The somatotropic axis: Effects on brain and cognitive functions

De somatotrope as:
Effecten op de hersenen en cognitieve functies
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op vrijdag 31 augustus 2012 des middags te 12.45 uur

door

Elisabetta Hendrika Quik

geboren op 21 augustus 1980

te Harderwijk, Nederland

Promotor:

Prof.dr. J.L. Kenemans

Co-promotoren:

Dr. P.S. van Dam

Dr. K.B.E. Böcker

De onderzoeken die in dit proefschrift worden beschreven werden (mede) mogelijk gemaakt dankzij financiële steun van de Stichting Neuroendocrinologie van Gedrag en Pfizer B.V.

The somatotropic axis: Effects on brain and cognitive functions

Elise H. Quik

PhD thesis, Experimental Psychology and Psychpharmacology, Utrecht University

ISBN: 978-90-889-1441-6

Printed and published by: Uitgeverij BOXPress || Proefschriftmaken.nl

Contents

Chapter 1

The somatotropic axis: Effects on brain and cognitive functions

Introduction

The somatotropic axis (GH/IGF-1 axis)

The somatotropic axis refers to the hormonal signaling from hypothalamus to anterior pituitary gland resulting in the release of growth hormone (GH), which in turn stimulates the production of insulin-like growth factor-1 (IGF-1) in the liver (Figure 1). The synthesis and release of GH from the pituitary are primarily controlled by the hypothalamic hormones GH-releasing hormone (GHRH) and somatostatin (SRIF), which in turn are regulated by feedback from blood GH and IGF-1 concentrations. The production of GH is stimulated by GHRH and by amino-acids such as arginine. Another way to increase GH and IGF-1 levels is by means of endogenous gastric secretion of acylated ghrelin (Kojima and Kangawa, 2010) or the administration of active GH secretagogues (Arwert et al., 2005) such as GH releasing peptides -2 or -6 (GHRP-2 or GHRP-6), which activate the ghrelin receptor.

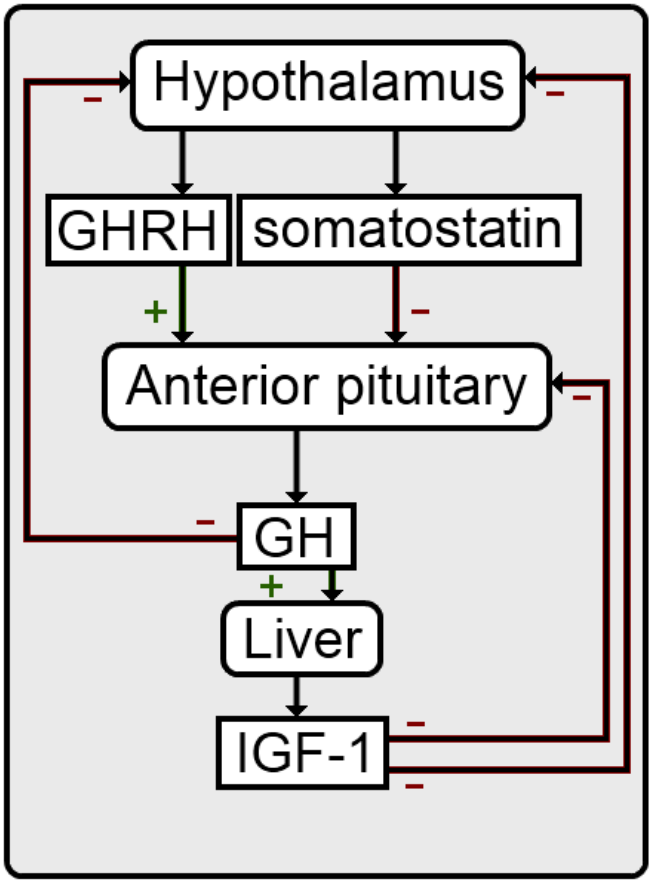


Figure 1 The somatotropic axis and its feedback loop
GHRH stimulates (+) growth hormone release from the anterior pituitary, which stimulates (+) the liver to produce IGF-1, IGF-1 inhibits (-) hypothalamic GHRH and pituitary GH release

GH or somatotropin is produced and released in a pulsatile manner, mostly at night during slow wave sleep by somatotroph cells, cells of the anterior lobe of the pituitary gland. As a consequence, GH plasma levels show high variability. In contrast, plasma IGF-1 levels are more stable, making this an acceptable marker of GH secretion despite several limitations such as a limited correlation between GH secretion and plasma IGF-1 under physiological conditions, especially in older adults (Aleman et al., 1999), or due to reduced IGF-1 synthesis in the liver in medical conditions such as type 1 diabetes, obesity or liver disease. Whereas GH stimulates the liver to release IGF-1, IGF-1 inhibits the release of GH from the anterior pituitary gland.

IGF-1 and GH can both cross the blood-brain barrier and bind to their receptors in the central nervous system (CNS). GH receptors have been found in several locations in the brain. Studies of the anatomical distribution of GH receptors in the human brain have demonstrated binding sites particularly in the cortex, hippocampus, amygdala, putamen, hypothalamus, and most prominently in the choroid plexus (Oertel et al., 2004; Nyberg and Burman, 1996; Johansson en Bengtsson, 1997).

IGF-1 receptors are localized in neurons and glia throughout the brain (Garcia-Segura et al., 1997), with the highest concentrations in the hippocampus, amygdala, and parahippocampal gyrus (van Dam et al., 2005).

GH deficiency: pathophysiological effects

The role of GH becomes especially apparent when looking at patients with GH deficiency (GHD). GHD is the medical condition of inadequate production of GH, usually as the consequence of pituitary or hypothalamic disease. To properly diagnose GHD, several GH stimulation tests are currently used, such as the GHRH-arginine test, the GHRH-GH releasing peptide-6 (GHRP6) test, and the insulin tolerance test (ITT). GHD has been found to produce different physical symptoms, such as decreased lean body and muscle mass, increased fat mass, reduced bone mineral density, lipid profile changes, and reduced vitality and energy levels (Arwert et al., 2005). Another study showed reduced muscle strength as well as reduced exercise capacity, energy expenditure, basal metabolic rate and myocardial function (Soares et al., 1999). In yet another study, symptoms such as hypertension, abdominal obesity, insulin resistance, dyslipidemia, enhanced activity of thrombotic factors and an increased risk for cardiovascular mortality were found (Abs et al., 2005).

GHD can be observed both in children and in adults. Adult GHD can have its onset in childhood or later in life in adulthood (AO GHD). Childhood onset GHD (CO GHD) is usually easier to detect because it normally results in retarded physical growth. In general, the peripheral and central effects of AO GHD may be more subtle than effects of GHD during childhood.

In addition to the physical symptoms listed above, GHD in adulthood (both CO and AO) is associated with adverse changes in: reports of a "sub optimal well-being", higher rates of unemployment, low levels of self-esteem, emotional instability, a tendency towards depression, and social isolation (Soares et al., 1999). Furthermore, CO GHD is associated with impaired intelligence (Almqvist et al. 1986; Degerblad et al. 1990; Sartorio et al., 1995).

GHD can occur either isolated (IGHD) or in combination with other pituitary or hypothalamic hormone deficits (multiple hormone deficiency, MHD). Of all GHD patients, 9.6% has been found to have IGHD (Abs et al., 2005).

While the effect of GH substitution on growth in children has been known for several decades, attention has been drawn to the symptomatology of GHD in adults and potential benefits of GH substitution therapy after the development of recombined human GH (RH-GH). The reversal of most of the symptoms associated with GHD in adults has been demonstrated in a number of studies (Dattani and Preece, 2004). For example, 6 months of daily GH treatment was significantly better than placebo at producing increased serum IGF-1 levels, reduced body mass index (BMI) and body fat, increased lean body mass and water, reduced waist/hip ratio and increased energy (Soares et al., 1999). At present, daily subcutaneous GH substitution therapy is generally accepted in symptomatic adult GHD patients for the prevention of long-term sequelae such as central obesity, osteopenia and cardiovascular disease, but also for the improvement of energy levels, general well-being and quality of life.

The somatotropic axis and quality of life

Both GH and IGF-1 contribute to cellular growth, development and recovery. In the CNS the somatotropic axis stimulates neuron growth, growth of glial cells and myelination. This has led to the hypothesis that GH contributes to mental function and development. Therefore, cognitive and behavioral functions have been studied in GHD subjects before and after GH substitution. Behavioral deficits associated with GHD are, among others, decreased mental energy, increased anxiety, and dissatisfaction with body image and reduction in quality of life (Abs et al., 2005). Quality of life (QoL) is defined as the total level of physical, emotional, social and psychosocial functioning assessed from the patients' perspective. The Quality of Life Assessment of Growth Hormone Deficiency in Adults (QoL-AGHDA) is especially designed to assess such aspects of GHD. The study of Koltowska-Häggström et al. (2006) reports QoL-AGHDA normative values for the population of England and Wales and confirms the extent of QoL impairment in patients with GHD in comparison with the general population. More severe impairment in QoL is reported by patients with AO GHD compared to CO GHD (Murray et al., 1999). In

contrast, other data indicate that cognitive functioning is disturbed to a larger extent in patients with CO- GHD than in those who become GH deficient during adult life (Van Dam et al., 2000).
With respect to GH therapy, Deijen et al. (2005) reported in a meta-analysis that 6-months GH replacement, relative to placebo, improved subjective well-being the most, followed by health status and QoL.

The somatotropic axis and cognitive function

GHD and cognition

Cognitive deficits globally associated with GHD concern memory, attention and executive functions (Abs et al., 2005; Falleti et al., 2006). The hippocampus, a brain structure crucial to learning and memory, the parahippocampal areas, and the prefrontal cortex have high concentrations of GH and IGF-1 receptors. This suggests that cognitive functions regulated by these areas, such as attention, memory, processing speed, and executive functions are affected the most by GHD and GH treatment (Falleti et al., 2006).
Executive functions are cognitive abilities that control and regulate goal-directed behaviors, such as planning, cognitive flexibility, abstract thinking, decision-making, rule acquisition, delaying a reward, initiating appropriate actions and inhibiting inappropriate actions, and selecting relevant sensory information. The latter process is also called selective attention, which enables focusing on specific information present in the stream of information that surrounds us. Its relation to GH is reviewed in chapter 2.
Other previous studies found associations between GHD and impairment in cognitive functions such as attenuated learning and memory performance (Baum et al., 1998; Peace et al., 1998; Bulow et al., 2002), long-term memory (Deijen et al., 1998), spatial learning (Bulow et al., 2002) and intelligence (Almqvist et al., 1986; Degerblad et al., 1990; Sartorio et al., 1995). GHD was also found to be associated with deficits in executive function (Peace 1998), perceptual-motor performance (Deijen et al., 1996), mental speed (Deijen et al., 1996) and attention (Lijffijt et al., 2003).
In the clinical domain, cognitive impairments of untreated GH-deficient adults can be reduced by GH replacement therapy. Using recombinant human GH, improvements in cognitive functions following 6 months of daily administration have been reported in adults with CO GHD in an open-label study (Sartorio et al., 1995). Similar global improvements have been found in placebo-controlled trials and in young adults with GHD (Almqvist et al., 1986), CO GHD (Deijen et al., 1998) and AO GHD (Soares et al., 1999). However, in two other placebo-controlled trials with adults with AO GHD, no changes in cognitive

functions were found after GH replacement therapy (Degerblad et al., 1990; Baum et al., 1998).
More specifically, improvements after GH replacement therapy have been reported for fluid intelligence (Almqvist et al., 1986; Sartorio et al., 1995), short-term memory, long-term memory, and iconic memory (Deijen et al., 1998). In a meta-analysis of current literature, Falleti et al. (2006) concluded that patients showed a sizable improvement after GH treatment (compared to placebo) in executive functions at 3–6 months, and a more modest improvement compared to placebo after 9–12 months of treatment.

IGF-1: effects on and relations with cognitive function

There are numerous reports addressing a possible relationship between serum IGF-1 levels and human cognitive function. In a 3-year longitudinal study of cognitive decline in 1318 subjects 65–88 years of age, Dik et al. (2003) observed an association between low serum levels of IGF-1 and deficits in information processing speed. Arwert et al. (2005) reported a positive correlation between IGF-1 and cognition, specifically orientation, attention, immediate and short-term recall, language and the capability to follow commands. However, Papadakis et al. (1995) reported no association between serum IGF-1 levels and age-adjusted cognitive status in 104 healthy older men. Aleman et al. (1999) observed statistically significant associations between both perceptual-motor performance and information processing speed and IGF-1 levels in a sample of 25 subjects 65–76 years of age. In another study the same group demonstrated an association between serum IGF-1 and fluid intelligence, a cognitive measure that is sensitive to aging (Aleman et al., 2001). The relation between IGF-1 and these cognitive tests was that higher levels of IGF-1 were associated with better performance. When cognitive performance, in particular mental processing speed, was evaluated in elderly men, higher levels of GH following GHRH-GHRP-6 stimulation were associated with poorer cognitive performance. In contrast, a positive association between IGF-1 and performance on the same cognitive tests (concept shifting and digit symbol substitution, which are known to be sensitive to aging) was found (Aleman et al., 2000). The authors conclude that a disruption of the relation between IGF-1 and GH secretion in older age may exist.

Thus, both IGF-1 as well as GH may have a positive effect on fluid intelligence. IGF-1 and GH were found to have a divergent impact on cognition. Higher levels of IGF-1 were found to be related to better information & mental processing speed, motor performance, recall, orientation, attention, and language, whereas improvement of different kinds of memory and executive functions were found after GH replacement therapy.

Effects related to or during aging

Aging is associated with a decline in the activity of the somatotropic axis (van Dam, 2006), and features of aging resemble those of GHD, suggesting that the GH–IGF-1 axis may play a role in age-related cognitive decline (Arwert et al., 2005). It has also been proposed that age-related alterations in the endocrine environment as a whole may modulate cognitive changes (Lamberts et al., 1997).
Age-dependent functional decline has been documented for a great variety of higher cognitive functions such as selective and divided attention, working memory and executive control (Kenemans et al., 1995) as well as mental processing speed (Leskelä et al., 1999). These impairments might be caused by a reduction both in levels of IGF-1 and GH, as well as in the density of GH and IGF-1 receptors, with increasing age (Sherlock & Toogood, 200; Nyberg, 1997; Lai et al., 1991).
Given the decline of specific cognitive functions with lower GH secretion, and the resemblances between functions affected by normal aging to those affected by GHD, it is reasonable to hypothesize that reduced GH levels may contribute to age-related cognitive decline (Arwert et al., 2005). Chapter 3 addresses this relation between GH secretion and cognitive function in men over 50 years old.
A study by Sathiavageeswaran et al. (2007) indicates that GH replacement may improve certain measures of cognitive function in elderly patients with GHD. Another study by Vitiello et al. (2006) observed a significant improvement in cognitive functions, particularly those involving problem solving, psychomotor processing speed, and working memory, after six months of daily GHRH treatments in healthy older men.

The somatotropic axis and brain function

Event related brain potentials (ERPs)

Brain activity involves changes in electric and magnetic fields upon which behavior depends. These changing fields can be recorded from the human scalp, resulting in the Electroencephalogram (EEG), but may also contain the response of the brain to specific events: Event-Related Potentials (ERPs) (Kenemans & Kähkönen, 2011). The scalp-recorded signal reflects volume conduction of the electrical component of neural activity, more specifically fluctuations in post-synaptic potentials throughout the cerebral cortex. Relative to the other measures of human brain activity, EEG has a high temporal resolution: Changes in activity can be followed on a millisecond basis. However, in comparison to some other methods, the EEG has a poorer spatial resolution: It is less accurate in indicating where in the brain the activity is located. The extraction of ERPs from the on-

going EEG rests on the fact that ERPs are time-locked to discrete events, such as stimuli. Thus, with a sufficient number of repeated measures, application of the method of signal averaging (sample-by-sample averaging of EEG values across repeated measures, or 'trials') yields a valid estimate of the ERP. The underlying idea is that the background EEG has no fixed temporal relationship with the point in time at which the stimulus was presented; on the other hand the ERP has a much more constant time course relative to the stimulus.

ERPs come in many colors. Brain potentials evoked from sensory cortices are strongly determined by physical stimulus characteristics. More genuine 'event-related' potentials reflect more the cognitive or affective context and may differ for one and the same stimulus depending on context (the most renowned example being the P300 or P3). Generally, sensory evoked potentials take less than 100 ms post-stimulus to evolve, while the more cognitive event-related potentials are marked by latencies of over 100 or hundreds of milliseconds. As in the present thesis, ERPs may also specifically reflect the preparation at cortex level of a motor act (e.g., the 'Lateralized Readiness Potential' or LRP). And rather than to a stimulus, ERPs may be synchronized to a behavioral response, such as in the case of the 'Error-Related Negativity' (ERN, see below). Finally, it should be noted that in many cases meaningful analysis of ERPs is only possible by taking into account the contrast between two (psychological) conditions, even to the extent that the two ERP waveforms corresponding tot the two conditions are subtracted from each other (see next section on selection potentials for an example).

Selection potentials: N2b

Selective attention refers to the focusing, and maintaining that focus, on a limited part of the available information. A common methodology is to present participants with streams of stimuli which differ in one or two features. Attention has to be selectively directed only to stimuli with one specific feature (e.g., attend to the blue patterns, ignore all the yellow ones; attend to tones in the left, ignore those in the right ear). ERPs are recorded to attended (relevant) and to ignored (irrelevant) stimuli, and the difference between these ERP indexes the effect of the attentional manipulation. Such difference or 'selection potentials' usually take the form of time-varying potential distributions, which reflect the sequential selective activation of different cortical areas (Kenemans & Kähkönen, 2011). For example, Kenemans and colleagues found that selective attention to specific visual spatial frequencies caused a sequence of selective activations in relatively dorsal-posterior cortex, followed by relatively ventral-posterior, followed by relatively medial-frontal cortex, all within an interval of 100 to 300 ms post-stimulus (Kenemans et al., 2002).

One component of these visual selection potentials arises at frontocentral scalp sites as a negative deflection at about 250-ms latency relative to stimulus onset and is called 'N2b'. It is thought to reflect selective processing of multiple stimulus attributes. This integrative

function is probably supported by the anterior cingulate cortex (ACC) and its extensive connections to the hippocampus, which are rich in GH receptors. It was found to be reduced in adults with CO-GHD (Lijffijt et al., 2003). This reduction could reflect functional deficits in the cingulate cortex (Kenemans et al., 2002). Reduced N2bs have also been reported for healthy senescent individual (Kenemans et al., 1995). The N2b will be further discussed in the upcoming chapters, also in relation to other ERPs, such as the error- or event-related negativity.

Error-related negativity (ERN)

The event-related negativity (ERN) is a negative ERP arising around the time that an incorrect motor response begins (Santesso et al., 2008). The ERN is proposed to reflect brain activity caused by dips in dopamine. This dopamine drop is a prerequisite for error processing and conflict monitoring by the ACC. An unexpected lack of reward leads to a phasic drop in dopaminergic input to the ACC. Previous ERP research has shown the presence of the ERN, immediately following responses on incorrect trials (Bruijn et al., 2006; Roelofs et al., 2006; Pailing et al., 2004; Ridderinkhof et al., 2002). Both components have consistently been reported as being generated by the ACC (Bruijn et al., 2006; Roelofs et al., 2006; Veen & Carter, 2006; Pailing et al., 2004; Ridderinkhof et al., 2002). According to the conflict theory, this ACC activity in the case of the ERN is a result of a conflict between the activation of the erroneous response and the somewhat slower activation of the correct response.
The relationship between dopamine-level and ERN has been intensively studied in patients and pharmacological studies and suggests a positive relationship between dopamine level, positive learning bias, and ERN amplitude. However, Frank et al. (2005; 2007) reported contradictory results. To clarify these opposite results, the same design as in Frank et al. (2005; 2007) was used for that study, in order to assess the relationship between learning bias and the amplitudes of the ERN. Chapter 6 describes this study and the relationship between dopamine, learning bias and the ERN in more detail. In Chapter 7 the ERN is compared with the N2b and another negative ERP which may be related to ACC activity, the NoGo N2.

NoGo N2

The NoGo N2 is a negative event related brain potential (ERP) component arising around 200 ms after stimulus onset specifically when the stimulus signals that a behavioral response is to be withheld, in a context of frequent ‘go stimuli’ (that signal that a behavioral response must be made). A coarse estimate of the cortical generator of the NoGo N2 has implicated the ACC (Bekker, Kenemans & Verbaten, 2005), much like the N2b and ERN,

described above. Chapter 7 compares the NoGo N2, N2b and ERN. Chapter 8 studies the same three ERP components and cognition in relation to an acute rise of GH, elicited by administrating GHRH into the bloodstream of elderly men.

P300 and GH replacement therapy

Another ERP which can be calculated using EEG is the positive potential 300ms after a stimulus. One study assessed the P300 event-related brain potential after 6 months of GH replacement, relative to placebo, in 14 patients with Sheehan's syndrome, a condition of short-term pituitary ischemia which leads to permanent hypopituitarism (Golgeli et al., 2004). It is normally assumed that P300 amplitude reflects the attentional mechanisms engaged to 'update' the neural representations of the stimulus context after early stimulus evaluation, and that P300 latency is a measure of the speed of task-related stimulus classification (Polich, 1998; Polich, 2003). GH replacement shortened P300 latency, down to normal levels, but did not affect P300 amplitude (Golgeli et al., 2004). Consistently, Braverman et al. (2007) found that decreases in IGF-1 were accompanied by increases in P300 latency.

Summary and aims of this thesis

Several studies indicate that a relation between GH secretion and general cognitive function exists. General cognitive functioning depends on core functions including selective attention, which have not been addressed specifically in relation to GH. Chapter 2 addresses current insights about specific effects of GHD on varieties of selective attention, as well as effects of GH suppletion.

As GH secretion decreases with age, this may contribute to cognitive changes associated with aging. Chapter 3 evaluates the relation between GH secretion and cognition in elderly men by assessing correlations between GH secretion and performance on cognitive tests in conjunction with the recording of ERPs in order to assess underlying neurophysiological mechanisms.

Circulating IGF-1 levels are associated with cognitive performance. The decrease of IGF-1 levels with age may contribute to cognitive changes associated with aging. The objective of chapter 4 is to investigate the relation between IGF-1 and cognition in older men by focusing on correlations between IGF-1 levels and performance on cognitive tests, and assessing the associations between IGF-1 and attention related cortical brain activity (ERPs).

Cranial irradiation may lead to pituitary dysfunction, in particular GHD (Appelman-Dijkstra et al., 2011). Shukitt-Hale et al. (2007) found that cranial irradiation was associated with reduced performance in tasks assessing spatial learning and memory, which could at least partly be due to a lack of GH. It remains unclear which specific functions, such as visual memory and selective attention, are impaired by GHD after external beam brain irradiation. Chapter 5 investigates the relation between GH and cognition in 19 patients who received external beam radiation therapy for treatment of brain tumors. Brain function was assessed using event-related potentials, including N2b and P300. Correlations were calculated between peak GH levels and cognitive functions. In addition, four patients who were diagnosed with GHD were compared to 15 non-GHD patients. It was hypothesized that low GH secretion in irradiated patients is associated with reduced attentional electro-cortical responses to task-relevant stimuli (N2b), decreases of target detections, slower speed of responding, increased P300 latencies, and impaired performance on neuropsychological tests.

GH has been found to be related to dopamine, therefore it would be interesting to study the relation between GH and dopamine related brain function. One way to study dopamine related brain functions is by recording the ERN, a specific ERP component. This component is related to error monitoring and is evoked from the ACC by the midbrain dopaminergic drop that follows error making. Chapter 6 describes a replication of a study on the relation between tonic dopamine level and ERN in healthy subjects. To understand the relation between the somatropic axis with brain electrophysiology better, in chapter 7 we comparatively analyze N2b, ERN, and NoGo N2. We hypothesize that N2b, ERN, and NoGo N2 are each a manifestation of ACC activity, and will therefore show strong within-subject correlations. Also, correlations between these ERP components and neuropsychological manifestations of cognitive control were expected.

In order to find out how hormones of the somatotropic axis influence cognition by direct receptor stimulation, the acute effect of GH on cognitive functioning and related brain physiology in healthy elderly men was studied by administration of growth hormone releasing hormone (GHRH) (chapter 8). Finally, chapter 9 will present a general discussion of all previous chapters followed by its translation in Dutch.

References

Aleman, A., Verhaar, H.J., De Haan, E.H., De Vries, W.R., Samson, M.M., Drent, M.L., van der Veen, E.A., Koppeschaar, H.P.F. (1999). Insulin-like growth factor-I and cognitive function in healthy older men. J. Clin. Endocrinol. Metab. 84, 471–475.

Aleman, A., Verhaar, H.J., De Haan, E.H., Verhaar, H.J.J., Samson, M.M., Koppeschaar, H.P.F. (2000) Age-sensitive cognitive function, growth hormone and insulin- like growth factor 1 plasma levels in healthy older men. Neuropsychobiology 41(2):73-78.

Aleman, A., de Vries, W.R., Koppeschaar, H.P.F., Osman-Dualeh, M., Verhaar, H.J.J., Samson, M.M., Bol, E., de Haan, E.H.F. (2001). Relationship between circulating levels of sex hormones and insulin-like growth factor-I and fluid intelligence in older men. Exp. Aging Res. 27, 283- 291.

Appelman-Dijstra, N.M., Kokshoorn, N.E., Dekkers, O.A., Neelis, K.J., Biermasz, N.R., Romijn, J.A., Smit, J.W.A., Pereira, A.M. (2011). Pituitary dysfunction in adult patients after cranial radiotherapy: systematic review and meta-analysis. J. Clin. Endocrinol. Metab. 96, 2330-2340.

Falleti, M.G., Maruff, P., Burman, P., Harris, A. (2006). The effects of growth hormone (GH) deficiency and GH replacement on cognitive performance in adults: A meta-analysis of the current literature. Psychoneuroendocrinology 31:681–691.

Garcia-Segura, L.M., Rodriguez, J.R., Torres-Aleman, I. (1997) Localization of the insulin-like growth factor I receptor in the cerebellum and hypothalamus of adult rats: an electron microscopic study. J Neurocytol 26:479–490.

Golgeli, A., Tanriverdi, F., Suer, C., Gokce, C., Ozemi, C., Bayram, F., Kelestimur, F. (2004). Utility of auditory event related potential latency in detecting cognitive dysfunction in growth hormone (GH) deficient patients with Sheehan's syndrome and effects of GH replacement therapy, Eur. J. Endocrinol. 150, 153–159.

Kenemans, J. L., & Kähkönen, S. (2011). How human electrophysiology informs psychopharmacology: From bottom-up driven processing to top-down control. Neuropsychopharmacology Reviews, 36, 26–51.

Kenemans, J. L., Lijffijt, M., Camfferman, G., & Verbaten, M. N. (2002). Split-second sequential selective activation in human secondary visual cortex. Journal of Cognitive Neuroscience, 14(1), 48-61.

Kenemans, J.L., Smulders, F.T., Kok, A. (1995). Selective processing of two-dimensional visual stimuli in young and old subjects: electrophysiological analysis. Psychophysiology 32, 108–120.

Kojima, M., Kangawa, K. (2010). Ghrelin: more than endogenous growth hormone secretagogue. Annals of the New York Academy of Sciences, 1200, 140-148(9).

Koltowska- Häggström M, Mattsson AF, Monson JP et al. (2006) Does long-term GH replacement therapy in hypopituitary adults with GH deficiency normalise quality of life? Eur J Endocrinol 155(1):109-119.

Leskelä, M., Hietanen, M., Kalska, H., Ylikoski, R., Pohjasvaara, T., Mäntylä, R., Erkinjuntti, T. (1999) Executive functions and speed of mental processing in elderly patients with frontal or nonfrontal ischemic stroke. Eur J Neurol. Nov;6(6):653–661.

Lijffijt, M., Van Dam, P.S., Kenemans, J.L., Koppeschaar, H.P., de Vries, W.R., Drent, M.L., Wittenberg, A., Kemner, C. (2003). Somatotropic-axis deficiency affects brain substrates of selective attention in childhood-onset growth hormone deficient patients. Neurosci. Lett. 353, 123–126.

van Dam, P.S., Aleman, A., de Vries, W.R., Deijen, J.B., Van der Veen, E.A., de Haan, E.H.F., Koppeschaar, H.P.F. (2000). Growth hormone, insulin-like growth factor-I and cognitive function in adults. GH IGF-1 Res. 10, S69–S73.

van Dam, P.S., de Winter, C.F., de Vries, R., van der Grond, J., Drent, M.L., Lijffijt, M., Kenemans, J.L., Aleman, A., de Haan, E.H., Koppeschaar, H.P., (2005). Childhood-onset growth hormone deficiency, cognitive function and brain N-acetylaspartate. Psychoneuroendocrinology. 30(4), 357-363.

van Dam, P.S. (2006). Somatotropic therapy and cognitive function in adults with growth hormone deficiency: A critical review. Treat Endocrinol. 5 (2), 1

Vitiello, M.V., Moe, K.E., Merriam, G.R., Mazzoni, G., Buchner, D.H., Schwartz, R.S. (2006). Growth hormone releasing hormone improves the cognition of healthy older adults. Neurobiology of Aging 27, 2, 318-323.

Chapter 2

Growth hormone and selective attention
A review

Elise H. Quik, P. Sytze van Dam & J. Leon Kenemans

Quik, E.H., van Dam, P.S., Kenemans, J.L. Growth hormone and selective attention: A review. Neurosci. Biobehav. Rev. 34 (2010) 1137–1143.

Abstract

Introduction

The relation between growth hormone (GH) secretion and general cognitive function has been established. General cognitive functioning depends on core functions including selective attention, which have not been addressed specifically in relation to GH. The present review addresses current insights about specific effects of growth hormone deficiency (GHD) on varieties of selective attention, as well as effects of GH suppletion.

Materials and methods

Studies investigating relationships between GH status and valid measures of selective or divided attention were reviewed.

Results and discussion

There are no indications that GHD is characterized by impaired attribute selection, interference control, or attentional switching. In contrast, a few studies point to a deficit in integrated processing of multiple dimensions, as well as speed of information processing. There is also weak evidence for beneficial effects of GH replacement in the opposite direction in these domains.

Conclusions

The function of integrated processing of multiple stimulus dimensions may be based on neural mechanisms in the anterior cingulate cortex and its extensive connections to the hippocampus, the latter being known to be rich in GH receptors.

Key words: growth hormone (GH) – growth hormone deficiency (GHD) – cognitive function – event related potentials (ERP) – selective attention – divided attention – anterior cingulate cortex.

Introduction

The somatotropic axis refers to the hormonal signaling from the hypothalamus to the anterior pituitary gland that normally results in the release of growth hormone (GH) and its secondary mediator insulin-like growth factor-1 (IGF-1). During childhood GH and IGF-1 correlate, but this correlation is less clear when physical development levels off, as both are affected differently by other variables, e.g., obesity, physical activity or sex steroids (Juul et al., 1997). Both substances contribute to development and recovery of diverse cells and organs, and stimulate neuron growth, growth of glial cells and myelination (Sonntag et al., 2005). It has been clearly demonstrated that both substances cross the blood-brain barrier and bind to their receptors in the central nervous system (CNS). GH reaches the cerebrospinal fluid (CSF) after subcutaneous injection and GH- and IGF-1 binding sites have been identified in several brain areas (Johansson en Bengtsson, 1997). Therefore, GH and IGF-1 may exert similar cognitive effects.

Experimental studies have identified numerous mechanisms through which GH and GH receptor activation affect brain development and functioning, including interactions with several neurotransmitters (Cuttler, 1996). GH binding sites have been identified in GH receptors (GH-R), which have been found in the brain in several mammals, including humans and rats, birds, fish, as well as in turtle and frog. These sites include cortex, basal ganglia (putamen), and amygdala (Johansson en Bengtsson, 1997), as well as, from highest to lowest density, in the choroid plexus, pituitary, hippocampus, putamen, thalamus, and hypothalamus in humans (Nyberg, 2000). In these latter areas, GH binding decreases with advancing age, and so does GH-R mRNA expression in human choroid plexus (Nyberg, 2000); as will be discussed, similar observations have been reported for human senescence. As we focus in the present review on selective attention, it is of particular importance to note that in the rat brain GH-Rs have been found in the parietal cortex (Möderscheim et al., 2007), pons, medulla oblongata, and also in oligodendroglial-like and glial cells (Scheepens et al., 2005). In addition, in chicks GH-R were found in the visual system (Baudet et al., 2007), suggesting a mediating role for GH in perception.

The GH system interacts with other hormones as well as with certain neurotransmitters. Higher insulin levels were found to enhance GH secretion (Galassetti and Davis, 2000) and administration of insulin increased plasma GH levels (Watson et al., 2009) even when plasma glucose levels remained normal. However, the interaction between insulin, glucose and the somatropic axis is extremely complex. Insulin-induced hypoglycemia stimulates the secretion of GH (Abs, 2003), while hyperglycemia, which also leads to a rise in insulin secretion, suppresses GH secretion (Tzanela, 2006). The interaction between insulin and IGF-1 is also extremely complex. Most studies regarding cognitive function in relation to insulin and glucose have been carried out in diabetic subjects, where chronic hyperglycemia and recurrent hypoglycemia are shown to have a significant impact on cognitive performance, independent from plasma insulin levels (Biessels et al., 2008). Moreover, GH administration has been shown to suppress cortisol production in man (Scheepens et al.,

2005). Furthermore, corticotropin-releasing hormone (CRH) directly stimulates pituitary GH release (in fish, Canosa et al., 2007). GH-releasing hormone (GHRH, also known as GH-releasing factor, GHRF) or somatocrinin, and somatostatin (also known as growth hormone inhibiting hormone, GHIH or somatotropin release-inhibiting factor, SRIF), are produced by neuroendocrine neurons of the periventricular nucleus of the hypothalamus, and travel through the blood vessels to the anterior pituitary gland. There SRIF inhibits and GHRH stimulates the secretion of growth hormone from somatotrope cells. In turn, ACTH indirectly stimulates pituitary GH release by inhibiting somatostatin (SRIF) secretion (in goldfish, Wong et al., 1998). In sum, activation of the CRH-ACTH axis promotes GH release, both directly (CRH receptor) and indirectly (ACTH receptor), while GH attenuates cortisol release, acting as negative feedback signal similar to cortisol itself.

It is known that cortisol reduces β-endorphins. β-endorphins have been shown to have an inhibitory effect on basal ACTH and cortisol secretion in humans (Inder et al., 1996), but can stimulate GH secretion in rats (Pagani et al., 1998). Mesocortical dopamine release acts as a negative feedback signal for hypothalamus-pituitary-adrenal (HPA) axis activation (Sullivan and Dufresne, 2006), possibly in concert with a similar effect of GH, suggesting a mutually inhibitory interaction between HPA axis and dopamine.

GH stimulates β-endorphins, which in turn stimulates dopamine neurons (in rainbow trout, Very and Sheridan, 2007). In turn, dopamine stimulates pituitary GH release in humans (Segal-Lieberman et al., 2006) and in fish (Canosa et al., 2007; Very and Sheridan, 2007) possibly through activation of dopamine subtype 1 receptors (D1R) (Wong et al., 1998), which shows that an interaction exists between the dopamine system and the somatotropic axis. This interaction is also suggested by another study that reported impaired body growth in mice that lack the dopamine D2 receptor (García-Tornadú et al., 2006). In humans, acute administration of 200 mg levodopa (a dopamine precursor) induces GH release in patients with Parkinson's disease (Müller et al., 2007). Furthermore, activation of the ghrelin receptor (GH Secretagogue receptor (GHS-R)) by ghrelin, known to promote GH release, amplifies DA/D1R-induced cAMP accumulation, as shown in mice (Jiang et al., 2006). Hence, the neurotransmitter dopamine and GH interact in a mutually excitatory fashion. These interactions between GH and dopaminergic transmission and amplification of dopaminergic effects may be important in relation to reward and conflict processing, as based in the Ventral Tegmental Area (VTA) - Nucleus Accumbens (NA) - hippocampus - cingulate cortex axis, as will be discussed later. They are also consistent with GH promoting effects of methylphenidate, to be discussed later, and suggests that GH deficiency may result in dopamine deficiency, which in turn may affect a number of cognitive functions, such as conflict monitoring (Holroyd and Coles, 2008), working memory (Abi-Dargham et al., 2002), and selective attention (Braver and Cohen, 1999).

Growth hormone deficiency in humans

GHD is the medical condition of inadequate production of GH. GHD can occur either isolated (IGHD) or in combination with other pituitary hormone deficits (multiple pituitary hormone deficiency, MPHD). Of all GHD patients, 9.6 % has been found to have IGHD (Abs et al., 2005). GHD can be observed both in children and in adults. In adults, GHD can have its onset in childhood (CO GHD) or later in life in adulthood (AO GHD). CO GHD is usually easier to detect because it normally results in retarded physical growth. Analogous effects of CO GHD may be expected for brain and brain-function development. In general, the peripheral and central effects of AO GHD may be more subtle. Most of the data regarding the association between selective attention and GH have been collected in adults with either CO GHD or AO GHD.

GHD is commonly associated with reports of a "suboptimal well-being", including impaired psychological functions (Dattani and Preece, 2004). These individuals have higher rates of unemployment than the general population, less involvement in leisure activities, and show low levels of self-esteem, emotional instability during specific stress, a fatalistic attitude with a tendency towards depression, and a very strong feeling of being isolated from the outside world (Soares et al., 1999). Sartorio et al. (1995) reported that children with isolated GHD have specific educational deficits, in particular learning disability and attention-deficit disorders. Shaywitz et al. (1990) studied the GH response to methylphenidate in children with attention deficit disorder. They found a significant correlation between the improvement in certain cognitive tests and GH response. Other data indicate that cognitive function is disturbed more significantly in patients with CO GHD than in those who become GH deficient during adult life (van Dam et al., 2000). Furthermore, within samples of healthy older men, correlations have been revealed between measures of fluid intelligence and IGF-1 levels (Aleman et al., 1999; Aleman et al., 2001).

One question is how independent and specific the effect of GH (deficiency) on cognition is in relation to other hormones, such as cortisol and thyroid hormones. One clue comes from hormonal replacement studies in multiple pituitary-hormone-deficient (MPHD) patients, in which other pituitary hormones (i.e., ACTH, TSH, gonadotropins) are substituted and psychological well-being is impaired (Arwert et al., 2005). In these individuals GH replacement improves cognition in a manner quite comparable to its effect in isolated GH-deficient (IGHD) (Soares et al., 1999).

Criteria for GHD in children and adults have been clearly defined (Casanueva et al, 2009; Gharib, 2003). In general, a GH provocative test such as an insulin tolerance test, GHRH-arginine test or GHRH-GHRP-6 test with an appropriate GH array is used. Plasma IGF-1 is considered to be less sensitive and specific for the diagnosis of GHD, and is not used for this purpose. However, 24h GH secretion and GH levels following a GH provocative test do correlate to some extent with plasma IGF-1 (Juul et al., 1997), and plasma IGF-1 levels are generally used to evaluate the treatment of GH substitution therapy in GHD.

Furthermore, the effects of GH seem to be qualitatively different from those of some other hormones. Cortisol modulates spatial working memory specifically for negative emotional expressions (Putman et al., 2007), while there are no indications of affectively mediated effects of GH on cognition. Samuels et al. (2008) investigated the effects of L-T4 (thyroid hormone) on different types of memory in hypothyroid individuals. They found an improvement in procedural memory, but no effects on declarative or working memory. This may be contrasted with reports of GH replacement improving declarative long-term memory (Deijen et al., 1998). On a related note, associations similar to those between GH and cognition are sometimes reported GH's secondary mediator IGF-1. Specifically, such associations have been reported for basic speed of information processing, verbal memory, and executive functions such as concept shifting (Aleman and Torres-Alemán, 2009). Furthermore, within samples of healthy older men, correlations have been revealed between measures of fluid intelligence and IGF-1 levels (Aleman et al., 1999; Aleman et al., 2001).

Cognitive function and intellectual capacity are often addressed in a very broad way, using read-out measures often constituted by a combination of specific neuropsychological test results. However, it is also generally believed that especially 'fluid intelligence' (Salthouse et al., 1998) or the 'don't hold' functions (Aleman et al., 1999) depend on core cognitive functions such as speed of information processing, selective attention, and working-memory capacity. GH has an effect on memory and learning processes, which probably is caused by stimulation of GH-R in the hippocampus (Nyberg, 2000).

Here we focus on selective attention, the central mechanism that enables us to select specific sources of information, over others, to govern behavior, and protects from distraction and incoherent or impulsive actions. This choice was in particular inspired by our findings of overlap in brain correlates of atypical attentional processing between senescent individuals (Kenemans et al., 1995) and CO GHD patients (Lijffijt et al., 2003). Adult hypopituitary patients with GHD, even when treated with adequate adrenal, thyroid and sex hormone replacement therapy, complain of attention disabilities (Oertel et al., 2004). Aging is associated with a decline in the activity of the somatotropic axis (van Dam, 2006), and features of aging resemble those of GHD, suggesting that the GH–IGF-1 axis may play a role in age-related cognitive decline (Arwert et al., 2005). It has also been reported that age-related alterations in the endocrine environment may modulate cognitive changes (Lamberts et al., 1997). Somatotropic supplementation was found to improve cognitive function in healthy older adults. GHRH treatment, which results in increases of both GH and IGF-1, improved cognitive function, especially problem solving, processing speed and working memory, functions known to be vulnerable with aging, in healthy older men and women (Vitiello et al., 2006).

Significant correlations were observed between IGF-1/GH ratio and cognitive performance in men of different ages, including measures of visual and verbal memory (Morley et al., 1997). Finally, a high density of GH receptors, and strong involvement in control of selective attention, overlap in at least one cortical region, viz., the parietal cortex (Kenemans et al., 2002; Möderscheim et al., 2007).

Varieties of selective attention

On a theoretical level, selective attention can be thought of as reflecting the balance between rigidity and flexibility. In the literature these components are referred to using such terms as top-down or goal-directed versus bottom-up or stimulus-driven attention or voluntarily versus involuntarily driven attention, or exploitation versus exploration (Aston-Jones & Cohen, 2005; Braver & Cohen, 1999; Cohen et al., 2004; Corbetta & Shulman, 2002). Rigidity is necessary to protect against distraction and interference from pre-potent but inadequate response tendencies. This necessitates adequate attribute selection (e.g., of color information over word information in a Stroop task) and may result in efficacious interference control (e.g., of a word-reading tendency in the Stroop task). Flexibility can be demanded in the face of salient, potentially biologically relevant stimuli outside the current task set, but also when the task demands continuous shifting of the focus of attention (such as in the Trailmaking B test, explained below). Finally, some tasks demand monitoring or integrating information from multiple sources (e.g., in a display or in working memory); in such conditions, the use of non-selective or divided attention may be the more efficacious mode of information processing.

Only a few tasks and tests have, in our opinion, been used appropriately to assess specific effects of GH or GHD on varieties of selective attention. A first one is the Stroop task (Stroop, 1935). Principally, the Stroop task assesses the ability to control interference from irrelevant sources of information (Lansbergen et al., 2007). The original set-up consisted of three conditions (Stroop, 1935). The first condition (word reading) requires participants to read a list of words as quickly as possible. The second condition (color naming) requires naming patches of colored ink out loud as quickly as possible. The third condition (color word interference) requires participants to name the ink color of the printed words (stimuli consist of color names that are printed in discordant colors, e.g., the word ‘blue’ printed in green letters). It is in this third condition that control of interference (from word information) and/ or ability to inhibit the pre-potent response (word reading), a selective attention sub-function, is taxed. Any score in this condition should be taken relative to ‘simple’ color naming as assessed in the second condition. This can be done by subtracting performance in the second condition from that in the third one, or (preferably, see Lansbergen et al., 2007), by computing the difference between them, and then comparing these derived scores between groups or drug conditions. In relation to GH and GHD, van der Reijden-Lakeman et al. (1997) deployed a so-called ‘focused-attention’ task, which, like the Stroop task assesses the ability to use visual information (the diagonal in a display on which digits were presented) to selectively direct attention, and to ignore items on other locations (i.e., the alternative diagonal). However, the diagonal also taxes a form of ‘divided’ attention, as the behavioral response is also determined by the identity of the letter on the relevant diagonal.

A second paradigm is the trail making test. The trail making test A provides a baseline measure for visual-conceptual and visual-motor tracking (scattered digits have to be

connected in ascending order). The trail making test B requires participants to switch between digits and letters, which, relative to A, specifically assesses the ability to switch selective attention between different sources of information. The difference score of these 2 tests then, is a measure of attentional-switching competence. As surveyed below, performance in the trail making A seems to be quite sensitive to GH differences and manipulations, and this will be discussed as a finding of interest in its own right.
Finally, one study (van der Reijden-Lakeman et al., 1997) used rather specific measures of 'divided' and 'focused' attention. This 'focused' attention reflects the ability to respond discretely to specific visual, auditory or tactile stimuli. 'Divided' attention implies the ability to respond simultaneously to multiple tasks or multiple task demands. Divided attention was probed in a task involving varying loads of working memory and can be considered at least partially the opposite of selective attention. Given a pattern of GH effects on measures of selective attention, one can ask whether a reverse pattern can be identified for measures of divided attention.

GHD patients versus healthy controls

Falleti et al. (2006) conducted a meta-analysis on measures of attention, memory, and executive function in GHD patients as compared to matched controls. General measures of 'attention' and 'executive functions' were derived, the former being based on, amongst others, digit span, digit cancellation, trail making A, and the latter on tasks like block design and digit symbol substitution, but also Stroop color word and trail making B. In contrast, the present analysis focuses on the selectivity aspect of selective attention (Table 1), emphasizing performance during tasks such as Stroop color word (relative to Stroop color, to isolate the ability to selectively attend to color and ignore word information) and trail making B (relative to A, to isolate the ability to alternatingly attend selectively to digit and ignore letter information, and vice versa). The effect sizes reported by Falleti et al. indicated moderate to large impairments in patients, but this was not further specified in terms of separate Stroop interference or trail making B/A. Therefore, this meta-analysis does not specifically address the association between GH and selective attention, but reflects the available data regarding attention in general. Van Dam et al. (2005) compared adult CO GHD patients after at least 3 months interruption of GH suppletion with matched controls on a number of tests, including Stroop and trail making. These authors did not report Stroop color/ color-word interference or trail making B/A switching difference scores. A re-analysis of their data revealed no difference in Stroop interference difference score ($p=.86$) or trail making B/A switching scores ($p=.90$).

