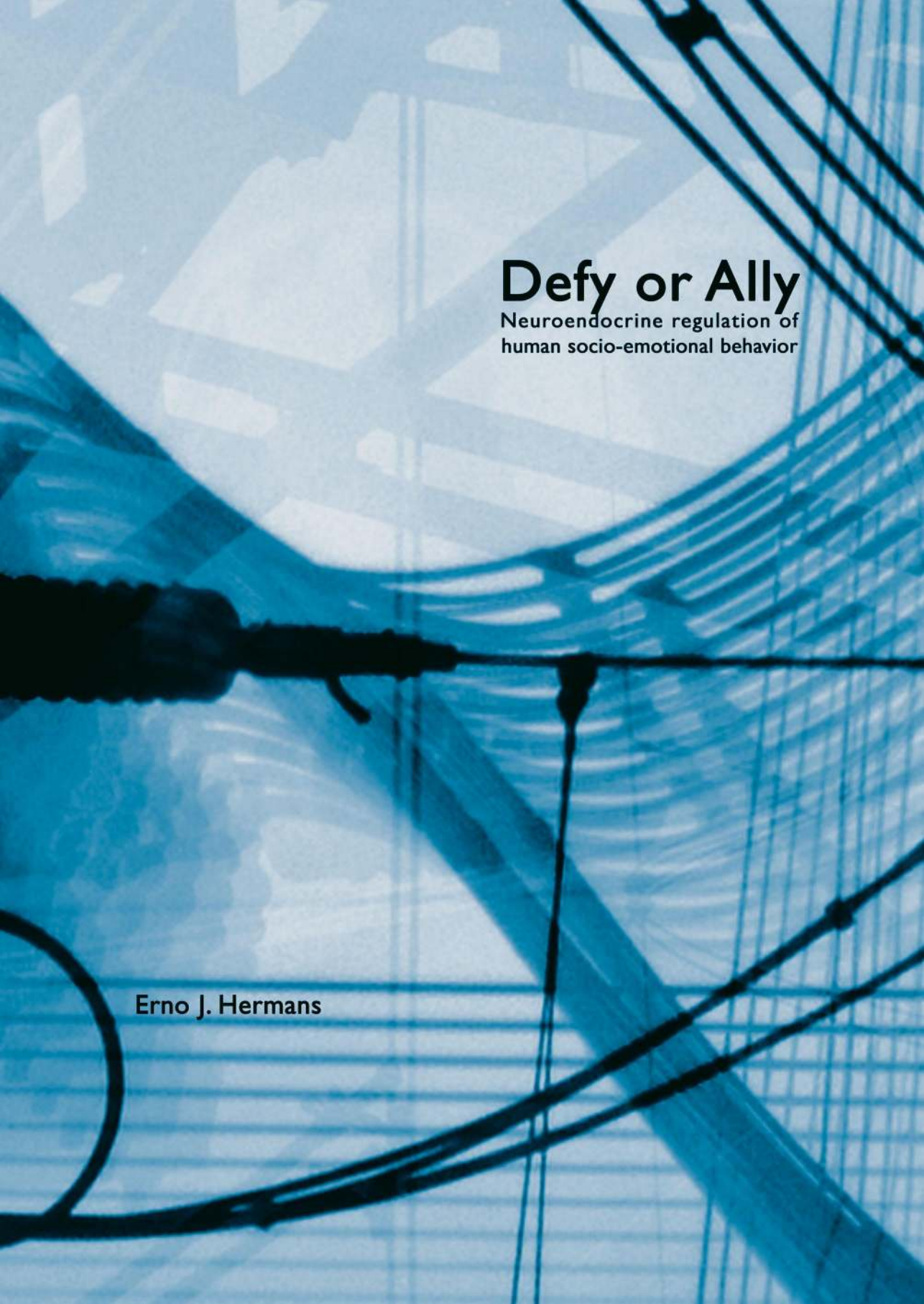

Defy or Ally

Neuroendocrine regulation of human socio-emotional behavior

Erno J. Hermans

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Trotseren of alliëren: Neuro-endocriene regulering van menselijk sociaal-emotioneel gedrag
(met een samenvatting in het Nederlands)

Proefschrift

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1 *Introduction*

1.1 *Phylogenetic roots of the socio-emotional brain*

"Our cortico-linguistic abilities are adept at generating concepts that may have little scientific utility" (Panksepp, 2006)

The central tenet of this thesis will be that humans are profoundly social animals, and therefore are social *by nature*. This is not to say that *social* is an attribute that covers entirely the essence of humanity, nor does it imply any moral judgment. It does imply that, among others, evolution has endowed human brains with specialized mental apparatus that generates uniquely human social behavior – which encompasses everything from affiliation to aggression (Mayr, 1974; Öhman & Dimberg, 1984). Moreover, as will become clear, social behavior is so fundamentally intertwined with emotional behavior, that one can justifiably refer to these jointly as "socio-emotional" (Adolphs, 2003).

Psychology and its related disciplines have come a long way since the behaviorist era of the first half of the twentieth century. This empiricist tradition held that man enters the world as a *blank slate* and that behavior can only be explained in terms of learning. This view confined psychology to the science of learning, and in doing so, set the behavioral sciences apart from the natural sciences: if behavior can only be explained by learning and cannot be reduced to component biological phenomena on a different level of explanation, it follows that underlying biological processes cannot be a causal force in shaping behavior (Tooby & Cosmides, 1992). The so-called cognitive revolution in the second half of the twentieth century tore down one of the pillars of behaviorism, namely, the doctrine of prohibiting reference to mental states. However, with its metaphor of the mind as a computer, cognitive psychology still implicitly adhered to the conception of the mind as a *blank slate*–like information processing device. This view has begun to erode with the application of modern evolutionary theory to psychology.

Toward a naturalist evolutionary psychology

Evolutionary psychology embraces the notion that evolution and its main principles of natural selection and inclusive fitness are the driving force behind the phylogeny of species (Dawkins, 1976), a process that necessarily includes brains. Environments exert selection pressures that, when imposed upon a gene pool that generates random variance, adapt species to optimally reproduce in their current environments. Therefore, the concept of adaptations – an evolved structure, trait, or process that somehow enhances reproductive success - is central to this approach, and is thought to apply to the psychological domain as much as it does to the physiological domain. This view should not be mistaken for a nativist position within a nature-nurture framework.

Instead, modern evolutionary theory allows transcending this classic distinction. Because natural selection causes genes to *incorporate* environments, it is impossible to separate nature and nurture as distinct causal factors in shaping behavior. A simple example can make this clear: species adapt to threats in their environments by evolving mental states of fear in order to motivate flight in appropriate circumstances (see, e.g., Rosen & Schulkin, 1998). Both nature - a brain that contains an adaptation that generates mental states of fear - and a fear-eliciting environment now are necessary conditions for fear states to occur. During evolution, the sensitivity of such a fear system is adjusted to the danger level of the current environment, much like setting a threshold for an alarm system. This process weighs the costs of misses against the costs of false alarms. If we now consider an individual that is excessively fearful, one way to conceive of this is that there is a mismatch between the *current* and the *genetically incorporated* environment (Cosmides & Tooby, 1999). Importantly, this demonstrates that a dichotomous choice between nature and nurture now becomes arbitrary: who can tell if the environment is too dangerous, or if the genotype is too fearful?

This type of analysis is of paramount importance when one tries to understand different kinds of socio-emotional psychopathologies from an evolutionary perspective. Such analyses should always start with the simple question if the given condition is a simple defect, or may be the result of (dys)functional defenses. These distinctions are common in medicine; it is, for example, of obvious importance to recognize that fever is a defense and is in itself not a defect. It has often been argued that similar analyses should precede considerations about psychopathology (Baron Cohen, 1997; Cosmides & Tooby, 1999; Nesse, 2000). Moreover, if not a simple defect, psychopathological conditions can simply be side effects of the intact workings of an evolved defense system, they can be a result of natural variation within a such a system, or a functional adaptation within an evolutionarily novel environment, among several other options (see Cosmides & Tooby, 1999 for a comprehensive discussion, and see chapter 2.1 for an application of this line of reasoning). Many of these questions can only be answered empirically (Stein, 2006).

From this point of view, it is important to investigate not only heritability of clinical disorders, but also the distribution of broader sub-syndromal traits across the population, because these are important clues as to whether the observed condition results from simple defects or not. Similarly, the search for endophenotypes, component markers of neuropsychiatric disorders between symptomatology and genotype (Gottesman & Gould, 2003; Panksepp, 2006), can provide important clues in this regard. Spectrum approaches seem especially appropriate in the case of disorders in the social domain, such as social phobia, antisocial personality disorders, and autism, which are all commonly interpreted as extremes on a spectrum of sub-clinical phenotypes

(e.g., Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001). There are also notable exceptions to this rule, such as fragile X syndrome and Williams syndrome, which clearly result from simple, genetic, defects.

The core heuristic strategy of the evolutionary psychology approach outlined above is to apply the principle of adaptationism to the human mind. Adaptations can be thought of as the "unit" of natural selection: a randomly generated genetic alteration in a species that coincidentally contributes to solving a problem currently faced by a species, and thus gains a higher chance of being promoted to next generations. The goal of the adaptationist program is not to merely describe the evolutionary background of the development of adaptations. Instead, it attempts to gain new insights into the workings of the mind by taking into account how it came about. In other words, adaptationism attempts to infer proximate design from ultimate causes (Tooby & Cosmides, 1992). Because the starting point of such analyses is a certain problem faced by a species, the solution, the adaptation, is a characteristic that has the function of solving this problem. Evolutionary psychology employs a top-down heuristic by inferring the existence of dedicated and content-specific mental "modules" from such functional evolutionary analyses. Subsequently, the existence of these is subjected to empirical scrutiny. Several such mental adaptations have been proposed in the socio-emotional domain: an intuitive psychological theory of other agents (Theory of Mind; Baron Cohen, Leslie, & Frith, 1985), a module for coalitional computation, or the automatic evaluation of allegiance of conspecifics (Kurzban, Tooby, & Cosmides, 2001), one for social exchange and detection of cheaters (Stone, Cosmides, Tooby, Kroll, & Knight, 2002), one for detection of and responding to imminent threat (Öhman & Mineka, 2001), and of course one for language (Chomsky, 1968; Pinker, 1997). Specialized computational apparatus has also been proposed to deal with non-goal directed causation, such as an intuitive theory of the physical behavior of non-agents (Theory of Bodies; Leslie, 1994).

This modular adaptationist approach, however, has been criticized on several grounds. First, it is important to appreciate the fact that adaptationism as applied to psychology shares some fundamental assumptions with a functionalist philosophy of mind. Functionalism holds that mental states can in principle be disembodied: mental states consist of information, and relate causally to one another. Therefore, it is thought, they can be implemented in multiple physical media, and are thus not restricted to brains but may theoretically be realized in non-biological systems as well. It has been argued that this view is dualistic, because it allows for two independent levels of causation, the mental and the physical. Psychological adaptationism implicitly adheres to this "multiple realizability" view by positing mental modules as functional information processing units the importance of whose specific neural implementation is not of primary interest.

The second criticism partly follows from the first and concerns the implicit assumption that

natural selection will favor *optimal* solutions (Gould & Lewontin, 1979). This is important, because proper design often forces to redesign core aspects of a system, taking a step back, in order to be able to continue development without reverting to ad-hoc solutions. Importantly, design through evolution cannot use this strategy, but is forced to create ad-hoc additions or functional shifts (i.e., exaptations; see Gould & Lewontin, 1979) to meet new environmental challenges. The attribute of design can therefore only be used metaphorically when applied to brain evolution. The brain, quite clearly, is *tinkered* rather than designed (see also, e.g., Vroon, 1992). The result of this is that it does not resemble a single unit information processing device in any meaningful way. Instead, the brain appears to be more of a multilayered conglomerate of specialized subunits, each of which has its own evolutionary background. Comparative studies are therefore essential for our understanding of brain function (Panksepp & Panksepp, 2000). Examination of the fossil record, and both phylogenetic and ontogenetic development, reveals that brain evolution has progressed through a series of distinct stages. These stages are reflected to a strong degree in the brains of different classes of extant species, although the parallel should not be taken too literally, as evolution has evidently not come to a complete halt at the lower stages. One influential classification of these stages is reflected in Paul Maclean's (1990) concept of the *triune brain* – consisting of protoreptilian, paleomammalian, and neomammalian subcomponents that are built upon the "neural chassis" of the lower brain stem areas and spinal cord.

Briefly, the protoreptilian formation, or the r-complex, comprises the most advanced brain structures that humans share even with reptiles. It includes structures built around the thalamus, such as the putamen, caudate, and nucleus accumbens, which are jointly referred to as the striatum. The r-complex regulates primordial ritualistic and compulsive behaviors.

The paleomammalian formation is the part of the brain that is common to humans and other mammals, and corresponds to what is known as the *limbic* system because it is wrapped around the r-complex structures like a girdle. It consists of areas such as the hypothalamus, hippocampus, and amygdala. These areas contain the circuits for regulation of primitive fight-flight, nurturing, and reproductive behaviors. All these are both affective and rudimentary intraspecific behaviors. Thus, there is a vast overlap between affective and social behaviors at this level, which appears to reflect the development of early mammals into social animals.

The neomammalian formation, finally, corresponds to the neocortex, and has emanated rapidly in the evolutionarily most recent development from non-human primates to humans. It is widely held that the neocortex makes humans distinctly human by implementing the capacity for rationality. It is thought-provoking, however, to observe that the rapid growth of the neocortex coincided with an evolutionary increase in sociality. For instance, comparative data indicate a correlation between social group size and neocortical volume, suggesting that neocortical growth

arose out of pressure to outwit conspecifics with what has been referred to as *Machiavellian* intelligence (Dunbar, 2003), rather than an increase in general problem solving abilities.

It is furthermore interesting to note that Paul Maclean did not conceive of this last stage of evolutionary development as the seat of the conscious mind: according to Maclean, each subcomponent of the triune brain has its own "mentation" or subjectivity. Thought-provokingly, but controversially, Maclean argued that subjectivity in lower parts of the brain has a more rudimentary form, also referred to as "affective consciousness" which is conceptually akin to affective forms of *core consciousness* (Damasio, 1999) or *primary consciousness* (see Panksepp, 2005) – a pure subjective sense of "being there", stripped of any form of reflective self-awareness.

The precise classification of structures into three distinct stages of phylogenetic development, and even more so, their specific functions, may be disputed. However, it is important to emphasize the following observations of the architecture of the human brain. First, functional specialization is ubiquitous. Conceptualizations of the human brain as a general purpose learning machine are therefore difficult to reconcile with what is known of functional anatomy. Second, the degree of specialization appears to vary as a function of evolutionary age: evolutionarily newer structures appear to be typified by increased plasticity (Panksepp & Panksepp, 2000). In terms of evolutionary psychology as put forward by Tooby and Cosmides (e.g., 1992), this means that the degree of dedication and content-specificity of mental adaptations seems to increase with evolutionary age. Consequently, evolutionary analyses are a safer venture for functions that are clearly localized. Third, the functions that are most clearly localized are the pathways that regulate socio-emotional behavior. Fourth, cross-species homology is evidently stronger for lower areas, and therefore, animal models are especially feasible within the domain of affect, when one entertains the notion that animals possess basic affective experiences that are parallel to ours (Panksepp, 2005). Finally, when studying more complex social processes such as altruism and pro-social cooperation, the starting point of such analyses should involve interactions between socio-cognitive systems and affect, in other words, this suggests that the capacity for empathy might underlie more complex forms of social behavior (Harris, 2003).

In sum, psychological adaptationism is a precarious enterprise when one ignores actual brain architecture, and requires strict ontological and epistemological discipline. Evolutionary psychology constrained, but also inspired, by these neuroanatomical limitations will benefit from moving beyond a functionalist view on the mind-body problem, towards a more naturalist conception of the relation between mental states and brain states, such as a relation of *supervenience* – which holds that no change in mental state can go unaccompanied by a change in brain state. Guided by neuroscientific data, as will become clear further on, one can resist the urge to conceive of other agents as applying a "folk psychology theory" to infer our mental states.

And with it, the necessity to invoke informational states of mind, or beliefs, as a level of causal explanation may gradually fade.

In the remainder of this chapter an account will be put forward of how increasing socio-emotional capacities in superimposed layers of the brain can heuristically be understood as implementing increasing levels of *intentionality* (Brentano, 1874; Dennett, 1989). The philosophical concept of intentionality refers to the level of mental reflection implicated in a mental process. For instance, computers are thought to possess no intentionality, or zero-order intentionality, because they have no subjective awareness of their ongoing "mental" processes. In contrast, first order intentionality roughly corresponds to self-awareness, and higher order intentionality to representations of the minds of a second person, third person, and so on.

In the following paragraph, it will be described how zero-order intentional, reflex-like, processes around the brainstem gradually transformed into first-order intentional mental systems with increasing limbic control. The subsequent paragraph will describe how specific neocortical socio-emotional "devices" may have promoted the development of higher order intentionality.

Zero to first order intentional socio-emotional behavior

Reflex-like homeostatic functions that are regulated through the autonomic nervous system and the neuroendocrine axes can justifiably be characterized as zero-order intentional. At this level, the integration of body and mind is particularly compelling: with their efferent connections and afferent feedback loops these systems firmly integrate mind and body in order to jointly counter external challenges (Öhman, Hamm, & Hugdahl, 2000), and maintain homeostasis (Cannon, 1929), forming a dynamic system that has the capacity to rapidly mobilize energetic resources, but also to down regulate activity afterwards for recuperative purposes (see also Rosen & Schulkin, 1998). It is important to understand how these phylogenetically shaped interfaces help to organize primitive socio-emotional behavior and have paved the way for the gradual emergence of increasingly complex social-emotional processes. In gaining an understanding of these systems, it is useful to emphasize that at all levels of organization, socio-emotional systems can meaningfully be divided into two functionally antagonistic super-categories of "approach-related" and "withdrawal-related" behaviors. Although this categorization is a simplification, this twofold organization appears to persist even throughout higher levels of organization in the neocortex, as will be described below.

Autonomic Nervous System (ANS): The ANS, which has retained its name from before it was recognized to be more centrally regulated than once thought (MacLean, 1990), is the part of the vertebrate nervous system that controls several types of physiologically supportive and

homeostatic functions (Cannon, 1929). Functionally, the ANS balances recruitment of resources for internal and external demands posed to the organism (Öhman, Hamm et al., 2000; Porges, 1995a). The two subcomponents of the ANS, the sympathetic (SANS) and parasympathetic (PANS) branches, usually, but not necessarily always, act in an antagonistic fashion.

The main function of the PANS, the phylogenetically oldest of the two branches, appears to be recuperation and energy conservation: for instance, the PANS suppresses heart rate through the vagus ("wandering") nerve, it promotes digestion by increasing blood flow to the gastrointestinal tract, and increases anabolism. In contrast, the SANS supports energetically expensive physiological processes that are required for adaptive responses to imminent environmental threats. It increases heart beat frequency, restricts blood flow to the gastrointestinal tract and the skin, and promotes catabolism. It furthermore supports action by dilating blood vessels around skeletal muscles and lungs, and at the same time promotes oxygen uptake by dilating the lung bronchi. Its activation results in stimulation of the adrenal medulla to release adrenaline, which in turn further stimulates sympathetically innervated organs through adrenergic receptors. Sympathetic afferent feedback connections furthermore cause sympathetic activation to increase with motor activity (see Öhman, Hamm et al., 2000, for a comprehensive discussion).

The concept of antagonistic activation of the sympathetic and parasympathetic branches, and its complementary concept of "autonomic balance", has eroded over the years, as more and more it became clear that both co-activation and co-inhibition occur in certain circumstances. For instance, during fearful responses strong sympathetic activation may be accompanied by parasympathetic drive to the gastrointestinal system. Coupled activation also accompanies sexual behavior. Examples of decoupled activation are observed in heart rate responses to discrete stimuli, which can change directionally in a multiphasic fashion as a function of relative sympathetic and parasympathetic involvement. These findings gave rise to the modern conception of a two-dimensional autonomic *space* (Berntson, Cacioppo, & Quigley, 1991). Porges (1995b) further refined this by showing that the parasympathetic drive through the vagus nerve is twofold and is controlled by distinct brainstem subnuclei, the dorsal motor nucleus of the vagus and nucleus ambiguous (NA). These two branches appear to control orienting (i.e., short-lived stimulus-coupled bradycardia), and respiratory sinus arrhythmia (i.e., respiratorily coupled bradycardia), respectively. The phylogenetic origin of this separation is argued to lie in the transition from reptiles to primitive social mammals, in order to meet the new metabolic demands of a shift from a primordial behavioral repertoire and survival strategy of foraging, feeding, and freezing towards a more complex mammalian strategy that requires rapidly alternating energetic mobilization and conservation during active fight-fight and social affiliation, respectively. This adjustive system, that came to be under control of limbic areas such as the amygdala, which evolved around the

same period, appears to function like a brake pedal that is released only when in need or when actively exploring (Porges, 1995b). It is fascinating to note that the vagus also controls somatic muscles of the larynx, pharynx, and the esophagus through its so-called *special visceral efferents*, voluntary motor efferents, and has strong neuroanatomical connections with facial nerves, which attest to the role of the NA vagal system in integrating vocalization, facial expression, and ingestive reflexes such as sucking and swallowing with energetic regulation through its cardiac and bronchial efferents. Together, these appear to form a "social engagement system" (Porges, 2003). Thus, the development of an active "smart" vagus next to the reptilian "vegetative" vagus coincided with the emergence of active *voluntary* motor system (Porges, 1995b). One may surmise that primitive volition, or decoupling of rigid "reptilian" stimulus-response couplings to allow for more flexible employment of environmental resources (Öhman, Flykt, & Lundqvist, 2000; Scherer, 1994), may have set the phylogenetic stage for the emergence of rudimentary affective mental states that instead of forcing the organism to follow its instinctual path, merely create a mental incentive to do so. Thus, this line of thinking implies that first order intentionality has arisen out of this process of decoupling stimulus and response.

It is furthermore striking to observe the level of integration of basic socio-emotional systems with facial expressions of emotion, which implies that these expressions are a constituent part of evolved socio-emotional behavioral repertoires. Darwin already acknowledged this fact by emphasizing the significance of facial expressions to the study of social behavior (Darwin, 1872), a view that has attracted a large following (Ekman, 2003a; Fridlund, 1994), and will recur throughout this thesis in discussions of aggression and fear.

Neuroendocrine axes: The adrenal and gonadal neuroendocrine axes form a second mind-body interface. Although their functions are largely complementary, there is little literature that provides a functionally integrated account of the autonomic and neuroendocrine systems, especially incorporating the gonadal axis. Below, a brief description will be given of the both the adrenal and gonadal neuroendocrine systems, followed by a discussion of their functions in regulating socio-emotional behavior.

The hypothalamic-pituitary-adrenal (HPA) axis has functions that parallel those of the SANS in terms of promoting energetically costly processes at the expense of disregarding long-term bodily maintenance. It is sometimes referred to as the *limbic* HPA axis to emphasize the fact that it is under dense control of the limbic areas in the amygdalar and hippocampal region (De Kloet, Joels, & Holsboer, 2005). When activated, the paraventricular nucleus of the hypothalamus secretes the neuropeptide corticotropin releasing hormone (CRH), which in interaction with arginine vasopressin (AVP) drives anterior pituitary release of adrenocorticotropic hormone

(ACTH), which in turn travels through the blood stream to the adrenal cortex where it stimulates the release of the glucocorticoid cortisol. Cortisol has a host of important peripheral effects, including elevation of blood glucose levels, immunosuppression and promotion of catabolism. Afferent negative feedback loops to amygdalar and hippocampal regions make the HPA axis into a complex dynamic system that is just beginning to be understood. HPA axis disturbances play a role in the pathophysiology of a wide range of psychopathological conditions, including posttraumatic stress disorder (Yehuda, 1997) and depression (De Kloet et al., 2005).

Gonadal steroid release is controlled through the release of gonadotropin releasing hormone (GnRH) from the hypothalamus, which in turn triggers the release of luteinizing hormone (LH) from the pituitary. The gonadal system is, obviously, highly sexually dimorphic. In males, LH stimulates the Leydig cells in the testes to produce testosterone. In females, testosterone is produced in much smaller amounts in the adrenal cortex and the ovaries. Furthermore, the HPG axis controls the estrous/menstrual cycles and thus the release of estradiol and progesterone.

Testosterone has a host of organizational effects that are the driving force behind gender differentiation, such as prenatal genital virilization and pubertal development of secondary sex characteristics. However, the HPG axis clearly also has afferent feedback loops that influence brain function in the short term. Manipulation studies in animals as well as correlational studies in humans strongly suggest that these activating effects of testosterone upon socio-emotional behavior are best characterized as promoting reproductive, competitive, and aggressive behaviors, and thus help to shape gender-specific socio-emotional behaviors (Mazur & Booth, 1998). However, the area of neurobiological backgrounds of gender differences in socio-emotional behavior remains profoundly understudied in contemporary literature, with human research being almost exclusively correlational and based on self-report (Archer, 1991). One of the main focuses of this thesis, therefore, will be to identify causal activating effects of gonadal steroids in molding human socio-emotional behavior.

Neuroendocrine feedback and limbic neuropeptidergic systems: There is an increasing body of research on the neuropeptidergic systems that integrate intraspecific behaviors at the level of the limbic system. The most important agents at this level are CRH, AVP, and oxytocin (OTC). All of these have multiple functions centrally and peripherally. For example, CRH and AVP regulate HPA axis activation in a synergistic fashion. Peripherally, AVP controls vasoconstriction to increase blood pressure and water reabsorption in the kidneys, among others. OTC stimulates lactation, and also plays an important role during parturition. Centrally acting CRH, AVP, and oxytocin are released from several locations throughout the limbic system, such as the paraventricular and supraoptic nuclei of the hypothalamus, but also in higher areas such as de medial amygdala and

bed nucleus of the stria terminalis.

Most research has been dedicated to the role of CRH as the central neuropeptide in synchronizing activity in central circuits of fear and anxiety (De Kloet et al., 2005). When activated, the basolateral and central nuclei of the amygdala and the bed nucleus of the stria terminalis drive efferent connections to various limbic and brainstem areas that control neuroendocrine and autonomic correlates of fear. Moreover, through the nucleus reticularis pontis caudalis, action readiness is increased through potentiation of the startle reflex (Walker, Toufexis, & Davis, 2003). On the other hand, actions of central oxytocin are functionally related to those described earlier for the vagal system, namely, to produce a calm, anti-stress, and affiliative state of mind (Porges, 2001). OTC is known from animal studies to be involved in pair-bond formation in monogamous animals (Young & Wang, 2004), and appears to serve similar goals in humans (Taylor et al., 2000). AVP, in contrast, has been implicated in aggression, among others, in animals (Ferris, 2005) and humans alike (Lee & Coccaro, 2001).

There are many indications that neuroendocrine systems act on these central neuropeptidergic systems in order to integrate mental states with physiological processes underlying intraspecific behaviors such as reproduction, social avoidance, aggression, and social affiliation. For instance, testosterone is known to potentiate AVP, which in turn synchronizes limbic activity to promote reproductive and aggressive behaviors (Giammanco, Tabacchi, Giammanco, Di Majo, & La Guardia, 2005), and to suppress CRH and the HPA axis to reduce fearfulness (Viau, 2002). Glucocorticoids produced through the HPA axis are known to enhance CRH expression in the amygdala in the long term (Rosen & Schulkin, 1998), and appear to disrupt reproductive behavior through suppression of the gonadal endocrine axis.

Current research efforts are directed towards gaining a better understanding of the role of these systems in the pathophysiology of various psychiatric conditions, and pharmacological manipulations of these mechanisms are increasingly viewed as potential novel therapeutic targets (Holmes, Heilig, Rupniak, Steckler, & Griebel, 2003). Therefore, more research into the acute effects of afferent feedback of the neuroendocrine systems through its steroid end products is of paramount importance, and will be the focus of this thesis. A thorough description of the complex interactions between neuropeptidergic mechanisms that regulate socio-emotional behaviors at the level of the limbic system is beyond the present discussion, but will be addressed in the relevant chapters in this thesis.

First to higher order intentionality: neocortical emotion regulation and empathy

Cortico-subcortical interactions and emotion regulation: One of the important phylogenetic developments that coincided with the evolution of the prefrontal cortex (PFC) is the ability to exert regulatory control over limbic areas such as the amygdala in order to mold affective states in a more flexible fashion. Higher dorsolateral parts of the prefrontal cortex, which are also implicated in working memory and executive function, appear to regulate volitional control of emotional processes and cognitive reinterpretation of emotional events (Ochsner & Gross, 2005). Lower parts of the PFC, i.e., the ventromedial and orbitofrontal areas, are thought to supply the prefrontal cortex with afferent visceral feedback of affective states in order to create so-called *somatic markers* (Damasio, 1999). Lesions to these areas result in uncontrolled affective behavior that is characteristic of acquired sociopathy (Blair, 2004). In agreement, hypometabolism in these areas is observed in patients diagnosed with antisocial and borderline personality disorders, both of which characteristically exhibit severe deficits in impulse control (Lee & Coccaro, 2001).
Interestingly, the aforementioned organizational division of affective systems into approach and withdrawal categories persists even at the level of the prefrontal cortex. A robust finding is that approach related affective states or dispositions are related to increased left-sided activation of the PFC, whereas withdrawal-related states are right-lateralized (Davidson, 2002). An important question is how socio-emotional constructs such as anger and aggression would fit into this framework. Although traditionally conceived of as a negatively valenced, and thus right-lateralized affective state, recent correlational data indicate that anger is left lateralized, and should be conceived of as an approach-related affective state (Harmon-Jones, 2003b). This issue will be addressed in this thesis, as will be the putatively parallel lateralization of control of the autonomic nervous system (see Öhman, Hamm et al., 2000).

Neocortical representation of higher order intentionality: How the mind accomplishes the key social skill of representing mental states of others, or *higher order intentionality*, has been a question that has haunted psychology and philosophy for a long time (e.g., Dennett, 1989). Two rivaling accounts have been put forward. A functionalist position, referred to as *theory* theory, holds that our cognitive systems are equipped with specialized folk-psychology *theories* that are used to infer intentions from the actions of other people, much resembling the way in which a scientist studies nature. This philosophical position is the precursor of what later became known as *Theory of Mind* (ToM) in psychology, and became popular as an explanatory model for the socio-cognitive deficits associated with autism (Baron Cohen et al., 1985). A rivaling position attempted to take higher order intentionality out of a functionalist framework of information processing by stressing the importance of imitation, and became known as *simulation theory*: representing another person's

state of mind not by describing and analyzing it in any way propositionally, but by recreating another person's state of mind within one's own mind as a means of establishing intersubjective understanding (e.g., Heal, 1986). This account has more appeal from a phylogenetic point of view, among others because it entails more evolutionary continuity in the distribution of socio-cognitive skills across primates by assuming a common core of simulative abilities. This avoids the unsatisfactory consequence of putting humans, so to speak, on an evolutionary pedestal, because other primates are typically denied mental capacities such as ToM (see Premack & Woodruff, 1978).

In strong support of this notion, newborns are able to mimic gestures on other's faces from a very early stage on, a phenomenon that seems hard to explain in terms of second order intentionality derived from systematically acquiring beliefs based on empirical interaction, as implied by *theory* theory. It is important to stress the fact that imitation of facial gestures not only entails the recreation of a motor program, it also implies *intermodal* mapping (Meltzoff & Moore, 1977): as the infant cannot see its own facial expression, it has to match its own tactile information with the visual input provided by the other.

Recent years have seen a substantial rise in popularity of the simulation account, which was fuelled by two key neuroscientific discoveries. First, a long lasting debate on the nature of mental representations underlying imagery was resolved with decisive findings indicating that mental imagery is intricately linked with ipsimodal perceptual representations. For instance, retinotopic visual representations have been shown to underlie visual imagery (Kosslyn, 1994). Second, a thought-provoking serendipitous discovery was made during a single-cell recording experiment when a neuron whose firing rate was being recorded reacted not only to the monkey reaching for a piece of fruit, but also to the researcher, presumably out of appetite, performing this same act. This discovery of these so-called *mirror neurons*, which respond equally to observation and production of actions, has had a large impact on current thinking on the nature of higher order intentionality, because it was the first to reveal a possible neural substrate of simulation. In many subsequent single cell recording studies in primates, this finding was reproduced systematically (see Iacoboni, 2005; Keysers & Perrett, 2004, for reviews). Because single cell recording is, for obvious reasons, too invasive for human research, other neuroscientific methods have been employed to investigate this notion in humans. For example, it has been shown that motor-evoked potentials induced by transcranial magnetic stimulation of primary motor areas are increased during observation of the corresponding movement performed by an actor (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995). Using functional magnetic resonance imaging, it has furthermore been shown that a network of cortical action representation areas is activated both during observation and execution of movement performed by actors. This network consists of

the superior temporal sulcus, posterior inferior frontal gyrus, premotor areas, and rostral parietal lobule (Iacoboni, 2005). Of these, the superior temporal sulcus appears to contain a dedicated mechanism for inferring biomechanically plausible motions (Giese & Poggio, 2003). The inferior frontal gyrus and parietal lobule are thought to exhibit "mirror" activity, thus, these appear to be involved in the recreation of the motor representation in the observer. Subsequently, the motor program itself is activated through the pre-motor areas.

It is easier to understand the significance of this these findings to our understanding of intersubjective representations and intentionality once this line of thinking is applied to socio-emotional higher-order intentionality, or empathy. This provides an explanatory framework within which findings of mimicry in neoneonates makes perfect sense: rather than applying theories to infer affective states of other actors in a propositional fashion, we possess specialized machinery that allows us to recreate affective mental states of others in our own brain, and thus to experience them *from a first-order perspective*. Much current research is being devoted to understanding the specific neural substrates of these competencies. For instance, we now know from several years of human functional neuroimaging research that a number of cortical areas are highly specialized to process distinct aspects of socio-emotionally relevant cues, such as facial motion in the superior temporal sulcus, detailed processing of faces and identity in the fusiform "face" area, and tagging of affective qualitities to faces in the amygdala (reviewed by Adolphs, 2003). Although the lion's share of this research has investigated processing of facial expressions, one may assume that the principles generalize to other affective modalities, such as prosody and postures (de Gelder, 2006). It is becoming more and more clear that these specialized "socio-neuroceptual" areas interact with the aforementioned areas comprising the mirror neuron system in order to recreate affective states and thus achieve *empathy* in a sense more literal than many have thought possible (Decety & Jackson, 2004). Thus, in order for true empathy to occur, activity cannot remain restricted to the level of the neocortex but implies a firm integration and interaction of our neocortical apparatus with the aforementioned primordial, subcortical areas that implement affective states (Harris, 2003). This process ultimately not only brings our mind in a state of affective *resonance*, but perhaps our entire *body*.

In sum, the scientific developments sketched above have given great impetus to the study of empathy and imitation. For instance, deficits in these mechanisms have recently been put forward as a novel framework for understanding autism (Williams, Whiten, Suddendorf, & Perrett, 2001), a hypothesis that will be explored in this thesis. Another line of reasoning that will be pursued is that gender differences in empathetic tendencies will need to be incorporated into this framework, and that this implies a role for the limbic neuropeptidergic and neuroendocrine systems in mediating these higher level processes as well.

Conclusion

This introductory chapter started with the observation that humans are an intrinsically social species. It was argued that human social behavior is best understood from within an evolutionary framework, and that such analyses should be constrained by neuroscientific facts. The socio-emotional brain is fundamentally multi-layered and multi-representational, and rests for a large part on structures that are strongly homologous with those in early mammals. Many large gaps in our knowledge base become evident once this approach is taken. For instance, the importance of neuroendocrine afferents in shaping species-specific and gender-specific socio-emotional behavior remains underappreciated. This observation strongly endorses the bottom-up approach that is taken in this thesis, which will be outlined in the next chapter.

1.2 *Outline of this thesis*

The goal of this thesis is to gain novel insights into human socio-emotional behavior and its neuroendocrine regulation by integrating theory and methodology from cognitive psychological, neuropharmacological, and neuroscientific traditions. The first part of the thesis will cover the neural and endocrine regulation of social aggression and social anxiety. The second part will concern neuroendocrine regulation of fear circuitry. Finally, the last part of this thesis concerns the higher order socio-emotional process of empathy and pro-social behavior. The main research aims and hypotheses of these three parts will be summarized below.

Psychoneuroendocrinology of social anxiety and aggression

This part of the thesis will start with a review on social anxiety and social phobia from the point of view of neuroevolutionary psychobiology that was detailed in the previous section. Assuming that processes underlying aggression and social anxiety have evolutionary roots in the organization of social groups, and can be conceived of as opposite ends of a continuum, a summary will be given of recent research and theory on social anxiety and its related psychopathological condition of social phobia.

Subsequently, a number of empirical studies are reported that sought to establish connections between traits and neurobiological substrates of aggression and reactions to angry facial expressions. The first three of these employed a research methodology that was adopted from cognitive-emotional psychology. Central to this tradition are the concepts of attentional bias and selective attention. Cognitive theories of psychopathology emphasize how patients diagnosed with different psychopathologies perceive the world through a negative filter which reinforces their negative stance by selectively processing congruent information. A large body of research has investigated these biases in healthy volunteers as well as in patients (Williams, Mathews, & MacLeod, 1996). An often used strategy to study such processes is to assess the degree to which processing of an emotionally charged stimulus disrupts execution of a primary task that participants are instructed to perform, such as naming the color of the stimulus. These so-called modified emotional Stroop tasks, modeled after the classic Stroop paradigm (Stroop, 1935), have proven to be a feasible laboratory model of the processes in which attentional biases are implicated. It has been argued, however, that tasks that use verbal stimuli lack the ecological validity that is necessary in order to study the processes that underlie neuroception and processing of true socio-emotional stimuli (Van Honk, Tuiten, de Haan, van den Hout, & Stam, 2001).

A modified version of this task was therefore devised for the present investigations that employed facial expressions as a more ecologically valid type of stimulus. Previous research has indicated that facial expressions are potent stimuli to recruit attentional resources (Vuilleumier, 2002). Moreover, it has become clear that facial expressions can evoke expression-specific effects even

when presented at durations that disallow conscious perception of these stimuli. This *subliminal* stimulus presentation is done by masking the stimulus with a subsequent scrambled stimulus after a very short (i.e., less than 30 ms) stimulus onset asynchrony. This procedure results in diminished verbally reportable identification of the facial expression while still eliciting measurable responses, which makes it a valuable method for studying unconscious emotional processes (Wiens et al., 2004). Furthermore, in chapter 2.1, it is argued that angry faces form a special category of stimuli that communicate social threat, and are therefore especially appropriate stimuli for studying processes underlying social anxiety and aggression, and less so to study fear, for which these stimuli have frequently been used. For instance, selective attention to angry faces has previously been shown to be predicted by strong anger traits (Van Honk, Tuiten, de Haan et al., 2001), high testosterone (Van Honk et al., 1999), and low cortisol (Van Honk et al., 1998). In chapter 2.2, an experiment is reported that investigated relations between selective attention to angry facial expressions and individual scores on the Behavioral Inhibition / Behavioral Activation Scales (Carver & White, 1994), which are questionnaires based on these two putatively orthogonal dimensions of behavioral disposition derived from Gray's (1982) model. From the point of view that anger is an approach rather than a withdrawal related emotion (Harmon-Jones, 2003a), the hypothesis was tested that *behavioral approach*, rather than *inhibition*, drives selective attention to the angry facial expression, In a similar vein, the experiment described in the subsequent chapter investigated selective attention for angry faces in social phobia. The prediction that was tested here is that, contrary to tasks that use verbal socially threatening material, socially phobic patients exhibit diminished selective attention to angry faces, or even avoidance. Furthermore, relations will be investigated between selective attention to facial threat and respiratory sinus arrhythmia, which is a measure of cardiac vagal tone, or nucleus ambiguous-driven inhibitory control of heartbeat frequency. Subsequently, as described in chapter 2.4, another patient group was investigated using the same task. These patients, who are diagnosed with a complex form of post-traumatic stress disorder, dissociative identity disorder (DID), exhibit such phenomenological fluctuations in affect that these are subjectively experienced as distinct identities. A masked version of the emotional Stroop task using angry facial expressions is used here to investigate selective attention in two such distinct identities, one that is self-reportedly unaware of trauma, and another that is trauma-fixed and subjectively anxious. Response patterns over these two identity states will be explored in comparison with a healthy control group.
In sum, jointly, the first four chapters center on the following broad hypothesis:

> *Hypothesis 1: Attentional bias for angry facial expressions is predicted not by generalized anxiety but by anger and behavioral approach tendencies.*

The experiment reported in the following chapter (2.5) employed a causal manipulation by means of transcranial magnetic stimulation (TMS) in order to investigate affective prefrontal asymmetry (see Davidson & Irwin, 1999). TMS makes use of a powerful magnetic field to transiently change local neuronal excitability. When applied repetitively (i.e., rTMS), this technique is capable of exerting effects that extend beyond the period of rTMS stimulation. Low frequency (< 1 Hz) stimulation typically results in temporary inhibition of an area. Thus, rTMS can be used to induce a shift in frontal lateralization (Schutter, van Honk, d'Alfonso, Postma, & de Haan, 2001).
Most accounts of prefrontal affective asymmetry adhere to the framework of the *valence hypothesis*, which states that positive and negative affects are left and right lateralized, respectively. However, recent correlational data indicate that a classification in terms of motivational direction (i.e., approach versus withdrawal) may be more appropriate, which implies that anger may be left lateralized (Harmon-Jones, 2003b). The experiment reported in chapter 2.5 therefore tested the following hypothesis:

> *Hypothesis 2: Within the framework of prefrontal lateralization of approach versus withdrawal, anger belongs in the approach category, and is thus left lateralized.*

Specifically, the prediction was tested that slow, inhibitory, rTMS over the right as compared to left dorsal part of the prefrontal cortex results in increased anger-related processing of angry faces. Furthermore, it was predicted that this increased selective attention would be accompanied by increases in sympathetic control of the heart, which is monitored by assessing the pre-ejection period using impedance cardiography (De Geus & van Doornen, 1996).

The final chapter of this part describes an investigation of effects of testosterone on human aggression proneness. Putative relations between testosterone and aggression are based on animal research and human correlational data (Archer, 2006), however, no causal evidence is available in humans. Here, an experiment is described that employs functional magnetic resonance imaging to explore the neural circuitry of human social aggression and its regulation by testosterone.
In a first session, responses to angry facial expressions were investigated as a function of endocrine parameters. It was expected that responding in limbic areas would be associated with an endocrine profile of high testosterone and low cortisol (Van Honk et al., 1998; Van Honk et al., 1999). Subsequently, in a double blind placebo-controlled crossover design, the prediction was tested that administration of testosterone would result in an enhanced response to angry faces in the areas that are implicated in reactive aggression (Blair, 2004), i.e., the amygdala and several of its

efferent structures such as the hypothalamus (see Van Honk, Tuiten, Hermans et al., 2001), and thus tested the following hypothesis:

Hypothesis 3: Testosterone potentiates reactive aggression circuits.

Psychoneuroendocrinology of fear circuitry

The first two chapters of this section concern studies that used a single administration of testosterone, the end-product of the hypothalamic-pituitary-gonadal axis, in healthy female volunteers in order to test effects of this gonadal steroid upon human fear circuits. Psychophysiological measurements of functioning of the autonomic nervous system, in combination with measurements of startle reflex modulation, are the most widely used methods to study fear-related processes in humans. An important reason for this is that these paradigms are often very similar to those used in animal research, which facilitates comparative research strategies.

In the experiments described in chapter 3.1 and 3.2, two such paradigms were applied in combination with placebo-controlled administrations of .5 mg of testosterone in female participants. The first study made use of a paradigm that was pioneered by Grillon and co-workers (1991). In alternating blocks, participants were informed that they either might, or would not, receive a mild electrical shock through electrodes attached to the wrist. This procedure results in very robust *fear* potentiation of the startle reflex. Startle eye-blink reflexes can be evoked using a burst of noise and are quantified using facial electromyography of the orbicularis oculi muscle. In a follow-up testosterone administration study reported in chapter 3.2, a closely related paradigm (see Lang, Bradley, & Cuthbert, 1997) was adopted that made use of standardized photographs from the International Affective Picture System (Center for the Study of Emotion and Attention, 1999) to elicit stimulus-locked emotional responses. This procedure allowed for analysis of event related electrodermal and heart beat frequency responses in order to monitor sympathetic and parasympathetic autonomic nervous system functioning. Moreover, startle probes were presented during picture viewing in order to replicate findings from the preceding chapter. Based on a line of animal research (Aikey, Nyby, Anmuth, & James, 2002; Bitran, Kellogg, & Hilvers, 1993; Boissy & Bouissou, 1994), here, the following hypothesis was tested:

Hypothesis 4: Testosterone reduces fear.

Previous research has shown that in these types of experiments, standard anxiolytic drugs such as benzodiazepines mainly affect baseline startle magnitudes, rather than fear potentiated startle. This suggests that these drugs are partly sedative and may partly also reduce unspecific anxiety

(Baas et al., 2002). As there is no indication that testosterone has sedative properties, it was expected that testosterone administration would result in attenuation of fear potentiated startle specifically, without affecting baseline measures. Moreover, sympathetic autonomic nervous system responses (as opposed to parasympathetically regulated decelerative heart rate responses) to aversive picture content were also predicted to be reduced after testosterone administration.

In the final chapter of this part, an experiment is reported that investigated feedback effects upon central fear circuitry of the hypothalamic-pituitary-adrenal axis through its end-product cortisol. Cortisol is strongly implicated in the pathophysiology of depression and anxiety disorders, with a consistent finding of chronically elevated cortisol levels in patients (Rosen & Schulkin, 1998). Recent findings, however, indicate that short term effects of cortisol may be different. For instance, it has been suggested that the negative feedback control that cortisol exerts on its own production, may be accompanied by an anxiolytic effect (Buchanan, Brechtel, Sollers, & Lovallo, 2001). Paradoxical findings that administration of cortisol shortly after trauma decreases incidence of PTSD (Schelling, Roozendaal, & De Quervain, 2004) can be interpreted as supporting this notion. Moreover, cortisol administration has very recently been shown to alleviate phobic fear (Soravia et al., 2006) and selective attention to fearful facial expressions (Putman, Hermans, Koppeschaar, Van Schijndel, & Van Honk, submitted), although conflicting results have also been reported (Tops, Wijers, van Staveren et al., 2005). Chapter 3.3 therefore employed the most widely adopted methodology to investigate fear systems, the threat of shock paradigm that was also employed in chapter 3.1, in order to investigate the following hypothesis:

Hypothesis 5: Cortisol acutely reduces fear.

In particular, we expected that a dosage that has been shown to reduce the acoustic startle reflex in a previous study (Buchanan et al., 2001), would attenuate not only unspecific baseline acoustic startle, but also fear potentiation of the startle reflex.

Psychoneuroendocrinology of empathy

In the last part of this thesis, two studies are reported on empathy, from the point of view, detailed above, that higher order social processes such as empathy are rooted in perspective taking through imitation. To investigate these notions, a paradigm was adopted that allows for quantification of spontaneous mimicry of facial expressions (cf. Dimberg, 1982) by recording facial electromyography (EMG) while participants view pictures of emotional expressions. Specifically, we measured EMG power in the zygomatic major and corrugator supercilii muscles,

which are implicated in posing facial expressions of happiness and anger, respectively. Healthy volunteers are known to exhibit rapid and automatic stimulus congruent EMG activity in response to facial expressions (Dimberg, Thunberg, & Elmehed, 2000; Dimberg, Thunberg, & Grunedal, 2002).

As already mentioned in the previous chapter, recent theoretical developments in understanding the pathophysiology of autism spectrum disorders suggest that deficits in automatic "mirroring" of motor programs may pave the way for the later development of the well-documented deficits in Theory of Mind (ToM) competencies (Williams et al., 2001). The following hypothesis was therefore investigated:

Hypothesis 6: Autistic traits are related to reduced spontenous mimicry of facial expressions.

As it is increasingly acknowledged that autistic disorders likely reside at the end of a spectrum of autistic traits (Baron-Cohen et al., 2001), healthy volunteers that scored either extremely high or extremely low on the autism spectrum quotient questionnaire were selected from a large database. The main prediction was that the high trait autism group would exhibit reduced mimicry of facial expressions.

Finally, the experiment reported in chapter 4.2 employed a similar paradigm in order to test effects of testosterone upon spontaneous mimicry of facial expressions. Testosterone is associated with aggression (Mazur & Booth, 1998) and anti-social personality disorders (Stalenheim, Eriksson, von Knorring, & Wide, 1998). Moreover, participants that score high on a self-report empathy questionnaire exhibit more spontaneous mimicry (Sonnby-Borgstrom, 2002). Men, who have testosterone levels about eight times higher than women, furthermore show decreased mimicry in response to facial expressions. The following hypothesis was therefore investigated:

Hypothesis 7: Testosterone reduces empathic behavior.

In a placebo controlled crossover design, female participants received sublingual administrations identical to those used in chapter 2.6, 3.1, and 3.2. In order to elicit a strong baseline mimicry effect, we made use of dynamic facial expressions. The rationale behind this was that perception of emotional expression is strongly facilitated by movement (Sato, Kochiyama, Yoshikawa, Naito, & Matsumura, 2004). Morphing software was therefore used in order to create two second duration movie clips of actors posing either happy or angry facial expressions. The prediction was tested that mimicry of facial expressions as measured by congruent EMG activity while watching the movie clips would be attenuated after testosterone administration.

II

Psychoneuroendocrinology of social anxiety and agression

2.1 *Toward a framework for defective emotion processing in Social Phobia*

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Abstract

Introduction: This paper explores and outlines an evolutionary approach to understanding Social Phobia (SP) as a developmental disorder in brain mechanisms that regulate socio-emotional behavior.

Method: A literature review of cognitive, neuronal, and endocrine correlates of SP is presented using an integrative approach.

Results: SP patients present with a specific and developmentally stable functional neuroanatomical and neuroendocrine profile that can be linked to findings of cognitive attentional abnormalities.

Discussion: It is argued that SP is the human counterpart to primate subordination stress and develops from clearly identifiable precursors in early childhood, the understanding of which requires fundamental insights into the regulation of socio-emotional behavior. The current state of knowledge speaks strongly in favor of a diathesis model, in which distorted cognitions that are characteristic of SP are secondary to hyperexcitability of fear circuits that set off at least as early as at pre-verbal ages and ultimately may lead to the development of SP.

