Reinforcement learning and risk-taking across the lifespan.

A comparison of adolescent and adult reward-related behavior in consideration of steroid hormones, stress, and test time.

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Thesis Examiners:

Jun. Prof. Dr. Esther K. Diekhof, Universität Hamburg

Prof. Dr. Jutta Schneider, Universität Hamburg

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Zusammenfassung

Belohnung und Bestrafung trägt maßgeblich zum Erwerb neuer Fertigkeiten sowie zur Anpassung von Verhaltensweisen bei. Belohnung fördert dabei die gezeigte Handlung, während das Ausbleiben einer Belohnung eine Anpassung von Verhalten provoziert. Neurophysiologisch werden diese Verhaltensweisen zum Großteil durch den Neurotransmitter Dopamin und dessen dopaminerge Verarbeitungspfade moduliert. Tier- und Humanstudien konnten bereits einen Einfluss von Steroidhormonen auf die Dopaminverfügbarkeit und Bioaktivität nachweisen. So scheint belohnungsbezogenes verstärkendes Lernverhalten dabei ebenso durch endogene Hormonkonzentrationen beeinflusst zu sein, wie Risikoverhalten. Mit dem Einsetzen der Pubertät während der frühen Adoleszenz beeinflussen Steroidhormone entscheidend Verhalten Adoleszenter aber auch Erwachsener.

Diese Dissertation untersucht unter Zuhilfenahme von computergestützen Verhaltensaufgaben den Einfluss von endogenen Steroidhormonen auf verstärkendes Lernverhalten und Risikoverhalten von der Adoleszenz bis ins frühe Erwachsenenalter. Dafür wurden in vier Studien die Einflüsse von unterschiedlichen Testzeitpunkten (Jahreszeit und Tageszeit), endogenen Hormonkonzentrationen (Estradiol, Progesteron, Testosteron und Cortisol), neurophysiologischen Verarbeitungsprozessen während des Erhalts einer Belohnung (positives Feedback) oder Bestrafung (negatives Feedback) und der Einfluss von psychosozialem Stress (hervorgerufen durch den Trier Social Stress Test) ausgewertet.

In der ersten Untersuchung konnte eine allgemein langsamere Antwortzeit am Morgen, jedoch keine weiteren Einflüsse von Photoperiodizität, Temperatur oder Jahreszeit auf verstärkendes Lernen beobachtet werden. Die weiteren Studien zeigten bei Adoleszenten und Erwachsen, dass Estradiol sehr wahrscheinlich Belohnungslernen stärkt. Hohe Testosteronkonzentrationen schienen hingegen einen negativen Einfluss sowohl auf Belohnungs- als auch auf Bestrafungslernen zu haben. In Studie drei konnte eine bessere Lernleistung der erwachsenen Testpersonen im Vergleich zu den adoleszenten beobachtet werden, jedoch auch die Tendenz einer stärkeren Hirnaktivität auf negatives Feedback bei Adoleszenten. Im Hinblick auf Stress konnten keine Auswirkungen auf das Lernverhalten beobachtet werden. Gestresste Testpersonen zeigten einen stärkeren Cortisolanstieg und weibliche Testpersonen berichteten von einer größeren Stresswahrnehmung. Mit stärkerem Cortisolanstieg konnte zudem eine vermehrte neurophysiologische Aktivität auf negatives Feedback beobachtet werden.

In der vierten Studie wurde der Einfluss des Verhältnisses von endogenem Testosteron- und Cortisolspiegel auf Risikoverhalten bei männlichen Versuchsteilnehmern mit einer herkömmlichen statistischen Analyse sowie mit einem computergestütztem Rechenmodell untersucht. Dabei konnte entsprechend der „*dual-hormone*“ Hypothese festgestellt werden, dass ein hoher Testosteronspiegel bei gleichzeitig niedrigem Cortisol vermutlich die Neigung zu riskantem Verhalten steigert. Allgemein zeigte sich ein positiver Zusammenhang zwischen erhöhten Testosteronwerten und vermehrtem Risikoverhalten, wobei auch ein quadratischer Zusammenhang zwischen Alter und Risikoverhalten aufgezeigt wurde. Hierbei wurde das riskanteste Verhalten während des frühen Erwachsenenalters beobachtet.

Insgesamt konnten im Lern- und Risikoverhalten Unterschiede zwischen Adoleszenten und Erwachsenen ermittelt werden. Auch ein Einfluss von Steroidhormonen auf Belohnungsverhalten wurde gemessen. Aufgrund von Erkenntnissen aus vorherigen Untersuchungen, könnte angenommen werden, dass sich die gezeigten Studienergebnisse unter anderem auf unterschiedliche dopaminerge Prozesse, aber auch eine entwicklungsbezogene Hirnreifung während der Adoleszenz zurückführen lassen. Diese und weitere Annahmen werden im Hinblick auf den aktuellen Forschungsstand in dieser Dissertation ausführlich diskutiert.

Die neurophysiologischen Vorgänge von Belohnungsverarbeitung sind nicht nur relevant für kognitive Verarbeitungsprozesse während des Lernens oder Treffens von Risikoentscheidungen. Zusammenhänge zwischen dopaminergen Funktionen und mentalen Störungen konnten zuvor in vorausgegangenen Patientenstudien beobachtet werden. Bereits während der Adoleszenz entstehen erste Störungsbilder (z.B. Depressionen oder Angststörungen), welche sich im Erwachsenenalter manifestieren. Eine gezielte Erforschung möglicher Einflüsse (z.B. von Stress, endogenem Hormonhaushalt, neurophysiologischen Prozessen) auf das dopaminerge Belohnungssystem kann zum besseren Verständnis der Störung der grundlegenden Verarbeitung von Belohnungsreizen beitragen. Dadurch könnten Kenntnisse über die Entstehung mentaler Störungen womöglich konkretisiert und eine Grundlage für weitere Präventions- und Behandlungsmöglichkeiten geschaffen werden.

Summary

Reward and punishment provide a meaningful contribution to the purchase of new capabilities and the adaptation of behavior. Thereby, reward supports the shown action, whereas the omission of reward evokes an adjustment of behavior. Dopamine is one of the main neurotransmitters which modulates these behavioral patterns via dopaminergic projections. Animal and human studies could already determine an impact of steroid hormones on dopamine availability and bioactivity. Thus, reward-related reinforcement learning, as well as risk-taking may be influenced by endogenous hormone concentrations. With the beginning of puberty during early adolescence, steroid hormones start to affect the behavior.

This thesis investigates the impact of endogenous steroid hormones on reinforcement learning and risk-taking with computer-based behavioral tasks from adolescence until young adulthood. The influence of steroid hormones will thereby be assessed alongside other modulating influences on dopaminergic processes such as psychosocial stress, and potential confounds like circadian rhythm. In four studies various aspects will be assessed: these include the influence of different periods of test time on reinforcement learning (season and daytime), endogenous hormone concentrations in the context of both reinforcement learning and risk taking (estradiol, progesterone, testosterone, and cortisol), neurophysiological processing during reward (positive feedback) or punishment (negative feedback) learning, and the influence of psychosocial stress (evoked by the Trier Social Stress Test).

In the first study (Study I), a generally slower response time during the morning but no impact of photoperiodicity, temperature, or season on reinforcement learning was observed. Further, Study II demonstrated that estradiol presumably improves reward learning in both adolescents and adults. On the contrary, high testosterone concentrations may diminish reward and punishment learning. In Study III, a better learning performance of adults compared to adolescents and a tendency towards a greater brain activity during negative feedback in adolescents became evident. Still, regarding the stress intervention, no effect on learning was detected. Stressed participants demonstrated a higher cortisol increase and female participants reported a greater stress perception during the test. Further, with increasing cortisol, an enhanced neurophysiological activity to negative feedback was shown.

The fourth study (Study IV) was conducted with male adolescents and adults. Here, the impact of endogenous testosterone and cortisol interactions on risk-taking behavior was investigated by using conventional statistical analyses and additionally a computational model. Following the

dual-hormone hypothesis, it was found that high testosterone level and simultaneously low cortisol level increased risk propensity. In general, a positive interaction between testosterone and increased risk-taking and a quadratic interaction between age and risk-taking was shown. Here, the riskiest behavior was observed during early adulthood.

In sum, differences between adolescents and adults emerged during reward-related behaviors, reinforcement learning and risk-taking. Further, an influence of steroid hormones on reward-related behavior could also be measured. Previous studies suggest that the demonstrated findings could inter alia trace to different dopaminergic processing but also indicate the development of brain maturation during adolescence. These and other assumptions are discussed in detail in this thesis.

Neurophysiological processes of reward processing are not only important for cognitive functions during learning or risky decision-making. Previous studies investigating patient groups observed interactions between dopaminergic functions and mental disorders. Already during adolescence, some pathologies emerge (e.g. depression, anxiety disorders) and fully manifest in early adulthood. An investigation of the possible influences (like stress, endogenous hormones, neurophysiological processes) on the dopaminergic reward system may contribute to a better understanding of the fundamental processing of reward and motivation. As a result, knowledge about the development of mental disorders could probably be concretized and create a basis for preventive possibilities and treatment options.

List of abbreviations

BIC (CHAPTER FOUR)	Bayesian Information Criterion
C (CHAPTER FOUR)	Cortisol
E_2 (CHAPTER THREE)	17β-estradiol
EEG	electroencephalography
ERP	event-related potential
fMRI	functional magnetic resonance imaging
GLM (CHAPTER FOUR)	General linear model
GPe	globus pallidus external
GPi	globus pallidus internal
pCi (CHAPTER FOUR)	percentage cortisol increase
SNr	substantia nigra pars reticulata
T (CHAPTER FOUR)	Testosterone
TSST	Trier Social Stress Test
VBA (CHAPTER FOUR)	Variational Bayesian Analysis

General introduction

Dopamine – the motor of reward

The need for reward

Reward evokes and reinforces approach behavior through learning. Therefore, behavior that leads to reward will be intensified, maintained, and reiterated. In addition, reward causes subjective positively connoted feelings. These functions of reward contribute to an elaborated individual and social behavior. Reward can be described as an appetitive environmental object which is particularly motivating through its effects on welfare, survival, and reproduction. In contrast, aversive objects can be punishments (Schultz, 1998). Not a single closed network processes rewarding behavior, rather do interaction of intricate neuronal mechanisms in the brain. The dopaminergic system has been discovered to be a key part of it. In 1957, Kathleen Montagu and colleagues first detected the neurotransmitter dopamine in the rat brain, almost half a century after the first laboratory dopamine synthesis (Hornykiewicz, 2002; Montagu, 1957). During the 1950s and 1960s, the working group of Arvid Carlsson developed a new fluorescent assay technique to verify dopamine in tissue (Fahn, 2008). Henceforth, they were able to demonstrate the neurophysiological relevance of dopamine as a neurotransmitter in the brain (Carlsson, 1959). From then on, dopamine became an immense research interest. Nowadays, the participation of the dopaminergic system in reward learning, risk-taking, and several diseases, is still extensively investigated.

Physiology and signaling of dopamine

Dopamine is a monoamine neurotransmitter with a catechol structure and an amine group added by an ethyl chain. Its biosynthesis begins with and is limited by tyrosine. Dietary L-phenylalanine can be converted to tyrosine by phenylalanine hydroxylase and tetrahydrobiopterin and molecular oxygen as cofactors in the central nervous system and the periphery (e.g. kidney and gut). Further, tyrosine can be hydroxylated to levodopa by tyrosine hydroxylase (defined as the rate-limiting enzyme) using the same cofactors and iron. The final synthesis to dopamine is catalyzed by L-amino acid decarboxylase with pyridoxal phosphate as a cofactor (Klein et al., 2019). A minor pathway is the conversion of dopamine from *p*-tyramine, a trace amine from tyrosine, through cytochrome P450 2D6 (Bromek et al., 2011).

In dopaminergic neurons, dopamine is stored and transported from the cytosol in synaptic vesicles until ejection into the synaptic cleft, mainly through exocytosis. In noradrenergic or adrenergic cells dopamine is a precursor of noradrenaline and indirect adrenalin (Klein et al., 2019). Extracellular dopamine concentrations can be affected by phasic and tonic mechanisms. Bursting events, inter alia after reward reception, elicit phasic (or synaptic) levels of dopamine from dopaminergic neurons. Hereby, action potentials lead to an increased presynaptic dopaminergic tone. Tonic (or extrasynaptic) levels of dopamine are inter alia affected by presynaptic limbic and cortical glutamatergic inputs but are also related to actions potentials. Unlike phasic levels, changes in tonic levels of dopamine release are less rapid and probably result in less dopamine release at the presynaptic terminal (Floresco et al., 2003).

Located pre- and postsynaptic at soma and dendrites in the central nervous system and peripherally, five G protein-coupled receptors (D_1 and D_5) and autoreceptors (D_2-D_4) were allocated to dopamine. According to their contrary functions, they are divided into D1-like (D_1 and D_5 $G_{\alpha s}$-coupled, excitatory function) and D2-like ($D_{2\ short}$/$D_{2\ long}$, D_3 and D_4 $G_{\alpha i}$-coupled, inhibitory function) receptors (Mishra et al., 2018). Additionally, dopamine can also bind to the trace amine-associated receptor 1 (Klein et al., 2019). Acting as an excitatory neurotransmitter, dopamine can stimulate the adenylyl cyclase activity, via D1-like receptors, resulting in increased cyclic adenosine monophosphate levels (Klein et al., 2019). Concurrently, D2-like receptors lead to a decrease of cyclic adenosine monophosphate and thereby dopamine can equally promote inhibiting processes (Mishra et al., 2018). A high density of dopamine receptors was found in the olfactory bulb, basal ganglia (including the striatum, globus pallidus, subthalamic nucleus, and substantia nigra), and amygdala (Jackson & Westlind-Danielsson, 1994; Trepel, 2015). Whereas middle to low expressions were determined in the cortex, the hippocampus (i.a. pyramidal cells), and hypothalamus (Jackson & Westlind-Danielsson, 1994). After the effect of dopamine on the receptors, it will be taken back into the presynaptic cell by the dopamine transporter and unspecifically binding monoamine transporters (Klein et al., 2019). Dopamine action is limited by the enzyme tyrosine hydroxylase and further through the breakdown enzymes monoamine oxidase and catechol-*O*-methyltransferase (Eisenhofer et al., 2004).

Neurophysiological projections

Heterogenous groups of dopamine neurons are mostly located in mammals' mesencephalic-diencephalic junction and to a lesser extent in the telencephalon (Hynes & Rosenthal, 1999). The

two partly overlapping major subgroups of dopamine neurons in the mesencephalon, which are important for dopamine-related behavior, are the substantia nigra pars compacta and ventral tegmental area. In the nigrostriatal system, involved in motor functions, dopaminergic fibers project from to the substantia nigra pars compacta predominantly to dorsal and ventral striatal regions (Wahlstrom et al., 2010; Wise, 2004). Dopaminergic projections from the ventral tegmental area to limbic structures (particularly nucleus accumbens, olfactory tubercle, amygdala, hippocampus, and septum) (see also Neurophysiological differencesNeurophysiological differences*)* and the cortex (especially transitional entorhinal, cingulate, orbitofrontal, and dorsolateral prefrontal cortex) are divided into the mesolimbic and mesocortical dopamine system (Wahlstrom et al., 2010). Summarized as the mesocorticolimbic system, it is of particular importance for reward mediation (Wise, 2004).

Seasonal and circadian fluctuations of dopamine

Human metabolism is regulated by the inner clock oriented towards solar time (Meyer et al., 2016). A natural circadian and seasonal fluctuation in dopamine concentration is conceivable and has been scarcely investigated by now. In rats, a diurnal increase of dopamine towards the night could be observed (Castañeda et al., 2004; Smith et al., 1992). To date, it is not feasible to investigate diurnal dopamine fluctuations in the living human brain without inversive measurement methods. Some seasonal measurements of the cerebrospinal fluid of healthy humans and patients with Parkinson's disease demonstrated greater concentrations during fall and winter than spring and summer (Eisenberg et al., 2010; Hartikainen et al., 1991; Kaasinen et al., 2012). However, the research group around Brewerton reported higher homovanillic acid concentrations in healthy human cerebrospinal fluid during summer and lower during spring in their initial study (Brewerton et al., 1988). In a more recent investigation, the opposite distribution became equally likely, since they observed significantly enhanced concentrations during spring compared to summer (Brewerton et al., 2018). A post mortem investigation revealed a higher quantity of midbrain neurons containing tyrosine hydroxylase in summer compared to winter. It was suggested that a higher dopamine concentration may occur during the summer, but the authors admitted that their finding could as well indicate higher concentrations during winter (Aumann et al., 2016). A single photon emission computed tomography in Taiwanese showed a greater D2-like receptor availability in participants with high-sunshine-exposure compared to a low-sunshine-exposure group (Tsai et al., 2011).

Nevertheless, the authors were not able to determine whether their finding indicates higher dopamine concentrations in summer or winter. Despite these insufficient and partly conflicting data, the observation of dopamine rhythmicity is an important factor which should be considered regarding reward-related behavior, not at least due to dopaminergic participation in this and numerous other cognitive processes.

Developmental changes from childhood through adolescence to adulthood

At birth D1- and D2-like receptors are detectable across cortical regions and dopaminergic neurons are widespread in all cortex areas. During the first postnatal days, the elaboration of dopaminergic projections throughout the dorsal prefrontal cortex and anterior cingulate cortex begins. While during early childhood, dopamine concentrations seem on an equal level throughout the cortex, in adolescent rhesus monkeys a greater expression is measurable in anterior regions (Wahlstrom et al., 2010). Besides, there is no general definition of adolescence and especially human adolescence in research. A rough classification places the human adolescence period from the age of 10 years to 17 years in girls and 12 years to 18 years in boys (Sinclair et al., 2014). Across different studies, the transition from adolescence to early adulthood appears to be blurred and has been only inconsistently defined yet. However, adolescence is a crucial period of emotional, cognitive, and (neuro-)physiological maturation and therefore extremely important for psychological and brain development.

Regarding age-related changes of the dopamine system, findings are currently insufficient. One human post mortem study (n = 56, age range = 1 day to 103 years) reported an increase in striatal dopamine level from childhood to adulthood (n = 35, age range = 1 day – 41 years) reaching its maximum levels in the age group from 3.5 to 14 years (n = 7) (Haycock et al., 2003). In contrast, another post mortem study (n = 26) was unable not demonstrate an age-related change of striatal dopamine concentration, but reported peaked concentrations of the metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid in infants (n = 4, age range = 11 - 24 months), as well as from childhood to young adulthood (n = 6, age range = 5 - 31 years) (Kalaria et al., 1993). But due to the rough age group classification and small sample sizes interpretations of these results should be treated with caution. Animal studies further demonstrate higher dopamine concentrations in the prefrontal cortex of adolescent rhesus monkeys (about 24-36 months) compared to monkey children (Goldman-Rakic & Brown, 1982).

Moreover, tyrosine hydroxylase immunoreactive axons and the density of varicosities presumably reach the maximum during adolescence and decrease to adulthood in this species (Rosenberg & Lewis, 1994).

In humans and rats, until puberty, a striatal dopamine receptor overproduction with a subsequent pruning process has been observed (Montague et al., 1999; Palacios et al., 1988; Seeman et al., 1987; Teicher et al., 1995). In adolescent rats, a maximum of receptor density relative to younger and adult rats has been detected (Gelbard et al., 1989; Tarazi et al., 1998, 1999; Teicher et al., 1993). Moreover, in young rats a lower striatal dopamine release and dopamine turnover occurred (Stamford, 1989). Prefrontal dopaminergic innervation in rats have been found to gradually increase from the prenatal period into early adulthood. Yet, this progression did not show any deviations during adolescence, in contrast to what has been observed in macaques (Kalsbeek et al., 1988).

Two contrary theories exist about the reward sensitivity of adolescents. The theory of hypersensitivity to reward postulates that adolescents need a lower intensity and frequency of rewarding stimuli for the activation of reward processing in comparison to adults. Thus, reward will be perceived faster, which probably results in a greater motivation to gain even more reward. The hyposensitivity theory, in turn, assumes that a higher intensity or frequency of reward is needed to activate the adolescent reward circuitry (Galván, 2014). Bjork and colleagues, who supported the latter theory, demonstrated twice, in a first study and a replication study, that adults compared to adolescents showed higher ventral striatal activity in to reward-predictive cues in a monetary incentive delay task (James M. Bjork et al., 2004, 2010).

However, the striatal hypersensitivity theory is supported by the majority of studies investigating reward-related behavior during adolescence. In a functional magnetic resonance imaging (fMRI) paradigm a reward-related peak with increased ventral striatum activation during reward receipt could be shown during mid adolescence (n = 18, age range = 14 - 15 years) compared to early adolescence (n = 17, age range = 10 - 12 years) and early adulthood (n = 15, age range = 18 - 23 years) (Van Leijenhorst et al., 2010). Other studies demonstrated increased behavioral and striatal activity to both appetitive and aversive stimuli (Galván & McGlennen, 2013) or during reward receipt in a monetary reward task in adolescents compared to adults (Ernst et al., 2005). Other studies demonstrated that adolescence is a period of hyperresponsiveness not exclusively to rewarding but also to social stimuli (Foulkes & Blakemore, 2016).

The limbic dopaminergic system of primates, unlike in rodents, is already well developed at birth (Wahlstrom et al., 2010). However, dopaminergic projections to the prefrontal cortex develop until early adulthood (Walker et al., 2017). Dopamine innervation thereby seems to be characterized by an increase during adolescence followed by a subsequent decrease into early adulthood (Wahlstrom et al., 2010). Given these data, changes in dopamine innervation from childhood into adulthood may be assumed. These neurophysiological alterations in the dopamine system may thereby probably entail behavioral differences between children, adolescents, and adults, especially in reward-related conditions.

Reinforcement learning

The basal ganglia Go-NoGo model

Reinforcement learning comprises learning through reward or positive feedback, but also learning from negative feedback, reward omission, or even via the punishment of one's actions. The effects of dopamine on positive and negative reinforcement can be described by the theory of the direct and stimulating "Go" pathway, and the indirect and inhibiting "NoGo" pathway that are located in the basal ganglia acting via D1- and D2-like receptors, respectively (Gerfen, 2000). During reinforcement learning, dopamine presumably acts via these two opponent pathways in the mesocorticolimbic dopamine system that connects the basal ganglia and cortical regions. According to theory, a high dopamine level is thereby associated with an activation of the Go pathway which mostly expresses D1-like receptors. Phasic dopamine bursts through reward and positive prediction errors result in a long-term potentiation mediated by D1-like receptors (Maia & Frank, 2011). The activation of Go neurons leads to an inhibition of the globus pallidus internal segment and the substantia nigra pars reticulata (GPi/SNr). These segments have a disinhibitory effect on the thalamus and consequently, excitatory signals reach the cortex (Maia & Frank, 2011) (Figure 1). An increase in dopamine following a rewarded action thus promotes learning through positive reinforcement.

In contrast, a reward omission or receipt of punishment (e.g., monetary loss) after an action leads to the suppression of tonic dopaminergic transmission, which activates the NoGo pathway and long-term depression through its action on D2-like receptors. NoGo neurons project to the globus pallidus external (GPe), which is connected to the GPi/SNr and reduces the tonic inhibition of the GPe on the GPi/SNr. Following, the inhibition of the thalamus will be enhanced, whereby no feedback will be sent to the prefrontal cortex (Maia & Frank, 2011) (Figure 1). This

pathway leads to the better future avoidance of the action that led to the negative outcome (Moustafa, Cohen, et al., 2008).

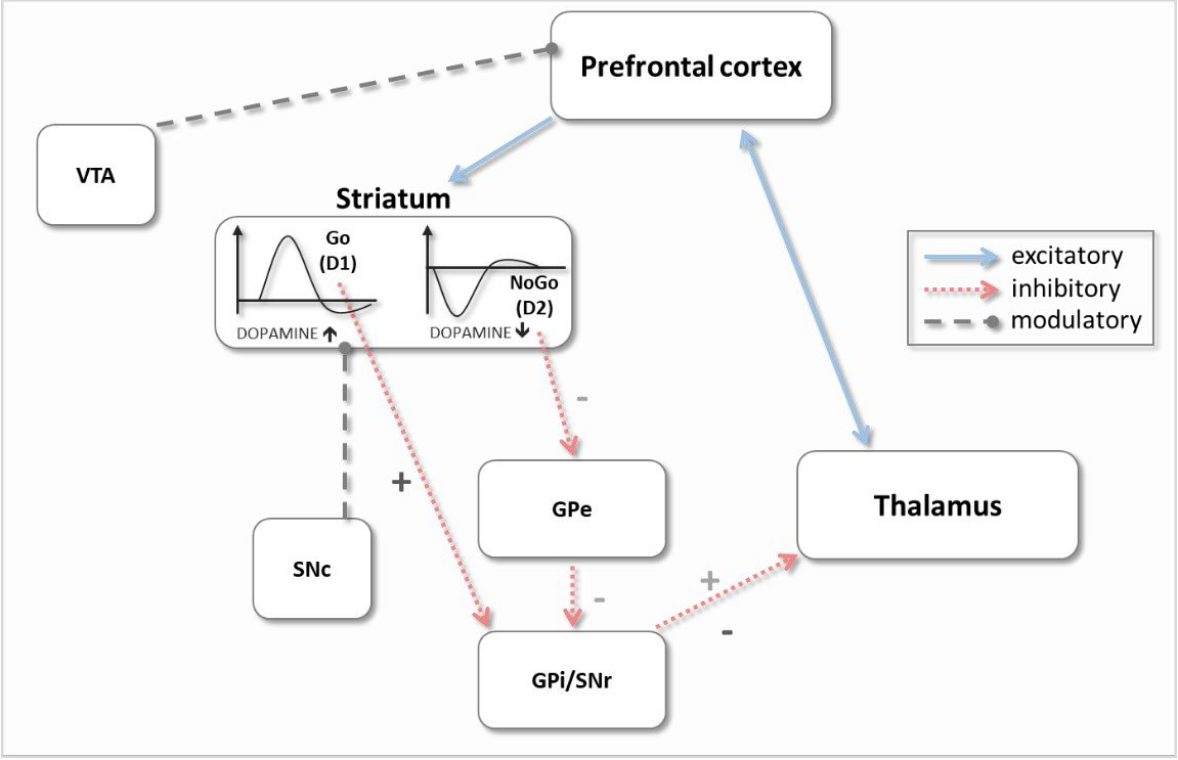

Figure 1: Anatomical basal ganglia Go-NoGo model

In the basal ganglia Go-NoGo model, GPi/SNr is directly inhibited by Go neurons (D1-mediated) and facilitates signaling from the thalamus to the prefrontal cortex. Activation of NoGo neurons (D2-mediated) indirectly suppress tonic inhibition of the GPi/SNr via GPe and prevents the feedback to the cortex (adapted from Frank, 2005).

Behavioral studies that assessed the influence of dopaminergic processes on reinforcement learning often studied patients with Parkinson's disease. These patients exhibit degenerations of dopaminergic neurons in the substantia nigra resulting in a diminished dopamine level (Sossi et al., 2002). In a probabilistic learning task, Parkinson patients, who were transiently deprived of their dopaminergic medication, demonstrated a better avoidance learning capacity, whereas patients on medication learned better through reward (Frank et al., 2004). A supporting study showed that medicated patients performed better in a feedback-based working memory task, but were worse in ignoring distracting stimuli compared to unmedicated patients (Moustafa, Sherman, et al., 2008). The authors argued that presumably the elevated Go signaling and suppressed NoGo learning in medicated patients compared to unmedicated patients led to

these behavioral differences. A better Go learning ability of medicated patients also emerged in a response time adjustment task (see Response time adjustment task) with the goal to maximize overall reward (Moustafa, Cohen, et al., 2008).

But even in healthy participants with a high baseline striatal dopamine synthesis, a better learning ability from unexpected rewards versus punishments became evident. In contrast, participants with a low baseline dopamine synthesis learned better from unexpected punishment than unexpected reward (Cools et al., 2009). Moreover, the administration of a single dose D_2 and D_3 receptor agonist "pramipexol" to healthy adults entailed an impaired response bias towards rewarding stimuli in a probabilistic reward task (Pizzagalli et al., 2008). In addition, a decreased reaction time could be observed which substantiates the findings from Moustafa, Cohen, and colleagues (2008) in the response time adjustment task (Moustafa, Cohen, et al., 2008; Pizzagalli et al., 2008). The agonist administration presumable has a blunting effect on phasic dopamine bursts and thereby impaired reward learning.

Steroid hormones and reward processing

Hormones have a great impact on physiological and behavioral processes. Individual hormone concentrations also affect transmission in the dopamine system and therefore exert an impact on dopaminergic reward processing. Especially steroid hormones are worthwhile to look at. Steroid hormones are lipophilic hormones synthesized from the common precursor, cholesterol. They contain four fused carbon rings and differ in their modified side chains (Strauss & FitzGerald, 2019). Five classes can be distinguished: progestins (e.g. progesterone), estrogens (e.g. estradiol), androgens (e.g. testosterone), glucocorticoids (e.g. cortisol), and mineralocorticoids (e.g. aldosterone) (Figure 2). Progestins (21-carbon atoms), estrogens (18-carbon atoms), and androgens (19-carbon atoms) can be summarized in the group of sex hormones, which are mostly secreted in the gonads but can also be synthesized in other tissues like the adrenal gland or the brain (Whirledge & Cidlowski, 2019). The sex hormones are integral for the maintenance of the human reproductive system. Glucocorticoids are primarily generated in the adrenal gland and induce the physiological stress response (Sinclair et al., 2014).

Sex hormone concentrations begin to rise with the onset of puberty that is initiated by the gonadarche, i.e., the maturation of the ovaries or testes, and the development of secondary sex characteristics. Hormone levels are regulated by the hypothalamic-pituitary-gonadal axis by the secretion of gonadotropin-releasing hormone in the hypothalamus. Then gonadotropins are

secreted from the anterior pituitary, which stimulates sex hormone release in the gonads (Sisk & Foster, 2004).

The adrenarche precedes the gonadarche which describes the maturation of the adrenal gland and also subsequent adrenal steroid hormone secretion (Dorn & Biro, 2011). Following stressful stimuli, cortisol secretion is regulated by the hypothalamic-pituitary-adrenal axis via the release of the corticotropin-releasing hormone as well as arginine vasopressin from the hypothalamus and subsequently the adrenocorticotropic hormone in the anterior pituitary (Sinclair et al., 2014). Both axes, the hypothalamic-pituitary-adrenal axis, and the hypothalamic-pituitary-gonadal axis interact with each other (Acevedo-Rodriguez et al., 2018).

