaly is due to effects of GH and IGF-1 excess on brain function, or whether these patients are distressed and are more prone to develop psychopathology, because of their illness, their associated morbidity and/or treatment. Interestingly, a recent study showed that reduction of quality of life is driven dominantly by psychopathology (anxiety and depression) rather than other biochemical factors in acromegalic patients [25]. This finding emphasizes our conclusion, adding importance to the measures of affective disorders rather than only biochemical variables, in order to improve patient management and control.

It is known that psychopathological variables are candidate modifiable factors to link pituitary disease, especially acromegaly, to a lower health-related quality of life [25]. A key clinical application of this research would be that depressive symptoms and anxiety, related with decision making, are essentially modifiable factors. A more complex treatment strategy including an extensive psychopathological evaluation and therapy may be an attractive possibility to improve patient management. The neuropsychology has reached an emerging role in the last decade, due to the numerous studies demonstrating neuropsychological impairments in these patients. Therefore, it would be worth to have a neuropsychologist in the multidisciplinary team who treat pituitary diseases. Finally, patient associations can give additional emotional support.

There are some limitations in our study. The cross-sectional design limits any causal inference. The patient group is small in size and clinically heterogeneous (i.e., cured after surgery or controlled with therapy, treated hypopituitarism), but acromegaly is a rare disease and it is difficult to attain large sample sizes. The possible effects of

prior radiotherapy and medical treatment could be postulated; however, radiotherapy and medical treatment were not predictors for decision making in the linear regression analysis. Moreover, current medical treatment and radiotherapy in acromegalic patients were not found to be related with memory and executive functioning [26, 27].

In conclusion, acromegalic patients show impairments of decision making and delayed memory related to anxiety and depressive symptoms, present even after disease control. Since mood alterations and affective disorders are frequently described in acromegaly, evaluation of cognitive function, such as decision making or delayed memory, may be recommended.

Acknowledgments We thank the patients and controls for collaborating in this study. This work was supported by a grant from the Instituto de Salud Carlos III, Spanish Ministry of Science and Innovation (MICINN, FISPI080302).

Disclosure The authors declare that they have no conflict of interest.