Introduction

The widely varying prevalence estimates (Cuthbert, 2002; Merikangas, Avenevoli, Acharyya, Zhang, & Angst, 2002) of Social Phobia (SP; also known as social anxiety disorder) can be seen as symptomatic for the not unquestioned status of this disorder in psychiatry today. It can be taken to merely signify a lack of unequivocal diagnostic criteria, but also, to indicate that SP is not a discretely definable condition. Indeed, it is reasonable to assume that the extreme social fears endured by diagnosed patients will to some extent overlap with the, perhaps controllable, but sometimes overwhelming stage fright that the majority of people has had to cope with when confronting a large audience for the first time. Unlike, for instance, psychotic psychiatric conditions, the symptoms of SP seem imaginable, at least, for everyone.

This is not the venue, however, to discuss the question if SP should be considered a "real" disorder. What this observation illustrates is that SP can plausibly be conceived of as the far end of a spectrum of normal fears (Rapee & Spence, 2004), which have adaptive value when within normal range (cf. Rosen & Schulkin, 1998). As a consequence, an understanding of the underlying problems associated with SP requires fundamental knowledge of the mechanisms underlying emotionality as well as social behavior. If seen as the end of a spectrum, studies on individual differences within the normal range, as well as on the other end of the spectrum (i.e., psychopathy), are indispensable for an understanding of this disorder, while SP is a very interesting subject of fundamental interest in itself because it gives the opportunity to observe a system "at its extreme". Reciprocally, fundamental knowledge gathered from this might foster new avenues in treatment.

Starting from this point of view, research has made considerable progress in recent years in delineating the neuroanatomical and neurochemical mechanisms behind socio-emotional functioning. As reviewing all studies in this field would be beyond the scope of this paper, we put our focus on some of our own work relevant to SP, along with a discussion of relevant findings from patient studies. Our aim will be to provide a link between cognitive, neural, and endocrine correlates of SP. But first, we will address the question what is special about social anxiety.

Are social fears any different from other fears?

A recurrent question in the study of any anxiety disorder is what sets this specific disorder apart from others, in terms of symptomatology, etiology, or biological markers. From the point of view we take in this paper, we argue that this question should be rephrased to ask what sets *social anxiety* apart from other *anxieties*, and even, what sets social behavior apart from other behavior. In the following we will argue that social behavior in humans is highly guided by primordial, dedicated social communication systems that have evolved to serve this purpose.

An interesting starting point in this regard is the observation that the objects of human fears and phobias are not arbitrary (Marks, 1969). Phobic stimuli seem to be largely restricted to certain categories, like animals (snakes, spiders, mice), heights, social interaction, etc. A striking common feature of all these is that even though some of these stimuli may still be associable with adverse events occurring in modern societies, the vast majority of actually threatening objects in the contemporary environment of man seems incapable of becoming the object of phobic fear. Intuitively, it would seem likely that the prevalence of car phobia, for instance, should be much higher than spider phobia, as it is reasonable to suppose that less people have suffered traumatic experiences with spiders than in traffic. Similar puzzling but striking observations have been reported in relation to SP: the mean age of onset of this disorder differs substantially from other anxiety disorders, and roughly corresponds to pubertal age, a developmental period when social hierarchies start to emerge (Öhman, 1986). Furthermore, SP also contrasts with other anxiety disorders, such as specific phobias, panic disorder, and generalized anxiety disorder, in the absence of a strong preference for the female sex (although epidemiological data indicate that SP occurs slightly more often in women; Merikangas et al., 2002), which can be taken to suggest a connection to the stronger hierarchical organization of male social order. However, the possible significance of these observations, if any, remains to be elucidated.

Many have argued that these observations make sense when viewed from a functional evolutionary perspective: fear serves to motivate behavior (e.g., fight or flight) that increases chances of survival (Marks & Nesse, 1994) or "makes us want to do what our ancestors had to do in order to survive" (Öhman, Flykt et al., 2000, pp 297). Thus, the evolutionary development of a characteristic such as fear can be viewed from within the same framework of evolutionary biology: a certain genetic change that gives one member of a species a slight edge over another in the competition for reproduction, may spread itself fast among the gene pool, especially when this alteration promises a solution to a pending problem for this species. It is this development of a new clearly definable characteristic of a species, an adaptation, that is of primary interest to evolutionary biology, and it is the tenet of evolutionary psychology that the same mechanisms also apply to the psychological domain (Tooby & Cosmides, 1992).

Drawing upon ethological studies, Mayr (1974) distinguishes between communicative and non-communicative animal behavior. The latter refers to behavior in relation to the inanimate physical world. According to Mayr, it is this type of behavior that relies most on open genetic programs, meaning that there is a less rigid relation between genotype and the phenotypic outcome of the development of this program. In order to be fully able to exploit natural resources in a dynamic environment, an animal has to be able to learn from its previous experiences.

In communicative behavior, however, the stakes are higher. This category refers to interactions

with other organisms. In interspecific communicative behavior, especially in dealing with natural predators, every learning experience is potentially fatal. Therefore, Mayr argues, relatively closed genetic programs likely guide communicative behaviors instead. However, the optimum in terms of survival chances lies in a trade-off between the certainty of rigid closed programs in recurrent situations and adaptability to changing environments of open programs. The balance between these determines, in the long run, the relative closure of genetic programs that guide these behaviors.

It is conceivable that relative closure of genetic programs is even higher when it comes to social behavior. Communication between primates mostly relies on postures, gestures, and facial expressions. All social animals show dominance hierarchies in which some members gain higher status than others. Formation of these classifications is said to take place at a highly symbolic level. Because of these symbolic acts there is no need to revert to actual violence between individual members of a group. When a subordinate individual reciprocates a gesture of dominance or aggression with the appropriate sign of appeasement, the dominant member has achieved his goal as in so doing his social status is reinstated. Displays of dominance include, for instance, showing ones teeth or raising the eyebrows. Averting the eyes and appeasing smiles, on the other hand, can be seen as signs of submission. Öhman (1986) hypothesizes that human social fears can be traced back to these primordial social systems. Following Mayr's (1974) supposition that intraspecific behavior bears heavily upon closed genetic programs, one may surmise that remnants of these primitive communicative systems are still part of the human behavioral repertoire.

In sum, arguably, the question if social fears are different from other fears should be answered affirmatively from a theoretical point of view. Over the last decade, empirical evidence that converges with these suppositions has accumulated following the advent of in vivo brain imaging techniques, which are especially suitable to investigate localization and specialization of functions and brain regions. Thus, we now know that "the social brain" relies on a network of relatively specialized brain areas (Adolphs, 2003). Not surprisingly, many of these regions overlap with those to which affective functions have been ascribed, to such an extent that it is justifiable to refer to this network as the "socio-emotional brain". Specific aberrations within this network are thought to underlie specific pathologies, among which autism and SP stand out as the prominent. The latter being the topic of this chapter, we will address three different lines of research: in the next section, we will discuss a somewhat older branch of cognitive research that has attempted to make one of the core symptoms of SP quantifiable: the attentional focus on social scrutiny, and has done so by pursuing a line of research that aimed at the quantification of information processing biases in this disorder. As this line of research has subsisted in relative isolation of other research into the biological and neural deficits associated with SP, we aim at providing a

direct link between these fields in the subsequent section, just as we have done in conducting our own research.

Selective attention in SP: vigilance or avoidance?

One can argue that the term "selective attention" is pleonastic: because we are incessantly flooded by sensory information, attention is selective by definition. Whereas we can, of course, select information by volition, some information appears to us to break its way into our focus of attention all by itself. It is this latter type of attention that the term selective attention refers to. An appealing example is how within a stream of unattended words coming from multiple surrounding speakers, the sudden mention of one's own name is able to rigorously draw attention to this one speaker. This example makes clear that attention allocation is not only guided by serial, conscious processes that we might use, for instance, to search an area for a specific object. Rather, attention allocation mechanisms work in an automatic, parallel fashion (McNally, 1995), continuously scanning the environment for potentially salient information. It is easy to see how such a mechanism might subserve a fear system: it is too late to flee a predator if you have to stumble upon it. Because of the high stakes involved, *false positives* must outnumber *misses*. But, as too much of anything is not good, excess false positives come at a cost. Hence, such attentional biases have been suggested to play a crucial role in the development, and especially the persistence, of anxiety disorders. The reason for this is that selective attention may become a circular, self-reinforcing mechanism when anxiety is triggered by the detection of threat, and vice versa, response thresholds are lowered by increased anxiety (Mathews & MacLeod, 1985).

These thoughts quickly gained momentum within cognitive psychology, supported by an ever growing body of empirical evidence that backs the notion that attentional mechanisms of patients with various anxiety disorders are specifically tuned to process cues relevant to their disorder (Williams, Mathews et al., 1996). The greater part of this research has employed cognitive interference paradigms, in essence based upon the original Stroop effect (Stroop, 1935). These paradigms allow assessment of selective processing of certain cues by determining their interference with a very easy, primary task, mostly color-naming of the stimulus. Thus, most studies presented patients with words relevant to their condition, and required them to name the color in which this word was printed as fast as possible. This procedure rests on the assumption that, when participants' attentional mechanisms automatically allocate processing capacity to the semantic aspects of the stimulus, less capacity is left for the primary task, which is reflected in longer response latencies. Typical data were reported by Mattia, Heimberg, and Hope (1993): SP patients show delayed color-naming to words pertaining to social scrutiny and rejection. In sum, using such cognitive emotional paradigms, one of the core symptoms of SP, rumination on social

scrutiny, became quantifiable in the psychological laboratory.

Several problems, however, arise from the use of verbal stimuli in these studies. For instance, an alternative explanation for these findings can be found in the relatively higher frequency of use for these words that patients can be thought to exhibit due to excessive rumination (see e.g. Abbott & Rapee, 2004), even though stimulus words used in these studies are mostly matched on general frequency of use (Williams, Mathews et al., 1996). Even though these problems might be overcome by a more careful selection, verbal descriptions of threat cues cannot be equated to the threat itself, and can instigate an emotional response only indirectly. It is for this reason that many researchers now opt for pictorial stimuli, often facial expressions, in their research (Clark, 1999; Van Honk, Tuiten, de Haan et al., 2001; Vuilleumier, 2002).

Early findings using facial expressions emphasize the preferential access of these stimuli to attention allocation mechanisms. For instance, in a visual search paradigm, participants were quicker to determine the presence of an angry facial expression amidst neutral distractors than conversely (Hansen & Hansen, 1988). Although this study may have been hampered by methodological problems (Purcell, Stewart, & Skov, 1996), the basic finding of this experiment has recently been replicated using carefully selected schematic faces (Öhman, Lundqvist, & Esteves, 2001). Moreover, in a similar paradigm, Byrne and Eysenck (1995) found detection speed to vary with measures of trait anxiety.

In comparable spatially oriented attentional tasks relations were found between personality characteristics and proneness to direct attention toward a significant stimulus. One such task is the facial *dot probe* task. In this paradigm, participants view long runs of trials in which they have to detect a probe, which is presented laterally onto the screen, that replaces either a threat cue (an emotional face) or a control stimulus (mostly a neutral face). Response latencies in this task putatively reflect the degree to which the participants' spatial attention has been reallocated in response to presentation of the threat stimulus. In a series of experiments, relations between trait anxiety (Bradley, Mogg, Falla, & Hamilton, 1998), levels of dysphoria (Bradley et al., 1997), and levels of social anxiety (Mogg & Bradley, 2002), and the strength of this attentional effect were established. However, results have not always been consistent. Mogg, Philippot, and Bradley (2004) reported that stimulus onsets asynchronies (SOA's) between cue and probe of 500 ms, SP patients are quicker to identify the probe than controls. Contrariwise, Chen, Ehlers, Clark, and Mansell (2002) found SP patients to be slower at the same SOA. One possible explanation for this discrepancy is a difference in control stimuli between these two reports: the former employed neutral faces, whereas the latter used household objects. Additionally, there is discussion about the question what the dot probe paradigm actually measures: the degree to which a stimulus is capable of drawing attention, as presumed originally, or the difficulty to disengage attention from

a threatening cue, as some have argued recently (Koster, Crombez, Verschuere, & De Houwer, 2004).

Besides this, it is important to recognize the fact that facial threat may elicit a process that is wholly different from semantic threat. Whereas persistent thoughts of social rejection and negative self-evaluation may play an important role in SP, everyday life of SP patients is characterized by avoidance of social scrutiny rather than excess attention towards it (cf. Clark, 1999), which is manifested in evading eye contact. Results consistent with this view were reported by Horley, Williams, Gonsalvez, and Gordon (2003), which demonstrate, using an eye-tracking device, that SP patients are prone to avoid processing facial features, especially eyes. In our own research, we have sought to capture this phenomenon in a pictorial version of the original Stroop task. In this task, participants have to name the color of the stimulus, which is either a colored emotional, or a neutral facial expression. Thus, one is said to exhibit selective attention towards a stimulus when the response latency for the emotional expression is longer than that for the neutral expression.

As has been laid out in the beginning of this chapter, submission gesturing such as gaze aversion, and provocation using angry or contemptous facial expressions, may subserve peaceful organization of primate social systems (Öhman, 1986; Sapolsky, 1990). Within this evolutionary framework, facial expressions are viewed as vehicles of communication, each of which has evolved to communicate a specific message (Fridlund, 1994). Thus, one cannot equate the threat that is communicated by the angry face to that signaled by the fearful face (Adams & Kleck, 2003), a fact that has often been overlooked in the past. The fearful face conveys the presence of threat somewhere in the environment (Whalen, 1998). Therefore, the degree to which one exhibits selective attention to fearful faces is related to state anxiety levels (Hermans et al., 1999; Van Honk, Schutter, d'Alfonso, Kessels, & de Haan, 2002).

Contrariwise, the angry facial expression signals provocation to the observer. While this can be viewed as a threat signal as well, it is important to recognize the fact that by means of gaze aversion, the subordinate individual is relieved of this threat. Therefore, selective attention to angry facial expressions may be determined by a different dimension, ranging from socially anxious to socially dominant, in which the former is expected to cause attentional avoidance of this facial threat. A study involving participants selected upon extremely high versus low trait anger questionnaire scores found support for this hypothesis (Van Honk, Tuiten, de Haan et al., 2001). Attentional bias toward angry facial expressions was not found in the low trait anger, conceptually similar to the subordinate individual, but in the high trait anger group. Subsequently, a second experiment showed that self-reported trait anxiety was not predictive of selective attention to angry faces. Moreover, Putman, Hermans, and Van Honk (2004, see chapter 2.2) recently found a positive relation between attentional bias in the same task and high scores on the behavioral activation

scale (Carver & White, 1994), a concept that encompasses a wider range of approach behavior than mere anger (Carver, 2004). The same data indicated that self-reported *social* anxiety was predictive of attentional avoidance of angry faces (i.e., shorter reaction times to angry faces). Finally, the most recent data from our laboratory indicate that this avoidance effect extends to SP patients (Hermans et al., submitted for publication, see chapter 2.3).

In conclusion, when compared with other, lexical, studies on attentional biases in SP, our findings with more ecologically valid pictorial stimuli are directly opposite. We argue this reflects a dissociation between primordial, reflex-like responses to social threat, and cognitively guided attentional responses that occur at a different level of verbal and conscious deliberation. While a specific pattern of cognitive rumination on social scrutiny may accompany SP, we hypothesize that these cognitions appear as a consequence of aforementioned primitive responses that have their roots in primordial subordination stress (see also Mathew, Coplan, & Gorman, 2001). In the following we will further explore this dissociation between cognitive and reflex-like responses and their possible connection to subordination stress in our discussion of neural and endocrine correlates of SP.

Central regulation of social anxiety and SP

The Amygdala: It is widely recognized that the fear system revolves around the amygdala. The involvement of this small bilateral limbic structure in different aspects of fear has been amply documented (see Davis & Whalen, 2001, for a review). The amygdala is crucially involved in the acquisition and expression of conditioned responses (Bechara et al., 1995), but also in unconditioned fear (Whalen, 1998). For instance, patients that have suffered strokes to this particular structure show impaired recognition of emotional expressions but can still correctly identify familiar faces (Adolphs, Tranel, Damasio, & Damasio, 1995). Although its specific role in social behavior is the subject of debate (Amaral et al., 2003), consensus is emerging that the function of this structure is best characterized as a protection device that is at least involved in emotional aspects of social behavior.

Afferent input to the central nucleus of the amygdala arrives from the hippocampus as well as many cortical areas. Moreover, it may receive sensory input through a pathway from the thalamus, through the superior colliculus and pulvinar (Morris, Ohman, & Dolan, 1999), which is suggested to be a remnant of a primordial mechanism for fast, makeshift selection of signals of imminent danger (amply described in LeDoux, 1996). Respectively, these account for activation of the fear system caused by events retrieved from memory or contextual conditioning, by cognitive ruminations or elaborate sensory analysis, and by sudden impending threat (Lang, Davis, & Ohman, 2000). Efferently, the central nucleus of the amygdala is highly involved in

modulating the peripheral physical changes associated with emotional responses. Some of the projected sites include the lateral hypothalamus which mediates sympathetic autonomic nervous system responses like heart rate increases and skin conductance responses, the nucleus reticularis pontis caudalis implied in startle potentiation, and the nucleus ambiguus which is involved in parasympathetic control of the heart. Hypothalamic projections also regulate the endocrine system, as will be described in the next section.

Not surprisingly, neuroimaging research in SP has for the greater part focused on functioning of this structure, and has, by and large, been successful in supporting the notion of hyperexcitablility of the amygdala in SP. For instance, SP patients exhibit exaggerated amygdalar responses to neutral faces (Birbaumer et al., 1998). Moreover, responses are enhanced in response to angry and contemptuous faces (Stein, Goldin, Sareen, Zorrilla, & Brown, 2002). Less surprisingly, the amygdala hyperresponds to symptom provocation (Tillfors et al., 2001), and during anticipation of public speaking (Tillfors, Furmark, Marteinsdottir, & Fredrikson, 2002). In sum, one can conclude that the conception that SP is characterized by hypervigilance to social threat cues at the level of the amygdala is well supported. This observation, however, begs the question as to what is the nature of this enhanced reactivity: is it cause or effect? In other words, is this effect a consequence of sensitization of this structure itself, or is it secondary to malfunctioning of another region that it fails to down regulate? We will first consider the latter option, before turning to the possible developmental causes of amygdalar sensitization.

Prefrontal Cortex: A wide array of functions has been ascribed to the prefrontal cortex (PFC), not all of which have direct bearing on socio-emotional functioning. Roughly, cognitive accounts hold this structure responsible for executive function and working memory, the more dorsal parts corresponding to higher levels of cognition. A more or less comparable hierarchical organization has been proposed concerning the emotional functions of the PFC, where goal-directed planning functions, including volitional regulation of emotional processes (Ochsner, Bunge, Gross, & Gabrieli, 2002), are attributed to dorsal parts, and lower level processes that subserve this goal-attainment may reside in more ventral areas. For instance, the ventromedial area is thought to participate in assessment of affective consequences of behavioral options by incorporating feedback from the peripheral nervous system (Bechara, Damasio, Damasio, & Anderson, 1994). The more lateral orbitofrontal area has been implicated in a great many functions, including reinforcement detection in terms of punishment and reward (Kringelbach & Rolls, 2004), the dysfunction of which may lead to antisocial acts (Raine, Lencz, Bihrle, LaCasse, & Colletti, 2000). It should be noted, however, that the functional specifics of all subcomponents of the PFC are just beginning to be disentangled.

A fascinating, but very robust, finding is the lateralization effect that is found in electroencephalographic (EEG) activation of the anterior PFC. During a long period of research, the nature of this anterior asymmetry has been refined more and more, from the valence hypothesis of hemispheric specialization, according to which the left hemisphere is specialized for positive emotion, and the right for negative emotion, to the approach-withdrawal account put forth primarily by Davidson (e.g., 2002), and further detailed to accommodate the emotion anger on the approach side (Harmon-Jones, 2003b; Van Honk, Hermans et al., 2002, see chapter 2.5). Evidence for this asymmetry abounds. To name a few, higher scores on the Behavioral Activation Scale (Carver & White, 1994) predict left dominance (Harmon-Jones & Allen, 1997). Contrariwise, depression is related to left PFC hypoactivity (Henriques & Davidson, 1991). Importantly, relations to other biological markers of an inclination towards inhibited temperament, such as high levels of the adrenal steroid cortisol (see next section), indicate that positive relations with right anterior dominance may appear at an age of only six months (Buss et al., 2003) suggesting firm dispositional roots of this asymmetry.

Several reports have appeared over the last years that have used neuroimaging to investigate prefrontal functioning in SP. First, EEG was used to assess the prefrontal asymmetry in these patients, and yielded results consistent with the above account (Davidson, Marshall, Tomarken, & Henriques, 2000). They recorded EEG data while patients were anticipating a public speech performance, and found that right hemispheric activity increased.

Neuroimaging techniques other than EEG, such as PET and fMRI, have yielded results that are by and large compatible with these ideas, although some inconsistencies remain. For instance, increased regional cerebral blood flow was detected using PET in the right dorsolateral region of the PFC of SP patients (Tillfors et al., 2002), but this right hemispheric dominance was not found by Stein et al. (2002) and Veit et al. (2002). The latter study did report very remarkable findings in more ventral, orbital areas of the PFC. SP was not only compared to healthy control subjects, but also to psychopathic individuals, and yielded results that are consistent with the functional specialization of this area as outlined above. SP and psychopathy were characterized by hyper- and hypoactivity, respectively, in this area, suggesting that incorporation of reinforcement contingencies into affective processes is relatively weak in psychopathy, and relatively strong in SP.

On the down side, different studies report many different cortical areas that are less easily linked to contemporary thoughts of brain function. Several reasons may account for this divergence. First, different neuroimaging techniques have different sensitivities. Functional MRI and PET are superior to EEG in terms of spatial resolution, and may thus be relatively insensitive to the rough anterior asymmetry as measured using EEG. Moreover, “activity” is defined in different

ways: for instance, decreased alpha frequency band oscillations in EEG versus hemodynamic responses to increased local oxygen use in fMRI. If further differences in paradigms, ranging from symptom provocation, and conditioning to passive viewing of pictures, are taken into account, it is hardly surprising that inconsistencies emerge.
Summarizing, one can defend the position that SP is accompanied by a specific profile of prefrontal lobe functioning. But again, the question remains to what extent this difference plays a causal role in SP.

Frontolimbic interactions: As has been outlined in the preceding two paragraphs, the state of knowledge at this point in time, as far as the evidence is concerned, is that SP is characterized by hyperactivity in the amygdala as well as several frontal sites. Based on these facts, it can be argued that the following course of events takes place when SP patients encounter a social cue: their amygdala will most likely exhibit an initial hyperreaction to the event. This will bring about peripheral autonomic nervous system reactions, in such a way that peripheral feedback will converge with slower, more cognitively guided assessment of the same event in the orbitofrontal and dorsolateral parts of the PFC, and bring about a distorted re-assessment in this area. As a consequence, there is less negative feedback to amygdala, which will result in sustained activation in this area.

Endocrine regulation of social anxiety and SP

The endocrine system can be conceived of as a means of communication by which body and mind can change each other's *modus operandi*. Evidently, it allows the brain to command the peripheral physical changes which it deems necessary to attain its goals. Interestingly, contemporary research indicates that the central nervous system, and especially the circuit involved in motivation and emotion, in its turn, is highly responsive to endocrine feedback. Thus, the body may, in its turn, strategically change the mind's priorities by means of endocrine communication. Hence, it is increasingly recognized that the endocrine system plays an important role in regulation of social behavior.
The hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes, and their respective end products, the glucocorticoid cortisol and the gonadal steroid testosterone, are important players in these processes. Both have organizational effects during development, as well as activating effects in the short run. Moreover, both are responsive to the social environment (Flinn, Baerwald, Decker, & England, 1998). Interestingly, the roles that these two systems play in regulation of social behavior appear antagonistic: whereas HPA activity is associated with withdrawal behavior, HPG axis is linked to the approach side. Accordingly, aberrations in

both axes have been implicated in the pathophysiology of different psychiatric disorders. In the following, we will discuss the contribution of each to social behavior and possible connections to SP.

The Hypothalamic-Pituitary-Adrenal Axis: The HPA-axis is a complex system that appears to serve as an interface between central fear states and the peripheral resources these recruit. It is implicated in regulating responses to stressful, impending events, as well as maintenance of states of vigilance (Rosen & Schulkin, 1998). The system has three main components. First, corticotropin-releasing hormone (CRH) is released from de paraventricular nucleus (PVN) of the hypothalamus. This peptide, along with, and in interaction with, Arginine Vasopressin (AVP), triggers the release of adrenocorticotropic hormone (ACTH) in the pituitary, which in turn activates synthesis of glucocorticoids, mainly cortisol, in the adrenal cortex. The mechanisms by which the HPA axis regulates fear have been well delineated in contemporary research. Apart from a host of peripheral effects, including effects on metabolism and immune function, glucocorticoids exert important effects in the brain. For instance, cortisol has been shown to down regulate the HPA axis by inhibiting CRH output of the PVN. This negative feedback is seen as crucial in restricting metabolically costly hyperactivation of the HPA axis. The dexamethasone suppression test (DST) is often used to test this feedback loop: under normal circumstances, cortisol levels drop after dexamethasone administration as this substance initiates the same inhibitory processes as excess levels of cortisol normally would. Contrariwise, there is evidence that cortisol increases CRH gene expression in the central nucleus of the amygdala (Rosen & Schulkin, 1998), which results in hyperactivation of this area, presumably to maintain a state of heightened vigilance while at the same time restricting demanding overactivation of the axis. Abnormalities in HPA axis functioning have been documented in several psychiatric disorders. Assessment of HPA functioning has largely relied on measurements of cortisol, as measurements of cortisol can be achieved non-invasively. Results are not consistent across disorders, suggesting specificity of HPA dysfunctions to various disorders. For instance, in (melancholic) depressive disorders, which have a high comorbidity rate with SP and other anxiety disorders, basal levels of cortisol are by and large above average. Moreover, dexamethasone administration does not result in suppression of cortisol levels in these patients (Carroll, 1984), suggesting aberrations in feedback control over HPA axis functioning.

Early suggestions concerning the role of the HPA axis in regulation of social behavior, indicating a possible connection to SP, stem from comparative research on social stress in primates. For instance, Sapolsky (1990) studied wild olive baboons, and observed hypercortisolism in animals low in the dominance hierarchy. Moreover, these animals exhibited decreased feedback inhibition

in the DST. Pursuing this line of thought, several investigations have been reported from our own lab in which relations between HPA axis functioning and subtle measures of social approach and withdrawal tendencies were investigated in humans, using the emotional Stroop paradigm as outlined above. In one study, high basal salivary measures of cortisol were shown to be related to avoidance of angry facial expressions (Van Honk et al., 1998), an effect that has also been found to be related to socially anxious traits (Van Honk, Tuiten, de Haan et al., 2001, see above).

As stated before, SP has been argued to have its evolutionary roots in subordination stress. Thus, not surprisingly, the hypothesis has been coined that HPA axis dysregulation (i.e., chronic overactivation) might be an important feature of SP. Results of research into this issue, however, have not been unequivocally affirmative. Hypercortisolemia, as found in socially submissive primates, has not been found in these patients. Several studies have documented a failure to find elevated basal cortisol levels (Condren, O'Neill, Ryan, Barrett, & Thakore, 2002; Martel et al., 1999; Potts, Davidson, Krishnan, Doraiswamy, & Ritchie, 1991; Uhde, Tancer, Gelernter, & Vittone, 1994). In addition, Uhde et al (1994) reported no differences between post-dexamethasone cortisol levels between patients and the control group.

However, baseline levels of cortisol do not provide a complete account of HPA functioning. First, responsivity of the HPA axis might be a better determinant of dysfunction than basal levels of its end product. Furlan et al (2001), and Condren et al (2002) used psychological stressors to induce a cortisol response. These studies were the first to report a significant difference between SP patients and control subjects on HPA axis functioning measures. SP patients proved hyperresponsive, and, importantly, this hyperresponsivity was specific to psychological stress (Furlan et al., 2001).

Second, the HPA axis is a dynamic system that is likely tuned in a very early developmental phase to respond adaptively to challenges posed by its environment. For instance, behavioral reactions of young infants (age three) to novel events have proved predictive of salivary cortisol levels at age 5.5 and 7.5 (Kagan, Reznick, & Snidman, 1988). Moreover, children classified as shy by their parents at age four exhibit hypercortisolemia (Schmidt et al., 1997). Furthermore, heightened adrenocortical responsivity has been found in seven year old children with low social competence as compared to highly competent (Schmidt, Fox, Sternberg et al., 1999), although results have not always been consistent (Schmidt, Fox, Schulkin, & Gold, 1999). Thus, the link between glucocorticoid exposure and behavioral inhibition and shyness is strongest during early life. These findings are consistent with animal studies that stress the importance of glucocorticoids during the development of the behavioral inhibition system in rats. These studies showed that overexposure to corticosterone has neurotrophic effects on the hippocampus (Takahashi & Kalin, 1999). This structure is highly involved in negative feedback regulation of the HPA axis, but also plays an

important role in conditioning: While the adjacent amygdala is thought to create associations between conditioned stimuli and conditioned responses, the role of the hippocampus seems to be to create a representation of the environment in which the conditioning experience has occurred (Sanders, Wiltgen, & Fanselow, 2003). Thus, deteriorated functioning of the hippocampus can first lead to hyperactivation of HPA-axis, and second, to overgeneralization of conditioned responses to other, irrelevant, contexts. Note that both increase activation of the amygdala and associated symptoms of fear, and may thus explain the hyperexcitability of the amygdala that has been found to be characteristic of SP in neuroimaging studies (see previous section).

The Hypothalamic-Pituitary-Gonadal axis: Like the HPA axis, effects of the HPG axis originate in the hypothalamus, which commands the release of Luteinizing Hormone (LH) in the pituitary by means of Gonadotropin releasing hormone (GnRH). LH, in turn, causes release of testosterone (T) from the Leydig cells in the testes. Although mostly considered an androgen hormone, T production is not limited to men, although female blood plasma T levels are on average about 10% of male levels. Women produce T in the ovaries and the adrenal cortex in similar amounts, and slight (20-25%) fluctuations occur within the menstrual cycle (Sinha-Hikim et al., 1998), which peak just before the LH surge preceding ovulation. Organizational functions of T include perinatal sex differentiation, and pubertal development of secondary sex characteristics. However, T continues to have activating effects on the brain throughout life.

High basal levels of T have traditionally been linked to aggression, dominance, and competitiveness, or, more generally, approach behavior. Besides the fact that basal levels of testosterone are strongly related to social rank in animals (Mazur & Booth, 1998), raising androgen levels by means of administration or otherwise has been shown to induce anxiolytic effects in a variety of species (Aikey et al., 2002; Boissy & Bouissou, 1994; Bouissou & Vandenheede, 1996). Thus, its possible relevance to SP lies in the fact that its effects are opposed to some of the symptoms of SP.

In humans, testosterone levels are higher in individuals with dominating personality styles. Interestingly, T levels prove responsive to social interaction as well. For instance, in anticipation of competitive events, levels of T increase and remain high until after the contest (Bateup, Booth, Shirtcliff, & Granger, 2002). These responses appear greater when competition occurs outside one's own group than inside (Wagner, Flinn, & England, 2002). Intriguingly, it has been observed that men's HPG axes respond to short interactions with women (Roney, Mahler, & Maestripieri, 2003). Thus, increases in T levels occur as a function of, and in support of, approach-related behaviors.

Only in recent years, studies are starting to be conducted in which behavioral effects of T are tested causally by means of T administration. Many of these studies have investigated the effects

of androgen replacement therapy in patients with endocrine dysfunctions. These studies mostly yielded positive effects in terms of restoration of sexual interest (see Apperloo, Van Der Stege, Hoek, & Weijmar Schultz, 2003). It must be noted, however, that none of these studies has addressed other measures than sexual interest. Interesting recent studies, however, are starting to give good indications that T might have therapeutic efficacy in patients suffering from refractory depression, especially those with low T baselines (Pope, Cohane, Kanayama, Siegel, & Hudson, 2003; Seidman & Rabkin, 1998).

The mechanisms by which T has enhancing effects are unclear due to longer periods of treatment which allow for changes to take place at multiple levels. Therefore, in our lab, several placebo-controlled experiments in which a single dose of testosterone was administered have been conducted. These studies yielded measurable effects on sexual arousal (Tuiten et al., 2000). Moreover, other experiments showed that not only sexual arousal is influenced: using a similar procedure, increased accelerative heart rate reactions to angry faces were found (Van Honk, Tuiten, Hermans et al., 2001). Additionally, participants shifted towards a pattern of more risky choice behavior in the IOWA gambling task (Van Honk et al., 2004). Results from the latter two studies may be summarized as indicating that T is capable of inducing a shift in the balance between punishment and reward sensitivity.

Interactions between HPA and HPG axes: At a functional level, HPA and HPG axes activity seems antagonistic. As mentioned before, end products of the HPG axis have been linked to an increase in types of behavior that are characterized as approach-related: increased reproductive activity, competitive and dominant behavior, and perhaps aggression (Mazur & Booth, 1998), whereas HPA activity is associated with withdrawal behavior, anxiety, decreased libido, and depression. It may therefore be worthwhile to conceive of the HPA and HPG axes as complimentary parts of an integrated system. Indeed, several studies now indicate that both interact at multiple levels.

High plasma levels of circulating glucocorticoids have, for instance, been associated with low basal levels of androgens, a pattern that has been observed in subordinate rodents (Blanchard, Sakai, McEwen, Weiss, & Blanchard, 1993). Correlational studies in humans yield similar results. For instance, high levels of stress, and thus, prolonged exposure to adrenocortical steroids, are detrimental to sexual functioning, presumably as a consequence of inhibition of the HPG axis. This is seen in depression patients with high cortisol levels (e.g., Michael & O'Keane, 2000), but has also been observed in individuals who endured prolonged exposure to glucocorticoids for other reasons, such as long duration exercise (MacConnie, Barkan, Lampman, Schork, & Beitins, 1986). These examples of HPG suppression by HPA end products raises the important question whether these effects are reciprocal. Indeed, recent experimental work on animals indicate that

this is the case. For instance, gonadectomy increases phasic activity of the HPA axis, whereas HPA function is gradually brought back to normal levels as a function of T substitution (Viau & Meaney, 1996). It seems that T exerts these effects centrally through enhancing negative feedback of the HPA axis (Viau, 2002).

In sum, much progress has been made in delineating the neuroendocrine events associated with social functioning. Especially animal models are increasingly detailed in their account of the influence of various hormones on social behavior. Human behavioral research lags behind in this regard, but we think the following conclusions may be drawn. Given the complexity of neuroendocrine systems, studying merely baseline activity of a single system is not very informative. A more promising enterprise is the examination of responsivity, as is becoming increasingly common in human behavioral research. Another step further is to not look at a single system in isolation, but to study interactions between them. Thus, we think a thorough understanding of dysfunctioning of the HPA system in a certain patient population will benefit from considering its interactions with other systems.

Regarding endocrine functioning in SP the following conclusions can be drawn. First, although baseline studies of cortisol have not revealed consistent elevations, the HPA axis has been found to be hyperresponsive in these patients. Second, functioning of the HPG axis in SP remains understudied. We hypothesize that SP is characterized by lower tonic HPG activity, and most importantly, decreased HPG-axis control over HPA activity during social stress (Viau, 2002). Future studies should address this possibility.

Discussion and conclusions

An evolutionary view on etiology: In this paper we sought to put forth the hypothesis that SP is best conceived of as a dysregulation of a defense mechanism, or as an adaptation *gone awry* (cf. Nesse, 2000). Like other fears, social anxiety is functional as long as it remains within boundaries. It has its specific function in regulating social order and the inhibition of inappropriate behavior and antisocial acts, it has its specific pathology when out of bounds, and it has its own neural substrate. It should be emphasized, however, that such an adaptationist argument in itself implies no specific claim on the etiology of SP, it merely establishes the theoretical grounds for treating social anxiety as a separable "mental faculty". Grounding the theoretical viability and utility of this concept allows one to infer specific and separable brain mechanisms that regulate social anxiety, which in turn provide a heuristic path for investigating SP. In other words, an adaptationist approach uses ultimate causes to infer proximate design (Tooby & Cosmides, 1992). The question of how such an adaptation can become a disabling condition in a minority thus needs to be addressed separately. The answer to this question cannot be simply framed in terms of a nature-nurture distinction.

Inevitably, every organism develops as an interaction between genes and environment, and every genotype develops over evolutionary time in interaction with ever changing environments, thus incorporating expected environmental invariants into the genotype. There are many paths within this mechanism by which adaptations can be rendered dysfunctional (see Cosmides & Tooby, 1999). Some possibilities relevant to SP are briefly summarized below.

The first option that can be considered is that SP may be best characterized as a simple functional defect in the mechanisms that regulate social anxiety. This option is not very plausible, because a simple defect would likely yield qualitative rather than quantitative differences with respect to the average population. However, almost everybody has experienced some mild form of the symptoms of SP, and diagnosed SP and severe shyness have proven to be hardly separable in several aspects (Turner, Beidel, & Townsley, 1990). An explanation in terms of a functional defect would therefore be contradictory to a spectrum approach that emphasizes the utility of social anxiety and treats SP as the far end of a broader dimension (Rapee & Spence, 2004).

Second, a possible reason why an otherwise functioning adaptation may become disabling is a mismatch between the current environment and the recurrent environmental constants to which the adaptation was attuned, often referred to as the *environment of evolutionary adaptedness* (EEA). Examples of this abound in other categories: humans for instance have an unhealthy preference for sweet and fat food presumably because of scarcity of certain nutrients in the EEA. An example more closely related to SP is the aforementioned affinity of the human fear system for small creepy animals that hardly pose any genuine threat in modern civilizations, which arguably accounts for the excess incidence of this type of specific phobia (Öhman & Mineka, 2001). One may defend that a similar argument holds for SP: human social behavior likely evolved in smaller and more closed communities than the one we live in now. As a consequence, we daily meet a manifold of the number of strangers that our ancestors met, and we may also speculate that these encounters have become less dangerous because of the way modern civilizations are organized.

The third option that deserves mention here is related to the above. If we assume that selection pressures have calibrated population-wise average social anxiety levels to the average EEA, and that evolution proceeds by generating variance, it can be deduced that such a stochastic process generates dysfunctional extremes at the end of this distribution. These extremes are sometimes referred to as *instance failure* (Cosmides & Tooby, 1999).

In sum, from an evolutionary viewpoint, social anxiety is regarded as an adaptation that, like others, develops during an organisms' lifetime in dynamic interplay between a genetic program and the environmental conditions it encounters. The more *open* a genetic program, the more it relies on "learning" mechanisms to fine-tune the adaptation to the current environment (Mayr, 1974). "Learning" in this sense may be taken to mean everything from acquiring cultural values

to setting a default level of activity of an endocrine system or an autonomic nervous system. Psychopathology such as SP, then, emerges as a consequence of an all too large discrepancy between the *expected* environment and the *real* environment. Mutations in each may underlie the etiology of SP in an individual, but the question on the relative weight of these contributions is one that needs to be addressed empirically. Although reviewing the entire literature concerning the etiology of SP (see for instance Rapee & Spence, 2004) is beyond the scope of this paper, we would like to emphasize that the current state of knowledge indicates that there is at least a significant genetic contribution to SP, with heritability estimates reaching up to .5 (Kendler, Karkowski, & Prescott, 1999). Recent years have witnessed the first attempts to link SP, and correlates of SP, to specific genetic polymorphisms. Although these studies have not yielded wholly consistent results, we will briefly touch upon them below because of their possibly large impact on future directions of research into SP.

There is a growing body of research on a polymorphism in the promoter region of the serotonin transporter gene, which is implicated in serotonin reuptake. The short allele variant, as opposed to the long variant, is associated with less serotonin transporter expression, causing reduced serotonin reuptake. The presence of at least one short allele has been related to self reported neuroticism (Lesch et al., 1996), and mood disorders such as depression (Caspi et al., 2003) and seasonal affective disorder (Rosenthal et al., 1998), although replication failures have also been reported (Flory et al., 1999). It has also been directly linked to brain functioning using an fMRI task that selectively engages the amygdala (Hariri, Mattay et al., 2002): carriers of the short variant exhibit a hyperreactivity of these fear circuits that is comparable to individuals with mood and anxiety disorders. In similar vein, Battaglia et al. (2005) reported that short allele carrying children exhibit a different electrophysiological response to pictures of angry facial expressions. However, attempts to link this polymorphism directly to SP have failed: Stein, Chartier, Kozak, King, and Kennedy (1998) found no evidence of a linkage. Nevertheless, social phobia patients with at least one short allele have been shown to exhibit increased self reported as well as amygdala response to symptom provocation (Furmark et al., 2004). Speculatively, one may argue that differences between short and long allele carriers surface more readily in the context of heightened emotional arousal. Investigations into other polymorphisms that are related to dopaminergic functioning have as yet remained equally inconclusive. Polymorphisms in the dopamine D2, D3, and D4 receptor gene, and the dopamine transporter do not predict SP (Kennedy et al., 2001). In conclusion, it can be argued that research into specific genetic factors that predispose towards SP is still in its infancy, but holds great promise for the future. In due course, it will likely yield an interacting set of polymorphisms rather than a single genotype of SP.

Concluding remarks: We think the data reviewed in this paper compellingly speak in favor of a diathesis model of SP. While SP has a characteristic average age of onset in mid-puberty (Marks, 1969), there are many indications that identifiable precursors of shyness and inhibited temperament occur as early as only few months after birth, which implies that there is a stable pattern of a temperament of behavioral inhibition, starting at a pre-verbal age, leading up to the development of SP (Hirshfeld et al., 1992; Schwartz, Snidman, & Kagan, 1999). For instance, inhibited infants exhibit hypercortisolemia when they reach pubertal age (Kagan et al., 1988; Schmidt et al., 1997), and even show hyperresponsivity of the amygdala to unfamiliar faces as grown ups (Schwartz, Wright, Shin, Kagan, & Rauch, 2003). At a biological level, an explanation for the maintenance of this pattern has been suggested to lie in the organizational effects of early glucocorticoid overexposure in young infants, which is thought to cause amygdalar hyperexcitability and overgeneralization of fear responses as a consequence of decreased hippocampal function (Takahashi & Kalin, 1999). Notwithstanding notable but rare exceptions, such as cases of severe disfigurement (Newell & Marks, 2000), the symptoms of SP generally forge through a pattern of increasing hyperactivity of central fear circuits that dates back to a pre-verbal age. Consequently, although SP may be characterized by excessive cognitive rumination on social scrutiny and negative self-evaluation, we argue that these cognitions are secondary to the mechanisms that ultimately lead to a hyperactive social anxiety system.

2.2 *Emotional Stroop Performance for Masked Angry Faces: It's BAS, Not BIS*

Peter Putman, Erno J. Hermans, & Jack van Honk
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Abstract

Theoretical models concerning selective attention to emotional stimuli predict heightened vigilance to angry faces in people with heightened trait anxiety or greater activity of the Behavioral Inhibition System (BIS). Recent evidence from electroencephalographic lateralization and affect studies and from studies assessing attentional biases to angry faces suggest, however, that heightened anger and activity of the Behavioral Activation System (BAS) should predict vigilant responding to angry faces. Social anxiety should predict avoidance of angry faces. Results from a masked emotional Stroop task verified these hypotheses, but an unmasked emotional Stroop provided no reliable relations. This dissociation confirms earlier claims that masked emotional Stroop performance is impervious to conscious control over the cognitive–emotional processes, as measured by the Stroop task.

Introduction

Research into selective attention to emotionally arousing material has shown that heightened anxiety is associated with selective attention (vigilance) to threatening cues (for overviews, see Mogg & Bradley, 1998; Vuilleumier, 2002; Williams, Mathews et al., 1996). Cognitive models (Mogg & Bradley, 1998; Williams, Watts, MacLeod, & Mathews, 1996) predict emotion-congruent influences in selectivity and vigilance for threatening material by anxious individuals, that is, an attentional bias toward threatening stimuli if anxiety is the prevailing mood state.

Usually, selective attention to emotional material is assessed with the emotional Stroop task (Williams, Mathews et al., 1996). Vigilant responding to an emotional stimulus causes an increase in latencies for color-naming that stimulus (interference), compared with the color-naming of a neutral stimulus. Conversely, a decrease in latencies for color-naming because of the stimulus' emotional content (facilitation) is thought to arise from avoidance of this content. The angry face can be a potent naturalistic threatening stimulus and has, in this context, been used as a fear-related stimulus. However, one should take into account the social signaling function this expression has likely evolved to serve: to challenge conspecifics to a dominance clash (Mazur & Booth, 1998; Öhman, 1986; Van Honk, Tuiten, de Haan et al., 2001). Socially anxious and submissive individuals will appraise such a display as threatening and will yield to the dominant conspecific by breaking eye contact, but aggressive or dominant individuals will be provoked to retaliate rather than be scared off by this social challenge.

Eckhardt and Cohen (1997) showed how an anger-induction procedure caused increased interference for insulting words in an emotional Stroop task. It is thus to be expected that angry and/or dominant individuals will show such vigilant responding to angry faces. Indeed, this has been found repeatedly (Van Honk, Tuiten, de Haan et al., 2001; Van Honk et al., 1998; Van Honk et al., 1999). Van Honk et al. (2001) even failed to show a relation between Stroop performance and anxiety in participants selected on extreme anxiety scores, whereas reallocation of these same participants to high and low-anger groups did reveal the relation between higher anger and interference for angry faces.

This dominance–submissiveness construct might be captured by the broader constructs of behavioral activation and inhibition (Carver & White, 1994; Gray, 1985). Sutton and Davidson (1997) reported relations between the behavioral activation system (BAS) and relative greater left-sided anterior electrocortical activity and between the behavioral inhibition system (BIS) and greater relative right-sided anterior activity, confirming a hypothesized left-right prefrontal lateralization for the major affective constructs of approach and withdrawal. Harmon-Jones and Allen (1997) confirmed the relation between BAS and left-sided anterior activity and found the same for trait anger (Harmon-Jones & Allen, 1998). d'Alfonso, Van Honk, Hermans, Postma,

and de Haan (2000) showed how transient manipulation of anterior asymmetry through repetitive transcranial magnetic stimulation (rTMS) results in shifts from facilitation (right anterior dominance) to interference (left anterior dominance) for angry faces in the emotional Stroop task. As the left anterior cortex is associated with BAS and not BIS activity, it follows that one should find relations between interference for angry faces and BAS, not BIS, even though BIS is very similar to general anxiety, and some models (e.g., Mogg & Bradley, 1998) predict vigilant attentional responding to angry faces in individuals higher in anxiety and BIS.

BAS, as measured by Carver and White's (1994) BIS/BAS Scale, consists of three subscales: BAS Fun Seeking, BAS Reward Responsiveness, and BAS Drive. Our BAS hypothesis is limited to the Reward Responsiveness and Drive subscales. The construct of BAS Fun Seeking is not the kind of approach behavior implicated in these processes. Also, in an experiment designed to validate the BAS subscales, data indicated that Fun Seeking is more sensitive to reflect the inclination to enter new situations of reward cues, whereas Drive and Reward Responsiveness seemed more predictive of affect during behavioral efforts in a context of potential reward (Carver & White, 1994). BIS is a construct very similar to and sometimes equated with general anxiety and is thus not expected to show any relation to an emotional Stroop bias score for angry faces (cf. Van Honk, Tuiten, de Haan et al., 2001). The construct of social anxiety, however, could well reflect the submissive pole of the dominance–submissiveness concept. In primates, such social submission is related to higher cortisol levels, especially in times of lesser stability in the social hierarchy when such potential clashes abound (Sapolsky, 1990). In humans, higher cortisol is associated with extreme shyness (Schulkin, Gold, & McEwen, 1998). Researchers have reported that greater right-sided anterior cerebral activity was related to higher levels of cortisol in rhesus monkeys (Kalin, Larson, Shelton, & Davidson, 1998) and human infants (Buss et al., 2003), further corroborating the hypothesized link between basal cortisol, withdrawal behavior, and right-sided anterior activity. Stroop responses to angry faces have also been related to cortisol levels in humans. Van Honk et al. (1998) showed how higher basal levels of cortisol predicted a more avoidant Stroop response to angry faces. In the present study, we thus expected that participants with higher levels of social anxiety would show a more avoidant response to angry faces.

There are indications that participants are capable of overriding emotional Stroop effects (e.g., Williams, Mathews et al., 1996), and occurrence of such override strategies seems mainly limited to unmasked presentation (MacLeod & Hagan, 1992; Van den Hout, Tenney, Huygens, Merckelbach, & Kindt, 1995; Van Honk et al., 1998; Williams, Mathews et al., 1996). Responding to masked stimuli seems to provide an index of more automatic responding. We thus expected any pattern of attentional bias scores from the unmasked task to be more clearly present in the masked task.

In summary, we predicted a positive relation between BAS Drive and BAS Reward Responsiveness, trait anger, and interference for angry faces and a positive relation between social anxiety and facilitation for angry faces. BIS and anxiety were hypothesized to be unrelated to an attentional bias to angry faces. We expected these patterns to be most pronounced in the masked data.

Method

Participants: Participants were 40 healthy students. Results of 5 participants had to be discarded because these participants scored too many correct responses on the awareness check, and Stroop results from 1 participant for the masked task were lost because of software failure. Reported results are for the remaining 34 participants who provided masked Stroop data (18 men and 16 women).

Material and Apparatus: Faces used in the Stroop task were digitized oval cutouts of 10 neutral, 10 angry, and 10 happy faces (5 male and 5 female actors), colored transparent red, blue, and yellow, from Ekman and Friesen's (1976) pictures of facial affect. Happy faces were included to check for the possible occurrence of biases for emotional faces in general instead of the valence-specific biases hypothesized. The Stroop tasks consisted of 90 trials, each face presented three times, with picture color-counterbalanced across the three face types. Onset of vocal color-naming response was recorded with a Lafayette voice key. Questionnaires used were the trait versions of the State–Trait Anger Scale (STAS; Spielberger, Jacobs, Russel, & Crane, 1983), the State–Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983), the BIS/BAS questionnaire (Carver & White, 1994), and the Willems Social Anxiety Scale (SA; Willems, Tuender-de Haan, & Defares, 1973).

Procedure: Participants performed either the masked task first and the unmasked task second or vice versa, counterbalanced across participants. Completion of a Stroop task always lasted less than 10 min. After completion of the last Stroop task, participants performed a forced-choice awareness check (masked recognition task for the faces) to objectively test for successful masking. Participants were given a 10-min break between performance of the two tasks. After completion of the last Stroop task, participants filled out the questionnaires.