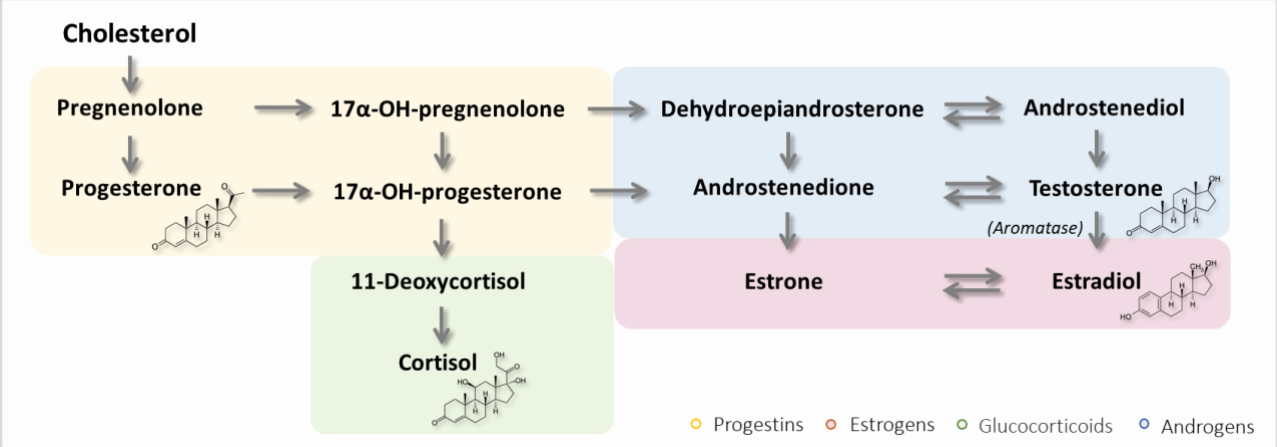

Figure 2: Biosynthesis of steroid hormones

Steroid hormones within the simplified biosynthesis pathway are classified in progestins (yellow shaded), androgens (blue shaded), glucocorticoids (green shaded), and estrogens (red shaded) (adapted from Strauss & FitzGerald, 2019).

Progesterone, which is concurrently a precursor of cortisol, testosterone, and estradiol, is produced in the female ovarian corpus luteum, the placenta, and the adrenal zona glomerulosa and fasciculata (Figure 2). In the female granulosa cells as well as in male testes and fetal-placental unit estradiol can be synthesized and in the adrenal zona fasciculata also estrone can be produced. Testosterone is generated in the testicular Leydig cells in men and lower concentrations by the ovarian theca cells and placenta in females. Further, testosterone is produced in the adrenal zona reticularis and metabolized to estradiol by aromatase in several tissues (i.a. testis, brain, adipose) (Norman & Henry, 2015a, 2015b).

All steroid hormones are transported from their endocrine glands to the target tissue via cognate plasma transport proteins, which are synthesized in the liver. Corticosteroid-binding

globulin transports progesterone and cortisol. The sex hormone-binding globulin is responsible for the carriage of estradiol and testosterone (Norman & Henry, 2015b). At the target cell, steroid hormones bind to specific intracellular or membrane receptors and form a ligand-receptor complex. The binding on an intracellular steroid receptor affects DNA transcription directly or indirectly, respectively, through transcription factors. A more rapid response is achieved by binding to an extracellular membrane receptor which activates an intracellular signaling cascade (Norman et al., 2004).

The impact of estradiol and progesterone

Researchers investigating the female menstrual cycle demonstrated that estradiol probably acts dopamine-agonistic, whereas progesterone may rather have a dopamine-antagonistic effect (see Diekhof, 2018 for overview). Pharmacologically manipulated estradiol concentrations in female ovariectomized rats potentiated striatal dopamine release (Becker, 1990; Castner et al., 1993), synthesis capacity (Pasqualini et al., 1995), and dopamine turnover (Lévesque & Di Paolo, 1988). Estradiol has also been observed to reduce D2-receptor binding affinity (Lévesque & Di Paolo, 1988) and enhanced D1-receptors density (Lévesque et al., 1989). Estradiol reduce the inhibitory effect of gamma-aminobutyric acid on dopaminergic neurons, whereas progesterone presumably increased the inhibiting effect (Hu et al., 2006; Majewska et al., 1986). Further, striatal dopaminergic activity was increased by estradiol and decreased by progesterone (Fernández-Ruiz et al., 1990).

In ovariectomized rats, a pre-treatment with estradiol and additionally a single administration of estradiol or progesterone led to an amphetamine-induced striatal dopamine increase. Without estradiol pre-treatment, this effect was validated for a single estradiol dose but not for the progesterone administration (Becker & Rudick, 1999). Moreover, progesterone diminished estradiol receptor density and in combination with estradiol administration, upregulated the monoamine breakdown enzyme monoamine oxidase (Luine & Hearns, 1990; Luine & Rhodes, 1983; Selcer & Leavitt, 1988). Accordingly, some progesterone effects occurred only in combination with estradiol. This suggests that the hormone interaction of estradiol and progesterone together modulates dopaminergic activity.

Diekhof and Ratnayake (2016) found evidence for a behavioral impact of the menstrual cycle phase on reinforcement learning in a probabilistic reward task (see also Diekhof et al., 2020). During the late follicular phase, which is characterized by high estradiol levels, young women

showed a better reward learning performance in a probabilistic learning task, whereas during the luteal phase, with enhanced progesterone concentrations, women were better to avoid punishment (Diekhof & Ratnayake, 2016; see also Diekhof et al., 2020). These findings indicated that estradiol may promote Go-learning ability during the follicular phase, and this might be supposedly achieved through a higher dopamine availability in the high estradiol state. On the contrary, a high progesterone level better supported the NoGo learning pathway, which may have been associated with the progesterone-mediated inhibition of dopamine (Diekhof & Ratnayake, 2016; see also Diekhof et al., 2020).

This study is substantiated by additional findings regarding the female menstrual cycle (for replication see also Diekhof et al., 2020). In a response time adjustment task, participants had to speed up or slow down to maximize their reward (Diekhof, 2015; Reimers et al., 2014). An elevated ability of speeding up in this task was associated with a better Go learning ability. The capability to slow down for reward was modulated by the NoGo learning pathway (Moustafa, Cohen, et al., 2008). In women, a positive correlation between estradiol and the ability to speed up (Go learning) was found in the follicular phase (high estradiol concentrations) and further, a worse performance during the slowing down sequences (NoGo learning) emerged (Diekhof, 2015; Reimers et al., 2014).

The impact of testosterone

In males, testosterone presumably modulates dopaminergic responses. Studies with male rats thereby supply evidence for an impact of testosterone on dopamine synthesis, transport and, metabolism (Sinclair et al., 2014). Intranasal and subcutaneous testosterone administration in gonadally intact male rats led to a significant dopamine increase in the nucleus accumbens (a part of the ventral striatum) and dorsal striatum (de Souza Silva et al., 2009). In castrated male rats, the concentration of dopamine and its metabolites was reduced. This effect could be reversed by testosterone administration. It was therefore assumed that testosterone increases metabolic activity in mesolimbic dopaminergic neurons (Alderson & Baum, 1981; J. B. Mitchell & Stewart, 1989). Further, castration led to a reduced tyrosine hydroxylase activity in the striatum which could be prevented by testosterone administration before castration (Abreu et al., 1988).

A study from Purves-Tyson and colleagues (2012) showed an elevated availability of tyrosine hydroxylase protein, catechol-*O*-methyltransferase, and monoamine oxidase mRNA in the

substantia nigra in gonadectomized adolescent male rats after receiving testosterone (Purves-Tyson et al., 2012). In a subsequent study, gonadectomized adolescent male rats had an increased dopamine turnover in the dorsal striatum, which could be prevented by testosterone replacement. Testosterone administration further increased dopamine transporter mRNA, dopamine receptor mRNA, and dopamine receptor gene expression (except for dopamine receptor D_3 mRNA) in the substantia nigra. (Purves-Tyson et al., 2014). But another study demonstrated that testosterone administration to gonadal-intact adolescent male rats significantly reduces tyrosine hydroxylase concentrations in the putamen (part of the dorsal striatum, e.g. important for motor control and learning) and showed no effects on the prefrontal cortex (Wood et al., 2013). Because of these conflicting findings, the results of studies with gonadectomized rats probably cannot easily be transferred to gonadal-intact human males.

Besides the observed dopaminergic impact of testosterone in rats, human findings of reward-related behavioral and neurophysiological activities also refer to an effect of testosterone on the dopaminergic system and therefore reward processing. A positive effect of testosterone on the ventral striatal activity during rewarding cues versus non-rewarding cues were demonstrated in women (Hermans et al., 2010). In healthy men higher serum testosterone was related to greater striatal activity during positive and negative reward-prediction errors (the difference between received and expected reward), which is associated with a greater dopamine activity (Morris et al., 2015). Further, the same study reported a positive correlation between androgen receptor mRNA and tyrosine hydroxylase mRNA in the substantia nigra of men's post-mortem tissues (Morris et al., 2015).

However, there are hardly any studies that investigated the influence of testosterone on reinforcement learning. Schultheiss and Rohde (2002) demonstrated that men with low impulse control showed a positive association between increased testosterone and enhanced implicit learning. The authors suggested that the demonstrated effect of testosterone on implicit learning might be dopamine-related (Schultheiss & Rohde, 2002). In a recent study, modeled data revealed an interaction between high salivary testosterone levels and high learning rates in middle-aged adolescents (Xia et al., 2021). Testosterone, similar to estradiol and progesterone, seems to modulate dopaminergic processes and probably has an impact on reward-processing and presumably reinforcement learning.

The impact of cortisol and stress

Besides the sex hormones also glucocorticoids demonstrate dopamine modulating effects. With a growing stress level, the concentration of cortisol increases measurably in humans (Kudielka & Kirschbaum, 2005). Converging evidence suggests that an increase of stress and cortisol enhances dopamine release in the mesolimbic dopamine system in humans and animals (Oswald et al., 2005; Saal et al., 2003). Stress triggered in mice by a Porsolt forced swim task increased the strength at excitatory synapses on midbrain dopamine neurons, even to a greater extent than drug administration (Saal et al., 2003). Additionally, in humans, Oswald and colleagues (2005) observed a greater amphetamine-induced dopamine increase with higher cortisol concentrations in healthy young adults (Oswald et al., 2005).

Acute stress enhances cortisol concentrations, but veterans with long-term, chronic stress exposition and a posttraumatic stress disorder had lower plasma cortisol levels (Boscarino, 1996). Moreover, it was shown that veterans with posttraumatic stress disorder had a hypersensitivity for punishment learning compared to control groups (Sawyer et al., 2016). Therefore, lower cortisol concentrations probably facilitate NoGo learning. Petzold and colleagues (2010) conducted the Trier Social Stress Test (TSST; consisting of a verbal speech and arithmetical task) and a probabilistic reward learning task with healthy young adults. They showed that stressed participants had a reduced sensitivity for a negative action outcome and a comprised avoidance learning capacity (Petzold et al., 2010). Presumably, the increased dopamine availability through high cortisol concentrations may strengthen Go learning ability in stressful situations, but NoGo learning, in turn, will be suppressed. Conversely, the stress-induced rise in overall dopamine level may render the transient dopamine drop, which normally occurs as a consequence of reward omission or punishment, less effective thus reducing the ability to learn from negative outcomes.

Risk-taking

Balancing risk taking and reward seeking

Risk-taking defines the willingness to engage in potentially rewarding situations which, at the same time, have a heightened tendency to result in a loss or even a harmful outcome (Kurath & Mata, 2018). Risk-taking thereby describes the reduced evaluation of negative relative to positive consequences of an action (Nigg, 2017). From an evolutionary perspective, risk-taking

is relevant for survival and reproduction success (Steinberg, 2008). In connection with dominance and status-seeking, taking risks presumably enabled our ancestors to achieve higher positions in social hierarchies (Steinberg, 2008). Pawlowski and colleagues (2008) showed that male adults are more prone to take risks compared to females. This behavior even increased if females were present, whereas female subjects did not make riskier decisions in the presence of males (Pawlowski et al., 2008). Regardless of age, male participants seem to have a higher risk-taking propensity than their female peers, which has been observed from childhood to adulthood (Van Leijenhorst et al., 2008).

Furthermore, a greater tendency for taking risks is reported among adolescents and young adults. Approximately until the age of 25, a generally unhealthier lifestyle (i.a. binge drinking, casual sex, engaging in violence, and especially more car accidents caused by risky driving or driving under the influence of alcohol) has been observed (Steinberg, 2008). An investigation revealed that risky decisions caused greater activity in the reward system (i.e., brain areas such as the ventral striatum and the orbitofrontal cortex) of adolescents compared to adults, especially in the presence of peers (Chein et al., 2012). Another study with male adolescents and young adults demonstrated a reduction of choice impulsivity and ventral striatum activation with increasing age (Christakou et al., 2011). The tendency to take more risks during adolescence could also be a consequence of a hypersensitivity to dopamine during that lifetime period (Galván, 2014). Accordingly, risky decisions occur probably more often, because of the potentially positive outcome, which causes a greater reward-related response that adolescents want to experience again and again.

Hormonal impact on risk-taking

The hormonal influence on reward-related behavior, which was reported in a previous paragraph, also refers to risk-taking. Previous studies examined the influence of single hormones on risk-taking, with testosterone of particular interest. In adults, riskier decision-making in gambling tasks were associated with higher salivary testosterone (Apicella et al., 2014; Stanton et al., 2011). A study investigating probabilistic decision-making in female adolescents observed increased risk-taking with higher salivary testosterone, whereas higher estradiol was related to decreased risk-taking (Op de Macks et al., 2016). However, a systematic summary of existing studies revealed a significantly positive correlation between risk-taking and testosterone as well as estradiol, respectively (Kurath & Mata, 2018).

As another steroid hormone involved in reward-related behavioral modulation, cortisol also appears to be important in risky decision-making. Administration studies demonstrated that cortisol and testosterone led to an increase in risk-taking in men, but not in women (Cueva et al., 2015; Kluen et al., 2017). Additionally, testosterone and cortisol may presumably interact in relation to risk-taking, but also status-seeking or bargaining performance (Mehta, Mor, et al., 2015; Mehta, Welker, et al., 2015; Van Den Bos et al., 2013). This has been outlined in detail in the "dual-hormone hypothesis"(Mehta & Prasad, 2015). Following the dual-hormone hypothesis, a low cortisol level with a simultaneous high testosterone concentration in adults is associated with risk-taking, but also a better performance in decision-making tasks (Mehta, Mor, et al., 2015; Mehta, Welker, et al., 2015). To date, results regarding these interactions are equivocal, and there are currently no results from endocrine studies with adolescents (Kurath & Mata, 2018).

Measurements and Design

Tasks

Response time adjustment task

Learning behavior was measured with two different computer tasks, presented with the NBS-Presentation software package (Neurobehavioral Systems Inc., Berkeley, CA). The first one was a response time adjustment task, which was established by Moustafa et al. (2008) and modified by Diekhof and colleagues (2015) (Diekhof, 2015; Kohne et al., 2021; Moustafa, Cohen, et al., 2008; Reimers et al., 2014). The so-called "clock task" is a sensitive measurement for dopamine availability in the context of reward learning. During the task, participants are required to stop a running clock arm at the most rewarding time. In the fast clock condition, the clock arm has to be stopped as soon as possible (see Figure 1 CHAPTER TWO). When confronted with the slow clock, the participant has to wait shortly before the clock arm completes a full rotation, which takes 5 s, to maximized reward outcome. Besides the fast and the slow clock condition a random clock condition serves as a baseline control. Thereby, different clock conditions are characterized by different colors. The participants were uninformed about the different clock types. In the task, the fast, slow, and random clocks are presented in 50 trials each, resulting in 150 pseudorandomized trials.

The ability to learn to stop the clock quickly for reward maximization is related to a better Go learning capacity, whereas the patience to wait and slow down for higher reward indicates better NoGo learning. Moustafa and colleagues (2008) conducted the task with patients with Parkinson's disease (Moustafa, Cohen, et al., 2008). Patients on dopaminergic medication and thus normalized dopamine were better at speeding up for reward maximization, whereas unmedicated, who were dopamine deprived, showed a better slowing ability for higher reward. These results suggest that the task is sensitive to dopaminergically-mediated reinforcement learning.

The working group around Diekhof used a modified version of the clock task and observed an association between estradiol and adjustment of response time. In two studies on the menstrual cycle, they observed that enhanced estradiol level presumably facilitated the ability to speed up for reward (Go learning), but simultaneously impaired the ability to slow down (NoGo learning) (Diekhof, 2015; Reimers et al., 2014). These findings have not yet been substantiated in studies with men or other age groups, including adolescents.

Probabilistic reward learning task

The second reinforcement learning task, which was conducted for this thesis, was a probabilistic social feedback task (Diekhof & Ratnayake, 2016; Frank et al., 2004). The goal is to choose the most frequently rewarded symbol. Participants are presented with different pairs of hiragana syllables and kanji symbols in the following named as "A", "B", "C", "D", "E", and "F". During the whole task, each symbol is probabilistically rewarded. A is rewarded 80% of the time, B 20%, C 70%, D 30%, E 60%, and F 40% (Figure 3). Pairs are shown until one of the two stimuli will be selected, but for a maximum of 1500 ms, followed by a feedback (male face or dot, 700 ms) and a blank screen of variable duration (400 - 1600 ms). The task consists of two sessions. In session I, the acquisition phase, the symbol pairs are fixed ("AB", "CD", and "EF"). Each pair will be presented 120 times resulting in 360 trials with a short break after 180 trials. The feedback is displayed in the middle of the two stimuli at the end of each trial. A smiling man represents positive feedback, whereas an angry-looking man represents negative feedback (Figure 3A). If no symbol is chosen in the given time of a maximum of 1500 ms a neutral-looking man appears. In session II, the transfer session, new combinations of the symbols are shown in pairs (e.g., "AD", "BE") in addition to the three fixed pairs of session I. After the response, a neutral, non-social feedback in form of a white dot appears signaling the registered keystroke (Figure 3B).

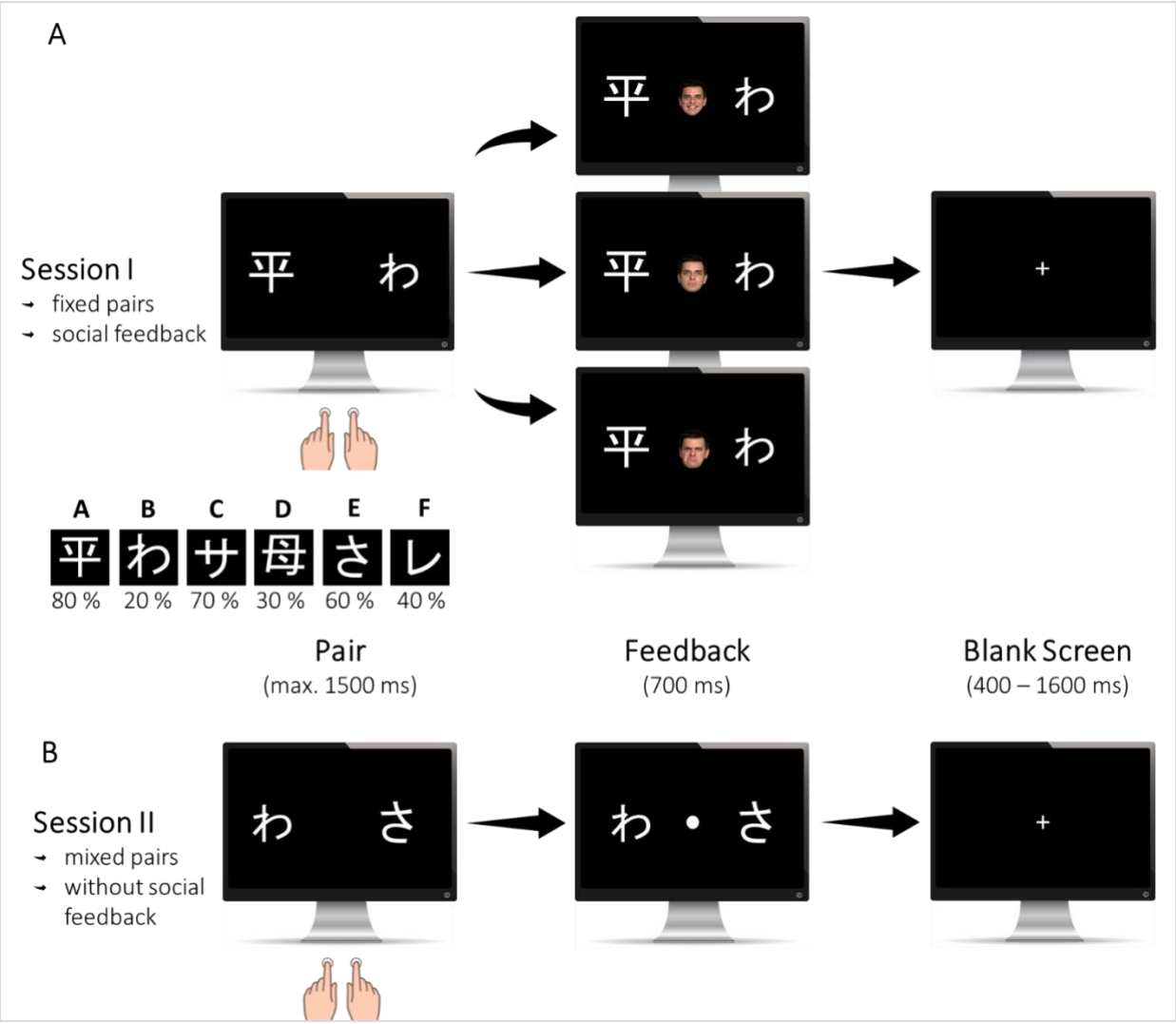

Figure 3: Probabilistic reward learning task

(A) In session I, the example trial starts with a fixed pair (pairs: "AB", "CD" or "EF") presented for a maximum of 1500 ms followed by a social feedback of 700 ms and a blank screen with a fixation point (400 - 1600 ms). During this session, subjects learn which stimuli more frequently lead to reward and punishment, respectively. (B) Following, in session II the pairs also consist of other stimulus combinations (e.g., "BE"; as shown here). Instead of a social feedback, a dot appears followed by the blank screen after which a new trial starts. (source: own illustration)

Whereas in the first session the participants learn which symbols are most rewarding, or which have the lowest reward probability, they are required to apply this knowledge in the second session. Here, especially new pairs that include the stimuli A, which has the highest reward probability of all symbols (80%), or B, for which reward is very unlikely (20%), allow conclusions on the learning type. A preference for symbol A in new pairs with A reflects individual reward learning capacity, while the successful avoidance of B in new pairs with B indicates avoidance learning ability.

Frank and colleagues conducted the task with Parkinson's disease patients and could observe that medicated patients exhibited a heightened preference to choose symbol A from new pairs, probably due to an enhanced dopamine availability and modulated via the Go pathway (Frank et al., 2004). The same, yet transiently unmedicated patients, and thus considerably reduced dopamine levels, on the contrary, were better at avoiding stimulus B in the new pairs, a behavior presumably modulated via the NoGo pathway. Studies from the working group of Diekhof also showed a connection between learning preference and the hormonal status of women in menstrual cycle studies (Diekhof et al., 2020; Diekhof & Ratnayake, 2016). While a high concentration of the dopamine-agonist estradiol in the late follicular phase was associated with a better reward learning capacity (i.e., increased choice of A in session II). A high progesterone level (mid-luteal phase) seemed to suppress dopaminergic transmission and supported NoGo learning via a better avoidance of the least rewarded stimulus B in session II.

The Balloon Analog Risk Task

Risky decision-making was analyzed with the Balloon Analog Risk Task, which seems to be a sensitive measurement for risk-taking in different age groups. Studies with adults observed an influence of the steroid hormones' testosterone and cortisol (Kessler et al., 2017; Mehta, Welker, et al., 2015). If this modulation can be transferred to other age groups has not been clarified so far.

During the task, participants have to pump up balloons via button clicks to earn money. With each click, the balloon gets more inflated and the probability to burst increases (see Fig. 1 CHAPTER FOUR). With each click a fixed amount of money is added to an imaginary bank account. If the participant decides to finish the inflation before the balloon bursts, the earned money will be added as yield. But if the balloon bursts before the money is saved the earnings for this trial are lost. There are three types of risk conditions: "risky", "moderately risky" and "not risky", associated with three different balloon colors and presented pseudo-randomly in 20 trials each. The participants have to maximize their outcome by finding out how many clicks they could risk until the balloon bursts. The more clicks or pumps and pops are recorded, the riskier is the behavior of a given participant.

Trier Social Stress Test (TSST)

The TSST originally consists of a verbal speech and arithmetical part and is a valid psychosocial stressor (Kirschbaum et al., 1993). In the version used in this thesis, participants introduce themselves, after a short preparation time, in front of an audience consisting of two research assistants. In the second part, the participants count backward from 1022 in steps of 13 without aids and as fast as possible. During the TSST, the audience remain neutral. They do not give confirmatory feedback (e.g. smiling or nodding their heads). The participants are told that the audience will take notes during the presentation and that the performance will be filmed and the behavior subsequently analyzed. Further, the audience strictly admonish the participant to continue with the speech if the time is not yet expired. In case adolescents end prematurely with the self-presentation without being able to continue it, the audience have prepared questions. A calculation error in the arithmetical part is directly addressed by the audience. Then, the participants have to start from the beginning.

Hormone analysis

Endogenous steroid hormone levels for this thesis were evaluated in saliva samples, which were independently collected from the participants after awakening in the morning and during the test. Samples were stored at -20°C until analysis. Saliva was thawed and centrifuged at RCF 604 x g for 5 minutes to separate it from mucin. Unbound estradiol, progesterone, and cortisol were analyzed with enzyme-linked immunosorbent assays, whereas free testosterone was determined with a luminescence immunoassay. Both assays are based on the competition principle of antigens and antibodies onto the wells. First, standard concentrations and a fixed amount of enzyme-labeled antigen (enzyme conjugate) were applied. In the following wells, the saliva sample with an unknown amount of antigen and enzyme conjugate competed for the binding sites of the assay, which was coated with antibodies. After incubation, washing, and the stop of the reaction, the measured color or luminescence intensity, was inversely proportional to the amount of antigen in the examined sample. Finally, results were determined with a standard curve. Saliva samples were assayed twice for more precise results. All steps of saliva preparation and analysis were done in our in-house laboratory. More detailed information on saliva collection, processing, and applied assay kits are included in the following chapters (see the Material and Methods paragraphs of the CHAPTERS ONE to FOUR).

Electroencephalography

Electroencephalography (EEG) is a neurophysiological method to record extracellular potential differences by electrical fields on the scalp which are caused mostly by synaptic electrical activity (Lopes da Silva, 2013). During synaptic activity of the pyramidal neurons of the cortex, ions flow across the membranes resulting in excitatory and inhibitory postsynaptic potentials. Both potentials contribute to measured EEG fields. Thereby, macroelectrodes dissipate a spatial summation of potentials resulting in a detectable voltage field (Lopes da Silva, 2013; Olejniczak, 2006). Even though EEG just delivers a macroscopic picture of neuronal activity, it provides a non-invasive measurement of neuronal electrical activity with a millisecond time resolution (Murakami & Okada, 2006).

Reinforcement learning is enabled through reward prediction error signals. These signals are presumably encoded inter alia by dopaminergic midbrain neurons (Arias-Carrián et al., 2010; Schultz & Dickinson, 2000). They occur if the expected feedback differs from the received feedback and thereby allows learning as well as an optimizing of behavior (Schultz & Dickinson, 2000). Previous EEG studies identified negative event-related potentials (ERP) after a participant committed an error. The feedback-related negativity (FRN), which represents a negative ERP, was observed after the participants received feedback and peaked between 200 and 300 ms after the feedback (Glienke et al., 2015). The FRN is presumably generated in the anterior cingulate cortex. Greater activation in this area is related to an increased FRN peak (Holroyd & Coles, 2002). The anterior cingulate cortex is connected to other affective, cognitive, and motor cortical areas like the dorsal prefrontal cortex. During the processing of conflicting inputs, the anterior cingulate cortex presumably has a monitoring function (Haber & Knutson, 2010).

In this thesis, the individual FRN amplitude was measured from frontocentral electrodes averaged across trials for each participant. Data acquisition was conducted with the BioSemi ActiveTwo System and recorded with 64 Ag/AgCl electrodes according to the international 10-20 system at 256 Hz. The head cap with electrodes was positioned in a standardized way oriented to the reference points of nasion and inion to ensure data comparability between measurements. The data was preprocessed with the FieldTrip toolbox implemented in Matlab (Oostenveld et al., 2011).

Aims of the thesis

By illuminating different aspects of reward-related behavior from adolescence into early adulthood, this thesis strived to gain an improved understanding of the dopamine-driven, developing reward processing during this lifetime period. At present, measurements of dopamine in the living human brain are just feasible with invasive methods. Therefore, behavioral tasks of reinforcement learning and risk-taking, endogenous hormone concentrations, and neurophysiological data were quantified and associated to indirectly investigate the dopaminergic aspects of reward processing.

Study I (CHAPTER ONE)

In the Study I, the impact of season and daytime on reinforcement learning was examined. Depending on how comprehensive a study design is constructed, studies are often conducted over a longer period. Therefore, it was an important aspect to clarify if potential seasonal or daytime-related differences in dopamine will be reflected in behavior. We employed the reward-related response time adjustment task to investigate a possible impact of dopamine fluctuations (see Response time adjustment task

Two groups with female and male young adults (Table 1) were tested twice in the morning and evening. One group was recruited and tested in the winter season the other one in the summer season. Based on previous animal and human findings, it was hypothesized that during enhanced dopamine activity in the summer season (compared to winter) and in the evening (compared to the morning) a better Go learning ability would be observed (see Seasonal and circadian fluctuations of dopamine).

Study II (CHAPTER TWO)

In a subsequent study, the reward-related response time adjustment task was conducted with adolescents (Table 1). The impact of endogenous sex hormones on reinforcement learning in an adolescent population has not been investigated yet. The main purpose of this study was to consider whether there is an effect of endogenous morning testosterone and estradiol on reward and punishment learning. An influence of estradiol and progesterone on Go/NoGo learning was detected in women with the same reinforcement learning task by the research group of Diekhof (Diekhof, 2015; Reimers et al., 2014) suggesting a modulating effect of these

hormones on dopamine availability in the reward system (see also Steroid hormones and reward processing). A similar effect of estradiol on adolescent Go-learning was assumed, especially for adolescent girls, because they exhibit a higher estradiol concentration compared to adolescent males. For testosterone, it was hypothesized that it might have a positive impact on Go-learning but it has to be mentioned that there is a lack of previous research. However, it was anticipated that any testosterone effect would be more pronounced in boys, due to larger endogenous concentrations.