Table 1 Results

Selective attention results

Author	Task	Selective Attentional form	Patients	Result
		GHD Patients versus healthy controls		
Van Dam	TMT B/A	Attentional shifting	CO-GHD	O
Van Dam	Stroop	Interference control	CO-GHD	O
Van der Reijden-L	FAT	Focused attention	IUGR children	O
Lijffijt	Go / no go	Sensory discrimination & selective attention	CO-GHD	O
		GHD patients: GH therapy versus placebo		
Van der Reijden-L	FAT	Focused attention	IUGR	O
Baum	Stroop	Interference control	AO-GHD	O
Cherrier	Stroop	Interference control	Older men	O

Other results

Author	Task	Attentional form	Patients	Result
		GHD Patients versus healthy controls		
Van Dam	TMT A	Basic processing speed	CO-GHD	X
Peace	TMT A, B	Basic processing speed, concept shifting	AO-GHD pituitary tumor	O
Lijffijt	Go / no go	N2b; Anterior (Fz) attentional process	CO-GHD	X
Van der Reijden-L	DAT	Divided attention	IUGR	X
		GHD patients: GH therapy versus placebo		
Oertel	TMT A	Basic processing speed	AO-GHD	X
Baum	TMT A, B	Basic processing speed, concept shifting	AO-GHD	O
Cherrier	TMT A	Basic processing speed	Older men	O
Golgeli	P300 latency	Basic processing/stimulus classification speed	Sheehan's syndrome	X
Braverman	P300 latency	Basic processing/stimulus classification speed	Patients > 40 Years	X

TMT = Trail Making Test, FAT = Focuses Attention Task, DAT = Divided Attention Task, X = significant, O = not significant.

This re-analysis of the data of the van Dam et al. study showed a trend, which may become significant if sample size would be increased, and may confirm a selective deficit in patients for trail making A (p=.09), as opposed to trail making B (p=.22). Table 2 displays the re-analysis mean scores in seconds of the adult CO GHD patients and matched controls on the Stroop task and trail making task.

Peace et al. (1998) examined trail making A and B in adult GHD patients who had been treated for a pituitary tumor by surgery (with or without additional radiotherapy) or medication, in comparison to matched controls. No GH suppletion was taken during the

study. There were no differences in trail making A or B performance between the patient group as a whole and controls. The authors note that performance was much more variable in the patients, one factor being the radiotherapy, which in itself was associated with superior performance on trail making A. The authors speculate that radiotherapy may have prevented tumor re-growth and associated endocrine disturbance.

Table 2 Results re-analysis of van Dam et al. (2005) data

Task / Group	TMT A *	TMT B	TMT B\A	Stroop word	Stroop color	Stroop interference	Stroop Color/int.
CO GHD	35.13	69.13	1.96	45.63	53.63	77.88	.70
Controls	25.82	56.46	2.21	45.44	52.78	80.33	.66

* p = .09.

Van der Reijden-Lakeman et al. (1997) compared intrauterine-growth-retarded (IUGR) children (mean age 9.5 years), who have probably been exposed to less GH during pregnancy, with matched controls. IUGR children were increasingly slowed down with increasing numbers of items to be held in working memory on a 'divided attention task', and made more incorrect responses for non-target letters on the relevant diagonal on a 'focused-attention task'. This suggests that IUGR children had no problems in selecting the relevant information (as in the Stroop task), but did have trouble integrating it with a second piece of information (letter identity).

A somewhat similar focused-attention design was used by Lijffijt et al. (2003) to compare adult CO GHD patients to matched controls. These authors used a go/ no go task, in which only a specific target conjunction of two visual attributes required an overt response. Patients made significantly more omission errors to these targets, but did not differ in commission error rates. In addition, Lijffijt and colleagues recorded event-related brain potentials to analyze the electro cortical substrate of attention-dependent (as well as attention-independent) processing. First, they found no group differences for visual-cortex activity, neither with respect to the bottom-up component (which purely reflects physical stimulus properties), nor with respect to activity in visual-cortex selectively elicited by task-relevant information. Second, they did report a so-called 'N2b' response to be smaller in patients. This N2b is thought to originate from the anterior cingulate cortex (ACC) and to reflect the integrated processing of the two relevant visual attributes, as was found by Kenemans et al. (2002). As such, the reduced N2b fits nicely in the pattern of results in the diagonal task described above. Reduced N2bs have also been reported in relation to normal aging (Kenemans et al., 1995), and a trend correlation between N2b amplitude and IGF-1 peak was observed (van Dam et al., 2005).

It should be noted that ACC activation is also commonly observed in conditions of Stroop interference, as revealed by functional Magnetic Resonance Imaging (MacDonald et al., 2000), as well as by event-related potentials (Lansbergen et al., 2007). From this it can be predicted that Stroop-related ACC activation would also be reduced by GHD, a proposition that has yet to be tested. Whereas the ACC is activated already when multiple stimulus attributes have to be integrated (e.g., Kenemans et al., 2002), this activation is especially vigorous when there is downright conflict between dimensions, as in the Stroop task. It is also generally accepted that this 'conflict monitoring' is only indirectly related to ongoing performance (MacDonald et al., 2000), which may explain the lack of GHD effects on behavioral Stroop interference.

In summary, there are no indications that CO GHD or AO GHD is characterized by impaired attribute selection (color versus word), interference control, or attentional switching. It is remarkable how this contrasts with the conclusions from meta-analyses that addressed broader function domains such as 'attention' and 'executive function' (Falleti et al., 2006). Impairments in trail making A, but not in B, may reflect that GHD manifests general slowing in many processes, but not attentional switching, which in itself slows down processing, but in such manner that differences between GHD and controls are masked (e.g., because the attentional switching process runs parallel to the processes that are slower in GHD). Some initial results do suggest, however, that at least CO GHD patients are impaired with respect to the processing of multiple sources of information, e.g., more than one visual dimension (rather than selective processing of one dimension). In contrast, attention-independent, 'bottom-up' processing is not affected by GHD.

GHD patients: GH therapy versus placebo

Clinical, psychiatric, as well as neuropsychological impairments of untreated GH-deficient adults can be decreased by recombinant human GH (rhGH) therapy (Soares et al., 1999). Improvements in cognitive functions following rhGH treatment have been reported in adults with CO GHD in an open-label study (Sartorio et al., 1995) and in young adults with GHD (Almqvist et al., 1986), CO GHD (Deijen et al., 1998) and AO GHD (Soares et al., 1999) in placebo-controlled trials, whereas in adults with AO GHD, in two other placebo-controlled trials, no changes in cognitive functions were found (Degerblad et al., 1990; Baum et al., 1998). The study of Sathiavageeswaran et al. (2007) indicates that GH replacement may improve certain measures of cognitive function in elderly patients with GHD. Moreover, if effects on cognitive function were found they were quite heterogeneous. Improvement after rhGH substitution therapy has been reported for fluid intelligence (Almqvist et al., 1986; Sartorio et al., 1995), short-term memory (Deijen et al. 1998), long-term memory (Deijen et al., 1998) and iconic memory (Deijen et al., 1998). In

a meta-analysis of current literature, Falleti et al. (2006) found that patients showed a large improvement after GH treatment (compared to placebo) in executive functions, including the trail making B, at 3–6 months, and a smaller improvement compared to placebo after 9–12 months of treatment. However, the specificity of these findings in relation to selective attention cannot be ascertained, as there was no reported attempt to relate trail making B performance to a baseline score from trail making A.

Van der Reijden-Lakeman et al. (1997) assessed attention in children with short stature following intrauterine growth retardation (IUGR), at baseline and after 2 years of rhGH treatment. As discussed above, at baseline, children with IUGR showed deficits in integrating visual information. After 2 years of rhGH treatment, this deficit was no longer apparent, to the extent that there was no significant difference between the IUGR group and a comparison group (different from the one used for the baseline comparison). This could be interpreted as that a specific deficit was specifically remediated by rhGH therapy, but this latter conclusion is based on a nil-result and needs further confirmation from placebo-controlled studies. The study by Van der Reijden-Lakeman et al. (1997) cannot be used to generalize the effect of medication. They used a different control group during baseline and post-treatment. The positive effect that they found could be solely due to a learning effect in the patient group that is absent in the control group.

Oertel et al. (2004) observed after 3 and 6 months of rhGH treatment a significant improvement of performance in patients with AO GHD. After six months, trail making test A scores, but not long-term verbal memory performance or non-verbal intelligence, was significantly better after RHGH than after placebo treatment. Performance on the trail making test A improved further continuously with time, over 12 months, in the RHGH treatment group. This result is very interesting in light of the rather specific deficits in trail making A performance discussed in the previous section. However, performance on this test reflects basic speed of a multitude of processes, rather than specifically selective or divided attention.

Baum et al. (1998) performed a randomized, double blind, placebo-controlled study of 18-months GH replacement in forty adult men with AO GHD and a history of pituitary disease. They found that in GHD adult men cognitive function, including performance on the trail making tests (A and B), and the Stroop task interference score, were not significantly influenced by chronic rhGH substitution. These authors used a relatively low dose of rhGH, which may have been insufficient to result in a significant beneficial effect.

Cherrier et al. (2004) conducted a placebo-controlled study in which older males (mean age 67.5) participated. In this study the potential changes in IGF-1 in response to testosterone administration and their relationship to cognitive functioning in healthy older man was examined. No correlation was found between IGF-1 and Stroop test scores in the interference condition. Soares et al. (1999) evaluated the impact of a clinical intervention with rhGH therapy on 9 GH-deficient adults and did not find a significant effect of 6 months rhGH therapy on the Stroop color word test scores, or on the trail making test A.

In all, GH replacement effects on selective-attention related performance seems to be mostly absent or inconsistent, and it may be again worthwhile to look at more direct reflections of brain functions. One study assessed the P300 event-related brain potential after 6 months of growth hormone replacement, relative to placebo, in 14 patients with Sheehan's syndrome, a condition of short-term pituitary ischemia which leads to permanent hypopituitarism (Golgeli et al., 2004). It is normally assumed that P300 amplitude reflects the attentional mechanisms engaged to `update' the neural representations of the stimulus context after early stimulus evaluation, and that P300 latency is a measure of the speed of task-related stimulus classification (Polich, 1998; Polich, 2003). GH replacement shortened P300 latency, down to normal levels, but did not affect P300 amplitude (Golgeli et al., 2004). Consistently, Braverman et al. (2007) found that decreases in IGF-1 were accompanied by increases in P300 latency. In line with these authors, P300 latency can be viewed as reflecting, amongst others, basic processing speed, which would be consistent with the trail making A results. While these P300 results may be a promising lead for a more direct way of assessing GH substitution-effects on brain function, it is again hard to interpret this result specifically in terms of selective attention, as variation in P300 latency may reflect a number of different perceptual and memory-related phenomena as well.
In sum, the range of paradigms measuring selective attention is limited, for instance spatial cueing paradigms are missing. Therefore, more research is needed to conclude that selective attention is affected by GH therapy. There were no effects on Stroop interference scores or trail making B (relative to trail making A) scores, although problems with non-optimal performance quantification cannot be completely ruled out (Lansbergen et al., 2007). One study found improvement on the trail making A test (Oertel et al., 2004), which mirrors the rather specific deficiency in GHD patients on this task, which however has only a loose relation with selective attention. Furthermore, there was weak evidence for an improvement in more divided-attention like functions, which were also disturbed in untreated patients, relative to controls (Van der Reijden-Lakeman et al., 1997).

Conclusions

The aim of this review was to clarify the effect of GH secretion specifically on selective attention, as reflected in task performance in GHD patients, as well as in the effects of GH replacement therapy. There are no indications for impairments on typical selective-attention sub-functions such as attribute selection, interference control, or attentional switching in either CO GHD or AO GHD. This also suggests that although there is a high density of GH receptors in parietal cortex, these receptors do not a play a role in the implementation of the control of selective attention as is known from neuro-imaging studies (e.g., Kenemans et al., 2002). Some initial results do suggest, however, that at least CO GHD patients perform

sub-optimally on cognitive tests when attention is less selective and has to include multiple sources of information, e.g., more than one visual dimension. This integrative process could have a cortical substrate in the ACC, and is perhaps also remediated by GH replacement therapy. In turn, the sensitivity of the ACC to GH could be related to its dense connections to the hippocampus (di Michele et al., 2005), an area known to be rich in GH receptors.
It has been suggested that the ACC is also part of another brain network, which involves the lateral prefrontal cortex as well as its projections to sensory cortex. Within this network, the ACC would play an indirect role in the control of selective attention, in that it serves as a monitor for response conflict as elicited by typical conflict stimuli, such as incongruent Stroop stimuli. This monitoring process would result in signals to lateral prefrontal regions, which in turn would be translated to enhanced selectivity of attention through the projections to sensory cortex. It remains to be tested whether this presumably ACC-based mechanism is also sensitive to GHD or GH in a more general sense. Activity in ACC and lateral prefrontal, as well as their interactions, may be particularly sensitive to dopamine; this suggests a further route through which GH may affect these processes, given the extensive interactions between the GH and dopamine system as discussed above. Both the hypotheses about specific effects on integrative processes, as well as the one about neuro-anatomical substrates, are in great need for more extensive and more direct further research.

References

Abi-Dargham, A., Mawlawi, O., Lombardo, I., Gil, R., Martinez, D., Huang, Y., Hwang, D., Keilp, J., Lisa Kochan, L., Van Heertum, R., Gorman, J.M., Laruelle, M., 2002. Prefrontal Dopamine D1 Receptors and Working Memory in Schizophrenia. The Journal of Neuroscience, May 1, 22(9):3708–3719.

Abs R., 2003. Update on the diagnosis of GH deficiency in adults. Eur J Endocrinol. 148(suppl 2), S3–S8.

Abs, R., Mattsson, A.F., Bengtsson, B., Feldt-Rasmussen, U., Góth, M.I., Koltowska-Häggström, M., Monson, J.P., Verhelst, J., Wilton, P, on behalf of the KIMS Study Group, 2005. Isolated growth hormone (GH) deficiency in adult patients: Baseline clinical characteristics and responses to GH replacement in comparison with hypopituitary patients. A sub-analysis of the KIMS database. Growth Hormone & IGF Research 15, 349–359.

Aleman, A., Verhaar, H.J., De Haan, E.H., De Vries, W.R., Samson, M.M., Drent, M.L., van der Veen, E.A., Koppeschaar, H.P., 1999. Insulin-like growth factor-I and cognitive function in healthy older men. J. Clin. Endocrinol. Metab. 84, 471–475.

Aleman, A., de Vries, W.R., Koppeschaar, H.P.F., Osman-Dualeh, M., Verhaar, H.J.J., Samson, M.M., Bol, E., de Haan, E.H.F., 2001. Relationship between circulating levels of sex hormones and insulin-like growth factor-I and fluid intelligence in older men. Exp. Aging Res. 27, 283- 291.

Aleman, A., Torres-Alemán, I., 2009. Circulating insulin-like growth factor I and cognitive function: Neuromodulation throughout the lifespan. Progress in Neurobiology. 89, 256–265.

Almqvist, O., Thoren, M., Saaf, M., Eriksson, O., 1986. Effects of growth hormone substitution on mental performance in adults with growth hormone deficiency: a pilot study. Psychoneuroendocrinology 11, 347–352.

Arwert, L.I., Deijen, J.B., Witlox, J., Drent, M.L., 2005. The influence of growth hormone (GH) substitution on patient-reported outcomes and cognitive functions in GH-deficient patients: a meta-analysis, Growth Horm. IGF Res. 15, 47–54.

Aston-Jones, G., Cohen, J.D., 2005. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. Annu Rev Neurosci 28, 403-50.

Baudet, M.L., Rattray, D., Harvey, S., 2007. Growth hormone and its receptor in projection neurons of the chick visual system: retinofugal and tectobulbar tracts. Neuroscience 148, 151-163.

Baum, H.B., Katznelson, L., Sherman, J.C. et al., 1998. Effects of physiological growth hormone (GH) therapy on cognition and quality of life in patients with adult-onset GH deficiency, J. Clin. Endocrinol. Metab. 3184–3189.

Biessels G.J., Deary, I.J., Ryan, C.M., 2008. Cognition and diabetes: a lifespan perspective. Lancet neurology 7(2), 184-90.

Braver, T.S., Cohen, J. D., 1999. Dopamine, cognitive control, and schizophrenia: The gating model. Progress in Brain Research, 121, 327-349.

Braverman E.R., Chen, T.J.H., Prihoda, T.J., Sonntag, W., Meshkin, B., Downs, B.W., Mengucci, J.F., Blum, S.H., Notaro, A., Arcuri, V., Varshavskiy, M., Blum, K., 2007. Plasma growth hormones, P300 event-related potential and test variables of attention (TOVA) are important neuroendocrinological predictors of early cognitive decline in a clinical setting: Evidence supported by structural equation modeling (SEM) parameter estimates. Age (Dordr). 29(2), 55–67.

Canosa, L.F., Chang, J.P., Peter. R.E., 2007. Neuroendocrine control of growth hormone in fish. General and comparative endocrinology 151, 1-26.

Casanueva, F.F., Castro, A.I., Micic, D., Kelestimur, F., Dieguez, C., 2009. New Guidelines for the Diagnosis of Growth Hormone Deficiency in Adults. Horm Res 71(Suppl.1), 112-115.

Cherrier, M.M., Plymate, S., Mohan, S. et al., 2004. Relationship between testosterone supplementation and insulin-like growth factor-I levels and cognition in healthy older men, Psychoneuroendocrinology, 29, 65–82.

Cohen, J. D., Aston-Jones, G., Gilzenrat, M. S., 2004. A sytems-level perspective on attention and cognitive control. Guided activation, adaptive gating, conflict monitoring, and exploitation versus exploration. In M. I. Posner (Ed.), Cognitive neuroscience of attention (pp. 71-90). New York: Guilford Press.

Corbetta, M., Shulman, G.L., 2002. Control of goal-directed and stimulus-driven attention in the brain. Nature Reviews Neuroscience, 3, 201-214.

Cuttler, L., 1996. The regulation of growth hormone secretion. Endocrinology & Metabolism Clinics of North America, 25: 3, 541-571

Dam, van, P.S., Aleman, A., de Vries, W.R., Deijen, J.B., Van der Veen, E.A., de Haan, E.H.F., Koppeschaar, H.P.F., 2000. Growth hormone, insulin-like growth factor-I and cognitive function in adults. GH IGF-1 Res. 10, S69–S73.

Dattani M., Preece M., 2004. Growth hormone deficiency and related disorders: insights into causation, diagnosis, and treatment. Lancet. 363, 1977-87.

Degerblad, M., Almkvist, O., Grunditz, R., Hall, K., Kaijser, L., Knutsson, E., Ringertz, H., Thoren, M., 1990. Physical and psychological capabilities during substitution therapy with recombinant growth hormone in adults with growth hormone deficiency. Acta Endocrinol. (Copenh), 185–193.

Deijen, J.B., de Boer, H., van der Veen, E.A., 1998. Cognitive changes during growth hormone replacement in adult men. Psychoneuroendocrinology. 45–55.

di Michele, F., Prichep, L., John, E.R., Chabot, R.J., 2005. The neurophysiology of attention-deficit/hyperactivity disorder. Int J Psychophysiol. 58(1), 81-93. Review.

Falleti, M.G., Maruff, P., Burman, P., Harris, A., 2006. The effects of growth hormone (GH) deficiency and GH replacement on cognitive performance in adults: A meta-analysis of the current literature. Psychoneuroendocrinology. 31, 681–691.

Galassetti, P., Davis, S.N., 2000. Effects of insulin per se on neuroendocrine and metabolic counter-regulatory responses to hypoglycaemia. Clin Sci (Lond) 99, 351-362.

García-Tornadú, I., Rubinstein, M., Gaylinn, B.D., Hill, D., Arany, E., Low, M.J., Diaz-Torga, G., Becu-Villalobos, D., 2006. GH in the dwarf dopaminergic D2 receptor knockout mouse: somatotrope population, GH release, and responsiveness to GH-releasing factors and somatostatin. J. Endocrinol. 190(3), 611 - 619.

Gharib H., 2003. American Association of Clinical Endocrinologists medical guidelines for clinical practice for growth hormone use in adults and children. Endocr Pract 9(1), 64-76.

Golgeli, A., Tanriverdi, F., Suer, C., Gokce, C., Ozemi, C., Bayram, F., Kelestimur, F., 2004. Utility of auditory event related potential latency in detecting cognitive dysfunction in growth hormone (GH) deficient patients with Sheehan's syndrome and effects of GH replacement therapy, Eur. J. Endocrinol. 150, 153–159.

Holroyd, C.B., Coles G.H., 2008. Dorsal anterior cingulate cortex intergrates reinforcement history to guide voluntary behavior. Cortex 44, 548-559.

Inder W.J., Livesey, J.H., Ellis M.J., Evans M.J., Donald R.A, 1996. The effect of β-endorphin on basal and insulin-hypoglycaemia stimulted levels of hypothalamic-pituitary-adrenal axis hormones in normal human subjects. Clinical endocrinology 44: 1, pp. 7-13 (1 p.1/4).

Jiang, H., Betancourt, L., Smith, R.G., 2006. Ghrelin Amplifies Dopamine Signaling by Cross Talk Involving Formation of Growth Hormone Secretagogue Receptor/Dopamine Receptor Subtype 1 Heterodimers Molecular Endocrinology 20(8):1772–1785.

Johansson, J.O., Bengtsson, B.A., 1997. Central nervous effects of growth hormone. Endocrinol. Metab. 4 (Suppl. B), 103– 107.

Juul, A., Kastrup, K.W., Pedersen, S.A., Skakkebæk, N.E., 1997. Growth Hormone (GH) Provocative Retesting of 108 Young Adults with Childhood-Onset GH Deficiency and the Diagnostic Value of Insulin-Like Growth Factor I (IGF-1) and IGF-Binding Protein-3[1]. The Journal of Clinical Endocrinology & Metabolism 82 (4),1195-1201.

Kenemans, J.L., Smulders, F.T., Kok, A., 1995. Selective processing of two-dimensional visual stimuli in young and old subjects: electrophysiological analysis. Psychophysiology. 32, 108–120.

Kenemans, J.L., Lijffijt, M., Camfferman, G., Verbaten, M.N., 2002. Splitsecond sequential selective activation in human secondary visual cortex. J. Cogn. Neurosci. 14, 48–61.

Lamberts, S.W., van den Beld, A.W., van der Lely, A.J., 1997. The endocrinology of aging. Science. 278, 419–424.

Lansbergen, M.M., Kenemans, J.L., van Engeland, H., 2007. Stroop interference and attention-deficit/hyperactivity disorder: a review and meta-analysis. Neuropsychology. 21(2), 251-262.

Lansbergen, M. M., van Hell, E., Kenemans, J. L., 2007. Impulsivity and conflict in the stroop task: An erp study. Journal of Psychophysiology, 21(1), 33.

Lijffijt, M., Van Dam, P.S., Kenemans, J.L., Koppeschaar, H.P., de Vries, W.R., Drent, M.L., Wittenberg, A., Kemner, C., 2003. Somatotropic-axis deficiency affects brain substrates of selective attention in childhood-onset growth hormone deficient patients. Neurosci. Lett. 353, 123–126.

MacDonald, A. W., Cohen, J. D., Stenger, V. A., Carter, C. S., 2000. Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. Science, 288(5472), 1835-1838.

Morley, J.E., Kaiser, F., Raum, W.J., Perry III, H.M., Flood, J.F., Jensen, J., Silver, A.J., Roberts, E., 1997. Potentially predictive and manipulable blood serum correlates of aging in the healthy human male: progressive decreases in bioavailable testosterone, dehydroepiandrosterone sulfate, and the ratio of insulin-like growth factor 1 to growth hormone. Proc. Natl. Acad. Sci. U.S.A. 94, 7537–7542.

Möderscheim, T. A. E., Christophidis, L. J., Williams, C. E., Scheepens, A., 2007. Distinct neuronal growth hormone receptor ligand specificity in the rat brain. Brain Research. 1137, 29.

Müller T., Welnic, J., Woitalla, D. Muhlack, S., 2007. Endurance exercise modulates levodopa induced growth hormone release in patients with Parkinson's disease. Neuroscience Letters 422: 119-122.

Nyberg, F., 2000. Growth Hormone in the Brain: Characteristics of Specific Brain Targets for the Hormone and Their Functional Significance Frontiers in Neuroendocrinology 21, 330–348

Oertel, H., Schneider, H., Stalla, G., Holsboer, F., Zihl, J., 2004. The effect of growth hormone substitution on cognitive performance in adult patients with hypopituitarism, Psychoneuroendocrinology. 29, 839–850.

Pagani F., Netti, C., Guidobono, F., Lattuada, N., Ticozzi, C., Sibilia, V., 1998. Effects of Amylin and Salmon Calcitonin on ß-Endorphin-Induced Growth Hormone and Prolactin Secretion in the Rat Neuroendocrinology 68, 220-228.

Peace, K.A., Orme, S.M., padayatty, S.J., Godfrey, H.P.D., Belchetz, P.E., 1998. Cognitive dysfunction in patients with pituitary tumour who have been treated with transfrontal or transsphenoidal surgery or medication. Clin. Endocrinol. 49, 3, 391-396.

Polich, J., 1998. P300 clinical utility and control of variability. J Clin Neurophysiol. 15, 14-33.

Polich, J., 2003. Theoretical overview of P3a and P3b. In: Polich J, editor. Detection of change: event-related potential and fMRI findings. Boston: Kluwer Academic. 83-98.

Putman, P., Hermans, E.J., van Honk, J., 2007. Exogenous cortisol shifts a motivated bias from fear to anger in spatial working memory for facial expressions. Psychoneuroendocrinology 32, 1, 14-21.

Salthouse, T.A., Fristoe, N., McGuthry, K.E., Hambrick, D.Z., 1998. Relation of task switching to speed, age, and fluid intelligence. Psychol Aging. 13(3), 445-461.

Samuels, M. H., Schuff, K. G., Carlson, N. E., Carello, P., Janowsky J. S., 2008. Health Status, Mood, and Cognition in Experimentally Induced Subclinical Thyrotoxicosis. J Clin Endocrinol Metabol 93 (5), 1730-1736.

Sartorio, A., Molinari, E., Riva, G., Conti, A., Morabito, F., Faglia, G., 1995. Growth hormone treatment in adults with childhood onset growth hormone deficiency: effects on psychological capabilities. Horm. Res. 6–11.

Sathiavageeswaran, M., Burman, P., Lawrence, D., Harris, A., Falleti, M., Maruff, P. and Wass, J., 2007. Effects of GH on cognitive function in elderly patients with adult-onset GH deficiency: a placebo-controlled 12-month study. European journal of endocrinology, 156, 4, 439-447.

Scheepens, A., Möderscheim, T.A., Gluckman, P.D., 2005. The role of growth hormone in neural development. Horm Res 64, 3, 66–72.

Segal-Lieberman, G., Rubinfeld, H., Glick, M., Kronfeld-Schor, N. and Shimon, I., 2006. melanin-concentrating hormone stimulates human growth hormone secretion: a novel effect of MCH on the hypothalamic-pituitary axis. Am J Physiol Endocrinol Metab 290: E982-E988.

Shaywitz, B.A., Shaywitz, S.E., Sebrechts, M.M., Anderson, G.M., Cohen, D.J., Jatlow, P., Young, J.G., 1990. Growth hormone and prolactin response to methylphenidate in children with attention deficit disorder. Life Sci. 46(9), 625-33.

Soares, C.N., Musolino, N.R., Cunha, N.M., et al., 1999. Impact of recombinant human growth hormone. A placebo-controlled trial (RH-GH) treatment on psychiatric, neuropsychological and clinical profiles of GH deficient adults. Arq. Neuropsiquiatr. 182–189.

Sonntag, W.E., Ramsey M., Carter, C.S., 2005. Growth hormone and insulin-like growth factor-1 (IGF-1) and their influence on cognitive aging. Ageing Research Reviews, 4: 2, 195-212.

Stroop, J.R., 1935. Studies of interference in serial verbal reactions. Journal of Experimental Psychology, 18, 643-662.

Sullivan, R.M., Dufresne, M.M., 2006. Mesocortical dopamine and HPA axis regulation: Role of laterality and early environment. Brain research, 1076, 49-59.

Tzanela, M., 2006. Dynamic tests and basal values for defining active acromegaly. Neuroendocrinology 83(3-4), 200-4.

van Dam, P.S., Aleman, A., de Vries, W.R., Deijen, J.B., Van der Veen, E.A., de Haan, E.H.F., Koppeschaar, H.P.F., 2000. Growth hormone, insulin-like growth factor-I and cognitive function in adults. GH IGF-1 Res. 10, S69–S73.

van Dam, P.S., de Winter, C.F., de Vries, R., van der Grond, J., Drent, M.L., Lijffijt, M., Kenemans, J.L., Aleman, A., de Haan, E.H., Koppeschaar, H.P., 2005. Childhoodonset growth hormone deficiency, cognitive function and brain N-acetylaspartate. Psychoneuroendocrinology 30 (4), 357–363.

van Dam, P.S., 2006. Somatotropic therapy and cognitive function in adults with growth hormone deficiency: a critical review. Treat Endocrinol. 5 (2), 1.

van der Reijden-Lakeman, I.E., de Sonneville, L.M., Swaab-Barneveld, H.J., Slijper, F.M., Verhulst, F.C., 1997. Evaluation of attention before and after 2 years of growth hormone treatment in intrauterine growth retarded children. J. Clin. Exp. Neuropsychol. 19 (1), 101–118.

Very, N.M., Sheridan, M.A., 2007. Somatostatin regulates hepatic growth hormone sensitivity by internalizing growth hormone receptors and by decreasing transcription of growth hormone receptor mRNAs. Am J Physiol Regul Integr Comp Physiol 292, R1956-R1962.

Vitiello, M.V., Moe, K.E., Merriam, G.R., Mazzoni, G., Buchner, D.H., Schwartz, R.S., 2006. Growth hormone releasing hormone improves the cognition of healthy older adults. Neurobiol. Aging 27, 318–323.

Watson, G.S., Baker, L.D., Cholerton, B.A., Rhoads, K.W., Merriam, G.R., Schellenberg, G.D., Asthana, S., Cherrier, M., Craft, S., 2009 Effects of Insulin and Octreotide on Memory and Growth Hormone in Alzheimer's Disease. J Alzheimers Dis., Jul 20.

Wong, A.O.L., Murphy, C.K., Chang, J.P., Neumann, C.M., Lo, A., Peter, R.E., 1998. Direct actions of serotonin on gonadotropin-II and growth hormone release from goldfish

pituitary cells: interactions with gonadotropin-releasing hormone and dopamine and further evaluation of serotonin receptor specificity. Fish physiology and biochemistry 19, 1, 23-34.

Chapter 3

Cognitive performance in older men is associated with growth hormone secretion

E.H. Quik, E.B. Conemans, G.D.Valk, J.L. Kenemans, H.P.F. Koppeschaar & P.S. van Dam

Quik, E.H., Conemans, E.B., Valk, G.D., Kenemans, J.L., Koppeschaar, H.P.F., & van Dam., P.S. (2012). Cognitive performance in older males is associated with growth hormone secretion. Neurobiology of Aging 34(8), 1137-43.

Abstract

Background and Aim

As GH secretion decreases with age, this may contribute to cognitive changes associated with aging. We evaluated the relation between GH secretion and cognition in elderly men by assessing correlations between GH secretion and performance on cognitive tests in conjunction with the recording of event-related potentials (ERPs) in order to assess underlying neurophysiological mechanisms.

Subjects and Methods

GH secretion of 17 male elderly participants was assessed by a GHRH-GHRP-6 test. Standardized neuropsychological tests were used to assess cognitive function. EEG/ ERPs were recorded to assess on-line electro-cortical correlates of sensory-cortical processing and selective attention.

Results

GH secretion was significantly correlated with target detections and speed of responding in the selection-potential task. Furthermore, GH peak was significantly correlated with the letter-digit span test performance.

Conclusion

The present data confirm that cognitive performance in elderly men is associated with GH secretion, with respect to target detection and speed of responding in conditions of selective attention, short-term memory and basic processing speed.

Key words: growth hormone (GH) – cognition – aging – somatotropic axis – event-related brain potentials (ERPs) – selection-potential task

Introduction

There is growing evidence of a relationship between growth hormone (GH) secretion and cognitive function (van Dam & Aleman, 2004; Quik et al., 2010). Studies comparing cognitive function in GH deficient (GHD) patients with that of matched controls have revealed impaired neuropsychological performance in the domains of memory and processing speed (van Dam et al. 2005; Falleti et al. 2006). Moderate improvements in these domains after GH therapy have also been reported (van Dam, 2006; Oertel et al., 2004).

Cognitive effects of chronic or acute variations in GH secretion may reflect the widespread presence of GH receptors throughout the brain. A significant decrease has been observed in the density of GH binding with increasing age (over 60 years old) in the choroid plexus, hypothalamus, hippocampus, pituitary and putamen (Lai et al., 1993; Nyberg, 1997). Furthermore, aging is thought to be associated with a decline in the activity of the somatotropic axis (van Dam, 2006). It is also known that circulating levels of growth hormone (GH) decrease with aging (Anawalt and Merriam, 2001). All this could at least partly account for the decline of specific components of cognitive function and would therefore suggest a relationship between GH and cognitive function. Moreover, features of aging resemble those of GHD, suggesting that the somatotropic axis may play a role in age-related cognitive decline (Arwert et al., 2005).

A rather elaborate analysis of possible neurocognitive deficits in patients with childhood onset (CO) GHD was provided by Lijffijt et al. (2003). These authors assessed behavioral and electro-cortical aspects of perception and selective attention, using a 'selection-potential' task in which participants had to attend to some stimuli and ignore others. Event-related brain potentials (ERPs) were recorded to derive the electro-cortical correlates of perception and attention. A specific abnormality was found in the form of a reduced 'N2b' in patients, which points to a functional deficit in the anterior cingulate cortex involved in integrated processing of different stimulus attributes (Kenemans et al., 2002). Behaviorally, patients were significantly worse in detecting a subcategory of to be attended stimuli ('targets'). These same patients were also significantly slower in the trail making A test, which assesses basic processing speed (going from a, to b, to c, etc.) (Van Dam et al., 2005). This result is consistent with a deficit in basic processing speed.

Reduced N2bs have also been reported for healthy senescent individuals (Kenemans et al., 1995). Given the decline of specific cognitive functions with lower GH secretion, and the resemblances between functions affected by normal aging to those affected by GHD, it is reasonable to hypothesize that reduced GH levels may contribute to age-related cognitive decline (Arwert et al., 2005). If so, especially in older individuals GH levels may predict anterior cingulate cortex (ACC) function as related to selective attention and as reflected in N2b.

The present study addresses the relation between GH secretion and cognitive function in men over 50 years old. Standard neuropsychological tests were used to assess the domains

of memory (15 words test), basic processing speed (trail making A), and concept shifting (trail making B). More specific neurocognitive aspects of perception and attention were again assessed using the selection-potential task described by Lijffijt et al. (2003), including event-related brain potential recording.
We specifically predicted that low GH secretion would be associated with reduced electro-cortical responses to task-relevant stimuli, reduced target detection, and impaired performance in tests assessing memory, processing speed, and possibly concept shifting.

Methods

Subjects

Seventeen healthy male participants (mean age 61.2 years, SD 7.9, range 50-78; mean education level was 10-13 years) were included. The mean waist/hip ratio was 0.94 (SD .05, range .88-1.03) and mean BMI was 26.3 kg/m^2 (SD 3.1, range 20.9-29.5). We excluded participants with neurologic or psychiatric disease, endocrine or internal disease, severe obesity (BMI > 32 kg/m^2), malnutrition (BMI < 18.5 kg/m^2), chronic alcohol (more than 3 units daily) or drug (use of any recreational drug) abuse, and use of medication that may affect cognitive functioning (e.g., benzodiazepines, antidepressants, anti-epileptics). Exclusion criteria were checked using a questionnaire including medical history, assessment of current and previous medication, and appropriate blood tests. In order to exclude participants with mental disorders such as dementia, participants with an MMSE score exceeding 24 points were included. Below this, scores may indicate severe (≤9 points), moderate (10-20 points) or mild (21-24 points) cognitive impairment. Therefore, one man with a MMSE score of 19 could not participate in our study. The local medical ethics committee approved the study protocol and all procedures were carried out with the adequate understanding of the participants and written consent according to the Declaration of Helsinki.

Tasks

Standardized neuropsychological tests included the trail making A, trail making B, the 15-Word test, and the Digit Span forward and backward. Briefly, the trail making task B is a test of planning of movement, visual-motor tracking and processing speed. The subject had to mark numbers and letters as fast as possible in a specific sequence: 1-A-2-B etc. The numbers and letters were randomly distributed in a circle. The 15 words Test (15 woorden

test; the Dutch version of the Rey Auditory Verbal Learning Task (RAVLT)) is a test for long term memory retention. Subjects learned a list of 15 words, and were asked to recall as many words as possible from memory after a delay of 15 min. For detailed descriptions, the reader is referred to Nelson (1982), Lezak (1995), Aleman et al. (1999), and Ball et al. (1999).

During the selective attention task (Kenemans et al., 1995; Lijffijt et al., 2003) participants viewed sequences of square, square-wave gratings that varied in spatial frequency (SF; 0.6 or 4.8 cycles per degree of visual angle, wide or narrow bars, respectively) and orientation (O; vertical or horizontal), making up four different stimuli. Stimulus onset asynchrony varied between 750 and 950 msec. Participants had to selectively press a button when perceiving a predefined combination of SF and O (Target), but had to ignore the other combinations, and were to emphasize speed over accuracy. In separate blocks, each of the four different stimuli was defined as Target, and each Target had to be responded to with either the left or right hand, thus obtaining eight blocks. Stimuli were mixed randomly within a block of 128 trials (4 x 32). A practice block containing 12 trials proceeded each new block.

Procedure and signal recording

All tests were performed individually and under identical circumstances. After signing informed consent subjects were tested individually in a quiet room. In the morning the GHRH-GHRP-6 test procedure was started. Neuropsychological testing began after 60 minutes following the start of the GHRH-GHRP-6 test and took approximately 45 minutes. On the same day after lunch, in the afternoon, EEG/ selective attention task measurement was scheduled. During this task, EEGs were recorded from several scalp sites using an electrode cap containing 30 tin electrodes, including Fz (frontal) and Oz (occipital). Horizontal and vertical electro-oculograms were recorded using four electrodes positioned around the eyes. The left mastoid was used as a reference. Skin impedance was kept below 5 kΩ. All signals were subjected to 30 Hz low-pass filters and a time constant of 3 s. The sampling rate was 200 Hz. One hour was needed to record EEG, including 25 min to complete the selective attention task.

GHRH-GHRP-6 test procedure and laboratory assessments

During the test day, subjects were fasting and they did not perform any strenuous activities before the test. All tests started between 08.30 and 09.00 h. a.m. After height and weight measurements, an intravenous catheter was placed in the forearm for blood sampling and drug administration. Initial blood samples were drawn at t = zero for measurement of basal

levels of GH. Subsequently, GHRH (100 µg; GHRH Ferring; Ferring Pharmaceuticals Ltd., Hoofddorp, The Netherlands) was administered intravenously as a bolus injection and followed by GHRP-6 (93 µg, His-DTrp-Ala-DPhe-Lys-NH_2, courtesy of Prof. F. Casanueva). Subsequently, blood was sampled at t = 30, 45, 60, 90, and 120 minutes after injection of GHRH and GHRP-6 for assessment of GH responses. The subjects remained fasting, non-smoking, and seated during the tests.
GH was measured using an immunometric technique on an Immulite Analyzer (Diagnostic Products, Los Angeles, CA). The lower limit of detection was 0.01 µg/L; the interassay variations were 9.7, 5.6, 4.4, and 5.2% at 0.13, 0.80, 4.2, and 15.4 µg/L, respectively (n = 69). One µg/L corresponds to 2.6 mIU/L (WHO International Ref. Prep 80/505). GH response was measured by assessment of the highest plasma level during the test (GH peak) and by calculating the area under the curve (GH-AUC).

Data analysis

The neuropsychological test scores were calculated according to standard procedures. ERPs were analyzed in Brain Vision Analyzer® version 1.05.0002 for Windows.
The present analysis focused on ERPs from the electrodes Fz and Oz, similar to previous studies (Kenemans et al., 1995; Kenemans et al., 2002; Kenemans et al., 1993), which showed that the time-varying cortical correlates of selective attention are best observed using these two leads. All signals were subjected to 30 Hz low-pass filters and a time constant of 3 s. The sampling rate was 200 Hz. Basic ERP analysis was conducted for Fz and Oz according to Lijffijt et al. (2003), including artefact rejections, ocular-artifact control, and baseline subtraction and this yielded occipital selection negativity (OSN), N2b, and sensory, stimulus-specific effects. Only the artifact-rejection procedure differed from the previous study in that an additional artifact scan was performed after ocular-artifact control to additionally check for subtle artifacts that had gone unnoticed when blinks had not been corrected yet.
Neuropsychological data were lost for one participant, who therefore is excluded from all further analysis, which leaves an N of 16 for the neuropsychological data analysis. Furthermore, due to technical problems, ERP data were not available for yet another six participants, which leaves us with an N of 10 for the data analysis.

Statistics

Bivariate Pearson correlations were calculated (two-tailed) using a standard version of SPSS® 12.0.1 for Windows between basal GH area under the curve and GH peak, and all cognition outcome parameters (neuropsychological tests and ERP data).

Results

GH secretion

Of all 16 participants included in neuropsychological data analyses the mean GH peak was 36.5 µg /l, with a minimum of 16.3 µg /l a maximum of 50.4 µg /l, and a standard deviation of 10.2. An evoked GH concentration of ≥ 15.0 µg /l distinguishes between healthy and GH-deficient adults (Popovic at al., 2000). Mean GH area under the curve (AUC) was 2276.8 µg /l x 120 min, the minimum of 963.9 µg /l x 120 min, a maximum of 3382.5 µg /l x 120 min, and a standard deviation of 219.9.

Of the 10 participants included in EEG analyzes mean GH peak was 36 µg /l, with a minimum of 16.3 µg /l a maximum of 50.5 µg /l, and a standard deviation of 11.3. Mean GH area under the curve (AUC) was 2170.7 µg /l x 120 min, the minimum of 963.9 µg /l x 120 min, a maximum of 3382.5 µg /l x 120 min, and a standard deviation of 695.6.

Selection-potential task performances

Mean target reaction time was 468.9 milliseconds, with a standard deviation of 62.1. The percentage of targets detected was 81.2%. The percentage of correct rejections of all non-targets (75% of all stimuli) was 96.1%.

Correlations

Table 1 Neuropsychological assessment correlations with GH secretion

Neuropsychological test	Function measured	Reference	GH secretion	
			GH Peak	GH AUC
Number-digit span test	Short-term & working memory	A		(r=.54, p=.03)
Information (WAIS)	General knowledge	A		
Digit Symbol Substitution (WAIS)	Cognitive and perceptual-motor processing speed	A		
Dutch National Adult Reading Test	Estimate of verbal intelligence	B		
Trail Making Task (TMT)				
Trail Making Task A	Processing speed & attention	B		
Trail Making Task B	Processing speed + attentional shifting	B		
TMT B minus TMT A	Attentional shifting	A		
TMT B vs. TMT A	Attentional shifting	E		
15 Words Test (RAVLT)				
Recall score	Verbal memory	A		
Immediate recall score	Immediate verbal memory	B/C		
Delayed recall score	Delayed verbal memory	B/C		
Recognition score	Recognition non-verbal memory	C		
Selective Attention Task				
Target detection	Sensory discrimination & selective attention	D	(r= .68, p=.03)	(r= .64, p=.05)
Mean reaction time, MRT	Speed of responding	D	(r=-.78, p=.01)	(r=-.70, p=.03)

A= Aleman et al. (1999), B= van Dam et al. (2005), C= van Zandvoort et al. (2005), D= Kenemans et al. (1995) E= new measure.

Table 1 lists the correlations between neuropsychological assessment and GH secretion. No significant age effect was found, and no correlations were found between level of education and neuropsychological test performances, nor with ERPs. Post hoc analyses revealed that GH response (GH peak, p = .33 GH AUC, p = .35) did not correlate with the MMSE score.

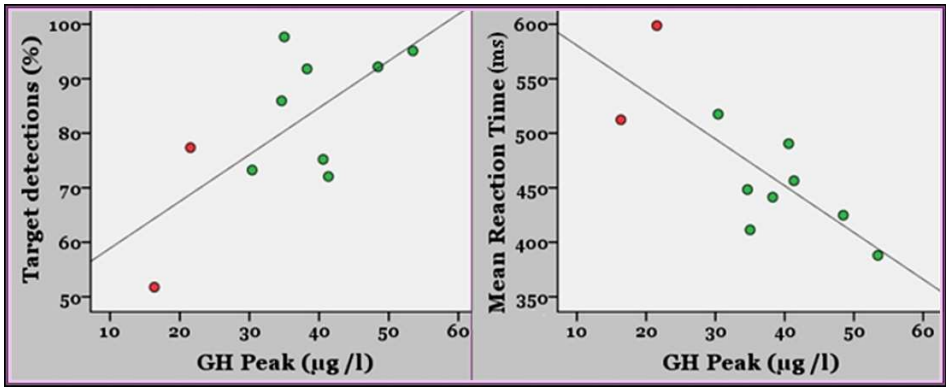


Figure 1 Correlations between percentage target detections and GH peak (a) and between mean reaction time and GH peak (b)

GH peak correlated significantly and positively with percentage target detections in the selection-potential task (r=.68, p=0.03; figure 1), but not with correct rejections. Target detections also correlated positively with GH AUC (r=.64, p=0.05). The speed of responding, or mean reaction time, in the selection-potential task correlated negatively with GH peak (r=-.78, p=0.01; figure 1) and with GH AUC (r=-.70, p=0.03). No significant correlation was found between GH secretion and N2b or other selection-potential amplitudes, or with sensory-specific activation. Figure 2 depicts N2b traces for the 5 lowest versus 5 highest GH-peak participants (F = 2.311; p = .172).

Although consistent with our expectations, this difference could not be statistically confirmed. The letter-digit span item scores correlated positively with the GH AUC (r=.54, p=0.03). In addition, trail making B (r=.59, p=0.01) and trail making B-A (r=.61, p=0.01) correlated positively with the GH peak, but both correlations became non-significant after exclusion of one outlier. There were no associations between GH response and performance on the 15 words test.