Stroop tasks. In the unmasked task, a fixation cross appeared for 1,000 ms, and after its disappearance, a face was presented and remained visible until computer registration of the vocal response (identification of the stimulus color). After an intertrial interval of 2,000 ms, the next trial commenced. The 90 trials were run in fixed random order. In the masked task, faces were presented for 25 ms and immediately replaced by a contrast-rich masking pattern in the

same color as the face, which remained visible until computer registration of vocal response. Participants were instructed to name the color of the faces or masks as fast as possible without making mistakes.

Data reduction. Outlier latencies (300 ms > latencies > 900 ms and >/< individual mean latency ± 3 standard deviations; 2.3%) were removed, and the remaining trials were used to calculate an individual's bias scores by subtracting mean latencies to neutral faces from mean latencies to emotional faces. A bias score greater than 0 thus indicates interference, and a bias score less than 0 indicates facilitation for that emotional face type.

Awareness check. To check whether the masking procedure had been successful, participants performed an objective forced-choice awareness check. The 30 masked faces were presented again, and participants were asked to indicate whether the presented masked face was neutral, angry, or happy.

Results

Awareness Check: Chance-level performance lies at 10 correct answers, and the binomial upper limit was set at 15 correct answers (one-sided alpha of 5% for n = 30; π = 1/3). Five participants scored 15 or more correct responses on this recognition test, and although each reported not to be able to identify the expressions, their data were discarded. The remaining participants scored an average of 37% correct identifications (against 33% chance-level performance). All other reported tests for the remaining 34 participants were performed with an alpha set at 5%, two-tailed.

Overall Latencies: For the masked condition, the mean latency to neutral faces was 668 ms (SD = 79), the mean latency to happy faces was 671 ms (SD = 78), and the mean latency to angry faces was 676 ms (SD = 79). For the unmasked condition, these mean latencies were 679 ms (SD = 91), 676 ms (SD = 94), and 678 ms (SD = 87), respectively. To test whether the masked condition resulted in stronger overall bias scores, we entered these raw latencies in a 2 (masking condition) × 3 (face type) repeated measures analysis of variance (ANOVA). There were no main effects of masking condition, $F(1, 33) = 0.292$, $P = .593$, or face type, $F(2, 33) = 2.060$, $P = .161$. The Masking Condition × Face Type interaction was significant, $F(2, 33) = 5.636$, $P = .024$. A repeated measures general linear model for the face types in the masked condition showed, $F(2, 33) = 4.494$, $P = .015$; for the unmasked condition, $F(2, 33) = 0.325$, n.s.

To further examine this, we conducted paired samples t tests between latencies to neutral and

emotional faces in both conditions.

For the masked condition, latencies to angry faces were a significant 8 ms longer than latencies to neutral faces, t(33) = 2.979, P = .005. Latencies for happy compared with neutral faces were, however, not significantly longer, t(33) = 1.237, P = .212. In the unmasked condition, neither bias scores for angry faces nor those for happy faces were significant, largest t(33) = 0.760, ns.

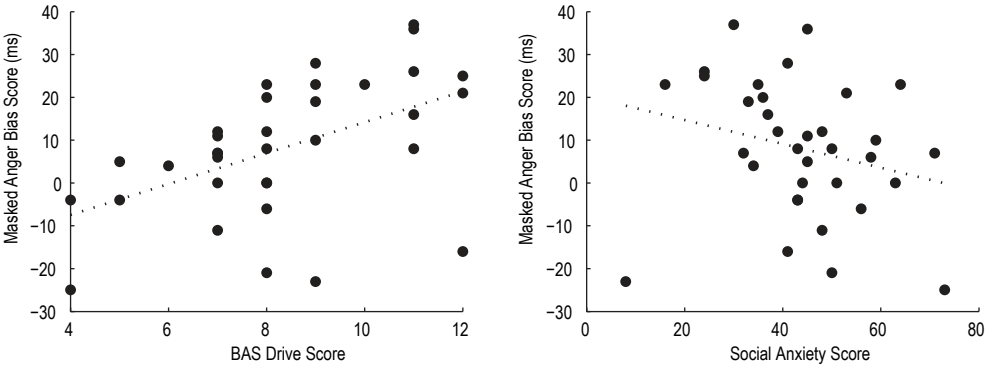

Figure 2.2.1: N = 34. Scatter plots for the relations between BAS (Behavioral Activation System) Drive and the bias score for masked angry faces (left panel) and the relation between social anxiety and the bias score to masked angry faces (right panel). Positive bias scores indicate interference. Not all data points are visible because of overlap.

Correlations Between Task Performance and Trait Measures: All Spearman's correlations between traits and bias scores for the masked and unmasked presentation conditions are shown in Table 2.2.1. We report these less powerful nonparametric tests for the relations between traits and bias scores for various reasons, most importantly because we are reluctant to perform parametric testing on questionnaire scores that likely do not meet the criterion of interval scaling.

We found significant correlations solely in the masked condition. As predicted, there were no significant correlations between either STAI (Spielberger, Gorsuch et al., 1983) or BIS and the bias for angry faces. The positive correlation between STAS (Spielberger, Jacobs et al., 1983) and the bias score for angry faces is as hypothesized, as is the positive correlation between BAS Drive and this bias. The hypothesized relation between BAS Reward Responsiveness and the bias was not confirmed. Although the overall BAS score is also related to the bias, it seems obvious how this effect is carried by the strong correlation between BAS Drive and this bias, whereas the other two BAS subscales show no significant correlation. A Steiger test (which tests whether dependent

Table 2.2.1: Correlations between emotional traits and bias scores for angry and happy faces in the masked and unmasked conditions

	Masked				Unmasked			
	Angry face bias		Happy face bias		Angry face bias		Happy face bias	
Trait measure	ρ	*p*	ρ	*p*	ρ	*p*	ρ	*p*
BIS	−.138	.436	−.093	.060	−.134	.451	.096	.588
BAS (total)	.344*	.046	−.130	.463	.060	.735	.065	.715
BAS Drive	.553**	.001	−.084	.636	−.145	.414	−.058	.744
BAS Reward Resp.	.264	.131	−.064	.721	.182	.302	−.029	.873
STAS	.410*	.016	−.064	.720	−.146	.411	−.188	.287
SA	−.349*	.043	−.141	.426	.059	.742	.171	.334
STAI	−.243	.167	.031	.862	−.220	.212	−.063	.722

Note. Positive correlations indicate greater bias scores are associated with stronger emotional traits. Note that significant correlations are only found between traits and angry faces, and only in the unmasked condition. BIS = Behavioral Inhibition System; BAS = Behavioral Activation System; STAS = State–Trait Anger Scale; SA = Social Anxiety Scale; STAI = State–Trait Anxiety Inventory.
** p < .05. **p < .01.*

correlation coefficients are significantly different in strength; see Steiger, 1980) confirmed that the stronger correlation between BAS Drive and the bias to angry faces differed significantly from the correlation between overall BAS (without the BAS Drive subscale) and the bias, t(31) = 2.447, P = .02. We also tested whether these data allow for the hypothesized conclusion that BAS Drive is a better predictor for the bias to angry faces than BIS, and that STAS (Spielberger, Jacobs et al., 1983) is likewise a better predictor than STAI (Spielberger, Gorsuch et al., 1983).

For BIS, BAS Drive, and the bias to angry faces, the test showed, t(31) = 3.310, P = .004. Thus, the relation between BAS Drive and the bias is significantly different from the relation between BIS and the bias. Running the same test for STAS (Spielberger, Jacobs et al., 1983) versus STAI (Spielberger, Gorsuch et al., 1983) resulted in t(31) = 3.696, P < .001. The predictors BAS Drive and STAS for the angry faces bias did not differ, t(31) = 0.833, P = .411.

Data also showed the hypothesized relation between facilitation for the angry face and SA (Willems et al., 1973; ρ = −.349, P = .043). A Steiger test for SA, STAI (Spielberger, Gorsuch et al., 1983), and this facilitation for angry faces could not confirm that SA is a better predictor of this bias, t(31) = 0.621, P = .54. See Figure 2.2.1 for scatter plots of the two most important correlations.

To test the hypothesis that relations between bias scores and trait measures were stronger in the masked than in the unmasked condition, we again performed Steiger tests. For the BAS correlations, there was no significant difference between the masked and unmasked condition, $t(31) = 1.309$, $P = .20$. However, for BAS Drive, $t(31) = 3.792$, $P < .001$, and STAS (Spielberger, Jacobs et al., 1983), $t(31) = 2.718$, $P = .011$, the correlations were stronger in the masked condition, and for SA (Willems et al., 1973), this was near significant, $t(31) = 1.9$, $P = .067$.

Discussion

An interesting aspect of these data is the contrast between results for the masked and unmasked task. We expected masking procedures to exclude more conscious override strategies to some extent and to provide a more direct measure of truly automatic responding, but we did not expect a complete absence of this selective responding. The total group showed interference for angry faces in the masked but not in the unmasked condition, and of the four significant correlations in the masked condition, three were significantly stronger (a trend for social anxiety) than their unmasked counterparts. Theoretical importance of this phenomenon was formulated by Williams, Mathews, and MacLeod (1996). They suggested that mood-controlling strategies capable of overriding Stroop effects might reflect an aptitude for control over emotional cognition. It is hypothesized that a lack of this ability to control the intrusion of emotion on cognition is of causal importance in psychopathology. These authors predicted that such override strategies should be unlikely in response to masked presentation, referring to data by MacLeod and Hagan (1992), replicated by Van den Hout et al. (1995) and most recently by Pury (2002) (all three studies used Stroop tasks with emotional words). These studies showed how masked Stroop data were valid predictors of real-life emotional behavior in response to stress, whereas unmasked Stroop data were not. Van Honk et al. (1998) also reported indications of this dissociation between masked and unmasked Stroop performance using emotional faces. The present data contribute to this growing body of evidence suggesting the methodological importance of using masking procedures to obtain less reactive measurements in this field of research.

Data for the Masked Task: None of the traits correlated with bias scores for the happy faces, allowing valence-specific interpretation of all bias scores for angry faces. The relation between trait anger and interference for angry faces confirms our hypothesis and replicates earlier findings (e.g., Van Honk, Tuiten, de Haan et al., 2001). As hypothesized, there was no significant relation between anxiety and Stroop performance, whereas social anxiety was significantly related to facilitation for angry faces. Our relation between responding to angry faces and social anxiety concurs with observations of relations between cortisol levels and behavior during social clash in

primates and the finding that, in humans, higher cortisol levels predict facilitation in response to angry faces (Van Honk et al., 1998). Thus, indeed these data seem to indicate that social anxiety is a good predictor of selective attention (i.e., avoidance) to angry faces, confirming our hypothesis and refining models predicting relations between anxiety and vigilant responding to generally threatening cues. We could not confirm that social anxiety is a better predictor than anxiety, but note that we did not find any relation between anxiety and a bias to angry faces. This cognitive avoidance of angry faces is the dimensional counterpart of the interference shown by more angry individuals. We argued that this dimension is social in nature, related to dominance and willingness-to-defend status. Along with aforementioned rTMS results (d'Alfonso et al., 2000), this notion of a basic motivational dimension of approach and withdrawal invited us to hypothesize similar results for anger versus anxiety, as for BAS versus BIS. Indeed, higher BAS, like higher trait anger, was associated with more interference for angry faces, confirming our conceptualization of the anger effect.

The just-significant correlation between total BAS score and interference for angry faces resides in only one of the three BAS subscales: BAS Drive. Note that we did not expect BAS Fun Seeking to show a relation to this interference. BAS Drive, which is conceptually most eligible for this relation, does indeed show the expected pattern. BAS Reward Responsiveness shows no relation either, although we did expect this approach-driven construct to show such a relation. It should be noted that in the Carver and White (1994) validation test of BAS subscales, BAS Drive was the best predictor of approach behavior in potentially rewarding situations (Carver & White, 1994, p. 330). A recent study by Harmon-Jones (2003b), relating various trait measures of anger to the BIS/BAS constructs, showed that BAS Drive was related strongest to anger, with no significant relations for BAS Reward Responsiveness and BAS Fun Seeking, which concurs with the behavioral data presented here. The present data indicate that BAS Drive is a better predictor than BAS overall, although BAS Drive is not a better predictor than anger. The confirmation of our BAS hypothesis is interesting because there are no items referring to anger or aggression in the BAS (Drive) scale. Verification of our hypothesis that BAS should be related to interference for angry faces (as is trait anger) supports the validity of our explanatory heuristic as well as hypotheses predicting vigilant processing of emotional faces whose valence concurs with prevailing emotional states (such as the mood congruency hypothesis).

BIS was expected not to be related to Stroop performance for angry faces, and this prediction was also confirmed. This lack of results for BIS is all the more interesting because BIS is conceptually related to anxiety (and strongly correlated to anxiety in this study; $\rho = .48$, $P < .004$). An important model (Mogg & Bradley, 1998), referring to Gray's (1985) BIS model, predicts vigilance to threatening cues in individuals higher in anxiety. This model is based substantially

on data from dot probe studies that showed such automatic shifts of attention toward angry faces in participants with higher levels of anxiety. We have no ready explanation for the discrepancy between results from this spatial dot probe task and the nonspatial Stroop task. The present data show that the constructs of interest with respect to selective attention to angry faces, as measured by the Stroop task, are BAS and anger, not BIS and anxiety.

Caution should be taken in interpreting specifically our null findings on anxiety and BIS because our sample size was relatively small. The reduced statistical power this implies reduces the chance of finding significant small correlations or differences between small correlations, and it is possible that we would have found some relations to anxiety and BIS in a larger sample.

In summary, this study extends confirmation of a heuristic to predict selective attention to facial threat from a more ecological conceptualization than models predicting such responding in terms of generally threatening stimuli. We do not question these models but stress the importance of considering the social nature of the angry facial expression in this research. It might be wise to use the fearful facial expression instead of the angry face when researching relations to anxiety (Vuilleumier, 2002; Whalen, 1998). These findings also provide further indications of the usefulness of masking procedures in research using tasks measuring selective attention.

2.3 *Reduced attentional processing of facial threat in social phobia*

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Herman G. Westenberg, & Jack van Honk (submitted for publication)

Abstract

Cognitive accounts of anxiety disorders assign a key role to the captivation of attention by stimuli relevant to current concerns. In social phobia (SP), however, an opposite form of attentional processing may be observed, namely, avoidance of socially threatening stimuli. In this study, medication free generalized SP patients were compared to a matched control group in an emotional Stroop paradigm tailored to assess attentional bias for threatening facial expressions. Baseline heart rate measures were also obtained. Results support the hypothesis by showing reduced rather than excess attention to angry facial expressions in SP patients, in comparison with controls. Furthermore, high baseline heart rate variability was found to predict attentional avoidance in the patient group. Together, these findings not only suggest that avoidance is the core direction of motivated attention in SP, but also that these withdrawal tendencies are mediated by mechanisms other than those that control regular fear responses.

Introduction

Biases in attention are thought to play a pivotal role in cognitive aspects of anxiety disorders (e.g., Mathews & MacLeod, 1985), including Social Phobia (SP). Although preferential processing of salient information is key to adaptive responding in threatening situations, excess attention to threat is said to reinforce itself through a spiral of ascending vigilance. Using laboratory assessment of biased attention, this notion of an attentional bias to threat has been verified both within and across anxiety disorders. Higher anxiety levels accompany stronger attention allocation to threatening cues, and importantly, to cues that are specifically relevant to one's major concerns (Williams, Mathews et al., 1996).

The vast majority of this research has relied on modified versions of the classic Stroop paradigm. In this task, affectively valenced words are presented in different colors, and affective processes are thought to interfere with the primary task of naming the color in which the word is printed. This interference effect has also been found in SP patients: compared to control participants, color-naming response latencies of patients are longer specifically to words pertaining to social scrutiny (Amir et al., 1996; Mattia et al., 1993).

The use of verbal stimuli in selective attention paradigms, however, is debatable. For instance, findings of interference in patients might arise solely from a relatively higher frequency of use that patients are thought to exhibit for words related to their condition. More importantly, semantic processing remains an indirect elicitor of emotional responding, and verbal descriptions of threat do not necessarily correspond to cues experienced as threatening in real life. Therefore, some researchers have chosen to resort to the use of pictorial stimuli, especially facial expressions, in selective attention paradigms (e.g., Clark, 1999; Van Honk, Tuiten, de Haan et al., 2001). It is doubtful, however, whether predictions pertaining to processing of semantic information generalize to pictorial stimuli. Whereas, for instance, SP patients can be expected to allocate more processing capacity to semantic information involving personal evaluation, it does not follow that they will also devote more attention to facial stimuli expressing social threat. Rather, clinical observations suggest that SP patients are prone to avoid attending to faces, especially with gaze directed at them (Clark, 1999). Similarly, in a study using eye-tracking apparatus, SP patients have been shown to avoid gazing at important facial features such as eyes (Horley et al., 2003).

Gaze aversion, among other submissive gestures, has been suggested to play an organizing role in social systems (Öhman, 1986; Sapolsky, 1990). From this viewpoint, keeping a straight gaze in reply to a provoking facial expression is seen as a sign of social dominance, whereas avoidance or gaze aversion is symbolic of submission. Accordingly, the threat value of different facial expressions cannot be taken for granted: facial expressions are social signaling mechanisms

that have evolved to communicate specific messages (Fridlund, 1994). In the case of anxious expressions, the message conveyed is that somewhere in the environment a threatening stimulus is present. Thus, it prompts for vigilance on the part of the observer (Whalen, 1998). The angry facial expression, however, signals provocation and dominance over the onlooker (Van Honk, Tuiten, de Haan et al., 2001). Rather than inducing vigilance, the response to this signal is thought to depend upon the motivational stance of the observer. In the case of SP, this is unlikely to result in attention being allocated towards this stimulus, as should be the case in dominant individuals, since maintaining eye contact does not relieve the onlooker of the threat conveyed by the provocative stimulus, whereas breaking eye contact would (Clark, 1999).

A series of experiments has been reported from our laboratory, in which these notions have been tested in healthy volunteers using a pictorial emotional Stroop task, in which a face-to-face encounter is simulated. In this task, participants are exposed to pictures of colored angry and neutral facial expressions and are required to name the color of these pictures. Attentional biases in this task are calculated by subtracting the color-naming latencies to neutral faces from those to angry faces. Thus, positive attentional bias scores reflect vigilance, whereas lowered scores signify reduced attentional processing of the threatening stimulus. On average, unselected samples of participants usually score above zero on this task (Putman et al., 2004). The main interest in these previous studies, however, has lain in explaining inter-individual variability on this measure.

Using this pictorial emotional Stroop task, Van Honk et al (2001) tested undergraduates selected on extreme scores on a trait anger questionnaire. Consistent with the above theoretical account, strong anger traits proved predictive of slow, vigilant responding, whereas low anger was associated with a more avoidant, fast response to angry facial expressions. A second experiment with participants selected on trait anxiety confirmed the hypothesis that this attentional bias is not related to generalized anxiety. Subsequent reallocation of these participants based on their trait anger scores again yielded a significant correlation between trait anger and attentional bias, also when stimuli were presented in conditions that preclude conscious perception using backward masking. Putman et al. (2004, see chapter 2.2) recently extended these findings by bringing attentional bias for angry facial expressions into the broader theoretical context of behavioral inhibition and behavioral activation systems (see Gray, 1982). Although generally considered a negatively valenced emotion, anger is subordinate to the concept of behavioral activation (Carver, 2004; Harmon-Jones, 2003b). In agreement with this, Putman et al. found a positive correlation between scores on a self-report index of behavioral activation and attentional bias for angry faces, i.e., higher scores on this questionnaire predicted more attention allocation to the angry face. Interestingly, the reverse relation was found specifically with self-reported *social* anxiety, but again not for scores on Spielberger's State Trait Anxiety Inventory, a questionnaire

tailored to assess *generalized* anxiety.

Relations have also been established between attentional bias for angry faces and physiological parameters related to social processes such as hormonal baseline measures. In animal research, reciprocal relations have been found between basal levels of the steroid hormones cortisol and testosterone. High cortisol levels are related to lower status, particularly in volatile social environments (Sapolsky, 1990), and have been related to social anxiety in humans as well, especially in children (Kagan et al., 1988). In contrast, baseline levels of the gonadal hormone testosterone appear to exhibit positive relations with measures of anger, aggression, and especially dominance (Mazur & Booth, 1998). In agreement with these notions, quicker, avoidant responding to angry facial expressions has been found to be related to high baseline cortisol levels (Van Honk et al., 1998), whereas the opposite relation has been reported with testosterone baselines (Van Honk et al., 1999).

In most of the aforementioned studies on attentional bias for facial expressions, stimuli were backwardly masked in order to remain below perceptual threshold levels. This was achieved by replacing the target stimulus with a scrambled photograph after less than 30 msec, which has the effect of blocking conscious perception of the target. Because under these circumstances participants are unable to identify the target stimuli, strategic effort to control attentional bias effects is unlikely to occur. Indeed, comparisons of data across experiments in which healthy volunteers were tested indicate that masked, compared to unmasked, versions of the emotional Stroop task have yielded more consistent results (Putman et al., 2004; Van Honk, Tuiten, de Haan et al., 2001). This observation is in line with evidence that masked, compared to unmasked, emotional Stroop performance is more predictive of actual coping with stressful life events (MacLeod & Hagan, 1992).

As stated above, avoidance of eye contact or social scrutiny is a characteristic symptom of SP. The present experiment therefore investigated attentional bias for angry facial expressions in patients with Social Anxiety Disorder. Based on previous research, the primary hypothesis was that SP patients, in comparison with a matched control group, would show reduced attentional processing of social threat. Specifically, this led us to predict reduced attentional bias scores in the SP group compared to controls. In addition to the regular pictorial emotional Stroop task, we included a masked version of this task, expecting that this effect would also appear when stimuli are presented below perceptual threshold levels. Moreover, we added happy facial expressions as control stimuli to control for non-specific emotionality effects. Because SP patients may be expected to respond differently to pictures of faces *per se*, regardless of expression (see e.g. Birbaumer et al., 1998), we also included scrambled pictures without facial features.

Exploratively, baseline electrocardiographic (ECG) data was collected in order to monitor baseline

autonomic nervous system (ANS) activity. Many psychiatric conditions have been associated with shifts in sympathetic/parasympathetic ANS balance towards sympathetic dominance. For instance, reduced parasympathetic control of the heart, as evidenced by decreased heart rate variability (see Porges, 1995a), has been found in generalized anxiety disorder and panic disorder (Friedman & Thayer, 1998). There is little evidence, however, that disturbances in baseline ANS balance exist in SP (Grossman, Wilhelm, Kawachi, & Sparrow, 2001), despite the fact that social stressors may elicit exaggerated sympathetic responses especially in the specific subtype of SP (Hofmann, Newman, Ehlers, & Roth, 1995). Therefore, our main interest lay in relations between baseline measures and task performance. If reduced processing of angry facial expressions in the emotional Stroop task is primarily motivated by fear, an emotional state accompanied by strong sympathetic activation, then SP patients with high sympathetic autonomic dominance should exhibit the strongest avoidant reduction of processing angry faces. In contrast, it can be argued that socially avoidant behavior is not primarily guided by fear, but serves instead to circumvent social stimuli and scrutiny, and thus to reduce fearfulness, which leads to the opposite hypothesis that a reduction in processing angry faces is accompanied by stronger parasympathetic dominance.

Materials and Methods

Participants: Twenty-five medication-free SP patients (14 female) were recruited from the Anxiety Clinic at the University Medical Center Utrecht. Twenty-one volunteers (11 female) served as control group. Groups did not differ in age ($t(43)=.39$, n.s.), and both groups had a median score of 6 on a 7 item ordinate scale of education level (Mann Whitney $z=-1.54$, $p=.12$). All participants received reimbursement of expenses.

All patients had a DSM IV (American Psychiatric Association, 1994) diagnosis of generalized SP, which was confirmed using a MINI-International Neuropsychiatric Interview (Sheehan et al., 1998). The Liebowitz Social Anxiety Scale (Heimberg et al., 1999) was used to assess severity of social anxiety symptoms in both groups (see table 2.3.1), which differed significantly ($t(40)=13.2$, $p<.001$). None of the control participants had any history of neurological or psychiatric disturbances. The following additional exclusion criteria applied for all participants: use of psychotropic drugs within four weeks of testing, alcohol dependency, current depressive episode, serious non-neurological diseases, color-blindness, and uncorrected sub-normal visual acuity. Due to apparatus failure, physiological data of four patients and four control participants were lost.

The local medical ethical counsel approved the protocol in accordance with the Declaration of Helsinki. All participants signed statements of informed consent.

Material and apparatus: Stimuli were photographs of 8 actors (4 male, 4 female) displaying happy, neutral, and angry expressions, selected from two photosets (Ekman & Friesen, 1976; Lundqvist, Flykt, & Öhman, 1998). Additionally, pictures containing random geometric shapes served as control stimuli without facial features. Masks were created from randomly cut and reassembled photographs of faces. Oval cutouts of these pictures were then equalized in luminance, and colored red, green or blue. The total set consisted of 8 (actors) * 4 (expression types) * 3 (colors) = 96 pictures, and 2 (different) * 3 (colors) masks. Presentation and response logging were controlled using E-Prime (Psychology Software Tools, inc).
Electrocardiographic (ECG) measurements were acquired using the Ambulatory Monitoring System (VU-Amsterdam), which calculates Inter-Beat Interval (IBI) time series online.

Procedure: Throughout the procedure, participants were seated in a comfortable chair, 60 cm from the computer screen, in a dimly lit chamber. Baseline ECG measures were acquired during a five minute resting period prior to the task. The emotional Stroop task consisted of four phases. During the first phase, participants performed ten practice trials in which only masks were used (i.e., without facial stimuli). Subsequently, participants completed a masked emotional Stroop task of 96 randomized trials. Each trial started with a 750 ms fixation cross, followed by a very brief (14.3 ms, one frame at 70 Hz) exposure to the target, which was replaced by the mask. Stimulus and mask had identical colors, and participants were required to vocalize this color. Responses triggered offset of the masks using a voice-activated relais. New trials started after a random inter-trial interval (2-4 seconds).
Subsequently, participants performed an unmasked version of the task, which differed only in absence of masks. Thus, the target stimuli remained visible until registration of responses.
The final phase of the task was a 30 trial forced choice awareness check in which participants were asked to determine or guess the emotional expressions of masked stimuli (happy, neutral, or angry).

Data reduction
Reaction times: Outliers were filtered using a <200 and >1300 ms cut-off, and subsequent removal of all RTs exceeding three SD from the mean. Remaining latencies (94.1%) were averaged over expression types. Statistical analyses were performed using repeated measures ANOVAs with stimulus type (angry vs. neutral) and exposure (masked vs. unmasked) as within subjects factors, and group as between subjects factor. ANOVAs were repeated for happy vs. neutral, and non-face vs. neutral comparisons, and alpha was set at .05.
Three alternative forced choice awareness check: The individual cut-off score for the number of

			Baseline[c]		Masked[d]				Unmasked[d]			
	Age[a]	LSAS[b]	HRA	MSSD	Angry	Neutr.	Happy	Non	Angry	Neutr.	Happy	Non
SAD (n=25)	37.5 (10.9)	69.8 (15.3)	74.0 (11.6)	34.2 (20.7)	540.2 (78.9)	546.8 (91.7)	544.8 (82.0)	546.5 (82.9)	585.7 (107.0)	588.4 (106.5)	591.8 (118.1)	603.4 (111.9)
Control (n=21)	38.9 (13.5)	13.8 (11.2)	81.4 (14.0)	33.6 (37.1)	530.0 (62.4)	525.3 (69.7)	524.3 (71.7)	526.5 (72.1)	569.5 (90.1)	557.3 (82.8)	564.3 (88.5)	574.0 (90.7)

Table 2.3.1: Means (and SD) of characteristics, physiological measures, and reaction time data from SAD and control groups. A) Age in years; B) Liebowitz Social Anxiety Scale scores; C) Baseline physiological measures (r-MSSD in msec and HRA in beats per minute); D) Reaction times in msecs to masked and unmasked presentations of angry, neutral, happy, and non-faces.

correct answers was calculated to be (larger than) 15 (with 10 correct answers being chance level), using a binomial distribution with alpha=.05, Π=.33, n=30. Participants scoring above this cut-off were to be removed from further analyses.

ECG: Heart rate average (HRA) and heart rate variability (HRV) were determined for baseline periods. For HRV, we calculated the root mean square of successive differences (R-MSSD), which is a reliable indicator of parasympathetic control of the heart, with higher values indicating higher parasympathetic control (De Geus, Willemsen, Klaver, & Van Doornen, 1995).

Results

Angry versus neutral: The repeated measures ANOVA with stimulus type (angry vs. neutral), exposure (masked vs. unmasked, and group (patient vs. control) revealed a main effect of exposure ($F(1, 44) = 27.33$, $P < .001$): RTs to masked trials were shorter than to unmasked trials. There was no main effect of group ($F(1, 44) = .64$, n.s.), which indicates that groups did not differ in overall mean reaction time. There was also no main effect of stimulus type ($F(1, 44) = .39$, n.s.), but the hypothesized interaction of group and stimulus type was significant: $F(1, 44) = 4.80$, $P = .034$ (see figure 2.3.1 and table 2.3.1). No interactions involving exposure reached significance (all $F < 1$). Separate ANOVAs only revealed a main effect of stimulus type ($F(1, 20) = 5.37$, $P = .031$) in the control group, whereas the patient group showed no such effect ($F(1, 24) = 1.06$, n.s.).

Happy versus neutral: The overall ANOVA for this comparison showed only a main effect of exposure ($F(1, 44) = 25.18$, $P < .001$; shorter RTs to masked trials).

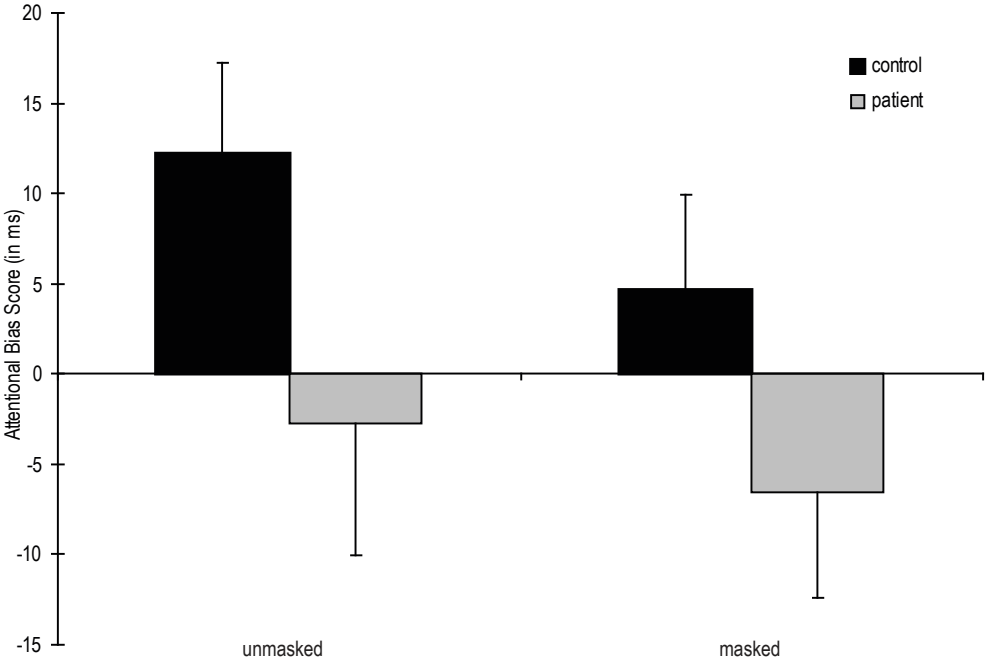

Figure 2.3.1: Mean attentional bias scores (angry minus neutral) and standard errors of the mean for the patient and control groups in the masked and unmasked task.

Non-faces versus neutral: The overall ANOVA again revealed a main effect of exposure ($F(1, 44) = 39.78$, $P < .001$). Also, a main effect of stimulus type (neutral face vs. non-face) was found ($F(1, 44) = 7.57$, $P = .009$), with longer reaction times to non-faces. Moreover, there was an interaction of exposure and stimulus type ($F(1, 44) = 7.22$, $P = .01$), but no interactions involving group were observed. Further analysis of this effect showed that it is absent in the masked task ($F(1, 44) = .009$, n.s., versus $F(1, 44) = 16.29$, $P < .001$ in the unmasked task).

Separate univariate ANOVAs were calculated to assess group differences in baseline R-MSSD and HRA. No difference was found for R-MSSD ($F < 1$, n.s.). A non-significant trend was found for HRA: $F(1, 34) = 2.93$, $P = .096$ (slightly lower heart rate in patients). There were, however, significant correlations between task performance (attentional bias score for angry facial expressions combined for masked and unmasked tasks) and these parameters. Within the patient group, there was a negative correlation between attentional bias for angry faces and R-MSSD (Pearson's $r(19) = -.69$, $P < .001$, Spearman's $\rho(19) = -.50$, $P = .022$), as well as a positive

correlation between attentional bias and HRA (Pearson's r(19) = .45, P = .039, Spearman's ρ(19) = .39, P = .083). These two correlations did not reach significance in the control group.

3 Alternative forced choice awareness check: No participants scored above the a priori cut-off score of 15 (see data reduction; grand mean was 9.91, with a chance level of 10). Thus, none were removed from analyses.

Discussion

Using an emotional Stroop task, the present study confirmed the hypothesis that SP patients do not exhibit increased attention towards angry facial expressions. Instead, compared with healthy control participants, SP patients show relative attentional avoidance of these social threat signals. Additionally, reduced processing of angry facial expressions was predicted by lower baseline heart rates and higher heart rate variability in the SP group.

Analyses of comparisons of neutral with happy facial expressions and non-faces stress the point that the group difference in attentional bias is specific to the angry facial expression and not due to an aberrant reaction to the neutral face control stimuli in the SP group. The apparent slow response in both groups to the non-faces in the unmasked task may be explained as an oddball effect because 75% of the stimuli were faces.

The control group replicates a previously reported effect of overall vigilance for angry facial expressions (Putman et al., 2004) in an unselected sample. We found no difference between results of the masked and unmasked task.

The pattern of decreased attention to socially threatening stimuli in SP patients adds to a body of evidence that links reduced processing of social threat to high levels of social anxiety (Putman et al., 2004), and high cortisol baselines (Van Honk et al., 1998). Conversely, selective attention towards social threat, i.e., vigilance, has been found in participants with strong anger traits (Van Honk, Tuiten, de Haan et al., 2001), high scores on the behavioral activation scale (Putman et al., 2004), and high baseline levels of testosterone (Van Honk et al., 1999). Thus, we interpret the latter as an indication of dominance and provocation, whereas the former effect might reflect a process related to gaze aversion and submission (cf. Öhman, 1986; Sapolsky, 1990). The present data are furthermore consistent with notions that suggest a linkage between SP and subordination stress in primates (see Mathew et al., 2001).

As expected, the present results using ecologically more valid pictorial stimuli stand in stark contrast to previous findings of Stroop interference to semantic stimuli (Amir et al., 1996; Mattia et al., 1993). It is furthermore interesting to compare the current findings with recent SP patient data from a spatial attention paradigm known as the dot probe task (Amir, Elias, Klumpp,

& Przeworski, 2003). The rationale of this task is that identification of peripheral probes is facilitated when preceded by threatening stimuli in the same location. Recently, however, a debate has arisen on how to interpret facilitation effects in this task (see Koster et al., 2004). The traditional interpretation is that threatening peripheral targets have an augmented capacity to draw attention, which facilitates responding on congruent trials. In contrast, an alternative explanation is that participants have difficulty disengaging attention from such stimuli, which would result in slower responding on incongruent trials. The results of Amir et al. (2003), who also used semantic stimuli, support the latter: responses to probes were slower on invalidly cued trials than on un-cued control trials. Results from the verbal emotional Stroop task and the verbal dot-probe task thus consistently show a difficulty to discard semantic information pertaining to social threat in SP. However, all these studies used words to convey social threat, a point that cannot be overstressed: the adaptive value of enhanced spatial detection of *printed verbal* material is not clear, nor would it be warranted to suppose that SP is characterized by higher sustained attention to social threat, e.g., maintaining eye contact, in a natural setting (cf. Clark, 1999).

Two recent studies, on the other hand, have employed a pictorial instead of a semantic version of the dot probe task in SP. First, Chen et al. (2002) reported that SP patients are prone to selectively attend away from faces, apparently regardless of emotions depicted by the faces. Conversely, Mogg et al. (2004) reported that at stimulus onset asynchronies equal to Chen et al., 500 ms, SP patients are quicker on validly cued trials. Mogg et al. thus draw the seemingly opposite conclusion that speeded detection of socially threatening material may enhance performance on these trials. Although it is doubtful if it is possible to reconcile these paradoxical findings, Mogg et al. point out that the divergence might arise from a difference in control stimuli: they used neutral facial expressions, whereas Chen et al. employed non-facial stimuli (household objects). Mogg et al. therefore conclude that species-specific, defensive, avoidant responses to social threat may readily occur in SP when there is an option of not attending to social stimuli. This also seems the most parsimonious account of these results when combined with the present findings of reduced processing of angry faces, where participants can remove the social stimulus by fast responding.

This distinction might be exemplary for a response mode to social stimuli in SP that is twofold: avoidance whenever possible and excess attention in those situations where social scrutiny is inevitable, such as in conversations with unfamiliar people or during public speaking. While these situations are likely endured with subjective feelings of distress and fear, avoidance of social threat, on the contrary, does not imply a strong emotional response. Theoretically, this is in line with notions that the evolutionary function of social submissiveness is to reduce costly dominance clashes within groups to a minimum by ritualization of submission gesturing

(Öhman, 1986). Thus, subordinate individuals are best served by an inhibited behavioral responses to social threat (Flinn et al., 1998). Physiologically, this response is characterized by lowered sympathetic versus parasympathetic autonomic nervous system dominance, and reduced responsiveness of the neuroendocrine system (Sapolsky, 1990), arguably comparable to the so-called conservation-withdrawal responses observed, e.g., in animals depleted of maternal care (Kaufman & Rosenblum, 1966). As such, this state is the antipode of the physiologically aroused state, characterized by elevated sympathetic responses, that is observed in specific SP patients in response to public speaking (Grossman et al., 2001; Hofmann et al., 1995). Thus, the present finding of a negative relation between baseline parasympathetic dominance and attentional bias for social threat within the patient group fits well into this picture. High parasympathetic activity has been linked to increased flexibility in responding to environmental demands (Friedman & Thayer, 1998), which may enable SP patients to navigate the social world without continuous metabolically costly fear responses. Only when attention cannot be disengaged from social threat, fear results.

In conclusion, the present data provide the first demonstration that compared to healthy controls, generalized SP patients show reduced attention allocation towards angry facial expressions. In sharp contrast with previous research using semantic stimuli, this suggests that avoidance, rather than excess attention, is the principal direction of motivated attention to social threat in SP when probed using more ecologically valid stimuli. Moreover, relations between ANS balance and this attentional bias suggest that this tendency is parasympathetically mediated and cannot be equated to regular, primarily sympathetic, fear responses.

2.4 *Identity state-dependent attentional bias for facial threat in dissociative identity disorder*

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Psychiatry Research 2006, 141(2), 233-236

Abstract

Biased attention for threatening stimuli has been associated with many forms of psychopathology. Here, attention for threatening faces presented below perceptual thresholds was assessed in patients diagnosed with Dissociative Identity Disorder using a pictorial emotional Stroop task. Patients were tested in two different identity states, in one of which they claimed strong awareness of trauma. Attentional bias for social threat proved state-dependent and deviated from controls.

Introduction

Chronic childhood traumatization – typically in the form of severe abuse and maltreatment – may lead to the development of Dissociative Identity Disorder (DID), a DSM-IV diagnostic category (American Psychiatric Association, 1994), also referred to as a complex form of posttraumatic stress disorder (Spiegel, 1984). DID is characterized by such fluctuations in affect that these become phenomenologically perceived as changes in identity.

Clinical observations suggest that DID involves several types of identity states. In one such identity state (here referred to as *trauma avoidant*), patients either claim unawareness of trauma, or they do reveal awareness of traumatizing events, but maintain that the trauma does not pertain to them. In this state, patients exert their functions of daily life and tend to hide their dissociative condition. However, when in identity states displaying strong trauma awareness (here denoted as *trauma fixated* states), patients report re-experiencing traumas and exhibit strong emotionality in response to reminders of trauma.

In recent years, we conducted a series of studies using a modified pictorial emotional Stroop task, aimed at investigating primary emotional responses. Subjects with dominance characteristics such as high basal levels of testosterone, high levels of self-reported anger, and high scores on the Behavioral Activation Scale (Carver & White, 1994) showed *longer* color naming latencies to angry faces as opposed to neutral faces (Putman et al., 2004; Van Honk, Tuiten, de Haan et al., 2001; Van Honk et al., 1999). Conversely, subjects with submissive characteristics such as high basal levels of cortisol or high levels of social anxiety showed *shorter* latencies (Putman et al., 2004; Van Honk et al., 1998). Effects were often more pronounced when emotional stimuli were presented below perceptual threshold levels by means of backward visual masking at stimulus onset asynchronies of maximally 30 ms. This pre-attentional bias was explained in terms of biological-evolutionary models of emotion, as unconsciously initiated reflexive signals of aggressive approach and fearful withdrawal (i.e., dominance and submission motives, Mazur & Booth, 1998; Öhman, 1986). Due to masking, participants are unable to modulate their emotional responses (MacLeod & Hagan, 1992; Williams, Mathews et al., 1996), resulting in an uncontaminated measure of the actual emotional state of an individual.

Since in the vast majority of DID patients, harm has been inflicted by close relatives, it is reasonable to assume that a social stimulus like the angry facial expression is an exemplary candidate to provoke emotional reactions in these patients. Therefore, we employed the pictorial emotional Stroop paradigm to investigate identity state-dependent (trauma avoidant vs. fixated) emotional reactivity in DID patients. Again, the backward masking technique was used to preclude strategic modulation of the emotional Stroop effect. We also added a control group of healthy participants that was required to feign these states (cf. Silberman, Putnam, Weingartner,

Braun, & Post, 1985).

As the emotional Stroop task employing threatening facial expressions has shown to be sensitive to a dimension ranging from dominance to social anxiety, we expected to find a shift towards the latter extreme (i.e., shorter reaction times) in healthy controls when simulating trauma fixated states. Regarding DID patients, mere extrapolation of existing data showing decreased response latencies to angry faces in socially anxious subjects (Van Honk, Tuiten, de Haan et al., 2001) led us to hypothesize a similar effect in DID patients in both states, but more pronounced in the trauma fixated state than in the trauma avoidant state.

Methods

Participants were nine female DSM-IV diagnosed DID-patients, and ten healthy female controls. Patients were medication free, had a medium to higher education, and their age range was 24-38. Controls were female students, ranging from 20-25 in age, and without history of psychopathology. State-anxiety induction for patients was established by guided switch to their trauma fixated states, coached by a clinical professional. Participants in the control group were instructed to mimic DID. They were given written information about DID, and they were subsequently asked to make up two imaginary "identities", one with memories of experienced trauma, and the other without memories of trauma. During the experiment, they were asked to "switch" between these identities. The local ethical committee approved the protocol, and all participants signed statements of informed consent.

Attentional biases for masked angry faces were indexed using an emotional Stroop task. In 80 trials, neutral and angry facial expressions, colored transparent red, green or blue, were displayed for 25 ms on a computer monitor before being replaced by color-congruent masking stimuli (randomly cut and reassembled photographs). Participants were asked to vocally identify the color of the pictures, and response latencies were recorded using a voice-activated relais and a microphone. Initiation of vocal responses terminated the trial (see Van Honk, Tuiten, de Haan et al., 2001, for details). Identical procedures were used in both identity states and identity state orders were counterbalanced. Attentional Bias scores were calculated by subtracting response latencies to neutral expressions from those to angry expressions. Data were analyzed using a repeated measures ANOVA. Alpha levels were set at 0.05, two-tailed, throughout.

Results

Data of 2 patients had to be discarded. Both patients reported awareness of facial expressions and strong fear, even rendering one of them unable to continue performance.

STATE (trauma avoidant vs. trauma fixated) was used as a within-subjects factor, while GROUP

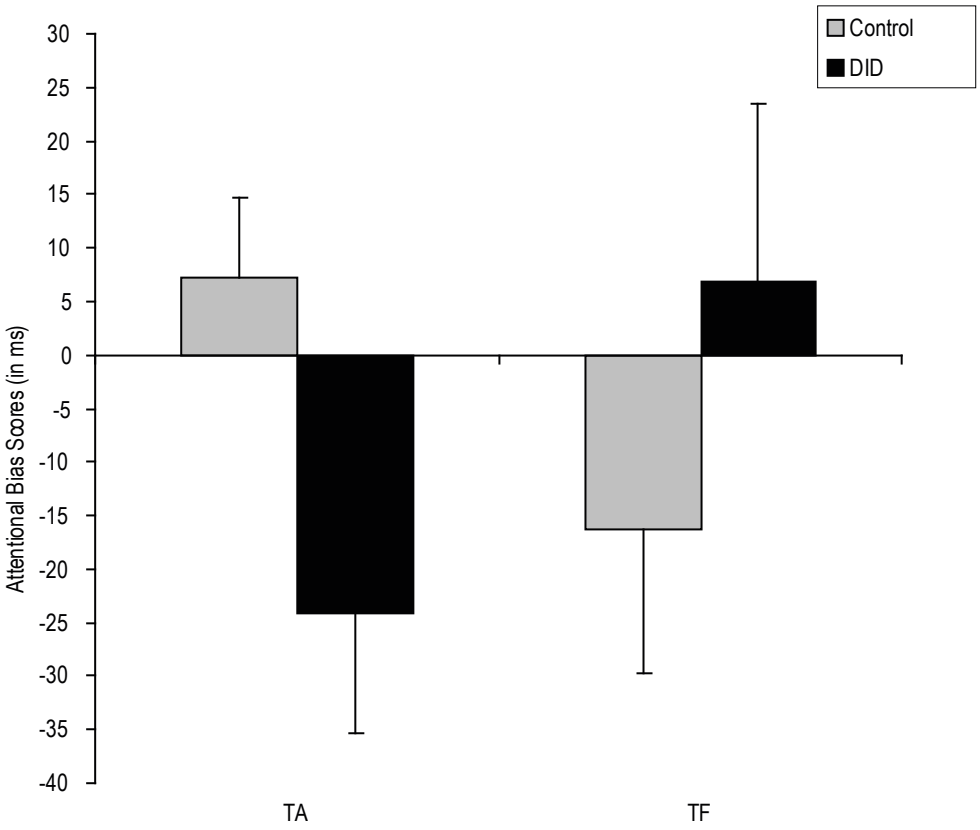

Figure 2.4.1: Mean (and SEM) attentional bias scores for DID-patients and controls in trauma avoidant (TA) and trauma fixated (TF) states.

(DID patients vs. controls) and ORDER (trauma avoidant vs. trauma fixated first) were used as between-subject factors. There were no significant interactions concerning ORDER ($P > 0.5$ throughout). Hence, data were further analyzed excluding the ORDER factor. Data revealed a significant GROUP X STATE interaction ($F(1,15) = 4.99$, $P = 0.041$). As can be seen from Figure 2.4.1, this interaction is due to a response reversal from the trauma fixated to the trauma avoidant state across groups.

To further investigate this effect, separate independent samples t-tests were computed for the different identity states. These revealed a significant effect for GROUP (DID vs. Control) in the trauma avoidant ($t(15) = 2.44$, $P = 0.028$) but not in the trauma fixated state ($t(16) = 1.09$, n.s.). As can be seen from Figure 2.4.1, this effect is due to patients showing shorter response latencies

than controls in the trauma avoidant state. Attentional bias scores of patients in the trauma avoidant state are almost significantly below zero ($t(6) = 2.16$, $P = 0.074$). Further post hoc t-tests revealed no significant effects.

Discussion

To the best of our knowledge, this study reports the first findings of preconscious attentional biases in this clinical group. Response patterns over different states in DID-patients deviated qualitatively from control subjects.

First, our control subjects showed the hypothesized effect. In their simulated trauma avoidant states no significant effect was observed. These data concur with our earlier research in non-clinical subjects where overall attentional biases for masked angry faces indicated marginal effects (Van Honk, Tuiten, de Haan et al., 2001; Van Honk et al., 2000). In further agreement, simulating trauma fixated states, controls shifted towards a response pattern similar to that observed in socially anxious individuals (Van Honk, Tuiten, de Haan et al., 2001; Van Honk et al., 1998).

On the other hand, as evidenced by a significant GROUP * STATE interaction, DID patients showed a reverse pattern of responding. In the trauma avoidant state, patients exhibited decreased color-naming latencies to angry facial expressions, whereas they showed slower color-naming in the trauma fixated state.

DID patients in a trauma avoidant state, to begin with, reacted to angry facial expressions in a way we have previously associated with high levels of social anxiety. That is, healthy volunteers with high scores on self-report social anxiety questionnaires, or with high baseline levels of cortisol tend to avoid processing of angry facial expressions. As mentioned above, we interpreted this avoidance in terms of submission, with reference to dominance clashes in primates, where often aggression is prevented by disengaging eye-contact, or other ritualized submissive gestures (Mazur & Booth, 1998). Note that, in submissive individuals, this disengagement response is adaptive as it relieves the threatened individual of the social threat conveyed by a conspecific (Clark, 1999). Thus, we interpret these shorter color-naming latencies in trauma avoidant DID patients as an adaptive response (cf. Nesse, 2000), preventing uncontrollable fear, which helps to cope with social threat cues in a way that enables them to function in daily life.

However, when in a putatively highly anxious, trauma fixated state, patients are apparently no longer able to use this strategy. Instead of showing an even stronger avoidant response, patients devote more processing capacity to the angry facial expressions, and thus show a slow response we previously found in healthy volunteers with high dominance characteristics such as high basal levels of testosterone, self reported anger traits, and high scores on the BAS scale. An interpretation in these terms, however, seems unlikely in the case of DID patients in a trauma

fixated state.

This paradoxical finding is, however, consistent with cognitive models of attentional bias that assume positive linear relations between perceived threat level and attentional bias at high state anxiety levels (Mogg & Bradley, 1998; Williams, Mathews et al., 1996). Possibly, as a result of persistent physical and sexual abuse, the angry facial expression is appraised as having such high threat value when patients are in a trauma fixated state, that a fear response is prompted. As a consequence, their reflexive avoidant responses, in which fear is not necessarily implied, no longer function.