Study III (CHAPTER THREE)

By combination of hormone analyses and a neurophysiological investigation, the impact of stress on reinforcement learning was considered in adults and adolescents (Table 1) in a third study (Study III). Whereas in the first two studies a response time task was used to assess reinforcement learning, now a decision-making task, the probabilistic reward learning task (see Probabilistic reward learning task), was applied to address the wider spectrum of reinforcement learning. Participants were randomly assigned to the stress or control group. Before the reinforcement learning task, the stress group had to complete the TSST (see Trier Social Stress Test), whereas the control group wrote a short introduction of themselves without having to present it to an audience and calculated a very easy multiplication task also in private. Both control tasks therefore did not elicit psychosocial stress.

For this study, morning progesterone, estradiol, cortisol, and testosterone were determined. During the actual test, participants had to collect three additional saliva samples, which were used to analyze the fluctuation of the physiological stress indicator cortisol. It was assumed that cortisol would increase significantly more in the stress compared to the control group and that the individual stress perception would also be greater in the stress group. Furthermore, the impact of sex hormones on reinforcement learning during stress was examined. For estradiol and progesterone supporting effects on Go and NoGo learning, respectively, were expected, like it had been observed before in adult women (Diekhof, 2015; Diekhof & Ratnayake, 2016; Reimers et al., 2014).

Moreover, it was presumed that the enhancing influence of stress on dopaminergic transmission would lead to better reward learning, while impairing avoidance learning capacity (see The impact of cortisol and stress). Previously, sex difference in the stress response of males and females have been observed (J. Goldstein et al., 2010). Therefore, it was hypothesized that

females will be more impaired by stress than males. Moreover, adolescence may be a vulnerable period regarding the impact of stress (Bale & Epperson, 2015). It was assumed that adolescents would be generally more susceptible to stress than adults. Finally, for the FRN on positive and negative feedback is was presumed that the peak would be larger in the stress group compared to the control group due to a greater activity of the anterior cingulate cortex (Wirz et al., 2017). Based on the findings by Cohen and colleagues (2010), striatal reward prediction errors peaked during adolescence, therefore a larger FRN was assumed for adolescents compared to adults (Cohen et al., 2010).

Study IV (CHAPTER FOUR)

In the last study, risk-taking as another aspect of reward-related behaviors that is also linked to dopamine was examined in male adolescents and young adults (Table 1). Based on previous findings regarding the impact of steroid hormones on male risk-taking (see Hormonal impact on risk-taking), the interaction between testosterone and cortisol was also considered and risk-taking was assess with the well-established Balloon Analog Risk Task (see Balloon Analog Risk Task). Behavioral analyses were enhanced by a computational model. It was hypothesized that the dual-hormone profile of high testosterone and low cortisol concentration promotes risk-taking in men but also in male adolescents.

Table 1 Number and age (mean ± SD) of participants of Study I to IV

Study I	men	women
n	37	40
age	18-25 years (mean ± SD: 21.97 ± 1.95)	18-27 years (21.75 ± 1.85)

Study II	boys	girls
n	37	52
age	11-18 years (14.84 ± 1.84)	11-18 years (14,67 ± 1.96)

Study III	men	boys	women	girls
n	31 (15 control)	25 (9 control)	31 (15 control)	22 (6 control)
age	20-26 years (23.13 ± 1.5)	10-16 years (13.16 ± 1.84)	20-29 years (23.16 ± 1.95)	11-16 years (13.82 ± 1.59)

Study IV	men	boys
n	60	28
age	20-34 years (26.14 ± 3.33)	12-18 years (15.21 ± 1.87)

CHAPTER ONE

Daytime and Season do not affect reinforcement learning capacity in a response time adjustment task

Sina Kohne, Luise Reimers, Malika Müller & Esther K. Diekhof

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Author contributions

The study was conceptualized by E.D. E.D. and L.R. designed the paradigm and experimental procedure. L.R. supervised the data collection. M.M. conducted the measurements and wrote on the first draft. The statistical analyses were performed by S.K. The published version of the manuscript was manly written by S.K.

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Date and Place

Signature of the supervisor
Jun. Prof. Dr. Esther K. Diekhof

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ORIGINAL ARTICLE

Daytime and season do not affect reinforcement learning capacity in a response time adjustment task

Sina Kohne, Luise Reimers, Malika Müller, and Esther K. Diekhof

Faculty of Mathematics, Informatics and Natural Sciences, Department of Biology, Institute of Zoology, Neuroendocrinology and Human Biology Unit, Universität Hamburg, Hamburg, Germany

ABSTRACT

Seasonal and circadian rhythms have a broad impact on physiological aspects, such as dopamine neurotransmission, and may be involved in the etiology of mood disorders. Considering this, studies on the influence of season and daytime on cognitive function are rare. The present study aimed to assess the impact of seasonal and diurnal effects on the ability to maximize reward outcomes by optimizing response times adaptively. For this purpose, a reward-based learning task that required an adaptation of response time to either a fast or a slow response was used. Eighty German participants (mean age ± SD = 21.86 ± 1.89 years, 41 women) were examined twice, in the morning and in the evening. Half of the participants were tested during the summer, while the other half performed the test in the winter. No impact of daytime, season or of the external factors photoperiodicity and temperature on reinforcement learning could be found. However, a generally slower response speed in the morning compared to the evening appeared. Previously conducted tasks could not display behavioral differences in both times of season and daytime, although neurophysiological findings suggest it.

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Introduction

The circadian clock system follows an approximately 24 hours-rhythm. It is regulated by zeitgeber, mainly photic signals (natural and artificial light), and possibly by other stimuli such as temperature, humidity or physical activity (Boivin & Boudreau 2014; Meyer et al. 2016). Controlled by clock genes located in the suprachiasmatic nucleus as the primary pacemaker and various other tissues, the circadian rhythm is sustained using intracellular feedback loops. Variations in light disposability based on the day–night rhythm are detected by retinal ganglion cells. These cells enable photic entrainment via the retinohypothalamic tract to the suprachiasmatic nucleus (Berson et al. 2002). The procedure allows indirect measurement of day length via photic signals managed by melatonin. This processing is essential for behavioral adaptions.

The daily dark–light cycle, which is additionally shaped by season, has a broad impact on behavioral and physiological aspects, that may also be involved in the etiology of mood disorders (Garbazza and Benedetti 2018). Nevertheless, investigations assessing seasonal and diurnal variations of cognitive function are scarce. Merikanto et al. (2012) demonstrated a seasonal difference in a vocabulary subtest of the WAIS-R (Wechsler Adult Intelligence Scale, Revised) (Wechsler 2014). Participants completed the task better in summer than early spring (Merikanto et al. 2012). Another study that created short-term exposures to warm or cold temperature in climatic chambers and varied light intensity in summer and winter observed a decrease in the accuracy in a simple response time task as well as an addition-subtraction task with increasing melatonin, which led to a worse performance with increasing signs of fatigue (Pääkkönen et al. 2008). In a similar experimental design, it was shown that mathematical performance in the addition-subtraction task was better in summer than in winter, whereas the accuracy on a repeated acquisition task was increased in winter. In the same study, season (winter or summer) did not interact with the short-term intervention and no general seasonal performance differences could be observed (Palinkas et al. 2005).

However, Brennen et al. (1999) tested the influence of season on cognitive performance with participants living in northern Norway (69°N). Instead of the expected worsening of performance during the winter, they observed enhanced performance compared to the summer seasons in some cognitive domains (e.g., word memory), while other domains were unaffected by

CONTACT Sina Kohne sina.korf@uni-hamburg.de Institute of Zoology, Neuroendocrinology and Human Biology Unit, University of Hamburg, Martin-Luther-King-Platz 3, Hamburg D-20146, Germany

season. Only verbal fluency became better in summer. In sum, most findings pointed to a slightly better cognitive performance during summer. But existing studies are barely comparable, because of the different cognitive aspects they investigated. Further no study considered seasonal variations in reward learning.

Regarding diurnal influences Byrne and Murray conducted several risk-taking tasks with men and could demonstrate a peak of risky decision making during the early afternoon compared to the morning and evening in men (Byrne and Murray 2017). In a probabilistic reward learning task Whitton et al. showed a diurnal pattern with the lowest performance in the middle of the day followed by an increase toward the afternoon (Whitton AE, Mehta M, Ironside ML, Murray G, Pizzagalli DA. 2018. Both studies addressed reward processing and speculated that fluctuations in dopamine, as the most important reward-related neurotransmitter, may have driven diurnal differences in reward-related behavior.

For this study, a modified version of a reward-related response time adjustment task was used. This task is a reinforcement learning task, in which subjects learn to optimize their responses in order to maximize reward (Diekhof 2015; Moustafa et al. 2008; Reimers et al. 2014). We assessed the individual differences in speeding as opposed to slowing responses for higher reward in two seasons (summer and winter) and at two times of the day (morning and evening). Based on the limited number of previous findings about behavioral differences we speculate that reward learning and therefore a better ability to speed up responses will probably be enhanced in the evening and during the summer season. In contrast, during the morning and the winter season participants are expected to be better at slowing down. We further consulted photoperiodicity and temperature as zeitgeber and assessed their influence on reinforcement learning capacity.

Materials & methods

Subjects

Thirty-nine healthy men and 41 women (undergraduate and graduate university students; mean age ± SD = 21.86 ± 1.89 years) were analyzed for this study. They had no history of psychiatric, neurological or hormonal disorders. Also subjects with chronic diseases, particularly those of the hormone system (e.g., diabetes, Hashimoto thyroiditis), were excluded. Further exclusion criteria were regular use of medication and excessive drug consumption. A special criterion for women to participate in this experiment was the use of hormonal contraceptives since studies using a comparable behavioral paradigm found changes in response time adjustments across the natural menstrual cycle (Diekhof 2015; Reimers et al. 2014). All subjects gave written informed consent and were paid for participation. This study conforms with international ethical standards for biological rhythm research as well as with *The Declaration of Helsinki of the World Medical Association* (Portaluppi et al. 2010) and was approved by the local ethics committee (Ärztekammer Hamburg).

Experimental procedure

Subjects were tested twice with a time lag of three to four days. Female subjects underwent both tests in their pill break. Forty subjects (20 women) were tested in summer (June 11 to September 30, 2015) and another 40 subjects (21 women) were tested in winter (November 30, 2015 to 31 March 31, 2016). Three subjects had to be excluded from further analyses due to a technical error that prevented the completion of the experiment. Every subject had to attend one test starting at 11:00 h. Subjects tested in summer, subsequently named as summer subjects, underwent the second test at 21:00 h, and subjects tested in winter, subsequently named as winter subjects, at 20:00 h. This change in evening test time was done because of the return to standard time and an earlier sunset during the winter months. The order of test times at both test days was counterbalanced between subjects. The procedure was the same on both days of testing and lasted one and a half hour. The experiment took place in a darkened room to minimize the influence of differences in daylight during the test. Subjects were given written instructions and a training version was conducted. If no questions remained, the experiment was commenced.

A main aspect of this study was to assess the influence of circadian rhythms on learning ability. To determine the individual preference of daytime psychological chronotype was evaluated with a specific questionnaire, the "Morningness-Eveningness-Questionnaire" (D-MEQ; Griefahn et al. 2001). Additionally, the Barratt Impulsiveness Scale-11 (BIS; Patton et al. 1995) was used to assess the trait impulsiveness of the respective subject before each testing. A comparison of the psychological chronotype and self-reported impulsiveness showed no differences between daytime (within subjects) and season (between subjects). Additionally, no influences on behavior were measured and further reported.

A demographic questionnaire was completed right at the beginning on the first day of testing. Subjects were asked for general demographical data (e.g., age and

nationality). Furthermore, a report on the amount of sleep during the night before the test and the time of awakening was requested. Additionally, external factors like photoperiodicity measured by the day length and temperature of the individual test day were protocolled as zeitgebers.

The clock task

Participants underwent a response time adjustment paradigm (adapted from the original version by Moustafa et al. 2008; see also Reimers et al. 2014; Diekhof 2015 for a similar test procedure), which will be called "the clock task" in the following. The task was originally developed to examine whether variations in central dopamine level modulate the capacity of speeding up versus slowing down for higher reward in patients with Parkinson's disease (Moustafa et al. 2008). The study revealed that Parkinson patients on dopaminergic medication were better at speeding up for reward maximization, while at the same time they displayed difficulties in slowing down to obtain higher rewards. In patients off medication, this behavioral pattern reversed, which led Moustafa et al. (2008) to infer that higher dopamine levels impair the ability to slow down for positive outcomes. This finding implied that variations of reward-related learning behavior in the clock task may reflect differences in central dopamine.

The clock task was displayed using the Presentation software package (Neurobehavioral Systems Inc., Berkeley, CA). The task consisted of 150 trials, in which subjects were presented with three different running clocks, with differently colored clock faces. On each trial the clock arm made a full rotation within five seconds. Subjects were instructed to explore the optimal time point for stopping the running clock arm via button press to win as many points as possible. It was thereby not possible to shorten the experiment by always stopping the clocks very fast, because the remaining time of the five seconds had to elapse before the next trial started. Reward value, which was associated with response time, varied between the three clocks. One clock required a fast response to maximize reward (fast clock condition). A second clock demanded the subject to slow down and be patient to yield maximum points (slow clock condition). Besides the fast and the slow clock, there was a third clock operating as baseline control (random clock condition). Reward value in this clock condition was randomly associated with response time by multiplying a random number with the difference between the maximum and minimum reward value and by adding the minimum reward value to this product. The exact score of a response made to the fast or the slow clock, respectively, was calculated with a cosine function and ranged between a minimum of 15 and a maximum of 60 points. Additionally, in all three clock conditions a random noise parameter with a range from −5 to +4 points was added to the calculated reward value to inhibit participants from linking a certain response time with a specific score (see also Reimers et al. 2014).

Feedback about the reward directly followed the response on every trial. The experiment consisted of three blocks of 50 trials with two short breaks in between. The different clock types were presented in a pseudorandomized order in each block. On the second test day the colors of the three clocks differed from the first day in order to prevent subjects from remembering the optimal response time related to each clock type.

Statistical analyses

The mean response time was calculated for each clock condition and subject and analyzed with IBM-SPSS27. A repeated-measures ANOVA with the within-subject factors "*daytime*" (early, late) and "*clock*" (fast, random, slow), as well as the between-subjects factor "*season*" (summer, winter) was conducted. Further, the same model was used in an ANCOVA with the covariates "*temperature*" and "*photoperiodicity,*" respectively, regarding the impact of daytime and seasonal variation on learning. Post-hoc Bonferroni-corrected *t*-tests and Pearson correlations were used. *P*-values smaller than .05, two-tailed, were considered significant. If the Mauchly-test was significant, Greenhouse-Geisser correction was applied when $\varepsilon < .75$, otherwise Huynh-Feldt correction was used.

Results

Seasonal and diurnal effects

The first ANOVA revealed a significant effect of *clock* ($F_{1.6,\ 117.1} = 445.27$, $p < .001$, $\eta^2_p = .86$) and *daytime* ($F_{1,\ 75} = 5.14$, $p = .026$, $\eta^2_p = .06$), though no effect of *season* could be observed. The three *clock* conditions were significantly different. As expected the participants adapted their response time to the different clocks fast < random < slow, which indicated successful learning ($p < .001$, Bonferroni corrected for 3 comparisons, *fast*: [mean ± sem] 1034.83 ± 46.27; *random*: 2092.87 ± 50.51; *slow*: 3660.78 ± 70.91; Table 1). A daytime comparison with the mean response time of all clocks in a paired *t*-test revealed a significantly slower response time in the morning compared to the evening ([mean ± sem] morning: 2293.77 ± 28.68, evening: 2231.88 ± 28.43, $t_{(df = 76)} = 2.26$, $p = .027$, $d_{Choen} = .26$, $n = 77$) (Figure 1).

Table 1. Comprehensive summary of post-hoc results.

	Morning vs. evening			Summer vs. winter			Summer vs. winter					
							Morning			Evening		
	$t_{(df = 76)}$	p	d_{Choen}	$t_{(df)}$	p	d_{Choen}	$t_{(df)}$	p	d_{Choen}	$t_{(df = 75)}$	p	d_{Choen}
Fast[*ab]	1.3	.592	.15	1.84_{67}	.213	.42	1.22_{65}	.569	.3	2	.148	.46
Random[*ac]	1.04	.91	.12	1.41_{71}	.49	.32	$.9_{75}$	1	.2	1.56	.37	.36
Slow[*bc]	.97	1	.11	$-.39_{75}$	1	−.09	$-.4_{75}$	1	−.09	−.28	1	−.07
All	2.26	.08	.26	1.7_{75}	.094	.39	1.02_{75}	.314	.23	1.97	.053	.45

*p-values Bonferroni corrected for 3 comparisons,
[a]t_{76}=−16.14, p<.001, d_{Choen}=−1.84; [b]t_{76}=−24.01, p<.001, d_{Choen}=−2.74; [c]t_{76}=−18,4, p<.001, d_{Choen}=−2.1

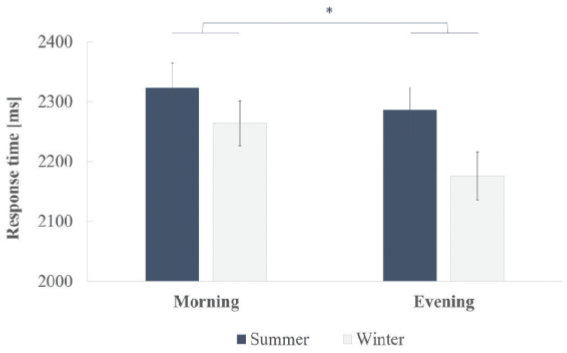

Figure 1. Independent of season, participants responded significantly slower in the morning compared to the evening ([mean ± sem] morning: 2293.77 ± 28.68, evening: 2231.88 ± 28.43, t76= 2.26, p = .027, dChoen= .26, n = 77).

Temperature effects

The second ANCOVA with the covariate *temperature* again showed the main effect of clock ($F_{1.6,\ 119.3}$ = 445.27, p < .001, η^2_p = .86), and two interactions with weak effect sizes for *clock* × *temperature* ($F_{1.6}$ = 3.49, p = .043, η^2_p = .05) and *clock* × *season* ($F_{1.6}$ = 4.39, p = .021, η^2_p = .06). Yet, post-hoc testing did not reveal a significant correlation between the temperature and individual clock conditions (all p-values > .19) (Figure 2, B), while the t-test also did not confirm significant differences between summer and winter (all p-values > .21, Bonferroni corrected).

Effect of photoperiodicity

Finally, in the last ANCOVA regarding the impact of *photoperiodicity* no significant effects emerged (Figure 2, A), except for a main effect of *clock* ($F_{1.6,\ 116.9}$ = 5.34, p = .01, η^2_p = .07).

Discussion

The study aimed to assess the impact of diurnal and seasonal effects on the ability to maximize reward outcomes across time in the sense of speeding up and slowing down for reward maximization. For this purpose, the reward-based clock task, that requires an adaptation of response time to either a fast or a slow clock, was applied. Additionally, we aimed to test whether the preference for speeding up or slowing down depends on external factors. We found an influence of daytime on the general response time in the clock task. This effect was independent of season and clock type, with participants exhibiting a slower response speed in the morning compared to the evening. However, we did not observe an influence of season, temperature or photoperiodicity on behavior.

Meyer et al. (2016) investigated seasonal influences on performance in a working memory and sustained attention task in healthy young adults and could not detect measurable behavioral effects, just as we could not find any relation between season and behavior. However, Meyer and colleagues could demonstrate neurophysiological changes with season. Working memory provoked a maximum response (inter alia in thalamus, prefrontal and frontopolar areas) around autumn and a minimum around spring. For the attention task, the greatest activity (inter alia in the thalamus, amygdala, frontal areas and hippocampus) was found around summer and it was reduced during the winter. Furthermore, neural activity in this task was closely related to photoperiodicity (Meyer et al. 2016). In addition to that, an impairment of the hippocampal long-term potentiation, learning and memory during simulated short days compared to long days was observed in male white-footed mice

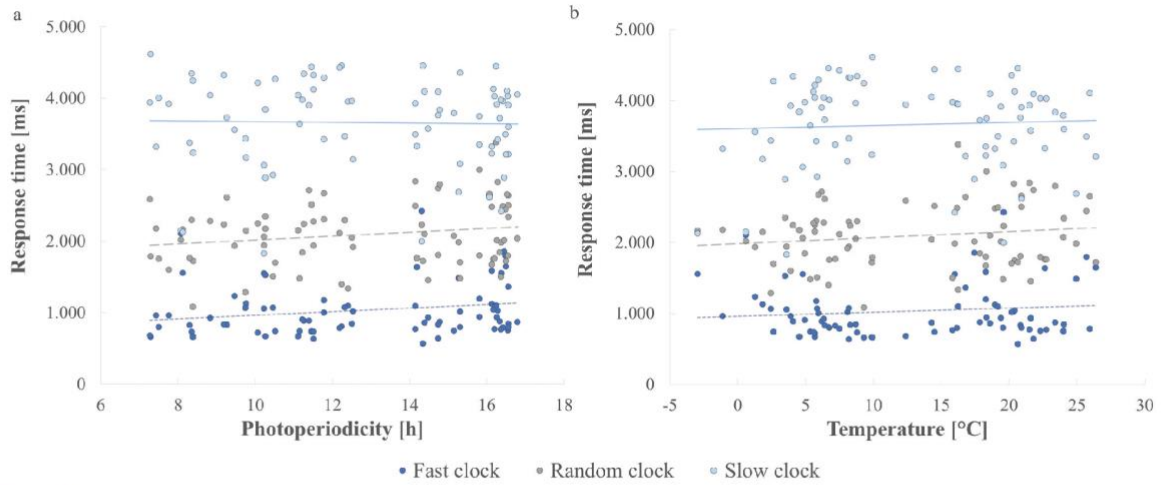

Figure 2. No correlations between the A. photoperiodicity (all p-values > .09, n = 77) and B. temperature (all p-values > .19, n = 77) emerged.

(Walton et al. 2011). This indicated that the impairment was mediated by photoperiod and the diminishing impact of melatonin on long-term potentiation and cognition (Walton et al. 2011). It may be assumed that season and probably photoperiod might have an influence on neurophysiological processing of various cognitive operations, which may not necessarily be measurable in behavior.

In a region of interest analysis, Hasler et al. (2014) reported greater striatal activity for monetary reward in the afternoon compared to the morning. The striatum plays a key role in reward processing and reinforcement learning (Balleine et al. 2007). Based on this finding, we expected a better reward learning ability during the evening compared to the morning, which could not be confirmed. Nevertheless, other reward-related studies demonstrated diurnal variations in risk taking in men as well as in a probabilistic reward task (Byrne and Murray 2017; Whitton AE, Mehta M, Ironside ML, Murray G, Pizzagalli DA. 2018.. However, differences in experimental design (e.g., the comparison between the middle of the day and the evening or morning) and the use of different behavioral tasks may explain divergent findings.

Previous reward-related investigations did not always point in the same direction. Masterson et al. (2016), who presented food-stimuli to their participants (i.e., a primary reward), found increased activation of the striatum in the morning compared to the afternoon. Whereas another study using a card game task could not observe greater reward-related striatal activity during the evening. In contrast, they found higher activation during the morning and evening compared to the early afternoon (Byrne et al. 2017). Thus, these previous studies show that increased striatal activation during reward processing could not be generally assigned to more efficient processing during a specific time of the day.

In a current review addressing the impact of circadian rhythms on human reward processing a modulation of human reward anticipation by daytime was assumed and evidence suggested that it might be even more pronounced during reward receipt (Byrne et al. 2019). Nevertheless, the limited number of studies (n = 15), of which two could not observe a circadian effect, and the heterogeneity of study designs precluded a definite inference on the nature of the circadian variation in reward processing capacity (Byrne et al. 2019).

A frequently cited explanation for any seasonal or diurnal variations in reward-related behavior are fluctuations in neurotransmitter levels that are sensitive to zeitgeber. In rodents a significant circadian fluctuation of dopamine could be observed in the striatum. Held under 12-hours-dark/ 12-hours-light conditions, the rats' dopamine level reached its maximum during the night (Castañeda et al. 2004). Comparable studies have demonstrated a similar circadian variation or even a seasonal rhythm of dopamine in rodents (Paulson and Robinson 1994, 1996; Rodriguez et al. 2013; Smith et al. 1992). But evidence for a possible circadian and seasonal rhythm of dopamine in the human striatum is scarce and mostly indirect. In humans, dopamine supposedly increases throughout the day (Castañeda et al. 2004; Matheson et al. 2015; Paulson and Robinson 1994; Rodriguez et al. 2013; Smith et al. 1992). The diurnal increase is thereby probably connected to the suprachiasmatic nucleus (Sleipness et al. 2007) and seems to be enhanced during fall and winter (Eisenberg et al. 2010; Hartikainen et al. 1991; Kaasinen et al. 2012;

Karson et al. 1984; Matheson et al. 2015; Praschak-Rieder et al. 2008). Nevertheless, previous data are mixed and partly inconsistent (Aumann et al. 2016; Brewerton et al. 2018b, 2018a; Kalbitzer et al. 2010; Tsai et al. 2011)

The present study just demonstrated a diurnal baseline difference in the general response speed, but no effect of diurnal or seasonal variations on reward learning behavior. Given this evidence, if response speed is an important parameter in a behavioral task, it thus seems to be sensible to check for circadian impacts or to test all participants during the same time of the day. Further studies are needed to gain additional insight into the significance of seasonal and diurnal rhythms in reward processing. For this purpose, not only behavioral but also neurophysiological and, if possible, endocrinological influences should be considered. Infact, endocrinological circadian fluctuations have been assumed (e.g. diurnal estradiol fluctuation: Rahman et al. 2019), which in turn could have an influence on reward processing (e.g. estradiol impact on reward processing: Diekhof 2018).

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CHAPTER TWO

Testosterone and estradiol affect adolescent reinforcement learning

Sina Kohne and Esther Diekhof

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Author contributions

The study was conceptualized by E.D and S.K. E.D. and S.K. designed the paradigm and experimental procedure. Data collection and hormonal analyses were done by S.K. and students support. Statistical analyses were performed by S.K. under supervision of E.D. The first draft of the paper was written by S.K. Both authors contributed to reviewing the manuscript. The authors would like to thank A.K. for her support in laboratory analyses.

Hamburg, 10.04.2022

Date and Place

Signature of the supervisor
Jun. Prof. Dr. Esther K. Diekhof

Testosterone and estradiol affect adolescent reinforcement learning

Sina Kohne and Esther K. Diekhof

Faculty of Mathematics, Informatics and Natural Sciences, Department of Biology, Institute of Animal Cell and Systems Biology, Neuroendocrinology and Human Biology Unit, Universität Hamburg, Hamburg, Germany

ABSTRACT

During adolescence, gonadal hormones influence brain maturation and behavior. The impact of 17β-estradiol and testosterone on reinforcement learning was previously investigated in adults, but studies with adolescents are rare. We tested 89 German male and female adolescents (mean age ± sd = 14.7 ± 1.9 years) to determine the extent 17β-estradiol and testosterone influenced reinforcement learning capacity in a response time adjustment task. Our data showed, that 17β-estradiol correlated with an enhanced ability to speed up responses for reward in both sexes, while the ability to wait for higher reward correlated with testosterone primary in males. This suggests that individual differences in reinforcement learning may be associated with variations in these hormones during adolescence, which may shift the balance between a more reward- and an avoidance-oriented learning style.

Subjects Developmental Biology, Neuroscience, Pediatrics, Psychiatry and Psychology
Keywords Adolescence, Learning, Reward, Estradiol, Testosterone

INTRODUCTION

Sex hormones have a great impact on adolescent (neuro-) physiological maturation. With the onset of puberty at 9 to 10 years in girls and 10 to 12 years in boys, respectively, sex hormone level increases rapidly (*Peper & Dahl, 2013*). Sex hormone levels are regulated *via* the reproductive hypothalamic-pituitary-gonadal axis initiated by the secretion of hypothalamic gonadotropin releasing hormone (GnRH). GnRH thereby stimulates the synthesis and secretion of luteinizing hormones and follicle stimulating hormones in the pituitary, which in turn contribute to the maturation of the gonads and sex hormone secretion (*Sisk & Foster, 2004*).

The rising sex hormone level during adolescence significantly contributes to pubertal development. With attainment of sexual maturity, sex hormones maintain reproductive function (*Sisk & Foster, 2004*). Neurophysiological investigations demonstrated a different impact of testosterone and 17β-estradiol (E_2) on brain maturation. Testosterone is related to an increase of global white and gray matter volume in male adolescents (*Peper et al., 2009*; *Peper et al., 2011*), whereas in female adolescents E_2 may be negatively associated with gray matter volume (*Peper et al., 2009*). Further, E_2 seems to predict white matter growth across the entire brain in both sexes (*Herting et al., 2014*). Moreover, neurophysiological developmental changes during adolescence could be better explained by hormonal and

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Corresponding author
Sina Kohne,
sina.korf@uni-hamburg.de

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Charles Okpala

Additional Information and Declarations can be found on page 15

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pubertal development (measured by the Pubertal Development Scale or Tanner Stages) than by chronological age (*Herting et al., 2014*; *Wierenga et al., 2018*).

Sex hormones are very important when it comes to behavior and cognitive function in animals and humans. Besides the impact of E_2 and testosterone on adolescent reward-related risk-taking (i.a. *Op De Macks et al., 2016*), an influence on reward-related learning and cognition has been assumed as well (*Diekhof, 2018*; *Hamson, Roes & Galea, 2016*). In adult women, E_2 may promote verbal memory and fluency (*Hamson, Roes & Galea, 2016*). In gonadectomized male and female rats, E_2 was found to improve learning and memory even after physiological or psychological stressors (*Hamson, Roes & Galea, 2016*; *Khaleghi et al., 2021*). Moreover, studies with castrated male rats suggested that learning may be improved by testosterone treatment (*Spritzer et al., 2011*). In healthy older men, a short-term testosterone administration improved cognitive performance significantly (*Cherrier et al., 2001*). Findings from children (6 to 9 years) further showed a relationship between moderate testosterone levels and an average intelligence (IQ between 70 and 130), whereas enhanced testosterone concentrations were related to high (IQ > 130), but also low intelligence (IQ < 70) (*Ostatníková et al., 2007*). Other studies also reported enhanced testosterone concentrations in children and young adolescents (6 to 13 years) with learning disabilities compared to peers without impairments (*Kirkpatrick et al., 1993*). Given this evidence, one may assume that during early adolescence balanced testosterone concentrations may be important for efficient cognitive processing.