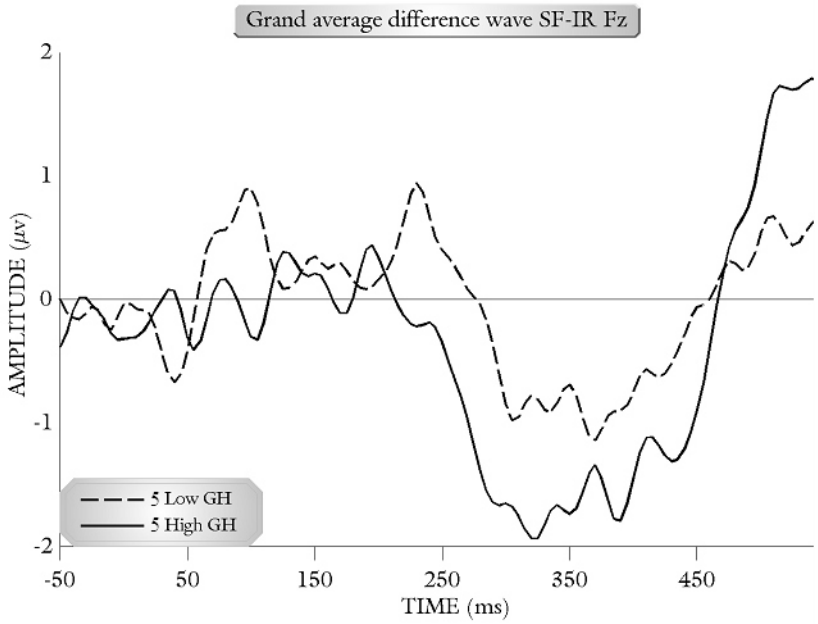


Figure 2 N2b difference waves of spatial frequency relevant (SF) minus irrelevant stimuli (IR) between 325-400 ms on electrode Fz for the 5 lowest versus 5 highest GH-peak participants

Discussion

We assessed the relation between GH secretion and cognition, as measured by performance on cognitive tests in older men, as well as the relation between GH secretion and attention related cortical brain activity during a selection-potential go/ no-go task. We expected to find deteriorated cognitive performance, reduced N2b amplitude, and a lower percentage target detections to be associated with lower GH secretion.

A positive correlation between selection-potential-target detections and GH secretion was found: Older men with lower GH levels made more omission errors. At the same time, there were no significant deficiencies in N2b with lower GH secretion, indicating that selective attention to stimuli that share spatial frequency with the target (but not necessarily the orientation) was not compromised. The reduced target-detection rate must then be attributed to a selection stage, in which the final decision about 'Targetness' is being made.

The present N2b results differ from the findings from Lijffijt et al. (2003) in CO-GHD patients, who observed that N2b was affected in these patients. The N2b-effects found in the study of Lijffijt et al. (2003) could be due to a direct effect of GH depletion. However, it could also result from lack of GH during development of the brain, which in turn may result

in deficient neuron and glia growth, or suboptimal myelination. The participants in the current study did not have a severe shortage of GH or a long-term deficiency, but were older men with physiologically reduced GH secretion. In addition, the difference in age could play a role, as the effect of GH could be less detectable in the elderly than in younger patients. It would be of interest to perform similar ERP studies in elderly subjects with pathologically reduced GH levels as a consequence of GHD. Future studies may also address the question whether the finding of the effect Lijffijt et al. (2003) found are caused by GHD directly or in indirect ways.

In the selection-potential task a significant negative correlation between GH secretion and speed of responding was observed: Low GH-peak participants reacted more slowly to correctly detected targets. In addition, we found a significant positive correlation between the Digit-span scores and GH AUC. This suggests reduced functioning of short-term memory with low GH, which may also be a mediating factor in reduced target detection, to the extent that the latter depends on intact short-term memory for what is relevant and what is not relevant (de Fockert et al., 2001).

In contrast, low GH-peak participants were actually faster in the trail making B test. However, this correlation became non-significant after removal of an outlier. Trail making A performance was not affected by lowered GH levels, which, together with the reduction in target detection for low GH, could suggest that basic processing speed, as manifest in trail making A, is not at all compromised with low GH, but detection of targets is either relatively impaired or slowed down when it does occur correctly. The latter may then reflect decelerated higher-order decision making, when multiple sources of information from a single stimulus must be integrated.

The present data confirm that cognitive performance in elderly men with respect to target detection and speed of responding in conditions of selective attention is associated with GH secretion. However, no specific neurophysiological alterations as measured by ERP were significant, whereas this was previously observed in young CO-GHD patients. The lack of an N2b effect in particular indicates that the neurophysiological mechanism underlying reduced target detection with low GH is different for the present sample of older men, relative to childhood onset GHD patients as assessed in the Lijffijt et al. (2003) study.

Acknowledgements

We thank Wouter M. Creyghton (MD) for including patients and Jeroen Gerritsen (MSc) for his help on collecting ERP data. GHRH was kindly provided by Ferring Netherlands. GHRP-6 was provided by Prof. Felipe Casanueva, Santiago de Compostela, Spain. UMCU provided funding for this study, but had no further role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

References

Aleman, A., Verhaar, H.J., De Haan, E.H., De Vries, W.R., Samson, M.M., Drent, M.L., van der Veen, E.A., Koppeschaar, H.P., 1999. Insulin-like growth factor-I and cognitive function in healthy older men. J. Clin. Endocrinol. Metab. 84, 471-475.

Aleman, A., de Vries, W.R., Koppeschaar, H.P.F., Osman-Dualeh, M., Verhaar, H.J.J., Samson, M.M., Bol, E., de Haan, E.H.F., 2001. Relationship between circulating levels of sex hormones and insulin-like growth factor-I and fluid intelligence in older men. Exp. Aging Res. 27, 283- 291.

Anawalt, B.D., Merriam, G.R., 2001. Neuroendocrine aging in men. Andropause and somatopause. Endocrinol Metab Clin North Am 30(3), 647-669.

Arwert, L.I., Deijen, J.B., Witlox, J., Drent, M.L., 2005. The influence of growth hormone (GH) substitution on patient-reported outcomes and cognitive functions in GH-deficient patients: a meta-analysis, Growth Horm. IGF Res. 15, 47-54.

Ball, L.J., Bisher, G.B., Birge, S.J., 1999. A simple test of central processing speed: an extension of the short blessed test. J. Am. Geriatr. Soc. 47, 1359–1363.

Falleti, M.G., Maruff, P., Burman, P., Harris, A., 2006. The effects of growth hormone (GH) deficiency and GH replacement on cognitive performance in adults: A meta-analysis of the current literature, Psychoneuroendocrinology 31, 681-691.

de Fockert, J.W., Rees, G., Frith, C.D., Lavie, N., 2001. The role of working memory in visual selective attention. Science 291, 1803-1806.

Kenemans, J.L., Smulders, F.T., Kok, A., 1995. Selective processing of two-dimensional visual stimuli in young and old subjects: electrophysiological analysis. Psychophysiology 32, 108-120.

Kenemans, J.L., Lijffijt, M., Camfferman, G., Verbaten, M.N., 2002. Splitsecond sequential selective activation in human secondary visual cortex. J. Cogn. Neurosci. 14, 48-61.

Lai, Z., Roos, P., Zhai, O., Olsson, Y., Fholenhag, K., Larsson, C., Nyberg, F., 1993. Age-related reduction of human growth hormone-binding sites in the human brain. Brain Res. 621, 260– 266.

Lezak, M.D., 1995. Neuropsychological assessment. Oxford University Press, New York.

Lijffijt, M., Van Dam, P.S., Kenemans, J.L., Koppeschaar, H.P., de Vries, W.R., Drent, M.L., Wittenberg, A., Kemner, C., 2003. Somatotropic-axis deficiency affects brain substrates of selective attention in childhood-onset growth hormone deficient patients. Neurosci. Lett. 353, 123-126.

Nelson, H.E., 1982. The National Adult Reading Test (NART): test manual. NFER Nelson, Windsor, UK.

Nyberg, F., 1997. Aging effects on growth hormone receptor binding in the brain. Exp. Gerontol. 32, 521– 528.

Popovic, V., Leal, A., Micic, D., Koppeschaar, H.P., Torres, E., Paramo, C., Obradovic, S., Dieguez, C., Casanueva, F.F., 2000. GH-releasing hormone and GH-releasing peptide-6 for diagnostic testing in GH-deficient adults. Lancet. 356(9236), 1137-1142.

Quik, E.H., van Dam, P.S., Kenemans, J.L. Growth hormone and selective attention: A review. Neurosci. Biobehav. Rev. 34 (2010) 1137–1143.

Shepard, R.N., Metzler, J., 1971. Mental rotation of three-dimensional objects. Science. 171(972), 701-703.

van Dam, P.S., Aleman, A., 2004. Insulin-like growth factor-I, cognition and brain aging, Eur. J. Pharmacol. 490, 87-95.

van Dam, P.S., de Winter, C.F., de Vries, R., van der Grond, J., Drent, M.L., Lijffijt, M., Kenemans, J.L., Aleman, A., de Haan, E.H., Koppeschaar, H.P., 2005. Childhood-onset growth hormone deficiency, cognitive function and brain N-acetylaspartate. Psychoneuroendocrinology. 30(4), 357-363.

van Dam, P.S., 2005. Neurocognitive function in adults with growth hormone deficiency. Horm Res. 64 Suppl 3, 109-114.

van Dam, P.S., 2006. Somatotropic therapy and cognitive function in adults with growth hormone deficiency: A critical review. Treat Endocrinol. 5 (2), 1.

van Zandvoort, M.J., Kessels, R.P., Nys, G.M., de Haan, E.H., Kappelle L.J., 2005. Early neuropsychological evaluation in patients with ischaemic stroke provides valid information. Clin Neurol Neurosurg. 107(5), 385-92.

Chapter 4

Insulin-like growth factor-I is associated with cognitive performance in older men

E.H. Quik, E.B. Conemans, G.D.Valk, J.L. Kenemans, H.P.F. Koppeschaar & P.S. van Dam

Abstract

Background and Aim

Circulating insulin-like growth factor-I (IGF-1) levels are associated with variation in cognitive performance. The decrease of IGF-1 levels with age may contribute to cognitive changes associated with aging. The objective of this study was to investigate the relation between IGF-1 and cognition in older men. We focused on correlations between IGF-1 levels and performance on cognitive tests, and we assessed the associations between IGF-1 and attention related cortical brain activity by recording event-related potentials (ERPs).

Subjects and Methods

We studied 10 healthy male subjects (mean age 61 years, range 50-78). Cognitive function was assessed by standardized neuropsychological tests. ERPs were recorded during a go/ no go selective attention task. IGF-1 was assessed by measurement of plasma IGF-1.

Results

A correlation was found between age and IGF-1, as expected. Higher IGF-1 levels were associated with faster Trailmaking A performance (r=-.86, p=00). No other correlations were found between IGF-1 and neuropsychological test performances. Higher IGF-1 levels were also associated with shorter mean reaction times during the ERP task (r=-.80, p=.005), as well with larger Lateralized-Readiness-Potential (LRP) amplitudes, an ERP measure of motor-cortex preparation (r=-.82, p=.00). No correlation was found between IGF-1 and N2b or any other attention-related ERPs.

Conclusion

Slower speed of responding, lower processing speed, and reduced motor-cortex preparation (LRPs) are associated with lower levels of IGF-1 in elderly men. IGF-1 was not significantly associated with attention-related brain potentials, which we previously found to be compromised in young GH deficient patients. Lower IGF-1 levels in older men may contribute to slower cognitive responses and may therefore hamper their performance on cognitive tests.

Key words: insulin-like growth factor-I (IGF-1) – cognitive function, cognition – EEG (ERP, LRP) – somatropic axis - aging

Introduction

There is growing evidence of a relationship between insulin-like growth factor-I (IGF-1) and cognitive function (van Dam & Aleman, 2004). The potential role of IGF-1in the central nervous system is suggested by the widespread distribution of IGF-1 receptors throughout the brain. The somatotropic axis refers to the hormonal signaling from hypothalamus to anterior pituitary gland, normally resulting in the release of growth hormone (GH) and IGF-1. Although a positive relationship has been documented between serum IGF-1 levels and spontaneous 24-h GH secretion in children, this relationship may be missing in older subjects (Aleman et al., 1999). Aleman et al. (2000) suggested a disruption of the relation between IGF-1 and GH secretion in older age. Important for the present discussion is that both substances cross the blood-brain barrier and bind to their receptors in the CNS. Decreased functioning of the somatotropic (GH-IGF-1) axis is found in GH deficient (GHD) patients and in normal aging adults (Anawalt and Merriam, 2001). As cognitive features of aging resemble those of GHD, it has been hypothesized that the GH–IGF-1 axis may play a role in age-related cognitive decline (Arwert et al., 2005). Several studies suggest an association between the GH-IGF-1 axis and cognitive functioning (van Dam & Aleman, 2004). Furthermore, a relation between GHD and cognitive functions is established, but it remains unclear whether this is mediated by IGF-1.
Aleman et al. (1999) found that IGF-1 levels in older men (range 65-76 years) correlated with age-sensitive, 'don't hold' tests, such as the Concept Shifting Task, but not with age-insensitive 'hold' tests. Their data suggest that cognitive functions sensitive to aging (fluid intelligence), particularly perceptual-motor performance and information processing speed, deteriorate at lower circulating plasma IGF-1 levels. In contrast, other cognitive functions, which are insensitive to aging (such as crystallized intelligence), were not affected by attenuated IGF-1 levels. The same group demonstrated a significant association between serum IGF-1 and fluid intelligence, a cognitive measure that is sensitive to aging (Aleman et al., 2001). In another study Aleman et al. (2000) observed that GH response to a challenge with GHRH was significantly associated with two age-sensitive cognitive tests, both measuring cognitive speed.
In a 3-year longitudinal study of cognitive decline in 1318 subjects 65–88 years of age, Dik et al. (2003) observed an association between low serum levels of IGF-1 and deficits in information processing speed (Dik et al., 2003). Rollero et al. (1998) observed a significant positive correlation between IGF-1 levels and cognitive function as measured by the mini-mental state examination (MMSE) in elderly subjects with varying degrees of cognitive impairment. Cherrier et al. (2004) conducted a placebo-controlled study in which older males, mean age 67.5, participated. In this study the potential changes in IGF-1 and IGF-related binding proteins in response to testosterone administration and their relationship to cognitive functioning in healthy older men was examined. Contrary to the results yielded by the study of Aleman et al. (1999), they found no correlation between IGF-1 and the test scores on the Stroop task (time to complete interference trial). A meta-analysis showed a

positive association between baseline plasma IGF-1 and cognition. Furthermore, individual increases in IGF-1 were associated with memory improvement (Arwert et al., 2005). All this data suggests an important role for IGF-1 in cerebral functioning, especially regarding cognitive performance.

Using event-related brain potentials (ERPs) recorded during a go/ no go task, in which only a specific target conjunction of two visual attributes required an overt response, Lijffijt et al. (2003) demonstrated that adults with childhood-onset GHD (CO-GHD) have attentional deficits in association with reduced attention-related electro cortical responses (N2b) amplitude to task-relevant stimuli. The altered N2b in CO-GHD was paralleled by more omission errors to the targets. The N2b originates from the anterior cingulate cortex and possibly reflects the integrated processing of the two relevant visual attributes (Kenemans et al., 2002). Reduced N2bs have been related to normal aging (Kenemans et al., 1995), and a trend correlation between N2b amplitude and IGF-1 peak has been observed (van Dam et al., 2005).

In this study, we focused on the relation between IGF-1 secretion and cognitive function in men over 50 years old. Cognitive function was assessed by standard neuropsychological tests sensitive and insensitive to aging. Inspired by findings of overlap between brain correlates of abnormal attentional processing between senescent individuals and CO-GHD patients (Lijffijt et al., 2003; Kenemans et al., 1995), an additional focus was on the relation between IGF-1 and ERPs. Our recent review (Quik et al., 2010), showed no GH effects on neuropsychological measures of selective attention (Stroop interference, Trailmaking B test). In addition, no association was found between GH and typical functions of selective attention, but a relation was found with basic perceptual-motor speed (Trailmaking A test). In the present study we therefore predict that IGF-1 affects basic perceptual-motor speed, including reaction times and target detection, rather than the neuropsychological measures of selective attention.

The ERPs as currently recorded during the go/ NoGo selective attention task also allow for the derivation of a measure of activation in the (vicinity) of the motor cortex specifically related to the ensuing overt reaction (Kenemans et al., 1995). This ‘lateralized readiness potential’ (LRP) may be of interest given the role of IGF-1 in maintaining the myelin that surrounds the axons to and from the motor cortex that may be instrumental in basic perceptual-motor speed. Specifically, LRP amplitudes have been shown to covary with demands on the behavioral motor response (Smulders et al., 1995) and therefore reflect the extent of motor and surrounding cortex recruited in service of producing speeded behavioral reactions.

Subjects and Methods

Subjects

Ten healthy male participants (mean age 60.6 years, range 50-78) were included. The mean waist/hip ratio was 0.95 (SD .05, range .88-1.03) and mean BMI was 25.3 (SD 3.0, range 20.9-29.5). Participants with neurologic or psychiatric disease (including dementia; MMSE scores below 24) scores, endocrine or internal disease, severe obesity (BMI > 32 kg/m^2), malnutrition (BMI < 18.5 kg/m^2), chronic alcohol (more than 3 units daily) or drug abuse, and use of medication that may affect cognitive functioning, were excluded.
Exclusion criteria were checked using a questionnaire including medical history, assessment of current and previous medication, and appropriate blood tests. The local medical ethics committee approved the study protocol and all procedures were carried out with the adequate understanding of the participants and written consent according to the Declaration of Helsinki.

Tasks

Standardized neuropsychological tests included the trail making A, trail making B, the 15-Word test, and the Digit Span forward and backward. Briefly, the trail making task B is a test of planning of movement, visual-motor tracking and processing speed. The subject had to mark numbers and letters as fast as possible in a specific sequence: 1-A-2-B etc. The numbers and letters were randomly distributed in a circle. The 15 words test (the Dutch version of the Rey Auditory Verbal Learning Task (RAVLT)) is a test for long term memory retention. Subjects learned a list of 15 words, and were asked to recall as many words as possible from memory after a delay of 15 min. For detailed descriptions, the reader is referred to Nelson (1982), Lezak (1995), Aleman et al. (1999), and Ball et al. (1999).
During the selective attention task (Kenemans et al., 1995; Lijffijt et al., 2003) participants viewed sequences of square, square-wave gratings that varied in spatial frequency (SF; 0.6 or 4.8 cycles per degree of visual angle, wide or narrow bars, respectively) and orientation (O; vertical or horizontal), making up four different stimuli. Stimulus onset asynchrony varied between 750 and 950 msec. Participants had to selectively press a button when perceiving a predefined combination of SF and O (Target), but had to ignore the other combinations, and were to emphasize speed over accuracy. In separate blocks, each of the four different stimuli was defined as Target, and each Target had to be responded to with either the left or right hand, thus obtaining eight blocks. Stimuli were mixed randomly

within a block of 128 trials (4 x 32). A practice block containing 12 trials proceeded each new block.

Procedure and signal recording

Plasma IGF-1 levels were drawn in the fasting state. On the same day, EEG/ selective attention task measurement was scheduled. During this task, EEGs were recorded from several scalp sites using an electrode cap containing 30 tin electrodes, including Fz (frontal) and Oz (occipital). Horizontal and vertical electro-oculograms were recorded using four electrodes positioned around the eyes. The left mastoid was used as a reference. Skin impedance was kept below 5 kΩ. All signals were subjected to 30 Hz low-pass filters and a time constant of 3 s. The sampling rate was 200 Hz. One hour was needed to record EEG, including 25 min to complete the selective attention task.

Laboratory assessments

IGF-1 was measured using an immunometric technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, CA). The lower limit of detection was 6.0 ng/mL and inter-assay variations were 8.7, 5.8, and 6.5% at mean IGF-1 plasma levels of 33, 174, and 445 ng/mL, respectively (n = 115).

Data analysis

The neuropsychological test scores were calculated according to standard procedures. ERPs were analyzed in Brain Vision Analyzer® version 1.05.0002 for Windows. The present analysis focused on ERPs from the electrodes Fz and Oz, similar to previous studies (Kenemans et al., 1995; Kenemans et al., 2002; Kenemans et al., 1993), which showed that the time-varying cortical correlates of selective attention are best observed using these two leads. All signals were subjected to 30 Hz low-pass filters and a time constant of 3 s. The sampling rate was 200 Hz. Basic ERP analysis was conducted for Fz and Oz according to Lijffijt et al. (2003), including artefact rejections, ocular-artifact control, and baseline subtraction and this yielded occipital selection negativity (OSN), N2b, and sensory, stimulus-specific effects. Only the artefact-rejection procedure differed from the previous study in that an additional artifact scan was performed after ocular-artefact control to additionally check for subtle artifacts that had gone unnoticed when blinks had not been corrected yet. Following previous studies, the present analysis focused on ERPs from the electrode sites Fz and Oz and on Lateralized Readiness Potentials (LRPs) from the electrode sites C3 and C4, (Kenemans et al., 1995; Kenemans et al., 2002; Kenemans et al.,

1993). Briefly, LRPs are derived by averaging the (C3 minus C4) ERP for right-hand responses and the (C4 minus C3) ERP for left-hand responses, so as to obtain a pure reflection of hand-selective cortical activation.

Statistics

Bivariate Pearson correlations were calculated (two-tailed) using a standard version of SPSS® 12.0.1 for Windows between plasma IGF-1 and all cognition outcome parameters (neuropsychological tests and ERP data).

Results

GH secretion

Mean IGF-1 was 117.1 ng/mL with a minimum of 60.7 ng/mL, a maximum of 145.3 ng/mL, and a standard deviation of 27.4 ng/mL.

Selective attention task performances

Mean target reaction time was 468.9 milliseconds, with a standard deviation of 62.1. The percentage of targets detected was 81.2%. The percentage of correct rejections of all non-targets was 96.1%. The percentage correct rejections of the spatial frequency relevant non-targets were 92.3%, of the orientation relevant non-targets was 98.1%, and of the irrelevant non-targets was 99.2%.

Correlations

No correlation was found between education and IGF-1. A significant correlation was noted between level of education and performance on the NVL (NART) (r=.61, p=.01). No other correlations were found between level of education and neuropsychological test performances. A correlation was found between age and IGF-1 (r=-745, p=.01), as expected. No significant correlations were found between age and neuropsychological test performances. A negative correlation between the Trailmaking A score and IGF-1 was found (r=-.86, p=00; Figure 1; Table 1), indicating that higher IGF-1 levels are associated

with faster performance on this task. No other correlations were found between IGF-1 and neuropsychological test performances (Table 1).

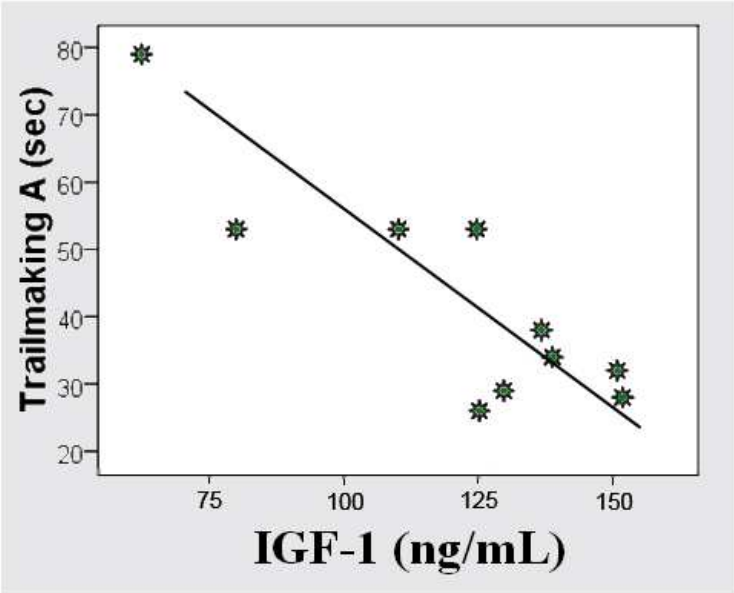


Figure 1 Correlations between the Trailmaking A score and IGF-1 (r=-.86, p=00)

Table 1 Neuropsychological assessment and correlations with plasma IGF-1

Neuropsychological test	Function measured	Reference	cor. IGF-1
Digit span test	Short-term & working memory	A	(r=-.04, p=.92)
Digit Symbol Substitution (WAIS)	Cognitive and perceptual-motor processing speed	A	(r= .33, p=.35)
Dutch National Adult Reading Test	Estimate of verbal intelligence	B	(r=-.26, p=.47)
Trail Making Task (TMT)			
Trail Making Task A	Processing speed & attention	B	(r=-.86, p=.00)*
Trail Making Task B	Processing speed + attentional shifting	B	(r=-.29, p=.41)
TMT B minus TMT A	Attentional shifting	A	(r=-.15, p=.69)
TMT B vs. TMT A	Attentional shifting	E	(r=-.16, p=.66)
15 Words Test (RAVLT)			
Recall score	Verbal memory	A	(r= .42, p=.23)
Immediate recall score	Immediate verbal memory	B/C	(r= .18, p=.62)
Delayed recall score	Delayed verbal memory	B/C	(r= .30, p=.40)
Selective Attention Task			
Target detection	Sensory discrimination & selective attention	D	(r= .55, p=.10)
Mean reaction time, MRT	Speed of responding	D	(r=-.80, p=.01)*
N2b	Anterior cingulate cortex activity	D	(r=-.23, p=.52)
Lateralized Readiness Potentials	Central activation of motor responses	D	(r=-.82, p=.00)*

A= Aleman et al. (1999), B= van Dam et al. (2005), C= van Zandvoort et al. (2005), D= Kenemans et al. (1995) Don't hold tests are sensitive and hold tests insensitive for aging (Aleman et al., 1999; Aleman et al., 2001).

Speed of responding (mean reaction time) in the go/NoGo task was higher with high IGF-1 levels (r=-.80, p=.005; figure 2). In addition, IGF-1 correlated with LRP amplitude (r=-.82,

p=.00; figure 3); high IGF-1 was associated with larger LRPs. No correlation was found between IGF-1 and N2b or any other ERPs.

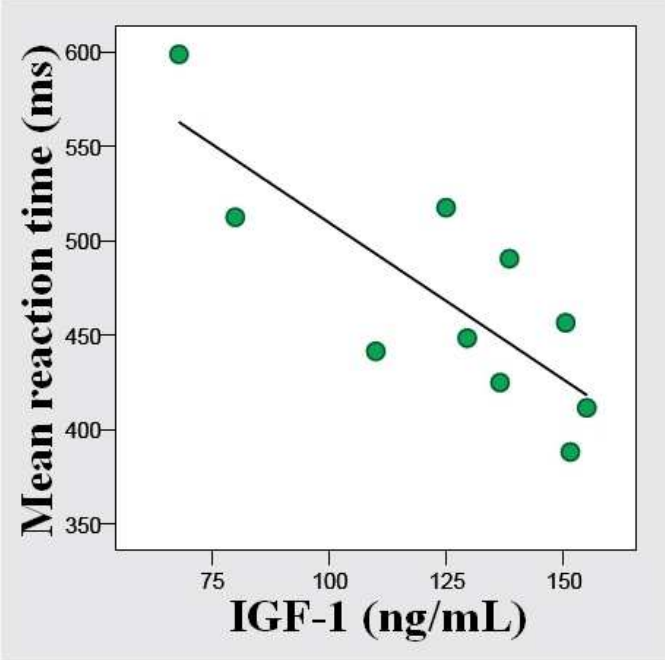


Figure 2 Correlations between IGF-1 (µg/mL) with mean reaction time (r=-.80, p=.005)

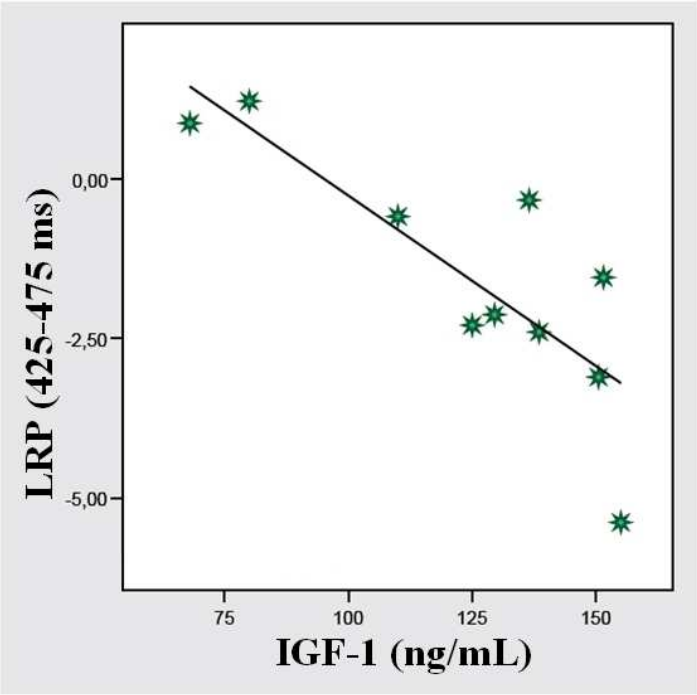


Figure 3 Correlations between selective electro-cortical motor preparation (LRP) and IGF-1 (r=-.82, p=.00)

Discussion

The relation between plasma IGF-1 levels and cognition, as measured via performance on age-sensitive and age-insensitive cognitive tests in older men, was evaluated as well as the relation between IGF-1 and attention related cortical brain activity during a selective attention task. We expected to find that variations in IGF-1 would be associated with perceptual-motor performance and information processing speed, memory, attentional electro-cortical responses to task-relevant stimuli (N2b), go/ no go commission or omission error rates, and performance in 'don't hold' tests.

A positive correlation was noted between years of education and performance on the NVL (NART), an estimate of verbal intelligence. As expected, a negative correlation was found between age and IGF-1.

A negative correlation between the Trailmaking A score and IGF-1 was found. Furthermore, speed of responding correlated negatively with IGF-1. IGF-1 also correlated negatively with LRPs. No correlation was found between IGF-1 and N2b.

The observed correlation between the Trailmaking A score and IGF-1 implicates a relation between IGF-1 and processing speed and attention. When higher levels of plasma IGF-1 were measured, performance on Trailmaking A was faster. The correlation between IGF-1

and Trailmaking A performance is in line with a previous study (van Dam et al., 2005), where performance on the Trailmaking A test was attenuated in CO-GHD patients. This suggests overlap between the cognitive deficits in GHD and elderly, both suffering from decreased functioning of the somatotropic axis (Arwert et al., 2005). Specifically, the fact that differences were observed for Trails A but not for Trails B, suggests that the deficit pertains to basic processing speed. Relative to A, the B version contains an additional component of switching between categories. As the more difficult switching performance in the B version was not affected by lower IGF-1 in our older male subjects, nor by the presence of CO-GHD (van Dam et al., 2005), deficits in basic processing speed may be masked in the B version and only visible in the A version. This is in line with the previous result in the studies of Aleman et al. (1999) and Dik et al. (2003) who both also observed an association between serum levels of IGF-1 and information processing speed.

The significant correlation between IGF-1 and the LRP measuring selective motor preparation was expected and found. This correlation may be related to the connection between IGF-1 and processing speed. IGF-1 maintains the myelin that surrounds the axons, and therefore may fasten communication between neurons and thus may increase the LRP and fasten processing speed. Our data may reflect that specific central nervous pathways, which are associated with selective motor preparation and processing speed, depend upon the presence of an intact somatotropic axis. This idea is supported by our finding that faster reaction times are related with higher IGF-1 levels.

There were no significant correlations between IGF-1 and N2b or other selection potentials, indicating that selective attention to stimuli that share spatial frequency with the target (but not necessarily the orientation) was not related with IGF-1 status. The present N2b results are in contrast with the findings from Lijffijt et al. (2003) in CO-GHD patients, who observed that N2b was affected in these patients. The N2b-effects found in the study of Lijffijt et al. (2003) could be due to a direct effect of GH and not IGF-1 depletion. The differences between our findings and the significant findings in CO-GHD patients (Lijffijt et al., 2003) can also be explained by differences in the study populations. The participants in the current study did not have a severe shortage of GH or a long-term deficiency, but were older men with physiologically reduced GH secretion. CO-GHD patients are confronted with a long term and severe GH deficiency, which started in their childhood, causing lower IGF-1 levels, which could in turn lead to a significantly changed N2b. This lack of IGF-1 during childhood may hinder the development of the brain, which in turn may result in deficient neuron and glia growth, or suboptimal myelination. Therefore, GHD during childhood may produce larger and thus significant defects in target detection and attention related cortical activity. It is very unlikely that our subjects suffered from an IGF-1 or GH deficiency during childhood. More EEG measurements are required in relation to IGF-1 to conclude that the effect found by Lijffijt et al. (2003) is related to circulating IGF-1 and/or GH, or could be the consequence of previous GHD during childhood. In addition, the difference in participants' age may play a role, as the effect of GH could be less detectable in the elderly, compared to younger patients. It would be of interest to perform

similar ERP studies in elderly subjects with pathologically reduced IGF-1. Moreover, the correlation between N2b amplitude and IGF-1 may well be significant in an experiment testing more subjects, in order to obtain a larger study sample.
In contrast to Rollero et al. (1998) no correlation between IGF-1 levels and cognitive function as measured by the mini-mental state examination (MMSE) was found. This can be explained by the exclusion criteria we used. Participants with an MMSE score lower than 24 points were excluded, to avoid effects of mental disorders such as dementia. Below 24 points, scores may indicate severe (≤9 points), moderate (10-20 points) or mild (21-24 points) cognitive impairment and could explain the findings of Rollero et al. (1998) being significant. Again, the correlation with IGF-1 may well be significant in a new experiment testing more subjects, in order to obtain a bigger sample size.
The present data confirm that cognitive performance in elderly men, in terms of speed of responding, and processing speed is associated with IGF-1. The decrease in IGF-1 status may cause elderly people to slow down mentally.
In contrast, we could not show specific neurophysiological alterations as measured by N2b which were previously observed in young CO-GHD patients. This discrepancy may be explained by differences in age of exposure to low circulating GH and/or IGF-1.

Acknowledgements

We thank Wouter M. Creyghton (MD) for including patients and Jeroen Gerritsen (MSc) for his help in collecting ERP data.

Role of funding source

The department of internal medicine, UMC Utrecht, provided funding for this study. UMC Utrecht had no further role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest

All authors declare that they have no conflicts of interest.

Contributors

P.S. van Dam designed the study and wrote the protocol. E.H. Quik managed the literature searches and analysis and undertook the statistical analysis. E.B. Conemans managed the recruitment of study participants and neuropsychological assessments. J.L. Kenemans supervised the go/ NoGo selective-attention ERP assessments and analysis. H.P.F. Koppeschaar and G.D. Valk were responsible for the endocrinological analysis and data collection. All authors have contributed to and have approved the final manuscript.

References

Aleman, A., Verhaar, H.J., De Haan, E.H., De Vries, W.R., Samson, M.M., Drent, M.L., van der Veen, E.A., Koppeschaar, H.P., 1999. Insulin-like growth factor-I and cognitive function in healthy older men. J. Clin. Endocrinol. Metab. 84, 471-475.

Aleman A, de Vries WR, de Haan EHF, Verhaar HJJ, Samson MM, Koppeschaar HPF. Age-sensitive cognitive function, growth hormone and insulin- like growth factor 1 plasma levels in healthy older men. Neuropsychobiology 2000; 41(2):73-78.

Aleman, A., de Vries, W.R., Koppeschaar, H.P.F., Osman-Dualeh, M., Verhaar, H.J.J., Samson, M.M., Bol, E., de Haan, E.H.F., 2001. Relationship between circulating levels of sex hormones and insulin-like growth factor-I and fluid intelligence in older men. Exp. Aging Res. 27, 283- 291.

Anawalt, B.D., Merriam, G.R., 2001. Neuroendocrine aging in men. Andropause and somatopause. Endocrinol Metab Clin North Am 30(3), 647-669.

Arwert, L.I., Deijen, J.B., Witlox, J., Drent, M.L., 2005. The influence of growth hormone (GH) substitution on patient-reported outcomes and cognitive functions in GH-deficient patients: a meta-analysis, Growth Horm. IGF Res. 15, 47-54.

Ball, L.J., Bisher, G.B., Birge, S.J., 1999. A simple test of central processing speed: an extension of the short blessed test. J. Am. Geriatr. Soc. 47, 1359–1363.

Cherrier, M.M., Plymate, S., Mohan, S., Asthana, S., Matsumoto, A.M., Bremner, W., Peskind, E., Raskind, M.A., LaTendresse, S., Haley, A.P., Craft, S., 2004. Relationship between testosterone supplementation and insulin-like growth factor-I levels and cognition in healthy older men. Psychoneuroendocrinology 29, 65– 82.

Falleti, M.G., Maruff, P., Burman, P., Harris, A., 2006. The effects of growth hormone (GH) deficiency and GH replacement on cognitive performance in adults: A meta-analysis of the current literature, Psychoneuroendocrinology 31, 681-691.

Iragui, V., Kutas, M., Salmon, D.P., 1996. Event-related brain potentials during semantic categorization in normal aging and senile dementia of the Alzheimer's type. Electroencephalogr Clin Neurophysiol 100, 392-406.

Kenemans, J.L., Kok, A., Smulders, F.T.Y., 1993. Event-related potentials to conjunctions of spatial frequency and orientation as a function of stimulus parameters and response requirements. Electroenceph. clin. Neurophysiol. 88, 51-63.

Kenemans, J.L., Smulders, F.T., Kok, A., 1995. Selective processing of two-dimensional visual stimuli in young and old subjects: electrophysiological analysis. Psychophysiology 32, 108-120.

Kenemans, J.L., Lijffijt, M., Camfferman, G., Verbaten, M.N., 2002. Splitsecond sequential selective activation in human secondary visual cortex. J. Cogn. Neurosci. 14, 48-61.

Lezak, M.D., 1995. Neuropsychological assessment. Oxford University Press, New York.

Lijffijt, M., Van Dam, P.S., Kenemans, J.L., Koppeschaar, H.P., de Vries, W.R., Drent, M.L., Wittenberg, A., Kemner, C., 2003. Somatotropic-axis deficiency affects brain substrates of selective attention in childhood-onset growth hormone deficient patients. Neurosci. Lett. 353, 123-126.

Popovic, V., Leal, A., Micic, D., Koppeschaar, H.P., Torres, E., Paramo, C., Obradovic, S., Dieguez, C., Casanueva, F.F., 2000. GH-releasing hormone and GH-releasing peptide-6 for diagnostic testing in GH-deficient adults. Lancet. 356(9236), 1137-1142.

Rollero, A., Murialdo, G., Fonzi, S., Garrone, S., Gianelli, M.V., Gazzerro, E., Barreca, A., Polleri, A., 1998. Relationship between cognitive function, growth hormone and insulin-like growth factor I plasma levels in aged subjects. Neuropsychobiology 38, 73– 79.

Shepard, R.N., Metzler, J., 1971. Mental rotation of three-dimensional objects. Science. 171(972), 701-703.

Smulders, F.T.Y., et al., 1995. The temporal selectivity of additive factor effects on the reaction process revealed in ERP component latencies. Acta Psychologica, **90**(1-3): 97-109.

van Dam, P.S., Aleman, A., 2004. Insulin-like growth factor-I, cognition and brain aging, Eur. J. Pharmacol. 490, 87-95.

van Dam, P.S., de Winter, C.F., de Vries, R., van der Grond, J., Drent, M.L., Lijffijt, M., Kenemans, J.L., Aleman, A., de Haan, E.H., Koppeschaar, H.P., 2005. Childhood-onset growth hormone deficiency, cognitive function and brain N-acetylaspartate. Psychoneuroendocrinology. 30(4), 357-363.

van Dam, P.S., 2005. Neurocognitive function in adults with growth hormone deficiency. Horm Res. 64 Suppl 3, 109-114.

van Dam, P.S., 2006. Somatotropic therapy and cognitive function in adults with growth hormone deficiency: A critical review. Treat Endocrinol. 5 (2), 1.

Appendix

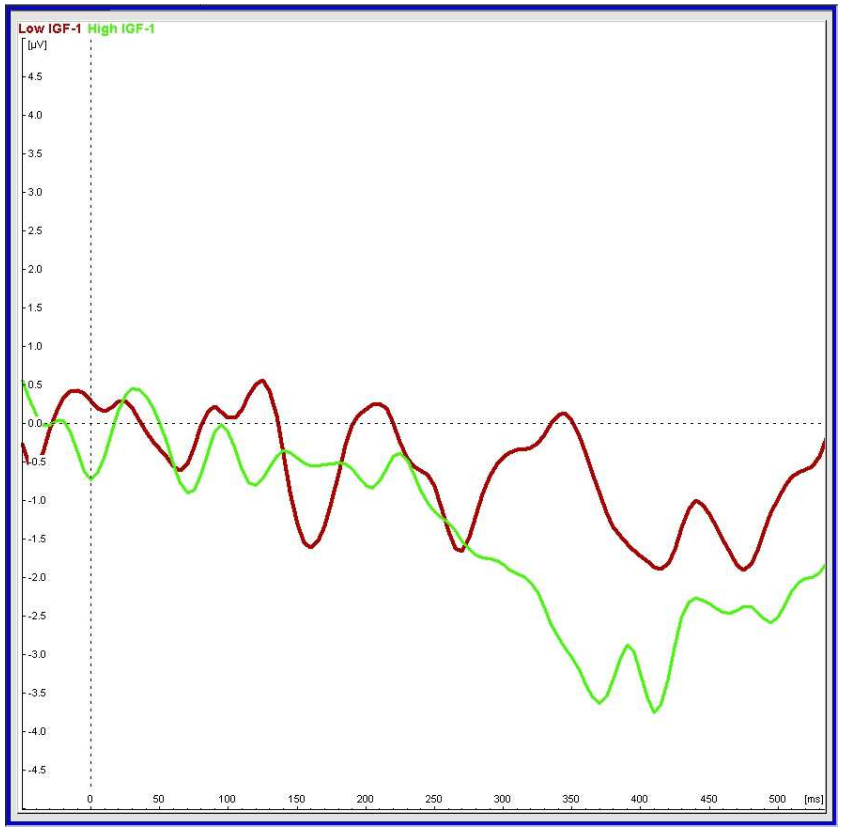


Figure 4 Lateralized Readiness Potentials, central activation of motor responses, of split half high and low IGF-1 group

Chapter 5

Reduced growth hormone secretion after cranial irradiation contributes to neurocognitive dysfunction

E.H. Quik, G.D. Valk, M.L. Drent, L.J.A. Stalpers, J.L. Kenemans, H.P.F. Koppeschaar & P.S. van Dam

Quik, E.H., Valk, G.D., Drent, M.L., Stalpers, L.J., Kenemans, J.L., Koppeschaar, H.P., van Dam, P.S. (2012) Reduced growth hormone secretion after cranial irradiation contributes to neurocognitive dysfunction. Growth Horm IGF Res. 22 (1), 42-7.

Summary

Cranial irradiation can lead to pituitary dysfunction, in particular growth hormone deficiency (GHD). Data from GHD patient studies suggest a relationship between cognitive function and growth hormone (GH) secretion. The objective of this study was to investigate the relation between GH and cognition in patients who received external beam radiation therapy for brain tumors. Hypothesized was that low GH secretion is associated with reduced attentional electro-cortical responses to task-relevant stimuli (N2b), decreases of target detections, slower speed of responding, increased P300 latencies, and impaired performance on neuropsychological tests. In addition, we hypothesized that low insulin-like growth factor 1 (IGF-1) is especially associated with reduced activation of the motor cortex responses (lateralized readiness potential, LRP) and slower speed of responding.

Cognitive function was assessed by neuropsychological tests in 19 patients who had received external beam radiation therapy for neurological tumors. Brain function was assessed using event-related potentials (ERPs) during a go/ NoGo selective-attention task, including N2b, P300 and selective motor preparation as reflected in the LRP. Correlations were calculated between peak GH levels after a standardized growth hormone-releasing hormone (GHRH)-arginine test, plasma IGF-1, and cognitive functions. In addition, four patients who were diagnosed with GHD according to the GHRH-arginine test results were compared to 15 non-GHD patients. All other pituitary functions were either intact or substituted.

Performance on WAIS digit span backward as well as the Rey-Osterrieth complex figure test correlated positively with GH peak. GHD patients performed worse than non-GHD patients on Stroop interference, trail making B/A attentional shifting and Rey-Osterrieth complex figure test. In contrast, trails A performance tended to be better (trend-level effect) in patients with lower GH levels and higher radiation doses. In the go/ NoGo selective attention task, GHD participants detected fewer targets (trend-level effect). N2b was not significantly altered by GH status. Furthermore, plasma IGF-1 was positively correlated with the sum of digit span forward and backward. No relations with P300 were observed.

In this study only 21% (4/19) of the patients who received fractionated radiotherapy for a non-endocrine brain tumor were diagnosed with GHD. GHD in these patients is associated with impaired interference control, attentional shifting, and visual long-term memory. The results for interference control and attentional shifting are inconsistent with precious reports and suggest an additional effect of the radiation history.

Introduction

Growth hormone deficiency (GHD) is due to insufficient production of growth hormone (GH) by the pituitary gland observed in both children (childhood-onset; CO-GHD) and adults (adult-onset; AO-GHD), usually as a consequence of congenital or acquired pituitary or hypothalamic disease. Some of the clinical characteristics of GHD in adults are central obesity, osteopenia, lack of energy and physical fitness, and decreased quality of life (Dattani & Preece, 2004; Koltowska-Haggstrom et al., 2006). In addition, GHD has often been associated with impaired psychological functions (Soares et al., 1999), and resembles some of the neuropsychological features of aging. Evidence is emerging that a relationship exists between the GH - insulin-like growth factor-1 (IGF-1) axis and cognitive function (van Dam & Aleman, 2004). The role of GH in the central nervous system (CNS) is supported by the widespread distribution of GH receptors through the CNS. GH can cross the blood-brain barrier, bind to its receptors in the brain and thereby may influence cognitive functions (Aberg et al., 2006; Johansson et al., 1995). Although reduced GH secretion may directly affect cognitive function, it is also likely that subsequent attenuation of systemic or local IGF-1 levels may be responsible for the observed effects (van Dam & Aleman, 2004; Aberg et al., 2006).

A review and meta-analysis of the data regarding cognitive functions in adult GHD patients, compared with matched controls, indicated that neuropsychological performance was impaired, predominantly in the domains of memory and executive functions, and that moderate improvements during GH therapy were found in particular also for these domains (van Dam, 2006; Falleti et al., 2006). Studies of event related brain potentials (ERPs) offer support for pathophysiological mechanisms responsible for the observed cognitive alterations in GHD patients. P300 latencies were found to be significantly prolonged in GHD patients and were significantly shortened after six months of GH therapy (Tanriverdi et al., 2009; Golgeli et al., 2004). Furthermore, Lijffijt et al. (2003) demonstrated reduced attention-related electro-cortical responses (N2b) to task-relevant stimuli in adults with CO-GHD, which may reflect functional deficits in the cingulate cortex (Kenemans et al., 2002). Previous research showed that the lateralized readiness potential (LRP), an ERP index for selective motor preparation was smaller in elderly men with low IGF-1 levels, for whom reaction times were also slower (Quik et al., *in submission*/chapter 4). At least part of the relation between GHD and cognitive functions then may be mediated by IGF-1.