In sum, the results of this study show a diverging pattern of responding in a pictorial emotional Stroop task between DID patients and healthy controls. Whereas controls shift towards an avoidant response pattern in a simulated state of trauma awareness, DID patients are apparently already extremely avoidant of social threat cues in their trauma avoidant state, a response that is not functional anymore when in a trauma fixated state.

2.5 *A left-prefrontal lateralized, sympathetic mechanism directs attention towards social threat in humans: evidence from repetitive transcranial magnetic stimulation*

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Abstract

The prioritized processing of threat is suggested to be motivated by anxiety, regulated by the parasympatheticus, and biased to the right hemisphere. However, according to an anterior dimensional model of negative affect this is unlikely to be true when threat is of social origin. Social threat is communicated by the angry facial expression, and recent research indicates that prioritized processing of angry faces is motivated by anger. Anger is a sympathetically dominated emotion, and for its expression and experience, neuroimaging data have demonstrated anterior lateralization to the left hemisphere. To scrutinize the above diverging statements, suprathreshold low-frequency repetitive transcranial magnetic stimulation (rTMS) was applied over the right and the left prefrontal cortex (PFC) of ten healthy subjects during 15 min continuously, and the subsequent effects on sympathetic and parasympathetic activity of the heart, and selective attention to angry facial expressions were investigated. Combined rTMS–neuroimaging studies have shown contralateral excitation after unilateral supratheshold low-frequency rTMS, hence the strengthening of contralaterally mediated emotion functions. The earlier reported increases in selective attention to angry facial expressions after right-PFC rTMS were found to be accompanied by and significantly associated with elevations in sympathetic activity. Our data suggest that a left-PFC lateralized, sympathetic mechanism directs attention towards the angry facial expression.

Introduction

The initiation of adaptive responses in threatening situations depends on a mechanism of attentional selectivity. Perceptual cues associated with danger are the most rapidly detected and attended to. Anxiety provides for a shift into a hyper-vigilance mode enhancing the scanning for threat in the environment (Matthews & Mackintosh, 1998).

Influential models of emotion and autonomic functioning presume right-hemispheric dominance in the above-noted indices of affective information processing. The Valence Hypothesis states that withdrawal-related emotions, such as anxiety, are biased to the right hemisphere, whereas approach-related emotions, such as joy and happiness are biased to the left hemisphere (Davidson & Irwin, 1999). Furthermore, according to the Polyvagal Theory, the selective response to threat is regulated by the autonomic nervous system. A short latency heart rate deceleration can be observed, which is part of the attentional orienting reflex and, crucially, primarily parasympathetically mediated along the right-sided vagus (Porges, 1995b). In summation, the prioritized processing of threat is motivated by anxiety, mediated by the parasympatheticus, and biased to the right hemisphere.

As might be expected, the prioritized processing of fearful faces is elevated in anxiety prone subjects (Hermans et al., 1999). The fearful face is a highly potent danger call and presumably evolved to function as noiseless predator alarm for the social group (Smith, McHugo, & Kappas, 1996). The question arises whether the story would hold when threat itself is of social origin. In humans, social threat is predominantly communicated by the angry facial expression, and recent evidence suggests that selective attention to angry faces is motivated by anger and not by anxiety (Van Honk, Tuiten, de Haan et al., 2001; Van Honk et al., 1999). This might lead to a reversal of the above proposal, since anger is not only a sympathetically dominated emotion (Bongard, Pfeiffer, al'Absi, Hodapp, & Linnenkemper, 1997; Kemper, 1990) but neuroimaging studies have also updated the Valence Hypothesis by providing evidence for an anterior dimensional model of negative affect.

Electroencephalographic (EEG) research showed relatively more right prefrontal activity for the expression and experience of fear (Coan, Allen, & Harmon-Jones, 1999; Kalin et al., 1998), but anger proved to be an approach-related emotion within the Valence concept, at least its expression and experience showed relatively more left prefrontal activity (Coan et al., 1999; Harmon-Jones & Allen, 1998).

In support of this dimensionality in negative affect, recently we demonstrated in a line of experiments, that suprathreshold low-frequency repetitive transcranial magnetic stimulation (rTMS) over the right prefrontal cortex (PFC) results in left PFC excitation as measured by EEG (Schutter et al., 2001), attenuated anxiety-related motivation observed as reduced selective

attention to fearful faces (Van Honk, Schutter et al., 2002), but elevated anger-related motivation observed as increased selective attention to angry faces (d'Alfonso et al., 2000). Arguably, the above-noted contralateral excitation observed after slow rTMS was due to reductions in transcallosal inhibition, since slow rTMS leads locally to transient inactivation (Pascual-Leone et al., 1998). Here, we present further data on sympathetic and parasympathetic activity of the heart from the latter rTMS study. The findings indicate that a left PFC-lateralized, sympathetic mechanism directs attention towards the angry facial expression.

Method

Participants: Ten healthy right-handed female subjects, age ranging from 18 to 30 years, were recruited for participation in the experiment. Those with a history of psychiatric or neurological disorder were excluded. Subjects were naive of rTMS and unaware of the aim of the study. They provided written informed consent and all procedures were approved by the local ethics committee of the Faculty of Social Sciences.

A Cadwell High Speed Magnetic Stimulator and a specially designed water-cooled eight-shaped figured coil (loop diameter = 7 cm) was applied over the right and left dorsolateral PFC. Subjects were stimulated for 15 min on separate days at 130% of the motor threshold (MT) with a frequency of 0.6 Hz, a method denoted as suprathreshold low frequency rTMS. MT was quantified using the method of visualization of the left and right thumb movement (Pridmore, Fernandes Filho, Nahas, Liberatos, & George, 1998). The position of stimulation was 5 cm anterior to the area where the MT was determined. The coil was held tangential to the stimulation point with the handle of the coil pointing posterior. A purpose-built coil holder was used to secure the coil position during stimulation. The position of stimulation was randomized and counterbalanced across subjects.

Stimuli and apparatus: Sympathetic and parasympathetic balance were assessed before stimulation (baseline activity) and during the emotional Stroop task (task-dependent activity) using an ambulatory electrocardiography/impedance cardiography (ECG/ICG) device and supplementary software package (Ambulatory Monitoring System, VU-Amsterdam). Changes in heart rate due to the respiratory cycle were filtered out of the inter-beat-interval (IBI) time series. The difference between the shortest IBI during inspiration and the longest IBI during expiration was used as a measure of respiratory sinus arrhythmia (RSA). This peak-to-trough method provides for a reliable index of parasympathetic activity. In addition, a standardized sympathetic measure was derived from the pre-ejection period (PEP), the interval between ventricular depolarization and ventricular ejection onset. Shortenings of this interval represent elevations in sympathetic activity

and are indexed by interactive visual detection of the B-point in consecutive ICG-complexes (De Geus & van Doornen, 1996). Both RSA and PEP measures were averaged over 4-min periods.
An emotional Stroop task was employed to index selective attention to angry faces. This task requires the participants to name (as quickly as possible) the color of pictures (red, green, blue, and yellow colored angry and neutral facial expressions) presented on a 160 Hz computer screen at a distance of 60 cm. One trial consisted of the presentation of a fixation-cross displayed for 750 ms, which was followed by the target picture. Initiation of vocal response was detected using a microphone connected to a voice-activated relay and terminated presentation of the target (i.e. a neutral or an angry face). Forty neutral and 40 angry faces were presented in random order with the restriction that the same color was never repeated more than twice consecutively. Data of one subject were lost due to apparatus failure. Attentional Bias scores were calculated by subtracting the individual mean response latencies for neutral faces from the individual mean response latencies for angry faces (Van Honk, Tuiten, de Haan et al., 2001; Van Honk et al., 1999).

Data analysis: A MANOVA with repeated measurements was used to compute lateralized task-dependent changes in sympathetic and parasympathetic activity with Stimulation Position (left vs. right) as a within-subject factor and both PEP and RSA (baseline vs. task-dependent) as measures. Stimulation Order (left–right vs. right–left) was used as a between-subject factor. For the emotional Stroop task, an ANOVA with repeated measurements was used for the Attentional Bias scores, with Stimulation Position as within-subject factor. Here again, Stimulation Order was used as between-subject factor. For post hoc computations, paired t-tests and Spearman rank-order correlations were assessed. The significance level was set at 0.05, two-tailed throughout.

Results

The MANOVA for PEP and RSA showed no significant interactions for Stimulation Order. There was, however, a crucial significant multivariate effect for Stimulation Position ($F(2, 7) = 7.3$, $P < 0.02$). Univariate tests showed no significant effects for RSA ($F(1, 8) = 1.0$, n.s.), but a significant effect for PEP ($F(1, 8) = 6.7$, $P = 0.03$). As can be seen from figure 2.5.1 this effect was largely due to a highly significant shortening of PEP during the Stroop task after right PFC rTMS ($t(8) = -4.1$, $P < 0.005$). After left PFC rTMS the pattern of PEP remained unchanged ($t(8) = 0.3$, n.s.). In short, elevations in sympathetic activity were found during task performance after right PFC rTMS exclusively.
The ANOVA for the Stroop task (d'Alfonso et al., 2000) showed no significant interactions concerning Stimulation Order. However, there was a crucial significant effect on Stimulation Position. Right compared to left PFC rTMS resulted in elevations in selective attention towards

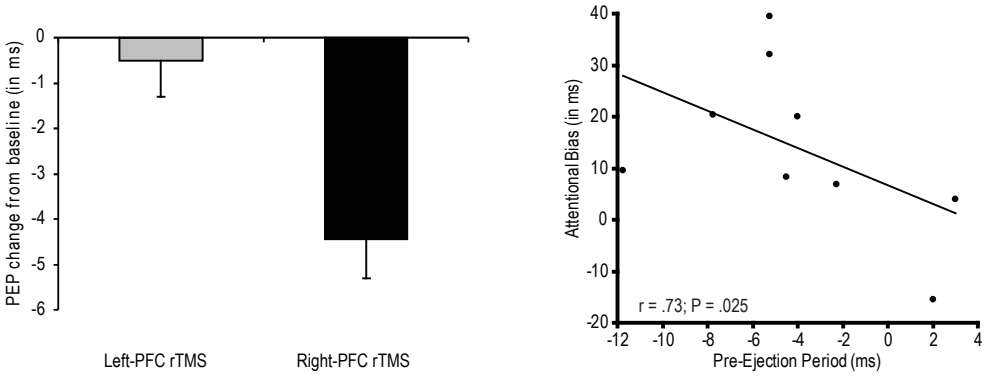

Figure 2.5.1: Left panel: stroop task-dependent changes in sympathetic activity (PEP) from baseline after left and right PFC rTMS (reductions in PEP represent elevations in sympathetic activity; see text). Right panel: scatter represents right relative to left rTMS-induced changes in PEP during the Stroop task vs. right relative to left rTMS-induced changes in Attentional Bias scores.

angry faces ($F(1, 7) = 11.22$, $P = 0.012$). To scrutinize possible relations between autonomic activity and selective processing, Spearman rank-order correlations were computed over task-dependent lateralized changes in PEP and lateralized changes in the Attentional Bias scores. As can be seen from figure 2.5.2, right relative to left PFC rTMS effects for PEP were significantly related to right relative to left PFC rTMS effects for Attentional Bias scores ($r(9) = -0.73$, $P = 0.025$).

Discussion

Selective attention to threat is argued to be an anxiety-driven change in information processing in potentially dangerous situations, evolutionarily descending from the fear-driven flight reflex (Matthews & Mackintosh, 1998). Evidently, in human primates, the processing of fear and anxiety is lateralized to the right PFC (Coan et al., 1999; Davidson & Irwin, 1999; Kalin et al., 1998). In very primitive animals, a cholinergically innervated exclusively parasympathetic brain circuit mediates the primordial response to threat (Campell, Wood, & McBride, 1997). When the reptilians evolved, sympathetic control of the heart came to provide for more flexible responses to impending danger (Porges, 1995b). The mediation of social behavior by means of emotional expressions can only be observed from the mammalian species onwards, and the emotion anger came to regulate the social hierarchy by means of its facial expression just very

recently, in the human primate (Mazur & Booth, 1998; Pridmore et al., 1998; Van Honk, Tuiten, de Haan et al., 2001).

By implementing angry faces in an emotional Stroop design in combination with autonomic measures, the present lateralized rTMS study scrutinized the claim of right-hemispheric, parasympathetic involvement in the prioritized processing of threat. Elevations in sympathetic activity were found after right PFC rTMS, and when compared to left PFC rTMS, these were accompanied by, and significantly associated with the earlier reported increases in selective attention to angry faces (d'Alfonso et al., 2000). This suggests that not the parasympathicus, but the sympathicus is the regulatory autonomic mechanism in the prioritized processing of angry facial expressions. Furthermore, as noted earlier, suprathreshold low-frequency rTMS over the right PFC results in elevated neural activity in the left PFC (Schutter et al., 2001). Concurring data are available from an interleaved rTMS-functional magnetic resonance imaging (fMRI) study (Nahas et al., 2001), and such contralateral changes in activity are arguably due to reductions in transcallosal inhibition (Pascual-Leone et al., 1998), though the exact mechanisms behind this distant effect of rTMS are not yet known (Speer et al., 2000). Notably, rTMS–neuroimaging studies not only show contralateral or focal changes in the activation of the targeted areas, but also other distant effects in anatomically interconnected regions. However, these distant changes in activation supervene on the initial focal effect, allegedly part of functionally connected networks in the brain (Fox et al., 1997; Paus et al., 1997). Nevertheless, rTMS–neuroimaging research suggests that the here-reported increases in sympathetic activity and motivated attention (i.e. after rTMS over the right PFC) are at least partly linked to elevations in neural activity in the left PFC.

In agreement, both heightened sympathetic activity (as indexed by PEP) and increased selective attention to angry faces (as indexed by the Stroop task) have been associated with the left PFC lateralized emotion anger (Burns, Friedman, & Katkin, 1992; Van Honk, Tuiten, de Haan et al., 2001; Van Honk et al., 1999). It is furthermore notable that the left hemispheric sympatheticus dominates the right in its adrenergic influence upon the ventricles (Zipes, 1991), since the cardiac sympathetic index of PEP is adrenergically innervated (Bongard et al., 1997). Finally, recent endocrine studies in humans have demonstrated interrelations between the steroid hormone testosterone (T), the emotion anger, and the attentional and physiological response to the angry facial expression (Van Honk, Tuiten, Hermans et al., 2001; Van Honk et al., 1999). Note that the sympathetic adrenergic neurotransmitter norepinephrine (NE) is associated with both T and anger, the T-NE-ANGER link (Kemper, 1990).

In conclusion, our data suggest that a left PFC lateralized sympathetic mechanism directs attention towards the angry facial expression. This result refines and elaborates the assumption

that an anxiety-motivated, parasympathetic, right-hemispheric brain circuit regulates the prioritized processing of threat, and provides further support for an anterior dimensional model of negative affect (Harmon-Jones & Allen, 1998). Future research into the processing of threat might benefit from taking into account the nature of the threat employed.

2.6 *Exogenous testosterone potentiates responses to angry facial expressions in the neural circuitry of reactive aggression in humans*

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Manuscript in revision

Abstract

Background: In a range of vertebrate species, the steroid hormone testosterone is known to potentiate brain circuits subserving intraspecific aggression. Disorders of impulsive aggression in humans have likewise been associated with high testosterone, but in humans evidence for the testosterone-aggression link remains correlational and inconclusive.

Methods: Twelve female participants underwent functional MRI during three sessions while viewing socially threatening stimuli: angry facial expressions. The first session was included to establish associations between baseline hormone levels and activation patterns. In a subsequent crossover design participants were tested again after receiving sublingual administrations of .5 mg of testosterone or placebo.

Results: Findings demonstrate consistent activation of areas involved in vertebrate reactive aggression, the amygdala and its efferent pathways in hypothalamic and brainstem subnuclei, but also in the orbitofrontal cortex, a region implicated in human impulse control. Baseline endocrine profiles of high testosterone and low cortisol were associated with stronger activation in subcortical structures. Importantly, responding predominantly in these subcortical regions was potentiated after testosterone administration.

Conclusions: The present data show that testosterone affects subcortical, i.e., amygdala and hypothalamus, to a larger degree than than cortical, i.e., OFC, components of the neural substrates of social aggression. These findings may have important implications for our understanding of the pathophysiology of disorders of impulsive aggression.

Introduction

Intraspecific aggression in vertebrates, including humans, appears to be controlled by an integrated molecular, neuroanatomical, and behavioral substrate which exhibits strong evolutionary stability (Nelson & Chiavegatto, 2001). This system is thought to underlie the establishment of hierarchies in social animals, often merely through ritualized species-specific behavioral displays of hostile intent, such as angry facial expressions (Blair, 2003; Öhman, 1986).

The hypothalamic-pituitary-gonadal (HPG) axis, through its end product testosterone, is an important agent in regulating this system. Across vertebrates, males are generally more physically aggressive than females, and gonadectomy reduces aggression (Giammanco et al., 2005; Lee & Coccaro, 2001). Moreover, animal research has unequivocally shown that testosterone elevation increases aggressiveness (Lumia, Thorner, & McGinnis, 1994; Melloni, Connor, Hang, Harrison, & Ferris, 1997; e.g., Rejeski, Brubaker, Herb, Kaplan, & Koritnik, 1988). In humans, there is correlational evidence for a link between testosterone and aggression within both sexes (Mazur & Booth, 1998). Disorders characterized by impulsive, reactive aggression, such as antisocial (APD) and borderline (BPD) personality disorders, have likewise been associated with high testosterone (Aromäki, Lindman, & Eriksson, 2002; Rasanen et al., 1999; Stalenheim et al., 1998; Virkkunen et al., 1994). Pending conclusive causal evidence (see, e.g., Archer, 2006), however, the exact role of testosterone in human aggression remains elusive.

Pathways controlling reactive aggression converge in amygdalar and hypothalamic regions. Animal research has shown that testosterone exerts its influence partly through interactions with arginine vasopressin (AVP) in the amygdala (de Vries & Miller, 1998; Ferris & Delville, 1994) and the (anterior) hypothalamus (Delville, Mansour, & Ferris, 1996). Also, testosterone down-regulates the hypothalamic-pituitary-adrenal (HPA) axis, resulting in chronically depressed cortisol (Viau, 2002). Hypocortisolemia in turn has also been associated with heightened aggression and social rank (Kalin, 1999; Sapolsky, 1990).

The capacity to inhibit reactive aggression, or impulse control, is generally attributed to the orbitofrontal cortex (OFC). Lesions to the OFC are known to result in socially aberrant behavior (e.g., Blair & Cipolotti, 2000; Eslinger & Damasio, 1985). Likewise, marked OFC hypometabolism has been observed in patients with personality disorders (Goyer et al., 1994; New et al., 2004). As indicated by low cerebrospinal fluid levels of 5HT catabolites, this hypometabolism appears to originate from reduced functioning of the serotonergic system (Lee & Coccaro, 2001), and testosterone has been suggested to play a role in the etiology of this abnormality (Giammanco et al., 2005).

Human neuroimaging findings have most consistently identified the amygdala and (lateral) OFC in responding to angry facial expressions (Blair, Morris, Frith, Perrett, & Dolan, 1999; Fischer

et al., 2005; Hariri, Tessitore, Mattay, Fera, & Weinberger, 2002; Sprengelmeyer, Rausch, Eysel, & Przuntek, 1998; Whalen et al., 2001). Behavioral experiments have furthermore shown that strong affective responding to angry faces is predicted by high testosterone (Van Honk et al., 1999) and low cortisol (Van Honk et al., 1998).

Using functional MRI, the present study first examined the pattern of brain responses to angry facial expressions in relation to levels of testosterone and cortisol. We scrutinized a hierarchical model consisting of (1) cortical inhibitory control, implemented in (lateral) OFC, and (2) subcortical reactive aggression circuits, i.e., amygdaloid region (medial/central nuclei, stria terminalis), hypothalamus, and brainstem subnuclei (periaqueductal grey; PAG) (cf. Blair, 2004). Subsequently, the same participants were tested in a placebo-controlled crossover design in order to test effects of testosterone administration on functioning of these circuits. We predicted that responding in subcortical reactive aggression circuits, contrary to OFC, would be associated with high testosterone/low cortisol, and that testosterone elevation would affect responding in a similar region-specific manner.

Methods and Materials

Participants: Participants were twelve healthy, adult female volunteers. Exclusion criteria were: history of endocrine or psychiatric disorder, left hand dominance, habitual smoking, current pregnancy, history of closed-head injury, and presence of metal objects in or around the body (braces, pacemaker, metal fragments). They were furthermore instructed not to use any (recreational) psychotropic drugs within 2 weeks of testing.

Ten women used standard estrogen/progestagen oral contraceptives. For the other two women who did not use oral contraceptives, testing was restricted to the pre-ovulatory phase in order to control for (minor) variations of androgen levels throughout the menstrual cycle.

All procedures were approved by the local institutional review board in accordance with the declaration of Helsinki, and each participant provided written informed consent. Afterwards, participants were debriefed and received payment.

Material and apparatus: Stimuli were selected out of two different photosets: the Karolinska Directed Emotional Faces (Lundqvist et al., 1998) and the Pictures of Facial Affect (Ekman & Friesen, 1976). Eight actors were chosen, and two photographs of each, happy (H) and angry (A) were included in the set. First, oval cut-outs containing only the face were made of all stimuli. Subsequently, all pictures were gray-scaled and equalized in appearance by adjusting luminance and contrast levels. Also, a fixation cross stimulus was made for baseline periods.

Stimulus presentation was controlled by an x86 notebook PC running custom software written

in E-Prime (Psychology Software Tools, Inc.). Using a data projector, stimuli were back-projected onto a transparent white screen hung from the ceiling near the participants' feet. Participants viewed the screen through a 45° angle mirror attached to the head coil.
A Philips ACS-NT scanner (Philips Medical Systems, Best, The Netherlands) with a 1.5 Tesla field strength was used for MRI.

Testosterone and placebo samples: Testosterone solutions consisted of .5 mg of testosterone, 5 mg of hydroxypropyl-beta-cyclodextrin (used as carrier), 5 mg ethanol, and 5 ml of water. Placebo solutions differed only in absence of testosterone. For details concerning this sublingual adminstation procedure, pharmacokinetics, and time-course of efficacy, see Tuiten et al. (2000). Identical procedures have repeatedly been applied successfully in our laboratory (Hermans, Putman, Baas, Koppeschaar, & Van Honk, 2006; Schutter & Van Honk, 2004; Van Honk, Peper, & Schutter, 2005; Van Honk, Tuiten, Hermans et al., 2001).

Procedure: Participants were tested with functional MRI on three separate days, the first of which did not involve any drug administration. Therefore, appointments for the first session were made at the MRI facility only. Participants were tested on afternoons (after 13.30) and were instructed not to eat within 1.5 hours prior to the appointment and to drink only water. Procedures started with collection of saliva samples (approximately 9 ml). Participants were offered sugar-free chewing gum in order to facilitate saliva production.
On the second and third testing day, participants arrived at the laboratory 3.5 hours before the appointment at the MRI facility. After providing saliva samples as described above, participants received the 5 ml drug sample, and were asked to keep this solution under the tongue without swallowing for a full minute in order for it to be fully absorbed. They were asked to come to the MRI facility 3.5 hours later, and refrain from physically or psychologically straining activities in the meantime. Further procedures were equal on all three days of testing and are detailed below.
At the MRI facility, participants first underwent an MRI safety screening in order to make sure that participants did not have metal objects present outside or inside of the body, e.g., metal fragments in the head or a pacemaker. Moreover, they were required to deliver a sample of urine for an instant pregnancy test. Following these safety procedures, participants first delivered another sample of saliva and were then escorted into the scanner room and made comfortable on the scanner bed. Care was taken to evenly distribute support of the head, and head stabilization was further increased using side-pads and a strap. Participants were instructed to make as little movements as possible. Further communication took place over an intercom system.

Scan sessions commenced with a short localizer scan (3 slices in all orientations, 2*2 mm in plane resolution) followed by a survey scan (19 sagittal slices, 1*1 mm in plane resolution) which was used for angulation of functional scans. Special care was taken to cover the same volume of the brain for each participant and each session. Scans with the following characteristics were obtained:

1. T2* weighted Blood Oxygenation Level Dependent (BOLD) images (segmented 3D EPI with navigator echo, flip angle 9.5°, TE/TR: 19.1/28.6 ms, FOV: 256*256*120 mm, matrix 64*64*30, voxelsize 4 mm isotropic, time per volume 3.26 s), with an oblique angulation with respect to the survey image in order to include the whole brain. This multishot 3D EPI pulse sequence was specially designed to minimize signal dropout and image distortion due to magnetic field inhomogeneity around air-tissue interfaces. See figure 2.6.1 for brain coverage and signal-to-noise ratio around the amygdala; see Van Gelderen et al (1995) for details on 3D functional imaging.
2. Reference image: identical to functional images except for flip angle: 30°, which results in more anatomical contrast. This image was used to facilitate registration of structural and functional images.
3. T1-weighted structural images (TE/TR 4.6/30 ms, flip angle 30°, FOV 256*180*208 mm, matrix: 256*256*150 mm, slice thickness 1.2 mm, voxel size 1*1*1.2 mm, scan duration: 8 minutes).

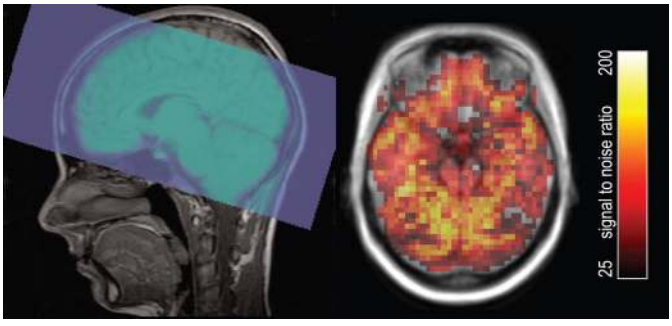

Figure 2.6.1: Localizer scan with an overlay showing the oblique angulation of the functional scans (left image). The right image shows a signal to noise ratio (mean intensity / standard deviation ratio) image overlay after high pass filtering with a 1.17e-2 Hz cut-off for a single subject. This image shows reasonable retention of signal and no readily detectable geometric distortions, around the amygdala and ventromedial prefrontal areas.

During the functional scan series, participants watched alternating 26.08 sec epochs (equal to eight scans duration). In each epoch, all 8 stimuli were presented in random order and repeated 7 times, comprising a total of 56 stimuli. Each stimulus was presented for a duration of 200 ms with a stimulus onset asynchrony of 467 ms. Baseline control epochs were also included, during which participants watched a fixation cross (+). Epochs were presented in the following order: +AH+AH+AHHA+HA+HA+ (or A and H reversed, counterbalanced across participants), which precludes task covariation with linear drifts of BOLD signal (see figure 2.6.2 for epoch order and example stimuli).

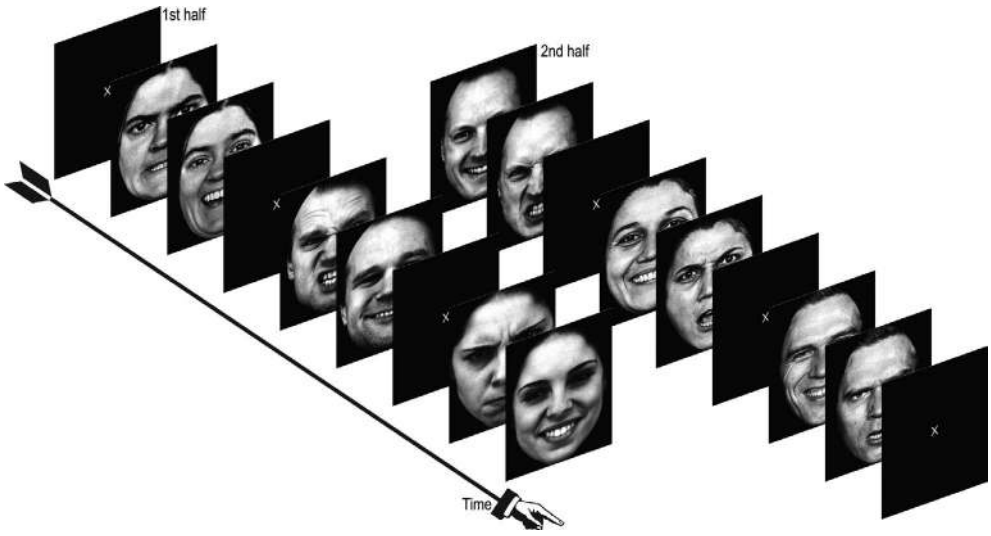

Figure 2.6.2: Order of epochs during the passive viewing task. Each epoch (fixation baseline, angry faces, or happy faces) lasted 26.08 seconds, and during each target epoch, 56 stimuli were flashed onto the screen. The second half of the task is mirrored with respect to the first half in order to preclude covariation with linear signal drifts.

Functional MRI image analysis: Preprocessing of fMRI data was performed with SPM99 (UCL London, UK). All functional scans were motion corrected by calculating rigid body transformation parameters yielding a minimal sum of squared differences with the reference scan. Cross-modal registration of the reference scan and the structural scan was achieved by estimating optimal full affine transformation parameters resulting in maximum mutual information. All

images were normalized to standard (MNI) space using affine transformations and non-linear deformations, and resliced at a 4 mm isotropic resolution. All resulting functional images were smoothed with an 8 mm full width at half maximum gaussian kernel. For visualization purposes, all normalized anatomical images were resliced at a resolution of 1 mm isotropic, and averaged across participants to serve as background for anatomical localization of activity.

Statistical analysis steps started with fitting session-specific general linear models containing box-car functions with hemodynamic delay for both task conditions (angry vs. happy), movement correction parameters (3 translations and 3 rotations), and a discrete cosine transform high pass filter with a cut-off of 1.17e-2 Hz. Contrast images (3 sessions * 12 participants) were calculated for the main comparison of interest (angry versus happy) images by subtracting parameter estimates for happy from those for anger. For the first session (no administration), a statistical map was then calculated testing for the EXPRESSION effect (angry versus happy facial expression) across participants using a statistical pooled variance approach (Ramsey et al., 1996). For our a priori regions of interest (ROIs), bilateral BA47 in the orbitofrontal cortex, bilateral amygdala, hypothalamus, and brainstem, the threshold for statistical significance was set at $p < .001$ uncorrected (i.e., $Z > 3.09$; one-sided). All adjacent voxels exceeding this threshold were subsequently clustered. Contrast values for each participant and for each ROI were extracted from the original angry versus happy contrast images and were averaged. These values were subsequently used for correlational analyses with hormone levels and between different areas. Significance threshold for voxels outside the ROIs was set using a Bonferroni correction for the whole brain, yielding a threshold of $\alpha = .05/24534 = 2.04e\text{-}6$; $z = 4.61$. For the second and third session, z-maps were calculated for the main effect of EXPRESSION and for the EXPRESSION*DRUG interaction, yielding areas that respond more to angry (versus happy) facial expressions in the testosterone condition.

For visualization purposes, both statistical group maps were thresholded at $Z > 3.09$ one-sided, and superimposed onto the averaged structural image of all participants (see figure 2.6.4 and 2.6.6).

Salivary measurements: All saliva samples were stored in plastic vials and frozen at -20° Celsius. Testosterone in saliva was measured after diethyl-ether extraction using a competitive radioimmunoassay employing a polyclonal antitestosterone antibody (J. Pratt, PhD, AZG 3290). [1,2,6,7-^{3}H]Testosterone (TRK402, Amersham Nederland B.V.) was used as a tracer following chromatografic verification of its purity (see Dabbs, 1990, for details). Testosterone levels in saliva samples taken four hours after sublingual administration were not determinable (all levels were strongly supraphysiological and not representative of actual levels).

Salivary cortisol levels were determined without extraction using a competitive radio-immunoassay employing a polyclonal anticortisol–antibody (K7348). Following chromatographic verification of its purity, 1,2-3H(N)–Hydrocortisone (NET 185, NEN Dupont, Dreiech, Germany) was used as a tracer. The lower limit for detection is 0.5 nmol/l and reference values for adults are 4–28 nmol/l. See Kirschbaum and Hellhammer (1994) for details concerning the validity and advantages of measuring cortisol in saliva.

To increase reliability of measurements, all three baseline levels were averaged for cortisol and testosterone (yielding 11.16 nmol/l, SD=2.80, and 79.28 pmol/l, SD=22.33, respectively). Subsequently, both distributions were standardized to T scores (mean 50; SD 10) and individual testosterone/cortisol ratio scores were calculated.

Results

Endocrine measures: Testosterone baseline levels on the three days of testing did not differ significantly ($F(2, 10) = 2.39$, n.s.). In the placebo condition, testosterone levels dropped slightly from pre-administration to post-scanning ($t(11) = 2.27$, $P = .044$).

Cortisol baseline levels were lowest on the first day of testing in comparison with the testosterone and placebo administration sessions ($F(1, 11) = 11.35$, $p = .006$ and $F(1, 11) = 9.01$, $p = .012$, respectively), which is explained by the fact that saliva samples were collected at a later time of day during the first session. Cortisol levels also dropped from the pre-administration to post-scanning samples for the second an third sessions ($F(1, 11) = 39.26$, $P < .001$), but no interaction with drug administration was found ($F(1, 11) = 1.45$, n.s.), indicating that exogenous testosterone elevation did not significantly decrease endogenous cortisol levels.

Figure 2.6.3: 3D rendering of the skin and the brain from a T1 weighted MRI scan (top, frontal, and left views, respectively). Clusters of suprathreshold activity in the main regions of interest are color-coded.

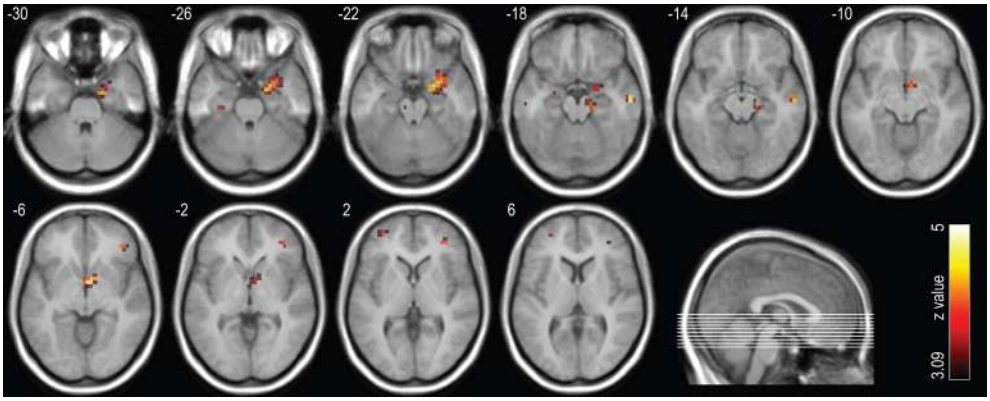

Figure 2.6.4: Ten axial slices at z=-30 to z=6 in MNI space from the averaged, normalized, anatomical scans from all 12 participants. All voxels exceeding the ROI-threshold of 3.09 (P < .001) from the contrast angry versus happy from the first scan session are overlaid onto these slices. See table 1 for additional data for each cluster.

Functional MRI results, first session: Results for the main contrast of interest (angry versus happy) are summarized in table 2.6.1. The main regions of interest (Brodmann area 47 in the orbitofrontal cortex, bilateral amygdala, hypothalamus, and brainstem) all showed evidence of suprathreshold activity. Amygdala responding was stronger in the right hemisphere. An additional focus of activity reaching a more conservative whole-brain corrected z threshold of 4.61 was found in the inferior temporal gyrus. Moreover, two clusters of activity, one in the brainstem and one in the insular cortex, were more activated during the happy than during the angry face conditions. Figure 2.6.3 shows a 3D rendering of clusters of activity in the main regions of interest. In figure 2.6.4, all voxels exceeding the z>3.09 region of interest threshold are plotted onto axial slices of the average normalized T1 weighted image of all participants.

Table 2.6.1: Summary of suprathreshold clusters of activation to angry vs. happy facial expressions in the first session:

Region	Side	X	Y	Z	Extent	Max z
Expression main effect: activations						
Hypothalamus	R	8	0	-8	23	4.91**
Amygdala	R	24	0	-24	55	4.80**
Inferior temporal gyrus (BA20)	R	60	-16	-20	9	4.80**
Superior Brainstem / Posterior Hippocampus	R	20	-24	-20	11	4.21*
Orbitofrontal Cortex (BA47)	R	40	40	-8	11	4.00*
Orbitofrontal Cortex (BA47)	L	-32	52	0	6	3.90*
Amygdala	L	-24	-8	-20	1	3.36*
Brainstem (Pons)	L	-8	-24	-24	1	3.14*
Expression main effect: deactivations						
Brainstem	R	16	-20	-4	25	4.71**
Insular Cortex	R	36	4	-4	51	4.70**

*Coordinates are defined in MNI space. *Activation significant at a P < .001 uncorrected threshold (one-sided and for regions of interest only). **Activation significant at a P < .05 whole brain bonferroni corrected threshold. Extent indicates the cluster size of adjacent voxels with P < .001, uncorrected.*

Table 2.6.2: Summary of rank order correlations between endocrine parameters and BOLD responses to angry facial expressions in activated clusters during the first session:

Region	Cortisol		Testosterone		T/CORT ratio	
	ρ	P	ρ	P	ρ	P
Amygdala	-0.32	0.308	0.12	0.721	0.68*	0.015
Left	-0.31	0.319	-0.09	0.787	0.51	0.090
Right	-0.28	0.379	0.22	0.498	0.69*	0.013
Orbitofrontal Cortex (BA47)	-0.20	0.527	-0.13	0.688	0.03	0.914
Left	-0.10	0.746	-0.34	0.285	-0.17	0.587
Right	-0.26	0.417	0.14	0.664	0.27	0.391
Hypothalamus	-0.58*	0.048	0.27	0.397	0.83**	0.001
Brainstem	-0.30	0.342	0.27	0.397	0.70*	0.011
Superior (/post hippoc.)	-0.32	0.308	0.22	0.498	0.56	0.059
Pons	-0.21	0.513	0.09	0.787	0.50	0.101
Inf. temporal gyrus (BA20)	0.08	0.795	0.80**	0.002	0.67*	0.017

*P < .05; **P < .01

Table 2.6.3: Summary of non-parametric cross-correlations between activated clusters in the first session:

Region	1)		2)		3)		4)	
	ρ	P	ρ	P	ρ	P	ρ	P
1)Bil. amygdala								
2)Bil. OFC (BA47)	-0.05	0.88						
3)Hypothalamus	0.60*	0.04	0.03	0.91				
4)Brainstem	0.76**	0.004	-0.18	0.57	0.57*	0.05		
5)Inf.tem.g.(BA20)	0.36	0.26	-0.33	0.30	0.21	0.51	-0.07	0.83

*P < .05; **P < .01

*Table 2.6.4: Summary of suprathreshold clusters for EXPRESSION maineffects and DRUG*EXPRESSION interactions in the 2nd/3rd sessions:*

Region	Side	X	Y	Z	Extent	Max z
Expression main effect: activations						
Orbitofrontal Cortex (BA47/10)	L	-32	60	-4	1	3.59*
Orbitofrontal Cortex (BA47)	R	40	48	-8	1	3.42*
Amygdala	R	20	4	-20	3	3.39*
Expression main effect: deactivations						
Parahippocampal gyrus (BA28)	R	20	-24	-9	24	6.51**
Brainstem (Pons)	L	-4	-17	-19	16	5.29**
Drug*Expression interaction: testosterone activations						
Amygdala	R	12	-4	-24	15	4.94**
Hypothalamus	R	8	0	0	17	4.45*
Brainstem (Pons)	L	-8	-20	-28	7	4.05*
Orbitofrontal Cortex (BA47/46)	L	-48	44	-8	6	3.99*
Orbitofrontal Cortex (BA47)	R	44	52	-12	2	3.88*

*Coordinates are defined in MNI space. *Activation significant at a p<.001 uncorrected threshold (one-sided and for regions of interest only). **Activation significant at a p<.05 whole brain bonferroni corrected threshold. Extent indicates the cluster size of adjacent voxels with p<.01.*

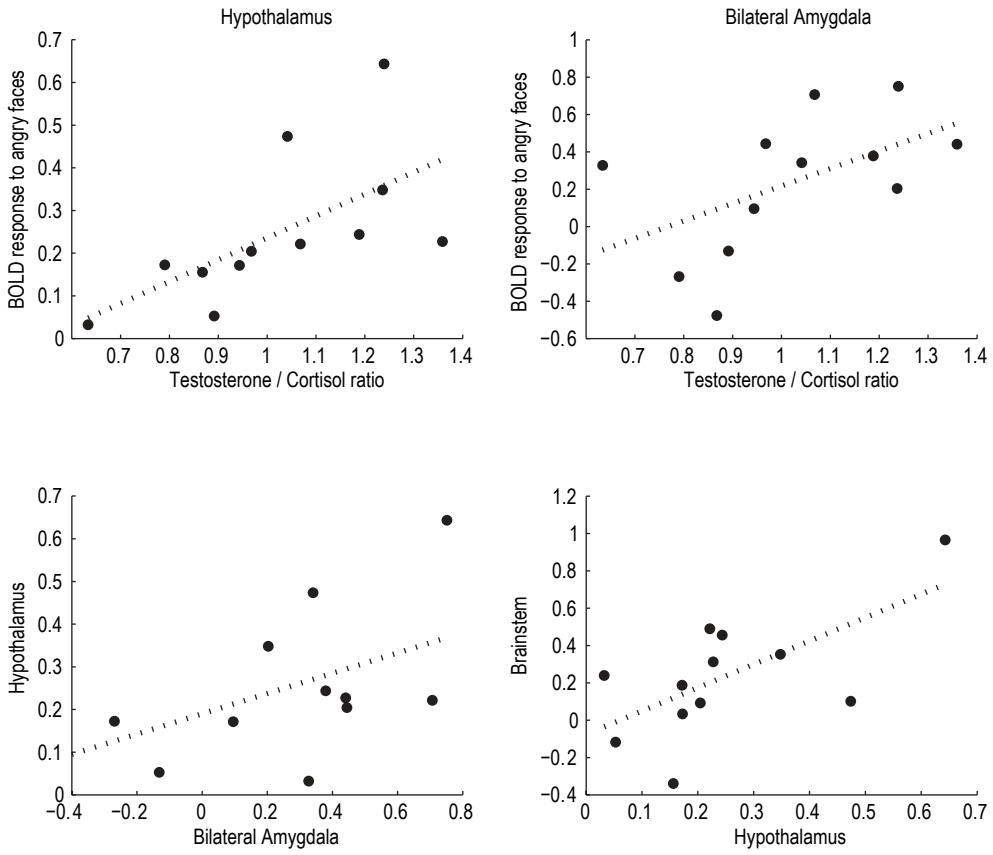

Figure 2.6.5: Top panels: scatterplots of the correlation between testosterone/cortisol ratios and the average magnitude of the BOLD response to angry versus happy facial expressions in the hypothalamus (A) and bilateral amygdala (B). Bottom panels: scatterplots showing interregional cross-correlations between the average BOLD responses to angry facial expressions in the bilataral amygdala and hypothalamus (C) and between hypothalamus and brainstem (D).

Correlational analyses: Averaged contrasts of parameter estimates for the angry versus happy comparison per cluster of suprathreshold activity were used for correlational analyses with endocrine measures. Non-parametric statistics (Spearman's rank order correlations) were applied because of the small sample size. Results of these analyses are summarized in table 2.6.2. Only hypothalamic activity in response to angry facial expressions exhibited a significant negative correlation with baseline cortisol levels. Baseline testosterone levels correlated positively with activity in the inferior temporal gyrus only. The testosterone/cortisol ratio, however, proved much more predictive of the response to angry faces: significant positive correlations were found with activity in the (predominantly right) amygdala, hypothalamus, brainstem, and inferior temporal gyrus. Scatterplots of the first two of these are depicted in figures 2.6.5A and 2.6.5B.
Subsequently, interregional non-parametric correlations across participants were calculated between activated clusters (see table 2.6.3). Responses to angry faces in the bilateral amygdala, hypothalamus, and combined brainstem clusters proved strongly interrelated (see figures 2.6.5C and 2.6.5D for scatterplots). However, there was no evidence for the predicted negative correlation between the bilateral orbitofrontal areas in Brodmann area 47 and any of the other ROIs. Moreover, combined brainstem clusters responses were positively correlated with inferior temporal gyrus activity.

Functional MRI results, second and third session: Results of the drug administration sessions are summarized in figure 2.6.6 and table 2.6.4, which contains both effects for the EXPRESSION main effect and the DRUG*EXPRESSION interaction effect. The EXPRESSION main effect contrast over these two sessions yielded a pattern of activated areas similar to the first session (i.e., bilateral Brodmann area 47 in the orbitofrontal cortex, and the right amygdala), although not all regions reached significance. Also, there was evidence of stronger activation to happy than to angry expressions in the brainstem (pons) and parahippocampal gyrus.
Crucial DRUG*EXPRESSION interaction effects are listed in the lower half of table 2.6.4. As predicted, responding in the greater part of the network specified in the first session increased in the testosterone administration condition (especially in the right amygdala and hypothalamus), although the peak location of the interaction effect in the amygdala appears to lie somewhat more medial than the main effects.

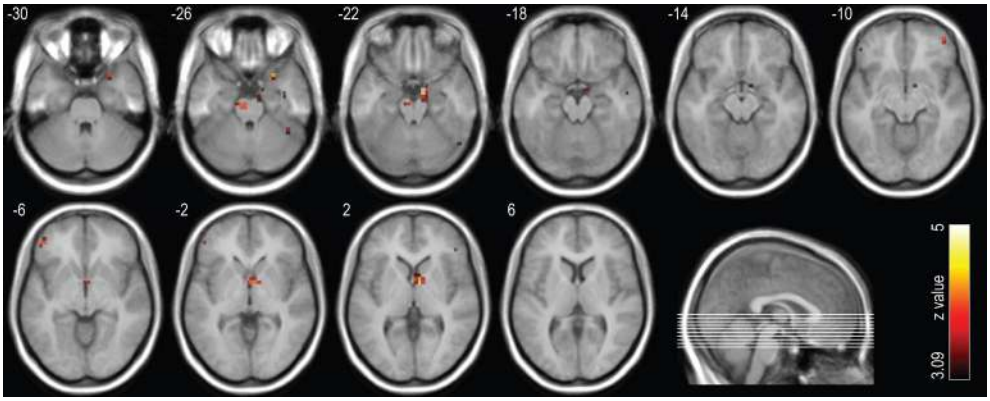

Figure 2.6.6: Ten axial slices at z=-30 to z=6 in MNI space from the averaged, normalized, anatomical scans from all 12 participants. All voxels exceeding the ROI-threshold of 3.09 (P < .001) for the DRUG FACE TYPE interaction (i.e., areas that respond more to angry faces during the testosterone session) from the second and third sessions are overlaid onto these slices. See table 4 for additional data.*

Discussion

The purpose of the present study was to gather insights into the neural regulation of human social aggression, in particular by investigating the regulatory role of steroid hormones. The main findings were, first, that cortical (the OFC), as well as subcortical (the amygdala and its efferents) circuits implicated in aggression can be identified functionally using fMRI in humans. Second, the degree to which these areas respond to social threat is dependent upon endocrine parameters, with strongest responses in participants with high testosterone/low cortisol. Third, critically, exogenous elevation of testosterone levels caused an increase in responding in these areas. Finally, activity in the OFC, which implements the more distinctively human competence of impulse control, appears to be less related to these endocrine variables.

The medial and central nuclei of the amygdala, the bed nucleus of the stria terminalis, and efferent structures such as the hypothalamus and brainstem areas (e.g., PAG) have traditionally been characterized as a defensive circuit that choreographs autonomic, endocrine, and behavioral fight/flight responses to impending threat (e.g., Kling & Brothers, 1992). Although this notion implies partly overlapping circuits for flight and fight, or fear and anger, contemporary human neuroimaging research has placed much more emphasis on the role of these pathways in fear than in anger. Neural responses to angry facial expressions are likewise sometimes interpreted in terms of fear. The present study replicated the existing data by showing responses to angry faces in the

bilateral amygdala (Fischer et al., 2005; Hariri, Tessitore et al., 2002; Sprengelmeyer et al., 1998; Whalen et al., 2001), as well as in some of the expected efferent pathways of the amygdala, the hypothalamus and brainstem subnuclei. However, the main findings of the present study place these responses in a different perspective.

Facial expressions of anger are a constituent part of socially aggressive behavior. Over the course of evolution, selection pressures have tended to moderate intraspecific conflicts over resources for survival and procreation, channeling them into ritualized dyadic exchanges of social signals of angry defiance (Öhman, 1986). Nonverbal social behavior is therefore thought to be regulated by relatively closed, pre-wired, genetic programs (Mayr, 1974) that integrate dedicated neural and molecular pathways with species-specific non-verbal behavior, the main vehicle of which is facial expression (Fridlund, 1991). Hence, angry facial expressions are presumed to play an important role in establishing and structuring social hierarchies (cf. Blair, 2004), and evoke affective responses in the observer that vary as a function of social status (Putman et al., 2004; Van Honk, Tuiten, Hermans et al., 2001; Van Honk et al., 1999).

Individual differences in functioning of the endocrine systems have proven to be reliable predictors of social rank. The HPA and HPG axes exhibit mutually inhibitory functional interactions (Viau, 2002) with opposite effects upon social dominance. Although aggressive episodes are accompanied by HPA-initiated phasic cortisol increases (Summers et al., 2005), a profile of low testosterone and chronically high cortisol is related to social submissiveness and low aggression in various species (Blanchard et al., 1993; Kalin, 1999). In agreement, previous research has shown that various measures of affective responses to angry facial expressions in human volunteers are predicted by high levels of anger, dominance, drive, and high testosterone levels (Putman et al., 2004; Van Honk, Tuiten, de Haan et al., 2001; Van Honk et al., 1999), and are oppositely related to social anxiety and tonic cortisol levels (Putman et al., 2004; Van Honk et al., 1998). Hence, correlations were calculated between BOLD responses within the activated areas and the testosterone/cortisol ratio. In line with predictions, results show that individuals with a high testosterone/cortisol ratio respond more to angry faces in the amygdala, hypothalamus, and brainstem areas. Note that this finding is at odds with an interpretation of activity in subcortical defense circuits solely in terms of fear, because both animal (e.g., Aikey et al., 2002) and human research (Hermans et al., 2006; Van Honk et al., 2005) has shown that testosterone has fear-reducing properties.