One way for sex hormones to modulate aspects of reward processing and reinforcement learning is through the neurotransmitter dopamine. Both estradiol and testosterone can act as natural dopamine-agonists, which promote dopamine release and dopaminergic transmission through various physiological mechanisms (*Becker, 1990*; *Castner, Xiao & Becker, 1993*; *Pasqualini et al., 1995*; *Sinclair et al., 2014*). This is in so far important, since dopamine plays a crucial role in reinforcement learning and determines how proficient individuals learn from positive or negative action outcomes. It has been assumed that changes in dopamine following so called reward prediction errors possibly act *via* two anatomically distinct pathways in the mesocorticolimbic dopamine system (*Maia & Frank, 2011*). The activation of the *Go* pathway after the dopamine burst that follows unexpected reward entails in a repetition of the same action. In turn, activation of the *NoGo* pathway results from a dip in the tonic dopamine level, which facilitates learning from unexpected reward reduction, omission, or even punishment. This optimally promotes an adaption of action choice to maximize overall reward (*Frank, Seeberger & O'Reilly, 2004*).

A study using a response time (RT) adaption task, the so-called "clock task", demonstrated this relation between dopamine and reinforcement learning by showing that patients with Parkinson's disease, but pharmacologically normalized dopamine concentration, were better in the *Go learning* aspect of the task. These medicated patients thereby showed an enhanced ability to speed up for a reward (*i.e.*, better ability to acquire a higher reward through quickly responding after trial onset). In comparison, in an unmedicated state and thus with pathologically lowered dopamine, the same patients, demonstrated a better *NoGo learning* ability. This was indicated by an increased capacity to

slow down responding for reward maximization (*i.e.*, enhanced capacity to wait for higher reward) (*Moustafa et al., 2008*).

With the same task, Diekhof and colleagues characterized the impact of periodically fluctuating sex hormones in women on *Go* as opposed to *NoGo learning* ability. They compared the RT adaption during three different menstrual cycle phases of late luteal phase, luteal phase and early follicular phase. During the late follicular phase E_2 is high and progesterone still remains low. In the luteal phase progesterone nears its maximum (*Reimers, Büchel & Diekhof, 2014*), whereas in the early follicular phase E_2 and progesterone are at their nadir (*Diekhof, 2015*). *Reimers, Büchel & Diekhof (2014)* concluded that heightened E_2 during the late follicular phase impaired the ability to slow down for reward maximization (*NoGo learning* ability), as opposed to the ability to speed up for higher reward (*Go learning* capacity). *Diekhof (2015)* extended these findings by showing a positive correlation between E_2 and the ability to speed up for reward during the early follicular phase. This latter study indicated a better *Go vs. NoGo learning* ability during the early follicular phase and assumed that the boosting influence of the still increasing, yet intermediate E_2 on dopamine probably optimally promotes *Go learning* ability.

Regarding the impact of testosterone on reward processing and reinforcement learning, data from humans are currently sparse. Also, rodent studies provide inconsistent findings about the influence of testosterone on reward processing. It has been observed that testosterone administration enhanced tyrosine hydroxylase (the rate-limiting enzyme catalyzing dopamine synthesis) in the substantia nigra of gonadectomized adolescent male rats (*Purves-Tyson et al., 2012*). Yet, testosterone may reduce tyrosine hydroxylase in gonadally intact adolescent male rats in the caudate putamen (*Wood et al., 2013*). Further, testosterone administration in gonadectomized adolescent male rats enhances mRNA of the dopamine degrading enzymes catechol-O-methyltransferase and monoamine oxidase in the substantia nigra (*Purves-Tyson et al., 2012*). In contrast, testosterone led to a significant increase of dopamine in the nucleus accumbens and dorsal striatum of gonadally intact male rats. Finally, in humans testosterone has been found to enhance striatal activity in the context of reward processing, while it decreased activation of the striatum during punishment processing (*Morris et al., 2015*).

Previous studies with early adolescents and young adults could not find a relation between testosterone and performance in cognitive or reward-related tasks (*Halari et al., 2005*; *Ladouceur et al., 2019*; *White et al., 2020*). Therefore, no clear assumptions can be made regarding the influence of testosterone on *Go* and *NoGo learning*. However, in light of its physiological significance for dopaminergic processing, a positive influence on reward processing and *Go* learning may be assumed.

Current study

In the present study, we assessed response time adjustments and learning behavior in the context of reward maximization in an adolescent sample. The salivary E_2 and testosterone concentration was measured on the test day, which enabled us to examine the effect of the two sex hormones on *Go* and *NoGo* learning capacity. The adolescents performed an RT adjustment task, the so-called *clock task* (modified by *Diekhof, 2015*; created by

Moustafa et al., 2008). In line with findings from adult research, we predicted that *Go learning*, associated with a better capability to speed up responding to maximize reward, would be related to "a higher E_2 concentration" (*e.g.*, *Diekhof, 2015*; *Reimers, Büchel & Diekhof, 2014*). Studies reporting a behavioral influence of testosterone on reward-related processing and especially reward learning are scarce. Whether higher testosterone would positively influence *Go learning* as well, could not be unconditionally hypothesized. Therefore, we examined the relation of testosterone and reinforcement learning capacity with the same analysis that was used to consider the impact of E_2. Finally, we hypothesized that the effects of sex hormones on reinforcement learning would be different in female and male adolescents, mostly due to higher E_2 concentrations in females and enhanced testosterone in males.

MATERIALS & METHODS

Participants

In total, 106 healthy German adolescents, between 11 and 18 years old, participated in this study. All participants had no history of psychiatric or neurological disorders and assured no regular medication intake. Fifteen adolescents were excluded from the analysis, because they showed a random response pattern throughout the task, which suggested that the task instructions had not been properly understood or that the respective participant lacked the motivation to perform the task properly. Another two participants were excluded because of technical problems that left the task unfinished. In sum, the data of 89 adolescents (mean age $\pm$ SD $=$ 14.74 $\pm$ 1.9 years; 52 females) were analyzed.

Every participant had to sign a written declaration of informed consent before participation. In the case of minority, a legal guardian (parent) also had to sign a written declaration of informed consent before the testing. The adolescents were recruited in sports and other leisure clubs. The study protocol was approved by the local ethics committee of the Ärztekammer Hamburg (Ref: PV3948) and the study was conducted in accordance with "The Code of Ethics of the World Medical Association" (Declaration of Helsinki).

On the test day, participants were screened for depressive symptoms with the validated German Depression Inventory for Children and Adolescents (*Stiensmeier-Pelster et al., 2014*). Individual cognitive capacity was tested *via* the Digit-Span Test by measuring both forward and backward span from the German version of the Wechsler intelligence scale for childen (*Wechsler, 2014*) by counting the numbers that were correctly recalled. Self-reported trait impulsivity was examined with the German Version of the Barratt Impulsiveness Scale (BIS-11) for adolescents (*Hartmann, Rief & Hilbert, 2011*). Finally, every participant and the corresponding legal guardian filled out a translated version of the Pubertal Development Scale (PDS) (*Petersen et al., 1988*). We then calculated a mean of both scores and used it as an indicator of the degree of physical pubertal development of the given participant.

Experimental task

A modified version of the clock task (see *Diekhof, 2015*), that had been introduced by *Moustafa et al. (2008)* was used. In the task, three differently colored clock faces were

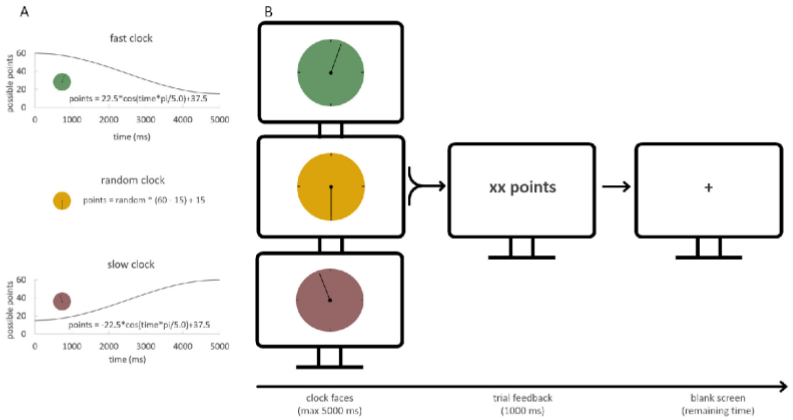

Figure 1 **Task design.** (A) Reward was calculated using cosine functions for the fast and slow clock. A time-independent function for the random clock was applied as control condition. (B) Clock faces were presented pseudo-randomly for 5,000 ms. Once a button press was made, the clock arm stopped, and immediate feedback was given. After that, a blank screen was shown for the remaining time that the clock arm would have needed to complete the 5,000 ms. Therefore, the blank screen ensures a constant time duration of a trial. A trial ended with the achieved points presented for 1,000 ms.
Full-size DOI: 10.7717/peerj.12653/fig-1

presented. A full rotation of the clock arm lasted 5 s. Each clock face was assigned to one of three conditions, namely the fast, the random, and the slow condition. Each of the three clock conditions was shown 50 times in three sessions of 50 trials each, resulting in a total of 150 trials. The sequence of clock faces was pseudo-randomized and balanced for trial-type transitions (see also *Diekhof, 2015* for further details on the clock task). The fast clock condition required a fast reaction once the clock arm started to move, in order to maximize reward outcome. The slow clock condition, in contrast, required the participant to postpone responding and slower RTs yielded higher reward. The random condition served as a control variant with no contingency between RT and reward outcome. It was used as an indicator of baseline response preference (see Fig. 1).

The participants had to adapt to the optimal response speed in each condition to maximize their overall reward. The exact reward value of each trial in the fast and slow condition was calculated with a cosine function, ranging between a minimum of 15 and a maximum of 60 points. The random reward value was calculated with the difference between minimum and maximum points of reward multiplied by a random number and added with the minimum reward value (see Fig. 1). In every condition, a random noise parameter (range between −5 to +4 points) was applied to the reward. This was done to disguise the relation of a specific reward outcome with a specific RT. Immediately after the response, the reward outcome was shown to the participant. For the remaining time of a full clock arm turn, a blank screen was shown. Thus, each trial had the same length. If the

participant did not respond within 5 s, no reward was presented, and the participant had to wait another 5 s before the next trial started.

Saliva collection and analyses

In the morning, three saliva samples were collected by the participant in 2 mL microcentrifuge tubes at home. Sample collection took place over the course of one hour (half-hourly samples) and started directly after awakening. The participants were allowed to drink water after the first sample up until 5 min before the second and third sample. They had to refrain from intake of food and beverages other than water during the sampling hour. Saliva samples were stored at −20 °C until further use. Before analysis, samples were thawed and centrifuged at room temperature at RCF 604 ×g (*i.e.,* 3,000 rpm in a common Eppendorf MiniSpin centrifuge) for 5 min to separate the saliva from mucins. For the E_2 analysis, a 17-β-Estradiol Saliva ELISA was used (Limit of Detection: 2.1 pg/mL), coated with anti-17-β-Estradiol antibody (monoclonal) with antibodies derived from donkey and sheep. For the testosterone analysis, a Testosterone Luminescence Immunoassay (both assays from Tecan/IBL International) was utilized (Limit of Detection: 1.8 pg/mL), coated with anti-mouse antibody. Intra-assay precision showed a mean CV of 8.8% (17-β-Estradiol Saliva ELISA) and 7.3% (Testosterone Luminescence Immunoassay). Inter-assay precision showed a mean CV of 11.8 (17-β-Estradiol Saliva ELISA) and 7.3% (Testosterone Luminescence Immunoassay).

The three morning samples were combined in an aliquot sample that consisted of an equal amount of saliva from every tube (100 μL). The analysis was done as described in the respective manual in our in-house laboratory. From the aliquot, two samples were assayed ($n = 2$). In addition, a high and a low control were analyzed. Subsequent behavioral analyses were done with standardized z-transformed values ($z_i = \frac{X_i - \bar{X}}{S_x}$) for each ELISA plate to standardize measurement inaccuracy of the plates.

Data preprocessing

For each subject, we calculated the mean RTs of each clock type. RTs under 200 ms were discarded, since they were very unlikely to reflect voluntary movements. In all, 125 trials (*mean* ± *sd*: 70 ± 72 ms) under 200 ms were excluded. We also calculated the mean RT of the initial 12 trials (called first block) and of the optimized last 12 trials (called last block) for each condition and participant (see *Diekhof, 2015*; *Kohne et al., 2021*; *Moustafa et al., 2008*; *Reimers, Büchel & Diekhof, 2014* for a similar procedure). At the beginning of the experiment (in the first block), the participant did not know which clock face was associated with faster or slower responses for higher reward. Hence, the participant had to try to achieve the optimal outcome *via* various reactions exploring the task structure. Conversely, at the end of the clock task (in the last block), the participant should have been well adapted and was expected to show optimal RTs that led to the highest reward outcome in relation to individual clock faces.

Apart from the mean RT for the three clock types, the actual learning preferences that reflected individual *Go* and *NoGo learning* ability, respectively, were calculated from the last block. They reflected the adaption to the optimal response speed to the slow and fast

clock, respectively, and allowed us to test the functional opponency of *Go versus NoGo learning*. For this, the RT of the slow and the fast clock were calculated in relation to the random clock, which provided information on the individual baseline response speed of a given participant. In order to calculate the optimized responses to the slow clock condition, we first subtracted the mean RT of the last 12 trials of the random clock condition from the mean slow clock RT of the last block. For standardization, this difference was then divided by the mean RT of the last 12 trials from the random clock. The resulting standardized relative RT reflects "optimized relative slowing". Correspondingly, the subtraction of the mean fast clock RT from the mean random RT and its division by the mean random RT was used as the "optimized relative speeding" value.

The individual learning-related change in RT for each clock condition was calculated by subtracting the RT of the first block from the RT of the last block.

Data analyses

The behavioral data were analyzed with IBM SPSS Statistics 25. First, we performed a repeated measures General Linear Model (GLM) with the factors "clock condition" (fast, random, slow), "block" (first, last), "sex" (female, male) and "age" to test for possible effects of these factors on the RT. In another two GLMs the factor "age" was replaced by either the covariate "pubertal development" (PDS-score) or the z-standardized sex hormone concentration of E_2 (zE_2) and testosterone (zT) (see Results section below). This was done to assess the impact of pubertal maturation and sex hormones level on reinforcement learning. Post hoc tests used paired and independent t-tests, which were Bonferroni-corrected for multiple testing. If Levene's test was significant, Welch's t-test instead of Student's t-test was used. The learning preference and effects of covariates were examined with a two-sided Pearson correlation. All effects and differences were considered as significant below a p-value of .05, two-tailed.

RESULTS

Learning preference

Studies with adults revealed a reverse capability for adaptive speeding *vs.* adaptive slowing of responses in the clock task (*Diekhof, 2015*; *Reimers, Büchel & Diekhof, 2014*). Our data demonstrate that this reverse relation in adjustment preferences to either the slow or the fast clock may also exist in adolescents. We found that optimized relative speeding and slowing were negatively correlated in both sexes (*females*: $r = -.48$, $p < .001$; *males*: $r = -.67$, $p < .001$) (see Fig. 2). Adolescents who were better adjusted to the last block of the slow clock had difficulties to speed up for reward. In turn, participants who responded faster to the fast clock in the last block were impaired in the ability to slow down for reward.

General group characteristics

The female and male adolescents did not differ in their age, impulsivity (BIS-11), and zE_2 concentration, which was determined by independent t-tests (see Table 1). The only significant differences between the two groups were a significantly higher zT level in males compared to females ($t_{43.95} = -6.82$, $p < .001$, $d = -1.56$) and a more advanced

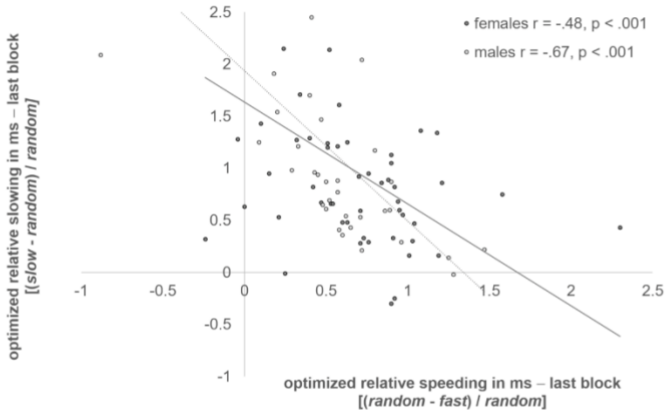

Figure 2 **Reverse relation of slowing and speeding.** Optimized relative speeding and slowing were negatively correlated in females, and males ($p < .001$).

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Table 1 **Group differences by sex.**

	Females		Males		Females *vs.* males			
	Mean ± *SD*	*n*	*Mean* ± *SD*	*n*	*t*	*p*	95% CI	
							lower	*upper*
Age (years)	14.67 ± 1.96	52	14.84 ± 1.83	37	−.4[c]	.689	−.98	−65
zE$_2$	.14 ± 1.11	49	−.2 ± .56	35	1.59[b]	.177	−.09	.77
E$_2$	5.89 ± 2.63 pg/mL	49	5.27 ± 2.08 pg/mL	35	.80	.425	−.64	1.49
zT	−.53 ± .42	52	.74 ± 1.07	37	−6.82[c]	**<.001**	−1.64	−.89
T	21.58 ± 14.1 pg/mL	52	89.61 ± 63.28 pg/mL	37	−6.43[d]	**<.001**	−89.45	−46.61
BIS-11	63 ± 6.45	52	63.83 ± 9.57	36	−.46[d]	.65	−4.5	2.83
PDS	3.03 ± .53	52	2.72 ± .56	37	2.67[a]	**.009**	.08	.55
DICA	11.58 ± 6.37	52	9.39 ± 3.94	36	1.99[e]	.05	−.01	4.39
Digit span forward	6.31 ± .9	52	6.31 ± .79	36	.01[f]	.991	−.37	.37
Digit span backward	4.85 ± 1.29	52	4.89 ± 1.13	37	−.17[a]	.862	−.57	.47

Notes.

[a] t_{87}.
[b] t_{82}.
[c] $t_{43.95}$.
[d] $t_{56.62}$.
[e] $t_{85.09}$.
[f] $t_{81.25}$
[g] $t_{38.55}$.

pubertal development of females compared to males ($mean_{PDSfemales}$ ± *se*: 3.03 ± .07; $mean_{PDSmales}$ ± *se*: 2.72 ± .09, $t_{87} = 2.67$, $p = .009$, $d = .57$).

Influence of age and sex on response time adjustments

In an initial step, we assessed the influence of "chronological age" and "sex" of the participant on learning performance. For this, we used a repeated measures GLM including the covariate "age", the between-subjects factor "sex" and the within-subject factors "clock condition" (fast, random, slow) and "block" (first, last). We solely found significant two-way interaction of "clock condition" x "block" ($F_{2,172} = 4.41$, $p = .014$, $\eta^2_p = .05$). This was reflected by a change in the RT from the initial to the optimized last block in the fast ($t_{88} = 11.08$, $p < .001$, $d = 1.17$, Bonferroni corrected for three comparisons) and in the slow condition ($t_{88} = -13.79$, $p < .001$, $d = -1.46$, Bonferroni corrected for three comparisons), but not in the random condition ($t_{88} = .14$, $p = 1$, $d = .02$, Bonferroni corrected for three comparisons) (Table 2).

Influence of pubertal development and sex on response time adjustments

The first GLM was repeated with the factor "pubertal development" (measured with the PDS) replacing the factor "age". A significant main effect of "clock condition" ($F_{2,172} = 7.28$ $p = .001$, $\eta^2_p = .08$), significant two-way interactions of "clock condition" x "pubertal development" ($F_2 = 3.4$, $p = .036$, $\eta^2_p = .04$) and "clock condition" x "sex" ($F_2 = 3.81$, $p = .024$, $\eta^2_p = .04$) emerged. Further, the interaction between "clock condition" and "block" remained significant ($F_{2,172} = 8.04$, $p < .001$, $\eta^2_p = .09$).

Post hoc t-tests showed a significant RT distinction between the three clock conditions (*fast vs. random*: $p < .001$, d $= -1.33$; *fast vs. slow*: $p < .001$, $d = -2.75$; *slow vs. random*: $p < .001$, $d = 1.61$, Bonferroni corrected for two comparisons) (see Table 2). Consequently, an adjustment to the varying clock conditions could be observed. Concerning the interaction between "clock condition" and "sex", a significant difference only arose in the slow clock condition. Males reacted significantly slower and thereby better to the slow clock in general than females did ($p = .048$, $d = -.43$) (see Table 2). The interaction of "pubertal development" and "clock condition" was reflected by a trend-wise positive correlation between the PDS and the RT of the random condition only ($r = .19$, $p = .068$) (see Table 2).

Influence of sex hormones and sex on response time adjustments

In a third GLM we investigated the modulatory influence of zE_2 and zT as a function of the participants' sex on RTs in the three clock conditions (fast, random, slow) and the two blocks (first, last). The main effect of "clock condition" ($F_{2,160} = 114.83$ $p < .001$, $\eta^2_p = .81$) and the interaction of "clock condition" and "block" ($F_{2,\ 160} = 7.28$ $p < .001$, $\eta^2_p = .59$) remained significant. Furthermore, an interaction of "block x clock condition x zE_2 concentration" ($F_2 = 4.9$, $p = .009$, $\eta^2_p = .06$) and a main effect of block ($F_{1,80} = 5.29$ $p = .024$, $\eta^2_p = .06$) and of "zT" ($F_1 = 5.28$ $p = .024$, $\eta^2_p = .06$) occurred.

The interaction of "block x clock condition x zE_2" was reflected by a negative correlation between zE_2 and the initial RT in the fast clock condition ($r = -.24$, $p = .03$) (see Fig. 3). In addition, we also examined the individual learning-related change in the RTs between first and last block, which demonstrated the adjustment from the initial to the optimized

Table 2 Comprehensive summary of RTs and post-hoc results.

		Mean RT ± SE		Females *vs.* males				Correlations of all participants					
						95% CI		*zT*		zE_2		*PDS*	
Block	*Clock*	*Females & males*	*Females*	*Males*	$t\ (df = 87)$	*p*	*lower*	*upper*	*r*	*p*	*r*	*p*	*r*	*p*
first & last	*FAST*	1264 ± 37ms[a], [b], ***	1302 ± 54 ms	1212 ± 293 ms	1.2	.234	−60 ms	241ms	−.12	.327	−.08	.497	−.12	.274
	RANDOM	2196 ± 65 ms[a], [c], ***	2157 ± 562 ms	2253 ± 695 ms	−.71	.477	−360 ms	170ms	**.23**	**.032****	−.02	.843	.19	.068*
	SLOW	3458 ± 67 ms[b], [c], ***	3346 ± 653 ms[d], **	3617 ± 593 ms[d], **	**−2**	**.048****	−539 ms	−2 ms	**.28**	**.009****	−.04	.731	.1	.359
	ALL CLOCKS	2307 ± 333 ms	2269 ± 311 ms	2360 ± 360 ms	−1.28	.203	−234 ms	50 ms	**.29**	**.007****	−.09	.412	.14	.185
first	*FAST*	1610 ± 58 ms[d], ***	1655 ± 571 ms	1547 ± 524 ms	.92	.363	−127 ms	345 ms	−.08	.441	**−.24**	**.03****	−.12	.249
	RANDOM	2203 ± 78 ms	2104 ± 685 ms	2343 ± 794 ms	−1.52	.132	−552 ms	73 ms	.18	.084*	.08	.469	.1	.354
	SLOW	2945 ± 87 ms[c], ***	2791 ± 807 ms**	3163 ± 803 ms**	**−2.15**	**.034****	−717 ms	−29 ms	**.3**	**.004****	−.06	.572	.1	.366
last	*FAST*	919 ± 36 ms[d]***	949 ± 382 ms	877 ± 275 ms	1.04	.3	−74 ms	219 ms	−.04	.718	.1	.383	−.04	.692
	RANDOM	2190 ± 80 ms	2211 ± 703 ms	2162 ± 834 ms	0.3	.765	−276 ms	374 ms	−.14	.195	−.12	.275	**.22**	**.038****
	SLOW	3972 ± 66 ms[c]***	3902 ± 694 ms	4071 ± 503 ms	−1.33	.188	−435 ms	97 ms	**.23**	**.03****	<.01	.991	.07	.491

Notes.

Equal letters mean significant paired t-Test results.

*** $p < .001$.

** $p < .05$.

* $p < .1$.

[a] $t_{88} = -12.51$; 95 CI −1080 ms, −784 ms.

[b] $t_{88} = -25.93$; 95 CI −2362 ms, 2026 ms.

[c] $t_{88} = 15.2$; 95 CI 1097 ms, 1427 ms.

[d] $t_{88} = -11.08$; 95CI −815 ms, −567 ms.

[e] $t_{88} = 13.79$, 95 CI 879 ms, 1175 ms

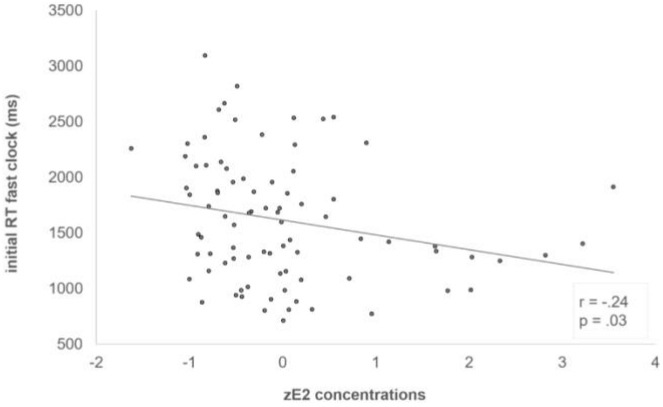

Figure 3 **Negative correlation between zE_2 and the initial fast clock.** Subjects who had higher zE_2 concentrations responded faster during the initial fast clock condition ($r = -.24, p = .03$).
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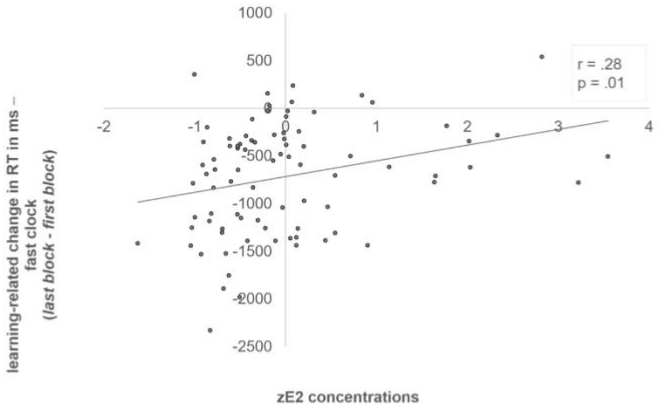

Figure 4 **Positive correlation between zE_2 and the learning-related change of the fast clock.** Subjects who had lower zE_2 concentrations showed a better adjustment from the initial to the optimized block in the fast clock condition, and became relatively faster in the last block, which resulted in as indicated by a more negative delta value of "last - first block" ($r = .28, p = .01$).
Full-size DOI: 10.7717/peerj.12653/fig-4

block (RT last block –RT first block). The learning-related change showed a significant positive correlation with zE_2 in the fast clock condition ($r = .28, p = .01$) (see Fig. 4). No correlation emerged with the slow ($r = .08, p = .497$) or random condition ($r = -.18, p = .096$).

A post-hoc comparison of the blocks evinced a slower response speed in the initial block compared to the last block ($t_{88} = -2.67, p = .009, d = -.28$). Further, zT was

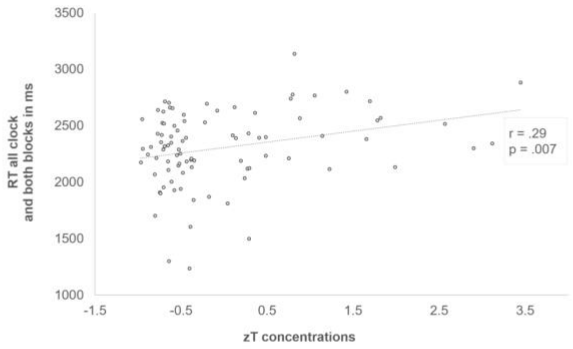

Figure 5 Positive correlation between zT and the response time of all clocks and both blocks. Subjects who had higher zT concentrations generally responded more slowly ($r = .29, p = .007$).
Full-size DOI: 10.7717/peerj.12653/fig-5

positively correlated with a slower RT independent of clock condition or block ($r = .29$, $p = .007$) (see Fig. 5). Since we found a significant difference in the zT of females and males, with higher concentrations in males (see Table 1), we additionally explored the zT effect separately for both sexes. From this, it became obvious that the correlation probably emerged from the male adolescents. Accordingly, the mean of both blocks across all clocks was positively correlated with zT in males ($r = .48$, $p = .002$), but not in females ($r = -.15$, $p = .298$). In males, a general slowing could also be observed with increasing zT in both blocks of all conditions (*first:* $r = .37$, $p = .025$, *last:* $r = .5$, $p = .002$) and especially in the slow ($r = .42$, $p = .01$) and the random ($r = .35$, $p = .032$), but not in the fast condition ($r = .09$, $p = .579$). Additionally, in the initial ($r = .35$, $p = .036$) and optimized block ($r = .44$, $p = .007$) of the slow clock positive correlations emerged. Again, these correlations could not be found in females.

DISCUSSION

This study examined the effects of adolescent E_2 and testosterone concentrations on RT adjustments in the clock task. Results indicate individual differences in the preference for either *Go* or *NoGo learning* (see Fig. 2) and an adaption to the different clock conditions from the initial to the optimized block. Both findings have already been demonstrated previously in studies with adults (*Kohne et al., 2021*; *Moustafa et al., 2008*; *Reimers, Büchel & Diekhof, 2014*). In addition, we also found that testosterone levels were significantly higher in males then females, while age, impulsivity and E_2 concentrations did not differ between the sexes. We also did not observe an age-dependent influence on the RT, and there was no association between individual pubertal development and *Go* or *NoGo learning*. Solely, a tendency towards a slower baseline response speed with increasing pubertal development emerged. Apart from that, we found a sex difference in the slow clock condition. Male adolescents responded significantly slower (better adapted) to the slow clock condition compared to females. E_2 and testosterone further appeared to modulate

learning ability in different ways. Whereas E_2 apparently enhanced initial *Go learning* (see Figs. 3 and 4), testosterone presumably promoted *NoGo learning* ability (see Fig. 5), yet primarily in males.