Long-term cognitive impairment is highly prevalent and burdensome in patients with brain tumors. Radiotherapy is a usual suspect for cognitive deterioration in brain tumor patients. However, in comparative studies wherein adult patients with a low-grade glioma or meningioma had been irradiated by state-of-the-art conformal radiotherapy using a low fraction dose (≤ 2 Gray (Gy)) no additional detrimental effect by irradiation was detected over damage by the tumor and by neurosurgery (Taphoorn & Klein, 2004; van Nieuwenhuizen et al., 2007; Dijkstra et al., 2009). Irradiation of pituitary and hypothalamic tumors is strongly associated with exhaustion of the pituitary axis and particularly with

GHD (Adan et al., 2001; Shalet & Brennan, 2002; Popovic et al., 2002). The tolerance of the pituitary gland for fractionated radiotherapy is dose dependent; hypopituitarism is virtually absent below 12 Gy but common above 35-40 Gy (Littley et al., 1989; Darzy & Shalet, 2009). Shukitt-Hale et al. (2007) found that cranial irradiation was associated with reduced performance in tasks assessing spatial learning and memory, which could at least partly be due to a lack of GH (Popovic et al., 2002; Appelman-Dijkstra et al., 2011). In the present study we report measures of cognitive functioning in relation to GH secretion, assessed by GH secretory capacity after GH releasing hormone (GHRH)-arginine, in patients who had previously undergone cranial irradiation for neurological tumors.
The aims of this study then are to investigate the prevalence of long-term pituitary dysfunction, particularly GHD, in brain tumor patients who had post-operative radiotherapy for a non-endocrine brain tumor, and to assess the effect of GHD on cognitive functioning. Our hypothesis was that low GH secretion would be associated with reduced attentional electro-cortical responses to task-relevant stimuli (N2b), reduced accuracy of performance, and slower speed of responding, increased P300 latencies, and impaired performance on neuropsychological tests of memory. Based on a recent review (Quik et al., 2010) we did not expect GH effects on typical neuropsychological measures of selective attention (Stroop interference, Trailmaking B test). As mentioned, for IGF-1 we specifically predict an association with selective motor preparation (LRP) and speed of responding (reaction time). We chose to study patients after cranial irradiation with the intention to assess whether the deficits sometimes found after radiation could be specifically due to a lack of GH and/or IGF-1. A comparison was made between patients with and without GHD, with respect to the measures mentioned previously. Our study design also gave us the possibility to study hormonal deficits after brain irradiation during adulthood; only limited data have been reported regarding the prevalence of hypopituitarism in these patients.

Materials and methods

Subjects

We studied 19 patients (mean age 43.3 years, SD 10.6, range 30–69 years; 8 females; Table 1) who had received therapeutic cranial irradiation for primary brain tumors in adulthood. GH secretion was assessed using a standard GHRH-arginine test. Other pituitary hormones (cortisol, thyroid hormone, gonadal hormones) were either normal or had been adequately substituted for at least three months. For this purpose, all subjects were seen by an endocrinologist and basal plasma hormone levels were measured shortly before the study. The average IQ was 115 (range 103-127) as measured by the Dutch National Adult Reading Test (DNART). Four participants (mean age 49.3 years, SD 14.2, range 36-69 years; 1 female) who had a GH peaks lower than 9 µg/l after GHRH-arginine stimulation were diagnosed as GH deficient (Aimaretti et al., 1998). The other 15 participants (mean

age 41.7 years, SD 9.3, range 30-62; 7 females) had an adequate GH response. We excluded participants with other neurological or psychiatric disease, other endocrine or internal disease, severe obesity (BMI > 32 kg/m2), malnutrition (BMI < 18.5 kg/m2), chronic alcohol (more than 3 units daily) or drug (any soft or hard drug) abuse, and use of medication that may affect cognitive functioning. No tumor progression or recurrence was detected and there was no history of pituitary or hypothalamic disease or other central neurologic disorders. There were no differences in age, sex distribution, and radiation dose or tumor characteristics between GHD and non-GHD subjects. As expected, the GHD subjects had higher outcome measures of central obesity (BMI 27.7 ± 1.8 vs. 24.7 ± 2.5 kg/m2, $p<0.05$; waist 104.8 ± 6.9 vs. 86.4 ± 8.1 cm, $p<0.01$).
Exclusion criteria were checked using a questionnaire including medical history, assessment of current and previous medication, and appropriate blood tests. The local medical ethics committee approved the study protocol. The experiments were undertaken with the understanding and written consent of each subject, and the study conformed to The Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the British Medical Journal (18 July 1964).

GHRH-arginine test procedure and laboratory assessments

On the test day, the subjects were fasting and were asked not to smoke or perform strenuous exercise before the test. All tests started between 08:30 and 09:00 a.m. After height and weight measurements, an intravenous catheter was placed in the lower arm for blood sampling and drug administration. Subsequently GHRH (100 µg; GHRH Ferring; Ferring Pharmaceuticals Ltd., Hoofddorp, The Netherlands) was administered intravenously as a bolus injection and followed by an arginine (0.5 g/kg body weight, maximum dose 30 g) infusion during 30 minutes. Blood was sampled at 30, 45, 60, 90, and 120 minutes after injection of GHRH and arginine for assessment of GH responses. The subjects remained fasting, non-smoking, and seated during the tests.
GH was measured using an immunometric technique on an Immulite Analyzer (Diagnostic Products, Los Angeles, CA). The lower limit of detection was 0.01 µg/l; the interassay variations were 9.7, 5.6, 4.4, and 5.2% at 0.13, 0.80, 4.2, and 15.4 µg/l, respectively (n= 69). One µg/l corresponds to 2.6 mIU/l (WHO International Ref. Prep 80/505). GH response was quantified as the highest plasma level during the test (GH peak).
IGF-1 was measured using an immunometric technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, CA). The lower limit of detection was 6.0 ng/ml and inter-assay variations were 8.7, 5.8, and 6.5% at mean IGF-1 plasma levels of 33, 174, and 445 ng/ml, respectively (n= 115). Other hormones (free thyroxin (FT4), cortisol, luteinizing hormone (LH) follicle stimulating hormone (FSH), testosterone, estradiol and prolactin) were also assessed using routine laboratory techniques.

Table 1
Characterization of individual patients

ient	Age (years)	Sex M/F	BMI kg/m^2	Waist (cm)	Diagnosis	Tumor localization	Radiation dose (Gray)	Radiation interval (months)	GH peak (μg/l)	IGF-I (μg/l)
	36	M	25.5	112	Astrocytoma grade II	Right postcentral	60	91	2.4	126
	43	M	27.5	97	Anaplastic mixed glioma	Left frontal	59	86	4.6	132
	57	F	29.8	109	Meningioma	Left orbital/frontal lobe	54	50	6.9	138
	49	M	27.8	101	Astrocytoma grade IV	Left frontal	60	68	8.1	141
	32	F	27	83	Anaplastic oligo-astrocytoma	Temporo-occipital	60	28	9.6	145
	35	M	26.3	99	Medulloblastoma	Left cerebellar	54	87	11.9	114
	39	M	25.8	91	pinealoblastoma	Pineal gland	54	84	12.3	99
	34	M	23.9	95	Germinoma	Pineal gland	45	51	13.8	165
	62	M	27.4	92	Meningioma	Right cavernous sinus	54	16	23.5	105
	41	F	29	86	Astrocytoma grade II	Left parieto-occipital	60	59	24.6	125
	48	M	23.4	94	Astrocytoma n.o.s.	Right parietal	54	11	25	107
	49	F	25.9	85	Astrocytoma n.o.s.	Right frontoparietal	60	87	25	116
	46	F	25.1	92	Medulloblastoma	Cerebellum	54	45	26.2	206
	42	F	25.8	81	Optic glioma	Left para/suprasellar with infrasellar extension	50	32	29.6	134
	42	M	21.9	86	Oligodendroglioma grade II	Right frontoparasagittal	45	117	35.8	93
	30	M	22.6	85	Astrocytoma grade IV	Posterior	60	98	46.2	133
	62	F	26.0	68	Meningioma	Left frontotemporal	50	87	53.8	115
	34	M	21.9	85	Medulloblastoma	Posterior fossa	54	68	55	170
	34	F	20.2	74	Astrocytoma grade III	Left frontal	60	61	96.2	135

reference levels: 30–35 years 90–275 (M), 92–280 (F); 36–40 years 85–250 (M), 85–260 (F); 41–50 years 74–220 (M), 75–220 (F); 51–60 years 64–200 (M), 65–200 (F); 61–
ars 58–175 (M), 60–170 (F).

Procedure

The GHRH-arginine testing took place during a morning session for approximately 2.5 hours. Afterwards, in the afternoon of the same day, the ERP task (N2b, P300) and the neuropsychological tests were scheduled. All these tests were performed individually and under identical circumstances, in a sound-attenuated room.

Tasks

Standardized neuropsychological tests used were WAIS III digit span subtest, 15 words test, Rey-Osterrieth complex figure test, Stroop color-word task, trail making test A&B, Brixton spatial anticipation task, WAIS III symbol substitution subtest, WAIS III similarities subtest, verbal fluency N, A and animals and the Dutch adult reading test, as listed in table 2. For detailed descriptions, the reader is referred to Nelson (1982), Lezak (1995), Aleman et al. (1999), and Ball et al. (1999).
Typical neuropsychological methods to assess selective attention are the Stroop color-word task (resistance against interference from distracting information), and the trail making task (shifting selective attention from one category to the other). ERPs were recording during a go/ NoGo selective-attention or 'selection-potential' task During the selection-potential task (Lijffijt et al., 2003; Kenemans et al., 1995) participants viewed sequences of square-wave gratings that varied in spatial frequency (SF; 0.6 or 4.8 cycles per degree of visual angle, wide or narrow bars, respectively) and orientation (O; vertical or horizontal), making up four different stimuli. Stimulus onset asynchrony varied between 750 and 950 ms. Participants had to selectively press a button when perceiving a predefined combination of SF and O (target), but had to ignore the other combinations and were to emphasize speed over accuracy. In separate blocks, each of the four different stimuli was defined as target, and each target had to be responded to with either the left or right hand, thus obtaining eight blocks. Stimuli were mixed randomly within a block of 128 trials (4 x 32). A practice block containing 12 trials preceded each new block.

Table 2

Neuropsychological assessment & results:
Correlation with IGF-1 (IGF-1), GH peak and Group Difference (GHD)

Neuropsychological test	Putative cognitive function	Reference	IGF-1 r; p	GH peak r; p	GHD F; p
Stroop Color Word Task					
Word	Word reading	1	.15; .54	-.38; .11	4.44; .05
Color	Color naming	1	.06; .80	-.25; .30	5.89; .03
Color & word	Color naming & interference control	1	.11; .67	-.30; .21	0.64; .01
Color & word minus Color	Interference control	1	.12; .64	-.28; .25	8.50; .01
Color / Color & Word	Interference control	1	-.15; .53	.14; .58	2.13; .16
Trail Making Task (TMT)					
Trail Making Task A	Processing speed, attention	2/3	.16; .51	.40; .09	1.22; .28
Trail Making Task B	Concept shifting	2/3	-.16; .51	.05; .84	.22; .64
TMT B minus A	Planning of movement/cognitive processing speed	4	-.33; .17	-.27; .27	2.41; .14
TMT B / A	Attentional shifting	5	-.26; .28	-.37; .12	5.14; .04
Rey-Osterrieth Complex Figure Test					
Immediate recall score	Immediate visual memory	3	.32; .18	.43; .06	2.03; .17
Delayed recall score	Delayed visual memory	3	.21; .39	.61; .01	3.94; .06
Delayed / Immediate recall score	Difference between delayed & immediate memory	3	-.50; .03	.64; .00	8.22; .01
Selective Attention Task					
Target detection	Sensory discrimination & selective attention	6	-.14; .58	.29; .22	3.94; .06
N2b (ERP)	Anterior (Fz) attentional process	6	-.39; .09	-.25; .30	2.17; .16
15 Words Test (RAVLT)					
Recall score	Verbal memory	4	.09; .72	.19; .43	1.27; .27
Immediate recall score	Immediate verbal memory	2/3	.02; .94	.15; .53	.29; .60
Delayed recall score	Delayed verbal memory	2/3	.21; .38	.31; .19	2.06; .17
Recognition score	Recognition memory	3	-.39; .10	.15; .55	.79; .39
Verbal Fluency					
Letter (N)	Language, executive function	3	-.19; .44	.08; .73	1.07; .32
Letter (A)	Language, executive function	3	-.24; .32	.03; .91	2.28; .15
Category Animal	Language, executive function	3	-.02; .94	.05; .83	.98; .34
Brixton Spatial Anticipation Task	Spatial learning & working memory	7	-.16; .51	-.10; .69	1.48; .24
Dutch National Adult Reading Test	Verbal intelligence	2	-.08; .73	.18; .46	1.29; .27
Wechsler Adult Intelligence Scale (WAIS)					
Digit Symbol Substitution	Cognitive and perceptual-motor processing speed	4	.01; .96	.15; .55	.46; .51
Similarities	Concept formation & abstract reasoning	3	-.19; .44	.16; .51	1.42; .25

1= Lansbergen (2007), 2 = van Dam et al. (2006), 3 = van Zandvoort et al. (2005), 4 = Aleman et al. (1999), 5 = new measure, 6 = Kenemans et al. (1995) & 7= Shallice & Burgess (1996)

ERP procedure and signal recording

During the selection-potential task, EEGs were recorded from several scalp sites using an electrode cap containing 30 tin electrodes, including Fz (frontal) and Oz (occipital). Horizontal and vertical electro-oculograms were recorded using four electrodes positioned around the eyes. The left mastoid was used as a reference. Skin impedance was kept below 5 kΩ. All signals were subjected to 30 Hz low-pass filters and a time constant of 3 s. The sampling rate was 200 Hz. It took about one hour to record the EEG, including 25 min to complete the task. Using Brain Vision Analyzer software, ERPs were derived as 'selection potentials', the differential electro-cortical correct response to task-relevant, relative to

irrelevant, visual stimuli. This logic yields the N2b (Lijffijt et al., 2003). More specific, the N2b was assessed from selective attention grating difference waves, also called selection potentials; the differential electro-cortical response to spatial frequency relevant non targets minus irrelevant non targets (SF-IR) at electrode Fz according to (Kenemans et al., 1995) and (Lijffijt et al., 2003). Furthermore, we calculated the LRP, and the P300 latency to targets (Kenemans et al., 1995). The lateralized readiness potential (LRP), i.e. an electrophysiological correlate of premotor activation in the primary motor cortex, is derived as the average difference in lateralization above the motor cortex associated with left- and right-hand responses, respectively. More specifically, the LRP was computed by subtracting potentials recorded over the left (electrode C3) and right side (electrode C4) of the scalp in the motor cortex (Kenemans et al., 1995). This voltage for the C4 is subtracted from C3 to yield a value that is then averaged over the course of all the subjects' responses for left hand button presses. The exact same procedure occurred for right hand button presses. The averaged potential is the LRP of which the most negative peak is obtained between 200 and 550 milliseconds. Only the trials in which the participant responded correctly were analyzed.

Statistics

Bivariate Pearson correlations were calculated (two-tailed) using a SPSS® 12.0.1 for Windows between the peak GH level during the GHRH-arginine test, plasma IGF-1 and all cognition outcome parameters (neuropsychological tests and ERP data). Furthermore, GHD-subjects were compared with non-GHD-subjects using an ANOVA. Statistical significance was given at a p-value < 0.05.

Results

GH secretion

Of all 19 patients (Table 1), mean GH peak was 26.9 µg /l, with a minimum of 2.4 µg /l, a maximum of 96.2 µg /l, and a standard deviation of 23.0. An evoked GH concentration of $\geq$ 9 µg/l accurately distinguishes between non-GHD and GH-deficient adults (17). No significant correlation was found between GH peak and IGF-1.

For the 4 GHD participants mean GH peak was 5.5 µg /l, with a minimum of 2.4 µg /l a maximum of 8.1 µg /l, and a standard deviation of 2.5. Mean IGF-1 was 134.3 ng/ml with a minimum of 126 ng/ml, a maximum of 141 ng/ml, and a standard deviation of 6.7 ng/ml.
For the 15 non-GHD participants mean GH peak was 32.6 µg /l, with a minimum of 9.6 µg /l a maximum of 96.2 µg /l, and a standard deviation of 22.7. Mean IGF-1 was 130.8 ng/ml with a minimum of 93 ng/ml, a maximum of 206 ng/ml, and a standard deviation of 30.5 ng/ml.
There was no significant difference in IGF-1 levels between GHD and non-GHD.

Radiation

The mean radiation dose of all 19 patients was 55.1 Gy, with a minimum of 45 Gy, a maximum of 60 Gy, and a standard deviation of 4.99 Gy. Patients received external beam conformal radiotherapy in daily fractions of 1.8 to 2.0 Gy. Except for two patients (table 1, patient 9 and 14), the pituitary gland and hypothalamus could be fully shielded from the high dose planning target volume (PTV). Particularly in the patients with GHD (table 1, patient # 1 to 4), these endocrine organs at risk did not receive more than 30% (15-20 Gy) of the prescribed dose.
The dose of total radiation did not differ between the GHD group (mean 58.3 Gy, range 54–60) and the non-GHD groups (mean 54.27 Gy, range 45-60). No significant correlations between radiation dose and GH peak were found (p=.16).

Neuropsychological tests

The 4 GHD patients performed significantly worse than non-GHD patients in all Stroop-test conditions (figure 1). GHD patients were slower when reading words (F=4.44, p=.05, Cohen's d=1.02), naming colors (F=5.89, p=.03, Cohen's d=1.18) and when naming colors of the incongruently colored words, the interference condition (F=10.64, p=.01, Cohen's d=1.56). The difference score between the interference condition and the color naming condition was higher for GHD patients (F=8.50, p=.01, Cohen's d=1.42).

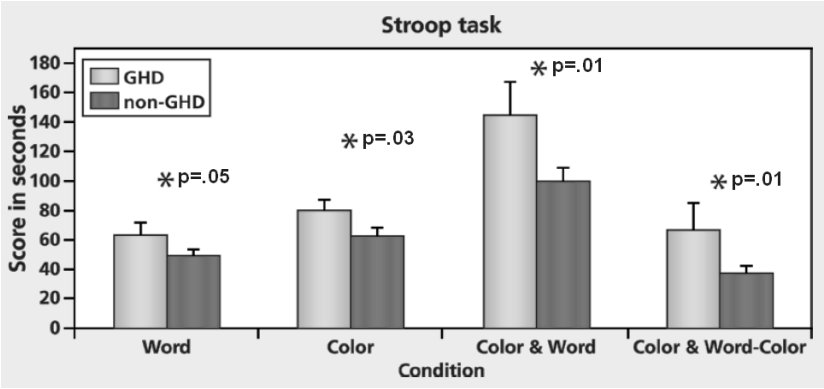


Figure 1 Mean (±SD) performance in three Stroop-task conditions (GHD vs. non-GHD subjects).The most right part depicts the differences in the interference score (color-word minus color)

Although, when separately analyzed, GHD subjects did not perform significantly differently on trail making A (time in seconds needed to connect numbers, figure 2a) and trail making B (attentional shifting, data not shown) than non GHD subjects, a significant difference in trail making B/A ratio between the two groups was found (F=5.14, p=.04, Cohen's d= 1.10; figure 2b). In addition, a positive trend-level correlation between trail making A score and GH peak (r=40, p=.09; figure 2c) was observed.

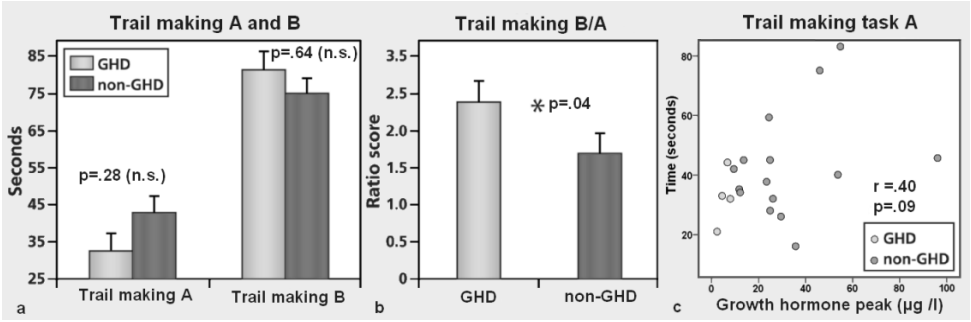


Figure 2 Mean (±SD) performance (GHD vs. non-GHD subjects, n.s.) on trail making task A & B (a) and on ratio score (TMT B/A) (b); correlation between GH peak and performance on Trails A task (c)

GHD subjects performed worse on the Rey-Osterrieth complex figure memory test, (as revealed by the ratio score F=8.22, p=0.01, Cohen's d=1.39; figure 3a). Furthermore, GH peak positively correlated with the WAIS III digit span backwards (r=.46, p<.05; data not shown). IGF-1 positively correlated with the total sum of the digit span forward and backward of the WAIS III (r=.51, p=.03; data not shown).

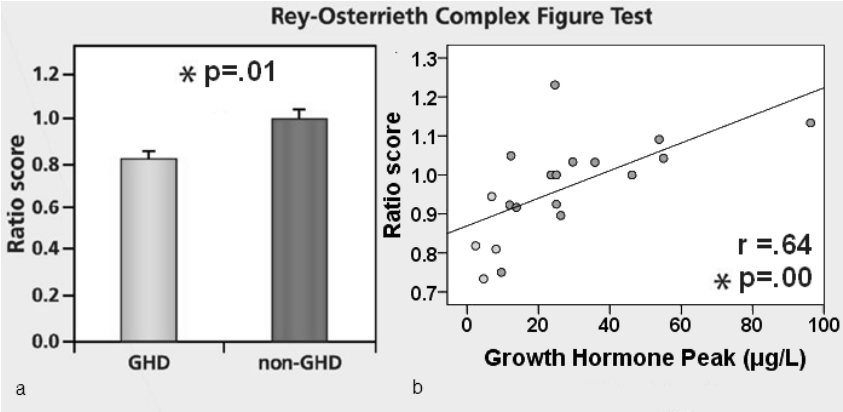


Figure 3 Mean (±SD) ratio scores (delayed/immediate recall score) for the Rey-Osterrieth complex figure test (GHD vs. non-GHD subjects) (a) and significant correlation between GH peak and the ratio score (b)

As to the other neuropsychological tests (table 2), all other calculated differences between GHD and non-GHD patients as well as correlations between GH peak and test outcomes were not significant. Furthermore, no significant correlation was found between GH secretion and performance on the DNART (reflects IQ). No significant correlation was found between years of education and performance on the neuropsychological tests.

Selection potential task performance and ERPs

Mean target reaction time (MRT) was 433.4 milliseconds, range 355.8 ms – 500.8 ms, with a standard deviation of 39.6 ms. Mean percentage targets detected was 98.6%, range 85.9% - 100%. Figure 4a shows that GHD participants detected somewhat fewer targets (trend-level, p=.06). Figure 4b shows that the N2b amplitude was smaller for GHD patients, but this difference was not significant. Mean reaction time (speed of responding) did not differ significantly between the GHD (442.9 ms) and the non-GHD (430.9 ms) group. P300 latency was not significantly different for GHD (448.8 ms) compared to non-GHD (439 ms).

GH peak was not significantly correlated with percentage target detections, nor with correct rejections. Speed of responding (mean reaction time) on irrelevant trials, on which no response was wanted, correlated with GH peak (r=.64, p=.05, data not shown). No significant correlation was found between GH secretion and N2b.

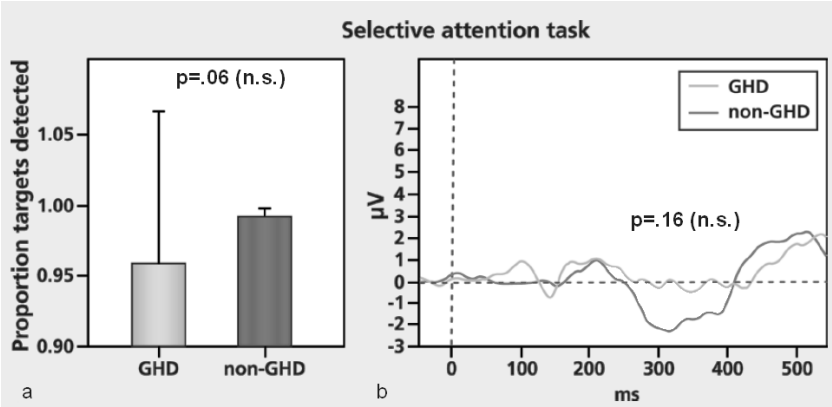


Figure 4 Mean (±SD) proportion of targets detected (GHD vs. non-GHD subjects) during the selective attention task (a) and mean activity area 280-400 ms (N2b) over anterior cortical areas (Fz) (b). No significant differences between GHD and non-GHD were observed

Selective motor preparation (LRP) was significantly correlated to IGF-1 (r=-.51, p=.03). Figure 5 illustrates the correlation by showing that the LRP was larger (more negative) for subjects with high IGF-1 levels than for those with low levels. N2b was somewhat more negative when IGF-1 levels were higher, but the correlation was not significant (r=-.39, p=.095). Plasma IGF-1 did not correlate with percentage target detections, correct rejections or with speed of responding (mean reaction time).

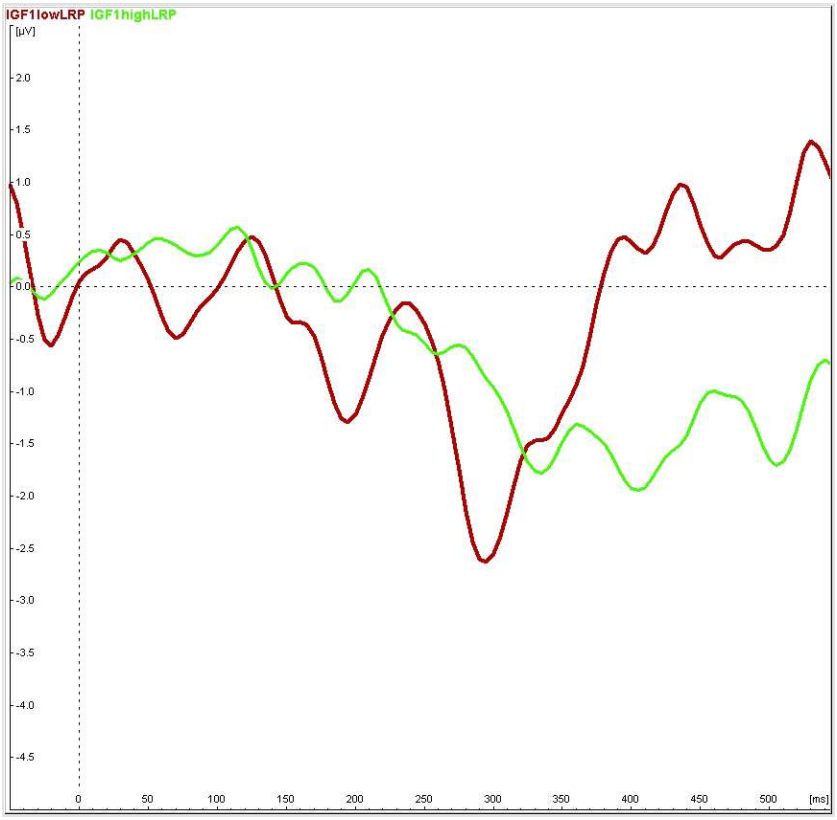


Figure 5 Lateralized Readiness Potentials, averaged across subjects separately for (split half) high (green) and low (red) IGF-1 levels

Discussion

The present study has focused on two points of interest. In the first place, pituitary function, with emphasis on GH secretion, in adults who had previously been irradiated during adulthood for intracranial tumors (pituitary and hypothalamic tumors excluded) was evaluated. Secondly, the relation between GH secretion and cognition was studied in these patients. In particular, the focus was on the correlation between GH secretion and the performance on a variety of neuropsychological tests (which represent different cognitive functions). Furthermore, we tried to relate changes in GH secretion to neurophysiological changes assessed by ERP.

The two main findings of this study are, first, that long-term GHD is relatively rare, in this study only 21% (4/19) in patients that received conformal fractionated radiotherapy for a non-endocrine brain tumor, but secondly, that GHD in these patients is associated with an additional impaired cognitive functioning.

As to pituitary function after brain irradiation during adulthood, GH was the only hormone that we found to be affected by radiation. In four out of 19 patients (21%), the GH peak after GHRH-arginine administration was below 9 μg/l, which is considered to be the cut-off threshold for severe GHD in patients with pituitary disease (Aimaretti et al., 1998). We did not observe any other clinically relevant deficiencies of pituitary hormones.

Only few studies have focused on pituitary function after cranial irradiation for non-pituitary tumors during adulthood. Our study population was comparable with the 56 patients studied by Agha et al. (2005) regarding age at irradiation, interval between radiation therapy and moment of testing, and radiation dose. In contrast, Agha et al. (2005) reported 32% of their patients having GHD as well as lower frequencies of defective other pituitary axes. The difference may be explained by different use of GH stimulation tests (they used the insulin tolerance test (ITT) or a combination of glucagon stimulation followed by an arginine stimulation test). The outcome of our study compared with the study by Agha et al. (2005) shows the dilemmas about GH testing in these patients: the ITT is often contraindicated because patients with a history of brain tumors also often have a history of seizures. We chose to perform a GHRH-arginine test, which may be a suboptimal GH stimulation test according to Darzy et al. (2003) for the detection of GHD as a consequence of hypothalamic dysfunction, and may detect GHD only at a later stage. Darzy et al. (2003) performed both an ITT and a GHRH-arginine test in 49 adults who had been irradiated mostly during childhood, and reported a significant correlation between the outcome of both tests, but a higher frequency of GHD when the ITT was used. They suggest that the ITT is a more appropriate test for the diagnosis of GHD in this patient group, as hypothalamic dysfunction is usually the cause of GHD. Formally, however, it should be noted that for the diagnosis of isolated GHD, a second GH stimulation test is required, and the GHRH-arginine test is one of the best validated tests in pituitary patients (Aimaretti et al., 1998). Popovic et al. (2002) used the ITT and the GHRH-GH releasing peptide-6 test in a group of 22 adults who had been irradiated either during childhood or

adulthood, and reported a defective GH releasable pool in 50% of their subjects, who were qualified as GHD by either of the tests. When we analyze our data in this context, we feel that the four patients in our study who were diagnosed as GHD certainly qualify for this diagnosis, and should be offered GH substitution therapy, while patients with GH peak levels between 9 and 16.5 µg/l should be retested within a few years or if symptomatic. This interpretation of the GHRH-arginine and its comparison to the ITT has also been proposed by Darzy et al. (2007; 2009) when they demonstrated that a compensatory overdrive of the somatotropic axis may exist in these patients.

A significant difference was found between the GHD and non-GHD group for delayed visual memory as assessed by the Rey-Osterrieth complex figure performance. This finding of an impaired delayed visual memory in GHD is consistent with that of an earlier report by Baum et al. (1998). In addition, we could demonstrate that a positive correlation exists between delayed visual memory and the GH peak in our entire study population, which may correspond with the presence of GH receptors in the hippocampus (Nyberg, 2000), and fits with previous studies reporting a general relation between GH and memory (Falleti et al., 2006). In addition to delayed visual memory, a significant correlation was found between GH peak and short-term and working memory as assessed by WAIS digit span backwards.

Significant differences were found for several Stroop-task variables, the most important one being the Color & Word minus Color difference score, which reflects the inverse of interference control. That is, GHD patients on average experienced more interference from incongruent color words when naming colors. In previous studies on CO-GHD (van Dam et al., 2005) and in elderly men (Vitiello et al., 2006), no significant impairment in Stroop-interference control was observed and GH suppletion studies did not reveal effects on Stroop interference control (Baum et al., 1998). A similar story holds for attentional shifting as assessed in the trail making test, in particular the B variety, in which participants continuously shift attention from letters to digits and back. Our GHD patients on average needed more time for trail making B, relative to A, while there was no correlation between trail making B performance and GH peak when we included the non-GHD subjects. As with Stroop interference, the previous study on CO-GHD did not find any differences in trail making B attentional shifting (van Dam et al., 2005), and GH therapy studies have failed to show beneficial effects on this variable (Baum et al., 1998).

A relevant point here may be the trend-level positive correlation between basic processing speed as assessed by trail making A, GH peak, and radiation dose. This essentially indicates better performance with lower GH levels; this is again, in contradiction with our previous study in CO-GHD subjects (van Dam et al., 2004), where CO-GHD was associated with reduced trail making A performance. However, a related finding was reported by Peace et al., (1998). These authors found that GHD patients who had received radiation therapy in the past actually performed better on the trail making A than GHD patients without past radiation exposure. At the same time, these radiation doses may be unfavorable for executive-function processes as probably based in prefrontal cortex and manifest in

interference control and attentional shifting. This would explain why low GH was associated with impairments in these latter domains in the present sample, but not for patients without a history of radiation therapy, as we reviewed previously (Quik et al., 2010).

As expected for the selection-potential task, GHD patients had smaller N2bs, decreased target detection and longer reaction times compared to non-GHD patients. However, these differences were not significant. The differences between our findings and the significant findings in CO-GHD patients (Lijffijt et al., 2003) could be explained by differences in study population. A GH deficiency starting during childhood may produce larger effects on cognition and brain function in general, and therefore also in target detection and attention-related cortical activity as assessed in our studies. This may explain the smaller, non-significant difference between our groups. Two other studies reported prolonged P300 latencies with severe GHD, suggesting a decelerating effect on certain aspects of stimulus processing. This was not confirmed in the present study, possibly due to the low number of GHD subjects in our sample.

In addition to the relationship between GH secretion and cognition after cranial radiotherapy, we assessed plasma IGF-1. As no significant differences in IGF-1 levels between the GHD and non-GHD subjects were observed, we studied correlations between IGF-1 and neuropsychological performance. A relation between IGF-1 and short-term and working memory, as well as with central motor activation, which possibly differs from the relation between GH and cognition, was found. This data is in line with previous observations (Aleman et al., 2000) and suggests a disruption between GH and IGF-1 mediated effects after brain irradiation, similar to the previously reported effects of aging. The correlation between IGF-1 and the LRP measuring selective motor preparation may be explained by the supportive effect of IGF-1 for the myelin that surrounds the axons, and may fasten communication between neurons.

In conclusion, the present data on GH secretion, in a specific population of subjects exposed to brain irradiation, are consistent with other reports on memory deficits in subjects with low GH secretion. In contrast, reductions in interference control and attentional shifting as observed in the present GHD patients are quite discrepant from previous reports and may rather reflect radiation effects. Plasma IGF-1 was positively correlated with the sum of digit span forward and backward and the present data confirm an association with selective motor-cortex activation.

Acknowledgements
Pfizer Inc. financially supported this study, but had no further role in the study design, in the collection, analysis, and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

Conflict of interest statement
All authors state that there are no financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work, all within years of beginning the work submitted.

References

Aberg ND, Brywe KG, Isgaard J. Aspects of growth hormone and insulin-like growth factor-I related to neuroprotection, regeneration, and functional plasticity in the adult brain. ScientificWorldJournal 2006; 6:53-80.

Adan L, Trivin C, Sainte-Rose C, Zucker JM, Hartmann O, Brauner R. GH deficiency caused by cranial irradiation during childhood: factors and markers in young adults. J Clin Endocrinol Metab 2001; 86(11):5245-5251.

Agha A, Sherlock M, Brennan S et al. Hypothalamic-pituitary dysfunction after irradiation of nonpituitary brain tumors in adults. J Clin Endocrinol Metab 2005; 90(12):6355-6360.

Aimaretti G, Corneli G, Razzore P et al. Comparison between insulin-induced hypoglycemia and growth hormone (GH)-releasing hormone + arginine as provocative tests for the diagnosis of GH deficiency in adults. J Clin Endocrinol Metab 1998; 83(5):1615-1618.

Appelman-Dijkstra NM, Kokshoorn NE, Dekkers OM, Neelis KJ, Biermasz NR, Romijn JA, Smit JW, Pereira AM. (2011) Pituitary dysfunction in adult patients after cranial radiotherapy: systematic review and meta-analysis. J Clin Endocrinol Metab. 96(8), 2330-40.

Aleman A, Verhaar HJ, de Haan EHF et al. Insulin-like growth factor-I and cognitive function in healthy older men. J Clin Endocrinol Metab 1999; 84(2):471-475.

Aleman A, de Vries WR, de Haan EH, Verhaar HJ, Samson MM, Koppeschaar HPF. Age-sensitive cognitive function, growth hormone and insulin-like growth factor 1 plasma levels in healthy older men. Neuropsychobiology 2000; 41, 73–78.

Ball LJ, Bisher GB, Birge SJ. A simple test of central processing speed: an extension of the Short Blessed Test. J Am Geriatr Soc 1999; 47(11):1359-1363.

Baum HB, Katznelson L, Sherman JC et al. Effects of physiological growth hormone (GH) therapy on cognition and quality of life in patients with adult-onset GH deficiency. J Clin Endocrinol Metab 1998; 83(9):3184-3189.

Darzy KH, Aimaretti G, Wieringa G, Gattamaneni HR, Ghigo E, Shalet SM. The usefulness of the combined growth hormone (GH)-releasing hormone and arginine stimulation test in the diagnosis of radiation-induced GH deficiency is dependent on the post-irradiation time interval. J Clin Endocrinol Metab 2003; 88(1):95-102.

Darzy KH, Pezzoli SS, Thorner MO, Shalet SM. Cranial irradiation and growth hormone neurosecretory dysfunction: a critical appraisal. J Clin Endocrinol Metab 2007; 92(5):1666-1672.

Darzy KH, Thorner MO, Shalet SM. Cranially irradiated adult cancer survivors may have normal spontaneous GH secretion in the presence of discordant peak GH responses to stimulation tests (compensated GH deficiency). Clin Endocrinol (Oxf) 2009; 70(2):287-293.

Darzy KH, Shalet SM. Hypopituitarism following radiotherapy. Pituitary. 2009;12(1):40-50.

Dattani M, Preece M. Growth hormone deficiency and related disorders: insights into causation, diagnosis, and treatment. Lancet 2004; 363(9425):1977-1987.

Dijkstra M, van Nieuwenhuizen D, Stalpers LJ, Wumkes M, Waagemans M, Vandertop WP, Heimans JJ, Leenstra S, Dirven CM, Reijneveld JC, Klein M. Late neurocognitive sequelae in patients with WHO grade I meningioma. J Neurol Neurosurg Psychiatry. 2009; 80(8): 910-5.

Falleti MG, Maruff P, Burman P, Harris A. The effects of growth hormone (GH) deficiency and GH replacement on cognitive performance in adults: a meta-analysis of the current literature. Psychoneuroendocrinology 2006; 31(6):681-691.

Golgeli A, Tanriverdi F, Suer C et al. Utility of P300 auditory event related potential latency in detecting cognitive dysfunction in growth hormone (GH) deficient patients with Sheehan's syndrome and effects of GH replacement therapy. Eur J Endocrinol 2004; 150(2):153-159.

Johansson JO, Larson G, Andersson M, et al. Treatment of growth hormone-deficient adults with recombinant human growth hormone increases the concentration of growth hormone in the cerebrospinal fluid and affects neurotransmitters, Neuroendocrinology 1995; 57–66.

Kenemans JL, Smulders FT, Kok A. Selective processing of two-dimensional visual stimuli in young and old subjects: electrophysiological analysis. Psychophysiology 1995; 32(2):108-120.

Kenemans JL, Lijffijt M, Camfferman G, Verbaten MN. Split-second sequential selective activation in human secondary visual cortex. J Cogn Neurosci 2002; 14(1):48-61.

Koltowska-Haggstrom M, Mattsson AF, Monson JP et al. Does long-term GH replacement therapy in hypopituitary adults with GH deficiency normalise quality of life? Eur J Endocrinol 2006; 155(1):109-119.

Lezak MD. Neuropsychological assessment. New York: Oxford University Press 1995.

Lijffijt M, Van Dam PS, Kenemans JL et al. Somatotropic-axis deficiency affects brain substrates of selective attention in childhood-onset growth hormone deficient patients. Neurosci Lett 2003; 353(2):123-126.

Littley MD, Shalet SM, Beardwell CG, Robinson EL, Sutton ML. Radiation-induced hypopituitarism is dose-dependent. Clin Endocrinol (Oxf). 1989;31(3):363-73.

Nelson HE. The National Adult Reading Test (NART): test manual. Windsor UK: NFER Nelson, 1982.

Nyberg F. Growth hormone in the brain: characteristics of specific brain targets for the hormone and their functional significance. Front Neuroendocrinol 2000; 21(4):330-348.

Peace KA, Orme SM, Padayatty SJ, Godfrey HP, Belchetz PE. Cognitive dysfunction in patients with pituitary tumour who have been treated with transfrontal or transsphenoidal surgery or medication. Clin Endocrinol (Oxf) 1998; 49(3):391-396.

Popovic V, Pekic S, Golubicic I, Doknic M, Dieguez C, Casanueva FF. The impact of cranial irradiation on GH responsiveness to GHRH plus GH- releasing peptide-6. J Clin Endocrinol Metab 2002; 87(5):2095-2099.

Quik EH, van Dam PS, Kenemans JL. Growth hormone and selective attention: A review. Neurosci. Biobehav. Rev. 34 2010; 1137–1143.

Quik EH, Conemans EB, Valk GD, Kenemans JL, Koppeschaar HPF, & van Dam PS. Cognitive performance in older males is associated with growth hormone secretion. Neurobiology of Aging 2012; 34(8), 1137-43.

Quik EH, Conemans EB, Valk GD, Kenemans JL, Koppeschaar HP, Dam PS. Insulin-like growth factor-I is associated with cognitive performance in older men. 2011 *In submission.*

Shalet SM, Brennan BM. Growth and growth hormone status after a bone marrow transplant. Horm Res 2002; 58 Suppl 1:86-90.

Shukitt-Hale B, Casadesus G, Carey AN, Rabin BM, Joseph JA. Exposure to 56Fe irradiation accelerates normal brain aging and produces deficits in spatial learning and memory. Adv Space Res 2007; 39(6):1087-1092.

Soares CN, Musolino NR, Cunha NM et al. Impact of recombinant human growth hormone (RH-GH) treatment on psychiatric, neuropsychological and clinical profiles of GH deficient adults. A placebo-controlled trial. Arq Neuropsiquiatr 1999; 182-189.

Tanriverdi F, Yapislar H, Karaca Z, Unluhizarci K, Suer C, Kelestimur F. Evaluation of cognitive performance by using P300 auditory event related potentials (ERPs) in patients with growth hormone (GH) deficiency and acromegaly. Growth Horm IGF Res 2009; 19(1):24-30.

Taphoorn MJ, Klein M. Cognitive deficits in adult patients with brain tumours. Lancet Neurol. 2004;3:159-68.

van Dam PS, Aleman A. Insulin-like growth factor-I, cognition and brain aging. Eur J Pharmacol 2004; 490(1-3):87-95.

van Dam PS, De Winter CF, De Vries R et al. Childhood-onset growth hormone deficiency, cognitive function and brain N-acetylaspartate. Psychoneuroendocrinology 2005; 30(4):357-363.

van Dam PS. Somatropin therapy and cognitive function in adults with growth hormone deficiency : a critical review. Treat Endocrinol 2006; 5(3):159-170.

van Nieuwenhuizen D, Klein M, Stalpers LJ, Leenstra S, Heimans JJ, Reijneveld JC. Differential effect of surgery and radiotherapy on neurocognitive functioning and health-related quality of life in WHO grade I meningioma patients. J Neurooncol. 2007; 84(3):271-8.

Vitiello MV, Moe KE, Merriam GR, Mazzoni G, Buchner DH, Schwartz RS. Growth hormone releasing hormone improves the cognition of healthy older adults. Neurobiol Aging 2006; 27(2):318-323.

Table references

Lansbergen, M.M., Kenemans, J.L., van Engeland, H., 2007. Stroop interference and attention-deficit/hyperactivity disorder: a review and meta-analysis. Neuropsychology. 21(2), 251-262.

van Dam, P.S., 2006. Somatotropic therapy and cognitive function in adults with growth hormone deficiency: a critical review. Treat Endocrinol. 5 (2), 1.

van Zandvoort, M.J., Kessels, R.P., Nys, G.M., de Haan, E.H., Kappelle L.J., 2005. Early neuropsychological evaluation in patients with ischaemic stroke provides valid information. Clin Neurol Neurosurg. 107(5), 385-92.

Aleman, A., Verhaar, H.J., De Haan, E.H., De Vries, W.R., Samson, M.M., Drent, M.L., van der Veen, E.A., Koppeschaar, H.P., 1999. Insulin-like growth factor-I and cognitive function in healthy older men. J. Clin. Endocrinol. Metab. 84, 471–475.

Kenemans, J.L., Smulders, F.T., Kok, A., 1995. Selective processing of two-dimensional visual stimuli in young and old subjects: electrophysiological analysis. Psychophysiology. 32, 108–120.

Shallice, T. and Burgess, P. W., 1996. The domain of supervisory processes and the temporal organisation of behaviour. Philosophical Transactions of the Royal Society of London B, 351, 1405-1412.

Chapter 6

Larger Error- and Feedback-related Negativity in Positive compared to Negative Learners

E.H. Quik, J. Gerritsen, J.L. Kenemans, P.S. van Dam, K.B.E Böcker

Larger Error- and Feedback-related Negativity in Positive compared to Negative Learners. E.H. Quik, J. Gerritsen, J.L. Kenemans, P.S. van Dam, K.B.E Böcker. Brain and Cognition. *In revision*

Summary

There are differences in probabilistic learning such that some people, the positive learners, learn from success, whereas others, the negative learners, learn more from avoiding failure. Studies in non-medicated and medicated Parkinson disease patients have demonstrated that tonic dopamine levels correlate positively with the ability to learn from success. Psychopharmacological manipulations in healthy controls suggest that high tonic dopamine levels also lead to large Error-Related negativity (ERN). Supposedly, ERN is an EEG manifestation of phasic drops in dopamine level that occur after making errors. Therefore it was hypothesized that positive learners, with high dopamine levels, would be characterized by high ERN amplitudes, relative to negative learners. However, the opposite result has been reported lately by Frank et al. (2005, 2007). In the present study, in line with the hypotheses derived from dopamine level manipulations, positive and not negative learners, showed larger ERNs after making an error. Furthermore positive learners also showed a larger Feedback-Related Negativity (FRN), the EEG response to negative feedback. We reconciled these findings by proposing an inverted U-shaped relationship between dopamine-level and the propensity to learn from errors compared to success. Detailed comparisons between the distributional characteristics of the present as compared to previous studied group of participants/patients support the conclusion that whereas the present studied group of participants might include negative learners with below average dopamine level, the previous samples might have included negative learners with above average dopamine levels. To test this interpretation, additional studies are needed that examine interindividual differences in ERN and FRN in relation to learning bias, and which also include independent correlates of dopamine activity, or dopaminergic drug manipulations.