Research on rodents suggests that testosterone exerts its effect upon reactive aggression in interaction with AVP. Apart from its role in osmotic regulation, the nonapeptide AVP has been shown to potentiate reactive aggression in various species (e.g., Winslow, Hastings, Carter, Harbaugh, & Insel, 1993). Higher levels of AVP in specifically the hypothalamus have been

related to aggression in rodents (Ferris et al., 1997). AVP is synthesized among others in the amygdalar (medial amygdalar nucleus and bed nucleus of the stria terminalis) and (ventrolateral) hypothalamic regions, where its effects are mediated by gonadal steroids (de Vries & Miller, 1998; Delville et al., 1996; Koolhaas, Van den Brink, Roozendaal, & Boorsma, 1990). In humans, high AVP levels have also been found in cerebrospinal fluid (CSF) of impulsively aggressive patients with personality disorders (Coccaro, Kavoussi, Hauger, Cooper, & Ferris, 1998), which is in accordance with findings of high testosterone in APD and BPD patients (Aromäki et al., 2002; Rasanen et al., 1999; Stalenheim et al., 1998; Virkkunen et al., 1994). Heuristically guided by the supposition that the angry facial expression has aggression-provoking properties (Van Honk et al., 1999), the present data provide the first causal support for the link between testosterone and reactive aggression in humans by showing an exaggerated response to angry facial expressions in the amygdalar/hypothalamic region after exogenous elevation of testosterone levels. Note that although the peak location of the drug interaction effect appears to lie medial with respect to the main effect, the underlying resolution of the statistical maps does not warrant inferences about functional subnuclei of the amygdala.

Other than the subcortical circuitry of reactive aggression, the role of the OFC in human social aggression appears to be inhibitory (see Blair, 2004, for a review). The present data replicate earlier findings of responses in this area to angry facial expressions (e.g., Blair et al., 1999). A theoretically expected negative interregional cross-correlation across participants (see table 2.6.3) between activity in OFC and subcortical structures could not be established in the present study. However, cross-correlations do show consistent responsiveness within subcortical reactive aggression circuits. Activity in the OFC thus appears relatively independent of these lower circuits.

Because the angry facial expression signals conspecifics to amend current behaviors, it has been argued that responding to angry faces recruits processes that are also implicated in response reversal and behavioral extinction, functions that have been ascribed to the OFC (Blair, 2004; Dias, Robbins, & Roberts, 1996). These notions are supported by neuropsychological observations of patients with OFC lesions. Often, these result in personality changes and impulsively aggressive, aberrant behavior (Blair & Cipolotti, 2000; Damasio, Grabowski, Frank, Galaburda, & Damasio, 1994). There is also evidence that the response reversal deficit in OFC lesioned patients is specific for social stimuli rather than a general cognitive impairment (Blair & Cipolotti, 2000). Moreover, volumetric analyses have shown sex differences in OFC volume that parallel sex differences in aggression: women have larger OFC volumes than men (Gur, Gunning-Dixon, Bilker, & Gur, 2002). In further agreement, neuroimaging has shown that impulsively aggressive individuals exhibit OFC dysfunctions (De La Fuente et al., 1997; Soloff et al., 2003), and studies that

used paradigms to enhance emotional states in BPD found augmented amygdalar responding (Herpertz et al., 2001) as well as reduced OFC activity (Schmahl, Vermetten, Elzinga, & Bremner, 2004), which fits into a picture of subcortical preeminence due to malfunctioning OFC impulse control.

Reduced serotonergic (5HT) neurotransmission may well underlie OFC hypofunctioning in impulsive aggression. This claim is supported by a body of evidence that has linked correlates of 5HT dysfunction (e.g., low 5HIAA in CSF and blunted prolactin response to d-fenluramine) to impulsive aggression in personality disordered patients or violent offenders (see Lee & Coccaro, 2001, for a review). In agreement, SSRI's reduce impulsiveness in personality disorders (Coccaro & Kavoussi, 1997), and alleviate OFC hypometabolism (New et al., 2004). Interestingly, gonadal steroids have a depressing effect upon 5HT systems (Martinez-Conde, Leret, & Diaz, 1985; Sundblad & Eriksson, 1997; van de Kar, Levine, & Van Orden, 1978), which is again consistent with aforementioned observations of high testosterone in personality disordered patients. In the present study however, a fairly subtle acute effect of testosterone upon OFC activity was found, with an increased response to angry faces in small subdivisions of the OFC, which may reflect increased inhibitory control to constrain subcortical activation. One explanation for this finding might be that testosterone effects via 5HT constitute a longer timescale process. Another explanation for this is that testosterone primarily interferes with cortico-subcortical communication, thus reducing efficacy of OFC impulse control (see Schutter & Van Honk, 2004). Further research is needed to resolve this issue.

In conclusion, through a testosterone-potentiated response to angry facial expressions, the present findings contribute importantly to identifying the neural circuitry through which gonadal steroids may regulate social aggression in humans. It was demonstrated that models of rodent reactive aggression generalize fairly well to humans passively viewing species-specific social threat conveyed through angry facial expressions. Furthermore, it was shown that testosterone, likely through interactions with AVP, predominantly affects excitability of subcortical reactive aggression circuits that converge in amygdalar and hypothalamic regions. These findings shed new light on an ongoing debate concerning the role of testosterone in human aggression. Correlations between testosterone levels and aggression in humans have often been moderate at best, especially when using self-report as dependent measure. This perception has caused hesitation among some to generalize the alleged testosterone-aggression link to humans (see Archer, 1991; Mazur & Booth, 1998). The present findings imply a direct link between testosterone and responsiveness of reactive aggression circuits to social threat, and thus suggest that the testosterone-aggression link in humans depends crucially upon the ability of the OFC to constrain these circuits.

III

Psychoneuroendocrinology of fear circuits

3.1 *A single administration of testosterone reduces fear potentiated startle in humans*

Erno J. Hermans, Peter Putman, Johanna M. Baas, Hans P. Koppeschaar, & Jack van Honk
Biological Psychiatry 2006, 59(9), 872-874

Abstract

Background: Ample evidence from animal research indicates that the gonadal steroid hormone testosterone has fear reducing properties. Human data on this topic, however, are scarce and far less unequivocal. The present study therefore aimed to scrutinize anxiolytic effects of a single dose of testosterone using a direct physiological index of fear in humans.

Methods: Twenty healthy female participants were tested in a double-blind placebo controlled crossover design involving sublingual administration of a single dose of testosterone. Four hours after intake, we assessed effects on baseline startle and fear potentiated startle in a verbal threat of shock paradigm.

Results: In accordance with predictions, testosterone administration resulted in reduced fear potentiated startle, without affecting baseline startle.

Conclusions: This study provides direct evidence that a single dose of testosterone reduces fear in humans. The relation of this effect to previous research on anxiolytic effects of benzodiazepines, and possible mechanisms of action are discussed.

Introduction

Research in a wide range of species indicates that the steroid hormone testosterone, the end product of the hypothalamic-pituitary-gonadal (HPG) axis, has anxiolytic properties both over longer periods of treatment (Bitran et al., 1993; Boissy & Bouissou, 1994; Bouissou & Vandenheede, 1996; Frye & Seliga, 2001) and after single dose administrations (Aikey et al., 2002; Bing et al., 1998). In man, however, evidence of anxiolytic efficacy of testosterone remains scarce and indirect. Some anecdotal indications exist of anxiolytic effects after long term testosterone supplementation (Cooper & Ritchie, 2000; Kasanin & Biskind, 1943). Also, self-reported mood improvements after testosterone supplementation therapy have been reported in hypogonadism (Burris, Banks, Carter, Davidson, & Sherins, 1992; Wang et al., 1996) and refractory depression (Pope et al., 2003). Recently, however, Van Honk et al (2005) reported that sublingual administration of a single dose of testosterone diminishes preconscious selective attention to threat, which was interpreted as resulting from fear-reducing properties of testosterone. The present study was designed to more directly scrutinize fear reducing efficacy of testosterone using an identical single administration.

A widely used laboratory model of fear in humans is potentiation of the startle reflex. In remarkable similarity with other species, the first line of defense against sudden threat in humans is a rapid contraction of the facial and skeletal musculature (Davis, Gendelman, Tischler, & Gendelman, 1982). This reflex is affected by psychological variables: aversive states such as fear and anxiety augment its amplitude. In humans, the eye-blink component of the startle reflex can be quantified conveniently using electromyography (EMG) of the orbicularis oculi muscle. A robust manipulation that potentiates the startle reflex is verbal threat of shock (Grillon et al., 1991). In this paradigm, alternating safe and threat blocks are presented during which startle reflexes are evoked acoustically at random time intervals. This procedure yields highly reliable startle potentiation, even when participants receive as little as one shock per session. Moreover, its reproducibility makes this paradigm particularly appropriate for crossover designs, which befit pharmacological studies because they control for inter-subject variability in the response measure.

Remarkably, human research using this paradigm has not found unequivocal support that typical anxiolytics such as benzodiazepines reduce fear potentiated startle. Instead, despite some positive results (Bitsios, Philpott, Langley, Bradshaw, & Szabadi, 1999; Graham et al., 2005), effects of benzodiazepines were often restricted to baseline measures (Baas et al 2002). One explanation for this observation is that separable neural substrates subserve cue-specific *fear* as opposed to background *anxiety* (Davis & Whalen, 2001). Reduced baseline startle effects of benzodiazepines have been suggested to reflect a specific effect upon the latter, partly due to reduced sensitivity

to the diffuse anxiogenic context of a psychological laboratory, and partly due to general sedative effects (Baas et al., 2002; Grillon, 2002). Furthermore, the clinical efficacy of benzodiazepines in treatment of specific phobias, putatively instances of exaggerated cue-specific *fear*, is low (Marks, 1987).

Contrary to benzodiazepines, testosterone has no sedative properties (O'Connor, Archer, Hair, & Wu, 2002; O'Connor, Archer, & Wu, 2004), yet produces anxiolytic effects in animal research. The present experiment therefore scrutinized the hypothesis that a single dose administration of testosterone would reduce fear potentiated startle in the threat of shock paradigm while leaving baseline startle unaffected.

Methods

Participants: Twenty female participants (age 18-29) granted informed consent as required by the medical-ethical counsel. Only women participated because the required administration parameters are unknown in men (see Tuiten et al., 2000, for details). Exclusion criteria were habitual smoking, history of psychiatric or endocrine illness, and use of medication other than single phase oral contraceptives. Participants were tested early in their menstrual cycles, on two separate afternoons, in a counterbalanced double blind crossover design.

Material and apparatus: Drug solutions for sublingual administration contained .5 mg testosterone, 5 mg ethanol, and 5 mg hydroxypropyl-beta-cyclodextrine, in 5 ml water. Placebos lacked testosterone but were otherwise identical. 4mm Ag/AgCl electrodes and a Psylab bioamplifier were used for EMG of the startle reflex (10 Hz high pass and 50 Hz hum filters, 1 kHz digitized). White noise (50 msec, 105 dB) through headphones evoked startles. Shocks were delivered to the inner wrist using a constant current stimulator.

Procedure: On both days, participants were tested four hours after administration in a dimly lit cabin. They first completed the Profile of Mood States (POMS) questionnaire (Shacham, 1983), after which all electrodes were applied (see Blumenthal et al., 2005). Subsequently, participants received 12 habituation startle probes in the absence of threat (shock electrodes were disconnected). Written instructions then informed participants they would receive three shocks across sessions maximally, that subsequent shocks would increase in strength, that strength of the next shock would increase with latency, and that they would certainly not receive shock when the word "safe" was presented. During threat, "danger" would appear. Shock electrodes were then connected, and another twelve baseline startle probes were presented while the screen showed "safe", followed by 16 alternating 50 second "safe" or "danger" blocks with three startle

probes per block. One probe during the 7th (first session) or 5th (second session) was replaced by shock (150 msec at intensities of 2.15 mA and 2.54 mA, respectively). Finally, questionnaires on subjective fear during safe and threat blocks were completed.

Data analysis: Raw EMG was 30 Hz high pass filtered, rectified, and filtered using a 40 msec moving average. Startle magnitudes were defined as peak EMG power between 20-90 msec minus average power 0-20 msec after probe onset, and averaged over three consecutive startles. An outlier in the crucial effect of testosterone on startle potentiation was removed (this participant had a difference score exceeding the criterion of 3*SD above the mean). Data were analyzed using ANOVAs with THREAT (danger vs. safe), DRUG (testosterone vs. placebo), and BLOCK (1-8) as within, and ORDER (testosterone on day 1 vs. 2) as between factor, with α=.05 throughout.

Results

A main effect of BLOCK ($F(7,11)= 3.57$, $P=.030$) indicates a reduction of startle magnitudes over time. A strong main effect of THREAT ($F(1,17)= 56.39$, $P<.001$) shows that the threat manipulation was successful. Crucially, this effect was moderated by DRUG (THREAT*DRUG: $F(1,17)= 5.98$, $P=.026$), indicating that startle potentiation was lower in the testosterone condition (figure 3.1.1C), although the THREAT effect was highly significant in both testosterone ($F(1,17)= 39.87$, $P<.001$) and placebo conditions ($F(1,17)= 56.65$, $P<.001$). The ORDER factor yielded only a DRUG*ORDER*BLOCK interaction ($F(7,11)= 3.3$, $P=.037$).

Separate ANOVAs were calculated for the habituation and baseline phases. Neither of these (all $F<1$) showed evidence of a main effect or interaction involving DRUG. Again, a BLOCK main effect indicates a reduction in responding over time (figure 3.1.1A/B; $F(3,15)= 51.77$, $P<.001$, and $F(3,15)= 7.64$, $P=.002$, respectively).

Questionnaires: An ANOVA over fear ratings confirmed that the threat manipulation resulted in subjective fear (THREAT main effect: $F(1,17)= 81.22$, $P<.001$), but revealed no DRUG interaction effect ($F<1$). Moreover, separate t-tests for none of the subscales of the POMS yielded significant effects of DRUG (all $t<1$).

Discussion

Our prediction that testosterone administration would result in reduced fear potentiation of the startle reflex was confirmed. Moreover, neither baseline measures of the startle reflex, nor self-reported mood was affected. This finding corroborates and extends previous results by Van Honk et al (2005) by showing that the diminished selective attention to unconsciously perceived threat after testosterone administration reported there is likely due to *fear* reduction specifically.

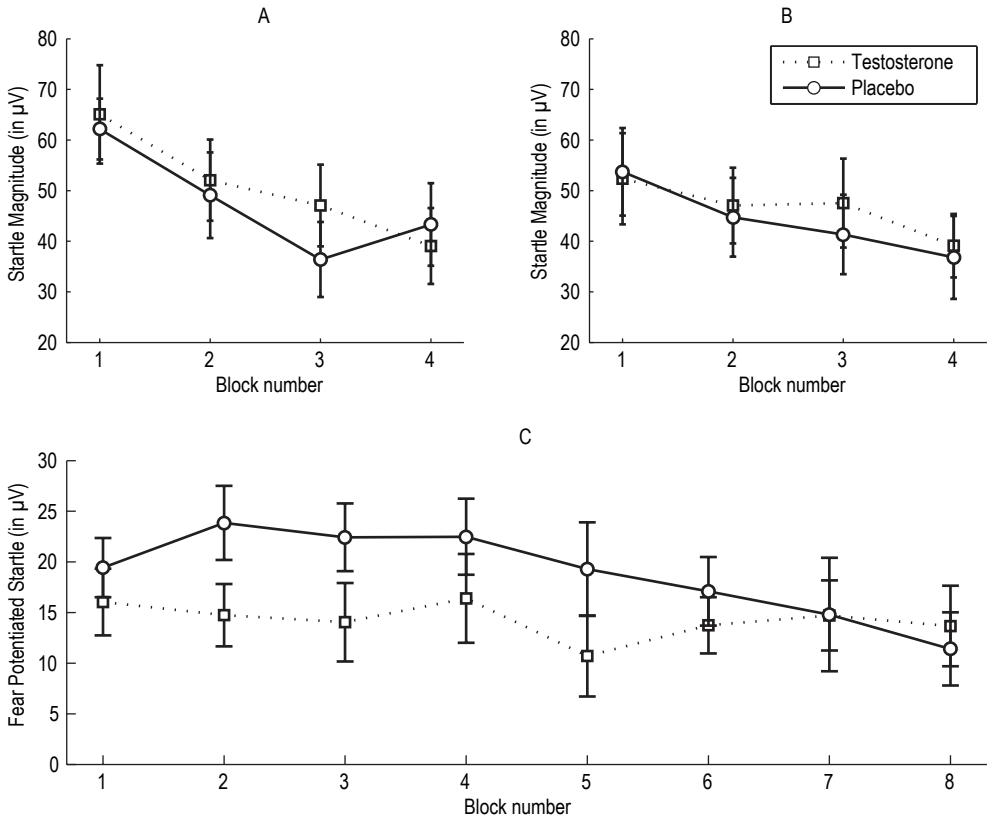

Figure 3.1.1: Traces showing startle reflexes decreasing in magnitude during the habituation phase (A; blocks represent means of three consecutive startles), a similar pattern in the baseline period after connecting the shock electrodes (B), and development of fear potentiated startle (C; threat minus safe) in the testosterone versus placebo conditions over consecutive blocks. All error bars represent standard errors of the means.

The present findings further lend support to notions that testosterone mediates sex differences in fears, which may in turn explain lower levels of aggression in females (Archer, 1999; Campbell, 1999).

The reduction in fear potentiated startle reported here stands in contrast to the effects of benzodiazepines, which predominantly affect baseline startle reflex magnitude, putatively indicative of reduced contextual anxiety (Baas et al., 2002). Also, presumably due to their amnestic and sedative side-effects, benzodiazepines interfere with the acquisition rather than the

expression of cue-conditioned fear (Scaife, Langley, Bradshaw, & Szabadi, 2005). However, an effect on fear potentiated startle very similar to the present was recently reported by Grillon et al (2003) using the glutamate receptor agonist LY354740.

Several neurobiological mechanisms may play a role in the anxiolytic effects of testosterone. Testosterone may serve as a prohormone for neuroactive steroids that act upon GABA-a benzodiazepine receptors (Bitran et al., 1993). However, this cannot explain the present divergence from findings with benzodiazepines. Another likely pathway involves genomic effects of 5α reduced metabolites such as dihydrotestosterone (Edinger & Frye, 2005). In agreement, androgen receptor blockers, and not GABA-a receptor blockers, reduce anxiolytic effects of testosterone (Fernandez-Guasti & Martinez-Mota, 2005). Finally, testosterone down regulates the hypothalamic-pituitary-adrenal (HPA) axis during stress (Viau & Meaney, 1996), which in turn may lower corticotrophin releasing hormone genetic expression in the amygdala and reduce fear through this pathway (Rosen & Schulkin, 1998).

In conclusion, the present study tested and confirmed the hypothesis that a single dose administration of the androgen steroid testosterone is capable of reducing fear potentiated startle in humans. A deeper understanding of the role of the HPG axis and its interplay with the HPA axis in regulating fear and anxiety may contribute importantly to finding avenues for more selective treatment of disorders of fear and anxiety.

3.2 *Exogenous testosterone attenuates the integrated central stress response in healthy young women*

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Manuscript submitted for publication

Abstract

Animal research has shown that the androgen steroid testosterone, the end product of the hypothalamic-pituitary-gonadal (HPG) axis, down regulates the integrated stress response at multiple levels. These effects have been demonstrated at the level of the amygdala and the bed nucleus of the stria terminalis (BNST), and along the different nodes of the hypothalamic-pituitary-adrenal (HPA) axis. The present study was designed to assess effects of exogenous testosterone upon reactivity of the autonomic nervous system and modulation of the acoustic startle reflex in humans. Twenty healthy female participants received double-blind, placebo-controlled sublingual administrations of .5 mg testosterone. Afterwards, measurements were made of phasic electrodermal activity, cardiac responses, and startle reflexes to acoustic probes while participants were exposed to pictures with strongly aversive, neutral, or positive content. Subjective reports of mood and picture evaluations were also obtained. Results support the hypothesis of a generally decreased responsiveness of the stress system by showing reduced skin conductance responses as well as reduced affective startle modulation in anxiety-prone participants. Candidate neurobiological mechanisms of action are outlined and discussed, and it is argued that androgens promote dynamic regulation of the stress system through actions upon central neuropeptidergic pathways that control corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) expression. The present findings additionally highlight the importance of further investigation of the possible role of the HPG axis in disorders that are associated with HPA axis dysfunctions, and may contribute to the development of neurobiologically embedded models of gender differences.

Introduction

Recent research suggests that the hypothalamic-pituitary-gonadal (HPG) axis, through its end-product testosterone, plays an important role in the down regulation of distinct components of the integrated stress response, such as central fear mechanisms (Hermans et al., 2006, see previous chapter; Van Honk et al., 2005) and the hypothalamic-pituitary-adrenal (HPA) stress response (Rubinow et al., 2005; Viau, 2002). Initially these notions were fuelled by findings of gender differences in HPA responsiveness in rodents (Kitay, 1963). Similar effects were later observed in humans, in terms of increased adrenal sensitivity to corticotropins in women (Horrocks et al., 1990; Roelfsema et al., 1993). Recently, the focus of attention has turned directly to the moderating role of androgen steroids on these processes. Evidence is mounting across various species that gonadal steroids attenuate central fear responses (Aikey et al., 2002; Bitran et al., 1993; Boissy & Bouissou, 1994; Bouissou & Vandenheede, 1996). In agreement, HPA axis functioning is down regulated after elevation of androgens (Kitay, 1963), especially phasic stress-related activity (Handa et al., 1994; Papadopoulos & Wardlaw, 2000; Viau & Meaney, 1996).

The integrated central response to stress (see De Kloet et al., 2005 for review) is thought to be regulated through the amygdalar region (basolateral and central nucleus) and the bed nucleus of the stria terminalis (BNST). These areas have efferent connections to, e.g., the nucleus ambiguous, which controls vagal cardiac innervation and induces bradycardia (Porges, 1995a), the nucleus reticularis pontis caudalis, which is implicated in startle modulation (Davis et al., 1982), and the hypothalamus, which mediates sympathetic autonomic responses through its lateral part, as well as endocrine responding via the paraventricular nucleus (PVN; see Walker et al., 2003). A human model for assessing the compound stress response is available through laboratory measurement of psychophysiological responding to affective content (Lang, Bradley, & Cuthbert, 1998). In agreement with the above notions, women have been shown to exhibit stronger startle modulation and autonomic responses to aversive content than men (Bradley, Codispoti, Sabatinelli, & Lang, 2001), which also suggests a similar attenuating role for androgens.

The present study scrutinized the hypothesis that androgens attenuate central stress responses by administering a single dose of testosterone to female participants, transiently raising their testosterone to an approximate male level. Using different categories of photographs as emotion provoking stimuli, we assessed both objective quantitative and subjective phenomenological effects of androgens upon the human stress system. Both baseline and phasic activity of sympathetic and parasympathetic branches of the autonomic nervous system were monitored using the dependent measures of electrodermal activity, heart rate, and startle reflex modulation. Subjective measures were obtained using affective picture ratings and mood questionnaires. Because affective startle modulation has been demonstrated to be positively related to fearful traits (Cook, Davis, Hawk,

Spence, & Gautier, 1992; Cook, Hawk, Davis, & Stevenson, 1991), and negatively to fearlessness (Lissek, Baas et al., 2005; Pastor, Molto, Vila, & Lang, 2003; Patrick, Bradley, & Lang, 1993), we assessed anxiety-proneness using Spielberger's Trait Anxiety Inventory (Spielberger, Gorsuch et al., 1983).

Experiments using this type of paradigm typically demonstrate that electrodermal activity varies as a function of generalized arousal, and is thus elevated independent of valence being positive or negative. In contrast, startle modulation is normally potentiated during aversive stimulation, and inhibited by positive stimuli. Furthermore, processing of strongly aversive material is commonly accompanied by sustained bradycardia (Lang et al., 1998). Instigated by previous findings using an identical testosterone administration procedure (Hermans et al., 2006; Van Honk et al., 2005), we anticipated a less pronounced effect in all physiological measures to aversive stimuli against a background of replication of these basic findings. In accordance with earlier findings, we expected no testosterone effects upon subjective reports.

Materials and methods

Participants: Twenty healthy female volunteers (age range: 18 – 23) were recruited through university campus flyers and received payment for participation in this study. All procedures were approved by the institutional review board in accordance with the declaration of Helsinki. All participants provided written informed consent. Exclusion criteria were: history of psychiatric or endocrine illness, left handedness, regular smoking, and use of any medication other than single phase oral contraceptives. Participants were tested in a double blind, placebo controlled, mixed factorial crossover design.

Testosterone administration samples: Testosterone solutions for sublingual administration consisted of .5 mg of testosterone, 5 mg of hydroxypropyl-beta-cyclodextrin (used as carrier), 5 mg ethanol, and 5 ml of water. Placebo samples differed only in absence of testosterone. The method of sublingual testosterone administration was established through extensive piloting in our laboratory as part of studies on the time course of effects of testosterone on sexual arousal (Tuiten et al., 2000). It was demonstrated that plasma levels of total testosterone peaked at a supraphysiological level (for females) of an approximate ten fold increase fifteen minutes after intake, without changes in sex-hormone binding globulin levels, whereas vaginal vasocongestion in response to sexual stimuli peaked 4 hrs after intake. In a line of research on the cognitive and emotional effects of acute testosterone we have successfully applied this delayed effect (Hermans et al., 2006; Postma et al., 2000; Schutter & Van Honk, 2004; Van Honk et al., 2005; Van Honk et al., 2004; Van Honk, Tuiten, Hermans et al., 2001). Therefore, the present study used the

same interval of 4 hrs between administration and testing.

Material and Apparatus: Photographs with emotional content were carefully selected from the International Affective Picture System (Center for the Study of Emotion and Attention, 1999) photoset based on normative ratings for women. In total, 2 (versions of the experiment) * 3 (emotional valence category; negative, neutral, or positive) * 19 (pictures per category) = 114, and an additional 2 * 3 = 6 neutral habituation pictures were selected. For the negative valence set, pictures were chosen on the basis of a high arousal and negative valence rating. The neutral set consisted of neutral valence and low arousal pictures. For the positively valenced picture set, pictures with high arousal and positive valence were selected. Because of the well established effects of testosterone on sexual arousal (Tuiten et al., 2000), we selected no pictures with erotic content. Pictures in the two different versions of the task were matched individually on valence and arousal ratings as well as content type (e.g., mutilation, physical threat). Mean valence and arousal ratings for the pictures used in this study, which were taken at the end of each session (see procedure), are summarized in table 3.2.1. All participants viewed both versions, and task version and order of drug administration was counterbalanced.

	Subjective valence			Subjective arousal		
	Set 1	Set 2	Overall	Set 1	Set 2	Mean
Negative	16.1(7.8)	16.8(7.7)	16.5(5.7)	56.1(17.4)	57.1(15.4)	56.6(10.3)
Neutral	50.1(3.3)	49.3(2.7)	49.7(2.3)	8.9(8.3)	8.0(6.0)	8.5(4.6)
Positive	74.2(9.4)	75.1(8.0)	74.7(6.0)	33.3(17.5)	37.2(16.6)	35.2(11.3)

Table 3.2.1: Mean and standard deviations of subjective valence and arousal ratings of the two different picture sets used in this study. Values represent percentages on a 0-100 visual analog scale. No significant differences in valence and arousal ratings between the two photosets were found. Moreover, there were no DRUG effects upon these ratings.

The experimental task was programmed in E-Prime (Psychology Software Tools, inc.) and run on an x86 PC. From an adjacent control room, stimuli were back-projected onto a 19 inch milk-white transparent screen using a data projector. Participants were seated in a comfortable chair approximately 1 meter from the screen in a dimly lit experimental cabin. All events and measurements were recorded using a second x86 PC and a Psylab system (Contact Precision Instruments).

Electromyography of eye blink startles was performed using bipolar placement of 4 mm diameter Ag/AgCl surface electrodes over the orbicularis oculi muscle, with one electrode placed below the left pupil and the other two cm lateral to the first, and a signal ground electrode on the contralateral forehead (see Blumenthal et al., 2005, for details). All were filled with high-conductivity electrolyte gel and attached using adhesive collars. Before applying the electrodes, the skin was prepared by cleaning with alcohol, rubbing gently using fine sandpaper, and a slightly abrasive gel. All electrode impedances were brought below 20 kΩ. Analog signals were 16 bit A/D converted at a sample rate of 1000 Hz. Online high and low pass filters were set to 10 Hz and 500 Hz, respectively, and 50 Hz notch filtering was used. Startle was evoked using 50 ms duration bursts of 105 dB white noise with instantaneous rise-time presented through headphones.

In order to measure skin conductance, 8 mm Ag/AgCl electrodes filled with K-Y Jelly (Johnson & Johnson) were attached to the palmar side of the distal phalanges of the index and middle finger of the non-dominant (left) hand. Raw signal was 24 bit A/D converted at 1000 Hz using a Psylab SC5 constant voltage skin conductance coupler (Contact Precision Instruments).

Finger pulse rate was recorded at 1000 Hz using a finger pulse photoplethysmograph unit on the ring finger of the left hand.

Questionnaires used were the trait version of the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch et al., 1983; Van der Ploeg, Defares, & Spielberger, 1980), and a shortened computerized version of the Profile of Mood States (Shacham, 1983). Furthermore, a computerized visual analog version of the Self Assessment Manikins (Bradley & Lang, 1994) was employed to measure subjective experience of the stimuli on the dimension of valence and arousal.

Procedure: After standardized short screening interviews over the telephone, participants received an information letter and informed consent form along with the questionnaire, which was completed before arrival in the laboratory. Appointments for drug/placebo administration (in the morning) and testing (in the afternoon) were made at the same hour of the day for both sessions in order to avoid confounds due to diurnal hormonal cycles. In order to control for endogenous hormone fluctuations, testing was restricted to the first ten days of the menstrual

cycle. Each participant was tested twice during this period, with at least 48 hrs between both sessions. Test sessions were identical on both days of testing.

Upon arrival in the lab, participants received a 5 ml solution containing either testosterone or placebo for sublingual administration (see Stuenkel, Dudley, & Yen, 1991, for details). They were instructed to refrain from physically demanding activities and return to the laboratory 3.5 hrs later for testing.

Testing sessions began with completion of the Profile of Mood States questionnaire (Shacham, 1983) using a visual analog scale on a test PC. Subsequently, the (female) experimenter placed all electrodes and the headphones on the participant and then left the experimental room to control all equipment from the adjacent room, communicating through intercom. While physiological recordings were being made, the participant was asked, using written verbal instructions, to breathe in deeply four times consecutively with an approximate 15 s delay and press a button at the onset of three seconds of breath holding. This procedure allows for an objective, non-motivational estimation of individual galvanic skin responsiveness (see data reduction). Participants were then given a five minute resting period during which baseline cardiac measurements were obtained.

After this period, a series of eight habituation startle probes was presented with a randomly varying time interval (8-15 s). Participants then received written instructions for the picture viewing paradigm on the projection screen. They were told to watch all pictures from onset until offset, to pay no attention to the noises they would hear, and to remain still unless movement would be necessary in order to remain seated comfortably. Before starting the picture viewing paradigm, they were given the opportunity to ask questions.

The actual task consisted of 3 (valence categories) * 19 (photographs) = 57 randomized presentations of stimuli, preceded by three neutral habituation pictures. All stimuli were presented with a duration of 6 s. During 3 (valence categories) * 14 = 42 trials, and one of the habituation trials, a startle probe was presented. The probe occurred either 3000 ms or 4000 ms after picture onset, both with a random margin of 300 ms. These stimulus onset asynchronies were chosen to allow assessment of skin conductance responses on each trial. Preceding as well as following the entire series of picture presentations, eight startle probes were presented. Moreover, during 15 inter-trial intervals (ITIs; 5 after each valence category) startle probes occurred. Without ITI startle probes, the ITI varied randomly between 14 and 22 s. With ITI startle probe, the ITI startle was preceded and followed by a random interval between 8 and 12 s.

After the picture viewing paradigm, all stimuli were presented again, now accompanied by a visual analog scale version of the Self Assessment Manikins (Bradley & Lang, 1994) for subjective valence and arousal measures. Participants used a computer mouse to rate all pictures. After completion of this task all electrodes were removed. At the end of the second session, participants

were debriefed and received payment.

Data reduction: All electrophysiological data were processed offline using custom software written in Matlab (The Mathworks, Inc.). Electromyographic recordings of the orbicularis oculi were cut out for a time window of -100 to +189 ms time-locked to startle probe onsets. These were first filtered additionally using a digital 30 Hz cut-off high pass filter, which attenuates skin movement related artifacts. Data were then rectified and low pass filtered using a 40 ms moving average window. Startle magnitudes were determined by subtracting mean baseline activity from 0-20 ms post-stimulus onset from the peak EMG power between 20-90 ms post-stimulus onset, and averaged per stimulus valence category.

Skin conductance responses were first scored for the breathing task at the beginning of each session. Skin conductance recordings were cut out time-locked to button presses indicating deep breath holding onset using a -5 to +7 second window. Phasic skin conductance responses were computer scored as the largest amplitude response during this period and square root transformed to reduce skewness. Because the mean level of this phasic skin conductance amplitude is a stable individual characteristic (cross-session correlation of $r(18) = .73$, $P < .0005$, without drug effect), these mean amplitudes were used for individual proportional scaling of skin conductance response amplitudes in the picture viewing paradigm.

Skin conductance data during picture viewing were cut out using a -7 s to +13 s window time-locked to picture onsets. These time windows were first signal drift corrected and subsequently computer scored using the following criteria: minimum onset latency of 750 ms, maximum onset latency of 3000 ms, minimum rise-time of 200 ms, maximum peak latency of 5500 ms. These parameters were chosen to maximize sensitivity with negligible risk of mistakenly scoring (relatively large) skin conductance responses to startle probes. Null responses, which occur commonly, were scored as zero. Resulting skin conductance response amplitudes were square root transformed and proportionally scaled to the average breath holding amplitude.

Raw photoplethysomograph signals were processed offline to calculate cardiac measures. First, a peak detection and artifact correction algorithm was used to calculate interbeat-intervals (IBIs), the results of which were carefully inspected for remaining artifacts. For baseline periods, heart rate average (HRA; in beats per minute) and heart rate variability (HRV) was calculated. HRV was calculated using the root mean square of successive differences (R-MSSD), a sensitive correlate of parasympathetic control of the heart (De Geus et al., 1995). Higher HRV indicates higher parasympathetic control of the heart, and indirectly, lowered stress (Porges, 1995a). Baseline cardiac data of one participant were discarded because of too many artifacts.

Cardiac responses during picture viewing were determined using a -1 to +9 second time window

with respect to picture onset. All responses were averaged over categories and baseline corrected using the -1 to 0 time window.
Trait anxiety self report questionnaires were used to create a group split factor STAI based on the median score on this measure. Questionnaire scores ranged from 25 to 56, median 34.5. Trait anxiety scores for the high and low groups after median split had means (and SDs) of 28.6(3.24) and 44.0(6.11).

Statistical analyses: Startle reflex magnitudes and skin conductance responses were subjected to statistical analyses using 2*3*2*2 mixed factorial repeated measures ANOVAs with DRUG (testosterone vs. placebo), CATEGORY (negative, neutral, or positive) as within group factors, and ORDER (testosterone on first vs. second session) and (trait) STAI (high vs. low) as between group factors. For assessment of baseline habituation startle reflex magnitudes and heart rate response curves, an additional within group factor TIME was added. Wherever sphericity assumptions were violated as indicated by Mauchly's test, Huynh-Feldt (HFε) or Greenhouse-Geisser (GGε; whenever HFε < .75) corrections were applied to adjust the number of degrees of freedom for within group effects (see Slagter, Kok, Mol, Talsma, & Kenemans, 2005). Alpha was set at .05 throughout.

Results

Startle Modulation: The overall ANOVA showed no main effects of DRUG, ORDER or STAI, but only of CATEGORY ($F(1.7, 27.7) = 12.84$, $P < .0005$, $HF\varepsilon = .87$). Both linear and quadratic contrasts of the CATEGORY effect were highly significant: $F(1, 16) = 14.14$, $P = .002$, and $F(1, 16) = 9.94$, $P = .006$, respectively. Further contrasts were calculated to test specifically startle potentiation during negative picture viewing (negative vs. neutral: $F(1, 16) = 14.65$, $P = .001$), and inhibition during positive picture viewing (positive vs. neutral: $F(1, 16) = .011$, n.s.). Thus, the present data support the hypothesis of a generally increased startle during negative picture processing, but fail to support startle inhibition during positive slides.
There were clear session repetition effects: a DRUG*ORDER interaction ($F(1, 16) = 10.92$, $P = .004$) shows that grand mean of startle magnitudes was larger on the first day. Moreover, a DRUG*ORDER*CATEGORY effect ($F(1.9, 30.3) = 7.01$, $P = .004$, $HF\varepsilon = .95$) indicates that the CATEGORY effect differed between the first and second day. This effect was further moderated by STAI: $F(1.9, 30.3) = 4.23$, $P = .026$.
No evidence was found of the predicted DRUG*CATEGORY effect ($F(1.9, 30.3) = 1.50$, n.s.). Crucially, however, there was a CATEGORY*STAI interaction effect ($F(1.7, 27.7) = 3.77$, $P=.041$, $HF\varepsilon = .87$), as well as a DRUG*CATEGORY*STAI three way interaction ($F(1.90$,

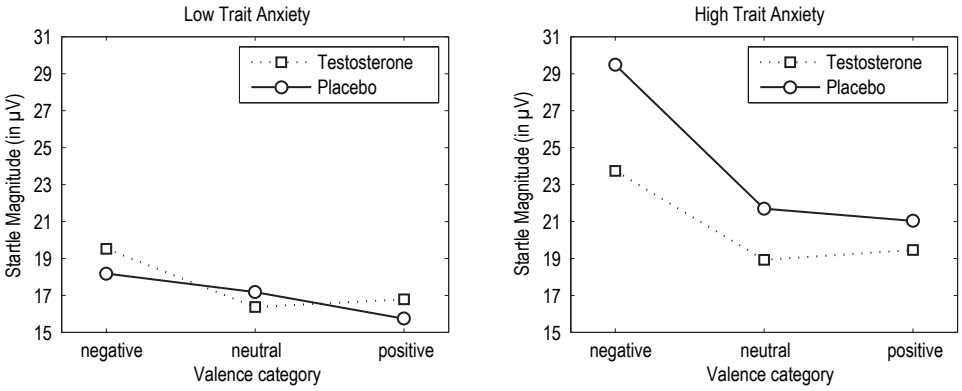

Figure 3.2.1: Mean startle reflex magnitudes (in µV) during affective picture viewing in the low (left graph) and high (right graph) trait anxiety groups, after administration of testosterone or placebo.

30.3) = 4.17, P = .027, HFε = .95). To further scrutinize the interaction effects involving STAI, separate repeated measures ANOVAs were run for both the high and low STAI groups (see figure 3.2.1).

In the low STAI group only a CATEGORY main effect was found (F(2, 16) = 4.99, P = .021; linear contrast: F(1, 8) = 5.66, P = .045), without a significant DRUG interaction (F(2, 16) = 1.34, n.s.). In high STAI group, there was also a CATEGORY main effect (F(2, 16) = 8.85, P = .003; linear contrast: F(1, 8) = 9.38, P = .016; quadratic contrast: F(1, 8) = 7.76, P = .024). Furthermore, the CATEGORY*DRUG interaction is marginally significant (F(2, 16) = 3.38, P = .06) with a significant CATEGORY*DRUG linear contrast (F(1, 8) = 5.91, P = .041). Thus, the high STAI group exhibited stronger startle potentiation, which was attenuated by testosterone administration.

To check for unspecific DRUG effects upon the acoustic startle reflex, a separate ANOVA involving DRUG, ORDER, STAI, and TIME (habituation startle 1-8) was calculated. This analysis revealed no indication of a DRUG main effect (F(1, 16) = 1.12, n.s.), but only a TIME main effect (F(7, 112) = 20.31, P < .0001) without interaction, indicating a general decline of startle reflex magnitudes over time during the habituation baseline phase. A similar ANOVA was calculated for the ITI startle probes, which also showed no evidence of a DRUG main effect (F(1, 16) = .20, n.s.), but only a similar TIME effect (F(5.6, 89.8) = 10.99, P < .0001, GGε = .19), also without interaction. Thus, there was no evidence of unspecific attenuation of startle reflexes after testosterone administration.

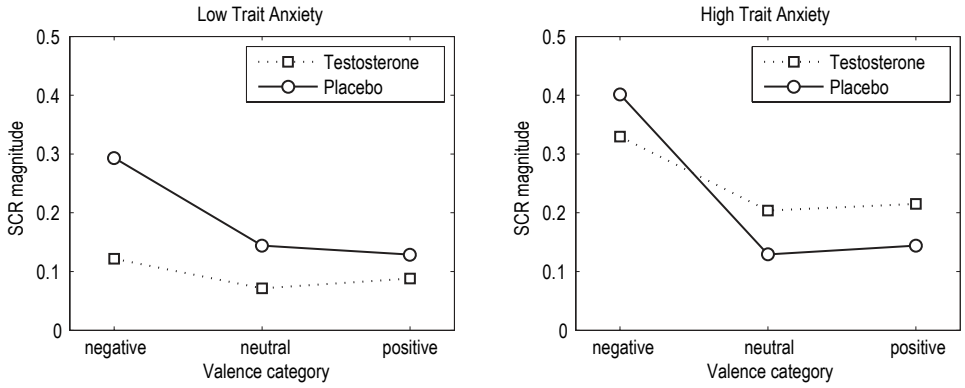

Figure 3.2.2: Mean skin conductance response amplitudes, in proportions of individual baseline respiratory response amplitudes, during affective picture viewing in the low (left graph) and high (right graph) trait anxiety groups, after administration of testosterone or placebo.

Skin Conductance Responses: The overall ANOVA for skin conductance responses yielded a main effect only for CATEGORY ($F(1.1, 18.4) = 10.13$, $P = .004$, $GG\varepsilon = .58$), and none for DRUG ($F < 1$), ORDER, or STAI. Linear and quadratic contrasts of the CATEGORY effect were also significant ($F(1, 16) = 10.21$, $P = .006$, and $F(1, 16) = 9.93$, $P = .006$, respectively). Further testing showed that responses to negative pictures are elevated as compared to neutral ($F(1, 16) = 11.01$, $P = .004$, whereas responses to positive pictures are not ($F(1, 16) = .21$, n.s. Thus, the hypothesis of overall increased skin conductance responses to negative slides is confirmed, but there is no evidence for the predicted increased response to positive slides.

Crucially, the predicted DRUG*CATEGORY interaction was highly significant ($F(1.9, 29.7) = 6.91$, $P = .004$, $HF\varepsilon = .93$), also with significant linear contrast over CATEGORY ($F(1, 16) = 11.05$, $P = .004$) as well as the contrast between negative and neutral slides ($F(1, 16) = 5.84$, $P = .028$). These effects, however, were not moderated by STAI (all $F < 1$). Testing separately within both STAI groups yielded the following statistics for the DRUG*CATEGORY effect (see figure 3.2.2): high STAI: $F(2, 16) = 5.57$, $P = .015$; low STAI: $F(2, 16) = 2.16$, n.s. Note, however, that conclusions regarding STAI are not warranted in the absence of any interaction with this factor, and that results thus support the hypothesis of an overall reduction of skin conductance responses to negative stimuli after testosterone administration.

There was furthermore no evidence for a session repetition effect in skin conductance responses (DRUG*ORDER; $F(1, 16) = 1.21$, n.s.).

Baseline cardiac measures: Analyses of baseline HRV yielded no significant results (DRUG main effect: $F(1, 15) = 1.96$, n.s.; STAI effect and all interaction terms $F<1$). HRA measures also showed indications of neither a DRUG effect ($F<1$) nor any interaction.

Cardiac responses during picture viewing: The overall repeated measures ANOVA yielded main effects of CATEGORY ($F(2, 32) = 8.92$, $P = .001$) and TIME ($F(2.2, 35.6) = 5.88$, $P = .005$, $GG\varepsilon = .13$), as well as a CATEGORY*TIME interaction ($F(4.9, 77.8) = 4.58$, $P = .001$, $GG\varepsilon = .14$). Together, these indicate that there were reliable content-specific heart rate responses (see figure 3.2.3). There was, however, no indication of any DRUG effect on these responses (DRUG*CATEGORY: $F<1$; DRUG*CATEGORY*TIME: $F(11.1, 177.3) = 1.23$, n.s.). Moreover, no significant interactions involving STAI were found.

To test more specifically for effects of heart rate deceleration during negative picture viewing, a separate ANOVA was run using trial specific maximum decelerations as dependent measures (thus excluding the TIME factor). This more sensitive measure yielded similar results with only a CATEGORY main effect ($F(2, 32) = 9.72$, $P = .001$), but again no DRUG or STAI interactions.

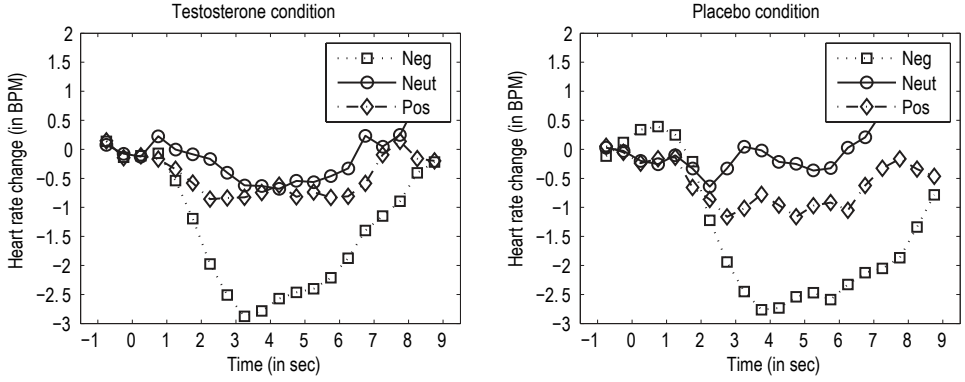

Figure 3.2.3: Heart rate change (in beats per minute) plots for the testosterone (left graph) and placebo (right graph) conditions during picture viewing. Separate lines represent traces for the negative, neutral and positive picture categories. All traces are baseline corrected using the -2 to 0 s time window with respect to picture onset.

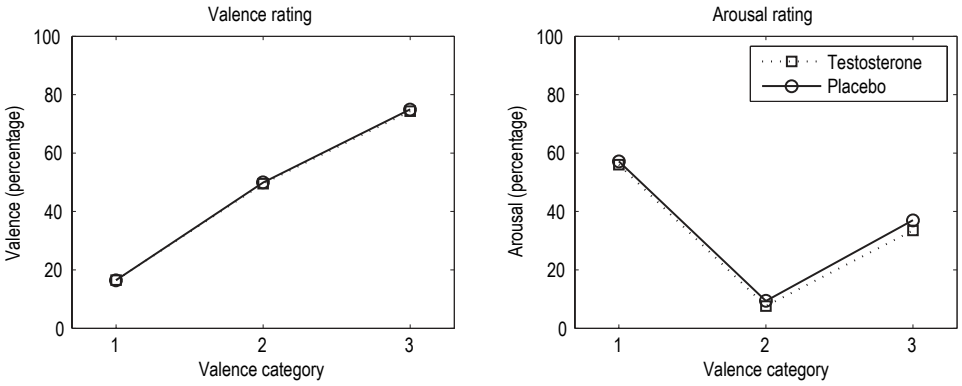

Figure 3.2.4: Subjective ratings of valence (left graph) and arousal (right graph) for the three categories of photographs. Scores represent percentage scores on a visual analog scale. All category differences were highly significant, without any effect of DRUG.

Subjective reports: Overall ANOVAs for the subjective valence and arousal picture ratings both show highly significant CATEGORY effects ($F(1.2, 18.7) = 278.9$, $P = .000$, $GG\varepsilon = .59$, and $F(2, 32) = 138.1$, $P = .000$, respectively). As expected, none of these showed any evidence of a DRUG*CATEGORY interaction ($F<1$; see figure 3.2.4). There were also no interactions involving STAI. Separate ANOVAs were furthermore run for all subscales of the POMS mood questionnaires, also none of which revealed a significant DRUG effect.

In a separate ANOVA, subjective valence and arousal ratings for the two different photosets (which were employed in a counterbalanced fashion) were compared. No significant differences were found between the two sets (see table 3.2.1).

Discussion

The main finding of this study is that testosterone attenuates sympathetically mediated components of the integrated central stress response, as evidenced by lowered phasic electrodermal activity. Affective startle reflex modulation was attenuated after testosterone administration in a subsample of highly trait anxious participants that was furthermore overall highly responsive to affective startle modulation. Concurring with earlier findings, testosterone effects upon subjective measures were found neither in mood questionnaires nor in ratings of the photographs. Moreover, no testosterone effects were found on any of the baseline measures.

Overall patterns of physiological responding in this study were comparable to results obtained in previous research. Physiological responses to negative versus neutral slides were markedly pronounced in both electrodermal measurements and startle potentiation. Startle was moreover significantly stronger in participants with high self-reported trait anxiety. Although some startle modulation was still found in low anxious participants, drug effects were limited to the highly anxious group. This finding is in line with previous reports of enhanced affective startle modulation in individuals high in self-reported fearfulness (e.g., Cook et al., 1992; e.g., Cook et al., 1991). The most plausible explanation for the absence of a drug effect in the low anxious group therefore is that startle modulation is already at a floor level in this group. In agreement, we recently reported overall attenuation of startle potentiation in response to verbal threat of shock – a more potent stressor than the presently employed pictures – after testosterone administration (Hermans et al., 2006, see previous chapter). It has been argued that more potent stressors such as verbal threat of shock impose a so-called strong situation onto the participant, leaving little room for inter-individual behavioral variability (Lissek, Pine, & Grillon, 2005). This implies that responses to milder stressors such as the present are more sensitive to individual differences, which in turn may explain why these have been more successful in detecting gender differences as well as exaggerated startle modulation in anxiety disorders (see Grillon & Baas, 2003).

The predicted startle inhibition combined with elevated skin conductance responses during positive pictures, putatively indicative of appetitive motivation (Lang et al., 1998), was however not evident in the present data. A plausible explanation for this finding is the fact that the present stimulus set did not include photographs with erotic content, which were omitted because testosterone is known to potentiate preparatory responding for reproductive behavior (Tuiten et al., 2000). Exclusion of this category of stimuli has previously been reported to diminish differences in psychophysiological responding to stimuli that are subjectively scored as appetitive versus neutral (e.g., Patrick et al., 1993). This observation has raised some concern whether a linear relation between appetitiveness and startle reflex magnitude may be an oversimplification (see Grillon & Baas, 2003).