Similar to studies with adults, our data confirmed the detection of a preference for *Go* or *NoGo learning* ability with a presumable supporting effect of E_2 on *Go learning* (*Diekhof, 2018*; *Moustafa et al., 2008*; *Reimers, Büchel & Diekhof, 2014*). Furthermore, we observed a relation between habitual testosterone and the ability to slow down for reward, which was especially evident in male adolescents. The observed divergence of females and males in the learning capability related to the slow condition could probably be ascribed to a hormonal sex-difference. Hormonal testosterone concentrations differed significantly between females and males who showed enhanced concentrations. The varying increase of gonadal hormones during puberty could thus be one reason for the different RT adjustments in the slow clock. Accordingly, testosterone was associated with a slower RT and enhanced *NoGo learning* in adolescents. An explorative analysis showed that this result could be traced back to the male adolescents, most likely because testosterone is the main acting gonadal hormone during male pubertal development and by far more variable in pubertal males than in females. In line with adult research, E_2 seemed to stimulate the initially faster responses and therefore *Go learning* in all adolescents. We speculate that the effect of E_2 could have been mediated by its modulatory impact on dopaminergic transmission, which has been assumed for similar findings in adult women (see i.a. *Diekhof, 2015*; *Reimers, Büchel & Diekhof, 2014*). Estrogen receptors can be found in the brain of both sexes via which E_2 presumably has modulating effects on neurotransmission and plasticity (*Gillies & McArthur, 2010*).

The correlation between *Go learning* and E_2 occurred exclusively in the initial block during which participants were still naïve regarding the temporal reward associations of the different clocks. This might indicate that E_2 has only a subtle effect on behavioral responding in the clock task. Once the RT had been optimized in later phases of the task, this correlation was no longer behaviorally measurable (see also *Reimers, Büchel & Diekhof, 2014*).

Alternatively, E_2 may also support learning through a promotion of signal transduction. E_2 administration in young and aged ovariectomized rhesus monkeys led to an increase in spine density in the dorsolateral prefrontal cortex (*Hao et al., 2003*). An increased spine density on pyramidal neurons is connected to an enhanced number of excitatory synapses per neuron which in turn might improve learning performance in general (*Mahmmoud et al., 2015*). Moreover, in ovariectomized rats E_2 administration provoked cell proliferation and an increase of dendritic spine density in the hippocampus (*Adams et al., 2002*; *Tanapat, NB & Gould, 2005*). In a previous study, Davidow and colleagues demonstrated the positive impact of hippocampal activity and its connectivity to the striatum on reinforcement learning in adolescents (*Davidow et al., 2016*). Therefore, the potentiating influence of E_2 on the hippocampus may improve reward learning as well. Besides E_2, androgens also positively affect prefrontal and hippocampal processing, but rat studies indicate a greater impact of androgens in males (*Hamson, Roes & Galea, 2016*).

Similar to E_2, testosterone can modulate dopaminergic transmission and may also impact transmission in other neurotransmitter systems (*De Souza Silva et al., 2009*; *Sinclair et al., 2014*). The enhancing effect of testosterone on slowing ability may additionally be explained through an interaction of testosterone and serotoninergic processing in males. In male rats, testosterone administration leads to an increase of cerebral serotonin and its metabolites (*De Souza Silva et al., 2009*; *Thiblin et al., 1999*). Moreover, a positive correlation between plasma testosterone and serotonin receptor 4 level emerged, leading to the suggestion that higher testosterone is accompanied by a higher cerebral serotonin tonus (*Perfalk et al., 2017*). Therapeutic approaches include selective serotonin reuptake inhibitors that increase synaptic serotonin levels and modulate neuroplasticity (*Kraus et al., 2017*). For learning and memory formation synaptic plasticity is exceedingly important. Serotoninergic impact on human behavior and neurophysiological processes is commonly investigated through a depletion of the serotonin precursor tryptophan. Studies with healthy humans using tryptophan depletion demonstrate a slowing of responses by pharmacologically increased serotonin (*e.g.*, (*Murphy et al., 2002*)). We observed a better slowing ability with habitually increased testosterone, which might indicate that this could have been an indirect effect of testosterone on serotoninergic transmission. This would also be in line with other studies, that found that the effect of behavioral slowing in punishment contexts, especially under high incentive motivation, disappeared, if serotonin was pharmacologically depressed (*e.g.*, *Crockett et al., 2012*). Lowered serotonin concentrations after depletion have further been associated with decreased neural sensitivity to punishment (*Helmbold, Zvyagintsev & Dahmen, 2015*). Hence, enhanced testosterone concentration might have driven *NoGo learning* and enabled a better slowing down for reward, through its interaction with the serotoninergic system.

Just as a recent study, we could not observe a relation between reward or punishment sensitivity and the pubertal stage (*Chahal et al., 2021*). A generally lowered response speed in further developed adolescents could be a consequence of reduced impulsivity, which may be an indicator of neurophysiological and cognitive maturation. Similar to others, we did not find an association with chronological age (*Wierenga et al., 2018*). Our results thus support the assumption that pubertal development is a better indicator regarding cognitive performance than chronological age.

To date, a non-invasive direct measurement of neurotransmitter processes like dopamine binding or synthesis in the adolescent human brain is not feasible. We used non-invasive measurements to determine steroid hormone concentrations and assessed the individual learning ability for *Go* and *NoGo learning*. By combining both parameters, we tried to apply them as indirect indicators of dopaminergic transmission. Besides E_2 and testosterone other steroid hormones are presumably attractive for future studies. For instance, the influence of progesterone as a counterpart to E_2 on dopaminergic action may be of increased future interest. Whereas E_2 is assumed to have an agonistic effect on dopaminergic transmission, progesterone supposedly reduces E_2 receptor density (*Selcer & Leavitt, 1988*) and apparently upregulates monoamine oxidase when it is administered together with E_2, which mimics the luteal phase of a natural menstrual cycle (*Luine & Hearns, 1990*; *Luine & Rhodes, 1983*). Additionally, progesterone enhances gamma-aminobutyric acid induced inhibition of

dopaminergic neurons (*Majewska et al., 1986*). Thus, an antagonistic and reducing effect of progesterone on dopaminergic transmission has been suggested (*Diekhof, 2018*). In future studies, the tracking of the developing menstrual cycle of the female adolescents could probably contribute to a better interpretation of the opposite effects of E_2 and progesterone.

Finally, genetic predisposition as such has already been observed to affect reward sensitivity (*Richards et al., 2016*), and may further interact with steroid hormone level as demonstrated previously (*Jakob et al., 2018*; *Veselic et al., 2021*). In addition to previous findings on receptor and transporter polymorphisms of dopamine, serotonin and sex hormones, future studies could examine genetic interactions *via* genome-wide associations.

CONCLUSION

Sex hormones modulate neurophysiological processes and behavior in the context of reward processing in both adult animals and humans. However, evidence from adolescent populations is sparse. The present study assessed the impact of E_2 and testosterone on adolescents' reinforcement learning. Similar to female adults (*e.g.*, *Diekhof, 2015*), E_2 promoted initial *Go learning* in both sexes in our adolescent sample. Testosterone, in turn, enhanced *NoGo learning* in males. It could be speculated that individual differences in reinforcement learning are associated with variations in these hormones during adolescence, which shift the balance between a reward and avoidance-related learning style.

Future investigations should consider further steroid hormones (*e.g.*, cortisol, progesterone) and neurophysiological processing to specify the impact of hormonal differences on the dopaminergic mechanisms of reinforcement learning.

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Sina Kohne conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Esther K. Diekhof conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Human Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

This study was approved by the local ethics committee of the Ärztekammer Hamburg (Ref: PV3948).

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the Supplementary File.

Supplemental Information

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CHAPTER THREE

A pilot EEG-study about the impact of psychosocial stress on reinforcement learning and the influence of sex hormones during adolescence and young adulthood.

Sina Kohne, Julian Keil and Esther Diekhof

Author contributions

The study was conceptualized by E.D and S.K. S.K. designed the paradigm and experimental procedure under supervision of E.D. Data was collected by S.K. and students support. S.K. conducted the hormonal analysis. S.K. and J.K. programmed the EEG analysis batches. Statistical analyses were performed by S.K. and E.D. The first draft of the paper was written by S.K. All authors contributed to reviewing the manuscript. The authors would like to thank A.K. for her support in laboratory analyses.

Hamburg, 10.04.2022

Date and Place

Signature of the supervisor
Jun. Prof. Dr. Esther K. Diekhof

Abstract

Stress enhances cortisol release and may affect reward processing via dopaminergic processes. This EEG-study investigated the impact of psychosocial stress (initiated by the Trier Social Stress Test; TSST) and endogenous steroid hormones (estradiol, progesterone, testosterone and cortisol) on event-related potentials (ERPs) during reinforcement learning in adolescents (13.5 ± 1.7 years) and young adults (23.2 ± 1.7 years). For this purpose, a probabilistic feedback learning task was used, which followed the TSST or a control task. The data showed that stressed participants had a more pronounced increase in cortisol than control subjects. Moreover, with increasing cortisol during the TSST a greater feedback-related negativity (FRN) to negative feedback was detectable. Adults showed better reward learning than adolescents. In comparison to males, females demonstrated a worse transfer performance and enhances vulnerability to the stressor. An explorative analysis of basal hormones concentrations evinced a supportive effect of high estradiol on reward learning, whereas high testosterone levels diminished positive and negative feedback sensitivity. No relationship between baseline hormone level and ERPs was measurable. In sum, no impact of psychosocial stress on probabilistic feedback learning was observed. Due to the significant influence of stress on the development of reward dysfunctions and connected mental disorders, it would be vital to expand this investigation by including children and older adults, as well as other brain imaging techniques like fMRI.

Introduction

During stress, glucocorticoids trigger the stress reaction in humans. The secretion of the human major stress hormone cortisol is regulated by the hypothalamic-pituitary-adrenal axis via the release of the corticotropin-releasing hormone as well as arginine vasopressin from the paraventricular nucleus of the hypothalamus and subsequently the adrenocorticotropic hormone in the anterior pituitary (Sinclair et al., 2014). Due to the expression of gonadal and adrenal steroid receptors in the paraventricular nucleus, sex hormones and the hypothalamic-pituitary-adrenal axis are thought to interact with each other (Handa & Weiser, 2014). Sex hormones showed effects on stress-related cortisol release, although the actual mechanisms by which sex hormones may act within the hypothalamic-pituitary-adrenal axis are not fully understood yet (Handa & Weiser, 2014).

In the domain of social stress, the Trier Social Stress Test (TSST) is an established intervention to provoke psychosocial stress by combining a verbal speech and arithmetical part (Kirschbaum et al., 1993). Two previous studies, which used the TSST, showed a positive impact of sex hormones in a paired associate test (Maki et al., 2015) and dampening effects on hypothalamic-pituitary-adrenal axis response (Stephens et al., 2016). Another study conducted the TSST with men and demonstrated that administered progesterone diminished psychological effects of stress like alertness, arousal, and negative mood (Childs et al., 2010). Moreover, the neuroprotective effect of 17β-estradiol (E_2) on hippocampal synapses from corticosterone-induced suppressed transmission was documented in male Wistar rats (Ooishi et al., 2012).

Differences in the neurological stress response have also been observed when comparing women and men, which has been assigned to different sex hormone concentrations. Goldstein and colleagues (2010) compared the stress response of men with that of women during the early follicular phase (characterized by low E_2 and progesterone levels) and women in the late follicular phase (characterized by high E_2, slightly increasing progesterone levels) by using visual stimuli. They found increased stressor-related activity in several brain regions (hypothalamus, left amygdala, or anterior cingulate gyrus) in men compared to women in general, whereby the male response was more similar to women in the early follicular phase (J. Goldstein et al., 2010). Moreover, between the luteal and follicular phase, Kirschbaum and Kudielka (2005) observed significantly different salivary cortisol concentrations (Kudielka & Kirschbaum, 2005).

From adolescence to adulthood, the vulnerability of women for affective disorders increases significantly when compared to men. Yet, barely any sex differences can be found between girls and boys before adolescence. For these reasons, it has been proposed that adolescence may be a time window of increased mental vulnerability for external influences such as stress (Bale & Epperson, 2015). With the onset of puberty, the risk for the development of psychiatric disorders increases markedly, which suggests a link between sexual and brain maturation (Paus et al., 2008). And indeed, both studies with humans and rodents demonstrated that stress could negatively affect the maturation of the prefrontal cortex, which resulted in a decreased gray matter volume and synaptic density (Andersen et al., 2008; Leussis et al., 2008). Furthermore, steroid hormone levels affect brain reorganization differently in males and females. For example, an investigation of cortical thickness of children and adolescents demonstrated a negative association between testosterone and cortical thickness in males' left hemisphere but a positive association in females' right hemisphere (Nguyen et al., 2013; see also Peper et al., 2011). It is also assumed that sex hormones affect stress responsivity inter alia via receptors at

the paraventricular nucleus in the hypothalamus and thereby modulate corticotropin-releasing hormone synthesizing neurons (Bale & Epperson, 2015).

Besides stress, this study focuses on reinforcement learning. According to theory, after (unexpected) receipt of positive feedback, a burst of the reward-related neurotransmitter dopamine occurs, while after a negative feedback or a reward omission, a dip in dopamine can be observed (Maia & Frank, 2011). Learning thereby occurs through either the reward of an action, which should enforce the previously chosen action or negative feedback /reward omission following an action, which should make it less likely in the future. In their seminal study, Frank and colleagues demonstrated this relation between dopamine and reinforcement learning in medicated (normalized dopamine level) and unmedicated (diminished dopamine level) patients with Parkinson's disease (Frank et al., 2004). Frank and colleagues substantiate the theory that enhanced dopamine, like it was observed in medicated patients, supports reward learning, while a reduction in dopamine as in unmedicated patients may promote learning from negative feedback/ reward omission. The stress intervention may also influence cognition and learning ability through its action on dopamine. A stress-induced increase of dopamine, which was probably caused by the concurrent increase of cortisol, was found in both humans and animals (Oswald et al., 2005; Saal, Dong, Bonci, & Malenka, 2003; but see also Montoya et al., 2014). The behavioral investigation of reinforcement learning under stress revealed significant impairment of avoidance learning and a better reward learning ability (Lighthall et al., 2013; Mather & Lighthall, 2012; Petzold et al., 2010).

The dopaminergic contribution to reinforcement learning relies on corticostriato-thalamocortical loops which involve the striatum, the globus pallidus (internal/external), the thalamus, and the prefrontal cortex (Frank, 2005). Brain maturation during adolescence also affects dopaminergic projections to the prefrontal cortex. Whereas dopaminergic projections to limbic regions are developed early in life, and partly already during the infant period, dopaminergic fibers to the prefrontal cortex and the prefrontal cortex itself maturate until early adulthood (Walker et al., 2017). The contrasting theories of a hyposensitivity (J. M. Bjork et al., 2004; James M. Bjork et al., 2010) as opposed to a hypersensitivity for reward (Galván, 2014) during adolescences strive to explain observed differences in reward processing between adults and adolescents. While the majority of studies supported the theory of an enhanced sensitivity for reward during adolescents (see Galván, 2014), inconsistent findings allow the assumption that none of the theories can be asserted sweepingly.

Steroid hormones do not just modulate the stress response, but may also impact reinforcement learning. In particular, E_2 showed a supporting effect on reward learning, while progesterone was found to enhance negative feedback sensitivity (Diekhof, 2018; Diekhof et al., 2020; Diekhof & Ratnayake, 2016). Animal studies confirmed these partly opposing effects of E_2 and progesterone on the dopaminergic system (Dluzen & Ramirez, 1984; E. Yoest et al., 2014). The former hormone thereby acted as a dopamine agonist, while the latter appeared to inhibit dopaminergic transmission, which would also explain their opponent effect on reinforcement learning (for further information see: Diekhof, 2018).

The feedback-related negativity (FRN) in medial-frontal scalp regions, which occurs 200-300 ms after receipt of informative feedback, is a non-invasive indicator of positive feedback and negative feedback sensitivity that is modulated by central dopamine (Glienke et al., 2015). Signals from the dopaminergic reward system seem to be crucially involved in the expression of the FRN in the anterior cingulate cortex. Thereby, the FRN trains the anterior cingulate cortex and shapes future decision-making (Holroyd & Coles, 2002). Wirz and colleagues conducted a probabilistic classification task after the TSST or a non-stress control condition (Wirz et al., 2017). They could not find a correlation between the FRN and learning in general, but observed a larger FRN to negative feedback in the stress compared to the control group (Wirz et al., 2017). Wirz and colleagues suggested that the stronger striatal activity during stress was responsible for the increased FRN. It could be hypothesized that higher dopamine availability due to enhanced hypothalamic-pituitary-adrenal axis function during stress could furthermore facilitate reward learning, which would be additionally expressed by a greater FRN. To date, there has been no investigation of the FRN during reinforcement learning in stressed adolescents. However, because of a still maturating brain during adolescence, and especially of the still developing prefrontal cortex, one may assume altered reward signaling in stressed adolescents, compared to fully matured adults. Also, an assumed hypersensitive dopaminergic reward system during adolescence could strengthen this effect (Galván, 2014). Moreover because of the assumed interaction between sex hormones and the hypothalamic-pituitary axis (Handa & Weiser, 2014) as well as reinforcement learning (Diekhof, 2018; Diekhof et al., 2020; Diekhof & Ratnayake, 2016) a hormonal impact on neuronal activity is presumed.

In this between-subjects study, we used the TSST as a reliable social stressor and contrasted male and female physiological stress reaction and task performance after the TSST in adolescents and adults to examine sex and maturational differences in the stress response. However, sex differences in reward learning during stressful situations have not been reported

so far, while differences in the stress response of men and women have been repeatedly observed (J. Goldstein et al., 2010; Hollanders et al., 2017; Uy & Galván, 2017). We assumed that stress will reduce negative feedback learning ability and may also increase reward sensitivity, presumably caused by an enhanced dopamine availability (Frank et al., 2004; Petzold et al., 2010).

We measured the FRN to positive and negative feedback to investigate the impact of social stress and age on reinforcement learning in adults and adolescents. Following the social stress or a control intervention, participants underwent EEG (electroencephalography) while performing a probabilistic feedback learning task with probabilistic positive or negative social feedback (happy or angry face).

Furthermore, because of a previously reported impact of steroid hormones on learning processes and the stress response (Diekhof, 2018; Diekhof et al., 2020; Diekhof & Ratnayake, 2016; Kudielka & Kirschbaum, 2005; Maki et al., 2015; Stephens et al., 2016), the impact of unbound endogenous steroid hormones (cortisol, E2, progesterone, and testosterone) on the FRN was assessed as well. Also, this interaction has never been addressed in a study which compared adolescents and adults before. Because of an elevated dopamine firing during stress, we expected a greater FRN to negative feedback in the stress relative to the control group. For cortisol, an promoting effect on the amplitude of the FRN was hypothesized (Paul et al., 2019). Because of missing research about the influence of sex hormones on the FRN, which indicative represents dopaminergic processes, we assume an impact of sex hormones on positive feedback and negative feedback sensitivity. Therefore, high E_2 levels probably interfere with negative feedback learning but presumably promote reward learning and thereby may affect the FRN to positive feedback, whereas enhanced progesterone concentrations presumably supply negative feedback sensitivity measurable in a greater FRN to negative feedback (e.g. Diekhof, 2018).

Material & Methods

Participants

109 healthy participants contributed to this between-subject study with 62 adults (mean ± SD: 23.2 ± 1.7 years; 31 women) and 47 adolescents (13.5 ± 1.7 years, 23 girls). Age and intervention groups were matched with validated German versions of the “Digit-Span” test (a part of the Wechsler intelligence scale, 2014) and an age-matched “Depression Inventory” (adults: *Beck Depression Inventory*, Beck et al., 1996; adolescents: *Depression Inventory for Children and*

Adolescence, Stiensmeier-Pelster et al., 2014). Individual stress perception of the stress group and a self-rated performance evaluation were assessed in all participants at the end of the test session.

Subjects were randomly assigned either to a control or stress group, whereby we tried to balance age and sex-proportion between the stress and control group. Unfortunately, the coronavirus pandemic led to an interruption of the test, which could not be continued until to date. This led to an insufficient size of the girls' control group.

Before participation, every participant, and in the case of minority a legal guardian (parent), had to sign a written declaration of informed consent. Upon completion of all tests, participants were comprehensively enlightened about the grouping during the testing and their assignment. The local ethics committee of the Medical Council of Hamburg (Ärztekammer Hamburg, Germany) approved this study. Participants were reimbursed for their participation.

Study design

During the test, our participants collected three saliva samples. The first sample served as a baseline sample and was collected right after the EEG preparation (Fig. 1). To reduce the stress of all participants at the beginning of the unfamiliar test situation and for lowering the cortisol level, mellow music played in the background while EEG was connected. Following this, the experimental design diverged for the control and stress group: After EEG preparation, the control group had eight minutes alone in the lab to make some notes about themselves and thereafter was asked to count upwards in steps of five for five minutes. Conversely, the stress group started a modified version of the TSST (Buske-Kirschbaum et al., 1997; Kirschbaum et al., 1993). Participants in the stress group had three minutes to prepare a five-minute speech without notes about themselves (adults: for a job interview, adolescents: for a new class). The speech was assessed by an audience unknown to the participant consisting of a male and female scientist. Before the speech started, the participant was told that the speech was to be recorded and analyzed by same-age peers regarding content, body language, and voice to enhance the level of social stress. If the participant stopped for more than 15 seconds the audience commented the situation with "Please continue.". In case the participant was unable to produce further speech, open-ended questions were asked about bad habits or weaknesses. After the speech, participants of the stress group had to subtract 13 continuously starting at 1022 for five minutes. If an error was made, the audience commented with "Stop!" and the participant had

to start from the beginning. The TSST and the control intervention lasted about 15 minutes. Subsequently, a second saliva sample was collected. Then the Digit-Span and the Computer task started. After completion, the third saliva sample was taken (Fig. 1).

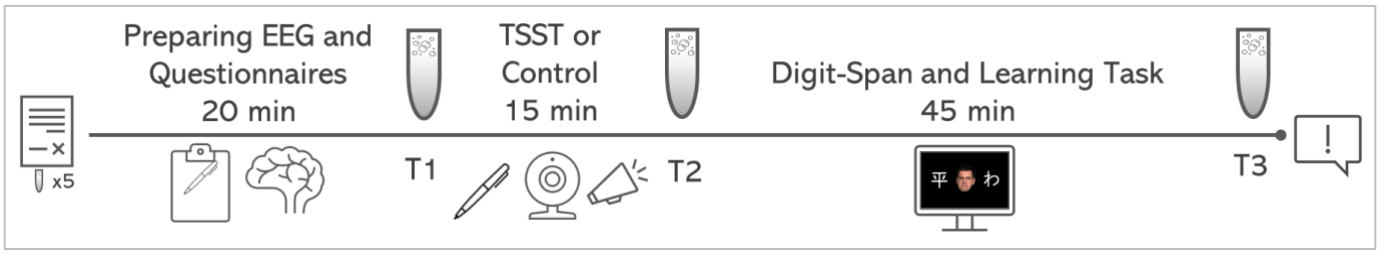


Fig. 1. Test procedure

Before the beginning of the test, participants submitted five morning saliva samples and a signed declaration of informed consent. The test started with questionnaires and the preparation of EEG recording. Then, the first saliva sample during the test session was collected. After the subsequent stress or control intervention, the second saliva sample was collected followed by the digital span and the learning task. In the end, a third saliva sample was collected and the participants were extensively informed about the study design.

Probabilistic Feedback Learning Task

Reward learning was investigated with a probabilistic learning task (see Diekhof and Ratnayake, 2016; Frank et al., 2004) consisting of an acquisition (session I) and a transfer session (session II). On the screen, stimulus pairs of hiragana syllables and kanji symbols were displayed pseudorandomly for a maximum of 1500 ms or until a response was made. Before a new trial started, a blank screen appeared (400-1600 ms). The participant was told to pick the rewarding symbol out of two presented symbols. Paired characters, henceforth named „A“, „B“, „C“, „D“; „E“, and „F“, were fixed in the first learning session (fixed pairs „AB“, „CD“, „EF“), and selection of a stimulus led to probabilistic feedback (see below). In the event of positive feedback, a smiling man appeared in the middle. Negative feedback showed an angry-looking man. If no decision was made within the 1500 ms, the male face displayed a neutral expression. In session II, with freely combined stimuli, a white point instead of the informative facial feedback appeared to inhibit further learning. Each feedback was presented for 700 ms.

The probability to receive positive feedback after choosing the symbol „A“ was 80% and therefore the chance to get negative feedback was only 20%. In contrast, the selection of „B“ was rewarded in 20% of the cases and punished in 80%. „C“ yielded reward in 70% and „D“ in 30%. A choice of symbol „E“ predicted positive feedback in 60% of the trials versus 40% for choosing „F“: Accordingly, choosing symbol „A“ provided the greatest chance of positive feedback, while „B“ was associated with the lowest probability. Session I contained 120 trials of

each pair („AB“, „CD“, „EF“), resulting in 360 experimental trials with a short break after 180 trials. Session II consisted of 180 trials, in which pairs were no longer fixed and all stimulus combinations were presented. In this session, new pairs with the best symbol “A” and those with the worst symbol “B” are of special interest, since the preferential choice of A from new pairs is an indicator of reward learning ability, while the extent avoidance of B in new pairs indicates avoidance learning capacity. We expected a training effect after session I, in that participants were expected to choose the more rewarding symbols „A“, „C“ and „E“ more often from the fixed stimulus pairs. Whereas in session II, with new pairs containing „A“ and „B“, a percentage preference for choosing „A“ pointed to a better reward learning and an avoidance of „B“ to a better avoidance learning.

Hormonal analysis

Besides the three saliva samples during the laboratory testing, participants collected five morning saliva samples in 2 mL Eppendorf tubes on the test day in the first hour after awakening. Participants were instructed to avoid animal products twelve hours before sampling. The first sample was collected lying down directly after awakening and was followed by four samples every 15 minutes. In this way, we were able to catch the morning testosterone peak and cortisol flow (see Matchock et al., 2007). After the collection of the first sample, the participants were asked to brush their teeth. Throughout the sampling, taking a meal or drinking other beverages than water was not allowed. Upon arrival in the lab, samples were stored until analysis at -20°C. Before analyzing, saliva was thawed and centrifuged at RCF 604 x g for five minutes (3000 rpm in a common Eppendorf MiniSpin centrifuge) to separate the saliva from the mucin. The morning samples were analyzed as aliquots containing an equal amount of all five tubes (100 µL). In our in-house lab, samples were assayed twice with enzyme-linked immunoassays according to the respective manuals. We utilized Testosterone Luminescence Immunoassay (IBL International LoD: 1.8 pg/mL) coated with anti-mouse antibody, Cortisol Saliva ELISA (IBL International; LoD: .003 µg/dl) coated with anti-rabbit antibody, Progesterone Saliva ELISA (IBL International LoD: 3.13 pg/mL) coated with anti-mouse antibody and 17β-Estradiol Saliva ELISA (IBL International LoD: 2.1 pg/mL) coated with anti-donkey and anti-sheep antibody. Intra-Assay precision showed a mean CV of 7.3% (Testosterone Luminescence Immunoassay), 4.3% (Cortisol Saliva ELISA), 4.9% (Progesterone Saliva ELISA), and 8.8% (17β-Estradiol Saliva ELISA). Inter-Assay precision showed a mean CV of 7.3% (Testosterone Luminescence Immunoassay), 13.2% (Cortisol Saliva ELISA), 6.7% (Progesterone Saliva ELISA), and 11.8% (17β-Estradiol Saliva ELISA). For better

comparability, concentrations were Fisher z-standardized for each assay plate. We calculated the standardized percentage cortisol increase (pCi = (C_{T1} – C_{T0}) ÷ C_{T0} × 100) of the raw concentrations between the second saliva sample after stress or control intervention (C_{T1}) and the baseline saliva sample, that was collected before the beginning of the test session (C_{T0}).

EEG preprocessing and FRN analysis

EEG was recorded using 64 Ag/AgCl electrodes according to the international 10-20 system at 256 Hz. Data were acquired with the BioSemi ActiveTwo System, implicit referenced on the left and right mastoid bones during recording, and re-referenced offline to an average of all 64 channels. Data were processed using the FieldTrip toolbox (Oostenveld et al., 2011) implemented in Matlab. Data were high-pass filtered at .1 Hz and low-pass filtered at 30 Hz using windowed sinc finite impulse response filters (Widmann et al., 2015) To reduce power line noise, a discrete Fourier transform filter at 50 Hz was applied. EEG epochs were extracted from 1 s before and to 1 s after the positive respectively negative feedback stimulus. Trials and channels were first visually inspected for artifacts and channels interpolated or removed if interpolation was not feasible (*removed channels*: mean ± SD = 3.5 ± 3). Eye-movement artifacts were corrected by fast independent component analysis (*removed components*: 4.7 ± 3.2). The amplitude of the event-related potentials was derived from participants' average waveform. We defined the FRN as the negative peak between 200 - 300 ms from frontocentral electrodes (Fz, FCz, and Cz) in response to negative feedback ($FRN_{negative}$) and positive feedback ($FRN_{positive}$) from each participant. To avoid an unequal distribution in the selection of trials, the same number of trials per subject was chosen for the determination of $FRN_{positive}$ and $FRN_{negative}$ (*selected trials:* 61.5 ± 14.9).

The $FRN_{negative}$ and the $FRN_{positive}$ were analyzed with a repeated measures ANOVA and the between-subject factors "*intervention*" (stress and control), "*age group*" (adolescents and adults), and "*sex*" (female and male). For post-hoc comparison, we utilized paired and independent t-test. Hormonal interactions were considered with Pearson (*r*) correlations.

Statistical analysis

All statistical analyses were conducted with IBM SPSS Statistic 27. First, we considered the physiological stress measured with the percentage cortisol increase (pCi) with a univariate ANOVA with the between-subject factors "*intervention*" (stress and control), "*age group*"

(adolescents and adults), and "*sex*" (female and male). Followed by univariate ANOVA of the perceived stress of the stress group with the factors "*age group*" and "*sex*".