Introduction

Error-related negativity (ERN) is an electrophysiological marker that occurs within 100 ms after participants make errors in cognitive tasks (Holroyd and Coles, 2002). The ERN is proposed to reflect brain activity caused by dips in dopamine. This dopamine drop is a prerequisite for error processing and conflict monitoring by the anterior cingulate cortex (ACC). Phasic dopamine input into the ACC has been assumed to form the basis for learning from rewards. A positive prediction error, that is, an unexpected reward, leads to phasic dopamine input into the ACC. In contrast, a negative prediction error, that is, an unexpected lack of reward, leads to a phasic drop in dopaminergic input to the ACC. At the scalp this drop in dopaminergic input manifests itself as a negative event-related potential (ERP) component when participants make incorrect responses, compared to the ERP component after correct choices (CRN) (Santesso et al., 2008).

ERNs have been reported to be enhanced in subjects showing preponderance for learning from negative as opposed to positive feedback. Subjects who avoid probabilistic non-reward showed larger ERNs than subjects that are biased to make choices that lead to probabilistic reward (Frank et al., 2005; 2007). This learning bias has been shown to be dopaminergically influenced. Parkinson's patients off medication were better at learning to avoid negative outcomes than they were at learning from positive outcomes (Frank et al., 2004). A dopamine agonist reversed this bias, so that patients learned from positive rather than negative outcomes.

Dopamine affects the ERN amplitude. ERN amplitude was found to be smaller after administering haloperidol, a D2 receptor antagonist (Zirnheld et al., 2004, De Bruijn et al., 2006). This was interpreted as a demonstration that the effects of the phasic dopamine dip that follows errors were blocked by haloperidol. The decrease in ERN was accompanied by impaired learning on a time estimation task (Zirnheld et al., 2004). In addition, ERN was found to be increased after the intake of amphetamine, a drug that increases the synaptic availability of dopamine (De Bruijn et al., 2004).

The data presented so far on patients and pharmacological manipulations suggest a positive relationship between dopamine level, positive learning bias, and ERN amplitude. Therefore, one would predict larger ERN amplitudes in positive learners. However, the opposite has actually been reported (Frank 2005; 2007). Haloperidol, a dopamine antagonist, increased positive learning in a probabilistic learning task (Frank and O'Reilly, 2006; Pizzagalli et al., 2008). In the same vein, the D2/D3 dopamine agonist's cabergoline (Frank and O'Reilly, 2006) and pramipexole (Pizzagalli et al. 2008) decreased positive learning. Frank and O'Reilly (2006) proposed that haloperidol and cabergoline had their net effects by stronger binding to presynaptic relative to postsynaptic receptors at the tested doses. In that case haloperidol would have increased dopamine by its presynaptic effect, and thereby positive learning. If the same mechanism was active in the pharmacological ERN studies (Zirnheld et al., 2004, De Bruijn et al., 2006), then the relationship between dopamine-level and ERN is opposite from what is generally assumed based on the

assumption of post-synaptic effects. In that case the relationship between learning bias (positive with dopamine) and ERN (negative with dopamine) would be as observed by Frank et al. (2005; 2007).

The feedback-related negativity (FRN) is an ERP component, which shares many similarities with the ERN. The FRN peaks negatively between 200 and 400 ms and originates in the dorsal ACC or medial prefrontal cortex (PFC) following error feedback (Frank et al. 2005; Gehring et al., 1993; Gehring and Knight, 2000; Muller et al., 2005; Nieuwenhuis et al., 2005; Van Veen et al., 2004). Like ERN, the FRN was found to be larger in negative compared to positive learners in a probabilistic learning task (Frank et al., 2005). Similarly, the FRN was found to be smaller in (positive) learners compared to non-learners (Santesso et al. 2008). This decreased FRN in positive learners was accompanied by increased current source density in dorsal ACC regions upon (positive) feedback.

The present study used the same design as Frank et al. (2005; 2007) study, in order to assess the relationship between learning bias and the amplitudes of the ERN and FRN. The FRN was recorded during the learning phase of a probabilistic learning task in which one (so-called A) stimulus was connected with positive feedback and the other (so-called B) stimulus was paired with negative feedback on 80% of the trials. C and D in CD pairs were followed by 70% positive and negative feedback respectively. Finally, E and F were probabilistically paired with positive and negative feedback on 60% of the trials (Figure 1).

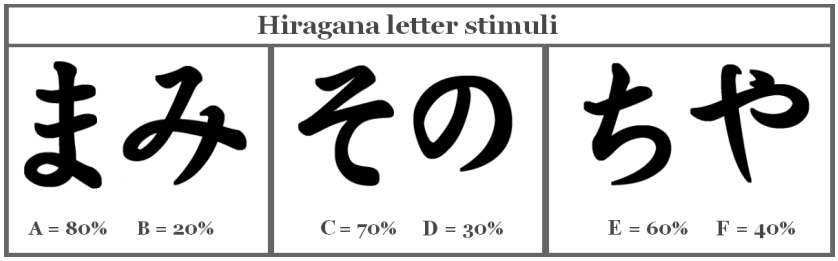


Figure 1 Exemplar Hiragana letter stimuli used in the probabilistic cognitive reinforcement learning task

These stimulus pairs minimize explicit verbal encoding. Each Hiragana stimuli pair is separately presented in different trials in random order, and participants have to select among the two stimuli; correct choices are determined probabilistically. The a priori probability of reward varies between stimulus pairs, as indicated below each stimulus.

In the test phase novel pairs were formed in which A and B stimuli were paired with other stimuli that were (non) rewarded more equivocally. The tendency to choose A rather than to avoid B in those novel pairs was used to characterize people as positive and negative learners, respectively. Errors during this phase evoked an ERN.

We tested whether the results either confirm those of Frank et al. (2005; 2007) or whether they are opposite and more in line with a postsynaptic interpretation of the pharmacological results outlined above. In the latter case we assume that positive learners, compared to negative ones, would have a higher tonic dopamine activity, in line with the results in treated Parkinson's patients in Frank et al. (2004). Higher dopamine level in positive learners would in turn enable larger dopamine dips upon errors and negative feedback, resulting in larger ERN and FRN amplitudes. In deviation from the Frank et al. (2005; 2007) studies we used inherently neutral geometrical stimuli as feedback in the learning phase. In that way the possible affective value of the feedback stimuli per se would not influence the FRN or its modulations.

Experimental Procedures

Sample

Fifty-eight healthy undergraduate participants (41 females, 17 males, 71% vs. 29%, age M = 20.6, range = 18-26, SD = 2.0) were recruited through the website of the psychology department of Utrecht University. They were given either a monetary reward of €7, - per hour or honorary points necessary to complete the curriculum. All subjects ascertained to have normal hearing and normal or corrected-to-normal vision. They signed an informed consent and were treated according to the Declaration of Helsinki.

Subjects were asked to refrain from drinking coffee or smoking one hour prior to the start of the experiment. Alcohol consumption on the testing day, prior to the experiment, was also prohibited. Furthermore, only those individuals who were unfamiliar with the Japanese language were included. We excluded participants with neurologic or psychiatric disease, endocrine or internal disease, severe obesity (BMI > 32 kg/m^2), malnutrition (BMI < 18.5 kg/m^2), chronic alcohol (more than 3 units daily) or drug (any soft or hard drug) abuse, and use of medication that may affect cognitive functioning. Participants signed informed consent before they were included. Data from 16 participants (28% of the original sample) who did not satisfy global accuracy measures during either the training or test sessions were discarded during analysis.

In total, 27 participants were found to be positive learners (mean age 20.5 years, SD 2.0, range 18-26 years; 19 females (70%)) who had a better performance at choosing the most rewarded stimulus (A, see below) relative to avoiding the least rewarded one (B, see below), when paired with more equivocally rewarded stimuli. These 27 positive learners were compared with 15 negative learners (mean age 20.5 years, SD 2.1, range 18-24 years; 9 female (60%)) who had a better performance at avoiding the least rewarded than choosing the most rewarded stimulus.

Task

Subjects performed a slightly modified version of the probabilistic reinforcement learning task Frank et al. (2005). Stimuli were Japanese letter characters known as Hiragana, which minimize explicit verbal encoding, randomly selected from a collection of relatively similar exemplars (Figure 1). Presentation of the stimuli and recording of the responses was controlled by ERTS software (BeriSoft, Frankfurt, Germany).

The task was divided into two phases, a training phase in which subjects learned which characters were 'correct', and a testing phase. In the training phase of the task, feedback regarding their choice was provided by means of a circle (correct response) or a triangle (wrong response). Three different stimulus pairs were presented during the training phase. For each pair the 'correct' character had a different probability of yielding positive feedback (figure 1: 80 % for A, 70 % for C, and 60 % for E), and the 'wrong' character a different probability of yielding negative feedback (figure 1: 20 % for B, 30 % for D, and 40 % for E). The three different stimulus combinations (AB, CD, and EF) were presented in random order. Subjects were instructed to choose the most consistently rewarded stimulus from each pair of Hiragana stimuli. The left or right Hiragana letter was selected by pushing one of two buttons, located on the left and the right of the response pad, with the index finger of either hand. The instructions stated to participants that on each trial they should choose the correct character, by initial guessing and later learning from feedback. It was emphasized that the feedback could be erroneous and that subjects should stick to the strategy of choosing the characters which were most likely to yield positive feedback, unless they discovered they had been consistently responding to the 'wrong' character.

All three combinations were presented 20 times, resulting in 60 trials. Each trial consisted of a fixation cross, the duration of which varied randomly between 250 and 750 ms, and a pair of Hiragana characters, presented for 750 ms, followed by response triggered feedback, presented for 600 ms. If participants failed to respond in time (reaction time > 1000 ms), a message appeared stating that no response was recorded. During the training phase FRN was recorded, which was evoked by intrinsically neutral, probabilistic (valid on 60-80% of the trials), feedback signals. These feedback signals informed the subjects whether or not they choose the correct member of each of three pairs of Japanese characters.

Before moving on to the testing phase, all participants were required to meet a performance criterion for each stimulus pair during the training phase. For AB pairs, 65% A responses were required, 60% C responses for CD pairs and 50% E for EF pairs. If after seven blocks the criteria were not met and no sign of improvement was present, the corresponding testing block was skipped and the next training block was presented. Twelve subjects were excluded because they did not reach the criterion at the end of seven training blocks. Additionally, 4 subjects were excluded because accuracy on either the A or the B pairs during the testing phase did not exceed 50%.

In the testing phase the participants were instructed that the same characters would be presented again, but often in novel pairs. They were told to continue to choose the most

rewarded character of the pair, but that no feedback would be given. ERN and the ERP component 100ms after correct responses (CRN) were assessed for trials with novel combinations of the previously trained characters. Correctness of the behavioral response was defined as the choice of the character that had been rewarded more consistently during the training phase. During the testing phase all training pairs and all novel combinations of stimuli were presented six times. This amounted to 90 trials, consisting of a fixation cross (same as training phase), and the Hiragana pair, presented for 1000 ms, followed by a blank screen for 950 ms.

Procedure

Upon arrival at the lab, informed consent was signed, and in- and exclusion criteria were checked. Next, a cap with 128 EEG electrodes was attached, after which participants were seated in a chair in a soundproof and electrically shielded room. All instructions and stimuli were presented on a computer screen. When a training block was finished, the participants' score was assessed, to determine whether or not the criterion to move on to the testing phase was reached. If so, a testing block was presented. Each participant completed three cycles of training and (most often a) testing phase, each of which featured different Hiragana characters for the stimulus pairs, chosen randomly from the aforementioned collection. After three cycles the electrode cap was dismounted and the subjects were paid, debriefed and dismissed.
Performance on all novel pairs containing A or B paired with either, C, D, E or F was then analyzed, and the proportion of choosing A and avoiding B in those novel pairs was calculated. Participants were classified as positive learners if they had greater accuracy in choosing A compared to avoiding B among those novel pairs. Negative learners were defined as those showing the opposite pattern.

Electrophysiological Signal Recording and Analysis

EEG signals were recorded using a 128-electode QuikCap and Neuroscan SynAmps amplifiers. The signals were then digitized at a sample rate of 250 Hz and filtered online (low-pass) at 100 Hz using Neuroscan Acquire software. Eye movements were recorded using horizontal and vertical electro-oculograms from four electrodes positioned around the eyes. EEG processing was conducted using Vision Analyzer version 1.05.0001. EEG signals were filtered offline (low-pass at 30 Hz, 12 dB/oct and additional high-pass with time constant 1 s, 24 dB/oct), after which epochs containing artifacts were removed. Signals were corrected for eye movements and blinks (Gratton, Coles & Donchin, 1983).
Response–locked ERPs were derived in the testing phase to obtain the ERN and its correct-response counterpart. Epochs ran from 800 ms prior to the response to 200 ms after the

response. A 15 Hz low-pass filter was applied and the baseline was then based on the first 100 ms of the epoch. For quantification a double subtraction procedure was followed. First, the correct-response ERP was subtracted from the error-related ERP (cf. Holroyd et al., 2002). Second, we subtracted the mean amplitude in the -100 to -50 ms pre-response interval from the mean amplitude in the 25-45 ms post response interval at Fz (compare Gehring et al., 1993). These intervals encompassed the grand average first negative peak after the response and the preceding positive peak respectively, which were measured on a subject-by-subject basis by Frank et al. (2005).
For the training phase feedback-stimulus-locked ERPs were derived for positive and negative feedback separately. FRN epochs started 100 ms prior to feedback until 1000 ms post-feedback. Baseline correction was based on the first 100 ms. For quantification the positive-feedback ERP was subtracted from the negative-feedback, cf. Holroyd et al. (2002), and FRN was defined as the mean amplitude in the 265-285 ms latency minus that in the 195-215 ms window at Fz. Like for the ERN these windows encompassed the grand average peaks that were assessed subject-by-subject in Frank et al. (2005).

Statistics

To assess differences between the positive and negative learners, analyses of variance (ANOVA) were used with group as the independent factor. All statistical analyses were calculated using a standard version of SPSS® (12.0.1 and 16) for Windows.

Results

Behavioral results

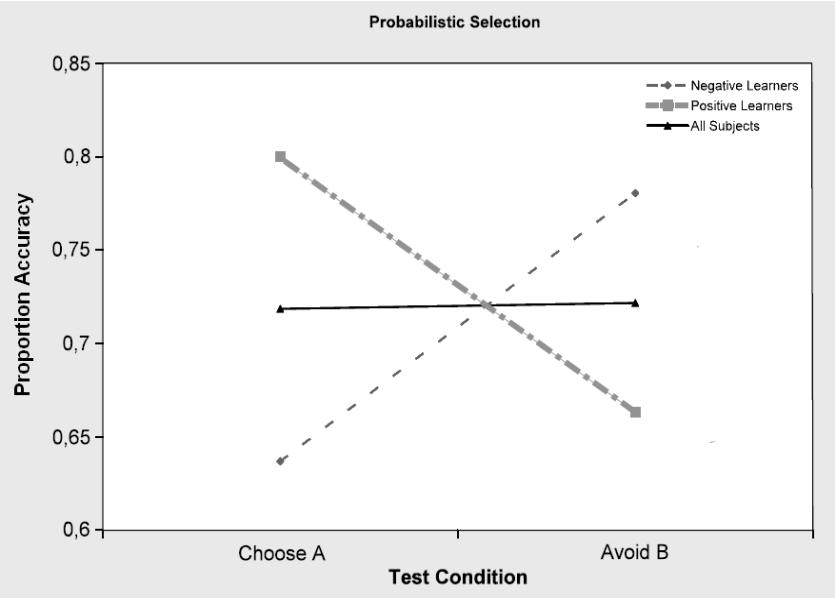


Figure 2 Negative learners were better at avoiding stimulus B, whereas positive learners were characterized by better performance at choosing stimulus A

Two groups of learners were formed and subsequently compared, i.e. negative (n=15) and positive learners (n=27), based on their tendency to avoid stimulus B (group difference: $t(30) = -4.63$; $p = .00$), relative to their tendency to choose stimulus A ($t(30) = 6.62$; $p = .00$; figure 2).

EEG results

During the training phase positive learners showed a larger FRN after negative than after positive feedback ($t(26) = 2.21$. $p < .05$; figure 3a), whereas the robust negative peak for negative learners did not discriminate between both types of feedback ($t(14) = 1.01$, n.s.; figure 3b).

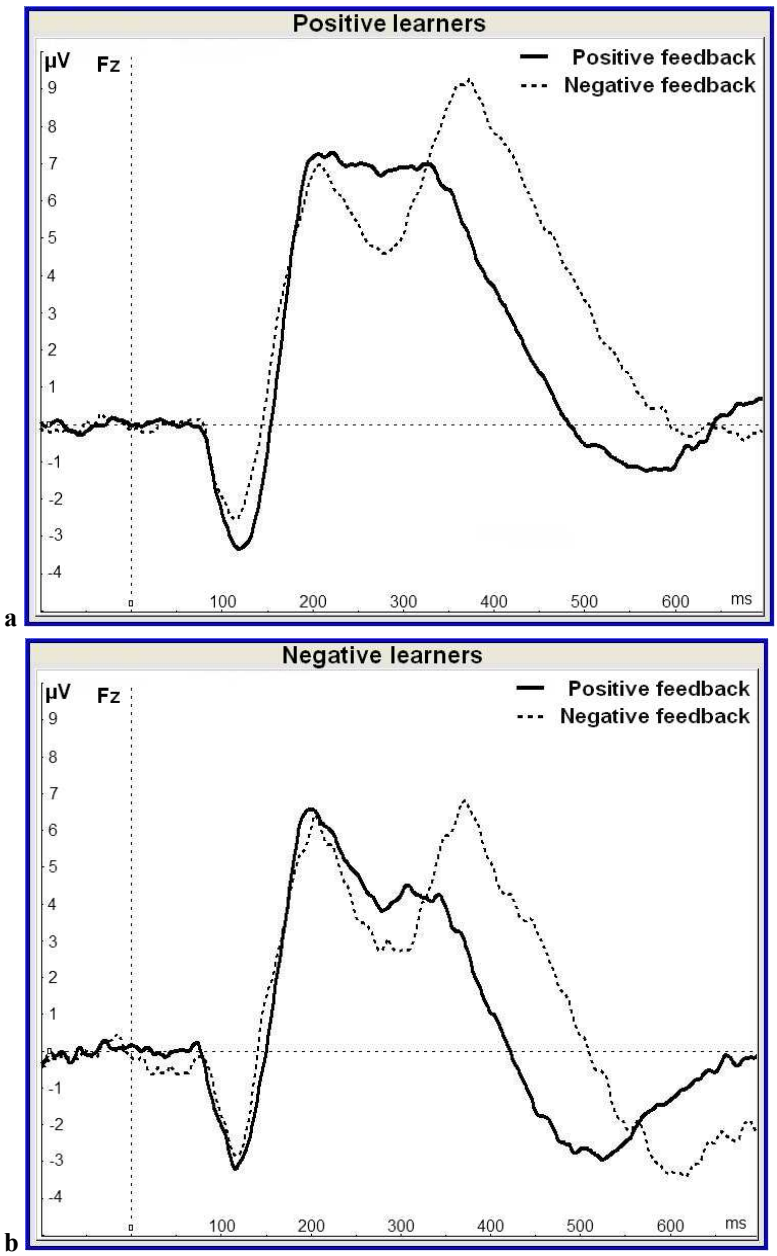
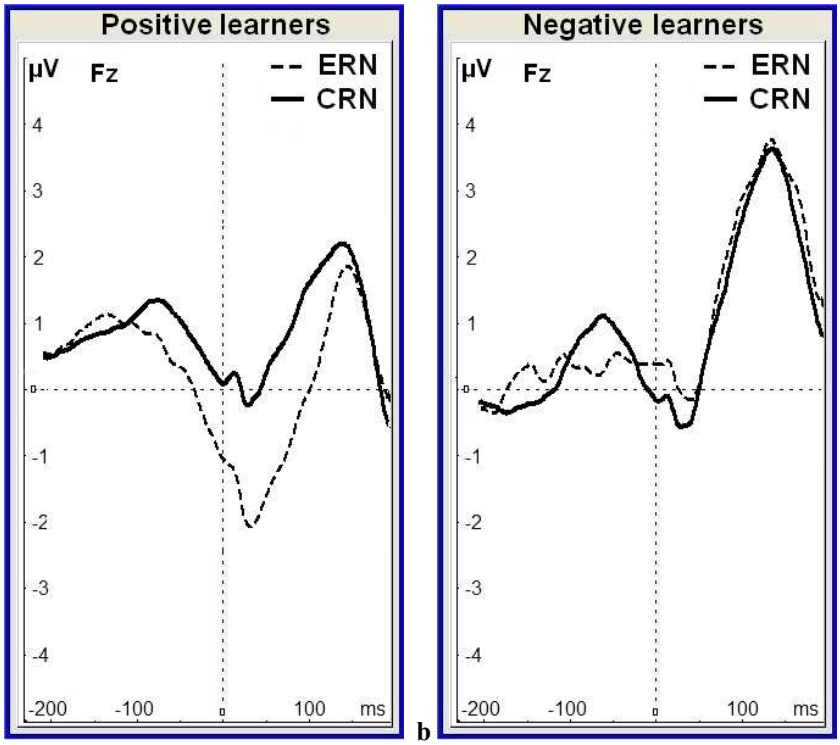


Figure 3a FRN, the mean amplitude in the 265-285 ms area minus that in the 195-215 ms area, for positive learners (n=27)

b The FRN for negative learners (n=15)

Positive learners showed a larger FRN after negative than after positive feedback, whereas the robust FRN for negative learners did not discriminate between the two types of feedback (Figure 3).

Similarly, during the testing phase positive learners showed a significantly larger negativity following errors than following correct responses (ERN; $t(25) = 2.50$; $p < .05$; figure 4a). In contrast the negativity was very small, and did not differ significantly between errors and correct responses, in negative learners ($t(14) = -1.62$; n.s.; figure 4b).



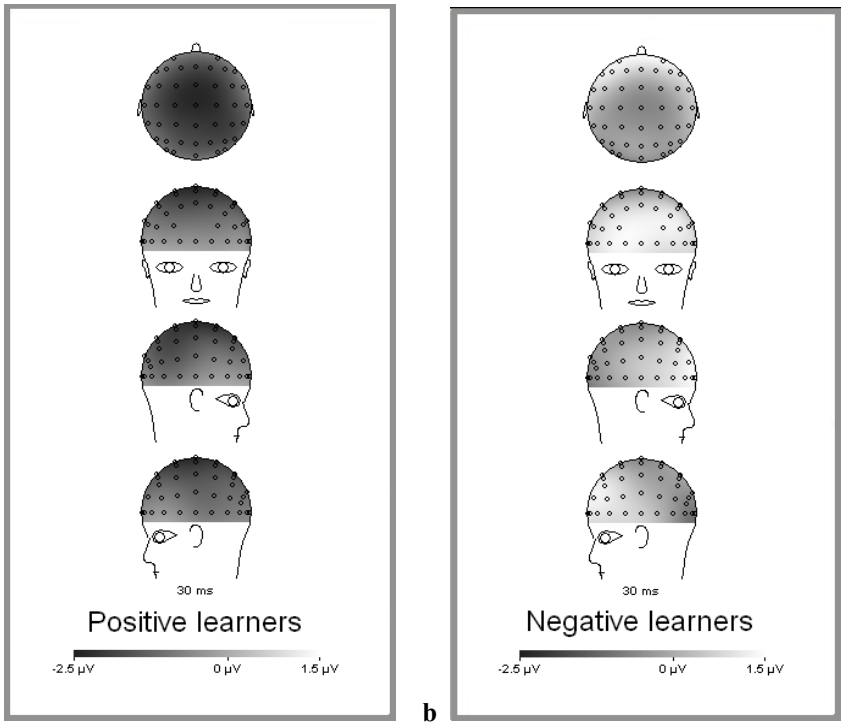
a **b**

Figure 4a ERN, the mean amplitude in the 25 to 45 ms area minus that in the -100 to -50 ms area, for positive learners (n=27)

b The ERN for negative learners (n=15). Positive learners showed a larger ERN after errors than after correct responses, whereas ERN and CRN were both small in negative learners

With regard to the scalp distribution of the ERN, positive learners showed a distribution that is compatible with a source in the Anterior Cingulate Cortex (ACC; figure 5). Positive learners showed a significantly larger negativity following errors (ERN) than following correct responses (CRN), whereas ERN and CRN were both small in negative learners (figure 5).

These results were confirmed by the correlation analysis. A bias to learn more from negative than positive feedback was associated with a smaller ERN (figure 6; $r = .33$, $p = .03$).



a **b**

Figure 5a ERN minus CRN scalp distribution for positive learners (n=27)
b ERN minus CRN scalp distribution for negative learners (n=15)

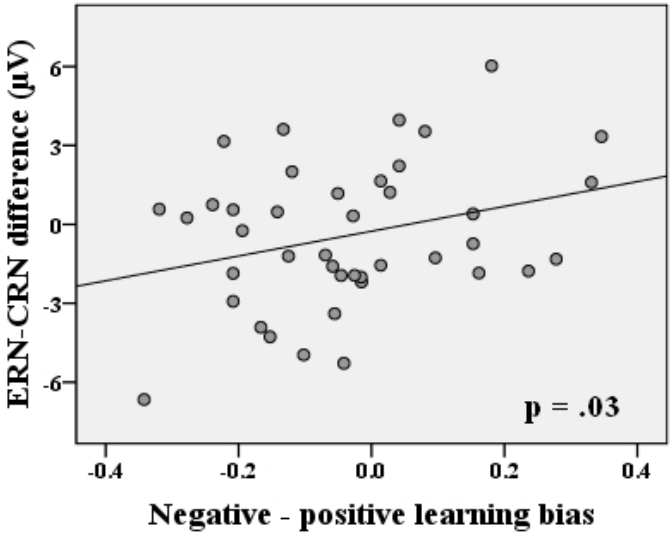


Figure 6 ERP correlates of learning; preferential biases to learn more from negative than positive feedback were associated with relatively less negative ERN minus CRN

Discussion

In the present study, the relation between ERN, FRN and learning bias, as measured by performance on the reinforcement learning task previously used by Frank et al. (2005; 2007) was evaluated. The difference between the positive and negative learners' performance and ERPs was analyzed, as well as the correlation between task performance and ERPs. One of two possible outcomes was expected. Either, following Frank et al. (2005; 2007) negative learning bias, i.e., learning to avoid negative outcomes, would be associated with increased ERN and FRN. Alternatively, positive learners might have larger ERNs and FRNs. The latter hypothesis was derived from performance on probabilistic learning in Parkinson's disease (Frank et al., 2004) and from pharmacological manipulations of the ERN/FRN (Zirnheld et al., 2004; Bruijn et al., 2004; De Bruijn et al., 2006).

A first important prerequisite finding of the present study was that erroneous responses induce larger negativity, or ERN, compared to correct responses. In the same vein, negative feedback elicited larger FRN than positive feedback. In the present study feedback was provided by affectively neutral geometrical forms. In previous studies this constituted a possible confound e.g., Frank et al. 2005 used yellow smiley face and red cross-out and Frank et al., 2007 the printed words "Correct!" and "Incorrect" Therefore we conclude that the difference in FRN evoked by negative compared to positive feedback is not dependent on the intrinsic affective quality of the feedback stimulus used.

Secondly, in the present study and contrary to Frank et al. (2005), ERN and FRN (for negative feedback) were larger for positive compared to negative learners. Similarly, correlations between positive learning bias and the absolute amplitudes of these ERPs were positive. As argued before, one might deduce from the fact that Parkinson's patients off dopamine agonistic medication show a negative learning bias that becomes more positive with such medication, that positive learning is associated with relatively high levels of tonic dopamine. Following the law of initial values, a relatively high level of tonic dopamine activity might lead to relatively large drops in phasic dopaminergic activity following unexpected non-reward (see Frank et al., 2007, p 304), and thus a larger ERN. This leads to a large ERN in positive (high tonic dopamine) compared to negative (low tonic dopamine) learners, as observed in the present study.

One might reconcile the conflicting results of Frank et al. (2005) and the present study, as well as the Parkinson's patients and pharmacological data, by assuming an inverted-U relationship between dopamine and positive learning. In this line of reasoning, first, the unmedicated Parkinson disease patients are expected to be far below optimum dopamine level, which makes them negative learners. Medication increases the tendency for positive learning. This forms the ascending limb of the inverted-U function. Positive-learning healthy individuals would have dopamine levels at the peak of the function, and negative-learning healthy individuals would have dopamine levels down either the ascending or the descending limb of the function. According to the computational model proposed by Frank

(2005), low-dopamine negative learners produce relatively small dopamine bursts to positive feedback (because of the low amount of available dopamine), and consequently they manifest as relatively negative learners. Also, consistent with the logic outlined in the introduction, due to their low tonic dopamine they have a limited dynamic range for dopamine dips, therefore they produce small FRNs and ERNs. High-dopamine negative learners have a small dynamic range for dopamine bursts to positive feedback, but a large one for dopamine dips to negative feedback. Hence they learn relatively little from positive feedback but produce relatively large ERNs and FRNs. Individuals with intermediate dopamine levels produce relatively high dopamine bursts and intermediate dopamine dips, therefore they reveal themselves as positive learners with intermediate FRNs and ERNs.

To resolve the conflict between the present results and those of Frank et al., we assume that in the present sample the negative learners have low tonic dopamine levels, while in the Frank et al. study they had above average tonic dopamine levels. Perhaps related to this, closer analysis revealed that our sample contained less negative learners (this being the smaller group, 36% of our sample) compared to the more even distributions in the previous studies (Frank et al., 2005; 2007). The present sample therefore might represent mainly the extreme more negative learners, including more participants with below optimal dopamine level. A low dopamine level with low dopamine dips is also suggested by the very small amplitudes of the non-subtracted response-locked ERPs in figure 3b (compared with fig 2b in Frank et al., 2005). A further indication that the selection bias might have been different in the present study comes from the number of excluded subjects. Whereas we lost 28% of the subjects because they did not pass the performance criteria during the training and testing phases, this was only 18% and 10% in the studies of Frank et al. (2005; 2007). A further potentially relevant finding was reported by Pailing et al. (2004). Here, uncertainty about whether an error has actually been committed reduced the difference between the negativities on error trials versus correct trials. One way to increase this uncertainty was to reduce the discriminability of the task stimuli. In the same vein the present negative learners may have found it more difficult to discriminate between the inherently neutral feedback stimuli than our positive learners. This would also explain the smaller number of participants included in the present negative learners group.

As far as the present study constitutes a replication, its main limitation is in the scoring of the ERP components. Here we followed the procedures proposed in the early ERN-papers by Holroyd, Gehring and Coles and the tradition of cognitive ERP research. That is, subtracted waveforms were used for scoring (Holroyd et al., 2002). This way, the measures mainly reflected how error processing differs from success processing (and, *mutatis mutandis*, how processing of negative feedback differs from processing of positive feedback), disregarding how response processing in general contributed to individual differences between positive and negative learners. Note that the non-subtracted waveforms in both papers by Frank et al. (2005, 2007) for the respective negative learner groups look quite different indeed, and again differ from the present ones. To evade the necessity of filtering or subjective decision making during peak-picking of noisy signals (see grand

average waveforms in Frank et al. 2007 and the present paper) we used mean amplitudes in short intervals around the grand average peaks instead (cf. Gehring et al., 1993). With peak-picking similar but less reliable patterns of results were obtained in the present study.
In sum, we have tried to replicate the finding that negative learning bias is associated with larger ERN and FRN amplitude. Contrary to previous findings, positive learners and not negative learners showed larger ERN and FRN amplitudes. This inconsistency in differential sensitivity is presumably related to different levels of tonic dopamine activity. In the present sample, negative learners might be assumed to have low tonic dopamine levels, cf. unmedicated Parkinson's patients in Frank et al. (2004), which gives small ERNs, both subtracted and not subtracted. The positive learners are assumed to have high tonic dopamine levels, cf. medicated Parkinson's disease patients in Frank et al. (2004), and show discriminative endogenous ERN and feedback triggered FRN. It seems improbable that the opposite group differences observed by Frank et al. (2005) and in the present study is due to the difference in feedback stimuli used, especially for the ERN that is recorded in the testing phase in the absence of these signals. Inconsistent results have also been published for the ERN in relation to other interindividual differences, e.g., obsessive-compulsive disorder (OCD) vs. normal controls (Gehring et al., 2000; Nieuwenhuis et al., 2005). These inconsistencies have also been attributed to a possible inverted-U relationship, between dopamine level and ERN amplitudes (Nieuwenhuis et al., 2005). To solve these issues, additional studies are required, which should include independent correlates of dopamine activity, and/or dopaminergic drug manipulations.

Conclusions

In the present study, 'positive-learning' individuals that learn more from positive than from negative feedback show a pronounced acute brain-potential response to self-emitted errors as well as negative feedback. This brain-potential response is absent, or not different for negative compared to positive feedback in negative learners. To accommodate these results with opposite results before, negative learning is hypothesized to result from either low tonic or excessively high tonic dopamine levels.

Acknowledgements

All authors, E.H. Quik, J. Gerritsen, J.L. Kenemans, and K.B.E Böcker, declare that they have no actual or potential conflicts of interest. All Authors disclose any actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence (bias) their work. There are no actual or potential conflicts of interest, including employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.
None of the author's institution has contracts relating to this research through which it or any other organization may stand to gain financially now or in the future.
UU provided funding for this study, but had no further role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.
The data contained in the manuscript has been submitted and accepted as a poster abstract to the Society of Neuroscience for the 2009 meeting in Chicago. All participants gave informed consent according to the Declaration of Helsinki.
All authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data.

References

De Bruijn, E.R.A., Hulstijn, W., Verkes, R.J., Ruigt, G.S.F., Sabbe, B.G.C. (2004). Drug-induced stimulation and suppression of action monitoring in healthy volunteers. Psychopharmacology *177*, 151–160.

De Bruijn, E. R. A., Sabbe, B. G. C., Hulstijn, W., Ruigt, G.S.F., Verkes, R. J. (2006). Effects of antipsychotic and antidepressant drugs on action monitoring in healthy volunteers. Brain Research, *1105*, 122-129.

Frank, M.J., Seeberger, L.C., and OReilly, R.C. (2004). By carrot or by stick: Cognitive reinforcement learning in Parkinsonism. Science *306*, 1940–1943.

Frank, M.J., Woroch, B.S. and Curran, T. (2005). Error-Related Negativity Predicts Reinforcement Learning and Conflict Biases. Neuron *47*, 495–501.

Frank, M.J. (2005). Dynamic dopamine modulation in the basal ganglia: A neurocomputational account of cognitive deficits in medicated and non-medicated Parkinsonism. J Cogn Neurosci *17*, 51-72.

Frank, M.J. (2006): Hold your horses: A dynamic computational role for the subthalamic nucleus in decision making. Neural Netw *19*, 1120-1136.

Frank, M.J., and O'Reilly, R.C. (2006). A mechanistic account of striatal dopamine function in human cognition: Psychopharmacological studies with cabergoline and haloperidol. Behav Neurosci *120,* 497-517.

Frank, M.J., D'Lauro, C. & Curran, T. (2007). Cross-task individual differences in error processing: Neural, electrophysiological and genetic components. Cognitive, Affective and Behavioral Neuroscience, *7*, 297-308.

Gehring, W.J., Goss, B., Coles, M.G.H., Meyer D.D., & Donchin, E. (1993). A neural system for error detection and compensation. Psychological Science, *4*, 385-390.

Gehring, W. J., Himle, J., & Nisenson, L. G. (2000). Action monitoring dysfunction in obsessive-compulsive disorder. Psychological Science, *11,* 1-6.

Gehring, W. J., & Knight, R. T. (2000). Prefrontal - cingulate interactions in action monitoring. Nature Neuroscience, *3,* 516-520.

Gratton, G., Coles, M.G., and Donchin, E. (1983). A new method for off-line removal of ocular artefact. Electroencephalography and clinical Neurophysiology, *55*, 468-484.

Holroyd, C.B., and Coles, M.G.H. (2002). The neural basis of human error processing: Reinforcement learning, dopamine, and the error-related negativity. Psychol. Rev. *109*, 679–709.

Holroyd, C.B., and Coles, M.G.H. (2008). Dorsal anterior cingulate cortex integrates reinforcement history to guide voluntary behavior. Cortex *44*, 548-559.

Muller, S.V., Rodriguez-Fornells, A., Munte, T.F. (2005). Brain potentials related to self-generated information used for performance monitoring. Clin Neurophysiol *116*, 63-74.

Nieuwenhuis, S., Slagter, H.A., von Geusau, A., Heslenfeld, D.J., and Holroyd, C.B. (2005). Knowing good from bad: Differential activation of human cortical areas by positive and negative outcomes. Eur J Neurosci *21*, 3161-3168.

Pailing, P. E., and Segalowitz, S. J. (2004). The effects of uncertainty in error monitoring on associated ERPs. Brain & Cognition, *56*, 215-233.

Pizzagalli, D.A., Evins, A.E., Schetter, E., Frank, M.J., Pajtas, P.E., Santesso, D.L., and Culhune, M. (2008). Single dose of a dopamine agonist impairs reinforcement learning in humans: Behavioral evidence from a laboratory-based measure of reward responsiveness. Psychopharmacology (Berl) *196*, 221-232.

Santesso, D.L., Dillon, D.G., Birk, J.L., Holmes, A.J., Goetz, E., Bogdan, R., Pizzagalli, D.A. (2008). Individual differences in reinforcement learning: Behavioral, electrophysiological, and neuroimaging correlates. Neuroimage *42*:807-816.

van Veen, V., Holroyd, C.B., Cohen, J.D., Stenger, V.A., and Carter, C.S. (2004). Errors without conflict: Implications for performance monitoring theories of anterior cingulate cortex. Brain Cogn *56*, 267-276.

Zirnheld, P.J., Carroll, C.A., Kieffaber, P.D., O'Donnell, B.F., Shekhar, A., Hetrick, W.P. (2004). Haloperidol impairs learning and error-related negativity in humans. J Cogn Neurosci *16*, 1098-112.

Chapter 7

The relation between ERN, NoGo N2, and visual N2b

E.H. Quik, I. van den Bosch, P.V. Nguyen, K.B.E. Böcker, J.L. Kenemans

The relation between ERN, NoGo N2, and visual N2b. E.H. Quik, I. van den Bosch, P.V. Nguyen, K.B.E. Böcker, J.L. Kenemans. International Journal of Psychophysiology. *In revision*

Abstract

Background and Aim

The N2 is a negative event related brain potential (ERP) component arising around 200 milliseconds after an event which may reflect activation of the anterior cingulate cortex (ACC). Several negativities around 200 milliseconds that are observed in different tasks might all be manifestations of a unified N2 component. These include NoGo N2, error related negativity (ERN), both reflecting conflict monitoring, and N2b, that reflects selective processing of multiple stimulus attributes. The objective of this study was to investigate the relation between NoGo N2, ERN, and N2b, in healthy participants. Our hypothesis was that these N2 manifestations are associated with each other and with performance on neuropsychological tests for executive function.

Subjects and Methods

We studied 38 adult participants (mean age 27 years, SD 12, range 17–68 years; 14 females) who performed three different cognitive tests: A visual selective attention task, a continuous performance task (CPT of Go/NoGo type), and an Eriksen flanker task (EFT). Electroencephalogram (EEG) was recorded during these tasks and event-related potentials (N2b, NoGo N2 and ERN, respectively) were derived. In addition, participants performed several neuropsychological tests for executive function (interference control and attentional shifting), long-term memory, and digit span.

Results

Spatial distributions of the NoGo N2, N2b and ERN were highly overlapping. Positive correlations between ERN and N2b amplitudes were observed, whereas the NoGo N2 amplitude showed no significant correlation with N2b or ERN. Higher ERN amplitudes, and to a lesser extent N2b amplitudes, were associated with speeded general processing and short-term memory, but not specifically with interference control or attentional shifting.

Conclusion

ERN and N2b had sizeable overlap in scalp topography, were significantly mutually correlated across subjects, and showed at least partially similar patterns of correlations with measures of general speed and short-term memory. Hence, these two electrocortical responses likely not only reflect the activation of the same neural ensembles in the anterior cingulate cortex, but these activations are also driven by partially the same signals from other brain regions. In contrast, NoGo N2 amplitude did not covary across subjects with either ERN or N2b amplitude, and was far more weakly associated with neuropsychological or other performance measures. Therefore, although NoGo-N2 scalp topography looked similar to that of ERN and N2b, NoGo-N2 activation is probably driven by a different set of brain signals. For neither N2 component we found any association with critical measures of executive function. The latter then, may to a large extent reflect sources of variance other than those associated with anterior-cingulate activation.

Introduction

Event-related brain potentials (ERPs) are widely used to study the brain correlates of cognitive processes. The high temporal resolution of ERPs allows for the separation of stages of information processing. These stages are manifested in several positive and negative components such as the N2. The N2 is a negative ERP component arising between 200 and 350 milliseconds after event onset (Folstein & van Petten, 2008). In the past, the N2 has been studied in many different experimental paradigms and it has been attributed many functional interpretations. First, Pritchard et al. (1991) distinguished between early and late N2 components, the N2a, N2b and N2c components. According to their scheme these were related to pre-attentional mismatch (evoked by unattended rare events), attentional mismatch (evoked by attended non-targets) and classification (evoked by attended targets), respectively. Alternatively, the N2 was thought to reflect the detection of response conflict (Donkers & van Boxtel, 2004), a non-motoric stage of inhibition, the recognition of the need for inhibition (Smith et al., 2008), a late attentional selection phase pertaining to integration of multiple features (Kenemans et al., 2002), or an error-detection (Falkenstein et al., 1991; Gehring et al., 1993). In a further division, Folstein and van Petten argued that the N2 is determined independently by mismatch/novelty and by the amount of cognitive control required by the evoking stimulus, and stated that the anterior cingulate cortex (ACC) contributes to the cognitive control N2-component (Folstein & van Petten, 2008).

The present study focused on three N2 varieties, each of which has been associated with ACC activation: NoGo N2, ERN, and N2b. The NoGo N2 has been argued to reflect conflict monitoring (van Veen & Carter, 2002; Niewenhuis et al., 2003; Bekker et al., 2004 & 2005). The same holds for the Error Related Negativity (ERN) (van Veen & Carter, 2002; Ridderinkhof et al., 2004). The ERN is a frontocentral negative wave peaking around 50-60 milliseconds post response, which is larger for errors (ERN) than for correct behavioral responses (Gehring et al., 1993). The N2b, possibly reflecting a mechanism involved in integrated processing of multiple features, has also been associated with ACC activation (Kenemans et al., 2002).

These N2 manifestations are generally recorded in different paradigms. First, a typical NoGo N2 has been reported from the AX form of a continuous performance task (CPT; Rosvold et al. 1956; Bekker et al., 2004). In this task subjects overtly respond to an 'X' only when it is preceded by an 'A', and must withhold this response when another (NoGo) letter follows 'A', a situation assumed to induce conflict. In case of a relatively high probability of the X following A, NoGo letters elicit a relatively large N2, consistent with the idea of increased conflict in case of a highly improbable NoGo event (Bekker et al., 2004). Accordingly, the ACC has been identified as an important generator of the NoGo N2 manifestation in this task (Bekker et al., 2005). Second, typical ERNs have been reported from Eriksen flanker tasks (EFT; Eriksen & Eriksen, 1974; Gehring et al., 1993;

Ridderinkhof et al., 2002). In such tasks, response-incongruent irrelevant flanker characters surrounding a target character result in a sizable number of choice errors which are associated with reliable ERNs. Third, N2bs are typically recorded and derived in so-called selection-potential paradigms (Lange et al., 1998; Kenemans et al., 2002). In such paradigms two visual features determine whether an overt reaction is needed (e.g., a blue color and a vertical orientation). N2bs are typically elicited when only one of the target features is presented (e.g., blue-horizontal) and the overt response must be withheld. This has been interpreted as N2b reflecting the extent of integrated processing of stimulus features (Böcker et al., 2001; Kenemans et al., 2002), which may in turn involve conflicting information or response tendencies.

In sum, the set of observed N2 manifestations in different tasks has been divided into different sets of N2 components by diverse authors. In the past, the division or unification of N2 manifestations has been based upon differential or communal sensitivity to a specific experimental manipulation, such as mismatch or conflict (for a review see Folstein and van Petten, 2008,). In the present study we tried to determine both the common and the unique interindividual variance in multiple manifestations of N2. In order to clarify their functional nature, the interindividual differences in N2 amplitudes were correlated with the variance in performance on N2 tasks and neuropsychological tests.
We hypothesized that all three N2 manifestations in this study, to a significant degree reflect the cognitive control N2 component originating from the ACC. Therefore they will show strong mutual within-subject correlations. In addition, we expected primary correlations of N2 amplitude with neuropsychological manifestations of cognitive control assessed by the trail making and Stroop color word tasks. Furthermore, secondary correlations were expected with neuropsychological indices of attention, short term memory and general intelligence, in as far as these reflect a variety of cognitive operations. Finally, no appreciable correlations with memory performance on the 15 words test and Rey-Osterrieth complex figure task were expected.

Methods and materials

Participants

Thirty-eight healthy participants were recruited (mean age 27 years, SD 12, range 17–68 years; 14 females). Participants were free of endocrine disorders, neurological disorders, psychiatric disorders, severe cognitive impairments, chronic medication, or medication influencing psychomotor functioning or mental capacity. Furthermore, they did neither use drugs of abuse, nor did they take in more than 3 units of alcohol per day. Participants gave a written informed consent before they participated in the study. Participation took 3 hours per subject, for which they received 18 euro. The study was approved by the local medical ethical committee.

Procedure

All tests were performed in a quiet room under identical circumstances. The standardized neuropsychological tests used comprised WAIS III digit span subtest, 15 words test, Rey-Osterrieth complex figure test, Stroop color-word task, trail making test A&B, and the Dutch adult reading test, as listed in table 2. For detailed descriptions, the reader is referred to Lezak (1995), Aleman et al. (1999) and Quik et al. (2010). After completion of these tests electrodes were applied. Then, participants performed 3 different tasks (see below), each consisting of several pseudo- random stimulus sequences. There were four sequences of the EFT, taking 5 minutes each to complete; two of the CPT, taking 5 minutes each to complete, and eight of the selection-potential task (SPT), each of which took 2.5 minutes to complete. During these tasks EEG was recorded.
During each EEG recording session, participants were seated in a comfortable chair in an electrically and acoustically shielded room. Stimuli were presented on a computer screen positioned in front of the participants at a distance of 100 cm.