The present study also reproduced the common finding of bradycardia in response to strongly aversive stimuli in both drug conditions (Bradley, Codispoti, Cuthbert, & Lang, 2001; Hamm, Cuthbert, Globisch, & Vaitl, 1997). It has been argued that this response may bear resemblance to freezing, and serves as an intermediate stage in the sequence of physiological events that comprise the so-called defense cascade (Fanselow, 1994; Lang et al., 1997). Bradycardia observed during this stage is accompanied by immobility which in turn is thought to facilitate sensory intake (Azevedo et al., 2005). Therefore, bradycardia is traditionally viewed as an index of orienting and attentiveness (Graham & Clifton, 1966). This suggests that sustained bradycardia

typically observed during picture processing may be more closely related to enhanced cognitive processing of stimuli then to fast rudimentary defense reflexes such as fear potentiated startle. It is therefore not surprising that bradycardia and subjective ratings of the photographs, if both instances of cognitive processing, were unaffected by the testosterone manipulation. This discrepancy highlights the advantage of implicit and physiological measures over subjective reports in psychopharmacological studies: (meta-)cognitive alterations necessary for altered cognitive evaluation likely take place on a much longer timescale as a result of complex internal cognitive-emotional interactions (see Harmer, Hill, Taylor, Cowen, & Goodwin, 2003).

It is interesting to note the similarity between the present evidence of causal effects of testosterone and results obtained from psychopathic patients. These patients exhibit a combination of reactive and instrumental aggression, callous disregard of social norms and others' interests, and fearlessness (Blair, 2004). Psychopathy has also been associated with elevated testosterone levels (Stalenheim et al., 1998). It has been proposed that hypoexcitability of subcortical defense circuitry is a critical factor in the pathophysiology of psychopathy (e.g., Veit et al., 2002). In support of this notion, psychopathy has been associated with reduced amygdalar responding during fear conditioning (Birbaumer et al., 2005). Moreover, two studies have been reported that have assessed startle reflex modulation using visual affective material. These have indeed found evidence of hyporesponsiveness parallel to what was found in the present experiment (Pastor et al., 2003; Patrick et al., 1993).

The present findings add to a growing body of evidence that the HPG axis affects functioning of the integrated stress system at multiple levels. Behavioral effects are borne out by multiple findings of testosterone effects upon fearful behavior that have been reported for a number of animal species (Aikey et al., 2002; Bitran et al., 1993; Boissy & Bouissou, 1994; Bouissou & Vandenheede, 1996). Two studies from our laboratory have reproduced this fear-reducing effect in humans using single administrations of testosterone. First, Van Honk et al (2005) found that testosterone diminishes selective attention to danger stimuli, which is an established correlate of fearfulness (e.g., Williams, Mathews et al., 1996). Second, testosterone was shown to reduce fear potentiated startle in verbal threat of shock paradigm (Hermans et al., 2006). At the peripheral end of the stress system, the adrenal cortex, testosterone has been shown to reduce adrenal sensitivity to adrenocorticotropin (ACTH) in men (Rubinow et al., 2005), a finding that is parallel with gender differences in ACTH/cortisol ratios (Horrocks et al., 1990; Roelfsema et al., 1993). Upstream along the nodes of the HPA axis, suppressing effects of testosterone have been reported upon adrenocorticotropin release from the anterior pituitary, and the release of hypophysiotropic CRH, and especially AVP, from the parvocellular neurosecretory neurons of the paraventricular nucleus (PVN) of the hypothalamus (Bao et al., 2006; Viau, 2002). However,

this attenuating effect of testosterone may partly originate upstream with respect to these regions, in areas that play a modulating role in affective and stress responsiveness such as the amygdala, the BNST, and other hypothalamic nuclei, regions that are common to the endocrine and autonomic arms of the stress system (Viau, 2002).

Although not yet fully understood, the neurobiological interactions by which testosterone exerts this effect in these higher regions are beginning to be identified. Many central as well as peripheral effects of testosterone are known to be dependent upon aromatization to estradiol (E2). However, estrogens appear to have a potentiating rather than depressing effect upon the HPA axis (Kirschbaum et al., 1996), and the non-aromatizable 5α and 3α reduced metabolites of testosterone, 5α-dihydrotestosterone (5α-DHT) and 3α-androstanediol (3α-diol) have been shown to have fear-reducing potency similar to testosterone (Edinger & Frye, 2004, 2005), suggesting a pathway independent of E2. Possibly, these metabolites act non-genomically by enhancing inhibitory effects of γ-aminobutyric acid (GABA) at GABA-a receptors (Aikey et al., 2002; Bitran et al., 1993), similar to mechanisms by which other neuroactive steroids such as allopregnanolone modulate neuronal excitability (see Rupprecht & Holsboer, 1999). However, androgen receptor blockers, but not GABA-a receptor antagonists, have been shown to suppress fear-reducing efficacy of testosterone (Fernandez-Guasti & Martinez-Mota, 2005). Genomic effects through intracellular androgen receptors, which are ubiquitously expressed in the limbic forebrain including the amygdala and BNST, therefore clearly play an important role.

Most probably through these genomic mechanisms, testosterone in turn can regulate the expression of the neuropeptides arginine vasopressin (AVP) and CRH, which are important in synchronizing activity across multiple subcomponents of the stress system (Holmes et al., 2003; Muller et al., 2003). Interestingly, the degree to which testosterone alters the expression of CRH and AVP appears to differ in a region-specific manner. Whereas expression of AVP is similarly affected in the amygdala and BSNT, attenuating effects upon CRH expression seem limited to the (central nucleus of the) amygdala, and are dependent upon glucocorticoids (Viau, Soriano, & Dallman, 2001). This distinction could gain particular significance in the light of several findings of functional dissociations between these two regions. A large body of research demonstrates that the amygdala is crucial in acquisition and expression of conditioned fear responses. In contrast with these rapid and short-lived activations which typically counter specific and imminent threats, sustained heightened activity of the stress system is thought to be regulated by the BNST (Walker et al., 2003). This discrepancy may parallel the distinction that clinicians make between fear and anxiety, respectively. It is interesting to note that in reports of acute fear-reducing effects of single administrations of testosterone in humans, effects were limited to selective attention to threat cues (Van Honk et al., 2005), fear potentiated startle (Hermans et al., 2006), and phasic

responses to aversive photographs (present study). All of these are putatively instances of cue-specific fear rather than free-floating anxiety. In contrast, baseline measures, which appear to be sensitive to the somewhat anxiogenic context of a psychological laboratory and may thus be a valid index of free-floating anxiety (Baas et al., 2002), were never affected by testosterone. These findings appear to dissociate effects of testosterone from effects of traditional anxiolytics such as benzodiazepines, which affect measures of contextual anxiety more than cue-specific fear (Baas et al., 2002; Grillon et al., 2006). As a cautionary note, however, it should be emphasized that in human experiments that tested fear-reducing effects of exogenous testosterone, only females were tested. The BNST is highly sexually dimorphic (Walker et al., 2003). Moreover, light-enhanced startle, a BNST-dependent rodent model of unspecific anxiety, has recently been shown to be attenuated by androgens, but only in males (Toufexis, Davis, Hammond, & Davis, 2005). Further research is necessary to determine whether this apparent discrepancy reflects gender differences, cross-species variability, or is a result of other methodological differences. In doing so, it will be of great importance to further narrow the conceptual gap between animal and human models of fear and anxiety by developing more closely related paradigms.

Because testosterone is released in a pulsed fashion with large natural fluctuations, it has been suggested that through these mechanisms, the HPG axis can exert dynamic control over central fear systems and the HPA axis, making the stress system more flexible in adapting to volatile environments and facilitating adaptation to repeated stress exposure (Gomez & Dallman, 2001; Williamson, Bingham, & Viau, 2005). These notions imply a role for testosterone in the etiology, and consequently also in the treatment of, different psychiatric conditions associated with HPA dysregulations, such as depression and anxiety disorders. Novel approaches towards targeting the HPA system are increasingly viewed as an alternative option to classic monoaminergic and GABAergic drugs in the treatment of these disorders (Berton & Nestler, 2006). Future research will benefit from systematically taking into account the role of the HPG axis in these mechanisms (Williamson et al., 2005), and should further explore and evaluate the therapeutic potency of androgen treatment in anxiety disorders and depression (see e.g., Pope et al., 2003; Schutter & Van Honk, 2004; Van Honk et al., 2005).

In conclusion, the present study demonstrates that exogenous testosterone reduces responsiveness of sympathetic autonomic components of the integrated stress system in human females. Moreover, affective startle modulation was attenuated in high anxiety-prone participants. Insights into the mechanisms by which testosterone exerts its effects are essential for the development of neurobiological models of gender differences in affective behavior and disorders, and may foster new avenues for treatment.

3.3 *Administration of a stress dose of cortisol does not affect fear potentiated startle in humans*

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Manuscript submitted for publication

Abstract

Background: The stress hormone cortisol down regulates its own production through negative feedback control on the hypothalamic-pituitary-adrenal axis. It is unclear, however, whether this effect is accompanied by additional anxiolytic-like effects on other components of the fear circuitry.

Methods: Eighteen healthy male participants were tested in a double-blind placebo controlled crossover design involving a single oral administration of 40 mg of cortisol. Effects on baseline startle and fear potentiated startle were assessed one hour after intake in a verbal threat of shock paradigm.

Results: Cortisol administration did not affect fear potentiated startle, which was equally strong in both cortisol and placebo conditions. Moreover, we found no cortisol effect upon baseline acoustic startle.

Conclusion: This study fails to provide support for recent notions that cortisol may have short-term anxiolytic effects. This null-finding is discussed in relation with other recent experimental data.

Introduction

In states of acute stress, sympathetic autonomic nervous system activation is supported by activation of the hypothalamic-pituitary-adrenal neuroendocrine axis, which results in release of corticosteroids from the adrenal cortex. These play an important role in supporting the metabolic demands of stressful situations by increasing energy turnover. A widely held view is that chronically elevated cortisol exhausts the body's energy reserves and results in what is referred to as *allostatic load* (Schulkin, 2003), a failure to maintain homeostatic balance due to over activation of otherwise adaptive stress responses. Thus, a timely down regulation of HPA axis and central fear circuits, rather than an *absence* of stress responses, is key to a healthy functioning stress system. Cortisol is known to exert such a negative feedback effect upon its own production by suppressing the corticotrophin releasing hormone (CRH) at the level of the hypothalamus. Likely, this negative feedback effect is mediated extrahypothalamically, but the underlying mechanisms, as well as other feedback effects that cortisol may have on central fear systems, remain elusive.

Distortions in HPA axis balance have been associated with several forms of psychopathology. For instance, post-traumatic stress disorder (PTSD) has been associated with chronically low cortisol in combination with enhanced cortisol responses (Yehuda, 1997). On the other hand, clinical depression (see Parker, Schatzberg, & Lyons, 2003 for a review), and also sub-clinical trait anxiety (Takahashi et al., 2005), is associated with elevated cortisol levels. Moreover, cortisol suppression in response to dexamethasone administration, which yields an index of the integrity of HPA feedback mechanisms, is blunted in major depressive disorder (see Heim & Nemeroff, 1999).

Animal research has shown that CRH gene expression in the amygdala, likely an anxiogenic-like pathway (Muller et al., 2003), is increased after prolonged glucocorticoid treatment (Rosen & Schulkin, 1998). However, short term feedback effects of cortisol at this level may instead have an anxiolytic profile. Indeed, there now is some data from experiments that administered cortisol to healthy volunteers indicating that such anxiolytic-like effects may occur (Buchanan et al., 2001; Putman et al., submitted; Soravia et al., 2006; Tops, Wijers, Koch, & Korf, 2005). From a functional point of view, it would seem plausible if attenuation of the HPA axis were accompanied by a reduction of responsiveness of other components of the fear circuitry, such as the sympathetic branch of the autonomic nervous system and potentiation of the startle reflex.

The startle reflex provides an excellent model for studying human fear in the laboratory (Grillon & Baas, 2003). When humans startle in response to a sudden stimulus, a connection over only few synapses sets off a protrusion of muscular contractions that rapidly unfolds throughout the body (Davis et al., 1982). Because the strength of this reflex is affected by psychological variables

such as states of fear, measurement of this reflex is often used as an index of activation of fear circuits. Among the fastest occurring components of the startle reflex is the eye-blink, which is controlled by the orbicularis oculi muscle. Using facial electromyography, short-latency muscular activation in response to a startle probe, e.g., a burst of noise presented through headphones, can be quantified. An effective and repeatable method of potentiating the startle reflex in humans is verbal threat of shock. This paradigm typically uses one minute duration blocks during which participants are informed to be either safe or in danger of receiving shock (Grillon et al., 1991). The present study therefore assessed effects of a single oral administration of a stress dose (40 mg) of cortisol on fear potentiated startle in healthy male volunteers.

Methods

Participants: Eighteen male participants recruited from university campus granted informed consent as required by the medical-ethical counsel. Only men participated in order to avoid confounding effects of hormonal cycle variation in hormone levels in women. Exclusion criteria were habitual smoking, history of psychiatric or endocrine illness, and regular use of medication. All participants refrained from use of alcohol for two days prior to testing and were tested on two separate afternoons, in a counterbalanced double blind crossover design.

Material and apparatus: Placebo and cortisol were administered using indiscernible oral capsules which contained only 360 mg primogel or both 320 mg primogel and 40 mg hydrocortisone. 4mm Ag/AgCl electrodes and a Psylab bioamplifier were used for EMG of the startle reflex (10 Hz high pass and 50 Hz hum filters, 1 kHz digitized). White noise (50 msec, 105 dB) through headphones evoked startles. Shocks were delivered to the inner wrist using a constant current stimulator.

Procedure: On both days, participants were tested one hour after administration in a dimly lit cabin. They first completed the Profile of Mood States (POMS) questionnaire (Shacham, 1983), after which all electrodes were applied (see Blumenthal et al., 2005). Subsequently, participants received 12 habituation startle probes in the absence of threat (shock electrodes were disconnected). Written instructions then informed participants they would receive three shocks across sessions maximally, that subsequent shocks would increase in strength, that strength of the next shock would increase with latency, and that they would certainly not receive shock when the word “safe” was presented. During threat, “danger” would appear. Shock electrodes were then connected, and another twelve baseline startle probes were presented while the screen showed “safe”, followed by 16 alternating 50 second “safe” or “danger” blocks with three startle

probes per block. One probe during the 7th (first session) or 5th (second session) was replaced by shock (150 ms at intensities of 2.15 mA and 2.54 mA, respectively). Finally, questionnaires on subjective fear during safe and threat blocks were completed.

Data analysis: Raw EMG was 30 Hz high pass filtered, rectified, and filtered using a 40 ms moving average. Startle magnitudes were defined as peak EMG power between 20-90 ms minus average power 0-20 ms after probe onset, and averaged over three consecutive startles.
Data were analyzed using ANOVAs with THREAT (danger vs. safe), DRUG (cortisol vs. placebo), and BLOCK (1-8) as within, and ORDER (cortisol on day 1 vs. 2) as between factor, with α=.05 throughout.

Results

A main effect of BLOCK ($F(7,10)= 6.44$, $P=.005$) indicates a reduction of startle magnitudes over time. A strong main effect of THREAT ($F(1,16)= 28.60$, $P<.001$) shows that the threat manipulation was successful. This effect was, however, not moderated by DRUG (THREAT*DRUG: $F(1,16)= .018$, n.s.), indicating that startle potentiation was indistinguishable statistically in both DRUG conditions (see figure 3.3.1C). The THREAT effect, moreover, was significant within both DRUG conditions ($F(1, 16) = 25.50$, $P < .001$ and $F(1, 16) = 21.07$, $P < .001$, for cortisol and placebo, respectively). The DRUG*ORDER and DRUG*ORDER*THREAT interactions were both not significant (both $F < 1$), indicating that overall startles were not lower on the second day of testing, nor was the fear potentiated startle effect.
Separate ANOVAs were calculated for the habituation and baseline phases. Both of these did not yield a significant effect of DRUG ($F < 1$), but only BLOCK main effects ($F(3, 14) = 17.92$, $P < .001$, and $F(3, 14) = 11.16$, $P = .001$, respectively). See figure 3.3.1A and B.
Questionnaires: An ANOVA over fear ratings confirmed that the threat manipulation resulted in subjective fear (THREAT main effect: $F(1,16)= 50.80$, $P < .001$), but revealed no DRUG interaction effect ($F < 1$). Moreover, separate t-tests for none of the subscales of the POMS yielded significant effects of DRUG.

Discussion

This experiment did not yield evidence of acute anxiolytic effects of cortisol. Fear potentiation of the startle reflex was robust, and equally strong within both drug conditions. Furthermore, both the habituation and baseline startle reflex magnitudes, which may partly index unspecific contextual anxiety because of the somewhat anxiogenic context of a psychological laboratory (Baas et al., 2002), were also unaffected by cortisol administration. Like a number of other

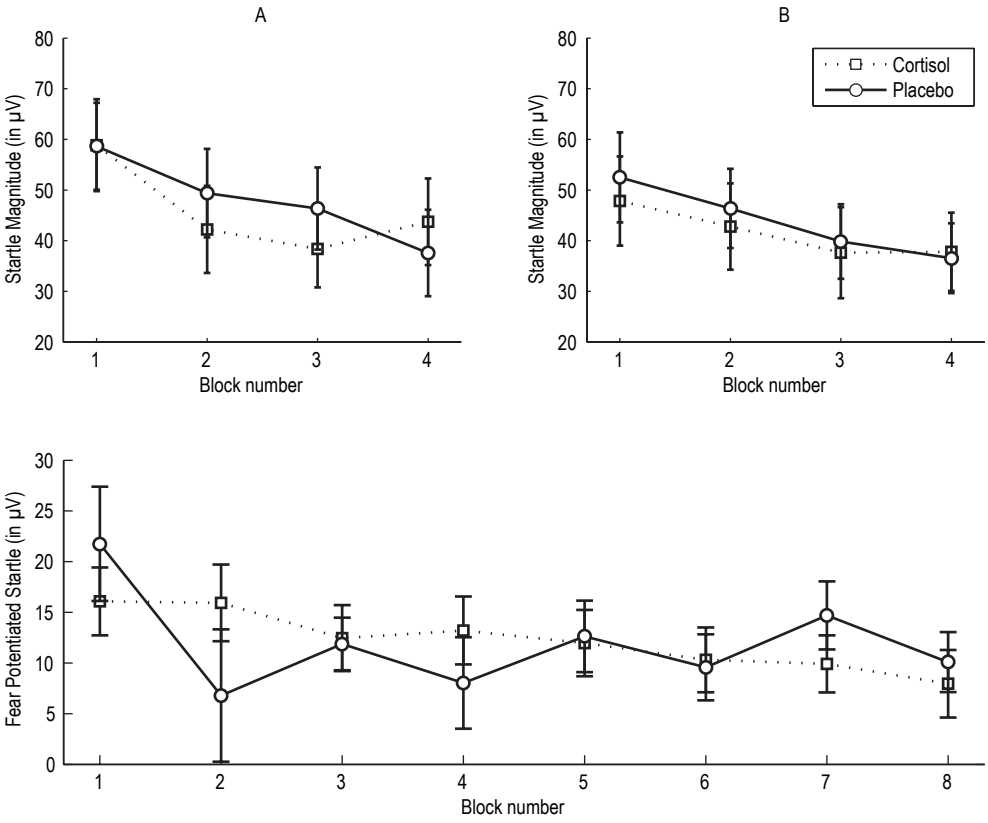

Figure 3.3.1: Traces showing startle reflexes decreasing in magnitude during the habituation phase (A; blocks represent means of three consecutive startles), a similar pattern in the baseline period after connecting the shock electrodes (B), and development of fear potentiated startle (C; threat minus safe) in the cortisol versus placebo conditions over consecutive blocks. All error bars represent standard errors of the means.

studies on single administrations of cortisol, no effects were found upon mood (Abercrombie, Kalin, Thurow, Rosenkranz, & Davidson, 2003; Buchanan et al., 2001; Kuhlmann, Kirschbaum, & Wolf, 2005; Tops, Wijers, Koch et al., 2005; Tops, Wijers, van Staveren et al., 2005).

Very few studies to date have investigated effects of cortisol on fear circuits in humans. Only one study has been reported that tested effects of cortisol on the acoustic startle reflex, and found that baseline startle, but not affective modulation using aversive photographs (see Lang et al.,

1997), was affected by administration of 20 mg of cortisol. A lower dosage, 5 mg, had no effect (Buchanan et al., 2001). Because baseline acoustic startle may be partly determined by contextual anxiety, this effect is indicative of anxiolytic efficacy. In agreement with this notion, positive effects upon a measure of approach behavior were reported in another study after administration of 35 mg of cortisol (Tops, Wijers, Koch et al., 2005). A recent study reported that exogenous cortisol reduces symptoms of phobic fear (Soravia et al., 2006). This effect was interpreted as a deleterious effect upon retrieval of stimulus-associated fear memory, because cortisol is known to interfere with memory retrieval (de Quervain, Roozendaal, Nitsch, McGaugh, & Hock, 2000). However, these data may alternatively be explained as an anxiolytic effect. Finally, recent data using the exact same procedure of cortisol administration as reported here indicates that cortisol acutely reduces selective attention to threat (Putman et al., submitted), which is a well established correlate of anxiety (Van Honk et al., 2005; Williams, Mathews et al., 1996). However, conflicting results have also been reported. For instance, effects have been reported of administration of 35 mg of cortisol upon prefrontal lateralization, where cortisol appeared to increase right hemispheric lateralization (Tops, Wijers, van Staveren et al., 2005), which is associated with anxiety, depression, and withdrawal related behavior (Davidson & Irwin, 1999).

Centrally, endogenous glucocorticoids act on mineralocorticoid (MR) and glucocorticoid (GR) receptors. Because MR receptors have a higher affinity for glucocorticoids, GR receptors are thought only to be occupied when levels are high, thus in highly stressful situations. It is therefore assumed that the GR system plays a key role in feedback regulation (De Kloet et al., 2005). Also for this reason, effects of cortisol on memory appear to exhibit an inverted u-shaped dose-response curve (Roozendaal, 2000). Dosages of 20 mg, which cause cortisol elevations that are comparable to moderate stress, appear to facilitate memory functioning, whereas the presently used dosage of 40 mg leads to cortisol levels that only occur during extreme stress, and have been shown to impair memory function (Abercrombie et al., 2003). These findings call for an assessment of fear potentiated startle also at different dosages than the currently used 40 mg.

In conclusion, the present experiment failed to provide support for a putative role of cortisol in down regulation of central fear circuitry. This null finding adds to a growing body of research on this important topic. If, or how, feedback mechanisms of the HPA axis exert effects that extend beyond attenuation of the HPA axis itself, remains an important question to be answered, all the more given the fact that glucocorticoid manipulations are increasingly seen as an alternative therapeutic strategy in the treatment of depression and anxiety disorders (De Kloet et al., 2005; Schelling et al., 2004).

IV

Psychoneuroendocrinology of empathy

4.1 *Reduced automatic facial mimicry in sub-clinical volunteers high in autistic traits*

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Abstract

Deficits in empathizing and perspective-taking are among the defining characteristics of autism spectrum disorders. Several converging lines of evidence suggest that these socio-emotional abilities are rooted in basic mechanisms that subserve automatic coupling of perception and action, and are implemented in so-called mirror-neuron circuits that attempt to map the mental state of a perceived individual onto the nervous system of the observer. The present study adds to a recent line of research that investigates the role of these mechanisms in the etiology of autism spectrum disorders by investigating automatic and voluntary imitation of facial expressions in sub-clinical volunteers high in autistic traits. Out of a large pool of volunteers that completed the autism-spectrum quotient questionnaire, participants were invited to take part in this study on the basis of an extremely high or low score. They were then tested using facial electromyography of the corrugator supercilii and zygomatic major muscles first while observing still frames of actors posing different facial expressions. Subsequently, photographs were shown again and participants were asked to imitate the expressions. Results show that individuals high in the autistic spectrum exhibit reduced automatic mimicry of angry facial expressions in the corrugator supercilii muscle. None of the groups showed evidence of zygomatic major activity in response to happy facial expressions. Moreover, all participants were able to perform the instructed mimicry task. These findings indicate that the degree to which individuals exhibit automatic mimicry varies along the putative autistic spectrum across the population as a whole, and is not restricted to ASD patients.

Introduction

Among the core characteristics of Autistic Disorder (AD) are pervasive deficits in socio-emotional competencies, such as empathy and perspective-taking, which can become apparent as early as during the first years of life. There is furthermore much reason to believe that AD is the extreme end of a broader spectrum of sub-syndromal autistic traits (Constantino & Todd, 2003). This conception implies a continuum of socio-emotional competency, or autistic traits, across the population, with an intermediate position for Asperger's syndrome (AS), or high-functioning autism (Baron-Cohen et al., 2001). Within this framework, AD and AS are often jointly referred to as Autism-Spectrum Disorders (ASD).

One of the most influential models of deficits in ASD describes these developmental socio-emotional abnormalities as resulting from a failure to acquire an intuitive "theory of mind" (ToM; Baron Cohen et al., 1985). ToM encompasses the attribution of mental states to other individuals as a necessary condition in order to develop an understanding of agency and intentionality. Using various experimental paradigms, ToM has been abundantly studied in ASD, and has undoubtedly stood the test of time (Hill & Frith, 2003). Despite its success in systematically describing some of the core abnormalities of ASD, however, ToM dysfunction has remained somewhat unsatisfactory as an etiological account of ASD (Williams et al., 2001). Apart from the fact that not all core symptoms fit well into the ToM framework, developmental abnormalities are evident prior to the age at which normal children develop this ToM ability. Therefore, the focus of attention is shifting towards precursors of ToM deficits.

One of the possibilities that are currently under investigation is that deficits in brain circuits that underlie perception-action coupling, or the automatic preparation of motor programs upon the perception of another individual performing that action, form the basis for later difficulties in developing mind-reading abilities (Gallese & Goldman, 1998; Williams et al., 2001). The theoretical reason for this is that perception-action coupling implies the existence of inter-subjective representations (Decety & Jackson, 2004), and thus, perspective taking. These notions have been inspired by a recent line of research in monkeys supporting the provocative statement that this ability is implemented at a level as low as single neurons, which have been dubbed "mirror neurons" for their property of responding equally to perception and execution of the same action (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992). Although electrophysiological recording of single neurons is not feasible in healthy human volunteers for obvious reasons, neuroimaging experiments have revealed a network of areas that appear to subserve perception-action coupling because they are jointly activated by viewing and producing actions. Several areas have repeatedly been found in these studies, including the superior temporal sulcus, inferior frontal gyrus, premotor cortex, and parietal lobule (Decety, Chaminade, Grezes, & Meltzoff,

2002; Iacoboni et al., 2001; Iacoboni et al., 1999; Keysers & Perrett, 2004; Koski, Iacoboni, Dubeau, Woods, & Mazziotta, 2003; Koski et al., 2002). These areas are often jointly referred to as action representation areas, and of these, the inferior frontal gyrus and the parietal lobule appear to form the center of the human mirror neuron system (MNS). Of particular interest for the present investigation is a study that required participants to either observe or mimic facial expressions. Among the jointly activated regions in this study were, in addition to this action representation network, regions that are known to be involved in affect and facial affect recognition in particular (Adolphs, 2003): the amygdala and the insula (Carr, Iacoboni, Dubeau, Mazziotta, & Lenzi, 2003). This finding suggests that perspective-taking and empathy may be rooted in automatic joint activation of affect and action representation areas (Gallese, 2003), a dysfunction of which is hypothesized to precipitate the development of autistic traits (Williams et al., 2001). In agreement, deficits in imitation in children with AD are well established (Smith & Bryson, 1994). An important question that follows from this analysis is if relations can be found between involuntary mimicry and autistic traits (Williams et al., 2001).

A feasible methodology to test this hypothesis is assessing facial muscle activity during viewing of facial expressions using electromyography (EMG; Dimberg, 1982). It is well established that people tend to copy other people's facial expressions, a fairly robust effect that occurs quickly (within 400 msec, Dimberg & Thunberg, 1998), is hard to counteract voluntarily (Dimberg et al., 2002), and is even activated when facial expressions are presented below the threshold for conscious visual perception using backward visual masking (Dimberg et al., 2000). In agreement with the aforementioned, recent investigations have shown that this facial mimicry effect is correlated with (self reported) empathy (Sonnby-Borgstrom, 2002), a result parallel with the finding that the perspective-taking subscale of the Interpersonal Reactivity Index predicts differences in posture mimicking (Chartrand & Bargh, 1999). Also, boys with disruptive behavior disorders, which are presumed to be characterized by a lack of empathy, exhibit reduced mimicry of angry facial expressions (de Wied, van Boxtel, Zaalberg, Goudena, & Matthys, 2006). Moreover, in line with notions that women are more empathetic than men, gender differences have been found in facial mimicry (Dimberg & Lundquist, 1990). Only one very recent study has assessed performance of ASD patients in this paradigm, and indeed found evidence in support of the notion that automatic mimicry of facial expressions is reduced in ASD (McIntosh, Reichmann-Decker, Winkielman, & Wilbarger, 2006).

The present study recorded facial electromyographic responses in a similar facial mimicry paradigm during instructed as well as spontaneous mimicry of facial expressions. Recordings were made of the zygomatic major and corrugator supercilii muscles in response to angry and happy facial expressions. In line with the aforementioned notion that autistic traits are continuously

distributed across the population, volunteers were invited to participate in this study that had either an extremely high or low score on the Dutch version of the Autism-Spectrum Quotient (AQ; Baron-Cohen et al., 2001), and were selected from a large pool of (mainly) university students that had completed the AQ. Although autism is much more prevalent in men than in women, it was attempted to equalize group sizes with respect to gender, which allowed us to use gender as an additional between group factor in statistical analyses.
Our main hypothesis was that a reduction in spontaneous imitation would be detectable in sub-clinical participants that are high in the autistic spectrum but exhibit no clinical autistic symptoms. We furthermore expected to replicate previously reported gender differences (i.e., stronger mimicry in women). In contrast, we anticipated no group differences in instructed voluntary mimicry.

Methods

Participants: 366 students were recruited from different faculties of Utrecht University to complete the Dutch version of the Autism-Spectrum Quotient (AQ; Baron-Cohen et al., 2001). This questionnaire consists of 50 four-point Likert items, which are scored in a dichotomous fashion (zero for "definitely disagree" and "slightly disagree"; one for "slightly agree" and "definitely agree"). Possible range of scores is therefore 0 to 50. Mean score across the total sample was 15.1 with a standard deviation of 5.75 and a range of 5 to 33.
Students that scored either extremely high or extremely low were invited to participate in the study, resulting in a low AQ group of 20 individuals (11 males, 9 females) which had an AQ-score among the lowest 30.7%. The high AQ consisted of 21 persons (11 males, 10 females) out of the 20.6% highest AQ scores. Due to technical failures, data of 7 participants were lost for both tasks, and for an additional two participants, data of instructed imitation were lost. The final groups for the spontaneous mimicry task consisted of 16 low AQ (10 male, 6 female) and 18 (10 male, 8 female) high AQ individuals. The mean age of the low AQ group (16 participants, 10 males, 6 females) was 21.1 years (range 18-25), and the mean age of the high trait autism group (18 participants, 10 males, 8 females) was 20.8 years (range 17-26).
Individuals with a history of psychiatric disorder were excluded from participation. Participants received payment for participation and provided written informed consent.

Material and apparatus: Photographs of 8 actors (half of which female) posing happy and angry expressions were carefully chosen from two different standardized photosets (Ekman & Friesen, 1976; Lundqvist et al., 1998). Stimuli were displayed on a 60 Hz CRT screen and controlled by software written in E-Prime (Psychology Software Tools, inc).

Electromyography of the facial muscles was performed using bipolar placement of 4 mm diameter Ag/AgCl surface electrodes over the zygomatic major and corrugator supercilii muscles, in accordance with the recommendations detailed by Fridlund and Cacioppo (1986). Raw signal was A/D converted to a 16 bit digital signal at 1000 Hz using a Contact Precision Instruments bioamplifier. Online band-pass filtering was set at 30 Hz to 500 Hz. Furthermore, 50 Hz hum filtering was used.

Procedure: Upon arrival in the lab, participants were first explained all procedures. Before electrode placement, the skin was gently rubbed with fine sandpaper and cleaned with alcohol. Subsequently, a slightly abrasive skin preparation gel (Nuprep) was used, after which the electrodes were filled with electrolyte paste and applied to the skin using adhesive collars. Care was taken to assure that impedance between all electrodes was below 20 kΩ. Otherwise, the full skin preparation procedure was repeated.
Participants were seated in a comfortable chair in a dimly lit cabin throughout the experimental procedure, 70 cm from a computer screen. The experimenter instructed the participant to restrict movement as much as possible, allowing only movement needed to remain comfortable. Participants were asked to keep watching the screen, and to look the photographed individuals in the eye. No reference whatsoever was made to emotional expression, and participants were not told that the objective of the study was to record facial EMG. After instruction, the experimenter left the experimental cabin and controlled all equipment from an adjacent room, communicating with the participant through an intercom.
Participants first viewed 4 habituation trials with different faces from the ones used in the experimental trials. Trials consisted of a fixation cross (1-3 seconds, random), followed by an emotional face which remained on screen for 5 seconds. Intertrial intervals (ITIs) varied randomly between 4-6 seconds. All 16 stimuli were presented in random order and repeated five times in subsequent blocks, comprising a total of 80 trials (40 happy, 40 angry).
After the experimental procedure, participants completed the Beck Depression Inventory (Beck, Rush, Shaw, & Emory, 1979), were debriefed and received payment for participation.

Data Reduction: Electrophysiological measurements were processed offline using custom software written in Matlab 7.0 (The Mathworks, inc.). Raw electromyographic recordings during the entire duration of the experiment were first standardized to z scores (i.e., standard deviations of all sessions were equalized to 1) in order to decrease inter-subject variability. Subsequently, the signal was rectified. For the instructed viewing task, means were calculated for each 100 ms interval. Responses to stimuli were cut out for a time window of 1 sec and were baseline

corrected using the -500 to 0 ms pre-stimulus onset. For the instructed imitation task, means were calculated for each 200 ms interval during the first two seconds of presentation. The reason for this difference between conditions is that instructed mimicry responses are known to have a longer latency and duration (Dimberg et al., 2002).

Statistical analyses were performed using mixed factorial repeated measures ANOVAs, separate for each muscle and condition, with TIME (10 time bins of 100 ms for viewing or 200 ms for imitation), EXPRESSION (angry versus happy), and STIMULUS GENDER (gender of the stimulus) as within group factors, and AQ (high versus low) and PARTICIPANT GENDER (gender of the participant) as between group factors.

In order to specifically test differences in response latencies between groups, the individual instructed imitation response latencies were determined for congruent responses to angry and happy facial expressions in the corrugator supercilii and zygomatic major, respectively. Latencies were defined as the 100 ms interval at which responses reached an average .1 standard deviation (of the entire raw EMG signal) increase with respect to baseline. Moreover, maximum amplitudes were defined as the average standardized EMG power during the peak 200 ms interval within the first 2 seconds of presentation.

Wherever sphericity assumptions were violated as indicated by Mauchly's test, Greenhouse-Geisser epsilon (GGε) corrections were applied to adjust the number of degrees of freedom for within group effects. Alpha was set at .05 throughout.

Results

Facial electromyography, corrugator supercilii, spontaneous mimicry condition: The overall ANOVA demonstrated that participants in general exhibited more corrugator supercilii activity in response to angry facial expressions, as evidenced by a main effect of EXPRESSION: $F(1, 30) = 15.08$, $P = .001$. A main effect of TIME was also found ($F(2.7, 81.9) = 14.45$, $P = .0000$, $GG\varepsilon = .30$), with a significant interaction with EXPRESSION ($F(2.8, 83.7) = 2.97$, $P = .040$, $GG\varepsilon = .38$). When testing specifically per 100 ms time bin, this yielded significant ($P < .05$) EXPRESSION effects for all intervals after 300 ms ($P < .005$ for 600-1000 ms). Thus, overall reaction patterns in the corrugator supercilii exhibited the expected early mimicry effect.

Figure 4.1.1 shows the graphs of corrugator responses when data are separated for the high and low AQ group. The crucial interaction between AQ and EXPRESSION was significant: $F(1, 30) = 5.62$, $P = .024$. Although there was no significant AQ*EXPRESSION*TIME interaction, further testing in separate 100 ms intervals indicated that AQ*EXPRESSION effects appear to start emerging at trend level ($F(1, 30) = 3.0$, $P = .09$) as early as in the 400-500 ms interval, and continue until the last interval.

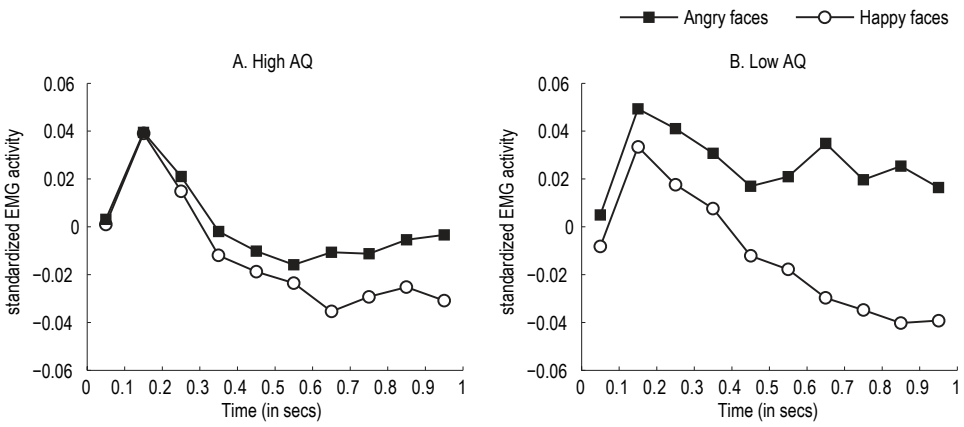

Figure 4.1.1: Mean standardized rectified EMG responses in the corrugator supercilii muscle in response viewing happy and angry facial expressions. Separate graphs are shown for participants selected for high (left graph) and low (right graph) scores on the AQ.

There furthermore was a main effect of PARTICIPANT GENDER: $F(1, 30) = 5.13$, $P = .031$, indicating that overall corrugator EMG activity was lower in women, regardless of stimulus. An EXPRESSION*PARTICIPANT GENDER interaction did not reach significance ($F < 1$). However, there was an EXPRESSION*PARTICIPANT GENDER*AQ three way interaction ($F(1, 30) = 8.15$, $P = .008$.

To further investigate these effects, separate ANOVAs were calculated for male and female participants. Figure 4.1.2 shows the traces for the four groups when split on gender and AQ. The ANOVA for the male participants yielded evidence of a differential response to angry and happy faces (main effect EXPRESSION: $F(1, 18) = 4.85$, $P = .041$), however, there was no evidence for the expected EXPRESSION*AQ interaction in this subgroup ($F(1, 18) = .12$, n.s.). The subgroup of female participants exhibited a strong differential response to angry and happy faces ($F(1, 12) = 12.68$, $P = .004$). This EXPRESSION effect was further moderated by AQ: $F(1, 12) = 15.52$, $P = .002$, indicating that, as predicted, low AQ women had a stronger differential response than high AQ women. Within these groups, low AQ women exhibited a significant differential response ($F(1, 5) = 13.98$, $P = .013$), but high AQ women did not ($F < 1$). Moreover, within the low AQ group as a whole, there was an almost significant EXPRESSION*PARTICIPANT GENDER effect indicating that mimicry is stronger in low AQ women also in comparison with low AQ men ($F(1, 14) = 4.35$, $P = .056$).

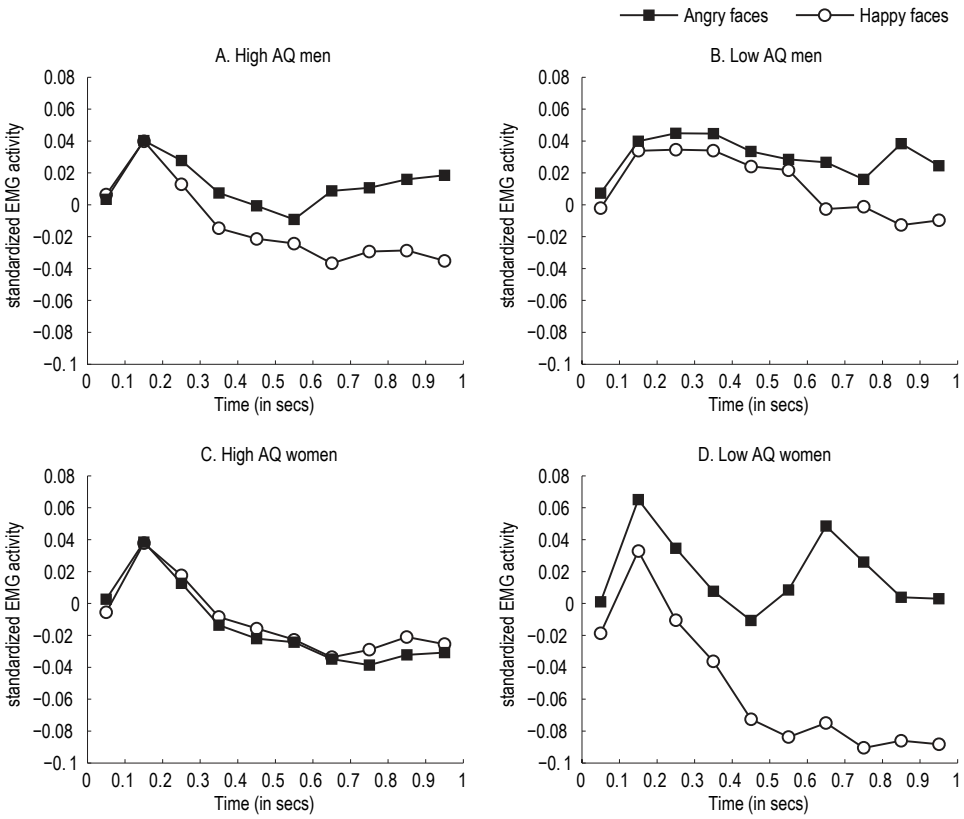

Figure 4.1.2: Mean standardized rectified EMG responses in the corrugator supercilii muscle in response to viewing happy and angry facial expressions. Separate graphs are shown for participants selected for high (left graphs) and low (right graphs) scores on the AQ, and for men (upper graphs) and women (lower graphs).

Exploratively, we also investigated effects of STIMULUS GENDER. There was a STIMULUS GENDER* PARTICIPANT GENDER effect ($F(1, 30) = 4.82$, $P = .036$). This indicates that men respond stronger than females to male stimuli ($F(1, 30) = 10.0$, $P = .004$), regardless of EXPRESSION. This gender difference is absent in responding to female stimuli ($F < 1$). Furthermore, there was a significant STIMULUS GENDER*EXPRESSION*PARTICIPANT GENDER*AQ complex four-way interaction, indicating that the aforementioned EXPRESSION*PARTICIPANT GENDER*AQ effect is stronger when using female stimuli ($F(1, 30) = 10.64$, $P = .003$) than when using male stimuli ($F(1, 30) = 1.88$, n.s.).

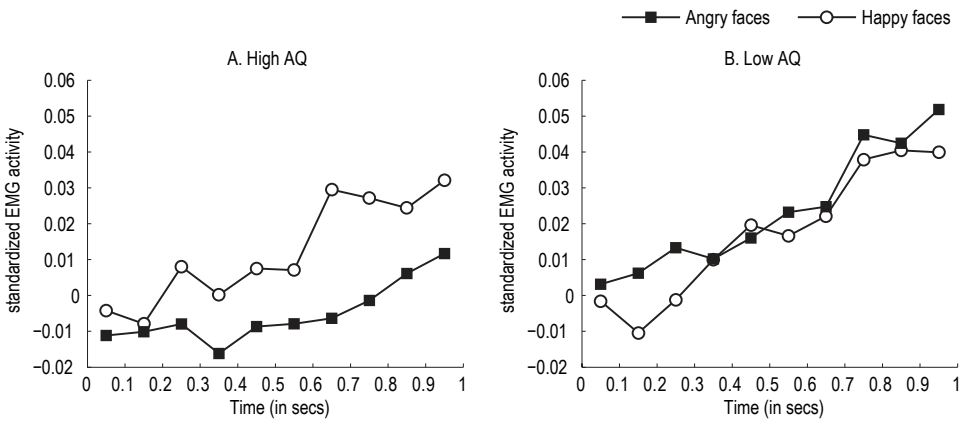

Figure 4.1.3: Mean standardized rectified EMG responses in the zygomatic major muscle in response to viewing happy and angry facial expressions. Separate graphs are shown for participants selected for high (left graph) and low (right graph) scores on the AQ.

In sum, the prediction of reduced mimicry of angry facial expressions in participants selected for high autistic traits was confirmed, as evidenced by an overall EXPRESSION*AQ interaction. This effect, however, is stronger in female participants, and is more pronounced also in response *to* female stimuli.

Facial electromyography, zygomatic major, spontaneous mimicry condition: Responses in the zygomatic major muscle are depicted in figure 4.1.3, separated for high and low AQ groups. Contrary to expectations, responses in the zygomatic major did not show an overall differential response to happy and angry faces (main effect of EXPRESSION: $F(1, 30) = .32$, n.s.), nor was there a differential response within the high or low AQ groups (both $F < 1$). There was a significant TIME main effect ($F(3.2, 94.5) = 12.36$, $P = .0000$, $GG\varepsilon = .35$), but this factor did not interact with EXPRESSION ($F < 1$). Instead, an interaction was found of TIME with PARTICIPANT GENDER ($F(3.2, 94.5) = 2.65$, $P = .051$, $GG\varepsilon = .35$), as well as a TIME*PARTICIPANT GENDER*AQ three way interaction ($F(3.2, 94.5) = 3.40$, $P = .019$, $GG\varepsilon = .35$). Testing within separate 100 ms intervals for effects of AQ or PARTICIPANT GENDER, did not yield any clear effects. There furthermore was a main effect of STIMULUS GENDER ($F(1, 30) = 6.01$, $P = .02$), demonstrating that overall responses were stronger to female stimuli.

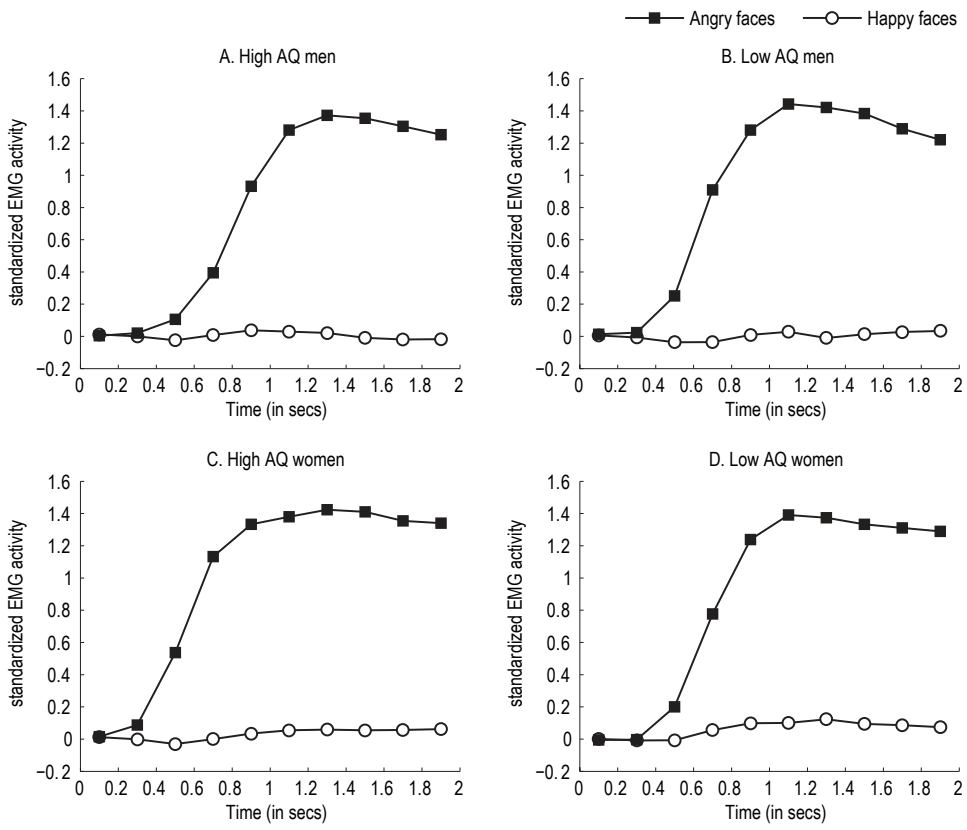

Figure 4.1.4: Mean standardized rectified EMG responses during the first two seconds in the corrugator supercilii muscle when participants were explicitly instructed to imitate either happy or angry facial expressions. Separate graphs are shown for participants selected for high (left graphs) and low (right graphs) scores on the AQ, and for men (upper graphs) and women (lower graphs).

Responses were, however, overall significantly above zero ($F(1, 30) = 5.61$, $P = .025$). An overall reduction in responsiveness in the high AQ group, which is suggested by figure 4.1.3, did not reach significance ($F(1, 30) = 2.79$, $P = .105$).

In sum, results for the zygomatic major muscle yielded no evidence of reduced mimicry in high AQ participants, but instead, failed to replicate the basic mimicry effect, making it difficult to infer anything from these findings.