References

1. N. Sonino, C. Ruini, C. Navarrini, F. Ottolini, L. Sirri, A. Paoletta, F. Fallo, M. Boscaro, G.A. Fava, Psychosocial impairment in patients treated for pituitary disease: a controlled study. Clin. Endocrinol. **67**, 719–726 (2007)
2. C. Sievers, C. Dimopoulou, H. Pfister, R. Lieb, B. Steffin, J. Roemmler, J. Schopohl, M. Mueller, H.J. Schneider, M. Ising, H.U. Wittchen, G.K. Stalla, Prevalence of mental disorders in acromegaly: a cross-sectional study in acromegalic patients. Clin. Endocrinol. **71**, 691–701 (2009)
3. J. Tiemensma, N.R. Biermasz, R.C. van der Mast, M.J. Wassenaar, H.A. Middelkoop, A.M. Pereira, J.A. Romijn, Increased psychopathology and maladaptive personality traits, but normal cognitive functioning, in patients after long-term cure of acromegaly. J. Clin. Endocrinol. Metab. **95**, E392–E402 (2010)
4. P. Pantanetti, N. Sonino, G. Arnaldi, M. Boscaro, The quality of life in acromegaly. J. Endocrinol. Invest. **26**, 35–38 (2003)
5. N.D. Aberg, K.G. Brywe, J. Isgaard, Aspects of growth hormone and insulin-like growth factor-1 related to neuroprotection, regeneration and functional plasticity in the adult brain. ScientificWorldJournal. **6**, 53–80 (2006)
6. J.F. Martín-Rodríguez, A. Madrazo-Atutxa, E. Venegas-Moreno, P. Benito-Lopez, M.A. Galvez, D.A. Cano, F.J. Tinahones, E. Torres-Vela, A. Soto-Moreno, A. Leal-Cerro, Neurocognitive function in acromegaly after surgical resection of GH-secreting adenoma versus naïve acromegaly. PLoS ONE **8**, e60041 (2013)
7. C. Sievers, P.G. Samann, H. Pfister, C. Dimopoulou, M. Czisch, J. Roemmler, J. Schopohl, G.K. Stalla, J. Zihl, Cognitive function in acromegaly: description and brain volumetric correlates. Pituitary. **15**, 350–357 (2012)
8. P. Brummelman, J. Koerts, R.P. Dullaart, G. van den Berg, O. Tucha, B.H. Wolffenbuttel, A.P. van Beek, Effects of previous growth hormone excess and current medical treatment for acromegaly on cognition. Eur. J. Clin. Invest. **42**, 1317–1324 (2012)
9. C.G. Yedinak, M. Fleseriu, Self-perception of cognitive function among patients with active acromegaly, controlled acromegaly, and non-functional pituitary adenoma: a pilot study. Endocrine **46**, 585–593 (2014)
10. K. Müssig, B. Besemer, R. Saur, S. Klingberg, H.U. Häring, B. Gallwitz, T. Leyhe, Deteriorated executive functions in patients with successful surgery for pituitary adenomas compared with other chronically ill patients. J. Int. Neuropsychol. Soc. **17**, 369–375 (2011)
11. L. Pessoa, On the relationship between emotion and cognition. Nat. Rev. Neurosci. **9**, 148–158 (2008)
12. M. Kirsch, S. Windmann, The role of anxiety in decision making. Rev. Psychol. **16**, 19–28 (2009)
13. M.P. Paulus, A.J. Yu, Emotions and decision-making: affects-driven belief systems in anxiety and depression. Trends Cog. Sci. **16**, 476–483 (2012)
14. O.J. Robinson, R.L. Bond, J.P. Roiser, The impact of stress on financial decision-making varies as a function of depression and anxiety symptoms. PeerJ. **3**, e770 (2015)
15. L.M. Williams, E. Gordon, Dynamic organization of the emotional brain: responsivity, stability and instability. Neuroscientist. **13**, 349–370 (2007)
16. T. Marzi, A. Regina, S. Righi, Emotions shape memory suppression in trait anxiety. Front. Psychol. **4**, 1001 (2014)
17. A.C. Miu, R.M. Heilman, D. Houser, Anxiety impairs decision-making: psychophysiological evidence from an Iowa gambling task. Biol. Psychol. **77**, 353–358 (2008)
18. C. Sievers, M. Ising, H. Pfister, C. Dimopoulou, H.J. Schneider, J. Roemmler, J. Schopohl, G.K. Stalla, Personality in patients with pituitary adenomas is characterized by increased anxiety related traits: comparison of 70 Acromegalic patients to patients with non-functioning pituitary adenomas and age and gender matched controls. Eur. J. Endocrinol. Metab. **160**, 367–373 (2009)
19. S. Melmed, A. Colao, A. Barkan, M. Molitch, A.B. Grossman, D. Kleinberg, D. Clemmons, P. Chanson, E. Laws, J. Schlechte, M.L. Vance, K. Ho, A. Giustina, Guidelines for acromegaly management: an update. J. Clin. Endocrinol. Metab. **94**, 1509–1517 (2009)
20. P. Whitney, J.M. Hinson, The role of source memory in gambling task decision making. J. Clin. Exp. Neuropsychol. **34**, 826–835 (2012)
21. M.T. Buelow, J.A. Suhr, Construct validity of the Iowa Gambling Task. Neuropsychol. Rev. **19**, 102–114 (2009)
22. A. Must, Z. Szabó, N. Bódi, A. Szász, Z. Janka, S. Kéri, Sensitivity to reward and punishment and the prefrontal cortex in major depression. J. Affect. Disord. **90**, 209–215 (2006)
23. J.A. Suhr, J. Tsanadis, Affect and personality correlates of the Iowa Gambling Task. Pers. Indiv. Diff. **43**, 27–36 (2007)
24. M.E. Toplak, G.B. Sorge, A. Benoit, R.F. West, K.E. Stanovich, Decision-making and cognitive abilities: a review of associations between Iowa Gambling Task performance, executive functions and intelligence. Clin. Psychol. Rev. **30**, 562–581 (2010)
25. V.J. Geraedts, C. Dimopoulou, M. Auer, J. Schopohl, G.K. Stalla, C. Sievers, Health outcomes in acromegaly: depression and anxiety are promising targets for improving reduced quality of life. Front. Endocrinol. **5**, 229 (2015)
26. P. Brummelman, M.G.A. Sattler, L.C. Meiners, M.F. Elderson, R.P.F. Dullaart, G. van den Berg, J. Koerts, O. Tucha, B.H.R. Wolffenbuttel, A.C.M. van den Berg, A.P. van Beek, Cognitive performance after postoperative pituitary radiotherapy: a dosimetric study of the hippocampus and the prefrontal cortex. Euro. J. Endocrinol. **166**, 171–179 (2012)
27. P. Brummelman, J. Koerts, R.P.F. Dullaart, G. van den Berg, O. Tucha, B.H.R. Wolffenbuttel, A.P. van Beek, Effects of previous growth hormone excess and current medical treatment for acromegaly on cognition. Eur. J. Clin. Invest. **42**, 1317–1324 (2012)