Tasks

Eriksen flanker task (EFT)
The ERN manifestation of N2 was recorded in the EFT (Eriksen & Eriksen, 1974; Ridderinkhof et al., 2002). The characters used for targets and flankers were < and > arrowheads. On each trial, a seven-arrowhead-string was presented. The central arrow served as target and indicated the correct response side. Participants were instructed to react as fast as possible (stressing speed over accuracy) with the hand indicated by the target. The remaining arrows served as possibly interfering flankers. On congruent trials, the target

arrow was the same as the flankers (<<<<<<< or >>>>>>>); on incongruent trials, the target arrow differed from the flankers (<<<><<< or >>><>>>). Each string was presented for 500 milliseconds and inter-stimuli intervals varied between 750 and 950 milliseconds. During the whole task a fixation cross remained visible above the target letter. Each block contained 220 stimuli in total in which each stimulus appeared with a frequency of 25%. The task was presented in 4 blocks of 5 minutes each.

Continuous performance task (CPT)
The NoGo N2 was recorded in the CPT following Bekker et al. (2004). Subjects viewed a series of letters (A, B, C, D, E, F, G, H, J, L, X), which were sequentially displayed, and had to respond to a target letter X, if and only if it followed letter A, by pressing a button with the right index finger. Letters were presented for 125 milliseconds with a 1400-1600 milliseconds inter-stimulus interval. Each block contained 40 Cues (A), 100 NoCues (B, C, D, E, F, G, H, J, or L not preceded by a Cue), 20 Go-stimuli (X preceded by a Cue (A)), 20 NoGo-stimuli (B, C, D, E, F, G, H, J, or L preceded by a Cue), and 20 X-only's (X not preceded by an A). To control for frequency differences the letters A, X and H always appeared with a frequency of 20% (Bekker et al., 2004). All remaining letters appeared with a frequency of 5%. The sequence of stimuli within each block was pseudo-randomized with the restriction that no physically identical stimuli ever preceded itself. The CPT consisted of 2 blocks containing 200 trials, taking 5 minutes each.

Selection-potential task (SPT)
The N2b was recorded in the selective attention grating task (SAT; Kenemans et al., 1995, 2002; Lijffijt et al. 2003), also called the selection-potential task (SPT). During the SPT participants viewed sequences of square, square-wave gratings that varied in spatial frequency (SF; 0.6 or 4.8 cycles per degree of visual angle, i.e., wide or narrow bars, respectively) and orientation (O; vertical or horizontal), which amounts to four different stimuli. Stimulus onset asynchrony varied between 750 and 950 milliseconds. Participants had to selectively press a button when perceiving a predefined combination of SF and O (target), but had to ignore the other combinations, and they were told to emphasize speed over accuracy. In separate blocks, each of the four different stimuli was defined as target, and each target had to be responded to with either the left or right hand, thus obtaining eight blocks. Stimuli were mixed randomly within a block of 140 trials (4 x 35). Each block of 140 grating stimuli took 2.5 minutes to complete.

Signal recording and analysis

EEGs were recorded from several scalp sites using an elastic electrode cap (BioSemi) containing 64 Ag-AgCl-electrodes, including Fz (Frontal) and FCz (Frontocentral) midline electrodes at which N2 manifestations normally peak. Eye movements were recorded using

four electrodes positioned around the eyes to measure horizontal and vertical electro-oculograms. Data acquisition was performed using the BioSemi ActiveTwo system, with a sampling rate of 2048 Hz. Signals were filtered with a 30 Hz (24dB/octave) high-pass filter. Ocular artefact correction was carried out using the procedure proposed by Gratton et al. (1983). Trials with other artefacts or AD-converter saturation were removed from further analysis (according to Lijffijt et al. 2003). For N2b baseline corrections were applied on the epoched data with respect to a 100 milliseconds time period before stimulus onset. For the NoGo N2 a baseline between 363 milliseconds and 403 milliseconds after the NoGo stimuli, to correct for overlapping P3 (Bekker et al., 2005) was applied. Response locked ERN was calculated using the area -50 till 0 milliseconds preceding the response for baseline correction.

Data Analysis

The neuropsychological tests scores were calculated according to standard procedures. In addition to the N2, performance was also measured in the experimental tasks. This included mean reaction time (MRT), within-subject standard deviation of the reaction time (SDRT), and proportion errors.
Following previous studies, the present analysis focused on N2s from the electrode sites Fz (Kenemans et al., 1993, Bekker et al., 2004) and FCz (Ridderinkhof et al., 2002). NoGo ERPs were subjected to average referencing (Bekker et al., 2005), and the NoGo N2 was quantified relative to the positive peak (cf. Nieuwenhuis et al., 2003; Bekker et al., 2005). The ERN was estimated at electrode FCz from the difference between response-synchronized ERPs during trials with incorrect choice responses and those during trials with correct responses (according to Ridderinkhof et al., 2002). The N2b was assessed from the selection potentials, which is the ERP difference between stimuli with target spatial frequency and the non-target orientation, and those with non-target features (according to Kenemans et al. (1995) and Lijffijt et al. (2003)). All ERP parameters were calculated using Brain Vision Analyzer® version 1.05.0005 for Windows.

Statistics

Bivariate Pearson correlations between N2 manifestations from all three tasks, behavioral and neuropsychological performance were calculated using a standard version of SPSS® 16.0 for Windows.

Results

Behavioral results

Eriksen flanker task performance

Mean target reaction time for congruent stimuli was 422 milliseconds and for incongruent stimuli 488 milliseconds. The 66 ms slowing in mean RT was highly significant ($t(35) = 24.1$, $p < .0005$). The mean standard deviation of the reaction time for incongruent stimuli was 28 milliseconds, which was significantly higher than for congruent stimuli (23.5, $t(35) = 3.0$, $p = .005$). The percentage correct choice reactions on congruent trials percentage was 96.5, compared to 84.0 on incongruent trials ($t(35) = 12.5$, $p < .0005$).

Continuous performance task performances

Mean target reaction time was 383 milliseconds. Percentage targets (X after A) detected was 97.3 %. False-alarms hardly occurred (A-notX) and were not analyzed.

Selection-potential task performance

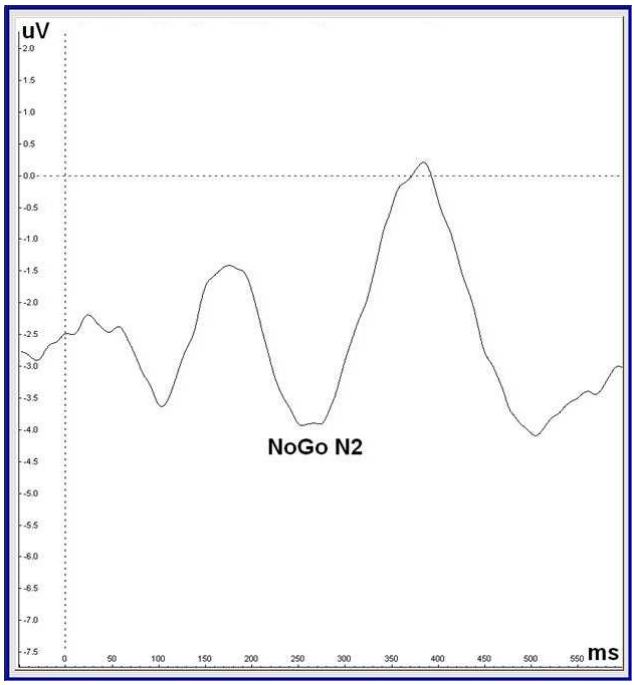
Mean target reaction time for targets was 395 milliseconds. Percentage targets detected was 96.5 %. The average correct rejection-rates were 97.5 % (target spatial frequency), and 99.6 % (for target orientation as well as for no target features); the difference between the first and the latter two NoGo stimuli was significant ($F(1, 35) = 37.7$, $p < .0005$).

N2 Components

Mean amplitudes for the NoGo N2, N2b and ERN are displayed in table 1. Waveforms for the three components are shown in figure 1. Spatial distributions of the NoGo N2, N2b and ERN are depicted in figure 2, which reveals considerable overlap in scalp topography among the three N2 components. Means with standard deviations are listed in table 1.
The positive correlation between N2b and ERN was .340 ($p < .05$; figure 3a), NoGo N2 amplitude showed no significant correlation with either N2b ($r=.059$; n.s.; figure 3b) or ERN ($r=.058$; n.s.; figure 3c).

Table 1 Mean peak amplitudes (μV) for NoGo N2, N2b and ERN

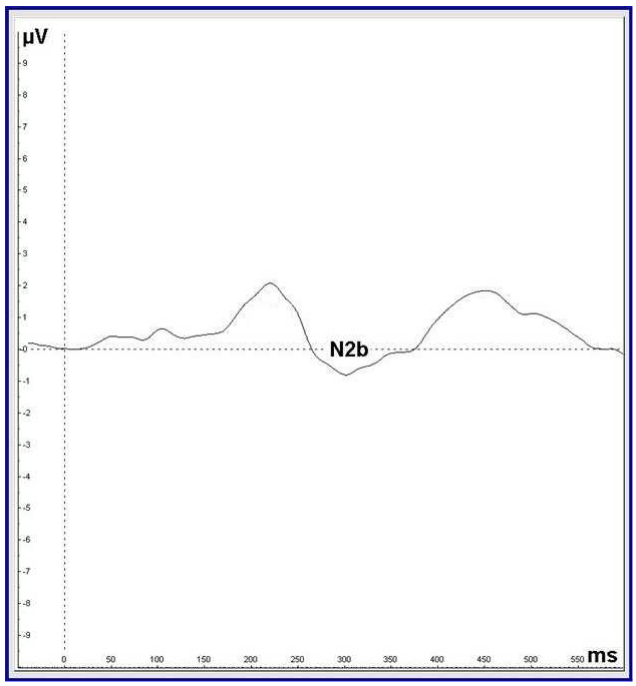
	Mean	Std. Deviation
NoGo N2 @ Fz	-5.42	2.54
N2b @ Fz	-2.18	2.32
ERN @ FCz	-4.23	5.52



a

Figure 1a NoGo ERPs from the CPT

b



c

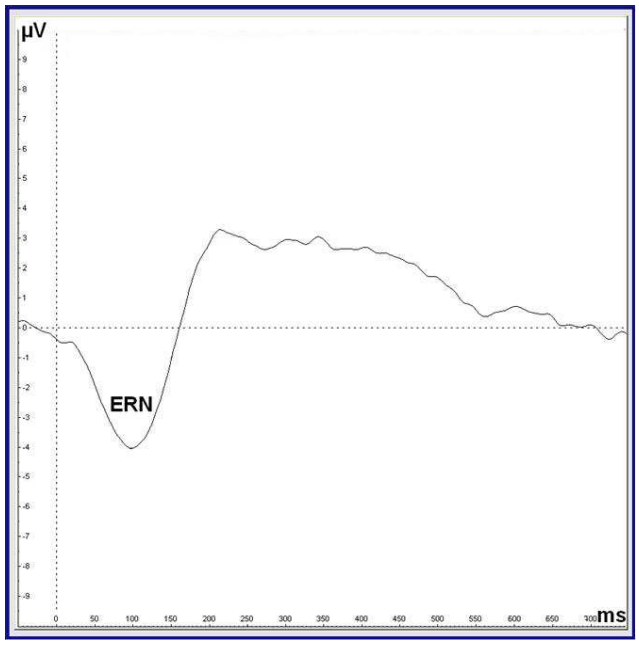


Figure* 1*b Difference waves from the SPT (selection potentials). The ERP to no-target stimuli was subtracted from that to target spatial-frequency stimuli

Figure* 1*c Difference wave (ERN) from the EFT. Time point zero corresponds to reaction time. The ERP on trials with correct responses was subtracted from that on trials with incorrect responses

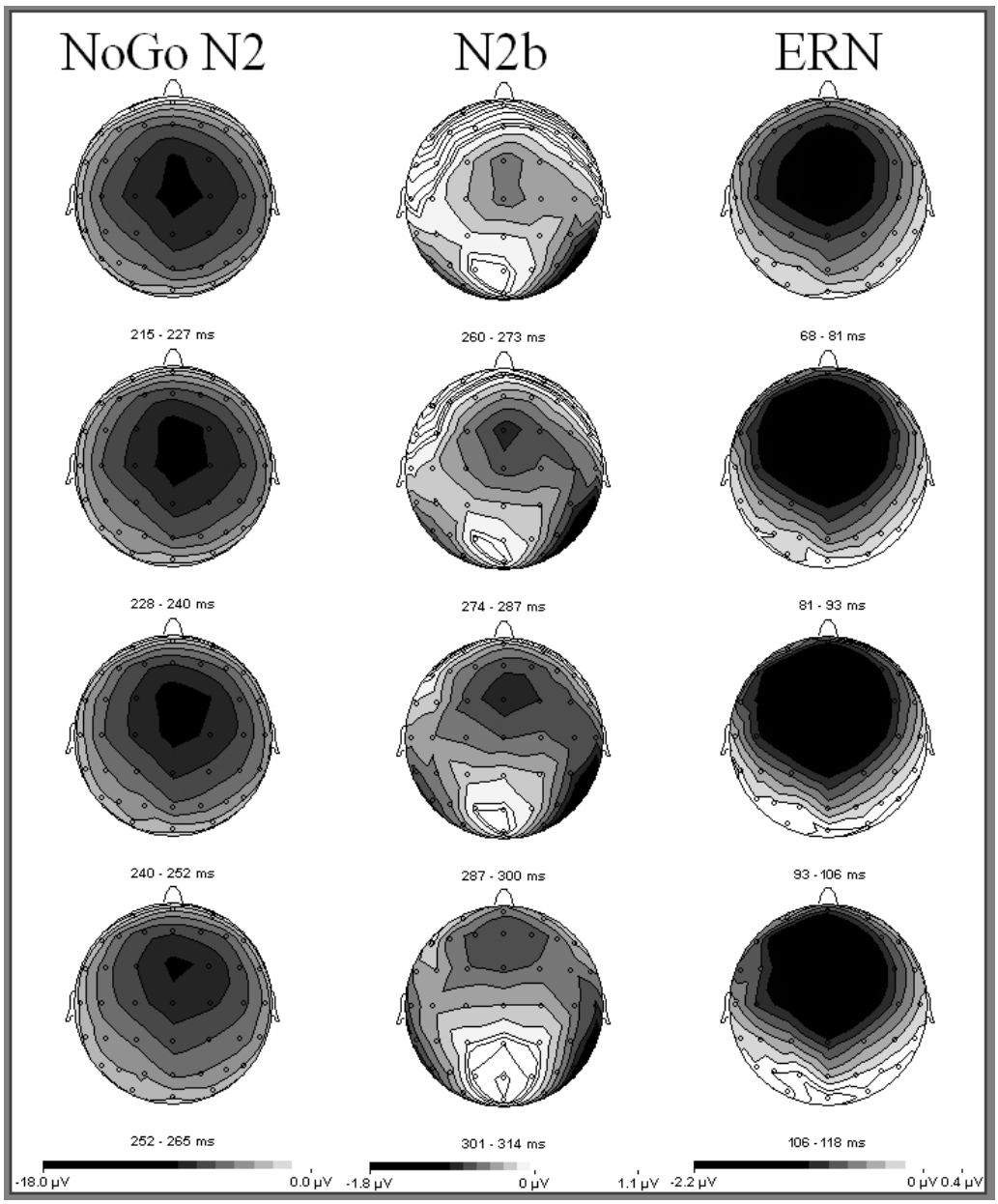


Figure 2 Scalp distributions corresponding to the waveforms in figure 1, at specific latencies

a NoGo N2

b N2b

c ERN

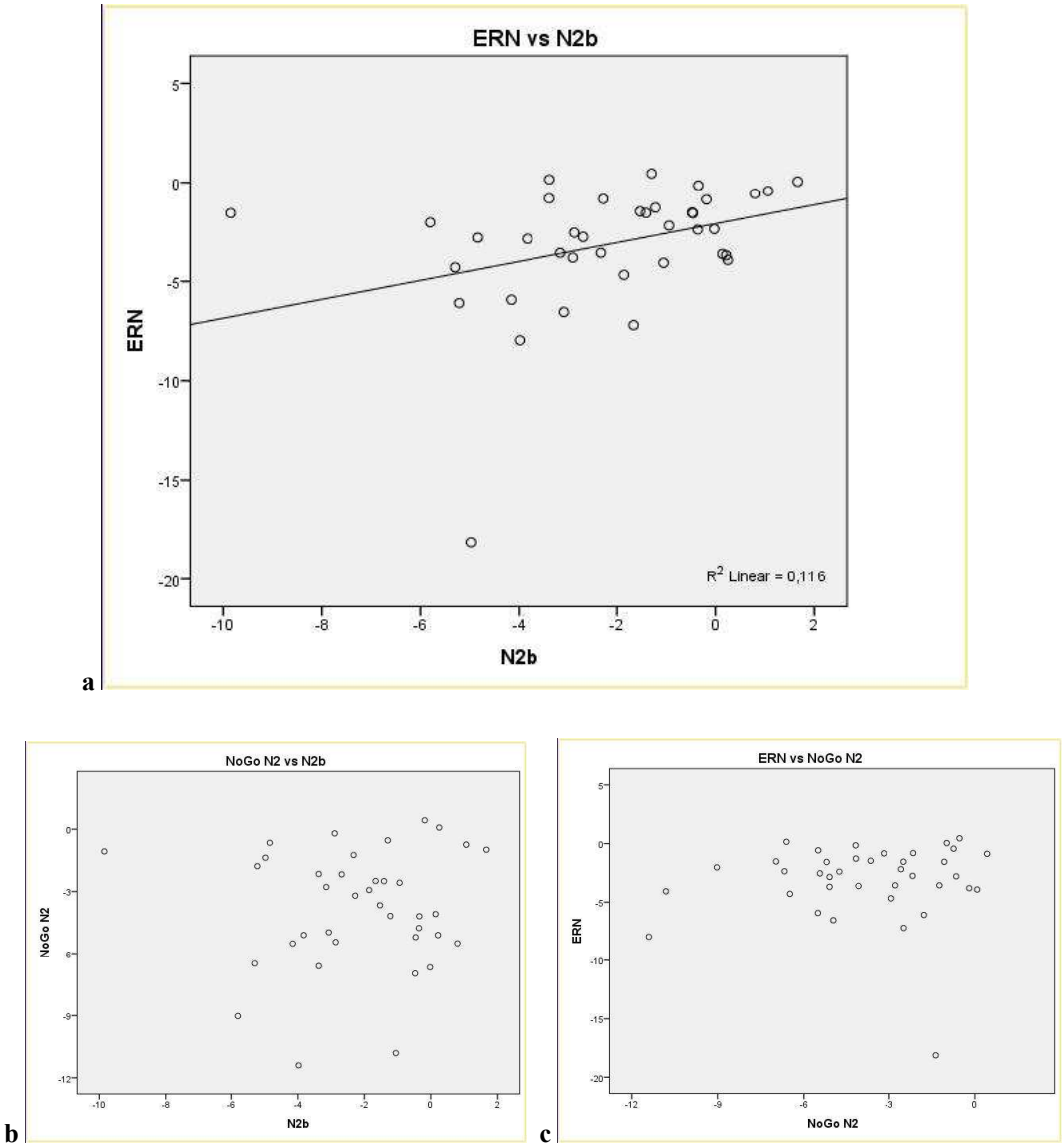


Figure 3 Individual N2 peak correlations, all values are in microvolts

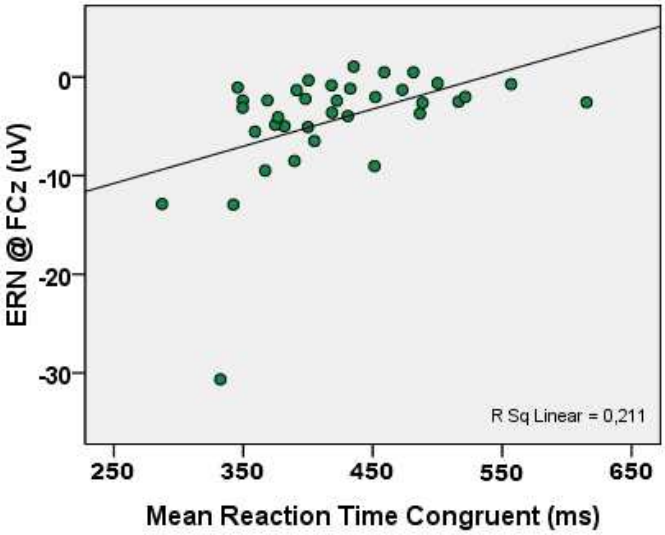
a ERN vs. N2b

b NoGo N2 vs. N2b

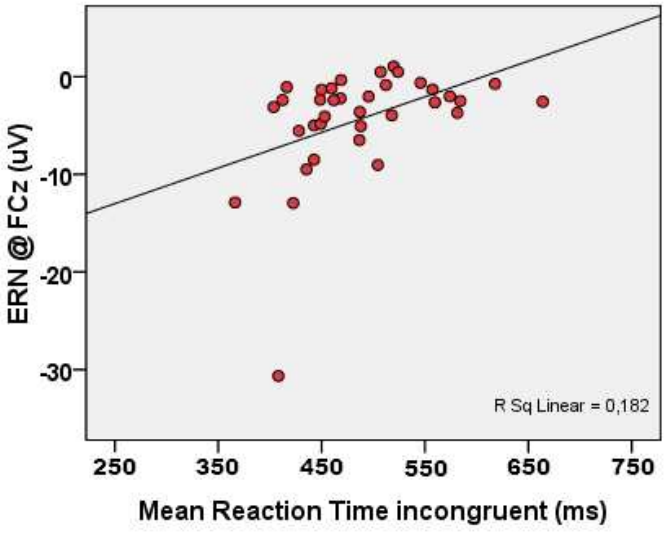
c ERN vs. NoGo N2

ERP -Performance correlations

Larger negative ERNs were associated with shorter reaction times for EFT congruent (r=.527, p=.00; Figure 4a) and EFT incongruent (r=.493, p=.00; Figure 4b) conditions.



a



b

Figure 4 Correlations between ERN and Mean Reaction Time in the EFT
a Congruent
b Incongruent

Faster color naming in the Stroop color condition was associated with more negative ERNs (p=.03; r=.351). Time on trail making B was shorter with larger ERNs (p=.02; r=.377). Span forward (p=.03; r= -.360) correlated negatively with ERN peak, reflecting that participants with larger ERNs recalled more digits in the right order. Immediate verbal memory correlated negatively with ERN peak (p=.01; r= -.450), thus more words were remembered by participants with larger ERN peaks.

NoGo N2 correlated negatively with the intraindividual variability of reaction time (SDRT) during the CPT (r= -.510, p=.00). The only significant neuropsychological correlation for NoGo N2 concerned immediate verbal memory (p=.03, r= -.391; higher scores for larger NoGo N2s). Larger N2bs were associated with faster color naming in the Stroop color condition (p=.03; r=.353) and in the color-word condition.

All correlations between neuropsychological assessment and N2 component peaks are listed in Table 2. In sum, only a few of those correlations were significant. Furthermore, the significance did not concern critical contrast variables for executive control (Stroop color-word minus color or rail making B relative to A), but rather reflections of general processing speed (of color naming) and short-term memory (including immediate verbal memory).

Table 2 Neuropsychological assessment: correlation (p value) with NoGo N2, N2b and ERN peaks

Neuropsychological test	Associated function	ngN2	N2b	ERN
Rey-Osterrieth Complex Figure Test				
Immediate recall score	Immediate visual I memory	.19	.41	.19
Delayed recall score	Delayed visual memory	.33	.21	.93
Delayed / Immediate recall score	Delayed relative to immediate memory	.48	.45	.15
15 Words Test (RAVLT)				
Recall score	Verbal memory	.29	.40	.49
Immediate recall score	Immediate verbal memory	.03	.35	.01
Delayed recall score	Delayed verbal memory	.19	.37	.29
Wechsler Adult Intelligence Scale (WAIS) Digit span				
Span forward	Short-term memory	.96	.18	.03
Span backward	Short-term & working memory	.51	.54	.10
Trail Making Task (TMT)				
Trail Making Task A	Processing speed	.76	.68	.23
Trail Making Task B	Processing speed plus attentional shifting	.76	.12	.02
TMT B minus A	Attentional shifting	.50	.17	.15
TMT B / A	Attentional shifting	.56	.40	.48
Stroop Color Word Task				
Word	Word reading	.96	.06	.13
Color	Color naming	.79	.03	.03
Color & word	Color naming with incongruent words	.89	.03	.07
Color & word minus Color	Interference control	.68	.09	.20
Color / Color & Word	Interference control	.54	.25	.39
Dutch National Adult Reading Test				
Word reading	Estimate of verbal intelligence	.47	.75	.46

Discussion

The N2 is a negative event related brain potential (ERP) component arising around 200 milliseconds after stimulus onset, which may reflect functioning of the anterior cingulate cortex (ACC). The present study used three different paradigms, specifically a CPT, an Eriksen flankers task (EFT) and a selection-potential task (SPT), to investigate the interrelation between the N2 varieties NoGo N2, ERN, and N2b. All three N2 manifestations are thought to originate in the ACC and therefore may be related.

N2b and ERN amplitudes were positively correlated to a modest degree (~11.6% common intersubject variance) and both were unrelated to the NoGo N2. This may lead to the conclusion that the ERN and N2b reflect similar processes implemented in the ACC (van Veen & Carter, 2002; Niewenhuis et al., 2003; Bekker et al., 2004 & 2005, Kenemans et al., 2002), such as monitoring of stimulus classification, action and error. Apart from this correlation, the NoGo N2, the N2b and ERN show large overlap in spatial distribution (Figure 2). The relatively modest correlation suggests that there is either a small direct contribution of ACC or indirect ACC modulation from probably non-overlapping brain areas. Stated differently, ERN and N2b may well reflect activation of the same ensemble of neurons, but this activation is driven by neural signals from other brain regions that are at least partly different for ERN and N2b, respectively. This difference is even more pronounced for the NoGo N2, relative to the other two, although there was also quite some overlap in scalp topography between NoGo N2 on the one hand, and ERN and N2b on the other.

An alternative interpretation of the finding of at most modest correlations between N2 manifestations might be that this measure is unreliable. This argument is mitigated by the finding that reliable group differences have been reported for ERN in OCD (Gehring et al., 2000; Nieuwenhuis et al., 2005) and N2b in growth hormone deficiency (Lijffijt et al., 2003). Furthermore, the NoGo N2 might be different from the other difference waves in that a relatively stronger posterior N2 is expected in this paradigm. Note (figure 2) that the distribution of the NoGo N2 is somewhat more biased towards posterior scalp areas, relative to the distributions of N2b and ERN.

The outcome of the analysis of the behavioral data suggests that the experimental tasks were validly implemented. For the SPT, the percentage of correct rejections was higher when the non-target was more distinct from the target. This is in line with previous studies using the SPT (Kenemans et al., 1993; 2002). In the EFT, mean reaction time for congruent stimuli was shorter than for incongruent stimuli and fewer errors were made for congruent trials, in line with previous studies using the EFT (Ridderinkhof et al., 2004).

If N2 components are related to cognitive control or executive function, then correlations between these components and neuropsychological indices of specifically executive-function may be expected. Faster color naming in the Stroop color condition was associated with more negative ERNs, as was shorter time on trail making B. In addition, larger N2bs were associated with faster color naming in the Stroop color condition as well as in the

color-word condition. However, neither for ERN, nor for N2b, were significant correlations observed with the contrast variables that critically index executive function (color naming in color-word relative to that in color-only for interference control; trail-making B relative to trail making A performance). It seems then that the possible involvement of the neural signals embodied in ERN and N2b in processes of interference control or attentional shifting is at best very modest and perhaps masked by a host of other factors that codetermine the behavioral manifestations of these functions. This holds all the more for the NoGo N2, which did not correlate at all with Stroop or trail making parameters.

These ERN and N2b correlations, along with those between ERN amplitude and mean reaction time to congruent and incongruent flanker stimuli, point to a more general association with basic processing speed. In addition, and further attesting to the non-specificity of N2-performance associations, ERN and NoGo N2 correlated with enhanced immediate-verbal memory, and ERN with digit-span performance. In all, ERP-performance correlations were overall weak and not specific for indices of cognitive control.

In sum, the ERN and N2b manifestations of the N2 may reflect similar activity from the ACC, which are partly driven by the same neural signals from other brain regions. There is no specific association between these manifestations of ACC activity and behavioral measures of executive function. The NoGo N2 both directly and indirectly reflects processes that are different from those that manifest in ERN and N2b.

References

Aleman, A., de Vries, W.R., de Haan, E.H.F., Verhaar, H.J.J., Samson, M.M., Koppeschaar H.P.F. (2000). Age-sensitive cognitive function, growth hormone and insulin- like growth factor 1 plasma levels in healthy older men. Neuropsychobiology 41(2), 73-78.

Assema, P. van, Brug, J., Ronda, G., Steenhuis, I. (2001). The relative validity of a short Dutch questionnaire as a means to categorize adults and adolescents to total and saturated fat intake. Journal of Human Nutrition and Dietetics 14, 377-390.

Bekker, E.M., Kenemans, J.L., Verbaten, M.N. (2004). Electrophysiological correlates of attention, inhibition, sensitivity and bias in a continuous performance task. Clinical Neurophysiology 115, 2001-2013.

Bekker, E.M., Böcker, K.B., Van Hunsel, F., van den Berg, M.C., Kenemans, J.L. (2005). Acute effects of nicotine on attention and response inhibition. Pharmacol Biochem Behav. 82(3), 539-48.

Bekker, E. M., Kenemans, J. L., & Verbaten, M. N. (2005). Source analysis of the N2 in a cued Go/NoGo task. Cognitive Brain Research, 22, 221-231.

Böcker, K. B. E., Baas, J. M. P., Kenemans, J. L., Verbaten, M. N., & Huizenga, H. M. (2001). Electrophysiological manifestations of fear-induced selective attention. Biomedizinische Technik, 46, 239-241.

Bruin, K.J., Wijers, A.A. (2002). Inhibition, response mode, and stimulus probability: a comparative event-related potential study, Clinical Neurophysiology 113, 1172–1182.

Donkers, F.C.L., van, Boxtel, G.J. (2004). The N2 in Go/No-Go tasks reflects conflict monitoring not response inhibition, Brain and Cognition 56, 165–176.

Eriksen, B.A. & Eriksen, C.W. (1974). Effects of noise letters upon the identification of target letters in visual search. Perception and Psychophysics 16, 143-149.

Falkenstein, M., Hohnsbein, J., Hoormann, J., & Blanke, L. (1991). Effects of crossmodal divided attention on late ERP components. II. Error processing in choice reaction tasks. Electroencephalography & Clinical Neurophysiology, 78, 447–455.

Folstein, J. R. & Van Petten, C. (2008). Influence of cognitive control and mismatch on the N2 component of the ERP: A review. Psychophysiology, 45, 152-170.

Gehring, W. J., Gratton, G., Coles, M. G. H., & Donchin, E. (1992). Probability effects on stimulus evaluation and response processes. Journal of Experimental Psychology: Human Perception & Performance, 18, 198-216.

Gehring, W. J., Goss, B., Coles, M. G., & Meyer, D. E. (1993). A neural system for error detection and compensation. Psychological Science, 4, 385-390.

Gehring, W. J., Himle, J., & Nisenson, L. G. (2000). Action monitoring dysfunction in obsessive-compulsive disorder. Psychological Science, 11, 1-6.

Holroyd, C. B., & Coles, M. G. (2002). The neural basis of human error processing: reinforcement learning, dopamine, and the error-related negativity. Psychological Review, 109, 679-709.

L.M. Jonkman (2006). The development of preparation, conflict monitoring and inhibition from early childhood to young adulthood; a Go/NoGo ERP study, Brain Research 1097, 181–193.

Kenemans, J.L., Kok, A., Smulders, F.T.Y., (1993). Event-related potentials to conjunctions of spatial frequency and orientation as a function of stimulus parameters and response requirements. Electroenceph. clin. Neurophysiol. 88, 51-63.

Kenemans, J.L., Smulders, F.T., Kok, A., (1995). Selective processing of two-dimensional visual stimuli in young and old subjects: electrophysiological analysis. Psychophysiology 32, 108-120.

Kenemans, J.L., Lijffijt, M., Camfferman, G., Verbaten, M.N., (2002). Splitsecond sequential selective activation in human secondary visual cortex. J. Cogn. Neurosci. 14, 48-61.

Kenemans, J. L., Jong, T. G., & Verbaten, M. N. (2003). Detection of visual change: mismatch or rareness? Neuroreport, 14, 1239-1242.

Kirmizi-Alsan, E., Bayraktaroglu, Z., Gurvit, H., Keskin, Y.H., Emre, M., Demiralp, T. (2006). Comparative analysis of event-related potentials during Go/NoGo and CPT: decomposition of electrophysiological markers of response inhibition and sustained attention, Brain Research 1104, 114–128.

Lange, J. J., Wijers, A. A., Mulder, L. J., & Mulder, G. (1998). Color selection and location selection in ERPs: differences, similarities and 'neural specificity'. Biological Psychology, 48, 153-182.

Lezak, M.D. (1995). Neuropsychological Assessment. Third ed. Oxford University Press, New York, NY.

Lijffijt, M., Van Dam, P.S., Kenemans, J.L., Koppeschaar, H.P., de Vries, W.R., Drent, M.L., Wittenberg, A., Kemner, C., (2003). Somatotropic-axis deficiency affects brain substrates of selective attention in childhood-onset growth hormone deficient patients. Neurosci. Lett. 353, 123-126.

Nieuwenhuis, S., Yeung, N., van den Wildenberg, W., & Ridderinkhof, K. R. (2003). Electrophysiological correlates of anterior cingulate function in a go/no-go task: effects of response conflict and trial type frequency. Cognitive, Affective & Behavioral Neuroscience, 3, 17-26.

Nieuwenhuis, S., Slagter, H.A., von Geusau, A., Heslenfeld, D.J., and Holroyd, C.B. (2005). Knowing good from bad: Differential activation of human cortical areas by positive and negative outcomes. Eur J Neurosci 21, 3161-3168.

Pritchard, W.S., Shappell, S.A., Brandt, M.E. (1991). Psychophysiology of N200/N400: a review and classification scheme. Adv Psychophysiol 4, 43–106.

Quik, E.H., van Dam, P.S., Kenemans, J.L. Growth hormone and selective attention: A review. Neurosci. Biobehav. Rev. 34 (2010) 1137–1143.

Quik, E.H., Conemans, E.B., Valk, G.D., Kenemans, J.L., Koppeschaar, H.P.F., & van Dam., P.S. (2012). Cognitive performance in older males is associated with growth hormone secretion. Neurobiology of Aging 34(8), 1137-43.

Ridderinkhof, K.R., Vlugt, Y. de, Bramlage, A., Spaan, M., Elton, M., Snel, J., Band, G.P.H. (2002). Alcohol consumption impairs detection of performance errors in mediofrontal cortex. Science 298, 2209 - 2211

Ridderinkhof, K. R., Ullsperger, M., Crone, E. A., & Nieuwenhuis, S. (2004). The role of the medial frontal cortex in cognitive control. Science, 306, 443-447.

Rosvold, H. E., Mirsky, A. F., Sarason, I., Bransome, Jr., E. D., Beck, L. H. (1956). A Continuous Performance Test of Brain Damage. Journal of Consulting Psychology, 20, 343-350.

Smith, J.L., Johnstone, S.J., Barry, R.J. (2008). Movement-related potentials in theGo/NoGo task: the P3 reflects both cognitive and motor inhibition, Clinical Neurophysiology, 119, 704–714.

van Veen, V., & Carter, C. S. (2002). The timing of action-monitoring processes in the anterior cingulate cortex. Journal of Cognitive Neuroscience, 14, 593-602.

Chapter 8

Acute effects of growth hormone on cognitive functioning and related brain function in healthy elderly men

E.H. Quik, I. van den Bosch, P.V. Nguyen, A.C. Sauër, I. Maitimu-Smeele, K.B.E. Böcker, P. S. van Dam, J.L. Kenemans

Acute effects of growth hormone on cognitive functioning and related brain function in healthy elderly men. E.H. Quik, I. van den Bosch, P.V. Nguyen, A.C. Sauër, I. Maitimu-Smeele, K.B.E. Böcker, P. S. van Dam, J.L. Kenemans. Neurobiology of Aging. *With editor*

Summary

Growth hormone (GH) is synthesized within the anterior pituitary gland. Its secretion is regulated by GH releasing hormone (GHRH), which excites GH synthesis and release. It is well known that the decline in the GH/insulin-like growth factor-1 (GH/IGF-1 or somatotropic) axis activity is age-related and may result in a decline in cognitive capacity. All available studies have focused on long-term effects of GH and IGF-1 on cognitive performance, but no data on immediate effects of a GH peak on cognition exist. Here, we evaluated whether a short-term rise in plasma GH following GHRH administration has positive effects on cognitive performance and brain function. We specifically focused on putative Event-Related brain Potential (ERP) indices of anterior-cingulate cortex (ACC) function.

Sixteen healthy males between 60 and 70 years old (mean age: 67 years) participated in this double-blind randomized placebo-controlled crossover intervention study. Following GHRH or placebo administration, cognitive performance (continuous performance, Eriksen flanker and selection-potential tasks) and GH secretion were assessed, while EEG was recorded.

A significant difference between GHRH and placebo was observed for the error-related negativity during the Eriksen Flanker test, with the negativity being more pronounced after GHRH. For N2b and NoGo N2 no significant differences were observed. Performance did not differ between the GHRH and the placebo condition. These findings are consistent with differentiated GH effects on ACC function, possibly thorough interactions with the dopaminergic system.

Introduction

Several studies have shown that decreased activity of the somatotropic or GH/IGF-1-axis is associated with decreased cognitive performance (van Dam & Aleman, 2004; Arwert et al., 2005; Quik et al., 2010/ chapter 2) particularly in old age. Correlations have been observed between the activity of the somatotropic axis and visual and verbal memory, processing and motor speed, cognitive performance, and executive functioning in elderly subjects. Similar associations between somatotropic axis activity and cognitive performance have been observed in GH deficient patients (Falleti et al., 2006). Such data are consistent with the observation that receptors for GH and IGF-1 are found in different regions of the central nervous system, in particular in areas which play a role in learning and memory (Adem et al., 1989; Zhai et al., 1994; Nyberg et al., 1996; van Dam et al., 2000; Hua et al., 2009). In a recent review we suggested that GH/IGF-1 variation may particularly affect functions of the anterior cingulate cortex (ACC; see also Lijffijt et al., 2003). There is also evidence that aging, which leads to reduced GH/IGF-1 axis activity, is accompanied by a functional impairment of the ACC (West & Moore, 2005). Here we present a more detailed view on the relation between somatotropic function and cognition-related ACC activation.

All studies evaluating the relation between somatotropic axis activity and cognition thus far have addressed the effects of long-term GH deficiency or suppletion. Effects of long-term deficiency may reflect two underlying factors: abnormal development due to chronically low GH levels, or the acute lack of stimulation of GH receptors. The present work focused on the latter possibility, by assessing the acute effects of GH.

For this study, we were especially interested in cognitive processing based in the anterior cingulate cortex (ACC) in elderly men. The available data regarding the contribution of the GH/IGF-1 axis to this system (Quik et al., 2010) are consistent with the above mentioned effects on general learning and memory, as one of the functional-anatomical characteristics of the ACC is its strong connection with the hippocampus. Here we extend the scope by assessing GH effects on different event-related potential (ERP) manifestations of information processing in the ACC. These include the so-called 'N2b' which has previously been revealed to be sensitive to GH-levels (Lijffijt et al., 2003); the 'error-related negativity' (ERN, Ridderinkhof et al., 1995); and the 'NoGo N2' (Bekker et al., 2004).

The N2b is typically observed in so-called 'selection-potential' tasks (SPT). The SPT yields electrocortical measures of selective visual processing. The main measure of interest here is the N2b, which has been suggested to reflect ACC-based (selective) integrated processing of multiple visual dimensions (Kenemans et al., 2002), and found to be sensitive to childhood onset GH deficiency (Lijffijt et al., 2003). Typically, N2b is preceded by frontal selection positivity (FSP) and followed by P300, both electrocortical reflections of other aspects of selective processing. Second, we administered a prototypical high-conflict, high error-prone task: the Eriksen flanker task. Errors in this task are typically accompanied by the error-related negativity (ERN) which is assumed to reflect brain activity caused by

transient dips in dopamine release. The ERN is a frontocentral negative wave, which is larger for errors (ERN) than for correct responses (Gehring et al., 1993).The underlying dopamine drops are thought to be a prerequisite for error processing and conflict monitoring by the ACC (Holroyd and Coles, 2002). Finally, the ERPs recorded during continuous-performance tasks typically reveal a NoGo N2 component specifically on no-go trials interspersed within a set of go-trials. The NoGo N2 has been shown to reflect processing of conflict (between the tendency for overt responding and the task demand to refrain from responding), and to be generated in the (vicinity of) the ACC (Bekker et al., 2004; 2005).
The aim of the present study was twofold. First, it was assessed whether the N2b that previously proved to be smaller in a GH-deficiency group, would also be sensitive to an acute GH manipulation. Secondly, the generalizability of the relation between GH and N2b to other electrophysiological measures of ACC activation was probed.

Materials and methods

Subjects

Sixteen healthy males (mean age 67, range 62-70 years; mean body mass index 26.5, range 22.0-32.3 kg/m^2) were recruited for this study. Subjects were recruited via media, advertisements in newspapers in and around Utrecht, and the internet. Exclusion criteria were a history of malignancy, pituitary or hypothalamic disease, or any history of other neurological disorders. Other reasons for exclusion were the incapacity to live independently due to severe cognitive deficits, bad vision not correctable by glasses, an alcohol consumption of more than 3 units per day, or a history of drug use (such as cocaine or cannabis) or the use of chronic medication that could affect cognitive function such as antidepressants and benzodiazepines. Subjects received a detailed information letter and gave informed consent before participating. The study was approved by the local medical ethical committee and was conducted in compliance with relevant laws and institutional guidelines.

Study design

The study was set up as a double-blind randomized placebo-controlled crossover intervention study. Subjects were tested twice, with exactly one week in between test days. Both tests took place between 8 and 12 A.M. Subjects were asked to refrain from any food or drink intake (except for water) or strenuous activity in the 12 hours before each

experimental day. On both test days, subjects had to perform a number of cognitive tests while EEG was recorded (see below). GHRH (100 μg GHRH; Ferring Pharmaceuticals Ltd., Hoofddorp, The Netherlands) or placebo (NaCl 0.9%) was administered through an intravenous catheter placed in the left forearm before performing the cognitive tasks. Blood samples to assess GH levels were drawn at baseline, before GHRH or placebo administration, and at 30, 60, 90, 120, and 150 minutes after GHRH administration. During each EEG recording session, subjects were comfortably seated in an acoustically shielded room. Stimuli were presented on a computer screen positioned in front of the subjects at a distance of 100cm.

Procedure and tasks

After participants signed informed consent electrodes were applied. At 9AM the intravenous catheter was placed, blood was drawn and GHRH or placebo was administered (time point 0). In the first 30 minute period, short practice blocks of the tasks were presented. In the 30-90 minute period, all three cognitive tasks, (CPT, the EFT, and SPT) were performed while EEG was recorded. The cognitive tasks were presented in a balanced order. After the last blood sampling the intravenous catheter was removed. Height, weight and circumference of the waist were assessed at the first visit.
The second test day was similar to the first. GHRH or placebo was administered in a pseudo-random order according to a balanced cross-over design.

Cognitive tasks

Continuous Performance Task (CPT)
The CPT consisted of three blocks containing 200 stimuli with a duration of 5 minutes each. Using Presentation software (Neurobehavioral Systems, Inc., Albany, USA), black capital letters (A, B, C, D, E, F, G, H, J, L, X) were presented between two continuously present vertical bars in the middle of the computer screen against a gray background. Each letter appeared for 125 ms and inter-stimulus intervals varied between 1400 and 1600 ms. Subjects were instructed to press the right button with their right index finger each time the letter X followed the letter A and to refrain from pressing a button on every other stimulus. Speed and accuracy were both stressed. Each block contained 40 Cues (A), 100 NoCues (B, C, D, E, F, G, H, J, or L not preceded by a Cue), 20 Go-stimuli (X preceded by a Cue (A)), 20 NoGo-stimuli (B, C, D, E, F, G, H, J, or L preceded by a Cue), and 20 X only's (X not preceded by an A). To control for frequency differences, the letters A, X and H always appeared with a frequency of 20%. All remaining letters appeared with a frequency of 5%. Furthermore, the probability that a Go-stimulus succeeded a Cue varied between 50-65% across blocks. Subjects first practiced a block containing 20 stimuli. Subsequently, three of

eight experimental blocks were presented in random order to participants in such manner that six different blocks (3 times 2 days) were presented to one participant, but averaged across subjects, all blocks were presented equally often in the two drug conditions. The sequence of stimuli within each block was pseudo-randomized with the restrictions that no physically identical stimuli were repeated and that no more than two Go-sequences (A-X) or NoGo-sequences (A-notX) were presented in succession (Bekker et al., 2004).

Eriksen Flanker Task (EFT)

The EFT (Eriksen & Eriksen, 1974, Ridderinkhof et al., 1995) consisted of four blocks with a 5 minute duration for each block and 220 stimuli within each block. Using Presentation software four possible strings of 7 white arrows were presented in the middle of the computer screen against a black background. Stimulus duration was 100 ms, and Inter-stimuli intervals varied between 750 and 950 ms. Subjects were instructed to respond as fast as possible to the central arrow of the presented string while ignoring the surrounding arrows. They had to respond by pressing a button on their right with their right index finger when the central arrow pointed to the right and by pressing a button on their left with their left index finger when the central arrow pointed to the left.

Each block contained 110 congruent stimuli (55 stimuli with all arrows pointing to the right: >>>>>>> and 55 stimuli with all arrows pointing to the left: <<<<<<<) and 110 incongruent stimuli (55 stimuli with the central arrow to the right and surrounding arrows to the left: <<<>><<< and 55 stimuli with the central arrow to the left and surrounding arrows to the right: >>><>>>). Each stimulus appeared with a frequency of 25%. Responding to congruent stimuli is relatively easy as all visual information primes the correct response. Therefore, these responses are typically faster than responses to incongruent stimuli. Responding to incongruent stimuli is more difficult resulting in slower reaction times and more errors on these trials, because the flanker information interferes with the processing of the central target.

Before administration of GHRH or placebo, subjects received a practice block. After administration, another three out of the remaining seven blocks were presented in a random order to fill 15 minutes of measurements during the GH peak. The sequence of stimuli within each block was pseudo-randomized with the constraints that they occurred with equal probabilities, and that no more than four physically identical stimuli were presented in succession.

Selection Potential Task (SPT)

The SPT consisted of four blocks with a duration of 2.5 minutes for each block and 140 stimuli within each block. Stimuli consisted of square, black-white, square-wave gratings which differed in their fundamental spatial frequency (0.6 and 4.8 or 0.6 and 2.4 c/d) and orientation (horizontal and vertical), resulting in four different feature combinations (Figure 1).