For behavioral analysis, we used a repeated measures ANOVA to analyze the learning performance of session I with the within-subject factor "*pair*s" (AB, CD and EF) and the transfer performance of session II with the within-subject factors "learning outcome" (Choose A and Avoid B performance). Both ANOVAs were fitted with the between-subject factors "intervention", "age group" and "sex". Post-hoc, paired and independent t-test were performed. If Levene's test was significant, we used Welch's t-test instead of Student's t-test. Tests were Bonferroni corrected for multiple testing by multiplying the p-values with the appropriate number of comparisons.

Results

Physiological and perceived stress

We first examined the impact of "*intervention*" (stress and control), "*age group*" (adolescents and adults), and "*sex*" (females and males) on the physiological stress marker cortisol in a univariate ANOVA of the individual percentage cortisol increase from C_{T0} to C_{T1} (pCi). A significant effect of *intervention* was detected ($F_{1, 100} = 4.99$, $p = .014$, $\eta^2 = .06$) with a deeper pCi increase in the stress group (stress: mean ± sem: 39.94 ± 13.07%) compared to the control group (control: mean ± sem: -2.13 ± 9.09%) (see Fig. 2 for the time course of cortisol of the stress and control group). No impact of age group or sex could be found.

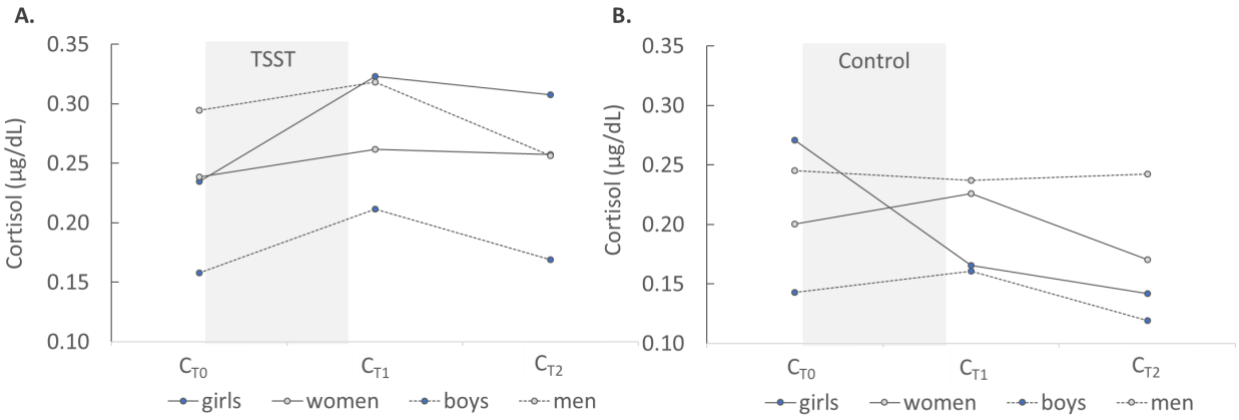


Fig. 2 Cortisol changes of (A.) stressed and (B.) control participants

A visual comparison of the cortisol concentrations from CT0 to CT2 demonstrated an increase of cortisol from CT0 to CT1 in the stressed group but not in the control group. A comparison of the mean percentage cortisol increase (pCi) revealed a greater increase in the stress compared to the control group (p = .014).

In the next step, we investigated the influence of *age group* and *sex* on self-reported stress perception of the stress group. The univariate ANOVA showed a significant effect of *sex* ($F_{1, 59}$ = 4.49, p = .003, pη^2 = .14), but not of *age group*. The female stress group appeared to be more vulnerable to the psychosocial stress elicited by the TSST and reported a significantly higher subjective stress perception than the male stress group (stress perception [mean ± sem]: females = 3.7 ± .16; males = 2.94 ± .18).

Learning performance in session I

The repeated measures ANOVA of the learning performance in session I with the within-subject factor "*pairs*" (AB, CD and EF) and the between-subject factors "*intervention*" (stress and control), "*age group*" (adolescents and adults) and "*sex*" (female and male) demonstrated a significant main effect of *pairs* ($F_{2, 202}$ = 27.73, p < .001, pη^2 = .22) and *age group* ($F_{1, 101}$ = 8.46, p = .004, pη^2 = .08). Moreover, significant interactions of *pairs* x *sex* ($F_{2, 101}$ = 4.05, p = .019, pη^2 = .04) and *pairs* x *sex* x *intervention* ($F_{2, 101}$ = 7.01, p = .001, pη^2 = .07) emerged.

Post-hoc t-tests demonstrated that the learning performance was significantly different between the pairs, as expected from the differences in reward probabilities (AB vs. CD: t_{108} = 4.98, p < .001, d_{Cohen} = .48; AB vs. EF: t_{108} = 6.83, p < .001, d_{Cohen} = .65; CD vs. EF: t_{108} = 2.44, p = .049, d_{Cohen} = .23; Bonferroni corrected for three comparisons). Participants selected the better option the most in pair AB > CD > EF (mean ± sem: AB = 68.37 ± 1.58%; CD = 60.4 ± 1.65%; EF = 55.17 ± 1.71%). A post-hoc comparison of age group showed that adults were generally better in choosing the best option compared to adolescents ($t_{106.81}$ = 2.9, p = .004, d_{Cohen} = .54; mean ± sem: adults= 64.19 ± 1.77%, adolescents = 57.52 ± 1.47%). Between the sexes there were no significant differences in the selection of the better option in the three pairs.

Regarding the interaction of *pair* x *sex* x *intervention,* a comparison between control and stress group within the female and male group, respectively, showed no results. An analysis of the sexes within the stress group also demonstrated no results, whereas in the control group male participants were significantly better in choosing the best option in pair EF compared to females ($t_{29.98}$ = 2.68, p = .036, d_{Cohen} = .83; Bonferroni corrected for three comparisons, mean ± sem: males= 62.64 ± 2.8%, females = 46.14 ± 5.49%).

Transfer performance in session II

The actual ability to learn via positive feedback (Choose A) and negative feedback (Avoid B) was measured in the second session. For this, a repeated measures ANOVA was conducted with the within-subject factor "*learning outcome*" (Choose A and Avoid B performance) and the between-subject factors "*intervention*", "*age group*" and "*sex*". The *learning outcome* showed a significant main effect ($F_{1, 101}$ = 8.94, p = .004, pη^2 = .08). Further, we found interactions between *learning outcome* and *age group* ($F_{1, 101}$ = 7.96, p = .006, pη^2 = .07) as well as *learning outcome* and *sex* ($F_{1, 101}$ = 5.79, p = .018, pη^2 = .05). No significant effects for the *intervention* emerged (main effect: F_1 = .76, p = .386, pη^2 = .01; interaction with *learning outcome*: F_1 = .9, p = .344, pη^2 = .07).

Post-hoc independent t-tests for the learning outcome demonstrated a better Choose A performance compared to Avoid B performance across participants (t_{108} = 3.95, p < .001, d_{Cohen} = .38; mean ± sem: Choose A = 68.73 ± 1.82%, Avoid B = 58.53 ± 1.66%). Thus, participants learned better through positive feedback compared to negative feedback. Between the age groups a significant difference for the Choose A performance (t_{107} = 2.41, p = .036, d_{Cohen} = .47; Bonferroni corrected for two comparisons), but not for the ability to Avoid B (t_{107} = -1.08, p = .566, d_{Cohen} = -.21; Bonferroni corrected for two comparisons) became apparent. Accordingly, adult participants were better at choosing A compared to adolescents (mean ± sem: adults = 72,47 ± 2.42%, adolescents = 63.81 ± 2.63%). Finally, the comparison between the sexes showed a better reward learning ability with a better Choose A performance of male compared to female participants (t_{107} = 2.31, p = .045, d_{Cohen} = .44; mean ± sem: males = 72.75 ± 2.38%, females = 64.49 ± 2.67%).

Explorative investigation of the hormonal impact on learning performance

We used t-tests to compare the ability to select the better option during session I as well as the Choose A and Avoid B performance in session II between the groups of low and high morning sex hormones (E_2, progesterone and testosterone). The analysis was conducted separately for the control and stress group. The control group showed no differences in learning performance in relation to basal morning hormones. However, in the stressed group, participants with higher than median E_2 concentrations compared to those with lower concentrations demonstrated a better learning ability during session I as indicated by a higher percentage of picking the better

option across the three pairs (t_{52} = -2.12, p = .039, d_{Cohen} = -.59; mean ± sem: low = 59.87 ± 2.05%, high = 66.72 ± 2.44%). Higher E_2 was also indicative of a better Choose A performance in session II (E_2: t_{52} = -2.4, p = .002, d_{Cohen} = -.67; mean ± sem: low = 63.7 ± 2.97%, high = 66.72 ± 2.44%). On the contrary, stressed participants with low testosterone concentrations compared to high concentrations showed a better learning ability (select the better option) during session I (t_{51} = 2.6, p = .012, d_{Cohen} = .81; mean ± sem: low = 64.35 ± 2.01%, high = 54.75 ± 2.54%), as well as a better Choose A performance in session II (t_{51} = 2.1, p = .042, d_{Cohen} = .65; mean ± sem: low = 71.15 ± 2.98%, high = 59.52 ± 4.11%) and a greater negative feedback sensitivity by more frequently avoiding stimulus B (t_{107} = 2.1, p = .041, d_{Cohen} = .65; mean ± sem: low = 59.55 ± 2.91%, high = 48.58 ± 3.2%). For progesterone no differences between hormone concentrations were found.

FRN of positive and negative feedback

For the FRN analysis, we performed a repeated measures ANOVA with the within-subject factor "*Feedback type*" ($FRN_{negative}$ and $FRN_{positive}$) and the between-subjects factors "*intervention*", "*age group*" and "*sex*" and could find a significant main effect of *Feedback type* ($F_{1, 82}$ = 141.2, p < .001, $p\eta^2$ = .63) and an interaction between *Feedback type* and *age group* ($F_{1, 82}$ = 5.95, p = .017, $p\eta^2$ = .07). No significant effect of *intervention* emerged (main effect: F_1 = 1.07, p = .303, $p\eta^2$ < .01; interaction with *Feedback type*: F_1 = .54, p = .466, $p\eta^2$ < .01).

The FRN peaks for negative and positive feedback were significantly different in a paired t-test comparison, with a more negative peak for negative feedback compared to positive feedback (t_{89} = -12.05, p < .001, d_{Cohen} = -1.27; mean ± sem: $FRN_{negative}$ = -1.88 ± .32 µV, $FRN_{positive}$ = 1.68 ± .32 µV).

A comparison between adolescents' and adults' FRN demonstrated a difference in the FRN for negative feedback. But after Bonferroni correction for two comparisons just a statistical trend with a slightly greater FRN for negative feedback could be seen in adolescents compared to adults (t_{88} = -2.09, p = .078, d_{Cohen} = -.45; mean ± sem: adolescents = -2.63 ± .51 µV, adults = -1.31 ± .39 µV).

Interactions of hormones and FRN

In the last analysis, we examined the interaction between the FRNs and endogenous hormone concentrations by performing correlations. We did not find any association between the FRNs and the morning steroid hormones (E_2, progesterone, testosterone and cortisol), but a negative correlation between the $FRN_{negative}$ and the pCi was observed (r = -.23, p = .028). Accordingly, the larger the negative peak of the FRN in response to negative feedback, the lower the pCi. The significant difference in pCi between the intervention groups, as described in the first analysis, let us explore the correlation between the $FRN_{negative}$ and the pCi separately for the control and the stress group. It became apparent that the negative correlation was only observed in the stress group (r = -.37, p = .006), but not in the control group (r = -.07, p = .681) (Fig. 3). Furthermore, a statistical trend for a correlation with the $FRN_{positive}$ was also found in the stress group, which indicated a weak negative correlation between $FRN_{positive}$ and pCi (r = -.25, p = .074).

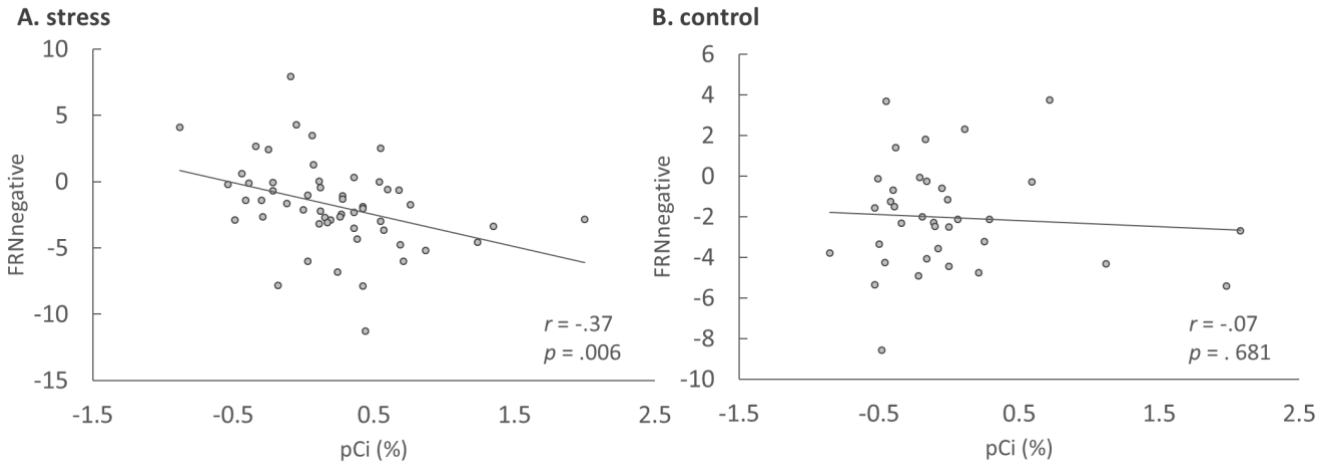


Fig. 3 Correlation between FRNnegative and pCi of (A.) stressed and (B.) control participants

The FRNnegative (FRN after negative feedback) correlated negatively with the pCi (percentage cortisol increase during TSST) in the stress (A. stress: r =-.37, p = .006), but not in the control group (B. control: r =-.07, p = .681).

Discussion

We investigated the impact of psychosocial stress on reward learning and the influence of endogenous steroid hormones on the FRN after positive and negative stimuli in adolescents and young adults. Overall, we observed a more pronounced cortisol increase after the TSST in the stress group compared to the control group, suggesting that psychosocial stress was successfully induced. Interestingly, only the female stress group reported a greater subjective stress perception compared to the male stress group.

In addition to that, adults had a generally better reinforcement-learning capacity compared to adolescents. Regarding sex differences, male controls selected the more rewarding stimuli of the most difficult pair EF more often than female controls, and also showed a significantly better transfer performance in session II.

An explorative consideration of the hormonal impact on the learning performance showed better reward learning in stressed participants with high morning E_2 levels and low morning testosterone concentrations, respectively. Moreover, high testosterone levels seemed to impair negative feedback learning ability, whereas for progesterone and control participants no differences between high and low hormone levels emerged.

Considering the neurophysiological feedback on positive and negative feedback, adolescents tend to have a greater $FRN_{negative}$ peak compared to adults. Between the FRN for positive and negative feedback no interactions with the morning hormones (E_2, progesterone, testosterone and cortisol) emerged. Only a negative correlation between the pCi and the FRN for negative feedback became apparent. Separate considerations of both intervention groups showed that this effect was only significant in the stress group. However, it should be noted again, that the control group, especially the one consisting of female adolescents, was underrepresented.

In comparison to boys and men, female participants seemed to be more vulnerable to psychosocial stress elicited by the TSST in our investigation. A higher stress response provoked by the TSST has been observed before in addicted women (Sherman et al., 2020). But also in healthy girls there is evidence of increased stress perception in real life compared to boys (Östberg et al., 2015). Psychosocial stress seems to play an important role in women's greater vulnerability to addiction (Sherman et al., 2020) and also mood disorders like posttraumatic stress disorder or depression (Albert et al., 2015). Animal studies reported a higher cortisol response to stress of females than males. Human studies are equivocal. No sex differences or higher cortisol responses in men were observed (Kudielka & Kirschbaum, 2005). Because women are more often affected by psychosocial stress, it is very likely that sex hormones also contribute to this sex difference.

Our findings also demonstrate a better reward learning capacity of adults compared to adolescents, which is different than a previous findings (Davidow et al., 2016; Lighthall et al., 2013). However, Davidow and colleagues (2016) noted reduced learning rates in adolescents compared to adults, in that adolescents needed more trials to update their learning process. In a another study, adults exhibited a better negative feedback learning ability than adolescents,

which could not be confirmed with our data (Javadi et al., 2014). Though, it should be noted that we investigated stressed and control participants whereas the working groups of Davidow (2016) and Javadi (2014) tested unstressed participants (Davidow et al., 2016; Javadi et al., 2014). Lighthall and colleagues (2013), who used the same probabilistic learning task and a physiological stressor (cold pressor stress) compared young adults with older adults. They reported that stress enhanced the ability of learning about cues with positive feedback and diminished feedback sensitivity (Lighthall et al., 2013). Between the age groups Lighthall did not find differences. Furthermore, also a worse prefrontal signal-to-noise ratio in adolescents, which is partly influenced by dopamine availability, may affected the reward learning performance (Diekhof et al., 2021).

The version of our conducted task might not be the most appropriate to investigate reward learning in adolescents. Unlike the fixed duration we had chosen, the learning phase in session I should probably have an individually suited number of trials until every participant achieved a learning plateau. Thereafter, in session II the learning preference could probably be better extracted in statistical analysis. However, the prior stress intervention might not be long-lasting enough and thus the participants must be stressed again within the learning phase or before the transfer session II in further investigations.

Male participants demonstrated a better transfer performance in session II that might be based on a better reward learning ability which, however, could not be observed in general in session I. In a previous study from Evans and Hampson (2015) no sex differences regarding punishment learning in adults were found (Evans & Hampson, 2015). It could be assumed that our observed sex differences were also based on the stress intervention but an interaction between the intervention and the learning performance was not detected. Besides, the small group size of the female control group could have a bias on the results.

Regarding sex hormones, we observed, that heightened basal E_2 compared to low concentrations seemed to attenuate the impact of stress on the reward learning performance. Albert and colleagues conducted the psychosocial Montreal Imaging Stress Task with women and detected higher reward-related hippocampal brain activity during enhanced E_2 concentrations (Albert et al., 2015). The hippocampus is a part of the dopaminergic limbic regions and is essential for learning and memory consolidation. An enhanced activity probably favors learning ability (Davidow et al., 2016). Further, in an emotional memory task after a TSST intervention an impairment of memory performance during the early follicular phase with low sex hormone level could be demonstrated (Maki et al., 2015). Both studies are consistent with

our findings that high basal E_2 concentrations presumably support dopamine-related reward learning during stress. Moreover, previous research on E_2 pointed to a positive impact on dopaminergic reward processing by enhancing dopamine availability and supporting neurophysiological dopaminergic actions (Diekhof, 2018). If an increasing activity of striatal areas and dopamine-promoting effects can be hypothesized at higher E_2 levels in stressful situations, this could entail better reward processing.

Stressed participants with high basal morning testosterone concentrations showed an impaired ability for reward learning but also negative feedback learning. An attenuating impact of testosterone on negative feedback sensitivity has been observed before (Van Honk et al., 2004). Because interactions between behavior and basal testosterone levels only occurred in the stress group, we assume that the results were most likely related to the enhanced cortisol concentrations during the test. Literature regarding testosterone and cortisol interactions mostly focuses on the dual-hormone hypothesis, according to which interactions between high testosterone and low cortisol concentrations are connected to obtain social status and thereby achieve a better task performance (Mehta & Prasad, 2015). After a TSST intervention with enhanced cortisol concentrations status-seeking behavior seemed to be diminished in high basal testosterone women and men (Prasad et al., 2019). In men, testosterone administration increased cortisol concentrations and negative affect (experience of negative emotions) after the TSST (Knight et al., 2017). Furthermore, testosterone was found to increase neuronal activity in response to threatening stimuli (Goetz et al., 2014). High testosterone seemed to reinforce the perception of threats (Knight et al., 2017). In connection to enhanced cortisol concentrations enhanced testosterone could probably lead to worse task performance, as we observed in our study. Another study using the TSST supports our findings. They observed a worsening effect of testosterone on interview performance (Knight & Mehta, 2017; but see also: Panizzon et al., 2018). One may speculate that during psychosocial stress, enhanced testosterone may promote the focus on the threatened status, which in turn may promote the worsening performance.

Two similar studies with men used the socially evaluated cold-pressure test for stress intervention followed by the probabilistic learning task, we conducted. (Glienke et al., 2015; Paul et al., 2019). One study reported a more negative FRN difference between positive and negative feedback in stressed participants (Glienke et al., 2015). The other demonstrated a steeper FRN with a greater cortisol increase (Paul et al., 2019). Both studies support our findings regarding the FRN. With a more pronounced stress-induced increase in cortisol, we observed a more negative FRN. A more negative FRN under stress could be related to increased striatal processing

(Glienke et al., 2015; Wirz et al., 2017). Further, as postulated at the beginning a greater dopamine availability during stress is related to a higher negative FRN (Glienke et al., 2015).

Our study only showed few differences between age groups. One of these was that in comparison to adults, adolescents had a more negative $FRN_{negative}$. This observation is in line with previous studies reporting a decreasing FRN to negative feedback from childhood to adulthood (Eppinger et al., 2009; Hämmerer et al., 2011). Therefore, adolescents seemed to be more sensitive to negative feedback than adults, whereas positive feedback did not show any group differences. It could be speculated that the enhanced neurophysiological activity to negative feedback in adolescents is reinforced by a more sensitive dopaminergic reward system (see also hypersensitivity theory in Galván, 2014). However, it should also be considered that the underrepresented female adolescent control group and thus, the larger number of stressed adolescents, probably encourage the finding of a higher $FRN_{negative}$ compared to adults.

CHAPTER FOUR

Testosterone and cortisol affect male risk-taking from adolescence into early adulthood in the Balloon Analogue Risk Task

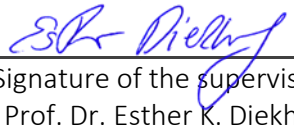
Sina Kohne, Rémi Janet and Esther Diekhof

Author contributions

The study was conceptualized by E.D and SK. S.K. designed the paradigm and experimental procedure under supervision of E.D. Data was collected by S.K. and students support. Hormonal and statistical analyses were performed by S.K. R.J. programmed the modeling batches. The first draft of the paper was written by S.K. All authors contributed to reviewing the manuscript. The authors would like to thank A.K. for her support in laboratory analyses.

Hamburg, 10.04.2022

Date and Place



Signature of the supervisor
Jun. Prof. Dr. Esther K. Diekhof

Abstract

Taking risks is an often-described behavior during adolescence and early adulthood. The dual-hormone hypothesis claims that more risk-taking could be explained by high testosterone in combination with low cortisol concentrations, especially in men. This is the first study that investigated the impact of the dual-hormone hypothesis on risk-taking in adolescent boys (n = 28) compared to young men (n = 60). In line with previous research, we found that high endogenous testosterone predicted riskier behavior in both adolescents and adults. Moreover, an inverted U-shaped relation between age and risk-taking could be observed. Regarding the dual-hormone analysis, a higher risk propensity could be shown in both, men and boys. Nevertheless, the sparse data on adolescents requires more studies including female participants to investigate the impact of steroid hormones on risk-taking more profound.

Introduction

Taking risks enable young people to explore their own physical and mental limits and risk-taking is often related to reward or recognition by peers. The development of adolescent boys into young men is characterized by both physical and psychological maturation. Testosterone plays a major role in male sexual maturation such as the development of secondary sex characteristics during puberty. Testosterone has also been identified as a critical part of risk-taking and impulsive behavior. Naturally enhanced testosterone concentrations appeared to support risk-taking in monetary games in men (Apicella et al., 2014) and boys, but also in girls (Peper et al., 2013).

Despite previously reported relations between enhanced testosterone level and increased risk-taking, it is more likely that testosterone does not act in isolation. Thus, interactions between different hormonal axis may even better explain risk-taking behavior. Among others, cortisol is one of the most promising hormones that interplay with testosterone. Testosterone secretion is regulated by the hypothalamic-pituitary-gonadal (HPG) axis. The physiological stress marker cortisol is released by the adrenal gland and regulated by the hypothalamic-pituitary-adrenal (HPA) axis. The HPG and HPA axes interact reciprocally. Cortisol presumably inhibits the HPG axis through reducing gonadotropin secretion (Dubey & Plant, 1985). Yet, the effect mechanism of sex hormones on HPA axis function has not been fully understood. The combined impact of testosterone and cortisol on behavior has recently been discussed within the framework of the

dual-hormone hypothesis. A dual-hormone profile with high testosterone in combination with low cortisol level was associated with increased adult risk-taking (Mehta, Welker, et al., 2015). Studies with adolescents assessing the interaction between the HPG and HPA axes are rare. Some demonstrated a relation of a dual-hormone profile with increased aggression (Platje et al., 2015). To the best of our knowledge, a relation between testosterone and cortisol in the context of risk-taking has not been investigated in adolescents, yet.

Previous studies investigating the impact of age on risk-taking behavior assumed a quadratic association between age and risk-taking with a peak during mid to late adolescence (B. R. Braams et al., 2015; Freeman et al., 2020). Besides behavior, also neurophysiological findings pointed to enhanced brain activity in response to risk-related reward during mid to late adolescence, which might be a reason for the observed increased risky decisions (B. R. Braams et al., 2015; Barbara R. Braams et al., 2014).

Considering the proposed influence of testosterone on risk-taking in boys and the impact of the dual-hormone profile in young men, we conducted the monetary reward-related *Balloon Analogue Risk Task* to assess risk-taking behavior according to the age (Mehta, Welker, et al., 2015; Peper et al., 2013). In addition, we used computational modeling to further identify the complex cognitive processes and their interplay with the hormonal profile that traditional performance indices failed to reveal. We predicted that a dual-hormone profile would entail increased risky-decision making with higher testosterone linked to higher risk-taking behavior only if combined with low cortisol level in men and probably also in boys. Moreover, we expected to see quadratic age-dependent risk-taking patterns with a peak of risky decision making during mid to late adolescence.

Materials & Methods

Participants and Procedure

For this study, 28 boys and 60 men (Tab. 1) were recruited. All subjects were from Germany, healthy, and had no history of a psychiatric disorder. Before participation, subjects and in the case of minority, also a legal guardian (parent), signed a written declaration of informed consent. The local ethics committee of the Medical Council of Hamburg (Ärztekammer Hamburg, Germany) approved this study in accordance with “The Code of Ethics of the World Medical

Association" (Declaration of Helsinki). Participants played the *Balloon Analog Risk Task* with three risk conditions: risky, moderately risky, and not risky.

The task

During the *Balloon Analog Risk Task* the participants had to pump up a computer-animated balloon (Fig. 1). With each pump a sound of an inflating balloon was audible, and a cent was added to the participants' imaginary bank account. The participant was free to choose how often he would like to click on the pumping button to inflate the balloon. But if he would like to earn money he had to stop and collect all the cents from the imaginary bank account before the balloon bursts. After the balloon bursts with an audible bang, the pumps and thereby the money of this trial was lost.

Balloons of three different colors were randomly presented. Unbeknownst to the subject, the color of a balloon was related to a high, moderate, or low risk to explode (Fig. 1). Each condition was presented in 20 trials with 60 trials in sum. The risk of explosion was calculated using a random parameter adjusted to the risk of explosion. During the experiment, the participant had to figure out how risky a balloon was and how many pumps he could chance to achieve as much money as possible. The participants were told that their final gain was to be calculated from the collected money of 20 randomly chosen trials. In fact, in the end, we added a fixed amount of money to the general payment.

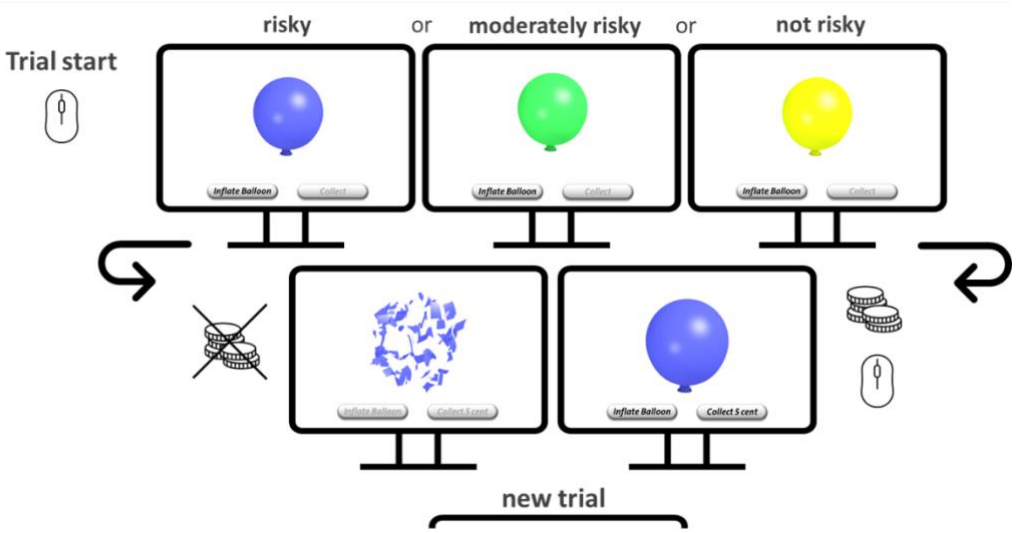


Fig. 1 The Balloon Analogue Risk Task

A trial started with a click on the "Inflate Balloon" button. Following, as long as the balloon is intact the participants could inflate the balloon again or collect the money via the "Collect X cent" button. If the balloon burst, the money for this balloon is lost and a new trial starts.

Saliva collection and analyses

Three morning saliva samples were collected independently by the participants in 2 ml Eppendorf tubes at home. Saliva collection started directly after awakening and took place over the course of one hour, with three collections in half-hourly intervals, on the day of testing. Participants were informed to avoid animal products 12 hours before saliva collection and to brush their teeth after the first sample. They were allowed to drink water five minutes before the collection. Only after the finished sample collection participants could take a meal. Before the start of the testing in the lab, samples were frozen at -20°C until analysis. For preprocessing, samples were centrifuged at RCF 604 x g (5 minutes at 3000 rpm in an Eppendorf MiniSpin centrifuge) to separate the saliva from the mucin. Following an aliquot from the three saliva residues was prepared using an equal amount from every tube (100 µl). The analysis was performed in our in-house laboratory with an enzyme-linked immunoassay according to the respective manual and each sample was assayed twice. Subjects' samples were equally distributed regarding age across the plates. A Testosterone Luminescence Immunoassay (IBL International, LoD: 1.8 pg/ml) coated with anti-mouse antibody and a Cortisol Saliva ELISA (IBL International; LoD: .003 µg/dl) coated with anti-rabbit antibody was utilized. Intra-Assay precision showed a mean CV of 7.3% (Testosterone Luminescence Immunoassay) and 4.3% (Cortisol Saliva ELISA). Inter-Assay precision showed a mean CV of 7.3% (Testosterone Luminescence Immunoassay) and 13.2% (Cortisol Saliva ELISA).