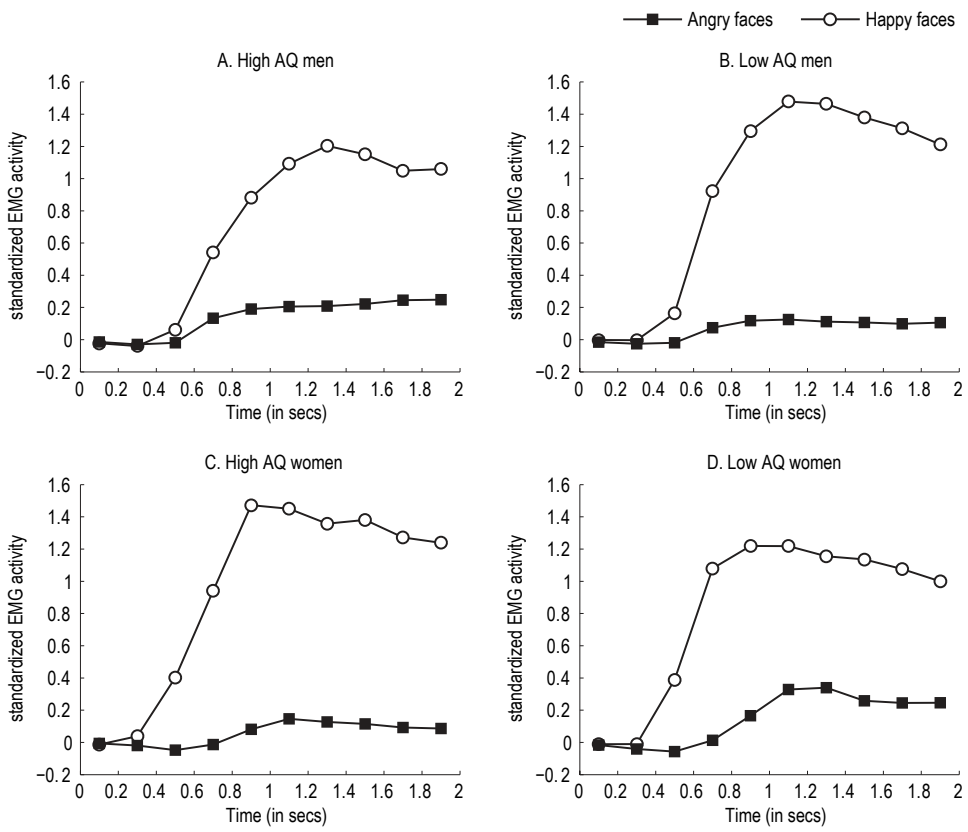

Figure 4.1.5: Mean standardized rectified EMG responses during the first two seconds in the zygomatic major muscle when participants were explicitly instructed to imitate either happy or angry facial expressions. Separate graphs are shown for participants selected for high (left graphs) and low (right graphs) scores on the AQ, and for men (upper graphs) and women (lower graphs).

Facial electromyography, corrugator supercilii, instructed mimicry condition: As can be seen in figure 4, responses in the corrugator supercilii muscle were, unsurprisingly, strongly differentiated for angry and happy facial expressions when participants were instructed to copy the expressions. Apart from a highly significant EXPRESSION main effect ($F(1, 28) = 409.07$, $P = .0000$, there was a TIME main effect ($F(2.9, 80.1) = 175.63$, $P = .0000$, $GG\varepsilon = .32$) as well as a TIME*EXPRESSION interaction ($F(2.7, 15.3) = 160.03$, $P = .0000$, $GG\varepsilon = .30$). To investigate

	Low AQ group		High AQ group	
	Men	Women	Men	Women
AQ	9.3(2.2)	7.8(1.2)	24.2(3.6)	26.1(4.3)
BDI	3.7(3.7)	2.3(2.3)	5.1(4.4)	6.0(4.7)

Table 4.1.1: Mean questionnaire scores (and SD) for the four groups on the Autism Spectrum Quotient and the Beck Depression Inventory.

the timing of this effect, separate tests were calculated for all subsequent 200 ms time bins, yielding significant (P < .005) differential effects as early as from the 200-400 ms time-bin (and onwards, highly significantly).

Effects of gender of the stimulus were markedly pronounced during instructed mimicry, as evidenced by a strong EXPRESSION*STIMULUS GENDER effect (F(1, 28) = 51.99, P = .0000), with larger responses to male actors, but the stimulus gender effects did not interact with any of the between group factors.

Interaction effects of EXPRESSION with AQ and PARTICIPANT GENDER (both F < 1), as well as an EXPRESSION*AQ*PARTICIPANT GENDER interaction (F(1, 28) = 2.32, n.s.) were not significant.

Facial electromyography, zygomatic major, instructed mimicry condition: Results for the zygomatic major muscle in response to instructed mimicry of happy and angry faces are depicted in figure 5. The instructed mimicry effect was again very strong (main effect EXPRESSION: F(1, 28) = 217.85, P = .0000). The TIME*EXPRESSION interaction (F(2.8, 77.5) = 63.11, P = .0000, GGε = .31) was also significant, and further testing per 200 ms interval yielded strongly significant EXPRESSION effects as of the 400-600 interval and onwards.

There was no EXPRESSION*AQ or EXPRESSION*PARTICIPANT GENDER effect (both F < 1). Only the EXPRESSION*AQ*PARTICIPANT GENDER three-way interaction was significant (F(1, 28) = 6.51, P = .016). When testing only the congruent zygomatic response to happy facial expressions separately, there was an AQ*PARTICIPANT GENDER effect (F(1, 28) = 4.27, P = .048). Further testing yielded no significant AQ effect in subgroups of men (F(1, 16) = 3.18, n.s.) or women (F(1, 12) = 1.51, n.s.), making this effect difficult to interpret. Because the mean response sizes may be confounded with differences in response latencies, group differences in onset latency and peak amplitude are tested separately below.

Furthermore, no interaction effects with STIMULUS GENDER were found.

Facial electromyography, imitation onset latencies and maxima: Response latencies for imitation of happy and angry facial expressions were subjected to a univariate ANOVAs with the between group factors AQ, and PARTICIPANT GENDER. This yielded a significant main effect of PARTICIPANT GENDER in both the zygomatic major ($F(1, 28) = 12.49$, $P = .001$) and the corrugator supercilii ($F(1, 28) = 4.26$, $P = .048$), indicating longer response latencies in men. No main effects of AQ (both $F < 1$) or interactions with AQ reached significance.
Maximum amplitude effects were investigated using a similar univariate ANOVAs. Main effects of PARTICIPANT GENDER and AQ were non-significant for both muscles (all $F < 1$). No other interaction reached significance.
Thus, imitative responses are slower in men, but are statistically undistinguishable in high and low AQ groups.

Questionnaires: Questionnaire scores were subjected to univariate ANOVAs with PARTICIPANT GENDER (male or female) and AQ (high vs. low) as between group factors. For the AQ questionnaire, this naturally resulted in a strong AQ main effect: $F(1, 30) = 244.1$, $P < .0001$, without GENDER effects (main effect and interaction $F<1$). The BDI scores yielded no significant effects, although there was a trend for higher BDI scores in the high AQ group ($F(1, 30) = 3.23$, $P = .082$), without any GENDER effects (see table 4.1.1).

Discussion

This study was designed to investigate the hypothesis that reduced socio-emotional capacities such as empathy and perspective-taking that are characteristic of autistic traits are related to dysfunctioning of basic mechanisms subserving coupling between perception and action, or the MNS (Williams et al., 2001). Results from the corrugator supercilii muscle are in line with predictions derived from this hypothesis by showing reduced automatic mimicry of angry facial expressions in participants selected for a high score on a self-report questionnaire of autistic traits (AQ). Findings from zygomatic major muscle were less clear-cut and are difficult to interpret, mainly because the overall mimicry effect was not replicated. Instead, overall, participants reacted with zygomatic major responses to both stimulus types. An apparent tendency in the high AQ for this effect to be reduced was not statistically reliable. Both groups were furthermore able to perform instructed mimicry of the facial expressions.
Our participant sample was selected from a large pool of students from different faculties, which as a whole scored very similar, in terms of mean and standard deviation, to a large group of students that completed the AQ in a previous study (Baron-Cohen et al., 2001). Our low AQ selection out of this participant pool scored on average over one standard deviation below the

total average. The high AQ group scored on average about 1.5 standard deviations above average. Scores were, however, not in the range of patients diagnosed with Asperger Syndrome, which previously have been shown to score almost three standard deviations above the mean with respect to an unselected student population (Baron-Cohen et al., 2001). Thus, even though our high AQ evidently is extreme within a student population, this sample is clearly a sub-clinical group.

Corrugator supercilii responses to angry and happy faces were overall, in shape and size, in close correspondence with the results obtained in previous experiments that investigated early mimicry responses (Dimberg & Thunberg, 1998; Dimberg et al., 2000; Dimberg et al., 2002), including an unspecific peak of activity in the 100-200 ms post-stimulus phase. This peak appears to arise from startle eye-blink reflexes to the sudden onset of stimuli, and may originate from a different source, presumably the orbicularis oculi muscle, which responds at about the same latency to startle probes of different modalities (see Blumenthal et al., 2005). Hence, during this period differential effects are absent, and start around 300 ms after stimulus onset both in the implicit and explicit mimicry conditions. This is the period during which automatic differential responses have been shown to occur in earlier experiments. The notion of automaticity is implied by a number of previous findings. Mimicry has been demonstrated to be stronger when participants are asked to copy the expressions of actors in comparison with conditions where they are asked to produce an incongruent expression such as smiling to an angry face (Dimberg et al., 2002). Moreover, participants have been shown to copy expressions unwittingly even when these are presented outside of conscious perception by means of backward masking (Dimberg et al., 2000). The present finding of a decreased differential corrugator response in the high AQ group therefore is consistent with the hypothesized view that upon the sight of an action, in this case a facial expression, the *automatic* preparation of an identical motor program in the observer is reduced in individuals high in the autistic spectrum.

Importantly, this attenuation of responding was stronger in female participants. When looking only at the low AQ group, women exhibited, almost significantly, stronger mimicry then men. This finding corroborates earlier findings of gender differences in facial mimicry (Dimberg & Lundquist, 1990), and is in line with the notion that women are more empathetic than men (e.g., Baron-Cohen, 2002). An abundance of studies has moreover demonstrated that women are more facially expressive than men (see Brody & Hall, 1993). Although autonomic nervous system responding to aversive stimuli is also increased in women (Bradley, Codispoti, Sabatinelli et al., 2001), this increased expressivity appears to be independent of subjective experience (Kring & Gordon, 1998).

Results for the zygomatic major muscle were much less clear-cut, mainly because of the failure to replicate a baseline mimicry effect in the entire sample of participants, or even within the low AQ

group. There was, however, an overall tendency to reciprocate every stimulus with a smile, but the apparent reduction in this tendency in high AQ participants did not reach significance. Reasons for this failure to replicate numerous other studies are difficult to pinpoint. One possibility is that some of the stimuli that were used in the present experiment were somewhat overacted and unnaturally strong, which may be experienced as humorous by some. Second, our participant sample consisted of individuals that were all selected for an extreme score on the AQ. Possibly, a differential response would have been obtained in a sample with average AQ scores, however, there seems to be no obvious reason why a low AQ should not exhibit a differential response.

Results for the explicitly instructed mimicry task were clear-cut: unsurprisingly, all participants were able to perform this task. This result is in line with the recent findings of unimpaired instructed mimicry in high functioning autism spectrum disorder patients (McIntosh et al., 2006). Impairments of voluntary imitation are common and well documented in ASD, but these are usually found in autistic children (Williams, Whiten, & Singh, 2004). Older samples may not exhibit this deficit anymore, or use different compensatory strategies to accomplish the same task. A novel finding is the delayed mimicry effect in male participants. There seems to be no ready explanation for this interesting finding, because if this is interpreted in terms of a delayed empathetic response, one would also expect this response to be delayed in high AQ participants. Comparisons with one earlier study that measured instructed mimicry responses (Dimberg et al., 2002) is hampered by the fact that in that study participants were explicitly instructed to copy the expressions as quickly as possible, and gender differences are not reported. Another study (McIntosh et al., 2006) tested almost exclusively male participants.

The present data are compatible with a number of recent findings in support of the hypothesis that deficits in the MNS underlie the socio-emotional impairments in ASD. First, as mentioned in the introduction, a very recent study employed an almost identical paradigm to the presently used in ASD, and found results that were highly compatible with the present (McIntosh et al., 2006). This study has shown that high-functioning patients with ASD exhibit reduced automatic mimicry, but are able to voluntarily mimic expressions. Mimicry was operationalized as the proportion of responses that were either congruent or incongruent, and thus data were not presented separately for zygomatic major and corrugator supercilii muscles, which makes it impossible to compare it with the present finding of an effect only in the corrugator. Second, a comparable design has recently been used in a functional neuroimaging study (Carr et al., 2003). In this study, participants were tested using functional MRI while either viewing or imitating facial expressions. This task activated in both conditions a network of action representation areas, namely, the superior temporal sulcus and inferior frontal cortex, in addition to activation of emotional circuits in the insula and the amygdala. Among these, of particular interest seems to be

the activation in the pars opercularis, part of the inferior frontal cortex. This area is thought to form the human MNS, together with the parietal lobule (Iacoboni et al., 1999). Interestingly, the pars opercularis coincides with Broca's area, which has led to claims that language may have evolved out of specialized systems of gesture imitation (Rizzolatti & Arbib, 1998). Another study in healthy volunteers indeed revealed that activation in this area is common to language production and several imitative tasks, such as facial imitation and hand gesture imitation (Leslie, Johnson-Frey, & Grafton, 2004). Several very recent studies have therefore investigated functioning of the MNS in ASD patients using functional imaging. One of these has employed a similar observation-imitation task in high functioning autistic children and healthy controls, and indeed found evidence of reduced activity in the pars opercularis, and activity in this region moreover correlated negatively with symptom severity in the patient group (Dapretto et al., 2006). This deficit in the MNS appears not to be specific to emotional imitation: comparable reduced MNS activity was found in high-functioning adolescent ASD patients, although effects were limited to the right parietal mirror area; the task yielded no activiation in the pars opercularis even in controls (Williams et al., 2006). Converging evidence has also been gathered using techniques other than functional MRI. For instance, suppression of mu-band activity in EEG, which is associated with mirror neuron activity, is absent in high functioning ASD patients (Oberman et al., 2005). Another EEG study tracked the time-course of spreading cortical activation during imitative actions, and found delayed and reduced inferior frontal activity in AD patients. Occipital and superior temporal activity, however, was similar to controls, suggesting that the apparent frontal mirror deficit in ASD is not merely due to impaired visual perception (Nishitani, Avikainen, & Hari, 2004).

More evidence has been gathered using transcranial magnetic stimulation (TMS): whereas in healthy volunteers, motor-evoked potentials evoked by TMS over the primary motor cortex are increased during observation of similar actions (Fadiga et al., 1995), this effect is indeed, as predicted by the MNS deficit theory, reduced in ASD (Theoret et al., 2005). Finally, structural MRI in adult ASD patients has revealed that cortical thickness is reduced specifically in the aforementioned MNS areas (Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2005). This field of research is currently developing at a fast pace, and will likely further advance our understanding of the pathophysiology of ASD in coming years (see Fecteau, Lepage, & Theoret, 2006). An interesting question for future research, for instance, would be to assess the predictive value of early mimicry deficits, because if automatic mimicry is indeed such an important process during the development of social competencies, it follows that automatic mimicry should be impaired at a very early stage of development, perhaps as early as during infancy, when normally developing children are already capable of spontaneous mimicry (Meltzoff & Moore, 1977).

In sum, this chapter started from the premise that the mere observation of an action increases the readiness to perform that action in the observer. This process appears to play an important part in social communication by facilitating perspective taking and the ability to infer mental states of others (Chartrand & Bargh, 1999; Hess, Philippot, & Blairy, 1999), and may also help establishing interpersonal relationships by communicating allegiance through imitation (Hatfield, Cacioppo, & Rapson, 1994). Because socio-emotional processes such as these are typically impaired in ASD, but may be distributed across the population as a continuum, we investigated processes of automatic mimicry in healthy volunteers that are either extremely high or low in the putative autistic spectrum. In accordance with predictions, reduced automatic mimicry of facial expressions, which has recently been found in ASD patients (McIntosh et al., 2006), is already observable within the sub-clinical range of the autistic spectrum.

4.2 *Testosterone administration reduces empathetic behavior: a facial mimicry study*

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Psychoneuroendocrinology 2006, 31, 859-866

Abstract

Although high baseline testosterone levels correlate with low empathy, there is no causal evidence for this association in humans. The present study tested the causality of this relationship by manipulating testosterone levels in a double-blind placebo controlled crossover design. 20 healthy female participants received either a sublingual administration of a single dose of testosterone or placebo on two days and were tested four hours after administration. Because research has shown that facial expression mimicry is a non-obtrusive index of empathy, facial electromyography was measured in response to dynamic facial expressions of happy and angry faces. Results showed that testosterone generally decreased facial mimicry. These findings are consistent with models that assign a critical role to mimicry in the ability to develop and communicate empathy towards conspecifics, and provide a potential causal mechanism of effects of testosterone on empathy.

Introduction

Recent research points to an important role of different neuroendocrine systems in empathy and pro-social behaviors (e.g., see Young & Wang, 2004). Evolutionary analyses suggest that these behavioral processes are rooted in parenting and nurturing behaviors (Taylor et al., 2000). For instance, the neuropeptide oxytocin plays an important role in both lactation and attachment (Kendrick, 2000).

Whereas oxytocin affects attachment, the androgen steroid testosterone (T) has been shown to potentiate anti-social behaviors. Animal research has demonstrated that T is positively related to the frequency of aggressive acts both within and between species (Mazur & Booth, 1998). In humans, high circulating blood plasma T levels have similarly been associated with criminal violence and delinquency (Dabbs & Morris, 1990), although the relation between T and aggression in humans may be more subtle, presumably because humans rarely need to revert to actual violence to assert their dominance (Mazur & Booth, 1998).

Whereas T enhances anti-social behavior, it may act antagonistically to oxytocin to decrease the incidence of pro-social behaviors (see Geary & Flinn, 2002). First, men have higher baseline T levels than women, and tend to engage less in pro-social empathetic behaviors (Baron-Cohen, 2002). Second, low levels of T have been found to predict pro-social attitudes, in both men and women (Harris, Rushton, Hampson, & Jackson, 1996). Third, lack of empathy, as clinical observations indicate, is one of the defining characteristics of psychopathy (Hare et al., 1990). Indeed, given the correlational links between antisocial tendencies and T levels, it is not surprising that high T has also been found to be a biological marker of psychopathy (Stalenheim et al., 1998) Fourth, relations between competitiveness and T levels are reciprocal in the sense that high baselines are related to success in conflicts, and success subsequently further increases T levels (Bateup et al., 2002). In sum, the nature of the relationship between T and human behavior likely lies in its propensity to amplify power motives and dominance, whilst attenuating empathy (Dabbs, 1997; Giammanco et al., 2005; Harris et al., 1996; Mazur & Booth, 1998). However, despite this evidence for a negative relationship between T levels and empathy, research in humans is only correlational.

Most definitions of empathy encompass taking someone else's perspective, or the ability to attribute mental states to others as a means of explaining agency and intentionality, often referred to as a "Theory of Mind" (Baron Cohen et al., 1985). It has been proposed that mimicry represents a rudimentary form of empathy and may be a factor in the development of perspective-taking by facilitating the ability to understand and predict other people's behavior (Chartrand & Bargh, 1999; Hess et al., 1999; Snodgrass, 1985; Williams et al., 2001). Moreover, mimicry may be a manner of expressing allegiance (Hatfield et al., 1994). Hence, therapists sometimes advocate the

use of mimicry as a means of communicating understanding and establishing rapport (Hess et al., 1999). Finally, mimicry might underlie the ability to learn vicariously. Indeed, vicarious learning is a ubiquitous process in human development. Examples of this abound, across various species and from human infants to adults: merely watching a specific action facilitates its reproduction in the observer and is so fundamental to brain function that single neurons have been shown to exist in non-human primates that discharge equally to observation and production of specific actions (e.g., di Pellegrino et al., 1992). Recent studies using functional neuroimaging in humans have delineated the neural substrates of imitation as composed of the superior temporal sulcus, posterior inferior frontal gyrus, premotor areas, and rostral parietal lobule (Iacoboni, 2005).

Research on mimicry has focused mainly on studying people's facial responses while watching other people's expressions. As posing facial expressions involves discrete patterns of specific muscle contractions, it is possible to quantify emotional expressions by means of electromyography (EMG). Most research has simultaneously recorded activity of the zygomatic major and corrugator supercilii muscles, which play a role in smiling and expressing anger, respectively. Using EMG, Dimberg (1982) showed that people generally tend to mimic facial expressions. That is, they smile when watching happy faces, and frown when watching angry faces. Subsequent research showed that this facial mimicry occurs quickly (within 400 msec, Dimberg & Thunberg, 1998), is under limited volitional control (Dimberg et al., 2002), and may even be initiated when emotional facial expressions have been prevented from reaching conscious awareness by means of backward visual masking (Dimberg et al., 2000).

Several studies found a correlation between mimicry and empathy. For instance, Sonnby-Borgstrom (2002) found that participants that scored high on the Questionnaire Measure of Emotional Empathy tended to mimic both angry and happy facial expressions to a greater degree. Also, individual differences in scores on the perspective-taking subscale of the Interpersonal Reactivity Index are related to differences in posture mimicking (Chartrand & Bargh, 1999).

The purpose of the present study was to test the effect of a single dose administration of T on the empathic measure obtained using a facial mimicry paradigm. Whereas previous studies were correlational, the present study manipulated T levels in a placebo controlled cross-over design to study effects of T on mimicry of dynamic happy and angry facial expressions. Our main prediction was that mimicry of facial expressions would be attenuated after T administration.

Methods

Participants: Twenty healthy females (age range 19-31, mean 21.5) were recruited from the student population at Utrecht University and received payment for participation. Individuals with a history of psychiatric or endocrine illness and habitual smokers were excluded from

participation. Care was taken to restrict testing to the pre-ovulatory phase (before the tenth day of the cycle), as androgen levels vary slightly during the menstrual cycle. No participants used any medication other than standard single phase estrogen/progestagen oral contraceptives. Finally, to control for diurnal cycles, testing was restricted to afternoons (i.e., administrations at nine in the morning or later, experimental session four hours later). All procedures were approved by the local medical ethical counsel, and all participants provided written informed consent.

Material and apparatus: Photographs of 8 actors (half of which female) displaying happy, neutral, and angry expressions, were carefully chosen from two different standardized photosets (Ekman & Friesen, 1976; Lundqvist et al., 1998). We then used freely available morphing software (WinMorph, version 3) in order to create dynamic facial expressions, resulting in 16 different movie clips, consisting of 41 frames, of neutral facial expressions changing into an emotional display of either anger or happiness (see figure 4.2.1). Movie clips were played at 20 frames/sec (i.e., two seconds duration) on a 60 Hz CRT screen, controlled by software written in E-Prime (Psychology Software Tools, inc). The final still frames of full emotional expressions remained on screen for an additional 4 seconds per trial. Each session consisted of 3 consecutive, individually randomized, runs of the 16 movie clips. Inter-trial intervals varied randomly between 6 to 9 seconds, and trials were preceded by a random 1-3 second presentation of a fixation cross directing the gaze of participants to look the upcoming photograph in the eye.
Electromyograms of the facial musculature were recorded using bipolar placement of 4 mm diameter Ag/AgCl surface electrodes over the zygomatic major and corrugator supercilii muscles, in accordance with the recommendations detailed by Fridlund and Cacioppo (1986). Raw signal was A/D converted to a 16 bit digital signal at 1000 Hz using a Contact Precision bioamplifier. Online high-pass filtering was set at 10 Hz, and 50 Hz hum filtering was used.

Procedure: Participants were tested on two separate occasions, in a double-blind, placebo-controlled mixed factorial crossover design. Upon arrival in the lab on each testing day, participants received a sublingual administration of T or placebo. T samples consisted of .5 mg of T, 5 mg of the carrier hydroxypropyl-beta-cyclodextrin, 5 mg ethanol, and 5 ml of water. Only T was omitted from the placebo samples. The time course of efficacy of sublingual T administration in previous work has shown a peak 4-6 hours post-administration (see Tuiten et al., 2000, for details). Therefore, participants returned to the laboratory after 3.5 hours, so that testing could start at exactly 4 hours post-administration.
All participants first completed a computerized, shortened version of the Profile of Mood States questionnaire (Shacham, 1983), with the sub-scales anger, tension, depression, vigor, and

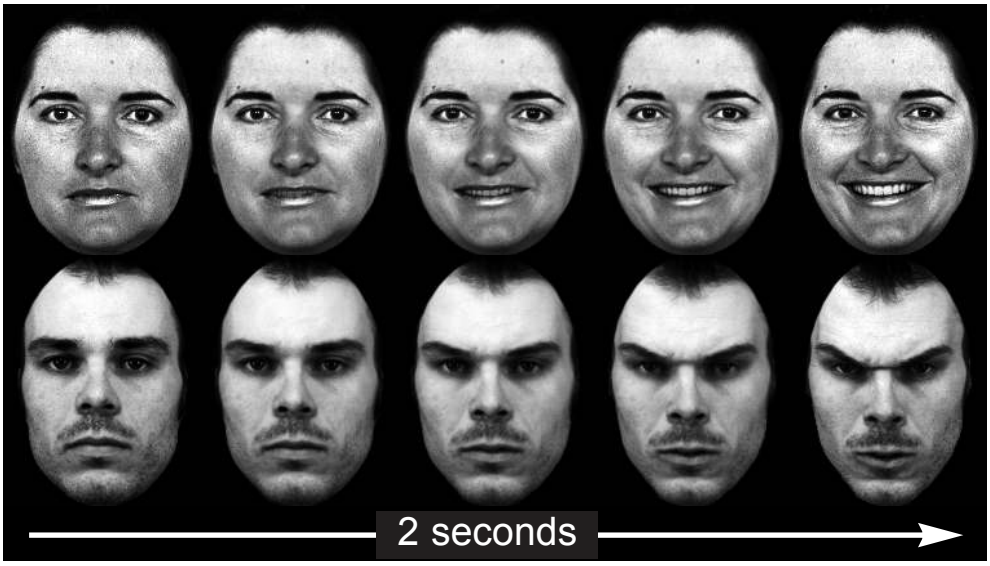

Figure 4.2.1: Examples of two morphed dynamic expressions. Clips were two seconds in duration and consisted of 41 frames.

fatigue.

Before electrode placement, the skin was gently rubbed with fine sandpaper and cleaned with alcohol. Subsequently, a slightly abrasive skin preparation gel was used, after which the electrodes were filled with electrolyte paste and applied to the skin. Care was taken to assure that impedance between all electrodes was below 20 kΩ (average: 14 kΩ). Otherwise, the full skin preparation procedure was repeated. A posteriori checks revealed no relation between signal strength and electrode resistance measurements.

Throughout the experimental procedure, the participant remained seated in a comfortable chair in a dimly lit room, 70 cm from a computer screen. The experimenter instructed the participant to prevent movement as much as possible, allowing only movement needed to remain in a comfortable position. Participants were asked to keep watching the screen, and to look the photographed individuals in the eye. No reference whatsoever was made to emotional expression, and participants were not told that the objective of the study was to record facial EMG.

After instruction, the experimenter left the experimental room and controlled all equipment from an adjacent room.

Data Reduction: Electrophysiological measurements were processed offline using custom software written in Matlab 6.5 (The Mathworks, inc.). A time window of 1 second pre-stimulus to 5 seconds post-stimulus onset was cut out of the raw data for each trial. Electromyographic recordings within this time window were filtered additionally using a digital 30 Hz cut-off high pass filter, in order to remove remaining (skin movement-related) artifacts. All data were rectified and baseline corrected by subtracting the average signal during the second preceding stimulus onset. Subsequently, mean EMG signal strength was calculated for each drug condition, stimulus type, and electrode site for the period during which facial movement was visible on screen (i.e., zero until two sec post-stimulus). Resulting data were subjected to statistical analyses using a repeated measures general linear model with SITE (zygomatic vs. corrugator), STIMULUS (angry versus happy), and DRUG (T versus placebo) as within group factors, and ORDER (T versus placebo on first day of testing) as between group factor.

Results

Facial EMG: No significant effects were found involving ORDER. Hence, this factor was omitted from further analyses. The remaining overall ANOVA contained the factors SITE, DRUG, and STIMULUS, and showed a main effect of SITE ($F(1, 19) = 4.54$, $P = .046$), indicating greater average activity in the zygomatic major muscle. A marginal non-specific main effect of DRUG ($F(1, 19) = 4.02$, $P = .06$) was also observed, with higher mean activity in the placebo condition. We found strong evidence for the predicted overall mimicry effect, as evidenced by a SITE*STIMULUS effect ($F(1, 19) = 13.39$, $P = .002$). Separate ANOVAs for both zygomatic major ($F(1, 19) = 7.10$, $P = .015$) and corrugator supercilii ($F(1, 19) = 17.55$, $P < .001$) substantiated this claim by showing stimulus-congruent muscle activity (i.e., STIMULUS main effects). Further testing showed a significant corrugator supercilii activity increase to angry faces ($F(1, 19) = 6.93$, $P = .016$, as well as a corrugator relaxation response to happy faces ($F(1, 19) = 15.90$, $P < .001$. For the zygomatic major muscle, no relaxation response to angry faces was found ($F = 1.8$, n.s.), but only a congruent response to the happy faces ($F(1, 19) = 6.34$, $P = .021$).

Crucially, in the overall ANOVA, the SITE*STIMULUS interaction was moderated by DRUG (3 way interaction, $F(1, 19) = 5.95$, $P=.025$), indicating that the SITE*STIMULUS mimicry effect was larger in the placebo condition than in the T condition, although both were highly significant ($F(1, 19) = 12.80$, $P = .002$, and $F(1, 19) = 11.77$, $P = .003$, respectively). Separate tests for both electrode sites indicated that congruent muscle activity was decreased in the T condition (STIM*DRUG interaction; $F(1, 19) = 4.80$, $P = .041$ for the corrugator supercilii, and $F(1, 19) = 3.92$, $P = .062$, for the zygomatic major). Finally, paired samples t-tests showed

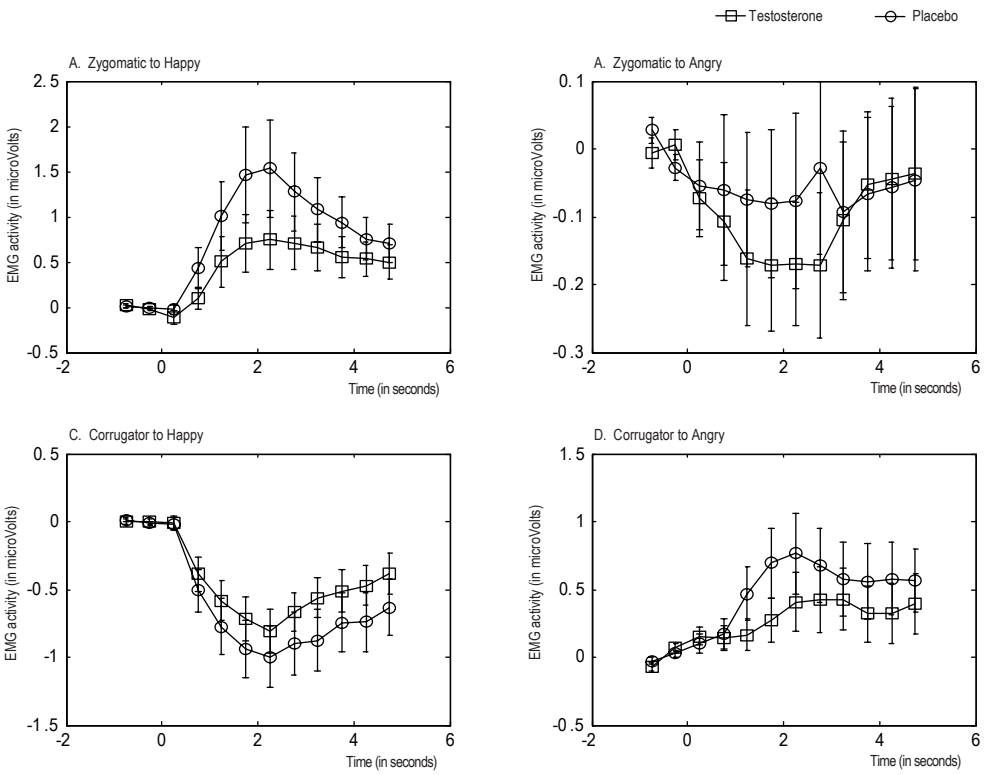

Figure 4.2.2: Graphs showing averaged rectified EMG responses to dynamic happy (left graphs) and angry facial expressions (right graphs) in zygomatic major (upper graphs) and corrugator supercilii (lower graphs) muscles plotted in 500 ms intervals. Error bars represent standard errors of the mean. Stimuli morphed to their respective expressions in the zero to two second time window, after which still images remained.

moderating DRUG effects on zygomatic major responses to happy faces ($t(19) = -2.11$, $P = .048$) and, marginally significant, corrugator supercilii responses to angry faces ($t(19) = -2.0$, $P = .06$), but no effects on incongruent responses (both t values n.s.). See figure 4.2.2.

Profile of Mood States questionnaire: Separate ANOVA's were performed over each of the subscales, with DRUG as within group factor, and ORDER as between group factor. No main effects of DRUG were observed (all F's < 1). A DRUG*ORDER interaction effect reached significance on the tension subscale exclusively, which indicates higher self-reported tension on the first day of testing ($F(1, 18) = 5.77$, $P = .027$; all other F's < 1).

Discussion

The main expectation of this study, that administration of a single dose of T would attenuate empathic mimicry of emotional facial expressions, was confirmed. Moreover, the present data provide a strong replication of the facial mimicry effect by making use of dynamic facial expressions. A T administration effect was evident in attenuation of the mimicry effect in the corrugator supercilii muscle and, near significant, the zygomatic major muscle. Self report measures of mood states did not show any effect of T. This latter null-finding is in line with a number of previous reports (Van Honk et al., 2004; Van Honk, Tuiten, Hermans et al., 2001), indicating that the subtle effects that are induced using this method of T administration take place outside of the realm of the consciously reportable. As has previously been argued, the main brain sites that are targeted by steroids are located in the limbic system (Wood, 1996), whereas self-report implies higher cortical processes. Therefore, steroid action primarily modulates rudimentary affective processes and responses, and may leak into conscious access only indirectly (Van Honk et al., 2004). Facial expression mimicry is likely an instance of such a rudimentary process, as it occurs largely outside of intentional control, even when voluntarily counteracted (Dimberg et al., 2002).

It is interesting to view the present findings in the light of some recent advances made in the field of social neuroscience, which has emphasized automatic coupling of perception and action. This process has been put forward as a foundation of empathy because it implies intersubjective representations (Decety & Jackson, 2004). Much evidence has been gathered in support of this notion. So called mirror neurons, which fire equally to observation and production of actions, have been discovered using electrophysiological recordings in monkeys (di Pellegrino et al., 1992). Subsequent human neuroimaging experiments have identified commonalities in the brain regions activated in response to viewing and producing actions. Regions that are consistently found in these studies are the superior temporal sulcus, inferior frontal gyrus, premotor cortex, and parietal lobule (Iacoboni, 2005). Interestingly, in a study that asked participants to either observe or mimic facial expressions, both conditions activated a similar pattern of areas. In addition to the aforementioned regions, these included the amygdala and the insula (Carr et al., 2003), areas that are known to be involved in emotional processing and expression recognition (Adolphs, 2003). These findings suggest that human empathy rests on an interaction between action representation areas and limbic structures. The present study indicates that an altered evaluation in the latter areas influences action readiness in the former.

The present data add to a growing body of evidence that administration of a single dose of T can have measurable short term effects. In previous studies, we have shown that testosterone administration reduces fear (Hermans et al., 2006; Van Honk et al., 2005) and punishment

sensitivity (Van Honk et al., 2004), but potentiates aggressive responding, measured as increased heart rate (Van Honk, Tuiten, Hermans et al., 2001) and amygdalar (Hermans, Ramsey, Tuiten, & Van Honk, 2004) responding to angry facial expressions. Based on these findings, one could argue that T administration should have resulted in increased reciprocation of anger displays. In theory, angry facial expressions communicate hierarchical relations (Blair, 2003; Knutson, 1996), and can be met with either social fear and appeasement, or defiance and retaliation, depending on the receiver's relative position in a dominance hierarchy (Van Honk, Tuiten, de Haan et al., 2001). Therefore, putative transient manipulation of this relative dominance position by elevating T could have resulted in an increased reciprocation of anger. The fact that quite the opposite occurred, however, is in accordance with a number of empirical findings. For instance, mimicry effects have shown to be strongly affected by social variables, such as whether there is a competitive context. In such cases, mimicry of facial expressions decreases (Lanzetta & Englis, 1989). People also more readily copy expressions of politicians they agree with (McHugo, Lanzetta, & Bush, 1991). Along similar lines, high social anxiety, rather than low social anxiety, is predictive of strong mimicry of angry facial expressions (Dimberg, 1997; Vrana & Gross, 2004). This relatively strong corrugator response to angry facial expressions seen in these socially anxious participants as well as in the placebo condition in the present experiment is highly unlikely to arise from elevated anger.

There are at least two possible explanations for these findings. First, there may be a discrepancy between the actual emotional state of an individual and the emotion that is displayed. This account would be consistent with notions that emphasize the communicative function of facial displays in manipulating behavior of conspecifics (e.g., Fridlund, 1991). An alternative explanation is that the electromyographic response to angry faces does not reflect anger. The medial part of the eyebrow can be controlled separately by the inner frontalis and corrugator muscles, which may not be distinguishable using the current electromyographic method. Also, corrugator activity is observed in expressions of fear and sadness. It can therefore not be ruled out that this activity is a sign of other emotions (see Ekman, 2003b), and may even be related to expressing empathy. Further research is needed to specify the nature of these responses to angry facial expressions.

As mentioned before, a reduced tendency to empathize is one of the core symptoms of psychopathy (Hare et al., 1990). Fairly strong associations exist between T levels and antisocial tendencies in men (see Archer, 2006) as well as women (Dabbs, Ruback, Frady, Hopper, & Sgoutas, 1988). High T has also proven to be a biological marker of psychopathy (Stalenheim et al., 1998) and antisocial personality disorder (Virkkunen et al., 1994). Moreover, previous results from our lab (Van Honk et al., 2004) indicate that females show a pattern of risky decision-making after T administration that is comparable to psychopaths (Mitchell, Colledge, Leonard, & Blair, 2002),

as well as boys with psychopathic tendencies (Blair, Colledge, & Mitchell, 2001). Interestingly, it was recently found that boys with disruptive behavior disorders, who are prone to develop psychopathy by adulthood, also mimic angry facial expressions to a smaller degree (de Wied et al., 2006). All these findings are consistent with the idea that the brain mechanisms involved in mimicry may provide a foundation upon which empathy can emerge, a developmental process that is likely disturbed in psychopathy.

As a limitation of this study, it should be mentioned that only female participants were tested. Future research is needed to specify the appropriate administration parameters for activating effects of T in males. However, there is little reason to assume that male subjects will show a qualitatively different response to T elevation in the present mimicry paradigm, despite the fact that on average, facial mimicry has been found to be stronger in females (Dimberg & Lundquist, 1990). Another question that remains unanswered in this study is whether the effects of testosterone on mimicry are limited to affective behaviors or may extend to non-emotional mimicry such as posture imitation (e.g., Chartrand & Bargh, 1999).

In conclusion, the present experiment indicates that T is causally involved in reducing empathy, which is manifested in attenuated facial mimicry.

V

Summary and conclusions

5.1 *Summary of findings*

The main research strategy of this thesis was to gain new insights into the neuroendocrine regulation of human socio-emotional behavior by adopting a multidisciplinary approach that combines theoretical and methodological approaches from cognitive psychology, psychopharmacology, and cognitive neuroscience. Below, each of the hypotheses that were put forward in chapter 1.2 will be discussed in the light of the findings reported in this thesis.

Hypothesis 1: Attentional bias for angry facial expressions is predicted not by generalized anxiety but by anger and behavioral approach tendencies

In the introduction and chapter 2.1 the outlines were sketched of an evolutionary approach towards understanding angry facial expressions as evolved communicatory signals of social defiance (e.g., Fridlund, 1991). It was argued that their threat value depends upon the social context in which the facial exchange takes place. A marked difference may therefore be expected between angry and fearful facial expressions, as the latter signal the observer that threat is present in the environment, without itself being the source. Using widely varying paradigms, it has indeed been shown that effects, such as selective attention, induced using fearful facial expressions are larger in participants with high trait anxiety. For instance, using comparable facial emotional stroop tasks, it has been shown that selective attention for fearful faces is related to trait anxiety (Hermans et al., 1999; Putman et al., submitted; Van Honk, Schutter et al., 2002). Similar findings have been reported using other cognitive-emotional tasks. For instance, gaze-cuing is facilitated when the actor displays a facial expression of fear, and this effect is stronger in anxious individuals (Mathews, Fox, Yiend, & Calder, 2003; Putman, Hermans, & van Honk, 2006; Tipples, 2006).

The studies reported in chapter 2.2, 2.3, 2.4, and 2.5 add to a growing body of evidence consistent with these notions. Earlier findings have shown associations of selective attention to threatening facial expressions with anger, even when controlling for anxiety (Van Honk, Tuiten, de Haan et al., 2001), with high testosterone (Van Honk et al., 1999), and with low cortisol (Van Honk et al., 1998). This led to the hypothesis tested in chapter 2.2 that within Gray's (1982) model of behavioral activation (BAS) and behavioral inhibition (BIS) systems, the broad concept of BAS should encompass anger (Carver, 2004) and thus predict selective attention to social threat, which was confirmed. In similar vein, social anxiety, and not generalized anxiety or BIS, predicted the opposite response of diminished selective attention to these faces. Moreover, it was shown that, likely because of the preclusion of counter-control strategies, a masked version of the task, in which participants are unable to verbally discriminate the angry facial expressions from neutral or happy control stimuli, yielded more consistent results.

In the next two chapters, this task was used to examine social threat processing in two different patient groups. First, in chapter 2.3, social phobia patients were tested. As predicted, these indeed exhibited a diminished selective attention effect on the same task, a finding that is remarkable in the light of cognitive models of social information processing in social phobia that have emphasized that social phobia is characterized by excessive cognitive rumination on social scrutiny. This process indeed seems to be reflected in emotional stroop tasks that employ verbal stimuli (Amir et al., 1996; Mattia et al., 1993). However, processing of more ecological valid pictorial stimuli of social threat appears to be distinct from this verbal level, and result in an opposite effect. This finding is consistent with clinical as well as experimental observations of avoidance of eye-contact by social phobia patients (Horley et al., 2003). These findings are moreover consistent with what is found in tasks that examine spatial attentional deployment cued by emotional facial expressions, which have yielded evidence of avoidance in social phobia (Chen et al., 2002), and recently also in maltreated children (Pine et al., 2005), although inconsistencies in the literature on social phobia remain (Mogg et al., 2004).

Our findings furthermore suggest that avoidance of social threat, and gaze aversion, is an adaptive response that may allow phobic individuals to find their way in the social world without constantly recurring metabolically costly fear responses. This notion is supported by the finding of a negative relation between selective attention to social threat and cardiac vagal tone, which is putatively an index of flexibility in psychophysiological responding to environmental challenges (Friedman & Thayer, 1998). This interpretation, however, is somewhat speculative and requires further research.

Chapter 2.4 employed a masked version of the same selective attention task in a rare group of patients that have been diagnosed with DSM IV category of dissociative identity disorder (American Psychiatric Association, 1994), which were tested in two distinct such identities and compared to a control group. Results show that, relative to a control group, patients exhibit a different response pattern over sessions, suggesting a diminished attention to, or avoidance of, social threat in the so-called *trauma avoidant* state, which disappeared in the *trauma fixated* state, with an opposite pattern for control participants. Effects were, however, rather weak, thus should be interpreted cautiously, but give some indication that patients were in a different anxious state over two sessions than controls.

Chapter 2.5 describes findings of increased selective attention to angry faces after manipulation of frontal asymmetry. This finding in relation to theories of frontal asymmetry will be discussed in the next section. Important to note here, however, is that an increase in selective attention was accompanied by increased sympathetic autonomic nervous system activation, as indicated by a decrease in pre-ejection period. It is interesting to compare this finding with the relation

that was found between a parasympathetic measure and decreased selective attention to angry faces in chapter 2.3. Together, these findings support the notion that increased selective attention is associated with an active, sympathetic fight/flight response, whereas avoidance is related to a parasympathetic withdrawal response.

The findings from emotional stroop tasks reported in this thesis call for a revision in the way emotional stroop tasks are traditionally conceived of. One can rightfully doubt whether the custom to refer to these tasks as *Stroop* tasks is tenable, because classic Stroop tasks are defined by the presence of a conflict at the level of the response. In emotional Stroop tasks, however, such response conflicts are absent, thus, models of classic Stroop interference effects (e.g., Cohen, Dunbar, & McClelland, 1990), may have limited explanatory value when applied to emotional stroop tasks, as some have argued (Algom, Chajut, & Lev, 2004). However, paradigms that use so-called emotional priming of positive and negative responses do seem to involve a response conflict, but these are rarely discussed in relation to emotional Stroop tasks (e.g., Hermans, Spruyt, De Houwer, & Eelen, 2003). Thus, the processes that cause interference in color-naming in the task used here appear to be more closely related to affective responses such as those measured with psychophysiological techniques (Algom et al., 2004; but see Dalgleish, 2005, for a different view). This interpretation of emotional stroop effects as resulting from emotional activation is compatible with the findings reported in the first experiment of chapter 2.6. By showing that baseline testosterone/cortisol ratio predicts amygdalar response to angry facial expressions, these findings closely replicate earlier findings of relations between selective attention to angry faces and baseline testosterone (Van Honk et al., 1999) and cortisol (Van Honk et al., 1998), and thus provide a plausible neural basis for the selective attention effects by showing that such effects likely arise from enhanced activation of subcortical fight-flight circuits.

Hypothesis 2: Within the framework of prefrontal lateralization of approach versus withdrawal, anger belongs in the approach category, and is thus left lateralized

Research on the hemispheric lateralization of experience and expression of emotion has a long and tormented history in psychology. Mainstream opinion has shifted gradually, from the classic right hemisphere hypothesis of emotional lateralization towards a valence-specific model in which positive and negative emotions are left and right lateralized, respectively (Davidson & Fox, 1982). Recent data, however, suggest that this valence model is ill-conceived, because it confounds motivational direction and affective valence. This problem has surfaced when researchers attempted to fit the emotion anger into this model: anger has an approach-related motivational direction. However, it cannot be considered a positive emotion. Harmon-Jones

(2003b) has performed a series of experiments that provide strong correlational evidence that anger is left-lateralized. These experiments have made use of prefrontal lateralization of alpha frequency power as a negative correlate of brain activity. A more recent non-invasive technique named transcranial magnetic stimulation, however, offers the opportunity to make causal inferences about lateralization, because it makes it possible to transiently manipulate hemispheric dominance. A series of experiments has exploited this potential by applying repetitive TMS over the prefrontal cortex in a lateralized fashion. For instance, slow rTMS over the right PFC results in decreased right-hemisphere dominance (Schutter et al., 2001). In accordance with both valence and motivational direction models, selective attention to fearful faces, a correlate of anxiety, is reduced after similar right hemisphere stimulation (Van Honk, Schutter et al., 2002). However, data reported in chapter 2.5 on selective attention to angry facial expressions after right versus left PFC rTMS, which elaborate on results reported by d'Alfonso, van Honk, Hermans, Postma, & de Haan (2000) by demonstrating the autonomic mechanisms involved, cannot be reconciled with a valence model. More converging evidence has since been reported by Van Honk and Schutter (in press). These findings strongly call for a revision of the mainstream conception of anterior asymmetry in terms of valence towards a dimensionality in terms of motivational direction. Moreover, these findings may have implications for treatment for depression that has typically aimed at increasing left hemisphere activity, either using biofeedback strategies (Rosenfeld, Cha, Blair, & Gotlib, 1995) or by multiple rTMS sessions (Paus & Barrett, 2004).

Hypothesis 3: Testosterone potentiates reactive aggression circuits

Although data from most species on this subject are quite compelling (Lumia et al., 1994; Melloni et al., 1997; e.g., Rejeski et al., 1988), the role of testosterone in *human* aggression has remained somewhat controversial (Archer, 2006). One of the problems is that there are many methodological and ethical complications in devising the studies necessary to shed more light on this issue. The experiments reported in chapter 2.6 build upon the previous chapters in assuming that responses to angry faces are related to anger and aggression proneness, and may thus provide an opportunity to study the brain mechanisms underlying aggression using functional magnetic resonance imaging without the obvious limitations attached to truly evoking aggressive behavior. Moreover, previous research has shown that single dose administrations of testosterone using the sublingual method employed here (see Tuiten et al., 2000) are capable of inducing measurable behavioral (Van Honk et al., 2004) and physiological (Van Honk, Tuiten, Hermans et al., 2001) effects after an interval of four hours between administration and testing. The first part of chapter 2.6 therefore specified the brain regions that respond to angry facial expressions, and the relation between these responses and testosterone and cortisol. Subsequently, a double blind placebo

controlled trial tested causal effects of testosterone upon responding in these regions in order to test the hypothesis that testosterone potentiates reactive aggression circuits.
Results show that a network of brain areas is activated during passive viewing of angry facial expressions. At the subcortical level, these areas largely coincide with the regions that are known to regulate fight-flight responding and reactive aggression, notably, the amygdala and hypothalamus. Cortical activity was found in the orbitofrontal cortex, which is known to be involved in impulse control and aggression regulation (Blair, 2004). Testosterone administration resulted in increased activation of the former, subcortical, areas. Based on animal research, it is argued that testosterone most likely regulates reactive aggression through interactions with arginine vasopressin. Furthermore, it is concluded that the fact that the link between testosterone and aggression in humans appears to be less direct than in animals may be explained by the fact that in humans the orbitofrontal cortex plays a pivotal role in suppressing overt aggression.

Hypothesis 4: Testosterone reduces fear

Apart from potentiating aggression, animal research has clearly indicated that testosterone has an attenuating effect upon functioning of fear circuitry at multiple levels (Aikey et al., 2002; Bitran et al., 1993; Boissy & Bouissou, 1994; Bouissou & Vandenheede, 1996). Two experiments using separate groups of healthy female volunteers, reported in chapter 3.1 and 3.2, provide the first direct tests of this notion in human volunteers by deploying well-established psychophysiological methods. Results from both experiments support the hypothesis.
Chapter 3.1 describes findings from a so-called "threat of shock" paradigm (Grillon et al., 1991). This procedure is the most robust method to induce fear potentiation of the startle reflex. Because of its robustness, it can be repeated with hardly any habituation effects, which makes it particularly appropriate for pharmacological studies that employ within group crossover designs. The experiment reported in chapter 3.2 used a slightly milder manipulation by exposing participants to standardized negatively, neutrally, and positively valenced photographs from the International Affective Picture System (Center for the Study of Emotion and Attention, 1999). Both experiments assessed startle reflex potentiation and subjective effects upon mood, and the latter also recorded electrodermal and cardiac responses to the photographs, as well as subjective ratings of the photographs.
First, results from both experiments demonstrated attenuating effects of testosterone upon fear potentiation of the startle reflex. In chapter 3.2, this effect was limited to a subgroup of participants that scored high on a self-report trait anxiety questionnaire (Spielberger, Gorusch, & Lushene, 1970). However, the low trait anxiety group exhibited only very little startle modulation even in the placebo condition. Thus, the absence of a testosterone effect in this subgroup may be

explained as a floor effect.