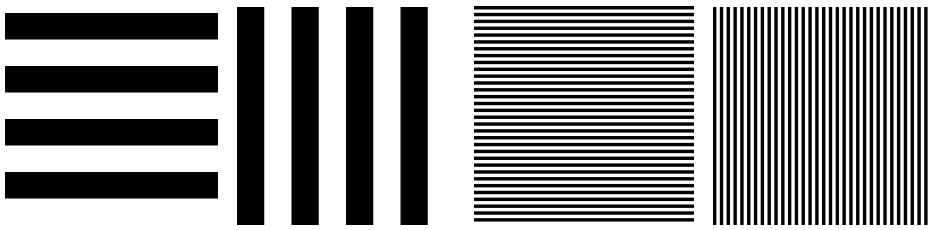


Figure 1 The stimuli used in the Selective Attention Task (SAT) low and high frequency orientated horizontally and vertically

Using Presentation software (Neurobehavioral Systems, Inc., Albany, USA), these stimuli were presented in the middle of the computer screen against a gray background for 50 ms, and inter-stimuli intervals varied between 750 and 950 ms. Subjects were instructed before each block to respond to one particular feature combination (target) by pressing either the right button with their right index finger or the left button with their left index finger. Thus one combination was the target, and non-targets shared with the target either spatial frequency ('spatial-frequency relevant'), or orientation ('orientation relevant'), or neither ('irrelevant'). Both speed and accuracy were stressed. Subjects first received a practice block with 12 stimuli. Subsequently they received four out of eight possible blocks. The eight possible blocks consisted of the eight combinations of the particular feature combinations (targets) and the response hand (right or left). Of these four blocks, two were to be performed with the right hand and two with the left hand. On their second visit, subjects were presented the four remaining blocks so that, on the whole, all possible target-response hand combinations were presented to each subject.

EEG Recording and Preprocessing

The EEG was recorded using 64 Ag-AgCl-electrodes mounted in an elastic electrode cap (BioSemi) according to the International 10-10 system, and referenced to a pair of active electrodes (Common Mode Sense and Driven Right Leg). The vertical electro-oculograms (EOG) was recorded bipolarly from electrodes placed above and below the left eye. The horizontal EOG was recorded also bipolarly from electrodes lateral to both eyes. Impedances of all electrodes were kept below 10 kΩ. The EOG and EEG signals were sampled at a rate of 2048 Hz. Signals were filtered off-line with a 30 Hz (24dB/octave) low-pass filter. Ocular artefact correction was carried out using the procedure proposed by Gratton et al. (1983). Trials with other artefacts or AD-converter saturation were removed from further analysis (according to Lijffijt et al. 2003). ERPs were derived using Brain Vision Analyzer® version 1.05.0005 for Windows.

Data Analysis

Continuous Performance Task

Correct button presses to X following an A were used to calculate mean reaction time (MRT). The EEG data was segmented off-line time-locked to stimulus onset, starting 100 ms before and ending 900 ms after stimulus onset for the NoGo-stimulus, similar to Bekker et al. (2004). ERPs were averaged over all stimuli from the same category to derive individual ERPs and over subjects to derive grand-average ERPs. The N2 was measured between 200ms and 350ms after stimulus onset at electrode Fz (Bekker et al., 2004). Following Bekker et al. 2005, we separated the NoGo N2 from surrounding peaks by using the positive peak succeeding the N2 (390 ms to 430 ms) after stimulus onset as baseline and re-referencing the data to average reference (Bekker et al., 2005).

Eriksen Flanker Task

Correct button presses were used to calculate mean reaction time (MRT). All responses with reaction times faster than 150 ms were seen as incorrect (i.e., pressing at chance) and were removed from the data sets for ERP analysis. ERNs were analyzed according to Ridderinkhof et al. (2002). The EEG data were segmented off-line time-locked to response onset for correct and incorrect responses to congruent and incongruent stimuli, starting 50 ms before and ending 1050 ms after response onset. Response-locked ERPs were computed for all response types (congruent/incongruent, correct/incorrect) at electrode FCz (Ridderinkhof, 2002). The resulting ERPs were averaged over blocks to derive individual ERPs and subsequently over subjects to derive grand-average ERPs. The ERN was derived by subtracting the ERPs to correct responses from the ERPs to incorrect responses (ERN). ERN amplitude was subsequently defined as the most negative peak in the 50 to 200 ms time window, time-locked to response onset. The area -50 ms to 0 ms before the response served as baseline.

Selection Potential Task

Correct button presses to the target were used to calculate mean reaction time (MRT). All responses with reaction times faster than 150 ms were seen as incorrect; i.e. pressing at chance. ERPs were analyzed in line with Quik et al. (2012/ chapter 3), using only trials with correct responses. The EEG data for the four different stimuli were segmented off-line time-locked to stimulus onset, starting 100 ms before and ending 900 ms after stimulus onset. The 100 ms prior to stimulus onset served as baseline. Selection potentials were derived by taking the ERPs to the non-target sharing spatial frequency with the target, and the ERPs to the irrelevant non-target (sharing neither spatial frequency nor orientation with the target), averaging these ERPs separately across blocks, and then subtracting the latter from the former. FSP was defined as the most positive peak in the 100-250 ms time-window and N2b as the most negative peak in the 200-400 ms time-window after stimulus onset at electrode Fz. P3 was quantified as the most positive peak between 400 and 550

ms-latency at the Fz electrode. For Pz P3, the difference ERP target minus orientation-relevant at the Pz electrode was determined in the 400-550 ms window.

Hormone assessments

GH was measured using an immunometric technique on an IMMULITE 1000 Analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, USA). The lower limit of detection was 0.12 mU/L and inter-assay variation was 10; 5 and 5% at 0.33; 2.2 and 24 mU/L respectively (n = 130).1 ng/mL = 2.4 mU/L (WHO International Ref. Prep NIBSC 2nd 98/574). GH response was measured by assessment of the highest plasma level during the test (GH peak).

Statistics

Individual averages of the behavioral data and ERP amplitudes were entered in paired-sample t tests or in a repeated measures General Linear Model (GLM) with order (first placebo vs. GHRH) as between-subject factor and Drug as within subject factor. All analyses were done using a standard version of SPSS® 16.0 for Windows.

Results

Growth Hormone peak differences between drug conditions

GH peak values after administration of GHRH or placebo are shown in table 1. After GHRH, the GH peak was significantly elevated compared to placebo ($F=20.89$, $p<.001$).

Table 1

Minima, maxima, mean and standard deviation across subjects for the two treatment conditions

	Minimum	Maximum	Mean	Std. Deviation
GH peak µg/L after GHRH	0.25	7.08	2.58	2.25
GH peak µg/L after placebo	0.01	2.42	0.29	0.64

Behavioral differences between drug conditions

Table 2 summarizes the behavioral data. No significant behavioral differences between GHRH and placebo conditions were found.
Additionally, in the selection-potential task, target-detection rates were 92.90% for GHRH, and 94.11% for placebo. For GHRH, the percentage correct rejections of the spatial frequency relevant non-targets was 93.08%, for the orientation relevant non-targets 97.86%, and for the irrelevant non-targets 98.08%. For placebo these values were 93.48%, 98.39%, and 98.48%. These figures indicate that spatial-frequency was the more compelling dimension for Go/ NoGo in both GHRH and placebo, a result consistent with all previous studies using this paradigm.

Table 2

Mean Reaction Times (RTs in ms), Within-subject Standard Deviation of the Reaction Time (SDRT), and Correct-response Rates (CRR, %), as function of Treatment and Task (condition)

Drug condition	GHRH		PLACEBO	
	Mean (ms)	Between-subjects SD	Mean (ms)	Between-subjects SD
CPT RT	224.55	69.31	232.63	63.88
CPT SDRT	37.75	19.50	43.69	17.07
CPT CRR	95.31	6.00	94.53	8.18
EFT congruent RT	497.46	77.90	503.19	82.77
EFT congruent SDRT	103.28	37.87	103.67	39.73
EFT congruent CRR	89.21	13.55	91.78	10.97
EFT incongruent RT	533.85	65.61	542.38	71.00
EFT incongruent SDRT	111.66	38.35	115.11	46.42
EFT incongruent CRR	74.69	15.30	76.15	17.33
SPT RT	425.16	55.80	433.93	57.09
SPT SDRT	75.48	19.01	73.67	15.16

ERP differences between drug conditions

N2b

SPT N2b peak at electrode Fz in the placebo condition was not significantly different from that in the GHRH condition (t=-.105, p=.918; Figure 2). The positive peak preceding the N2b at electrode Fz, the FSP (p=.729), the Fz positive peak P3 following the N2b (p=.851), and the P3 (difference wave target minus orientation relevant) at electrode Pz (p=.868), did not differ between drug conditions.

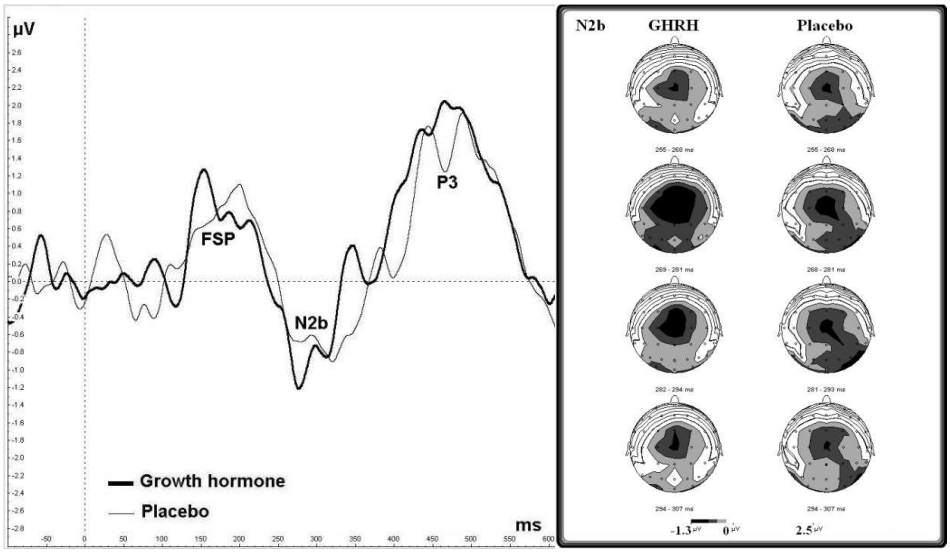


Figure 2 The grand average difference waves at electrode Fz and maps 'spatial-frequency relevant minus irrelevant' (N2b) for GHRH and Placebo

NoGo N2

The CPT NoGo N2 peak at electrode Fz was at trend-level significance smaller during the placebo condition than during the GHRH condition (F=3.75, p=.07; Figure 3).

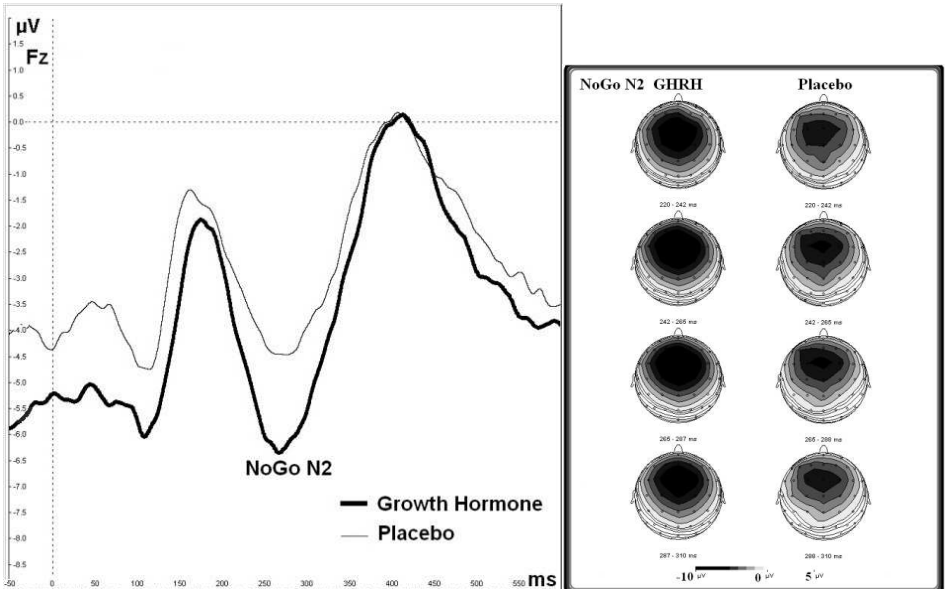


Figure 3 CPT grand average difference waves revealing NoGo N2 for GHRH and Placebo

ERN

The EFT ERN (Figure 4) at electrode FCz was significantly smaller during placebo than during GHRH (F=6.04; p=.03), as was the ERN mean voltage in the 100-150 ms-latency window (F=9.54; p=.01).

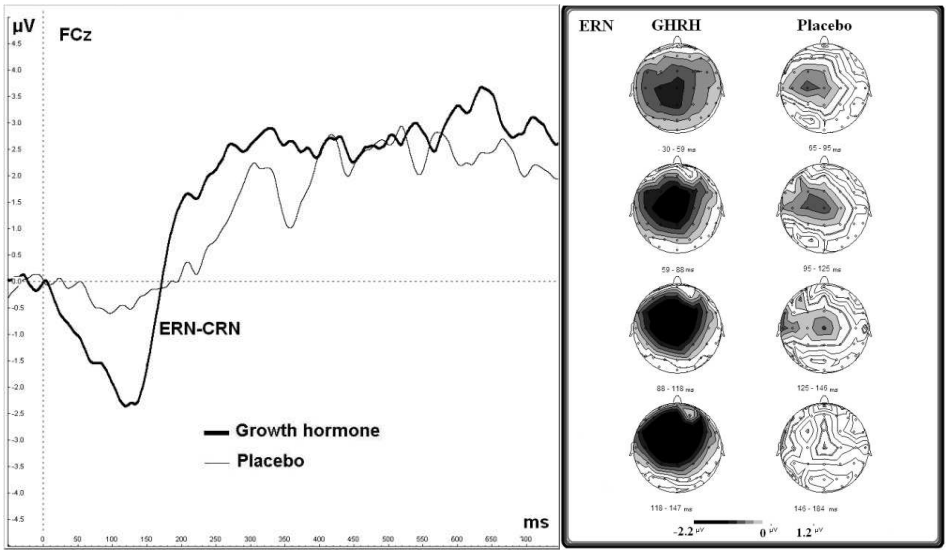


Figure 4 The grand average difference waves ERN-CRN for GHRH and Placebo

Discussion

The present study was motivated by earlier research in GH deficient (GHD) patients and elderly subjects and by the assumption that the anterior-cingulate cortex (ACC) is a critical structure involved in the relation between cognitive effects and GH. This study was designed to investigate whether an acute rise of GH has an effect on cognitive performance in healthy older men when compared to a placebo condition. More specifically, we examined the acute behavioral and electrophysiological modulations, the latter presumably stemming from ACC, caused by a short GH peak after a GHRH challenge. We hypothesized that cognitive performance would improve and ACC-generated ERP components increase during or shortly after the GH peak.

First, the challenge did induce the expected rise in GH. Second, contrary to our expectation no significant differences were found between the placebo and GHRH condition regarding behavioral performance. Third, the amplitude of one the putatively ACC-based electrocortical responses; the Error-Related Negativity (ERN) was significantly larger after the GHRH challenge. In addition, this also held for the NoGo N2 component at trend level. This difference could not be reduced to differences in performance. ERN amplitudes are

directly related to the correct-choice response rates (larger ERNs go with fewer errors; Ridderinkhof et al., 2002). However, the present GHRH and placebo conditions did not differ with respect to performance. It seems then that the ACC-based error-monitoring mechanism itself was boosted by GHRH, and not so much the expectancy of making fewer errors.

Thus, although the GHRH challenge did induce a GH peak that contrasted clearly with GH levels under placebo, only one ERP component and none of the behavioral measures were significantly affected by this manipulation, although some of them showed a change in the expected direction. First, this pattern of results shows that first ERPs are more sensitive to acute GHRH challenge than behavioral measures. Second, from the ERP components only the ERN was sensitive to acute GH effects to a significant degree. This is probably related to differences in the generating mechanism, that also lead to only modest ERN-N2b correlations in younger adults and none with NoGo N2 (Quik et al., in submission/Chapter 7). ERN, NoGo N2 and N2b share that they are all assumed to be generated in the ACC (Coles and Holroyd, 2002) and are all related to conflict monitoring; success vs. failure (Ridderinkhof et al., 1995), Go vs. NoGo (Bekker et al., 2004), and integration of conflicting Go vs. NoGo stimulus features (Lijffijt et al., 2003). Furthermore, both ERN and NoGo N2 probably include dopaminergic mechanisms, and GH peaks induce tonic increases in dopamine (Burman et al., 1996, Johansson et al., 1995). However, in this latter respect the ERN stands out as far as it is response related and the generative process of only this ERP component has so far been related to phasic dopamine dips (Coles and Holroyd, 2002). The results suggest that this mechanism might be more sensitive to GH induced changes in dopamine level than the neurotransmission underlying the N2b and NoGo N2.

Another possibility is that ERN, NoGo N2, and N2b, all reflect similar mechanisms in the ACC, but differ with respect to the sources in other brain regions of signals that control these ACC-based mechanisms (see also Quik et al., in submission, chapter 7). The most likely signal source for the ERN comprises the ventral striatum, and it is possible that especially (dopaminergic) mechanisms in this region are affected by variation in GH level. In contrast, signals that drive the NoGo N2 and the N2b may originate from quite different areas, include cortical ones.

The effects of GH on cognitive performance may also result from interactions with or concurrent increases of other hormones or neurotransmitters. For example, GH probably affects dopaminergic activity in the brain, as it has been shown that the dopamine metabolite homovanillic acid is decreased following GH treatment in GHD patients (Burman et al., 1996, Johansson et al., 1995). The ERN has been attributed to phasic changes in firing of dopaminergic projections from the mesencephalon to the ACC (Holroyd & Coles, 2002). Dopaminergic activity in the brain thus could influence ERP components measured in this study and may be modulated by GH. Cognitive performance in this study was measured between 30 and 90 minutes after GHRH administration. In this time window, secretion of GH is most pronounced. In turn, it may take some time for GH to modulate dopaminergic activity. It may well be possible that the trend level effects found

in the 30-90 minute time window indicate that dopaminergic activity started to increase, which explains the significantly larger ERN in the GHRH condition. In this case, significant cognitive differences between the GHRH and placebo condition related to dopamine other than ERN may be expected later in time. The trend level effects for the NoGo N2 may indicate that the onset of dopaminergic activity in the ACC is increased by a GH peak. Further studies will be needed to see if the N2b and NoGo N2 are influenced significantly by GH later in time.

The inability to observe any significant behavioral effects of GH can be accounted for by multiple explanations. First, immediate effects of GH on cognitive performance have never been described. Under normal circumstances, plasma levels are low but frequent short peaks occur spontaneously, mostly during sleep but also following stress or fasting. It is unlikely that these short peaks play a predominant role in overall cognitive function. In addition, the lack of significant differences could be due to the fact that older and more obese subjects have lower absolute levels of GH compared to younger subjects. The activity of the GH/IGF-1 axis declines with age and in the elderly lower levels of GH are available (Sherlock & Toogood, 2007, Nyberg, 1997). Upon administration of GHRH, the secretion of GH is stimulated. However, with lower GH-levels, a less pronounced effect of the administration of GHRH is consequently noticeable. While the age-related decline in GH/IGF-1 axis activity is associated with the age-related decline in cognitive performance; this decline may contribute to a less pronounced GH secretion which makes it harder to notice significant GH effects following GHRH administration on cognition. Finally, the study may have lacked sufficient statistical power needed to draw reliable conclusions concerning behavior following GHRH administration.

Furthermore, we cannot exclude the possibility that the observed effects that we attribute to GH are a direct effect of the short plasma GHRH rise that we induced. Until now, no major clinical effects of systemic GHRH have been reported independent from its effect on GH. Further studies should therefore also include other mechanisms to induce a GH peak (e.g., arginine, GH-releasing peptides) to evaluate whether similar effects of the GH rise can be observed. In addition, although synthetic GH is only registered for subcutaneous administration and has therefore different pharmacokinetics, intravenous administration might also be feasible in an experimental design.

Taken together, this study yielded significant effects of an acute rise of plasma GH levels on cognitive correlates in healthy older men. Despite not having found significant differences in behavioral performance, NoGo N2 and N2b activity, the ERN was found to be more negative during the EFT task following a GHRH-induced rise in plasma GH. This suggests that a short rise in plasma GH may influence signaling between the ventral striatum and the ACC, possibly as a consequence of alterations in dopamine release or metabolism.

Acknowledgements:
GHRH was provided by Ferring, the Netherlands.

References

Adem, A., Jossan, S.S., d'Argy R, et al. Insulin-like growth factor 1 (IGF-1) receptors in the human brain: quantitative autoradiographic localization. Brain Res (1989) 503(2):299-303.

Aleman, A., de Vries, W.R., de Haan, E.H., Verhaar, H.J., Samson, M.M., Koppeschaar, H.P. Age-sensitive cognitive function, growth hormone and insulin-like growth factor 1 plasma levels in healthy older men. Neuropsychobiology (2000)41: 73–78.

Aleman, A., de Vries, W.R., Koppeschaar, H.P., Osman-Dualeh, M., Verhaar, H.J., Samson, M.M., Bol, E., de Haan, E.H. Relationship between circulating levels of sex hormones and insulin-like growth factor-1 and fluid intelligence in older men. Experimental Aging Research (2001); 27: 283–291.

Aleman, A., Verhaar, H.J., de Haan, E.H., De Vries, W.R., Samson, M.M., Drent, M.L., Van der Veen, E.A., Koppeschaar, H.P. Insulin-like growth factor-I and cognitive function in healthy older men. Journal of Clinical Endocrinology & Metabolism (1999); 84: 471-475.

Almqvist, O., Thoren, M., Saaf, M., Eriksson, O. Effects of growth hormone substitution on mental performance in adults with growth hormone deficiency: a pilot study. Psychoneuroendocrinology (1986) 11(3):347-352.

Arwert, L.I., Deijen, J.B., Witlox, J., Drent, M.L. The influence of growth hormone (GH) substitution on patient-reported outcomes and cognitive functions in GH-deficient patients: a meta-analysis, Growth Horm. IGF Res. (2005) 15, 47-54.

Assema, P. van, Brug, J., Ronda, G., Steenhuis, I. The relative validity of a short Dutch questionnaire as a means to categorize adults and adolescents to total and saturated fat intake. Journal of Human Nutrition and Dietetics (2001) 14:377-390.

Badzakova-Trajkov, G., barnett, K.J., Waldie, K.E., Kirk, I.J. An ERP investigation of the Stroop task: The role of the cingulate in attentional allocation and conflict resolution. Brain research (2009) 1253: 139-148.

Bekker, E.M., Kenemans, J.L., Verbaten, M.N. Electrophysiological correlates of attention, inhibition, sensitivity and bias in a continuous performance task. Clinical Neurophysiology (2004) 115: 2001-2013.

Bekker, E. M., Kenemans, J.L., Verbaten, M.N. Source analysis of the N2 in a cued Go/NoGo task. Cognitive Brain Research (2005) 22, (2): 221-231.

Bengtsson, B.Å., Koppeschaar, H.P.F., Abs, R. et al. Growth hormone replacement therapy is not associated with any increase in mortality. J Clin Endocrinol Metab (1999) 84, 4291-4292.

Bruijn, E.R.A. de, Grootens, K.P., Verkes, R.J., Buchholz, V., Hummelen, J.W., Hulstijn, W. Neural correlates of impulsive responding in borderline personality disorder: ERP evidence for reduced action monitoring. Journal of Psychiatric Research (2006) 40: 428-437.

Burman, P., Hetta, J., Wide, L., Månsson, J.-E., Ekman, R., Karlsson, F.A. Growth hormone treatment affects brain neurotransmitters and thyroxine. Clinical Endocrinology (1996) 44: 319 – 324.

Burman, P., Deijen, J.B. Quality of life and cognitive function in patients with pituitary insufficiency. Psychother Psychosom (1998) 67(3):154-167.

Carroll, P.V., Christ, E.R., the members of Growth Hormone Research Society Scientific Committee:, Bengtsson, B. Å., Carlsson, L., Christiansen, J. S., Clemmons, D., Hintz, R., Ho, K., Laron, Z., Sizonenko, P., Sönksen, P. H., Tanaka, T., Thorner, M. Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. J Clin Endocrinol Metab (1998) 83(2):382-395.

Deijen, J.B., de Boer, H., Blok, G.J., van der Veen, E.A. Cognitive impairments and mood disturbances in growth hormone deficient men. Psychoneuroendocrinology (1996) 21(3):313-322.

Deijen JB, de Boer H, van der Veen EA. Cognitive changes during growth hormone replacement in adult men. Psychoneuroendocrinology (1998) 23(1):45-55.

de Vries, B.B., Robinson, H., Stolte-Dijkstra, I., Tjon Pian Gi, C.V., Dijkstra, P.F., Van Doorn, J., Halley, D.J., Oostra, B.A., Turner, G., Niermeijer, M.F. General overgrowth in the fragile X syndrome: variability in the phenotypic expression of the FMR1 gene mutation. Journal of Medical Genetics (1995) 32: 764-9.

Dik, M.G., Pluijm, S.M., Jonker, C., Deeg, D.J., Lomecky, M.Z., Lips, P. Insulin-like growth factor I (IGF-1) and cognitive decline in older persons. Neurobiology of Aging (2003) 24: 573–581.

Donahue, C.P., Kosik, K.S., and Shors, T.J. Growth hormone is produced within the hippocampus where it responds to age, sex, and stress. Proceedings of the National Academy of Sciences of the United States of America (PNAS) (2006) 103,15: 6031-6036.

Eriksen, B.A. & Eriksen, C.W. Effects of noise letters upon the identification of target letters in visual search. Perception and Psychophysics (1974) 16: 143-149.

Falleti, M.G., Maruff, P., Burman, P., Harris, A. The effects of growth hormone (GH) deficiency and GH replacement on cognitive performance in adults: A meta-analysis of the current literature. Psychoneuroendocrinology (2006) 31: 681-691.

Faul, F., Erdfelder, E., Lang, A.-G., Buchner, A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods (2007) 39: 175-191.

Friedlander, A.L., Butterfield, G.E., Moynihan, S., Grillo, J., Pollack, M., Holloway, L., Friedman, L., Yesavage, J., Matthias, D., Lee, S., Marcus, R., Hoffman, A.R. One year of insulin-like growth factor I treatment does not affect bone density, body composition, or psychological measures in postmenopausal women. Journal of Clinical Endocrinology & Metabolism (2001) 86: 1496–1503.

Gehring, W. J., Goss, B., Coles, M. G., & Meyer, D. E. (1993). A neural system for error detection and compensation. Psychological Science, 4, 385-390.

Gilchrist, F.J., Murray, R.D., Shalet, S.M. The effect of long-term untreated growth hormone deficiency (GHD) and 9 years of GH replacement on the quality of life (QoL) of GH-deficient adults. Clin Endocrinol (Oxf) (2002) 57(3):363-370.

Golgeli, A., Tanriverdi, F., Suer, C., Gokce, C., Ozesmi, C., Bayram, F., Kelestimur, F. Utility of P300 auditory event related potential latency in detecting cognitive dysfunction in growth hormone (GH) deficient patients with Sheehan's syndrome and effects of GH replacement therapy. European Journal of Endocrinology (2004) 150: 153-159.

Gratton, G., Coles, M.G., Donchin, E. A new method for off-line removal of ocular artifact. Electroencephalography and Clinical Neuropsychology (1983) 55: 468-484.

Hatrick, A.G., Boghalo, P., Bingham, J.B., Ayres, A.B., Sonksen, P.H., Russell-Jones, D.L. Does GH replacement therapy in adult GH-deficient patients result in recurrence or increase in size of pituitary tumours? Eur J Endocrinol (2002) 146(6):807-811.

Holroyd, C.B., & Coles, M.G. The neural basis of human error processing: reinforcement learning, dopamine, and the error-related negativity. Psychological Review (2002) 109: 679 – 709.

Hua, K., Forbes, M.E., Lichtenwalner, R.J., Sonntag, W.E., Riddle, D.R. Adult-onset deficiency in growth hormone and insulin-like growth factor-I alters oligodendrocyte turnover in the corpus callosum. Glia. (2009) ;57(10):1062-71.

Johansson, J.-O., Larson, G., Andersson, M., Elmgren, A., Hynsjö, L., Lindahl, A., Lundberg, P.-A., Isaksson, O.G.P., Lindstedt, S., Bengtson, B.-Å. Treatment of growth hormone-deficient adults with recombinant human growth hormone increases the concentration of growth hormone in the cerebrospinal fluid and affects neurotransmitters. Neuroendocrinology (1995) 61: 57–66.

Jonkman, L.M., Kenemans, J.L., Kemner, C., Verbaten, M.N., Engeland, H. van. Dipole source localization of event-related brain activity indicative of an early visual selective attention deficit in ADHD children. Clinical Neurophysiology (2004) 15: 1537-1549.

Kenemans, J.L. Split second sequential selective activation in human secondary visual cortex. J. Cogn. Neurosci. (2002) 14, 48–61.

Lai, Z., Emtner, M., Roos, P., Nyberg, F. Characterization of putative growth hormone receptors in human choroid plexus. Brain Research (1991) 546: 222-226.

Lijffijt, M. Van Dam, P.S., Kenemans, J.L., Koppeschaar, H.P.F., Vries, W.R. de, Drent, M.L., Wittenberg, A., Kemner, C. Somatotropic-axis deficiency affects brain substrates of selective attention in childhood-onset growth hormone deficient patients. Neuroscience Letters (2003) 353: 123-126.

Maruff, P., Falleti, M. Cognitive function in growth hormone deficiency and growth hormone replacement. Hormone Research (2006) 64: 100-108.

Nyberg, F. Aging effects on growth hormone receptor binding in the brain. Experimental Gerontology (1997) 32: 521-528.

Nyberg, F., Burman, P. Growth hormone and its receptors in the central nervous system--location and functional significance. Horm Res (1996) 45(1-2):18-22.

Nyberg, F. Growth hormone in the brain: characteristics of specific brain targets for the hormone and their functional significance. Frontiers in Neuroendocrinology (2000) 21: 330-348.

Pailing, P.E., & Segalowitz, S.J. The effects of uncertainty in error monitoring on associated ERPs. Brain and Cognition (2004) 56: 215 – 233.

Quik, E.H., van Dam, P.S., Kenemans, J.L. Growth hormone and selective attention: A review. Neurosci. Biobehav. Rev. 34 (2010) 1137–1143.

Quik, E.H., Conemans, E.B., Valk, G.D., Kenemans, J.L., Koppeschaar, H.P.F., & van Dam., P.S. (2012). Cognitive performance in older males is associated with growth hormone secretion. Neurobiology of Aging 34(8), 1137-43.

Quik, E.H., van den Bosch, I., Nguyen, P.V., Böcker, K.B.E., Kenemans, J.L. The relation between ERN, NoGo N2, and visual N2b. Int. j. of Psychophysiology *in submission.*

Ridderinkhof, K.R., van der Molen, M.W. and Bashore, T.R. Limits on the application of additive factors logic: Violations of stage robustness suggest a dual-process architecture to explain flanker effects on target processing Acts Psychologica (1995) 90: 29-48.

Ridderinkhof, K.R., Vlugt, Y. de, Bramlage, A., Spaan, M., Elton, M., Snel, J., Band, G.P.H. Alcohol consumption impairs detection of performance errors in mediofrontal cortex. Science (2002) 298: 2209 – 2211.

Roelofs, K., Bruijn, E.R.A. de, Van Galen, G.P. Hyperactive action monitoring during motor-initiation in conversion paralysis: An event-related potential study. Biological Psychology (2006) 71: 316 – 325.

Rollero, A., Murialdo, G., Fonzi, S., Garrone, S., Gianelli, M.V., Gazzerro, E., Barreca, A., Polleri, A. Relationship between cognitive function, growth hormone and insulin-like growth factor I plasma levels in aged subjects. Neuropsychobiology (1998) 38: 73–79.

Sherlock, M., & Toogood, A.A. Aging and the growth hormone/insulin like growth factor-1 axis. Pituitary (2007) 10: 189-203.

Sonntag, W.E., Ramsey, M., Carter, C.S. Growth hormone and insulin-like growth factor-1 (IGF-1) and their influence on cognitive aging. Ageing Research Reviews (2005) 4: 195-212.

van Dam, P.S., Aleman, A., de Vries, W.R. et al. Growth hormone, insulin-like growth factor-I and cognitive function in adults. GH and IGF-1 Res (2000) 10:S69-S73.

van Dam, P.S. & Aleman, A. Insulin-like growth factor-I, cognition and brain aging. Eur J Pharmacol 2004; 490(1-3):87-95.

van Dam, P.S. Neurocognitive function in adults with growth hormone deficiency. Hormone Research (2005) 64 (suppl 3): 109-114.

Veen, V. van & Carter, C.S. Error detection, Correction, and Prevention in the Brain: A Brief Review of Data and Theories. Clinical EEG and Neuroscience (2006) 37: 330-335.

van Veen, V. & Carter, C.S. Conflict an Cognitive Control in the Brain. Current Directions in Psychological Science (2006) 15: 237-240.

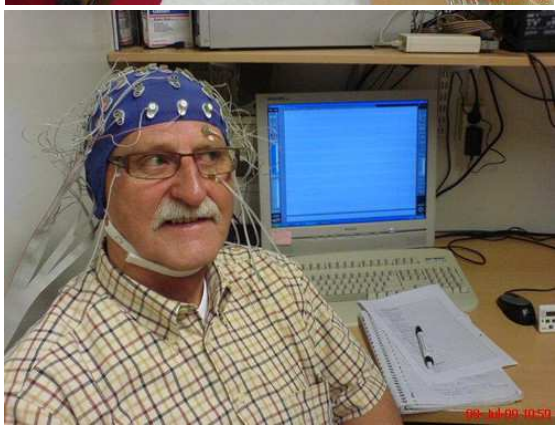
Verhelst, J., Abs, R., Vandeweghe, M. et al. Two years of replacement therapy in adults with growth hormone deficiency. Clin Endocrinol (Oxf) (1997) 47(4):485-494.

Verhelst, J., Abs, R. Long-term growth hormone replacement therapy in hypopituitary adults. Drugs (2002) 62(16):2399-2412.

Vitiello, M.V., Moe, K.E., Merriam, G.R., Mazzoni, G., Buchner, D.H., Schwartz, R.S. Growth hormone releasing hormone improves the cognition of healthy older adults. Neurobiology of Aging (2006) 27: 318-323.

West, R. & Moore, K. Adjustments of cognitive control in younger and older adults. Cortex (2005) 41: 570 – 581.

Zhai, Q., Lai, Z., Roos, P., Nyberg, F. Characterization of growth hormone binding sites in rat brain. Acta Paediatr Suppl (1994) 406:92-95.



Chapter 9

Summary and discussion

Discussion and summary

Both hormones of the somatotropic axis, insulin-like growth factor-1 (IGF-1) and growth hormone (GH) can cross the blood-brain barrier and bind to their receptors in neurons and glia throughout the brain. These receptors have been localized in humans and other animals in numerous cerebral regions, including prefrontal, parietal, and parahippocampal cortex, hippocampus, amygdala, putamen, hypothalamus, and choroid plexus (Oertel et al., 2004; Nyberg and Burman, 1996; Johansson and Bengtsson, 1997 (van Dam, 2005). It may be expected that cognitive functions regulated by these areas, such as attention, memory, processing speed, and executive functions are affected by GHD and GH treatment (Falleti et al., 2006).

Growth hormone (GH) deficiency (GHD) is a well-defined clinical syndrome concerning inadequate production of GH, observed both in children and in adults. Its pathogenesis has been explained in the first chapters of this thesis, and includes congenital causes, hypothalamic or pituitary diseases, brain irradiation and traumatic brain injury. Cognitive deficits globally associated with GHD concern memory, attention and executive functions (Abs et al., 2005; Falleti et al., 2006). Neuropsychological performance improvements during GH therapy in GHD subjects were found in particular for memory and processing speed (van Dam, 2006; Falleti et al., 2006; Oertel et al., 2004; see chapter 2).

Features of aging resemble those of GHD (Arwert et al., 2005) and aging is also associated with a decline in the activity of the GH–IGF-1 axis (van Dam, 2006). A significant decrease has been observed in the density of GH binding with increasing age (over 60 years old) in the choroid plexus, hypothalamus, hippocampus, pituitary and putamen (Lai et al., 1993; Nyberg, 1997). This may suggest that the somatotropic axis plays a role in age-related decline of a great variety of higher cognitive functions such as selective and divided attention, working memory and executive control (Kenemans et al., 1995) as well as mental processing speed (Leskelä et al., 1999). These impairments might be caused by a reduction both in levels of IGF-1 and GH (Anawalt and Merriam, 2001), as well as in the density of GH and IGF-1 receptors, with increasing age (Sherlock & Toogood, 2007; Nyberg, 1997; Lai et al., 1991). A study by Lijffijt et al. (2003) revealed a comparable resemblance at the level of event-related selection brain potentials of differences between childhood onset GHD (CO-GHD) and controls on the one hand, and older and younger healthy subjects on the other. In the present thesis this ERP paradigm was included in several comparisons between higher and lower levels of GH.

These findings prompted us to zoom in on ‘selective attention’ as a possible core deficit in GHD, including its specific varieties of interference control, attribute selection, and attentional switching (chapter 2). In parallel observational studies we addressed relations between on the one hand GH and IGF-1 levels and on the other (1) cognition in general (neuropsychology); (2) performance indices of selective attention (target detections, false-

positive responses); and (3) attention-related brain function, the so-called selection potentials (as in Lijffijt et al., 2003, and Kenemans et al., 1995; chapters 3, 4, and 5)
The most conspicuous result in the Lijffijt et al. (2003) study was the reduction in childhood onset GHD of the N2b selection potential. N2b has previously been associated with anterior-cingulate-cortex based integrated processing of different stimulus attributes (Kenemans et al., 2002). The anterior cingulate cortex (ACC) receives extensive connections from the hippocampus, which in turn is rich in GH receptors. Disinhibition of the hippocampus-ACC circuit is one possible mechanism for the generation of ACC based electrocortical responses, such as N2b (and ERN, see below; see chapter 2).
Activity in the hippocampus-ACC circuit is also sensitive to dopamine, suggesting a further route through which GH may affect cognitive processes, since it is known that dopamine stimulates pituitary GH release in humans (Segal-Lieberman et al., 2006) and GH stimulates β-endorphins, which in turn stimulates dopamine neurons (Very and Sheridan, 2007). Like GH, dopamine also decreases with age (Volkow et al., 1996) and both may influence cognitive decline associated with aging. Brain function that has typically been associated with dopamine includes the ERN, but also the NoGo N2 and perhaps even the N2b. Chapter 6 looked at a specific hypothesis on the relation between ERN and reward sensitivity. Furthermore, in chapter 7 the hypothesis was evaluated that N2b, ERN, as well as another putatively ACC-based ERP component, NoGo N2, all reflect the activity of the same neurons with the same functions in the ACC. Finally, several perspectives were lumped together in chapter 8. Cognitive and brain-function effects of chronically low GH levels may rest on abnormal development with chronically low GH, and /or on reduced acute GH-receptor stimulation. In chapter 8 we attempted to shed light on this issue by investigating the acute effects of a single administration of GH releasing hormone, on N2b, ERN, NoGo N2, as well as performance indices of selective attention.

Is selective attention really implicated in effects of GH? (Chapter 2)

The second chapter reviews the effects of GH on selective attention, especially in GHD patients, as well as the effects of GH replacement therapy. We found no indications that GHD is characterized by impairments in the processes implicated in selective attention such as attribute selection, interference control, or attentional switching. However, a few studies do point to a GHD-related deficit in cognition, in particular implicating integrated processing of multiple dimensions, as well as speed of information processing. In addition, there is weak evidence for beneficial effects of GH replacement therapy in the opposite direction in these domains. With respect to integrated processing of multiple stimulus dimensions Lijffijt and colleagues (2003) recorded event-related brain potentials and reported that the so-called N2b response was smaller in GHD patients. We concluded that

the function of integrated processing of multiple stimulus dimensions may be based on neural mechanisms in the anterior cingulate cortex and its extensive connections to the hippocampus, the latter being known to be rich in GH receptors. Of note, reductions in interference control and attentional shifting were found in GHD attributable to radiation therapy (chapter 5). These reductions are quite discrepant from previous reports and may rather reflect radiation effects.

GH and Insulin-like growth factor-I levels, cognitive performance, selective attention and brain function in older men (chapters 3 & 4)

In a first observational study, the relation between GH secretion and cognition was studied in elderly men between 50-78 years old. Standard neuropsychological tests were used to assess the domains of memory (15 words test), basic processing speed (trail making A), and concept shifting (trail making B). More specific neurocognitive aspects of perception and attention were again assessed using the selection-potential task described by Lijffijt et al. (2003). This pertained to target detection and ERPs, including the N2b and the Lateralized Readiness Potential (LRP), which reflects cortical motor preparation. A correlation between GH secretion and target detections was found: older men with lower GH levels detected fewer targets. Also, GH levels correlated negatively with reaction times for these detected targets: Faster responses with higher GH levels. N2b was smaller for low-GH individuals, but not significantly. This study could not confirm a relation between GH and basic processing speed as reflected in the trail making A test. Thus, the more subtle fluctuations in GH in healthy older men (compared to patients) were only associated with performance measures in the context of the relatively complex selection-potential task, in which information from two stimulus dimensions has to be integrated.
In contrast to GH, IGF-1 levels were positively correlated with trail making A performance as well as with speed of responding to selection-potential targets. These effects may reflect an influence of IGF-1 levels on basic processing speed. This relation may be further qualified by taking into account the also observed positive effect of IGF-1 on the amplitude of the lateralized readiness potential (LRP), a measure of electrocortical preparation of motor responses. These combined data suggest that IGF-1 affects especially the motor-preparation aspects of basic processing speed. A possible mechanism for this is the supportive role of IGF-1 in maintaining the myelin sheets for axons that provide synaptic input in, e.g., the apical dendrites in the motor cortex.
The combined pattern of GH and IGF-1 relations with cognition and brain function suggest at least a partial dissociation between GH and more ‘higher’ forms of cognition on the one hand, and IGF-1 and more basic (motor) processing on the other. However, future studies

employing especially samples that are larger than the present one (N=10) should confirm this.

Reduced somatotropic and cognitive functions after cranial external beam radiation therapy for brain tumors (chapter 5)

A second observational study evaluated the influence of GHD on cognition and correlations between GH secretion and cognition in patients who underwent cranial external beam radiation (Chapter 5). Standardized neuropsychological tests used included the WAIS III digit span subtest, 15 words test, Rey-Osterrieth complex figure test, Stroop color-word task, trail making test A&B, and the Dutch adult reading test. Brain function was assessed using ERPs, including N2b, P300 and LRP. This study also gave us the possibility to evaluate hormonal deficits after brain irradiation during adulthood. This is of interest because only limited data have been reported regarding the prevalence of hypopituitarism in these patients. The GHD subgroup in the patients who received external beam radiation therapy showed impairments in interference control (Stroop color-word) and attentional shifting (trail making B), as well as in visual and long-term memory. The lower target-detection rate and the smaller N2b for the GHD subgroup in the selection-potential task were in the expected direction but did not reach significance. The impairments in Stroop-interference control and attentional shifting were quite unexpected, given our previous studies (chapter 3, and the review in chapter 2). In chapter 5 it was suggested that the association between low GH and interference control and attentional shifting was mediated by higher radiation doses for lower GH levels. Future research outside the context of GHD should clarify this.

Also the patients who received cranial radiation exhibited a discordant GH and IGF-1 pattern. A relation between IGF-1 and short-term and working memory, which differs from the relation between GH and cognition, was found. Furthermore, confirming the results of chapter 4, the relation between IGF-1 and the LRP (reflecting cortical motor preparation) was again found and may be explained by the supportive effect of IGF-on myelin.

As to pituitary function after brain irradiation during adulthood, GH was the only hormone that we found to be affected by radiation. Four out of 19 patients (21%) were classified as having severe GHD. We did not observe clinically relevant other deficiencies of pituitary hormones. This low number of GHD subjects may have contributed to the lack of statistically significant differences between the subject groups (N2b, target-detection parameters).

Positive or negative minds: how do we learn from errors? (Chapter 6)

Mutual enhancing effects between the somatotropic axis and the dopamine system have been described in chapter 2. Brain function that has typically been associated with dopamine includes the ERN, but also the NoGo N2 and perhaps even the N2b. Chapter 6 looked at a specific hypothesis on the relation between ERN and reward sensitivity. Chapter 7 investigated the relations between ERN, NoGo N2, and N2b. Finally, chapter 8 assessed the effects of acute GH-level enhancement on these three ERP components.
Learning bias has been shown to be dopaminergically influenced. Parkinson's patients off medication were better at learning to avoid negative outcomes than they were at learning from positive outcomes (Frank et al., 2004). A dopamine agonist reversed this bias, so that patients learned from positive rather than negative outcomes. ERN is an EEG manifestation of phasic drops in dopamine level that occur after making errors. Therefore it was hypothesized that positive learners, with high dopamine levels, would be characterized by high ERN amplitudes, relative to negative learners. However, the opposite result has been reported by Frank et al. (2005, 2007). Thus, we attempted to replicate this study, and found the opposite results (Chapter 6), in line with our assumptions. We reconciled these contradictory findings by proposing an inverted U-shaped relationship between dopamine level and the propensity to learn from errors compared to success. Detailed comparisons between the distributional characteristics of our participants as compared to previous participants support the conclusion that whereas our participants might include (more) negative learners with below average dopamine level, the participants used in previous studies might have included (more) negative learners with above average dopamine levels. To test this interpretation, additional studies are needed that examine interindividual differences in ERN in relation to learning bias, and which also include independent correlates of dopamine activity, or dopaminergic drug manipulations.

ERN versus N2b: Different paradigms, one mechanism? (Chapter 7)

Both the N2b as well as the ERN have been interpreted as ACC correlates of conflict monitoring (van Veen & Carter, 2002; Niewenhuis et al., 2003; Bekker et al., 2004, 2005), and also the N2b has been proposed to reflect ACC activity. (Kenemans et al., 2002). If these three components really reflect the same ACC function as well as activity of the same neuronal ensembles, then their amplitudes should be correlated, and they should show similar scalp topographics. The latter was indeed found. With respect to correlations, we observed that the N2b and ERN amplitudes were positively correlated and that both were unrelated to the NoGo N2. This combined pattern of results was interpreted as the Nogo-N2

reflecting ACC-based neural activity similar to that reflected in the ERN and N2b, but driven by signals from parts of the brain that are different from those that send the signals to ACC that control ERN and N2b. Furthermore, manipulations that effect ERN should in principle always also affect N2b. This hypothesis, however, was not supported by the results of chapter 8, in which acute GH releasing hormone (GHRH) manipulation affected ERN but not N2b (nor NoGo N2).