Computational models

The four-parameter-model

The first "four-parameter model" is based on the assumptions that the participants learned from each trial and accordingly adapted their behavior, but also decided the number of pumps before each trial.

In the first assumption, the participant's perceived probability p_k^{burst} that pumping the balloon on trial k will make the balloon explode, is constant during the trial k. The participant observes each trial and updates the initial prior belief about the probability of burst:

$$p_k^{burst} = 1 + \frac{\alpha + \sum_{i=0}^{k-1} n_i^{success}}{\mu + \sum_{i=0}^{k-1} n_i^{pumps}} \; with \; 0 < \boldsymbol{\alpha} < \boldsymbol{\mu} \qquad (1)$$

The decision about the optimal number of pumps l before each trial k, vk, is given by the following equations based on the first derivative of the expected utility (Park et al., 2020) for pump l equals zero:

$$vk = \frac{-\gamma}{\ln(1-p_k^{burst})},\ with\ \boldsymbol{\gamma} \geq 0 \qquad (2)$$

Using vk the probability of pumping the balloon p_{kl}^{pump} can be calculated:

$$p_{kl}^{pump} = \frac{1}{1+e^{\tau(l-vk)}}\ with\ \boldsymbol{\tau}\ \geq 0 \qquad (3)$$

The inverse parameter $\boldsymbol{\tau}$ defines how deterministic (higher values) or random (lower values) a choice was made. This model results in four parameters to be estimated: $\boldsymbol{\alpha}$, $\boldsymbol{\mu}$, $\boldsymbol{\gamma}$ and $\boldsymbol{\tau}$.

The three-parameter-model

In the second model ("three-parameter model") it is assumed that the participants did not learn during the *Balloon Analogue Risk Task* and the fixed parameter $\boldsymbol{\theta}$ will be used as the believed exploding probability. The optimal number of pumps v will be calculated similarly to equation (2):

$$v = \frac{\gamma}{\ln(1-\theta)}\ with\ \boldsymbol{\gamma}\ \geq 0 \qquad (4)$$

Followed by the probability that the participant will inflate the balloon (cf. (3)), three parameters will be estimated from this model $\boldsymbol{\theta}$, $\boldsymbol{\gamma}$, and $\boldsymbol{\tau}$:

$$p_{kl}^{pump} = \frac{1}{1+e^{\tau(l-v)}}\ with\ \boldsymbol{\tau}\ \geq 0 \qquad (5)$$

The reparametrized four-parameter-model

The third model ("reparametrized four-parameter model") is almost equal to the first model. Except that the parameters $\boldsymbol{\alpha}$ and $\boldsymbol{\mu}$ will no longer be interpreted together and instead reparametrized. Thereby the initial belief that pumping will burst the balloon $\boldsymbol{\phi} = \alpha/\mu$ and $\boldsymbol{\eta} = \frac{1}{\mu}$ as a coefficient of the participants' belief, which will be updated by the observed data, were calculated (cf Park et al., 2020); Embed in equation (1):

$$p_k^{burst} = 1 + \frac{\phi + \eta\sum_{i=0}^{k-1} n_i^{success}}{1+\eta\sum_{i=0}^{k-1} n_i^{pumps}}\ with\ 0 < \boldsymbol{\phi} < 1, \boldsymbol{\eta} > 0. \qquad (6)$$

The exponential-weight-model

This model is created to provide more information about the learning process and to evade the issue of a participants' potentially strong prior belief for the optimal number of pumps before each trial k. Here, the learning process was modified with an initial value of p_k^{burst}: $\psi = 1 - \phi$. Resulting in the new equation, adapted from (6) with the updating exponent $\boldsymbol{\xi}$:

$$p_k^{burst} = e^{\Sigma_{i=0}^{k-1} n_i^{pumps}} \psi + \left(1 - e^{\Sigma_{i=0}^{k-1} n_i^{pumps}}\right) P_{k-1} \; with \; 0 < \boldsymbol{\psi} < 1, \boldsymbol{\xi} > 0 \; (7)$$

The observed probability that pumping the balloon has made the balloon explode up to trial $k - 1$ is represented in $P_{k-1} = \frac{\Sigma_{i=0}^{k-1}(n_i^{pumps} - n_i^{success})}{\Sigma_{i=0}^{k-1} n_i^{pumps}}$. The parameter $e^{\Sigma_{i=0}^{k-1} n_i^{pumps}}$ is added for a better estimation of the prior belief. It represents how much weight is given to the prior belief that the balloon explodes on trial k and approximates the hyperbolic weight with an exponential function (further details in Park et al., 2020). Furthermore, instead of assuming the participant previously considers the number of pumps before a trial, a subjective utility with the prospect theory for pumping or not pumping the balloon before each pump l on trial k with the actual reward r for each successful pump was calculated (cf. Park et al., 2020):

$$U_{kl}^{pump} = \left(1 - p_k^{burst}\right) r^{\rho} - p_k^{burst} \lambda \{(l-1)\} r^{\rho} \; with \; 0 < \boldsymbol{\rho} < 2, \boldsymbol{\lambda} > 0, \; (8)$$

$$U_{kl}^{transfer} = 0 \quad (9)$$

Then the probability of pumping the balloon p_{kl}^{pump} could be calculated:

$$p_{kl}^{pump} = \frac{1}{1 + e^{\tau\left(U_{kl}^{transfer} - U_{kl}^{pump}\right)}} \; with \; \boldsymbol{\tau} \geq 0. \quad (10)$$

This model has five parameters to be estimated: $\boldsymbol{\psi}$ (prior belief of burst), $\boldsymbol{\xi}$ (updating exponent), $\boldsymbol{\rho}$ (risk propensity), $\boldsymbol{\tau}$ (inverse temperature) and $\boldsymbol{\lambda}$ (loss aversion).

The exponential-weight-mean-variance-model

The exponential-weight-mean-variance-model uses the mean-variance analyses to calculate the subjective utility after l pumps on trial $k(U_{kl})$ as followed with the amount of reward r for each successful pump, the risk propensity $\boldsymbol{\rho}$ as a coefficient of the variance term ($\boldsymbol{\rho} < 0$: preferring an option with a large variance of the potential outcome, $\boldsymbol{\rho} = 0$: the subjective utility is determined by the expected value of an option, $\boldsymbol{\rho} < 0$: preferring a small variance option) and loss aversion $\boldsymbol{\lambda}$:

$$U_{kl}^{pump} = \left(1 - p_k^{burst}\right)r - p_k^{burst}\,\lambda(l-1)r + \rho p_k^{burst}(1-p_k^{burst})\{r + \lambda(l-1)r\}^2 \; with \; \boldsymbol{\lambda} > 0, \quad (11)$$

$$U_{kl}^{transfer} = 0. \quad (12)$$

The probability of pumping the balloon p_{kl}^{pump} on trial k for l pumps was calculated with the subjective utilities and the inverse temperature $\boldsymbol{\tau}$ like in equation (10). The model resulted in five parameters: $\boldsymbol{\xi}$ (updating exponent), $\boldsymbol{\psi}$ (prior belief of burst), $\boldsymbol{\rho}$ (risk propensity), $\boldsymbol{\tau}$ (exploratory behavior) and $\boldsymbol{\lambda}$ (loss aversion). Parameters were compared between boys and men with U-tests and correlated using Spearman rank correlation.

The random-bias-model

This model was used to control that our previous models fit the data better than the chance level. It is assumed that the participants did not learn during the *Balloon Analogue Risk Task* but could be biased toward pumping or transferring money. Therefore, there is no updating value, as in the three-parameter-model. The decision about pumping or not is modeled using only a bias parameter ($\boldsymbol{\delta}$). The resulting action decision is:

$$U_{kl}^{pump} = \boldsymbol{\delta}$$

We constrained the $\boldsymbol{\delta}$ parameter between 1 and 0 using a sigmoid function. If $\boldsymbol{\delta}$ > .5, then the participant is biased toward pumping. Otherwise, if $\boldsymbol{\delta}$ < .5, the participant is biased toward not pumping and transfers money.

Analyses

The data were analyzed with IBM SPSS Statistics 27. For the behavioral analysis, the means of pumps and sum of pops of all trials for each balloon condition (risky, moderately risky, and not risky) were calculated separately.

Concentrations one standard error of mean above the mean were assigned to the high testosterone and high cortisol group, respectively, whereas concentrations one standard error of the mean below the mean were assigned the low testosterone and low cortisol group.

General linear model (GLM) repeated measures were conducted with the three different "*balloon condition*" (risky, moderately risky, and not risky balloons) of the number of pumps,

respectively pops, as dependent within-subject factors, the covariate *"age"*, the *"testosterone group"* (low testosterone and high testosterone) and the *"cortisol group"* (low cortisol and high cortisol) as between-subject factors. We corrected GLM results with Greenhouse-Geisser ($\varepsilon < .75$) or Huynh-Feldt ($\varepsilon > .75$) if sphericity was not given by Mauchly. Independent and paired t-tests (Levene and Bonferroni corrected) were performed for group comparisons and Cohen's D was calculated as an effect size. Non-linear regressions (R^2) were used post-hoc to consider the impact of *age*.

We tested 6 different computational models to capture behavior during the *Balloon Analogue Risk Task*. Five models were generated according to Park and colleagues (2020) and the last model, called "random-bias-model" (used to control for bias choices toward pumping or not). Models were compared with Bayesian model selection implemented with the Variational Bayesian Analysis (VBA) toolbox in MATLAB. Only the model with the highest exceedance probability using the Bayesian Information Criterion (BIC) was analyzed. Model parameters as dependent within-subject factors were analyzed with univariate GLMs using the covariate "*age"* and the between-subject factors *"testosterone group"* (low testosterone and high testosterone) and the *"cortisol group"* (low cortisol and high cortisol). Post-hoc Mann-Whitney U test for independent samples and a non-linear regression was performed to analyze the influence of *age*.

Results

Group comparison

Before behavioral analyses, boys and men were compared regarding age and hormonal concentrations. Except for age, no differences could be observed (Tab. 1). Moreover, cortisol and testosterone concentrations ($r = .56$, $p < .001$) were correlated positively.

Analysis of pumps

In the GLM for the number of pumps (Tab. 2), the *balloon condition* (risky, moderately risky, and not risky balloons) missed significance after Greenhouse-Geisser correction ($F_{64.72,\ 1.14} = 3.56$, $p = .059$, $\eta^2_p = .06$). Further, the main effects of *age* ($F_{1,\ 57} = 7.44$, $p = .008$, $\eta^2_p = .12$) and of *testosterone group* (low testosterone and high testosterone) ($F_{1,\ 57} = 4.21$, $p = .045$, $\eta^2_p = .07$) were significant. No statistical interaction between the testosterone and cortisol groups have

been observed ($F_{1, 57}$ = 1.05, p = .309, η^2_p = .02) (but see Fig. 2 A for the exploration of response patterns by groups).

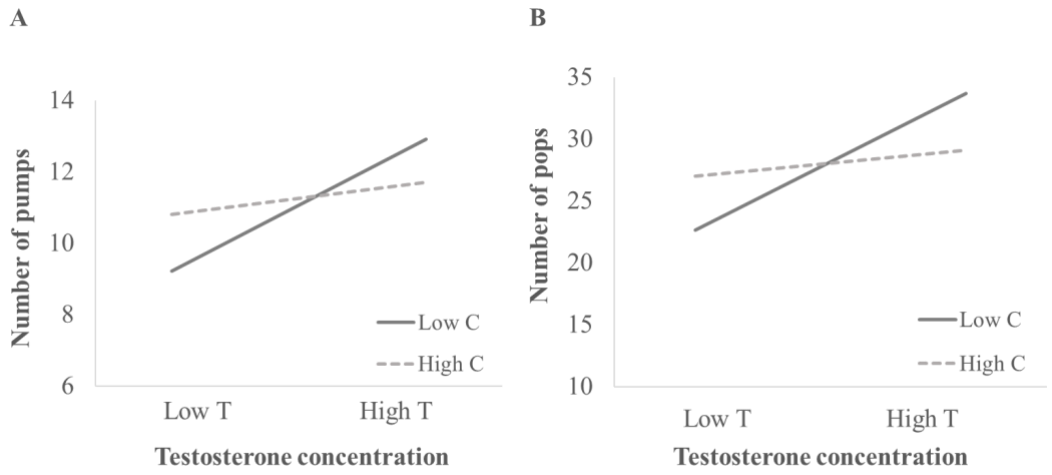


Fig. 2 Visual exploration of the non-significant dual-hormone interaction of testosterone (T) and cortisol (C) with the number of pumps A) and pops B).

No statistical interactions between the high (= 1 sem above mean) and low (= 1 sem below mean) testosterone (T) and cortisol (C) groups were found for pumps and pops. However, visual comparisons indicate the highest number of pumps A) and pops B) during high testosterone and low cortisol concentrations.

Explorative post-hoc tests for the *balloon condition* confirmed an increasing number of pumps with decreasing risk (number of pumps: risky < moderately risky < not risky, p < .001) (Tab. 2). A significant quadratic relation was found between *age* and the mean number of pumps regardless of condition ($F_{2, 85}$ = 3.35, p = .04, R^2 = .07) (Fig. 3 A). An increase of pumps with rising age could be observed until the mid-twenties followed by a decrease thereafter. The regression equation was found to be:

$$estimated\ mean\ number\ of\ pumps = -.75 + (.96) * (age) - .02 * (age^2)$$

Regarding the hormonal impact, participants with high testosterone made more pumps compared to participants with low testosterone (*mean ± sem:* high testosterone: 11.49 ± .48, low testosterone: 9.51 ± .54; t_{69} = 2.53, p = .014, d = .62).

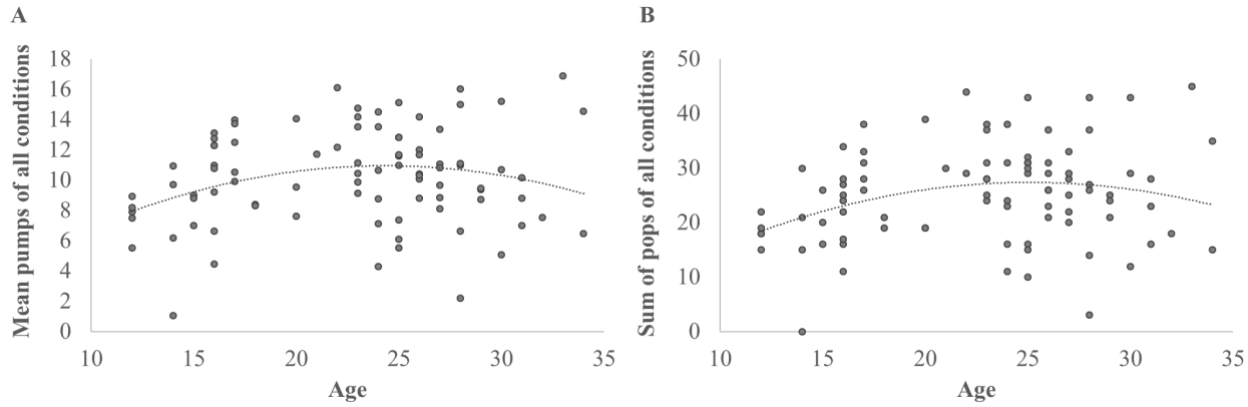


Fig. 3 Inverted U-shape relation of age with pumps A) and pops(B).

A quadratic relation between the participants' age and A) the mean number of pumps (F2, 85 = 3.35, p = .04, R2 = .07) and B) the sum of pops (F2, 85 = 3.83, p = .026, R2 = .08) of all balloon conditions was observed with an increase of pumps and pops until early adulthood and a subsequent decrease.

Analysis of pops

In the GLM for the number of pops (Tab. 2), the interaction between *balloon condition* (risky, moderately risky, and not risky balloons) and *age* ($F_{1.98,\ 112.75}$ = 7.27, p < .001, η^2_p = .11) was significant. In addition, main effects of *age* ($F_{1,\ 57}$ = 10.52, p = .002, η^2_p = .16) and *testosterone group* ($F_{1,\ 57}$ = 4.65, p = .035, η^2_p = .08) emerged. Again, no statistical interactions between the testosterone and cortisol groups emerged ($F_{1,\ 57}$ = 1.43, p = .236, η^2_p = .03) (but see Fig. 2 B for the exploration of response patterns by groups).

An inverted U-shape relation was found for the sum of pops and *age* ($F_{2,\ 85}$ = 3.83, p = .026, R^2 = .08) (Fig 3 B). Like it was observed for pumps, with increasing age the sum of pops increased until the mid-twenties and decreased thereafter. The regression equation for the sum of pops was:

$$estimated\ sum\ of\ pops = -5.55 + (2.63) * (age) - .05 * (age^2)$$

The number of pops for the risky balloon condition also showed a significant quadratic relation with *age* ($F_{2,\ 85}$ = 5.64, p = .005, R^2 = .12) and there was a statistical trend for the association between moderately risky and *age* ($F_{2,\ 85}$ = 2.77, p = .068, R^2 = .06) but no interaction with not risky ($F_{2,\ 85}$ = 1.5, p = .23, R^2 = .03).

A comparison between the testosterone groups showed more pops of the high compared to the low testosterone group (*mean ± sem:* high testosterone: 28.7 ± 1.47, low testosterone: 23.27 ± 1.48; t_{69} = 2.46, p = .016, d = .6).

Model analysis

We conducted a univariate GLM for all modeled parameters of the best model with *age*, the *testosterone group* (low testosterone and high testosterone) and *cortisol group* (low cortisol and high cortisol). The parameter risk propensity was influenced by the *testosterone group* ($F_{1, 57}$ = 4.77, p = .033, η^2_p = .08), the *cortisol group* ($F_{1, 57}$ = 4.49, p = .038, η^2_p = .07), and their interaction ($F_{1, 57}$ = 11.62, p < .001, η^2_p = .17).

Post-hoc the risk propensity was compared between the cortisol groups (low vs. high), separately for both testosterone groups to investigate the impact of testosterone and cortisol interactions on risk propensity. The *cortisol groups* showed a significant difference in risk propensity for the low *testosterone group* (U = 57, p = .044) and a statistical trend for the high *testosterone group* (U = 9, p = .055) (Fig. 4). In the low *testosterone group* participants with a low cortisol level had a lower risk propensity (*mean ± sem:* 1.11 ± .03) compared to participants with a high cortisol level (1.18 ± .02). In the high *testosterone group*, the risk propensity was reversed. Participants with low cortisol level had a greater risk propensity (*mean ± sem:* 1.45 ± .28) compared to participants with high cortisol level (1.11 ± .03).

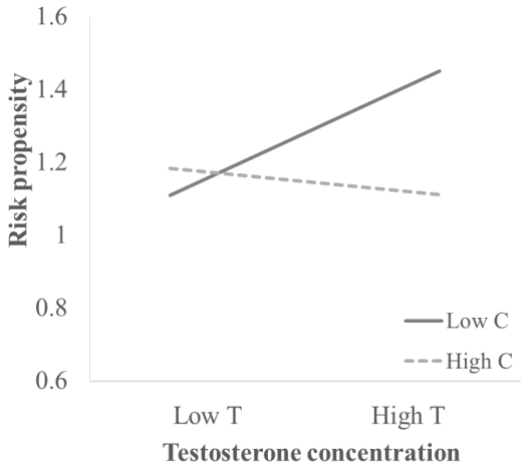


Fig. 4 Dual-hormone interactions of testosterone and cortisol with the modeled parameter risk propensity.

During low testosterone (= 1 sem below mean) a significantly higher risk propensity was observed in the high (= 1 sem above mean) compared to the low (= 1 sem below mean) cortisol group (U = 57, p = .044). Whereas during high testosterone (= 1 sem above mean) a statistical trend for a lower risk propensity in the high cortisol group compared to the low cortisol group was found (U = 9, p = .055).

An impact of age on the prior belief of burst showed a quadratic relation ($F_{2, 77}$ = 4.32, p = .017, R^2 = .1) (Fig. 5). The regression equation for the prior belief of burst was:

$$estimated\ prior\ belief\ of\ burst = 1.22 + (-.024) * (age) + .0005 * (age^2)$$

No further influences of age or hormones on parameters could be observed.

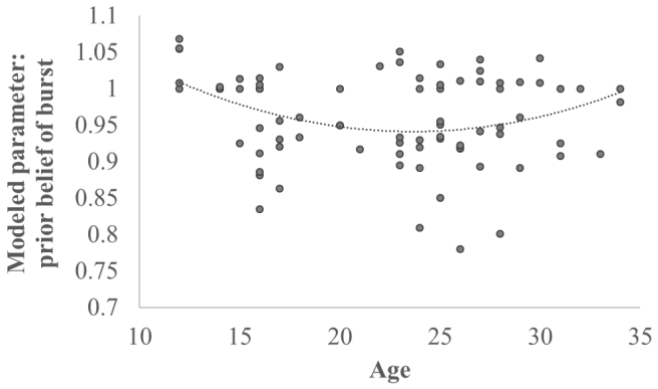


Fig. 5 Quadratic relation of age and the prior belief of burst

With increasing age, the modeled parameter of the prior belief of burst decreased until the mid-twenties and increased again thereafter (F2, 77 = 4.32, p = .017, R2 = .1).

Discussion

This study investigated the interaction of testosterone and cortisol on male adolescent and adult risk-taking behavior in the Balloon Analog Risk Tasks. First, we confirmed previous results showing that a dual-hormone profile with high testosterone and low cortisol concentrations enforced risk-taking behavior in men. We extended this result by observing similar effects for boys. The examined groups of men and boys differed significantly in age, but not in mean hormone concentration. As assumed, with increasing risk fewer pumps could be observed regardless of age or hormone concentration. Further, the analysis of pumps and pops revealed a quadratic relation between age and both pumps and pops (Fig. 2 A/B). The highest number of pumps and pops could be observed in men during their early twenties. Participants with high testosterone level made more pumps and had more pops, than participants with low concentrations. However, we found no evidence for an interaction of testosterone and cortisol levels with respect to the total number of pumps or pops, that would indicate a dual-hormone effect on risk-taking behavior. Only when considering the computational model, the parameter "risk propensity" was greater for males with a combination of high testosterone and low cortisol

compared to individuals who exhibited both high testosterone and cortisol (Fig. 4). Further, a quadratic relation between age and the parameter "prior belief of burst" became apparent, with men in their early twenties having the least pronounced belief that the balloon would burst, which supplements the reported finding of more pumps during this age (Fig. 5).

The observed quadratic relation between age and risk-taking tendency (more pumps and pops) showed that the willingness to take risks was greatest in younger men compared to adolescent boys and men in their mid-twenties and older. In an international sample of adolescents and young adults an increased sensation seeking, which might be related to enhanced risk-taking behavior, was evinced during late adolescence and early adulthood (Steinberg et al., 2017). One reason for this could the still progressing brain maturation. Especially the maturation of the prefrontal cortex (important for reward-related behavior and cognitive control) until early adulthood appears to play a crucial role in this context (Walker et al., 2017).

Moreover, the relation between age and risk-taking is related to the computational modeling results, as we found a quadratic relationship between prior belief of burst and age. Young adults tend to have less prior belief that the balloon will burst, which drives the higher number of pops and pumps, respectively, observed within the young adults. Increased flexibility is attributed to young adults (Gopnik et al., 2017). It has been shown that they pay less attention to prior belief and more to empirical evidence.

Both risky decision-making and an increased sensation-seeking propensity may be related to an enhanced reward drive. During the transition phase from late adolescence to early adulthood enhanced reward-related reactivity in the nucleus accumbens was associated with increased sensation seeking (Hawes et al., 2017). Moreover, prior studies which conducted the *Balloon Analogue Risk Task* found a greater risk-taking propensity mid- to late adolescents (14-17 years) compared to older adults (35-55 years) as well as an increase of risky behavior from pre to post puberty (S. H. Mitchell et al., 2008; Peper et al., 2013). Both findings are in line with the results of the present study.

Further consistent with previous findings (Apicella et al., 2014; Peper et al., 2013), our participants showed a greater tendency to take risks if their testosterone level was higher and this effect appeared independent of age. For instance, Op de Macks and colleagues (2011) found a positive correlation between salivary testosterone and activation of the nucleus accumbens, which is a crucial brain area of reward processing (Haber & Knutson, 2010; Op De Macks et al.,

2011). This finding, triggered by a monetary reward in a slot machine task, demonstrated the relation between testosterone and reward (Op De Macks et al., 2011).

Our behavioral data did not statistically support the dual-hormone hypothesis in the context of this risk-taking task. However, a visual inspection of the distribution of pumps and pops in relation to testosterone and cortisol suggests riskier behavior in both men and boys, when the endogenous testosterone concentration was high and cortisol level was low (see Fig. 3 A/B). This observation was further statistically supported by our computational model, which might detect rather subtle behavioral differences. It demonstrated a connection between a dual-hormone profile and a greater risk propensity in both adolescent and adult males. This makes our study the first that investigated and reported a connection between the dual-hormone profile and riskier behavior in male adolescents. Adult studies showed evidence of the dual-hormone-hypothesis in social status relevant behavior, aggression, dominance (Mehta & Josephs, 2010) and risk taking (Mehta, Welker, et al., 2015). For example, a dual-hormone profile predicted better task performance in financial games, with higher earnings in a status relevant competitive negotiation and an ultimatum game in adult males. In contrast to that, the combination of high testosterone and increased cortisol was associated with weak earning (Mehta, Mor, et al., 2015). Studies with adolescents are rare. Some demonstrated a positive relation between aggression and testosterone in individuals with a simultaneously low cortisol level (Grotzinger et al., 2018; Platje et al., 2015). Another study underpinned, that low cortisol and testosterone promoted behavioral problems in boys (attention problems and symptoms of oppositional defiant disorder). Further, antisocial behavior and behavioral problems in boys were significantly enhanced if high cortisol level occurred in combination with high testosterone (Susman et al., 2017).

The sparse data on adolescents will require additional studies including adolescent girls and neurophysiological examinations. Further investigations should probably consider additional steroid hormones, e.g., estradiol and progesterone.

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General discussion and conclusion

This thesis aimed to investigate the dopamine-mediated aspects of reinforcement learning and risk-taking behavior from early adolescence into young adulthood. A particular emphasis was placed on the modulatory influence of the gonadal steroid hormones estradiol, progesterone, and testosterone, as well as the glucocorticoid cortisol as they are known to affect dopaminergic processes (Diekhof, 2018; Oswald et al., 2005; Sinclair et al., 2014). An initial study (see CHAPTER ONE) addressed the influence of daytime and season on reinforcement learning in an adult group. This study was conducted to observe if a different daytime or season as well as the recruitment of participants over a longer period of time will bias the study results. In the second study (Study II), the impact of estradiol and testosterone on reinforcement learning was further assessed in adolescents (see CHAPTER TWO). Then, a related aspect of reinforcement learning was addressed to compare the learning performance of adolescents and adults after a stressful experience compared to a control situation while EEG was simultaneously recorded (see CHAPTER THREE). Finally, risk-taking as another aspect of reward-related decision-making was investigated in male adolescents and young adults, and also in relation to testosterone and cortisol (see CHAPTER FOUR).

Measurement of reinforcement learning

Feedback

Processing positive and negative feedback is essential to learn from actions from early childhood through late adulthood. Feedback in the conducted studies was provided in different ways. Like previous studies, we mostly used social and or monetary feedback to reproduce real-life situations and investigate reinforcement learning appropriately. The first study (Study I) and the second study (Study II) of this thesis concentrated on monetary feedback, whereas in the third study (Study III) of this thesis primary social feedback was applied. Social feedback is already significant during childhood and is for example involved in language acquisition (M. Goldstein et al., 2003). In previous studies, both children and adolescents reported a high motivation from social reward, and adolescents compared to both children and adults even showed greater activity in the putamen and the motor cortex when receiving positive social feedback (Jones et al., 2014; Wang et al., 2017). Wang and colleagues demonstrated that social reward was more important than monetary reward during childhood (7.9 to 8.5 years old) and adolescence (12.9–

13.5 years old) but not adulthood (20.1–29.4 years old) (Wang et al., 2017). However, monetary reward becomes more meaningful during adolescence (Wang et al., 2017) and is an incentive reward in adults (Lea & Webley, 2006).

The stressed participants of Study III were further put under pressure by telling them that they would get the opportunity to receive an additional prize if they performed better during the verbal speech and arithmetical task (both parts of the TSST) than the previous participants. After the stress intervention, they were told that the performance was not good enough and that they would not receive the prize. This was done to further increase the experienced stress level. (After the experiment all participants were informed about the task design and received the prize, obviously without a comparison with previous participants.) In order to generate a high learning motivation in both intervention groups (stress and control) of adolescents and adults, besides the social feedback during the probabilistic reward learning task, the entire learning performance was associated with a possible monetary gain depending on the performance in the probabilistic reward learning task.