Measurements of autonomic nervous system parameters in chapter 3.2 revealed a specific effect upon the sympathetically mediated electrodermal response to the negative photographs. Heart rate deceleration in response to negative pictures, which is a parasympathetically mediated response, was not affected. Arguably, the absence of a testosterone effect upon this parasympathetic measure is due to the fact that vagally mediated heart rate decelerations are associated with attention (Öhman, Hamm et al., 2000), and may thus be more strongly associated with enhanced cognitive processing of the stimuli than with a fear response. In agreement, no drug effects were found on subjective ratings of the photographs and on mood questionnaires.

In sum, the results of these two experiments convincingly demonstrate that testosterone affects multiple components of the human stress response. This can be taken to suggest that, although effects may be obvious even at peripheral nodes of the HPA axis, these effects are mediated centrally. Based on data from animal studies, chapter 3.1 and 3.2 explore the possible mechanisms by which testosterone exerts these effects, and come to the conclusion that most likely, testosterone acts on intracellular androgen receptors which are widespread throughout the limbic forebrain including the amygdala and the bed nucleus of the stria terminalis (Fernandez-Guasti & Martinez-Mota, 2005). Involvement of non-genomic mechanisms that enhance inhibitory effects of γ-aminobutyric acid (GABA) at GABA-a receptors cannot be ruled out (Aikey et al., 2002; Bitran et al., 1993). However, traditional GABAergic drugs such as benzodiazepines typically have sedative side-effects and more consistently attenuate baseline acoustic startle reflex than its potentiation by fear (Baas et al., 2002).

It has been suggested that the HPG axis plays a key role in down regulation of the central fear circuits and the HPA axis, which allows for more flexibility in adaptation to environmental challenges such as repeated exposure to stressors (Gomez & Dallman, 2001). This conception calls for a more thorough investigation of HPG axis function in neuropsychiatric disorders that have also been associated with HPA axis dysfunction. Such an integrated view on functioning of the two neuroendocrine axes may pave the way for more specific treatment strategies (Williamson et al., 2005).

Hypothesis 5: Cortisol acutely reduces fear

The experiment reported in chapter 3.3 used exactly the same paradigm as the one reported in chapter 3.1, but failed to find evidence of anxiolytic effects of cortisol. Fear potentiated startle was very robust across sessions. However, this fear potentiation, baseline startle, and self-reported mood all were unaffected by cortisol administration. Given its significance and relevance for diverse neuropsychiatric disorders, it is surprising that so few other studies have examined

effects of exogenous cortisol on fear circuits in humans. Only a single study used a startle reflex methodology comparable to the one used in chapter 3.2. This study only found attenuation of baseline startle at a dosage of 20 mg. No effects were found on startle modulation caused by processing of aversive photographs. Also, a lower dosage of 5 mg yielded no effects (Buchanan et al., 2001). Another study found compatible results on a measure of approach behavior after administration of 35 mg of cortisol (Tops, Wijers, Koch et al., 2005). Furthermore, it was reported recently that exogenous cortisol reduces phobic fear (Soravia et al., 2006). Finally, a study from our own laboratory, which used the same dosage and administration procedure as reported in chapter 3.3, demonstrated that selective attention to danger cues, fearful facial expressions, is diminished after cortisol administration. Interestingly, a similar effect has been reported after testosterone administration (Van Honk et al., 2005). However, the experiment reported in chapter 3.3 is not the only negative report on this putative anxiolytic-like effect of cortisol. For instance, a seemingly opposite effect of cortisol was demonstrated in a study that assessed effects of cortisol upon prefrontal lateralization, in which 35 mg of exogenous cortisol increased right hemisphere activity, which is associated with anxiety and depression (Davidson & Irwin, 1999).

Because it is known that glucocorticoids exert their central effects through different receptor systems, mineralocorticoid (MR) and glucocorticoid (GR) receptors, that have differing affinities for glucocorticoids, cortisol may have qualitatively different effects at different dosages. Indeed, such effects have been found in cortisol effects upon memory function (Roozendaal, 2000). Thus, because the dosage used in chapter 3.3 is one that results in cortisol levels that are comparable to extreme stress situations, it is possible that other effects are found at lower dosages. This possibility should be explored in future research, because the question if, or how, cortisol affects fear circuits remains pertinent given the role of the HPA axis in neuropsychiatric disorders.

In conclusion, results from chapter 3.3 in combination with findings from other studies yield a mixed picture. Although functionally and theoretically still plausible, evidence for an anxiolytic feedback effect of cortisol is modest. Based on the data presented in chapters 3.1 to 3.3, it appears that down regulating effects of the neuroendocrine systems upon central fear circuits is more robust for the HPG axis.

Hypothesis 6: Autistic traits are related to reduced spontenous mimicry of facial expressions

In part 4 of this thesis, two studies are reported that investigate empathy as a higher order social process that is rooted in perspective taking and imitation. Both use a so-called *facial mimicry* paradigm (Dimberg, 1982), in which participants are required to passively view photographs of

emotional faces while, unbeknown to the participant, spontaneous mimicry is monitored using facial electromyography (EMG).

The experiment described in chapter 4.1 was designed to investigate relations between autistic traits and spontaneous mimicry. Recently, growing dissatisfaction with the explanatory power of *Theory of Mind* - accounts of autistic impairments of socio-emotional capabilities led to the notion that deficits in automatic mimicry, or mirroring of motor programs, may play an important role in the etiology of autism (Williams et al., 2001). Chapter 4.1 therefore tested if autistic traits are predictive of a diminished spontaneous mimicry response to facial expressions. Towards this end, participants were selected on the basis of having either an extremely high or extremely low score on the autism spectrum quotient (AQ; Baron-Cohen et al., 2001). Results are largely in support of the hypothesis. Mimicry of angry facial expressions, as measured by congruent corrugator supercilii activity, was greater in the low AQ group. No robust mimicry of happy faces was found in either group. In contrast, when explicitly instructed to imitate facial expressions, both high and low AQ groups were able to perform this task. These findings suggest that the so-called *mirror neuron system* (MNS) may be less active in participants with strong autistic traits. Other recent studies have yielded evidence converging with this notion. Importantly, in a comparable mimicry paradigm, autism spectrum disorder patients exhibited reduced congruent muscle activity (McIntosh et al., 2006). A functional MRI study that employed a similar task in a group of high-functioning autistic children, but did not measure facial EMG concurrently, found reduced activation of an important cortical MNS area, the inferior frontal gyrus (Dapretto et al., 2006). In further agreement, other recent studies that investigated MNS functioning in ASD have yielded compatible results (see Fecteau et al., 2006).

Hypothesis 7: Testosterone reduces empathic behavior

High testosterone is thought to be associated with decreased pro-social tendencies. For instance, antisocial personality disorders are characterized by high testosterone levels (Stalenheim et al., 1998). There is, however, no causal evidence for this link. The experiment reported in chapter 4.2 therefore tested effects of testosterone administration on spontaneous mimicry of facial expressions, assuming that this automatic imitative response reflects empathy (Sonnby-Borgstrom, 2002; see also chapter 4.1). In a placebo controlled crossover design, sublingual testosterone identical to that used in chapters 2.6, 3.1, and 3.2 was administered to 20 healthy female participants. The task was comparable to that used in chapter 4.1, except for one methodological improvement: in this version of the task morphing was used to create dynamically emerging facial expressions, because dynamic presentation is known to facilitate perception of emotional expressions (Sato et al., 2004). Results show, first, that this alteration was successful. Very robust mimicry was found

for both happy and angry facial expressions. Second, exogenous elevation of testosterone levels resulted in a blunted mimicry response for both stimulus types. This finding is the first to establish a causal link between testosterone and a measure of empathy. It is furthermore consistent with the observations that, in comparison with women, men, who have up to ten times higher levels of testosterone, exhibit reduced facial mimicry (Dimberg & Lundquist, 1990), and are less prone to pro-social behavior and empathy (Baron-Cohen, 2002).

5.2 *Discussion and perspectives*

This thesis started with the observation that humans are an intensely social species. It was argued that this social character of what one might call *human nature* is best understood from an evolutionary framework. Far from being a general purpose learning machine, the human brain contains many evolved dedicated social devices which generate typically human social behavior. Throughout this thesis, the word social has been used to refer to all intra-specific behaviors, ranging from affiliation to aggression. In order to stress its close ties with basic affective systems, it was chosen to refer to these processes jointly as "socio-emotional" (see Adolphs, 2003).

It was furthermore argued that psychological adaptationism as articulated by its main proponents Tooby and Cosmides (1992) has a functionalist signature. The reason for this is that it employs a top-down heuristic that infers proximal design from ultimate selection pressures and adaptations to these. In doing so, it remains at a functional level of explanation, and the specific neural implementation of the evolved mental *modules* it posits is often regarded secondary. This position is untenable when one considers the fact that evolution has created a brain that appears not to be properly or thoughtfully designed but rather consists of several superimposed layers which each have their own phylogenetic origin. Evolutionary approaches towards human socio-emotional capacities need to be constrained by these neuroanatomical facts. Nonetheless, when it embraces this position of an inescapable embodiment of the mind, psychological adaptationism can be a powerful research heuristic. Used in a prudent fashion, it has the potential of fostering new directions of research and treatment for the interdisciplinary fields of evolutionary affective neuroscience and evolutionary psychiatry alike (Panksepp, 2006; Stein, 2006).

An integrated evolutionary heuristic framework of regulation of human socio-emotional behavior was presented, loosely based on Paul Maclean's (1990) concept of the triune brain. It was argued that the three phylogenetic stages in brain evolution roughly coincide with the emergence of ever more complex forms of social behavior, and the concept of *intentionality* was invoked as an explanatory guideline to elucidate how primordial reflex-like behaviors may gradually have evolved into social emotions and higher order intentional processes such as empathy. It is important to emphasize the fact that cross-species homologies are strongest for more basic socio-emotional systems (Panksepp, 2005), and thus, that these systems form the building blocks from which evolution has built complex human social behavior. This conception endorses the bottom-up approach that was pursued throughout this thesis: we cannot come to a full understanding of socio-emotional behavior until we understand the fundamentals upon which it was built, most of which we share with other mammals. Thus, we first need to understand how the autonomic nervous system and the neuroendocrine system mount adaptive responses to challenges by synchronizing bodily and psychological processes. We then need to understand

how these systems are integrated by neuropeptidergic systems at the level of the limbic system. Finally, we need to learn how the neocortex exerts control over these lower areas. Although gaps in our knowledge warrant increased research efforts in all these areas, the focus of this thesis has been on the gonadal and adrenal neuroendocrine systems and their regulation of socio-emotional behavior through actions upon central neuropeptidergic systems, because especially here knowledge was sparse. The most important empirical findings reported in this thesis will be summarized below.

It is intriguing to observe how psychotropic effects of testosterone – which are popularly regarded as well established, if not taken for granted – have actually never been investigated systematically in humans. This is surprising all the more given the fact that all scientific efforts to build biobehavioral models of gender differences are bound to fail when effects of the prime driving force in gender differentiation – testosterone – are not taken into account. The experiments reported in the chapters 2.6, 3.1, 3.2, and 4.2 therefore all applied rigorous double blind methods to investigate these assumptions. The clearest evidence was found for fear-reducing effects of testosterone, but also indirect measures of empathy and aggression were affected, which supports the general conclusion that the hypothalamic-pituitary-gonadal axis, through testosterone, promotes a behavioral profile characterized by fearlessness, aggression, and blunted empathy. All of these are highly sexually dimorphic behaviors (Baron-Cohen, 2002; Mazur & Booth, 1998) and have been related correlationally with high testosterone levels (Giammanco et al., 2005; Stalenheim et al., 1998; Van Honk et al., 1999). Chapter 3.3 furthermore tested the hypothesis that the adrenal steroid cortisol serves a function in downregulation of central fear systems, as suggested by a recent findings (Buchanan et al., 2001; Putman et al., submitted; Schelling et al., 2004; Soravia et al., 2006), but found no effects, suggesting that if these effects of cortisol exist, they might take another form, or are less robust than those of testosterone.

Perspectives

In order to grasp complex reality, researchers are inclined to make use of heuristic models as a framework for guiding research. The literature on emotion is ridden with such models. Although the labels vary from field to field, the most influential of such models have generally carved up affective processes into two opposing super-categories, such as positive versus negative *valence* (Davidson & Irwin, 1999; Lang et al., 1998), behavioral activation versus behavioral inhibition (Gray, 1982), and approach versus withdrawal (Harmon-Jones, 2003b). In the introduction, a preference was articulated for models that are based on motivational direction rather than valence. Although the difference appears subtle, valence models are on unsafe theoretical ground by invoking a normative construct such as *valence* as an organizing principle around which

behavior and affect is organized. Nature cannot be understood from such a normative perspective, in particular because values are not *natural kinds*. This problem is illustrated by the difficulties that this model has faced when attempting to incorporate affective behaviors that we humans value as *negative*, such as anger, but which clearly involve behavioral approach and activation. There is now increasing theoretical agreement and substantial empirical evidence that anger and aggression are conceptually subordinate to behavioral activation rather than inhibition (Harmon-Jones, 2003a; chapter 2.2) and that these are subserved neuroanatomically by approach-related pathways such as the left PFC (Harmon-Jones, 2003b; chapter 2.5) and the HPG axis (Van Honk, Tuiten, Hermans et al., 2001; chapter 2.6).

However, despite its heuristic value, some of the findings presented in the last part of this thesis suggest that a motivational direction model may obscure the fact that some socio-emotional behavior may not be much *directed* at all. With its emphasis on energetically expensive affective processes such as fight-flight, research into socio-emotional behavior has tended to overlook the calming emotional states that subserve pro-social behaviors such as empathy, affiliation, and nurturing (Taylor et al., 2000). However, all evolutionary arguments, in terms of supporting survival and procreation, apply equally to these pro-social behaviors as they do to other presumed socio-emotional adaptations such as states of fear. Therefore, pro-social behaviors conceptually stand on equal footing with all of these.

In the introductory chapter of this thesis, several hierarchically organized components of this pro-social network were introduced (Porges, 2003). For instance, it was described how the dual control of the parasympathetic vagus from the nucleus ambiguous emerged as a means of down-regulating physiology in order to promote socially affiliative states (Porges, 1995b). Subsequently, oxytocin was identified as a key neuromodulator that integrates pro-social behaviors (Lim, Bielsky, & Young, 2005), among others through exerting control over the vagal system (Carter, 1998). A possible mechanism was furthermore outlined by which the neocortex implements the core pro-social capacity of representing mental states of other individuals, namely, by attempting to bring itself into the exact same mental state. Finally, in chapter 4.2, data were presented that suggest that manipulations at lower levels – testosterone is known to act antagonistically to oxytocin (Jezova, Jurankova, Mosnarova, Kriska, & Skultetyova, 1996) – interact with this higher level simulative process.

Importantly, because approach-withdrawal models are inspired by, and largely based on, research into energetically expensive processes such as fight-flight behavior, these calm affiliative states are profoundly difficult to incorporate into these models. Figure 5.2.1 therefore illustrates how a model that separates energetically calming pro-social behaviors from the active fight-flight dimensions would look. Three systems, prefrontal, neuroendocrine, and autonomic nervous

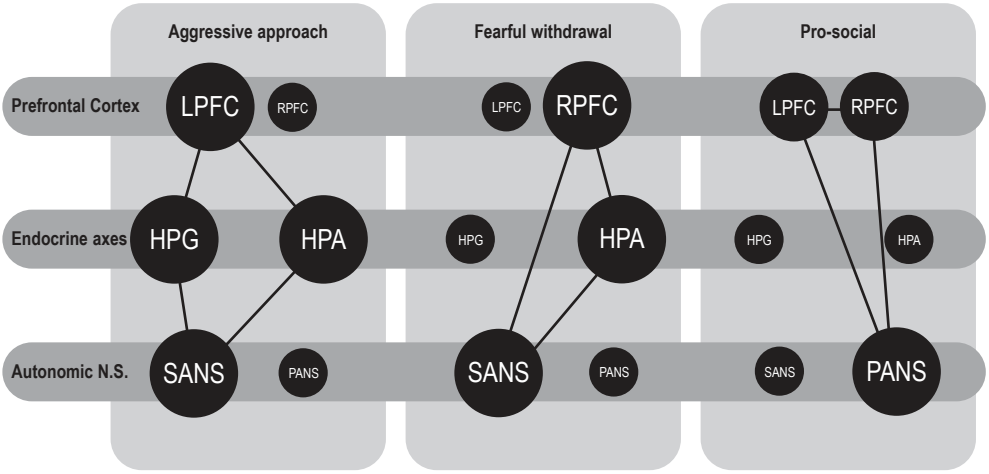

Figure 5.2.1: Model of cortico-neuroendocrine-autonomic networks that subserve the broad socio-emotional categories of aggressive approach, fearful withdrawal, and pro-social behavior. See text for explanation. Abbreviations: LPFC: left prefrontal cortex; RPFC: right prefrontal cortex; HPG: hypothalamic-pituitary-gonadal axis; HPA: hypothalamic-pituitary-adrenal axis; SANS: sympathetic autonomic nervous system; PANS: parasympathetic autonomic nervous system.

systems are shown, and lines are drawn between the areas that form co-activated networks when this socio-emotional state occurs.

First, aggressive approach is evidently prefrontally left lateralized (see, e.g., chapter 2.5), and is promoted by the HPG axis end product testosterone (e.g., chapter 2.6), which most probably acts on vasopressin to organize aggressive behavioral responses (Giammanco et al., 2005). This process also acutely requires increased metabolism which is supported by the HPA axis (Summers et al., 2005) and the sympathetic autonomic nervous system (SANS; see chapter 2.5).

Fearful withdrawal, in contrast, exhibits right prefrontal lateralization (Davidson, 2002), is associated with increased expression of the neuropeptide CRH, both extrahypothalamically (see De Kloet et al., 2005) and hypothalamically in order to activate the HPA axis, which in turn suppresses the HPG axis (see Viau, 2002), and is coactivated with the SANS, although joint activations with the parasympathetic branch (PANS) may also occur during fear (Öhman, Hamm et al., 2000).

Finally, pro-social physiological profiles are calmer and rest mostly on the neuropeptide oxytocin and the PANS (Porges, 2003), are accompanied by suppression of the HPA axis and SANS

(Lim et al., 2005), and are counteracted by the HPG axis (see chapter 4.2). Lateralization of the prefrontal cortex in these processes is a matter of speculation, as there is no conclusive evidence on this topic. However, valence-oriented approaches would predict left-dominance, whereas motivational direction-oriented accounts of prefrontal lateralization may imply a balanced activation of both hemispheres (see, e.g., Van Honk et al., 2005).

In conclusion, this thesis has explored the neuroendocrine regulation of different socio-emotional pathways from a perspective that these socio-regulatory systems are phylogenetically shaped adaptations that are so basic to our brain function that strong cross-species homologies exist, primarily with other mammals (Panksepp, 2005). Gaining a more thorough understanding of these endocrine systems and the neuropeptidergic systems they interact with is gradually becoming a major research focus not only in affective neuroscience but also in neuropsychiatry (Berton & Nestler, 2006). An important reason for this is that an understanding of these basic systems will prove to be a prerequisite for developing a new generation of more targeted treatments for social and affective disorders, which hold great promise for replacing today's relatively unspecific neurotransmitter-based interventions (Panksepp, 2006).

Summary in Dutch

Introductie

De mens is een buitengewoon sociale soort. Dit sociale karakter, dat men vrij van enig waardeoordeel zou kunnen omschrijven als de menselijke natuur, krijgt bij uitstek betekenis wanneer deze bekeken wordt vanuit een evolutionair kader. Heel anders dan een computer, de afgelopen decennia in zwang als een metafoor voor de werking van het menselijk brein, vormen de hersenen geen algemene, *inhoudsonafhankelijke* informatieverwerkingseenheid die pas gedurende de ontwikkeling inhoud opdoet. Zij bestaan eerder uit een veelzijdige, gelaagde opeenstapeling van gespecialiseerde *inhoudsafhankelijke* eenheden die gedurende de evolutie gevormd zijn om specifiek menselijk sociaal gedrag te genereren. Omdat een neuro-anatomische analyse van deze eenheden ons leert dat affectief en sociaal gedrag in de laagste, lees oudste, delen van de hersenen fundamenteel met elkaar verwoven zijn, is er in dit proefschrift voor gekozen naar deze twee processen gezamenlijk te refereren als *sociaal-emotioneel* (Adolphs, 2003).

Evolutionair psychologische analyses van menselijk sociaal gedrag zijn veelal gebaseerd op het psychologisch adaptationisme. Deze benadering, zoals verwoord door haar meest voorname proponenten Tooby en Cosmides (1992), heeft een sterk functionalistische signatuur. De reden hiervoor is dat hierbinnen een onderzoeksheuristiek wordt toegepast die gebaseerd is op het toetsen van proximate predicties die worden afgeleid van op ultimate gronden veronderstelde *adaptaties*: functionele mentale modules die geëvolueerd zouden zijn als oplossing voor een bepaald probleem waar een soort zich in het verleden mee geconfronteerd wist. Dit zorgt ervoor dat deze benadering veelal blijft steken op een functioneel verklaringsniveau. Aan de specifieke neurale implementatie van de mentale modules die worden verondersteld wordt vaak slechts secundair belang gehecht. Deze positie is echter onhoudbaar wanneer men constateert dat de evolutie een brein heeft ontwikkeld dat verre van bedachtzaam ontworpen is, maar meer weg heeft van een gekunstelde, van toevalligheden aan elkaar hangende opeenstapeling van functionele subcomponenten die elk hun eigen fylogenetische oorsprong hebben. Evolutionaire benaderingen van menselijk sociaal-emotioneel gedrag zullen zich moeten zich bewegen binnen de marges die

gesteld worden door deze neuroanatomische feiten, en daar heeft het in het verleden soms aan ontbroken. Een dergelijke benadering is in staat nieuwe theoretische en empirische wegen te bewandelen in de interdisciplinaire velden van evolutionaire affectieve neurowetenschappen en evolutionaire psychiatrie (Panksepp, 2006; Stein, 2006). Dit gezichtspunt vormt de rode draad van dit proefschrift.

Aan de hand van Paul Macleans (1990) concept van de 'drie-eenheid' van het brein wordt in de inleiding van dit proefschrift uiteengezet hoe deze drie stadia in de evolutie van het brein grofweg samenvallen met het ontstaan van steeds complexere vormen van sociaal gedrag. Complexiteit van sociaal gedrag zou men kunnen afmeten aan haar orde van *intentionaliteit* (zie bv. Dennett, 1989). In de meest basale delen van de hersenen, die via het autonome zenuwstelsel en de neuro-endocriene assen sterk geïntegreerd zijn met lichamelijk functioneren, wordt gedrag veelal op een reflexmatige, niet-intentionele wijze gereguleerd. Met de evolutie van limbische structuren wordt verondersteld dat zelf-bewuste, eerste orde intentionele, sociale emoties zijn ontstaan (Panksepp, 2005). Tenslotte heeft de evolutionaire groeisprint van de neocortex de fundamenten gelegd voor het ontstaan van hogere orde intentionele processen zoals empathie, een vermogen dat representatie, of preciezer gezegd re*creatie*, impliceert van de mentale toestand van andere individuen (Harris, 2003).

De hoofddoelstelling van dit proefschrift is het verwerven van nieuwe inzichten in de neuro-endocriene regulering van menselijk sociaal-emotioneel gedrag. Hiertoe wordt een multidisciplinaire aanpak omarmd die theoretische en methodologische benaderingen van de cognitieve psychologie, de psychofarmacologie en de cognitieve neurowetenschappen met elkaar verenigt. Ingebed in het hierboven geschetste theoretisch kader wordt een reeks empirische studies beschreven die zich richt op de hieronder beschreven drie hoofdthema's.

Psychoneuro-endocrinologie van sociale angst en agressie

Dit deel van het proefschrift begint met een overzicht van de wetenschappelijke literatuur op het gebied van sociale fobieën vanuit het hierboven geschetste perspectief van neuro-evolutionaire psychobiologie. Uitgangspunt is de aanname dat processen als agressie en sociale angst evolutionaire wortels hebben in de organisatie van sociale groepen (Öhman & Dimberg, 1984) en kunnen worden opgevat als uiteinden van een continuüm. Een overzicht van beschikbare literatuur op dit gebied leidt tot de conclusie dat sociale fobieën zich ontwikkelen uit aanwijsbare precursors, zoals endocriene parameters, in de vroege kindertijd (Takahashi & Kalin, 1999). Verstoorde cognities die karakteristiek zijn voor sociale fobieën zouden derhalve secundair kunnen zijn aan hyper-exciteerbaarheid van angstcircuits in de hersenen. Dit zorgt voor een pathologisch stabiel functioneel neuro-anatomisch en neuro-endocrien profiel dat uit kan monden in de ontwikkeling

van een sociale fobie.

In de vijf hierop volgende hoofdstukken worden empirische studies beschreven die beoogden verbindingen te leggen tussen verschillende karaktertrekken en het functioneren van neurobiologische substraten van agressie en sociale angst aan de hand van reacties op geëvolueerde signalen van sociale provocatie: boze gelaatsuitdrukkingen. De eerste drie studies maakten gebruik van een onderzoeksmethode die ontleend is aan de cognitief-psychologische traditie. Centraal in deze methode staat het concept van selectieve aandacht. Cognitieve theorieën van psychopathologie hechten grote waarde aan dit fenomeen als verklaring hoe patiënten met verschillende psychopathologieën, vaak angststoornissen, in een vicieuze cirkel terecht komen waarin zij hun negatieve kijk op de wereld herbekrachtigen door informatie met een dreigend karakter selectief te verwerken. Er is gedurende de afgelopen decennia veel onderzoek gedaan naar dit verschijnsel (Williams, Mathews, & MacLeod, 1996). Een veel gebruikte methode om dergelijke processen te bestuderen is het bepalen van de mate waarin een emotioneel geladen stimulus in staat is de uitvoering van een primaire taak als het benoemen van de kleur van een stimulus te verstoren. Deze zogenaamde gemodificeerde emotionele Strooptaken hebben zich bewezen als een plausibel laboratoriummodel voor de processen waarin selectieve aandacht een rol speelt. Het is echter de vraag of men met het gebruik van verbale stimuli niet de ecologische validiteit mist om daadwerkelijk de processen te bestuderen die ten grondslag liggen aan sociale interactie (Van Honk, Tuiten, de Haan, van den Hout, & Stam, 2001). Omdat voorgaand onderzoek heeft laten zien dat gezichtsuitdrukkingen krachtige stimuli zijn in het aantrekken van aandacht (Vuilleumier, 2002), is bij de in dit proefschrift gerapporteerde studies gebruik gemaakt van een versie van deze taak waarin gelaatsuitdrukkingen in plaats van woorden als stimuli gebruikt worden. Daarnaast is uit eerder onderzoek bekend dat expressies zo vroeg verwerkt worden dat zij zelfs differentiële effecten kunnen bewerkstelligen wanneer zij aangeboden worden met een duur die bewuste registratie van de stimulus niet toelaat (Esteves, Dimberg, & Ohman, 1994). Met deze techniek van achterwaartse maskering wordt de stimulus na een duur van vaak minder dan 30 ms vervangen door een masker, meestal een verknipte foto. Dit resulteert in verstoorde verbale reporteerbaarheid van de stimulus, terwijl differentiële effecten in impliciete taken intact blijven (zie bv. Wiens et al., 2004). In hoofdstuk 2.1 wordt beargumenteerd dat boze gelaatsuitdrukkingen een speciale categorie van stimuli vormen die sociale dreiging communiceren. Zij zijn derhalve bij uitstek geschikt voor het bestuderen van processen die ten grondslag liggen aan sociale angst en agressie. Selectieve aandacht voor boze gelaatsuitdrukkingen is in voorgaand onderzoek dan ook gerelateerd aan een sterke zelfgerapporteerde neiging tot boosheid (Van Honk et al., 2001), hoge testosteronniveaus (Van Honk et al., 1999) en lage cortisolniveaus (Van Honk et al., 1998). In hoofdstuk 2.2 wordt

een onderzoek gerapporteerd dat dit selectieve aandachtseffect voor boze gelaatsuitdrukkingen relateert aan individuele scores op de zogenaamde *Behavioral Inhibition Scale* (BIS) en *Behavioral Activation Scale* (BAS; Carver & White, 1994). Dit is een vragenlijst die gebaseerd is op deze twee, vermeend orthogonale, gedragsdisposities die zijn afgeleid van het theoretische model van Gray (1982). Vanuit het oogpunt dat boosheid binnen dit kader van toenaderings- versus terugtrekkingsgedrag een toenaderingsmotivatie is (Harmon-Jones, 2003a), werd de hypothese getoetst en bevestigd dat scores op de BAS schaal, en niet de BIS schaal, voorspellend zijn voor selectieve aandacht voor boze gelaatsuitdrukkingen. Het daaropvolgende hoofdstuk bouwt voort op deze bevinding door de tegengestelde hypothese te onderzoeken dat sociale *angst* juist gepaard gaat met *vermijding* van verwerking van sociale dreigstimuli als boze gezichtsuitdrukkingen. Deze predictie werd getoetst in een patiëntengroep die gediagnosticeerd is met een sociale fobie. In tegenstelling tot de verhoogde selectieve aandacht die in eerder onderzoek is gevonden voor *verbaal* materiaal dat betrekking heeft op sociale evaluatie, en in overeenstemming met de predictie, lieten deze patiënten in vergelijking met een controlegroep een patroon van relatieve vermijding van dreigende gelaatsuitdrukkingen zien. Bovendien werd er een relatie gevonden tussen deze neiging tot vermijding en de variabiliteit van de hartslag in rusttoestand, hetgeen een maat is voor het functioneren van de parasympathische tak van het autonome zenuwstelsel. Hoofdstuk 2.4 beschrijft vervolgens een onderzoek met een andere patiëntengroep. Deze groep, die gediagnosticeerd is met een complexe vorm van een posttraumatisch stresssyndroom, de DSM IV (American Psychiatric Association, 1994) categorie dissociatieve identiteitsstoornis, laat zulke sterke fenomenologische fluctuaties in affect zien dat deze subjectief worden ervaren als gescheiden identiteiten. Een gemaskeerde versie van de bovengenoemde emotionele Stroop-taak werd ingezet in deze groep terwijl zij twee verschillende van deze identiteiten ervaarden, één die volgens eigen zeggen niet bewust is van een trauma en één die juist angstig is en gefixeerd op een trauma. Responspatronen in deze twee identiteitstoestanden werden onderzocht en afgezet tegen een controlegroep die gevraagd werd dergelijke identiteiten te simuleren. Resultaten laten zien dat, relatief aan een controlegroep, patiënten een afwijkend responspatroon laten zien dat wijst op een verminderde selectieve aandacht voor dreigende stimuli in de traumavermijdende identiteit, welke verdween in de traumagefixeerde identiteit. Bij het interpreteren van deze bevindingen moet gezien het exploratieve karakter van de studie enige voorzichtigheid worden betracht, maar deze geeft een indicatie dat er sprake was van een verschil in affectieve staat tussen de twee sessies in de patiëntengroep.

Het onderzoek dat gerapporteerd wordt in hoofdstuk 2.5 richt zich op prefrontale lateralisatie in relatie tot boosheid. Hiertoe werd gebruik gemaakt van een causale manipulatie van deze lateralisatie door middel van transcraniële magnetische stimulatie (TMS). TMS maakt gebruik

van een sterk magnetisch veld om neuronale exciteerbaarheid kortdurend te veranderen. Wanneer TMS pulsgewijs repetitief wordt toepast (i.e. rTMS) is deze techniek in staat effecten te sorteren die aanhouden tot na de periode van rTMS. Laagfrequente (i.e. < 1 Hz) rTMS resulteert in een tijdelijke inhibitie van een hersengebied (Schutter, van Honk, d'Alfonso, Postma, & de Haan, 2001).

In de literatuur wordt veelal uitgegaan van een zogenaamd *valentie*model van prefrontale lateralisatie, waarin positief en negatief affect worden verondersteld links, respectievelijk rechts, gelateraliseerd te zijn (Davidson & Irwin, 1999). Recente correlationele bevindingen wijzen er echter op dat een classificatie in termen van motivationele richting, i.e. toenaderings- versus terugtrekkingsgedrag, meer toepasselijk zou kunnen zijn. Dit impliceert dat de emotie boosheid links gelateraliseerd zou kunnen zijn (Harmon-Jones, 2003b). Het experiment dat gerapporteerd wordt in hoofdstuk 2.5 biedt steun aan deze theorie door te laten zien dat selectieve aandacht voor sociaal dreigende stimuli als gelaatsuitdrukkingen toeneemt na rechtszijdige versus linkszijdige laagfrequente rTMS. Deze toename bleek gerelateerd aan verhoogde activiteit van de sympathische tak van het autonome zenuwstelsel. De hier gerapporteerde bevindingen, samen met recente convergerende causale evidentie van Van Honk en Schutter (in druk) en correlationele bevindingen van Harmon-Jones (2003b), roepen om een revisie van het gevestigde beeld van frontale asymmetrie in termen van valentie, ten faveure van een classificatie op basis van motivationele richting. Een beter begrip van de frontale affectieve asymmetrie zou implicaties kunnen hebben voor de behandeling van onder andere depressie, die zich soms richt op het verhogen van links-gelateraliseerde activiteit, bijvoorbeeld door middel van *biofeedback* strategieën (Rosenfeld, Cha, Blair, & Gotlib, 1995) of langdurige rTMS behandeling (Paus & Barrett, 2004).

In het laatste hoofdstuk van dit deel wordt een onderzoek beschreven naar het effect van het geslachtshormoon testosteron op geneigdheid tot agressie in mensen. Vermeende relaties tussen testosteron en agressie zijn gebaseerd op dierstudies en correlationele bevindingen in mensen (Archer, 2006). Goed gecontroleerde causale studies in mensen ontbraken echter tot op heden. De in hoofdstuk 2.6 beschreven studie maakte gebruik van een functionele beeldvormingstechniek, *functional Magnetic Resonance Imaging* (fMRI), om effecten van testosteron te bepalen op circuits in de hersenen die verondersteld worden betrokken te zijn bij de regulering van agressie. Proefpersonen werden getest in drie verschillende sessies terwijl zij in een MRI-scanner keken naar sociale dreigstimuli en controlestimuli. Hersenactiviteit gemeten tijdens de eerste sessie bleek gerelateerd aan endocriene parameters. In lijn met voorgaand onderzoek met andere methoden bleek een sterke reactiviteit in limbische gebieden als de amygdala en hypothalamus voorspeld te worden door een profiel van hoge testosteron en lage cortisol (Van Honk et al., 1998; Van

Honk et al., 1999), terwijl activiteit in de orbitofrontale cortex, een gebied dat geassocieerd wordt met impulscontrole, hier relatief los van stond. Vervolgens werd in een dubbelblind placebogecontroleerde studie de predictie getest dat toediening van testosteron zou leiden tot een verhoging van de reactiviteit in voorgenoemde limbische gebieden, hetgeen bevestigd werd. Gebaseerd op dieronderzoek wordt beargumenteerd dat deze effecten van testosteron gemedieerd worden door de neuropeptide vasopressine op het niveau van het limbisch systeem. Daarnaast wordt benadrukt dat de link tussen testosteron en agressie in veel diersoorten sterker is dan in mensen, hetgeen verklaard zou kunnen worden uit de belangrijke rol die de orbitofrontale cortex speelt in het onderdrukken van agressieve impulsen in mensen.

Psychoneuro-endocrinologie van angstcircuits

De eerste twee hoofdstukken (3.1 en 3.2) van dit deel beschrijven opnieuw studies waarbij wordt gekeken naar psychoactieve effecten van het geslachtshormoon testosteron, het eindproduct van de hypothalamus-hypofyse-gonadale (HPG) neuro-endocriene as. Hierbij wordt de hypothese onderzocht dat testosteron een angstremmende[1] werking heeft. Deze predictie is gebaseerd op vele bevindingen uit dieronderzoek (Aikey, Nyby, Anmuth, & James, 2002; Bitran, Kellogg, & Hilvers, 1993; Boissy & Bouissou, 1994). Het derde hoofdstuk test een zelfde predictie voor het eindproduct van de hypothalamus-hypofyse-bijnierschors (HPA) neuro-endocriene as, het 'stress-hormoon' cortisol. Deze predictie is gebaseerd op recente bevindingen dat korte termijneffecten van cortisol, anders dan lange termijneffecten, onder andere zouden kunnen bijdragen aan negatieve terugkoppeling naar centrale angstcircuits, parallel aan het bekende remmende effect van cortisol op zijn eigen productie. Zo is gebleken dat toediening van cortisol kort na een trauma de kans op ontwikkeling van en posttraumatisch stresssyndroom zou kunnen verlagen (Schelling, Roozendaal, & De Quervain, 2004). Daarnaast is uit recent onderzoek gebleken dat toediening van cortisol fobische angst zou kunnen verminderen (Soravia et al., 2006).

Dergelijke processen worden vaak bestudeerd met behulp van psychofysiologische technieken. Deze maken het mogelijk het functioneren in kaart te brengen van de sympathische en parasympathische takken van het autonome zenuwstelsel. Een andere veelgebruikte maat is de sterkte van de oogknipperingsreflex in reactie op een plotselinge stimulus als een hard geluid. De sterkte van deze reflex wordt gemoduleerd door de momentane affectieve toestand van het individu. Dergelijke paradigmata zijn niet in de laatste plaats zo populair omdat vrijwel identieke

[1]In de engelstalige literatuur wordt veelal een onderscheid gemaakt tussen *fear* en *anxiety* om het verschil te duiden tussen aan een stimulus gekoppelde, respectievelijk aspecifieke, angst. Omdat deze begrippen moeilijk vertaalbaar zijn en het onderscheid niet direct relevant is voor deze samenvatting zal hier voor beide het Nederlandse woord *angst* worden gebruikt.

procedures kunnen worden toegepast in dieronderzoek, hetgeen translationeel onderzoek vereenvoudigt.

In de experimenten die beschreven worden in hoofdstuk 3.1 en 3.2 werden twee zulke paradigmata toegepast in combinatie met placebogecontroleerde toedieningen van 0,5 mg testosteron in vrouwelijke deelnemers. De eerste studie gebruikte een paradigma dat werd ontwikkeld door Grillon, Ameli, Woods, Merikangas, & Davis (1991). In afwisselende blokken werd deelnemers verteld dat zij al dan niet lichte elektrische schokken zouden kunnen krijgen. Deze procedure resulteert in zeer robuuste potentiatie van de akoestische schrikreflex, hetgeen meetbaar is via elektromyografie van de *orbicularis oculi*. Een soortgelijk paradigma werd toegepast in hoofdstuk 3.2 (Lang, Bradley, & Cuthbert, 1997) waarbij gebruik werd gemaakt van een gestandaardiseerde set foto's, het *International Affective Picture System* (Center for the Study of Emotion and Attention, 1999), om reacties van de sympathische en parasympathische takken van het autonome zenuwstelsel op te wekken, alsmede potentiatie van de akoestische schrikreflex te bewerkstelligen. In hoofdstuk 3.3 wordt hetzelfde paradigma toegepast als in hoofdstuk 3.1, echter hier in combinatie met placebogecontroleerde toediening van cortisol.

Resultaten van hoofdstuk 3.1 en 3.2 laten verlagende effecten zien van testosteron op potentiatie van de akoestische schrikreflex. In hoofdstuk 3.2 was dit effect beperkt tot een subgroep van deelnemers die hoog scoorden op zelfgerapporteerde angstige trekken (Spielberger, Gorusch, & Lushene, 1970). Omdat de subgroep die laag scoorde in angstige trekken ook weinig potentiatie liet zien in de placeboconditie kan dit uitgelegd worden als een bodemeffect. Metingen van reacties van het autonome zenuwstelsel in hoofdstuk 3.2 lieten zien dat vooral reacties die gemedieerd worden door de sympathische tak, zoals de elektrodermale respons, verlaagd zijn na toediening van testosteron. Een parasympathische reactie als een verlaging van de hartslagfrequentie liet echter geen effect van testosteron zien. Een verklaring hiervoor kan liggen in het feit dat deze hartslagverlagingseffecten meer gerelateerd zijn aan aandacht dan aan affectieve reacties (zie Öhman, Hamm, & Hugdahl, 2000). Zij zouden derhalve meer gerelateerd kunnen zijn aan versterkte cognitieve verwerking van de gepresenteerde stimuli, processen waarop geen invloed van testosteron zou worden verwacht. In overeenstemming hiermee is het feit dat geen effecten werden waargenomen op een andere cognitieve maat, namelijk subjectieve beoordeling van de foto's.

De resultaten van hoofdstuk 3.3 bieden geen evidentie voor de hypothese dat cortisol een angstremmende werking heeft. In de cortisol toedieningsconditie werd een even sterke potentiatie van de akoestische schrikreflex waargenomen tijdens dreiging met het krijgen van een elektrische schok als in de placeboconditie.

Samengevat kan worden gesteld dat de resultaten van deze twee onderzoeken aantonen dat

testosteron, duidelijker dan cortisol, effecten sorteert op meerdere componenten van het menselijke stresssysteem. Dit zou erop kunnen wijzen dat, hoewel effecten van testosteron op perifere componenten van de HPA as evident zijn, deze effecten centraal gemedieerd worden. Gebaseerd op dieronderzoek wordt in hoofdstuk 3.1 en 3.2 de mogelijke neurobiologische mechanismen besproken die ten grondslag zouden kunnen liggen aan dit effect. Er wordt geconcludeerd dat angstremmende effecten van testosteron waarschijnlijk optreden via intracellulaire androgeenreceptoren die wijdverspreid zijn in limbische gebieden als de amygdala en de bed nucleus van de stria terminalis (Fernandez-Guasti & Martinez-Mota, 2005). De betrokkenheid van non-genomische effecten die de inhibitoire werking van γ-aminoboterzuur (GABA) op GABA-a receptoren versterken kan echter niet worden uitgesloten (Aikey et al., 2002; Bitran et al., 1993). Traditionele GABAerge anxiolytica als benzodiazepines hebben echter sterk sederende bijwerkingen en hebben in de voorgenoemde paradigmata vaak robuustere aspecifieke effecten op basale metingen van de akoestische schrikreflex dan op de angst-potentiatie hiervan (Baas et al., 2002), hetgeen in de huidige onderzoeken met testosteron niet gebeurde. Deze bevindingen, in combinatie met recente andere literatuur op dit gebied, suggereren dat de HPG as een belangrijke rol speelt in de negatieve terugkoppeling naar centrale angstcircuits. Een dergelijk mechanisme zou meer flexibiliteit toelaten in adaptatie aan omgevingsfactoren zoals herhaalde stressoren (Gomez & Dallman, 2001). Dit geeft het belang aan van meer onderzoek naar de rol van de HPG as in neuropsychiatrische stoornissen die ook geassocieerd worden met afwijkingen in de HPA as. Een geïntegreerd beeld van de functies van de HPG en HPA assen in stress- en angstregulatie zou kunnen leiden tot de ontwikkeling van meer specifieke behandelingsstrategieën van dit soort stoornissen (Williamson, Bingham, & Viau, 2005).

Psychoneuro-endocrinologie van empathie

In het laatste deel van dit proefschrift worden twee studies gerapporteerd naar empathie, vanuit het gezichtspunt dat hogere orde sociale processen, zoals het vermogen zichzelf te verplaatsen in het perspectief van een ander, geworteld zijn in het vermogen tot imitatie. Om deze processen te onderzoeken werd een paradigma toegepast dat het mogelijk maakt spontane imitatie van gelaatsuitdrukkingen te kwantificeren met behulp van elektromyografie (zie Dimberg, 1982). Hierbij werd activiteit gemeten van de zygomaticus major en de corrugator supercilii, welke respectievelijk betrokken zijn bij gelaatsuitdrukkingen van blijdschap en boosheid. Vrijwilligers laten in dergelijke paradigmata snelle en automatische stimuluscongruente EMG-activiteit zien in reactie op foto's van gezichtsuitdrukkingen (Dimberg, Thunberg, & Elmehed, 2000; Dimberg, Thunberg, & Grunedal, 2002). Bovendien is uit eerder onderzoek gebleken dat vrijwilligers die als sterk empathisch worden geclassificeerd op basis van zelfrapportage, ook sterkere imitatiereacties

laten zien (Sonnby-Borgstrom, 2002).

Recente theoretische en empirische ontwikkelingen suggereren dat afwijkingen in het automatisch 'spiegelen' van neurale motorprogramma's, gereguleerd door zogenaamde *spiegelneuronen*, een belangrijke rol zouden kunnen spelen in de etiologie van autisme. Deze spiegelneuronen zouden de basis vormen voor de ontwikkeling van sociaal-emotionele cognitie, ook wel *Theory of Mind* genoemd (Williams, Whiten, Suddendorf, & Perrett, 2001). In de studie die beschreven wordt in hoofdstuk 4.1 werd daarom de hypothese onderzocht dat autistische trekken gerelateerd zijn aan verminderde spontane imitatie van gelaatsuitdrukkingen. Omdat autistische stoornissen zich waarschijnlijk bevinden aan het uiteinde van een spectrum van autistische trekken (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001) werden uit een groot bestand van gezonde vrijwilligers deelnemers geselecteerd met een extreem hoge, dan wel extreem lage, score op een vragenlijst voor autistische trekken (autisme spectrum quotiënt; Baron-Cohen et al., 2001). De resultaten bevestigden in grote lijnen de hypothese. Verminderde imitatie in vrijwilligers met sterke autistische trekken werd gevonden in de corrugator supercilii in reactie op dreigende gelaatsuitdrukkingen. De sterkte van expliciet geïnstrueerde imitatiereacties bleek echter niet te verschillen. Deze bevindingen bieden steun aan de notie dat afwijkingen in de circuits van spiegelneuronen (het zogenaamde *mirror neuron system*; MNS) een rol zouden kunnen spelen in de etiologie van autisme. Ander zeer recent onderzoek is hiermee in overeenstemming. Zo is in een vergelijkbaar paradigma gevonden dat patiënten met een autisme spectrum stoornis een vergelijkbaar effect laten zien (McIntosh, Reichmann-Decker, Winkielman, & Wilbarger, 2006). Een ander onderzoek heeft met behulp van functionele MRI laten zien dat activiteit in een belangrijk deelgebied van het MNS, de inferieure frontale gyrus, in kinderen met een autisme spectrum stoornis minder actief is dan in een controlegroep tijdens het kijken naar gezichtsuitdrukkingen (Dapretto et al., 2006). Andere onderzoeken naar MNS functies in autisme spectrum stoornissen leverden soortgelijke resultaten op (Fecteau, Lepage, & Theoret, 2006).

In hoofdstuk 4.2 wordt tenslotte een studie gerapporteerd waarin een soortgelijk paradigma werd gebruikt om de hypothese te toetsen dat testosteron empathisch gedrag vermindert. Deze hypothese is gebaseerd op bevindingen van relaties tussen hoge testosteron en antisociaal gedrag, bijvoorbeeld in antisociale persoonlijkheidsstoornissen (Stalenheim, Eriksson, von Knorring, & Wide, 1998). Bovendien vertonen mannen, die ongeveer tien maal zo hoge niveaus van endogene testosteron hebben als vrouwen, minder spontane imitatie van gezichtsuitdrukkingen (Dimberg & Lundquist, 1990) en een mindere neiging tot pro-sociaal gedrag en empathie (Baron-Cohen, 2002). Causaal bewijs voor deze associatie tussen testosteron en empathie is echter nooit gerapporteerd. In de studie die wordt beschreven in hoofdstuk 4.2 werd daarom een

placebogecontroleerde toediening gebruikt van testosteron identiek aan hoofdstuk 2.6, 3.1 en 3.2. Het gebruikte paradigma was in grote lijnen identiek aan dat in hoofdstuk 4.1. Echter, dit maal werden met behulp van *morphing* software korte films gemaakt van bewegende gezichten door foto's van een neutrale uitdrukking te laten overgaan in een emotionele uitdrukking. Het is bekend dat dynamische presentatie de perceptie van emotie in gezichten faciliteert (Sato, Kochiyama, Yoshikawa, Naito, & Matsumura, 2004) en deze procedure resulteerde dan ook in zeer sterke imitatieresponsen. Bovendien bleek verhoging van testosteron door middel van toediening te resulteren in verminderde imitatie van beide stimulustypes. Deze bevinding is de eerste om een causaal verband aan te tonen tussen testosteron en een objectieve maat voor empathie.

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Curriculum Vitae

Erno Hermans werd geboren op 28 december 1974 in Oss. In 1993 rondde hij daar zijn VWO af aan het Titus Brandsma Lyceum. Binnen zijn studie psychologie specialiseerde hij zich in de theoretische en experimentele psychologie en vervulde als student enkele respresentatieve functies en bestuursfuncties binnen de Utrechtse opleiding psychologie en studenteninspraak. Hij voerde vervolgens zijn afstudeeronderzoek uit op het gebied van psychofysiologie en aversieve conditionering binnen het Karolinska Instituut te Stockholm, Zweden. Na enkele (onderzoeks-) assistentschappen begon hij in 2002 aan een promotietraject bij de capaciteitsgroep Psychonomie binnen het Helmholtz Instituut aan de Universiteit Utrecht. Dit project werd uitgevoerd in nauwe samenwerking met de functionele neuroimaging groep binnen de afdeling Psychiatrie van het Universitair Medisch Centrum Utrecht, hetgeen resulteerde in het voorliggende proefschrift. Vanaf september 2006 is hij als wetenschappelijk onderzoeker verbonden aan het F.C. Donderscentrum voor Cognitieve Neuroimaging te Nijmegen.

Erno Hermans was born on December 28 of 1974 in Oss, the Netherlands. In 1993, he completed his secondary education at the "Titus Brandsma Lyceum" in Oss. He subsequently studied psychology, within which he specialized in theoretical and experimental psychology, and held several positions within the undergraduate student representation within the Utrecht psychology degree course. He obtained his psychology degree after a research internship at Karolinska Institute in Stockholm, Sweden, which concentrated on psychophysiology and aversive conditioning. After several (research) assistantships, he started his PhD project in 2002 at the Psychonomics Department, Helmholtz Institute, Utrecht University. Within this project, he closely cooperated with the functional neuroimaging group within the Psychiatry Department at the University Medical Center Utrecht. This project resulted in the present thesis. As of September 2006, he holds a research position at the F.C. Donders Centre for Cognitive Neuroimaging in Nijmegen, The Netherlands.