Effects of GH: Acute or chronic (Chapter 8)?

The effects discussed so far concerned long-term GH shortage, either as a consequence of disease or associated with normal aging. Effects of long-term deficiency may reflect two underlying factors: abnormal development due to chronically low GH levels, or the acute lack of stimulation of GH receptors. Chapter 8 focused on the latter possibility, by assessing the acute effects of administration of GH releasing hormone versus placebo. Previous studies have demonstrated a relationship between GH status and specific cognitive functions, but no data exist regarding acute effects of GH on cognition. In a double blind cross-over study we injected healthy older men with either GHRH or placebo and then they perform several cognitive tasks during which EEG was measured. The acute rise of plasma GH levels caused by an injection of GHRH resulted specifically in an increased ERN, but did not affect N2b, NoGo N2, nor performance measures. This increase of ERN under GHRH leads to the conclusion that part of the acute effects of GH is probably mediated by its effect on dopamine activity. Our results also implicate that GH has dissociative effects on the N2b and ERN, although their amplitudes covarried in the absence of a GHRH challenge (chapter 7). Specifically, a short rise in plasma GH may influence signaling between the ventral striatum and the ACC, possibly as a consequence of alterations in dopamine release or metabolism, thought to be crucial for elicitation of the ERN (in the ACC). In contrast N2b generation by ACC neurons may be driven by signals from other brain regions, perhaps from cortical areas implicated in the analysis of different visual stimulus attributes (Kenemans et al., 2002). Reductions in N2b with chronically low GH status as reported before (Lijffijt et al., 2003; chapters 3 and 5) may rather reflect developmental effects of chronic GH shortage.

Conclusion

The main topic of this thesis is to specify the influence of GH on cognition and brain function. In the studies that are presented, cognition is assessed by neuropsychological tests, which are covering multiple domains of cognition, experimental psychological tasks, which mainly concern selective attention and electrophysiological recordings. The effects of GH are studied both observationally and experimentally. The observational studies concerned the correlations between GH levels, including GH deficiency or GHD, in healthy older men and patients that underwent radiation therapy for brain tumors. The experimental study involved a pharmacological trial of acute GH releasing Hormone (GHRH) with cognitive assessments as outcome measures.

Previous studies have indicated that the effects of GH on cognition might pharmacologically involve the neurotransmitter dopamine and from an anatomical stance the VTA-hippocampus-ACC axis. We combined these notions in an integrative hypothesis stating that the effects of GH might for a significant part be due to GH stimulated projections from the hippocampus that modulate dopamine activity in the ACC. Therefore the thesis also includes more basic cognitive neuroscience research concerning the interrelations between electrophysiological correlates of ACC activity, and their relationship to interindividual differences in dopamine dependent learning from (probabilistic) feedback.

IGF-1 and GH were not found to be significantly associated with the attention-related brain potential N2b in older men, which we previously found to be compromised in young GHD patients. The present N2b results differ from the findings from Lijffijt et al. (2003) in CO-GHD patients, who observed that N2b was smaller in these patients. The N2b-effects found in the study of Lijffijt et al. (2003) could be due to a direct effect of GH depletion and not IGF-1 depletion. However, it could also result from lack of GH during development of the brain, which in turn may result in deficient neuron and glia growth, or suboptimal myelination. The differences between our findings and the significant findings in CO-GHD patients (Lijffijt et al., 2003) may be explained by differences in the study samples. The participants in our studies did not have a severe shortage of GH or a long-term deficiency, but were elderly males with physiologically reduced GH secretion, brain radiated adult patients or healthy older men. CO-GHD patients are confronted with a long term and severe GH deficiency which started in their childhood, causing lower IGF-1 levels, and may lead to a significantly changed N2b. It would be of interest to perform similar ERP studies in elderly subjects with pathologically reduced GH and IGF-1 levels as a consequence of GHD. Moreover, the correlation between N2b amplitude and GH or IGF-1 may well be significant in an experiment testing more subjects, in order to obtain a larger study sample.

As concluded in chapter 2, it has been suggested that the ACC is part of a brain network, which involves the lateral prefrontal cortex as well as its projections to sensory cortex. Within this network, the ACC may play an indirect role in the control of selective attention,

in that it serves as a monitor for response conflict as elicited by typical conflict stimuli, such as incongruent stimuli. This monitoring process results in signals to lateral prefrontal regions, which in turn are translated to enhanced selectivity of attention through the projections to sensory cortex. It remains to be tested whether this presumably ACC-based mechanism is sensitive to GHD or GH in a more general sense. Activity in ACC and lateral prefrontal cortex, as well as their interactions, may be particularly sensitive to dopamine and suggests a further route through which GH may affect these processes, given the extensive interactions between the GH and dopamine system as discussed in chapter 2.

Previous research has documented the decline of the somatotropic system as well as the dopaminergic neurotransmitter system in the aging human brain and, more specifically, the loss of dopamine receptors in the striatum and extrastriatal regions, which has been associated with basic impairments in motor functions (Yordanova et al., 2004).

Studies showing that cerebrospinal fluid concentration of the dopamine metabolite homovanillic acid is affected by GH treatment support the hypothesis that GH significantly affects neurotransmitters, such as dopamine and neural cell metabolism in adult men (van Dam et al., 2005; Deijen et al., 1998; Lijffijt et al., 2004). Therefore, the hypothesis arises that GH influences behavior and cognition by influencing dopamine. Executive functions, which are impaired in GHD, have been associated with the dopaminergic system. For example, application of methylphenidate in children with ADHD performing the stop task resulted in improved stopping performance (Lijffijt et al., 2006). Stopping performance is typically seen as a reflection of the executive-functions system. In the Lijffijt et al. (2006) study, stopping performance was also specifically positively correlated with dopamine-metabolite (homovanillic acid) levels. In chapter 2 the relation between dopamine and GH was described in further detail, but more research is needed to clarify this relation.

In sum, relations between somatotropic-axis function, and cognition and brain function were found:

- There is an acute effect on ACC-based brain function which is probably dependent on dopaminergic signaling;
- Pathologically low levels of GH result in reductions in basic processing speed, as well as impairments in (brain function related to) ‘higher’ forms of cognition such as the integrated processing of various sources of information;
- Even in healthy aging, there is a relation between IGF-1 levels and motor processing.

References

Abs, R., Mattsson, A.F., Bengtsson, B., Feldt-Rasmussen, U., Góth, M.I., Koltowska-Häggström, M., Monson, J.P., Verhelst, J., Wilton, P, on behalf of the KIMS Study Group, 2005. Isolated growth hormone (GH) deficiency in adult patients: Baseline clinical characteristics and responses to GH replacement in comparison with hypopituitary patients. A sub-analysis of the KIMS database. Growth Hormone & IGF Research 15, 349–359.

Adan, L., Trivin, C., Sainte-Rose, C., Zucker, J.M., Hartmann, O., Brauner, R., 2001. GH deficiency caused by cranial irradiation during childhood: factors and markers in young adults. J Clin Endocrinol Metab 86(11), 5245–5251.

Anawalt, B.D., Merriam, G.R., 2001. Neuroendocrine aging in men. Andropause and somatopause. Endocrinol Metab Clin North Am 30(3), 647-669.

Arwert, L.I., Deijen, J.B., Witlox, J., Drent, M.L., 2005. The influence of growth hormone (GH) substitution on patient-reported outcomes and cognitive functions in GH-deficient patients: a meta-analysis, Growth Horm. IGF Res. 15, 47–54.

Bekker, E.M., Kenemans, J.L., Verbaten, M.N. Electrophysiological correlates of attention, inhibition, sensitivity and bias in a continuous performance task. Clinical Neurophysiology (2004); 115: 2001-2013

Bekker, E. M., Kenemans, J. L., & Verbaten, M. N. (2005). Source analysis of the N2 in a cued Go/NoGo task. Cognitive Brain Research, 22, 221-231.

Deijen, J.B., Arwert, L.I., Witlox, J., Drent, M.L. (2005) Differential effect sizes of growth hormone replacement on Quality of Life, well-being and health status in growth hormone deficient patients: a meta-analysis. Health Qual Life Outcomes. 19;3:63.

Falleti, M.G., Maruff, P., Burman, P., Harris, A., 2006. The effects of growth hormone (GH) deficiency and GH replacement on cognitive performance in adults: A meta-analysis of the current literature. Psychoneuroendocrinology. 31, 681–691.

Frank, M.J., D'Lauro, C. & Curran, T. (2007). Cross-task individual differences in error processing: Neural, electrophysiological and genetic components. Cognitive, Affective and Behavioral Neuroscience, 7, 297-308.

Golgeli, A., Tanriverdi, F., Suer, C., Gokce, C., Ozemi, C., Bayram, F., Kelestimur, F., 2004. Utility of auditory event related potential latency in detecting cognitive dysfunction

in growth hormone (GH) deficient patients with Sheehan's syndrome and effects of GH replacement therapy, Eur. J. Endocrinol. 150, 153–159.

Johansson, J.O., Bengtsson, B.A., 1997. Central nervous effects of growth hormone. Endocrinol. Metab. 4 (Suppl. B), 103– 107.

Kenemans, J.L., Smulders, F.T., Kok, A., 1995. Selective processing of two-dimensional visual stimuli in young and old subjects: electrophysiological analysis. Psychophysiology. 32, 108–120.

Kenemans, J.L., Lijffijt, M., Camfferman, G., Verbaten, M.N., 2002. Splitsecond sequential selective activation in human secondary visual cortex. J. Cogn. Neurosci. 14, 48–61.

Lai, Z., Emtner, M., Roos, P., Nyberg, F. Characterization of putative growth hormone receptors in human choroid plexus. Brain Research (1991) 546: 222-226

Leskelä M, Hietanen M, Kalska H, Ylikoski R, Pohjasvaara T, Mäntylä R, Erkinjuntti T. Executive functions and speed of mental processing in elderly patients with frontal or nonfrontal ischemic stroke. Eur J Neurol. 1999 Nov;6(6):653–661.

Lijffijt, M., Van Dam, P.S., Kenemans, J.L., Koppeschaar, H.P., de Vries, W.R., Drent, M.L., Wittenberg, A., Kemner, C., 2003. Somatotropic-axis deficiency affects brain substrates of selective attention in childhood-onset growth hormone deficient patients. Neurosci. Lett. 353, 123–126.

Lijffijt, M., Bekker, E.M., Quik, E.H., Bakker, J., Kenemans, J.L., Verbaten, M.N. (2004) Differences between low and high trait impulsivity are not associated with differences in inhibitory motor control. J Atten Disord 8:25–32.

Lijffijt, M., Kenemans, J.L., Wal, A. ter, Quik, E.H., Kemner, C., Westenberg, H.G.M., Verbaten, M.N. & Engeland, H. van (2006). Dose-related effect of methylphenidate on stopping and changing in children with attention-deficit/hyperactivity disorder. European Psychiatry, 21(8), 544-547.

Möderscheim, T. A. E., Christophidis, L. J., Williams, C. E., Scheepens, A., 2007. Distinct neuronal growth hormone receptor ligand specificity in the rat brain. Brain Research. 1137, 29.

Nieuwenhuis, S., Yeung, N., van den Wildenberg, W., & Ridderinkhof, K. R. (2003). Electrophysiological correlates of anterior cingulate function in a go/no-go task: effects of

response conflict and trial type frequency. Cognitive, Affective & Behavioral Neuroscience, 3, 17-26.

Nyberg F, Burman P. Growth hormone and its receptors in the central nervous system--location and functional significance. Horm Res (1996) 45(1-2):18-22.

Nyberg, F. Aging effects on growth hormone receptor binding in the brain. Experimental Gerontology (1997) 32: 521-528.

Oertel, H., Schneider, H., Stalla, G., Holsboer, F., Zihl, J., 2004. The effect of growth hormone substitution on cognitive performance in adult patients with hypopituitarism, Psychoneuroendocrinology. 29, 839–850.

Popovic V, Pekic S, Golubicic I, Doknic M, Dieguez C, Casanueva FF. The impact of cranial irradiation on GH responsiveness to GHRH plus GH- releasing peptide-6. J Clin Endocrinol Metab 2002; 87(5):2095-2099.

Sherlock, M., & Toogood, A.A. Aging and the growth hormone/insulin like growth factor-1 axis. Pituitary (2007) 10: 189-203.

Soares, C.N., Musolino, N.R., Cunha, N.M., et al., 1999. Impact of recombinant human growth hormone. A placebo-controlled trial (RH-GH) treatment on psychiatric, neuropsychological and clinical profiles of GH deficient adults. Arq. Neuropsiquiatr. 182–189.

Shukitt-Hale, B., Casadesus, G., Carey, A.N., Rabin, B.M., Joseph, J.A., 2007. Exposure to ^{56}Fe irradiation accelerates normal brain aging and produces deficits in spatial learning and memory. Advances in space research 39(6), 1087-1092

van Dam, P.S., de Winter, C.F., de Vries, R., van der Grond, J., Drent, M.L., Lijffijt, M., Kenemans, J.L., Aleman, A., de Haan, E.H., Koppeschaar, H.P., 2005. Childhoodonset growth hormone deficiency, cognitive function and brain N-acetylaspartate. Psychoneuroendocrinology 30 (4), 357–363.

van Dam, P.S., 2006. Somatotropic therapy and cognitive function in adults with growth hormone deficiency: a critical review. Treat Endocrinol. 5 (2), 1.

van Veen, V., & Carter, C. S. (2002). The timing of action-monitoring processes in the anterior cingulate cortex. Journal of Cognitive Neuroscience, 14, 593-602.

Very, N.M., Sheridan, M.A., 2007. Somatostatin regulates hepatic growth hormone sensitivity by internalizing growth hormone receptors and by decreasing transcription of growth hormone receptor mRNAs. Am J Physiol Regul Integr Comp Physiol 292, R1956-R1962.

Vitiello, M.V., Moe, K.E., Merriam, G.R., Mazzoni, G., Buchner, D.H., Schwartz, R.S., 2006. Growth hormone releasing hormone improves the cognition of healthy older adults. Neurobiol. Aging 27, 318–323.

Volkow, Nora D., Ding, Yu-Shin, Fowler, Joanna S., Wang, Gene-Jack, Logan, Jean Gatley, S., John Hitzemann, Robert, Smith, Gwenn, Fields, Suzanne D. and Gur, Ruben (1996) Dopamine Transporters Decrease with Age The Journal of Nuclear Medicine, 37, 4, 554-559.

Yordanova, J., Kolev, V., Hohnsbein, J., Falkenstein, M., 2004. Sensorimotor slowing with ageing is mediated by a functional dysregulation of motor-generation processes: evidence from high-resolution event-related potentials. Brain 127, 351–362.

Hoofdstuk 10

Samenvatting en conclusies

Beide hormonen van de somatotrope as (GH-IGF-1 as), te weten insuline-achtige-groeifactor-1 (IGF-1) en groeihormoon (GH), kunnen de bloed-hersen barrière passeren en daarna binden aan hun receptoren in neuronen en glia in de hersenen. Deze receptoren zijn zowel bij de mensen als bij andere dieren gelokaliseerd in tal van hersengebieden, inclusief prefrontale, pariëtale en parahippocampale cortex, hippocampus, amygdala, putamen, hypothalamus en plexus choroideus. De aanwezigheid van deze receptoren maakt het aannemelijk dat cognitieve functies die gereguleerd worden door deze gebieden, zoals aandacht, geheugen, verwerkingssnelheid en executieve (hogere controle) functies, beïnvloed worden door verandering in de activiteit van de GH-IGF-1 as of door behandeling met GH.

Groeihormoon deficiëntie (GHD) is een duidelijk omschreven klinisch syndroom dat veroorzaakt wordt door onvoldoende productie van GH, waargenomen bij zowel kinderen als volwassenen. De pathogenese van GHD is uitgelegd in de eerste hoofdstukken van deze dissertatie en omvat congenitale oorzaken, hypothalame of hypofysaire aandoeningen, hersenbestraling en traumatische hersenbeschadiging. De cognitieve afwijkingen die voornamelijk geassocieerd worden met GHD zijn afwijkingen van het geheugen, aandacht en executieve taken. Verbeteringen van de neuropsychologische prestaties gedurende GH therapie in GHD patiënten werden vooral voor het geheugen en de verwerkingssnelheid gevonden (zie hoofdstuk 2).

Kenmerken van veroudering lijken op die van GHD en veroudering wordt ook geassocieerd met een daling van de activiteit van de GH-IGF-1 as. Een aanzienlijke afname in de dichtheid van GH receptoren werd waargenomen naarmate de leeftijd van proefpersonen steeg, voornamelijk na het 60ste levensjaar, in de plexus choroideus, hypothalamus, hippocampus, hypofyse en putamen. Dit suggereert dat de somatotrope as een rol speelt bij de aan leeftijd gerelateerde achteruitgang van een grote verscheidenheid aan hogere cognitieve functies, zoals selectieve en verdeelde aandacht, werkgeheugen en besluitvorming, en mentale verwerkingssnelheid. Deze achteruitgang zou deels kunnen worden veroorzaakt door een daling van het peil van zowel IGF-1 alsook GH, en deels door de daling van de dichtheid van GH en IGF-1 receptoren met toenemende leeftijd. Een studie uit 2003 liet een afname zien op het niveau van de elektrische hersenactiviteit (de zogenaamde event-related potentials, ERPs) zoals gemeten tijdens het uitvoeren van een aandachtstaak bij jong volwassenen met tijdens de kindertijd verworven GHD (childhood onset/CO-GHD) vergeleken met gezonde proefpersonen. In de huidige dissertatie was deze bevinding een belangrijke basis om effecten van condities met meer of minder circulerend GH op cognitieve functies te bestuderen.

De eerder genoemde studies spoorden ons ertoe aan om in te zoomen op selectieve aandacht als een potentieel belangrijk defect bij patiënten met GHD, inclusief de bijbehorende specifieke veranderingen van interferentie controle, selectie van eigenschappen en switchen van aandacht (hoofdstuk 2). In parallelle observationele studies hielden we ons bezig met relaties tussen aan de ene kant GH en IGF-1 niveaus en aan de andere kant (1) cognitie in het algemeen (neuropsychologie); (2) taak-prestaties in relatie

tot selectieve aandacht (target detectie, vals-positieve responsen); en (3) aandacht-gerelateerde hersenfunctie, de zogenoemde selectie potentialen (hoofdstukken 3, 4 en 5).
Het meest opvallende resultaat in de literatuur was de vermindering van de N2b selectie potentiaal in de patiënten met in de kindertijd verworven GHD. N2b is voorheen geassocieerd met het geïntegreerd verwerken van verschillende stimulus attributen via de anterieure cingulate cortex (ACC). De ACC heeft veel neuronale verbindingen met de hippocampus, die op zijn beurt rijk is aan GH receptoren. Ontremming van het hippocampus-ACC circuit is een mogelijk mechanisme voor het moduleren van in ACC gegenereerde electro-corticale responsen, zoals N2b (en ERN, zie onder, zie hoofdstuk 2).
Activiteit in het hippocampus-ACC circuit is ook gevoelig voor dopamine, wat een verdere route suggereert waarlangs GH cognitieve processen kan beïnvloeden, aangezien bekend is dat dopamine het vrijkomen van hypofysair GH in mensen stimuleert en GH vervolgens β-endorfines stimuleert, die op hun beurt dopamine neuronen stimuleren. Net als GH neemt dopamine af met de leeftijd en beide kunnen cognitieve achteruitgang, geassocieerd met leeftijd, beïnvloeden. Hersenpotentialen of ERPs die voornamelijk met dopamine worden geassocieerd zijn de ERN, maar ook de NoGo N2 en misschien zelfs de N2b. Hoofdstuk 6 keek naar een specifieke hypothese over de relatie tussen ERN en gevoeligheid voor beloning. Verder werd in hoofdstuk 7 de hypothese geëvalueerd, dat zowel N2b, ERN, en de NoGo N2, allemaal de activiteit van dezelfde neuronen met dezelfde functies in de ACC reflecteren. Tot slot werden in hoofdstuk 8 verschillende perspectieven samengenomen. Cognitieve effecten en effecten op de hersenfunctie van chronisch lage GH niveaus zijn waarschijnlijk gebaseerd op een abnormale ontwikkeling met een chronisch laag GH en/of op gereduceerde acute stimulatie van de GH-receptor. In hoofdstuk 8 hebben we dit onderwerp onderzocht door de acute effecten van een eenmalige toediening van GH releasing hormoon (GH-RH), op N2b, ERN, NoGo N2, en op selectieve aandacht te onderzoeken.

Beïnvloedt GH selectieve aandacht? (hoofdstuk 2)

Het tweede hoofdstuk bespreekt de effecten van GH op selectieve aandacht, vooral in GHD patiënten met of zonder GH substitutietherapie. We hebben geen aanwijzingen gevonden dat GHD wordt gekenmerkt door aandoeningen/problemen bij de processen die betrokken zijn bij selectieve aandacht, zoals selectie van attributen, interferentie controle of het switchen van aandacht. Er wijzen echter wel enkele studies op een GHD-gerelateerd gebrek in cognitie, met name betrekking hebbend op geïntegreerde verwerking van meerdere dimensies en snelheid van informatieverwerking. Verder is er een zwak bewijs voor gunstige effecten van GH substitutietherapie in de tegenovergestelde richting in deze gebieden. Ten aanzien van geïntegreerde verwerking van meerdere stimulidimensies,

werden ERPs van de hersenen gemeten en bleek dat de zogenaamde N2b respons kleiner was bij CO-GHD patiënten. We concludeerden dat de functie van geïntegreerde verwerking van meerdere stimulidimensies mogelijk gebaseerd is op neurale mechanismen in de ACC en zijn uitgebreide verbindingen met de hippocampus, waarvan laatstgenoemde erom bekend staat rijk te zijn aan GH receptoren. Opmerkelijk is dat er verminderingen in interferentie controle of switchen van aandacht gevonden werden in GHD, toe te schrijven aan bestralingstherapie (hoofdstuk 5). Deze verminderingen zijn nogal tegenstrijdig met voorgaande bevindingen en reflecteren eerder de effecten van bestraling.

GH en IGF-1 spiegels, cognitieve prestatie, selectieve aandacht en hersenfunctie bij oudere mannen (hoofdstukken 3 en 4)

In een eerste observationele studie werd de relatie bestudeerd tussen de secretie van GH (na een GH stimulatietest) en cognitie bij oudere mannen tussen de 50 en 78 jaar oud. Standaard neuropsychologische tests werden gebruikt om onderdelen van het geheugen te testen (15 woorden test), fundamentele verwerkingssnelheid (trail making A) en veranderen van concept (trail making B). Meer specifieke neurocognitieve aspecten van perceptie/waarneming en aandacht werden weer bepaald door de selectieve aandachtstaak te gebruiken. Dit had betrekking op target detectie en ERP's, inclusief de N2b en de LRP (lateralized readiness potential; een maat voor het voorbereiden en uitvoeren van een actie met of de linker-, of de rechterhand). Er werd een correlatie tussen GH secretie en target detectie gevonden: oudere mannen met lagere GH spiegels detecteerden minder targets. Ook correleerden GH spiegels op positieve wijze met reactiesnelheden voor deze gedetecteerde targets: snellere reacties met hogere GH niveaus. N2b was kleiner voor individuen met een lage GH secretie, echter niet significant. Deze studie kon geen relatie bevestigen tussen GH en fundamentele verwerkingssnelheid, zoals in de trail making A test weerspiegeld. Aldus werden de meer subtiele fluctuaties in GH bij gezonde oudere mannen (vergeleken met patiënten) alleen geassocieerd met prestatiemetingen in de context van de relatief complexe selectie-potentiaal-taak, waarin informatie van twee stimulus dimensies moet worden geïntegreerd.
Net als GH correleerden IGF-1 spiegels positief met de reactiesnelheid in de selectieve aandachtstaak. In tegenstelling tot GH waren ze bovendien positief gecorreleerd met Trail Making A prestatie (Hoofdstuk 4). Deze effecten kunnen een invloed van IGF-1 op fundamentele verwerkingssnelheid weerspiegelen. Deze relatie kan verder worden gekwalificeerd door rekening te houden met het tevens waargenomen positieve effect van IGF-1 op de omvang van de LRP. Deze gecombineerde gegevens suggereren dat IGF-1 vooral de aspecten van motor-preparatie en van fundamentele verwerkingssnelheid beïnvloedt. Een mogelijk mechanisme hiervoor is de ondersteunende rol van IGF-1 in het

handhaven van myeline lagen voor axonen, die synaptische input leveren in bijvoorbeeld de apicale dendrieten in de motor cortex.

Verminderde somatotrope activiteit en cognitieve functies na craniële uitwendige radiotherapie (external beam radiotherapie/EBRT) voor hersentumoren (hoofdstuk 5)

Een tweede observationele studie evalueerde de invloed van GHD op cognitie en correlaties tussen GH secretie en cognitie bij patiënten die eerder craniële uitwendige radiotherapie hadden ondergaan (hoofdstuk 5). De gebruikte gestandaardiseerde neuropsychologische tests bevatten de WAIS III sub-test digit span (aandachts spanne voor cijfers), 15 woorden test, Rey-Osterrieth complex figuur test, Stroop kleur-woord taak, trail making test A&B en de Nederlandse lees-test voor volwassenen. De hersenfunctie werd bepaald door het gebruik van ERP's, inclusief N2b, P300 en LRP. Deze studie gaf ons ook de mogelijkheid om hormonale tekorten na bestraling van de hersenen gedurende volwassenheid te evalueren. Dit is van belang omdat alleen beperkte gegevens zijn gerapporteerd met betrekking tot de aanwezigheid van hypopituïtarisme bij deze patiënten. De GHD subgroep bij de patiënten die uitwendige radiotherapie ondergingen vertoonde deficiënties in de interferentie controle (Stroop kleur-woord), aandacht verschuiven (trail making B), en in het visuele en lange-termijn geheugen. Het verminderde aantal gedetecteerde targets en de kleinere N2b voor de GHD subgroep in de selectie potentiaal taak waren in de verwachte richting, maar bereikte geen significantie. De verzwakte prestaties bij Stroop-interferentie controle en shiften van aandacht waren nogal onverwacht, gegeven onze voorgaande studies (hoofdstuk 3 en de bespreking in hoofdstuk 2). In hoofdstuk 5 wordt gesuggereerd dat de associatie tussen laag GH en interferentie controle en shiften van aandacht mede wordt beïnvloed door hogere stralingsdoses die kunnen leiden tot lagere GH secretie. Toekomstig onderzoek buiten de context van GHD zou dit moeten verduidelijken.
Patiënten die schedelbestraling ontvingen, vertoonden een discongruent GH en IGF-1 patroon. Er werd een relatie tussen IGF-1 en korte termijn- en werkgeheugen gevonden, die verschilt van de relatie tussen GH en cognitie. Verder werd, als bevestiging van de resultaten van hoofdstuk 4, opnieuw de relatie tussen IGF-1 en de LRP (die corticale motor preparatie reflecteert) gevonden, wat kan worden verklaard door het ondersteunende effect van IGF-1 op myeline.
Wat betreft de functie van de hypofyse na bestraling van de hersenen gedurende de volwassenheid, was de GH-IGF-1 as de enige hypofyse-as die door radiotherapie was aangetast. Vier van de 19 patiënten (21%) werden geclassificeerd als ernstig GH tekortkomend. We hebben geen klinisch relevante andere tekorten van hypofyse hormonen gevonden. Dit lage aantal GHD subjecten kan hebben bijgedragen aan het gebrek aan

statistisch significante verschillen tussen de (non)GHD groepen (N2b, target detectie parameters).

Positieve of negatieve breinen: hoe leren we van fouten? (hoofdstuk 6)

Wederzijds verhogende effecten tussen de somatotrope as en het dopamine systeem zijn beschreven in hoofdstuk 2. Een hersenfunctie die typisch wordt geassocieerd met dopamine omvat de ERN, maar ook de NoGo N2 en misschien zelfs de N2b. Hoofdstuk 6 betrof een specifieke hypothese over de relatie tussen ERN en gevoeligheid voor beloning. Hoofdstuk 7 is gewijd aan de relaties tussen ERN, NoGo N2 en N2b. Tot slot werden hoofdstuk 8 de effecten van acute verhoging van het GH-niveau op deze 3 ERP componenten onderzocht.
Het is gebleken dat individuele verschillen in de gevoeligheid voor positieve respectievelijk negatieve feedback door dopamine worden beïnvloed. Parkinson patiënten zonder medicijnen waren beter in het leren vermijden van negatieve uitkomsten dan in het leren van positieve uitkomsten. Een dopamine agonist deed deze gevoeligheid omkeren, zodat patiënten eerder van positieve dan van negatieve uitkomsten leerden. ERN is een EEG manifestatie van fasische daling in het dopamine niveau die optreedt na het maken van fouten. Derhalve werd verondersteld dat mensen met een positieve leerstijl, met hoge dopamine niveaus, zouden worden gekarakteriseerd door hoge ERN amplitudes, vergeleken met mensen met een negatieve leerstijl. Echter, het tegenovergestelde resultaat is ook gerapporteerd. Zodoende probeerden we om deze studie te repliceren en vonden dit keer wel de theoretisch verwachte resultaten. Deze tegenstrijdige bevindingen zijn onzes inziens te verklaren door een omgekeerde U-vormige relatie tussen het dopamine-niveau en de neiging om van fouten in plaats van succes te leren. Gedetailleerde vergelijkingen tussen de resultaten van onze deelnemers vergeleken met die in de voorgaande studies ondersteunen de conclusie dat, terwijl onze steekproef meer deelnemers met een (meer) negatieve leerstijl bevatten met een lager dan gemiddeld dopamine niveau, de voorgaande studies meer deelnemers met een (meer) negatieve leerstijl met bovengemiddelde dopamine niveaus zouden kunnen hebben bevat. Om deze interpretatie te testen, zijn aanvullende studies nodig die interindividuele verschillen in ERN in relatie tot leer-bias onderzoeken, en die ook onafhankelijke correlaten bevatten van dopamine activiteit, dan wel gebruik maken van dopaminerge drug-manipulaties.

ERN versus N2b: verschillende paradigma's, één mechanisme? (hoofdstuk 7)

Zowel de NoGo N2 als ERN kan worden geïnterpreteerd als ACC correlaten van conflict monitoring, maar ook de N2b wordt verondersteld ACC activiteit te reflecteren. Als deze drie componenten, NoGo N2, ERN & N2b, inderdaad dezelfde ACC functie reflecteren alsook activiteit van dezelfde neuronale ensembles, dan zouden hun amplitudes met elkaar moeten correleren en dan zouden ze vergelijkbare schedelverdelingen vertonen. Dit laatste werd inderdaad ook gevonden. Met betrekking tot correlaties namen we waar dat de N2b en ERN amplitudes positief zijn gecorreleerd en dat beide niet gerelateerd waren aan de NoGo N2. Dit gecombineerde patroon van resultaten wijst erop dat alle drie de ERP-componenten gegenereerd worden door eenzelfde structuur, waarschijnlijk de ACC, maar dat ze gemoduleerd worden door signalen afkomstig van delen van het brein die per N2 component verschillen.
Verder zouden manipulaties die de ERN beïnvloeden in principe ook de N2b moeten beïnvloeden. De correlaties zijn echter van matige omvang en in hoofdstuk 8 zullen we zien dat acute manipulatie van GHRH wel de ERN beïnvloedde, maar niet de N2b (noch NoGo N2).

Effecten van GH: acuut of chronisch? (hoofdstuk 8)

De effecten die tot nu toe werden bediscussieerd, betroffen een tekort aan GH op lange termijn ofwel als een gevolg van ziekte of geassocieerd met normale veroudering. Effecten van lange termijn GH tekort kunnen twee onderliggende factoren reflecteren: abnormale ontwikkeling als gevolg van chronisch lage GH niveaus of het acute gebrek aan stimulatie van GH receptoren. Hoofdstuk 8 concentreerde zich op de laatstgenoemde mogelijkheid, door de acute effecten van toediening van GH vrijgevend hormoon versus placebo te bepalen. Voorgaande studies hebben een relatie aangetoond tussen GH status en specifieke cognitieve functies, maar er bestaan geen gegevens betreffende acute effecten van GH op cognitie. In een dubbelblinde cross-over studie injecteerden we gezonde oudere mannen met GHRH of placebo. Daarna voerden ze verschillende cognitieve taken uit, gedurende welke elektro-encefalogram (EEG) werd gemeten. De acute stijging van plasma GH niveaus, veroorzaakt door een injectie van GHRH, resulteerde specifiek in een verhoogd ERN, maar beïnvloedde niet N2b, NoGo N2, noch prestatiemetingen. Deze verhoging van ERN onder GHRH leidt tot de conclusie dat een deel van de acute effecten van GH waarschijnlijk wordt gerealiseerd via een effect op de activiteit van dopamine. Onze resultaten impliceren ook dat GH dissociatieve effecten heeft op de N2b en ERN, ook al co-varieerden hun amplitudes bij de afwezigheid van een GHRH stimulus (hoofdstuk 7). In het

bijzonder kan een korte stijging in het GH plasma de signalering tussen het ventrale striatum en de ACC beïnvloeden, waarschijnlijk als een gevolg van veranderingen in de release of het metabolisme van dopamine, die cruciaal worden geacht voor de generatie van de ERN (in de ACC). Daarentegen kan de generatie van N2b door ACC neuronen worden aangedreven door signalen vanuit andere hersengebieden, misschien van corticale gebieden die betrokken zijn bij de analyse van verschillende visuele stimulus attributen. Verlaging van N2b als gevolg van chronisch lage GH status zoals eerder gerapporteerd (hoofdstukken 3 en 5) zou in het licht van de huidige resultaten eerder ontwikkelingseffecten reflecteren.

Conclusie

Het belangrijkste onderwerp van deze dissertatie is het onderzoeken van de invloed van GH op cognitie en hersenfunctie. In de in dit proefschrift beschreven studies werd cognitie bepaald door neuropsychologische tests, die verscheidene gebieden van cognitie dekken, en experimentele psychologische taken, die hoofdzakelijk selectieve aandacht en de bijbehorende elektrofysiologische aspecten betreffen. De effecten van GH werden zowel observationeel als experimenteel bestudeerd. De observationele studies betroffen de correlaties tussen GH secretie, inclusief GH tekort en GHD, bij gezonde oudere mannen en patiënten die radiotherapie voor hersentumoren ondergingen. De experimentele studie betrof een farmacologische trial met GHRH, leidend tot een korte GH piek, met cognitieve assessments als uitkomstmaten.
Voorgaande studies hebben laten zien dat de effecten van GH op cognitie vanuit een farmacologisch oogpunt betrekking kunnen hebben op de neurotransmitter dopamine en anatomisch gezien gerelateerd zouden kunnen zijn aan de VTA-hippocampus-ACC as. We combineerden deze veronderstellingen in een geïntegreerde hypothese, die stelt dat de effecten van GH voor een significant deel verklaard zouden kunnen worden door GH gestimuleerde projecties van de hippocampus die dopamine activiteit in de ACC moduleren. Daarom bevat dit proefschrift ook meer elementair cognitief neurowetenschappelijk onderzoek betreffende de onderlinge relaties tussen elektrofysiologische correlaten van ACC activiteit en interindividuele verschillen in dopamine-afhankelijk leren op basis van (probabilistische) feedback.
IGF-1 en GH bleken niet significant te worden geassocieerd met de aandacht-gerelateerde hersenpotentiaal N2b bij oudere mannen, welke eerder aangetast bleek te zijn bij CO-GHD patiënten. De huidige N2b resultaten verschillen van eerdere bevindingen dat N2b kleiner was bij CO-GHD patiënten. De N2b-effecten die gevonden werden zouden kunnen worden verklaard door een direct effect van GH depletie en niet IGF-1 depletie. Echter, het zou ook kunnen voortvloeien uit een gebrek aan GH gedurende de ontwikkeling van de hersenen, dat op zijn beurt kan leiden tot een gebrekkige groei van neuronen en glia, of suboptimale

myelinatie. Om tot een definitieve conclusie te komen zouden vergelijkbare ERP studies bij oudere proefpersonen met pathologisch tekort aan GH en IGF-1 als een gevolg van GHD moeten worden uitgevoerd. Bovendien kan de correlatie tussen N2b amplitude en GH of IGF-1 significant blijken te zijn in een experiment, waarbij meer proefpersonen worden getest.

Zoals geconcludeerd in hoofdstuk 2, is er gesuggereerd dat de ACC onderdeel is van een hersennetwerk met verbindingen met de laterale prefrontale cortex als ook projecties naar de sensorische cortex. Binnen dit netwerk kan de ACC een indirecte rol spelen bij de controle van selectieve aandacht, daarin dient het als een monitor voor een respons conflict ten gevolge van bijvoorbeeld incongruente stimuli. Dit controle-proces resulteert in signalen aan laterale prefrontale gebieden, die op hun beurt worden vertaald naar versterkte selectiviteit van aandacht door verdere projecties naar de sensorische cortex. Het moet nog onderzocht worden of de ACC binnen dit netwerk gevoelig is voor GHD of GH in een meer algemene zin. Activiteit in ACC en de laterale prefrontale cortex, alsook hun interacties, kunnen zeer gevoelig zijn voor dopamine. Dit suggereert een alternatief mechanisme voor de invloed van GH op deze processen.

Voorgaand onderzoek heeft een verslechtering van het somatotrope en het dopaminerge neurotransmitter systeem gedocumenteerd in het verouderende menselijke brein en, meer specifiek, het verlies van dopamine receptoren in het striatum en extrastriatale gebieden, dat geassocieerd is met elementaire beschadigingen in motor functies.

Studies die aantonen dat liquorconcentraties van de dopamine metaboliet homovanillic zuur worden beïnvloed door behandeling met GH ondersteunen de hypothese dat GH neurotransmitters aanzienlijk zou kunnen beïnvloeden, bijvoorbeeld in het dopaminerge systeem en het neurale cel metabolisme bij volwassen mannen. Dit leidt tot de hypothese dat GH gedrag en cognitie niet direct, maar indirect beïnvloedt via dopamine, Executieve functies, die gecompromitteerd zijn in GHD, zijn geassocieerd met het dopaminerge systeem. Gebruik van methylfenidaat (Ritalin) bijvoorbeeld, bij kinderen met ADHD die de stoptaak uitvoeren, resulteerde in verbeterde stopprestatie. Stopprestatie wordt vooral gezien als een reflectie van het executieve functies systeem. Eerder werd stopprestatie ook specifiek positief in verband gebracht met dopamine metaboliet (homovanillic zuur) niveaus. In hoofdstuk 2 werd de relatie tussen dopamine en GH in verder detail beschreven, maar meer onderzoek is nodig om deze relatie te verduidelijken.

In het kort, er werden relaties tussen de activiteit van de somatotrope as, cognitie en hersenfunctie gevonden:

- Er is een acuut effect van de somatotrope as op ACC-gebaseerde hersenfunctie, dat waarschijnlijk afhankelijk is van dopaminerge signalering;
- Pathologisch lage niveaus van GH resulteren in afname van fundamentele verwerkingssnelheid, alsook in aantasting van (executieve) hersenfuncties m.b.t. hogere vormen van cognitie, zoals de geïntegreerde verwerking van verschillende bronnen van informatie;
- Zelfs bij gezond ouder worden is er een relatie tussen IGF-1 niveaus en motor verwerking.

Dankwoord

Een proefschrift kan alleen tot stand komen door de bijdragen en steun van een groot aantal personen. Velen hebben dan ook een indirecte of directe bijdrage geleverd aan de totstandkoming van dit proefschrift en de onderzoeken welke er in beschreven zijn. Mijn grote dank daarvoor!

Het is onbegonnen werk om al deze mensen een voor een te bedanken, maar enkele personen wil ik echter in het bijzonder bedanken, omdat die mij de afgelopen jaren gesteund en/of geholpen hebben en daarmee een speciale bijdrage aan dit proefschrift hebben geleverd.

Om te beginnen wil ik mijn promotor en mijn copromotoren bedanken voor de mogelijkheid tot promoveren en de deskundige begeleiding die ze mij de afgelopen jaren gaven.

Leon Kenemans wil ik bedanken voor de langdurige begeleiding. Leon, ondanks alle drukte die jouw banen met zich mee brengen, vond je de tijd om constructief commentaar te leveren op manuscripten en om nieuwe onderzoeksideeën te bespreken.

Mijn co-promotor, Koen Böcker, wil ik bedanken voor zijn inzet en enthousiasme bij de totstandkoming van dit proefschrift. Koen, hartelijk dank voor je betrokkenheid en interesse in mijn promotieonderzoek. Ik wil je bedanken voor het vertrouwen dat je altijd in mij gesteld hebt. Jouw laagdrempelige manier van begeleiden zorgde ook voor een fijne en open werksfeer.

Mijn co-promotor, Sytze van Dam wil ik bedanken voor het mogelijk maken van dit proefschrift. Sytze, zonder jou was dit proefschrift er niet geweest. Je had nog een onderzoeker plek beschikbaar voor het groeihormoonproject van het UMCG afdeling endocrinologie en zo kon ik beginnen als onderzoeker in samenwerking met jullie en bij de afdeling experimentele psychologie en psychofarmacologie. Je support en vertrouwen zijn van grote invloed geweest op mijn ontwikkeling als onderzoeker.

Mijn voormalig stagebegeleider Marijn Lijffijt heeft mijn interesse in de invloed van hormonen op de hersenen en gedrag aangewakkerd en mij laten kennismaken met afdeling

endocrinologie. Dit heeft de uiteindelijk geresulteerd in dit proefschrift. Marijn, mijn dank daarvoor.

Mijn dank gaat ook uit naar Hans Koppeschaar voor het delen van zijn kennis en medewerking aan onze onderzoeken, boekhoofdstuk en artikel. Dit heeft mij vele malen goed op weg geholpen. Dank u wel daarvoor.

Jeroen Gerritsen, Elfi Conemans, Madeleine Drent, Inge Maitimu-Smeele, Anne-Mette Sauër, Gerlof Valk en Lucas Stalpers, bedankt voor jullie energieke medewerking aan het artikel tevens hoofdstuk, aan het onderzoek bij bestraalde patiënten / gezonde mannen, en aan het screenen van de patiënten. Jullie inzet heeft me verder op weg geholpen en ik wist me hierdoor telkens erg gesteund.

Chantal Kemner wil ik bedanken vanwege het feit dat ik al vanaf het uitvoeren van mijn afstudeeronderzoek welkom was bij je op afdeling kinder- en jeugdpsychiatrie en gebruik mocht maken van je onderzoeksmeetruimte.

Gert Camfferman wil ik bedanken voor alle technische ondersteuning en leermomenten, en je medeleven toen ik met holteontstekingen te kampen had.

Graag wil ik ook alle proefpersonen en patiënten bedanken die hebben meegedaan aan de onderzoeken en daardoor de totstandkoming van dit proefschrift hebben mogelijk gemaakt. In het bijzonder wil ik Ome Jan bedanken voor zijn bijdrage en de foto's.

Verder wil ik de gehele vakgroep psychofarmacologie en experimentele psychologie bedanken voor de prettige samenwerking, hun inspiratie en het plezier dat in overvloed aanwezig was. Met name wil ik Stijn, Valentina, Monique, Ivo, Alexander en Sophie bedanken voor alle inzichten, geduld, warmte en alle plezier die zij daar nog bij voegden. Marga, jou wil ik in het bijzonder bedanken voor al je administratieve werkzaamheden en vooral ook je luisterend oor en steun.

Ook wil ik alle medewerkers van psychonomie bedanken voor hun warme welkom en fijne werksfeer. In het bijzonder wil ik alle (m)eet-en-greet-ers bedanken voor alle inspirerende gezelligheid. Niet alleen waren jullie gezellige collega's, maar ook vrienden, we hebben het samen, ook internationaal in Italië en Gent, heerlijk gehad.

Verder wil ik alle voormalige studenten graag bedanken voor al hun inzet, wetenschappelijk werk en gezelligheid, vooral Fatima, Monique, Iris en Phan Vu: hartelijk dank daarvoor.

Ook mijn nieuwe collega's in Groningen wil ik bedanken voor hun medeleven tijdens de laatste loodjes. Paul, bedankt voor deze mooie kans, waardevolle tips en warme welkom in de wereld van HTA.

Mijn paranimfen, hartelijk dank voor de stimulans die jullie me bleven geven om door te gaan wanneer het wat moeilijker ging. De laatste loodjes wegen het zwaarst, maar waren dankzij jullie dragelijk. Henk en Freek, wat geweldig dat jullie mijn paranimfen willen zijn.

Buiten de vakgroepen zijn er veel vrienden en vriendinnen die mij op meer en minder cruciale momenten terzijde hebben gestaan om er plezier in te krijgen en te houden, bedankt daarvoor. Vooral Erik, Jolanda, Ronald, Richard, Vincent, Judith, Caspar en Marieke ben ik erg dankbaar en in het speciaal Bertina, bedankt voor alles, je steun, alle LOL, maar vooral je onvoorwaardelijke vriendschap. Mijn familie heeft ook altijd achter mij gestaan, ook op momenten dat ik het zelf niet meer zag zitten. Ik ben jullie allen erg dankbaar.

Last but not least, wil ik mijn ouders bedanken voor de onvoorwaardelijke steun die ik van hen kreeg; jullie waren en bleven altijd geïnteresseerd en betrokken. Bedankt voor alles. Ik ben er trots op dat jullie mijn ouders zijn!

Al is het zo dat dit proefschrift maar een klein steentje bijdraagt aan het doorgronden van de werking van het menselijk brein, toch stemt het mij tevreden dat ik dat ene kleine steentje heb kunnen leveren.
Gedachtig aan "De Steen" van Bram Vermeulen (en mooi bezongen door Stef Bos)*:*

"Ik heb een steen verlegd in een rivier op aarde, het water gaat er anders dan voorheen. Omdat, door het verleggen van die ene steen, het water nooit dezelfde weg zal gaan.."

Elise Quik

Biografie

De auteur van dit proefschrift, Elise Quik, werd geboren op 21 augustus 1980 te Harderwijk. Aansluitend aan het behalen van het MAVO diploma aan de Brouwerskamp te Nunspeet en MBO-SPW diploma aan Landstede te Harderwijk, startte zij in 1998 de HBO studie MWD. Na een jaar haar propedeuse behaald te hebben en na een gesprek met de decaan besloot zij biologische psychologie te gaan studeren. In 2000 begon zij haar studie psychologie aan de universiteit Utrecht. In 2004 studeerde zij af in de psychologie met als afstudeer richting bio- en neuropsychologie. In 2004 begon zij tevens aan de studie naar groeihormoon en cognitie waardoor de basis werd gelegd voor een PhD traject onder leiding van Prof. dr. J.L. Kenemans, dr. P.S. van Dam en dr. K.B.E Bocker. Sinds 2010 is ze werkzaam als postdoc bij de afdeling epidemiologie van de universiteit van Groningen, universitair medisch centrum Groningen waar ze zich bezig houdt met health technology assessment.