It was considered that the reward was not too low, but also not too high because motivation also plays a decisive role, which inter alia is described by the “choking-effect”. This effect describes the phenomenon that reward can have a negative impact on performance and is probably affected by a dopamine “excess” especially in people with high baseline dopamine availability (Aarts et al., 2014; Zhuang et al., 2017). In adolescence, the hypothesized hypersensitivity of the dopaminergic reward system could presumably be overstimulated via reward which in turn may have a detrimental influence on performance (Galván, 2014; Zhuang et al., 2017). Thereby, the quadratic relation between dopamine concentration and task performance probably follows an “inverted U-shape trajectory” (Figure 4), which has previously been described in connection to dopamine-related drug administration studies (Cools & D’Esposito, 2011). Thereby, both a dopamine deficit, but also a dopamine concentration that is too high can impair task performance. This is probably also caused by a reduction of the prefrontal signal-to-noise ratio during reinforcement learning which could be affected by dopamine concentrations (Diekhof et al., 2021)

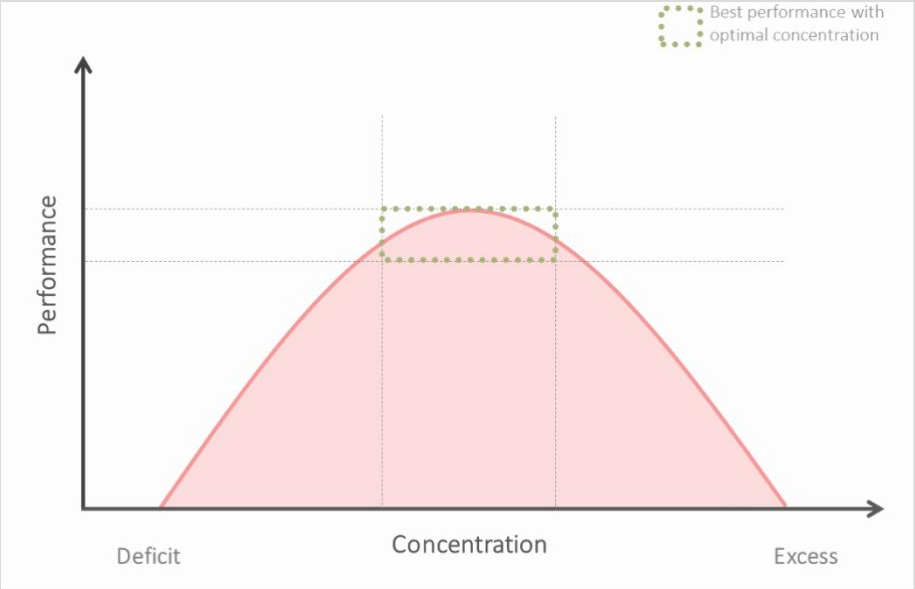

Figure 4: The inverted U-shape trajectory

The increase of a low concentration (e.g. of dopamine, see also Chapter Neurophysiological differences) up to an optimum promotes task performance. While any further increase of the concentration leads to a performance decline. This is best described by the inverted U-function, but not by a linear relationship. (adapted from Cools & D'Esposito, 2011). (source: own illustration)

Season and daytime

Reinforcement learning is enabled by the neurotransmitter dopamine, and dopamine itself is presumably subject to seasonal and daytime fluctuations (see Seasonal and circadian fluctuations of dopamine). Still, no previous study examined a seasonal impact on feedback learning and only one study investigated reward learning throughout the day. There, the lowest performance was reported in the middle of the day and an improvement of reward-learning towards the evening was observed (Byrne & Murray, 2017). Study results regarding seasonal or diurnal influences on cognition are difficult to compare, because they focused on different aspects of cognition. Whereas two studies rather support a better cognitive performance (verbal and arithmetical skills) during the summer than winter (Merikanto et al., 2012; Pääkkönen et al., 2008), another study observed a better performance (e.g. reaction time, word memory) during winter than summer (Brennen et al., 1999). Because of physiological findings regarding dopamine concentrations during different seasons and different daytimes, a better Go learning ability was hypothesized during the summer season and in the evening.

Nevertheless, daytime and season and the zeitgeber photoperiodicity and temperature in the first study (Study I) did not influence reinforcement learning in the response time adjustment task. Thus, different from previous expectations, the presumed dopaminergic fluctuations over

the day and between seasons did not affect reinforcement learning behavior. Although, a study with adults demonstrated neurophysiological differences with maximum brain responses (i.a. thalamus, amygdala, frontal areas and hippocampus) during summer and minimum during winter in an attention task (Meyer et al., 2016). In children (7 to 11 years old), one study found a time-of-the-day influence in neural reward responsiveness. Compared to younger children, older children thereby showed a stronger neurophysiological response to gains versus losses during the middle of the day and in the afternoon (Tsypes & Gibb, 2020). In our study, we were not able to investigate neurophysiological differences and focused on processes in the reinforcement learning system. Even if our study did not observe a behavioral difference there are possibly neurophysiological differences during task performance between summer and winter. In future studies, it will be of interest, to further assess whether a seasonality in reward processing can be demonstrated (e.g. neurophysiological differences in the response time adjustment task) in adults and adolescents.

The recruiting of children and adolescents takes significantly more time and effort compared to the recruitment of young adults. Therefore, it was vital to investigate the impact of daytime and season in young adults. It was assumed that a possible fluctuation of dopamine also influences the learning behavior across the day and the seasons. However, since only an influence of daytime on the general response speed could be observed, similar test times were considered in the second study (Study II) with adolescents. Here, the same task was used.

Developmental differences

Behavioral differences

A comparison of the first study (Study I) and second study (Study II) conducted with adults and adolescents, respectively, demonstrated that participants who better learned through reward had difficulties in punishment learning and vice versa. In the third study (Study III), all participants showed a better reward than punishment learning in the probabilistic reward learning task. However, adults were generally better in reward learning compared to adolescents in the acquisition phase (i.e., learning session I) and also in the ensuing transfer session II in the third study (Study III).

Modeled data of a trial-by-trial probabilistic learning task demonstrated a lower learning rate, though a better reinforcement-based updating and reward-related memory of adolescents (13 to 17 years old) compared to adults (20 to 30 years old) (Davidow et al., 2016). It was concluded

that adolescents learned better, but over a longer period of time than adults. However, Peters and Crone (2017), who scanned participants between 8 and 25 years, observed a peak in feedback-learning at the end of adolescence and the beginning of adulthood (16 to 20 years) (S Peters & Crone, 2017). Moreover, they observed a peak in activity (middle frontal gyrus, , parietal cortex and supplementary motor area) related to informative value. Both findings led to the conclusion that the transition between adolescence and adulthood is a potentially important developmental phase, which probably is an optimal period for feedback learning (S Peters & Crone, 2017). For a better differentiation between the adolescent and adult subject groups, the range between 17 and 19 years was not examined in the third study (Study III) of this thesis. Instead, only boys and girls from early to mid-adolescence (11 to 16 years old) were included here. Therefore, the development of reinforcement learning during the transition period from late adolescence to young adulthood could not be assessed. Nevertheless, young adults (20 to 29 years old) generally showed a better learning performance compared to the adolescent group. This finding would be consistent with the observations on young adults in the investigation of Peters and Crone (2017) (S Peters & Crone, 2017).

With a guess and application task (adapted from a previous study of Zanolie et al., 2008), van Duijvenvoorde et al. (2008) as well as Zhuang et al. (2017) compared feedback-based learning in children, adolescents, and adults (Van Duijvenvoorde et al., 2008; Zanolie et al., 2008; Zhuang et al., 2017). Here, participants had to choose between two pictures. During the first session, the guess trial, the participants learned the rule set (color rule or shape rule) by selecting shape or color. In the second trial, the application trial, they had to apply the rule based on the previous feedback during the guess trial. Again, adults demonstrated the best learning performance, which was also observed in the probabilistic feedback task of this thesis and in the study by Peters and Crone (2017) (S Peters & Crone, 2017). Moreover, after receiving positive feedback compared to negative feedback all age groups responded faster and more accurately in the guess and application task (Van Duijvenvoorde et al., 2008; Zhuang et al., 2017). This result is comparable to the finding of Study III, where a better reward than punishment learning in all age groups was observed.

Furthermore, Zhuang and colleagues (2017) confirmed previous findings from an adult subject group of Zanolie and colleagues (2008) (Zanolie et al., 2008; Zhuang et al., 2017). The studies from Zanolie et al (2008) and Zhuang et al. (2017) reported a significant reduction in learning after negative feedback in all participants (Zanolie et al., 2008; Zhuang et al., 2017). This effect was even most pronounced in adolescents, when compared to children and adults (Zhuang et

al., 2017). Van Duijvenvoorde and colleagues (2008) further showed an impairment of learning from negative feedback in the guess and application task in children compared to young adults, whereas young adolescents performed at an intermediate level (Van Duijvenvoorde et al., 2008). However, in Study III, no significant differences in punishment learning were found between the two age groups. Only a descriptively better avoidance learning was observed in adolescents compared to adults. This observation is supported by previous studies using similar probabilistic reward learning tasks. One study demonstrated a decreased punishment sensitivity with increasing age (Van Den Bos et al., 2012) and others a greater punishment sensitivity in children (9 – 11 years old) and older adults (65 – 75 years old) relative to adolescents (13 to 14 years old) (Frank & Kong, 2008; Hämmerer et al., 2011).

As we observed a better reward learning in both age groups in Study III, a difference in reward or punishment sensitivity between late adolescents (16 – 17 years old) and early adulthood (18 – 29 years old) could also not be observed with the self-reported "*Sensitivity to Punishment and Sensitivity to Reward*" Questionnaire (Torrubia et al., 2001) (Santesso et al., 2011). Though, Harden and colleagues (2018) observed an increased reward sensitivity with enhanced self-reported pubertal development measured with the "Pubertal Developmental Scale" (Harden et al., 2018; Petersen et al., 1988). The reward or punishment sensitivity might also be dopamine-dependent.

The presently available behavioral findings on reinforcement learning, favor neither of the two opposing theories of hypersensitivity or hyposensitivity of the adolescent dopamine reward system (see Developmental changes from childhood through adolescence to adulthood

). Although two studies observed a deficit in learning after negative feedback in adults and even more so in adolescents, these studies did not find a general difference in learning capacity with regard to rewarding or punishing outcomes between the age groups of children, adolescents, and young adults (Zanolie et al., 2008; Zhuang et al., 2017). One study demonstrated a deficit in punishment learning in children compared to young adults (Van Duijvenvoorde et al., 2008). Presumably, it is also crucial which tasks are used. Thus, in the probabilistic reward learning tasks, that are similar to the one we conducted, it is important to use the given feedback to estimate the expected value of the available choice (Van Den Bos et al., 2012). In this task, it was shown that punishment sensitivity seems to be more pronounced in children and older adults than in adolescents (Frank & Kong, 2008; Van Den Bos et al., 2012).

It must be considered that the still maturating neurophysiological connectivity in adolescents had a vital impact on the observed punishment sensitivity in previous studies. Further, neurophysiological findings in humans and animals rather suggest an increase in dopaminergic processing from childhood to adolescence (see Developmental changes from childhood through adolescence to adulthood

). Dopamine availability possibly peaks during late adolescence/young adulthood and declines again in older adults (65 to 75 years) (Hämmerer et al., 2011). The ability for better punishment learning during childhood or late adulthood should thus be enhanced, since dopamine concentration may be lower compared to adolescence. Unmedicated Parkinson's disease patients with a pathologically reduced dopamine level also demonstrated better punishment learning and punishment avoidance, respectively (see The basal ganglia Go-NoGo model) (Frank et al., 2004; Frank & Hutchison, 2010). Since we examined adolescents and young adults, no difference was expected.

Accordingly, the nonsignificant and only descriptively observed better punishment performance in adolescents in Study III might be connected to probably lower dopamine concentrations which sensitize punishment learning and thereby improve punishment avoidance (see Developmental changes from childhood through adolescence to adulthood

). In general, the reward processing system of young adults seems to lead to a better performance in reinforcement learning, which was also supported by the results of Study III. A reason for this could be the differentiated and matured neurophysiology especially between the medial prefrontal cortex and the striatum in adults compared to adolescents and children (Van Den Bos et al., 2012).

Neurophysiological differences

From childhood through adolescence into adulthood structural and functional brain maturation is in continuous progress. The human brain areas maturate heterochronously from posterior to anterior. Thus, the prefrontal cortex maturates until early adulthood (Liu et al., 2012). With increasing age, a decrease in cortical grey matter volume and thickness and an increase in cortical white matter volume until mid to late adolescence have been observed (Mills et al., 2016; Tamnes et al., 2017). Subcortical gray matter structures did not show a consistent development. Whereas the volume of the caudate nucleus, putamen, and nucleus accumbens decreased with increasing age from childhood to early adulthood, the volume of the

hippocampus, amygdala, globus pallidus, and cerebellum demonstrated an inverted U-shaped trajectory during maturation (Wierenga et al., 2014) (Figure 5). Studies often report heterogeneous findings between the subjects. Besides age-related development, brain maturation also seems to be affected by genetic predispositions, environmental factors (like social factors: family, friends, school), gender or nutrition, and also pubertal stage, which cannot be equated with chronological age (Foulkes & Blakemore, 2018).

In Study III, despite worse reward learning capacity and transfer performance of both reward and punishment learning, adolescents tended to have a greater neuronal activity by a more negative FRN peak compared to adults regardless of the intervention group (stress or control group). This finding is consistent with previous studies investigating reinforcement learning across the lifespan. Two previous studies, which conducted a probabilistic reward learning task with children (9 – 12 years old), adolescents (13 – 14 years old), younger adults (20 – 30 years old), and older adults (65 – 75 years old), also reported decreasing FRN peaks with increasing age in non-stressed participants from childhood to older adulthood (Eppinger et al., 2009; Hämmerer et al., 2011). However, Santesso and colleagues (2011) examined the FRN in response to positive and negative feedback in a card gambling task and did not find any differences between late adolescents (16 – 17 years old) and young adults (18 – 29 years old) (Santesso et al., 2011). Eppinger (2009) and Hämmerer (2011), who did not investigate mid to late adolescence, observed age-related FRN differences, whereas Santesso et al. (2011), who only investigated late adolescents, did not observed age-related differences in the FRN amplitude (Eppinger et al., 2009; Hämmerer et al., 2011; Santesso et al., 2011). Age-related differences in reward-related neurophysiological feedback are probably rather measurable between childhood/early adolescence and early adulthood than between middle/late adolescence and early adulthood. Brain maturation, which is already more advanced during late adolescence, presumably results in fewer differences in the transition period between late adolescence and young adulthood.

Studies that have used neuroimaging such as fMRI also highlighted both late adolescence and early adulthood as an efficient phase for feedback learning. Peters and Crone (2017) investigated striatal activity in response to reward in a longitudinal study with a feedback learning task. They found that striatal activity to feedback peaked during late adolescence/early adulthood (between 17 and 20 years). Furthermore, with increasing age, stronger striatal activity to negative feedback in the caudate nucleus and the nucleus accumbens was observed (S Peters & Crone, 2017) (Figure 5). Also, an investigation of the reward-prediction error in a probabilistic

learning task showed a striatal peak during adolescence (14 to 19 years old) compared to childhood (8 to 12 years old) and adult (25 to 30 years old) age (Cohen et al., 2010). In the hippocampus, which is primarily relevant e.g. for memory consolidation (see Physiology and signaling of dopamine), an increased prediction error-related blood oxygenation level dependent signal during surprisingly positive versus negative feedback in adolescents compared to young adults could also be observed (Davidow et al., 2016; Jackson & Westlind-Danielsson, 1994). Further, during reinforcement, the hippocampus and the putamen showed a stronger functional connectivity in adolescents but not young adults (Davidow et al., 2016). The connectivity correlated positively with the extent to which memories for positive reinforcing events were increased (Davidow et al., 2016). In both, adults and adolescents, bilateral amygdala and nucleus accumbens demonstrated a stronger activation during reward receipt than reward omission, but there was a stronger activation of the amygdala in adults and of the nucleus accumbens in adolescents (Ernst et al., 2005; Galvan et al., 2006).

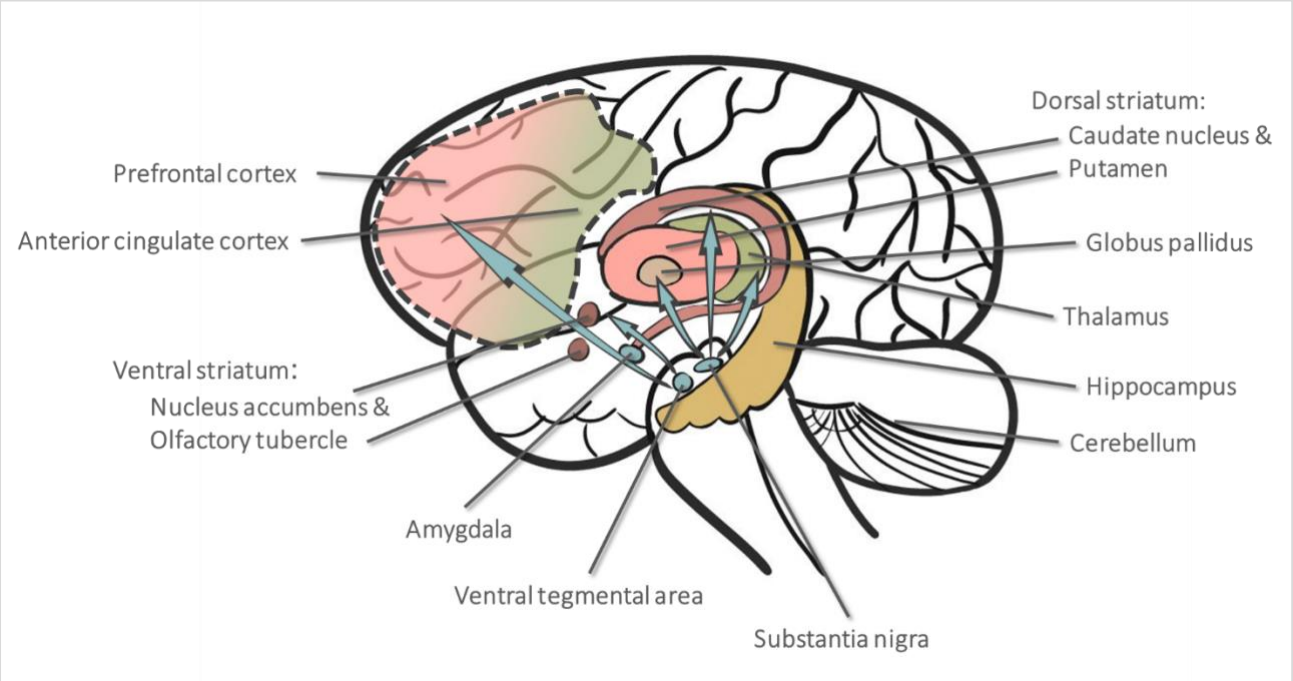

Figure 5: Two-dimensional illustration of important reward-related brain areas

Two major pathways from the ventral tegmental area and substantia nigra project dopamine neurons into different regions of the brain (see Neurophysiological projections). The dorsal striatum (caudate nucleus and putamen), the thalamus, and the globus pallidus receive dopamine projections from the substantia nigra (Maia & Frank, 2011; Wahlstrom et al., 2010). The ventral tegmental area releases dopamine in the ventral striatum (nucleus accumbens and olfactory tubercle), the limbic structures (e.g. amygdala and hippocampus), and also the prefrontal cortex (Wahlstrom et al., 2010). (source: own illustration)

In sum, in line with our findings regarding the greater neuronal activity in adolescents than adults, the presented findings of mostly greater activation in striatal regions during adolescence compared to children and adults is probably an indication of a more active dopaminergic reward processing, which may support the dopamine-related hypersensitivity theory during adolescence (see also Ernst et al., 2005). However, relations between dopamine-related neurophysiology and behavior requires further examination.

Sex differences

In the second and third study (Study III), sex differences in reinforcement learning were specifically examined. Although, Study II failed to demonstrate an influence of sex on reinforcement learning, a general slowing of responses was found in male adolescents. The learning session of Study III also demonstrated no differences in the general choice of the better option between males and females. Solely in the selection of the more frequently rewarding stimulus of the pair with the most similar reward association (i.e., pair "EF" with a 60:40 reward association), male subjects performed better than females. Further, in the transfer phase of the task males selected the rewarding stimuli more often than the female participants and thus presented a better reward learning performance.

Regarding the results of Study III, it should be considered that the findings include both stressed and control participants. Moreover, the female adolescent control group was noticeably underrepresented (adolescent control group: male n = 9; female n = 6). Although the influence of the intervention was statistically controlled and showed no significant impact, the observed sex differences in reward learning of Study III could still be a result of the stress intervention combined with the imbalance of the group sizes. In adult humans, sex-specific reactions to stress have been demonstrated (Kluen et al., 2017). Tests using decision-making tasks during stressful situations showed hormonal (stronger cortisol response of girls than boys, and neurophysiological (e.g. reduced prefrontal activation in adolescent boys compared to girls; greater blood oxygenation level dependent signals in men compared to women in the follicular–midcycle menstrual phase in anterior cingulate cortex, orbitofrontal cortex, medial prefrontal cortex) sex differences in adolescent and adult participants (J. Goldstein et al., 2010; Hollanders et al., 2017; Uy & Galván, 2017). It has been assumed that girls and boys may also react differently to different types of stressors (physiological vs. social), like women and men (Bale & Epperson, 2015). In Study III, no behavioral effect of stress or the stress-related rise in cortisol

on reinforcement learning was found. But, as supposed, a greater stress perception of female compared to male participants became evident which also could be a reason why male participants demonstrated a better reward learning performance.

Studies investigating cognitive inhibition demonstrated a better task performance of males than females (Halari et al., 2005; Halari & Kumari, 2005). The observed slower response time of males may have also resulted from greater cognitive inhibition. Halari and Kumari (2005) assumed that there is a sex-dependent relation between a better cognitive inhibition of adult males and the processing in prefrontal areas but they were unable to find a connection (Halari & Kumari, 2005). Animal studies suggested a sex difference in prefrontal cortex maturation, which is probably originated from sex hormones (McEwen & Milner, 2017). Especially, the different progress of the maturation of prefrontal cortex of male and female adolescents (see Neurophysiological differences) might have been a possible reason for differences in response time and learning behavior of males and females. Yet, this needs to be further assessed in future studies, since no difference in response time was found between boys and girls in the second study (Study II).

Unlike the better reward learning capacity of adolescent and adult males in the transfer phase of Study III, a previous study that used the same probabilistic reward learning task observed a better reward learning performance in female adults compared to males (Evans & Hampson, 2015). However, Evans and Hampson (2015) also did not find a sex difference in punishment learning or during the learning phase (Evans & Hampson, 2015).

Whereas sex differences in response to reward or punishment could not be observed in a monetary incentive task (Warthen et al., 2020), women reported a higher sensitivity to punishment in the *Sensitivity to Punishment and Sensitivity to Reward* Questionnaire, whereas men reported a higher sensitivity to reward (Dhingra et al., 2021). Also, young and middle-aged adolescent males reported a higher sensitivity to reward in the same questionnaire compared to female peers (Chahal et al., 2021). In a recent study with rats, a faster punishment avoidance learning of female than male rats occurred, but no sex differences emerged in reward learning (Chowdhury et al., 2019). Different from the results from Evans and Hampson (2015), these findings rather support our finding of a better reward learning capacity of male participants and concurrently indicate a probably greater sensitivity to punishment learning in females.

Differences between the adult human male and female dopamine system were researched in some studies by using positron emission tomography or single-photon emission computed tomography (see also Woodcock et al., 2020). This method allows to image physiological

functions via the tracking of radioactive tracers in the human body. Here, women presented a significantly higher striatal dopamine synthesis capacity (Ernst et al., 1998; Laakso et al., 2002) and lower D2-receptor affinity in the left striatum (Pohjalainen et al., 1998). One study observed a greater female dopamine D2-like receptor binding potential in the anterior cingulate cortex (Kaasinen et al., 2001), another reported the opposite in the frontal cortex (Glenthoj et al., 2006). Nevertheless, the most studies could not observe sex differences in the dopamine binding potential (e.g. Munro et al., 2006; Pohjalainen et al., 1998) or receptor density (Farde et al., 1995; Pohjalainen et al., 1998). In contrast, the majority of studies observed a significantly higher evoked dopamine release in the ventral striatum of males compared to females (e.g. Munro et al., 2006; C. Smith et al., 2019).

Altogether, no clear assertions on dopamine availability in males and females can be derived from previous findings. Most studies, which examined differences in reward and punishment learning as an indirect indicator of dopamine or actual physiological dopamine processing between males and females, did not control for endogenous hormone concentrations. This is a great limitation of comparative studies between the sexes, and particularly so since female punishment and reward learning ability has repeatedly been demonstrated to depend on the menstrual cycle and thus variations in sex hormones (see Diekhof et al., 2020; Diekhof & Ratnayake, 2016; Reimers et al., 2014). In our studies (except in Study I), we included endogenous hormone concentrations.

Hormonal impact

An association of sex hormones on reinforcement learning was shown in the second and a tendency in the third study (Study III). Higher estradiol in adolescents was associated with significantly faster responses to the fast clock at the beginning of the clock task in Study II. Moreover, a higher testosterone concentration was related to a generally slower response time, but a separate consideration of the sexes showed that this influence derived from the boys. In the third study (Study III), no correlations between the morning concentration of salivary hormones (estradiol, progesterone, testosterone, and cortisol) and neurophysiological activity during reward or punishment receipt was observed. Nevertheless, a supportive influence of estradiol on reward learning and an impairing impact of testosterone on reward and avoidance learning became evident in the exploratory analysis. Also, the cortisol increase during the TSST

of Study III was related to a more pronounced negative peak to negative feedback, and this effect seemed to originate in the stress group.

The results from studies two and three are consistent with previous findings by Diekhof and colleagues (Diekhof, 2015; Diekhof et al., 2020; Diekhof & Ratnayake, 2016; Reimers et al., 2014) and confirm our hypothesis of a support of estradiol on Go-learning. Therefore, the ability to adapt to the fast clock and reward learning is probably improved by higher estradiol concentrations in both sexes, which may presumably reflect enhanced dopaminergic transmission (see The impact of estradiol and progesterone). Because of the lack of publications regarding the impact of testosterone on reinforcement learning, it was not possible to rely on previous findings. Testosterone is higher in human males than in females and this sex difference starts during adolescence (Morley et al., 1997). Hence, it was expected that testosterone would have a greater impact on male adolescents of Study II, which was indeed observed. An influence of testosterone on dopaminergic and serotoninergic processes has already been assumed, yet no concrete influence on reinforcement learning has been demonstrated (de Souza Silva et al., 2009). This is in contrast to the domains of reward-related risk-taking and impulsivity, for which findings on the association with testosterone already exist. These findings support the assumption that testosterone can positively modulate dopaminergic processes (Apicella et al., 2014; Stanton et al., 2011) (see Risk-taking and Male risk-taking in the Balloon Analog Risk Task). As discussed in CHAPTER THREE, a link between the observed worsening in learning behavior during states of high endogenous testosterone was also related to social status maintenance (Mehta & Prasad, 2015). The observations from studies two and three indicate that enhanced testosterone may promote an adverse dopamine ratio. Thus, both the slowing in response time observed in Study II and the worse learning performance in Study III could be indicative of a lower dopamine concentration.

In this thesis, only a few hormonal interactions with the task performance were observed. Two previous studies, which examined the influence of blood sex hormone levels of estradiol, progesterone, testosterone, luteinizing hormone, and follicular stimulating hormone on cognitive performance, did not find any interaction between hormone concentrations and task performance of cognitive tasks (e.g. verbal, spatial or inhibition tasks) with adult participants (Halari et al., 2005; Halari & Kumari, 2005). In early adolescents, more recent studies investigated the impact of salivary dehydroepiandrosterone, testosterone, and estradiol on the performance (measured by accuracy and reaction time) in a reward cue processing task and also found no associations (Ladouceur et al., 2019; White et al., 2020).

Furthermore, the correlation between a more pronounced negative FRN and the increased cortisol level in Study III is in line with findings from Glienke et al. (2015) and Paul et al. (2019) that reported a greater FRN peak with increasing cortisol and the physiological-induces stress level (via cold-pressor test) (Glienke et al., 2015; Paul et al., 2019). Previous studies showed that sex hormones influence cognitive and neurophysiological development (Peper et al., 2011). Thus, it has been assumed that the FRN differ depending on sex hormone concentrations, which could not be observed.

Besides endogenous steroid hormone concentrations, most studies did not include menstrual cycle data of the female participants like it was done by Diekhof and colleagues (Diekhof, 2015; Diekhof et al., 2020; Diekhof & Ratnayake, 2016; Reimers et al., 2014), which could indirectly inform about current hormonal state. Moreover, different task designs to investigate cognition and reinforcement learning and varied measurements of endogenous hormones could be a reason for the heterogenous findings and even null-findings in some studies. Another limitation is the measurement of steroid hormones. Studies, which examined salivary hormones report the impact of unbound and thereby bioactive hormones. Bioactivity means that the measured hormone level represents the number of hormone concentrations, which can enter cells and bind to receptors (Herting & Sowell, 2017). Blood hormone measurements, however, report the completeness of unbounded and bounded hormone concentrations, whereby less than 2% of the steroid hormones are bioactive (Dunn et al., 1981). Therefore, it is recommended that in blood samples, besides hormone concentrations, also the plasma transport protein concentrations (see Steroid hormones and reward processing) should be analyzed to be able to extrapolate the ratio of unbounded to bounded hormone levels, which is rarely done. Analyzing blood hormone concentrations provide the opportunity to determine low hormone concentrations during prepuberty or in early adolescents, where hormone concentrations are still low and unbound hormone levels are sometimes below the detection limit (Herting & Sowell, 2017). But at the same time, the collection of blood samples is an invasive method, unlike saliva collection, and thus can act as a physiological stressor, which should be avoided when testing children and adolescents.

Male risk-taking in the Balloon Analog Risk Task

Previous studies, which used the Balloon Analog Risk Task observed a relation between a dual-hormone profile with high testosterone and simultaneously low cortisol concentrations and

enhanced risk-taking behavior in adults (Dekkers et al., 2019; Mehta, Welker, et al., 2015). The dual-hormone hypothesis regarding endogenous testosterone and cortisol has not been examined in adolescents before. In this thesis, the impact of baseline testosterone and cortisol on male adolescent and young adult risk-taking was examined. Thereby, four main results were documented in Study IV. First, in line with previous studies, increased testosterone was related to a heightened risk-taking propensity (e.g. Apicella et al., 2014; Peper et al., 2013). Second, between age and risky decisions, a quadratic relation was observed. Accordingly, risk-taking seemed to be highest during the early to mid-20s, but declined towards higher age and was also lower during adolescence. Third, the dual-hormone profile with high testosterone and low cortisol level presumably supported a greater computationally modeled risk-propensity, whereas the consideration of the number of pumps and pops was not associated with this testosterone-cortisol interaction and risk-taking. However, a visual consideration suggested a possible connection between a dual-hormone profile and riskier decisions.

Peper and colleagues (2013) hypothesized that an enhanced testosterone concentration leads to more sensation seeking and risk-taking behavior in boys (Peper et al., 2013). Besides the assumed positive influence of testosterone on dopamine availability (see The impact of testosterone), testosterone may also promote reward-related risk-taking by its effect on the associated neurophysiological connectivity. A study that examined risk-taking, as indicated by adolescent alcohol consumption, found a negative effect of enhanced testosterone on amygdala and orbitofrontal cortex connectivity, which was associated with an increased alcohol intake in boys. By diminishing this connectivity alcohol might lead to a reduced cognitive top-down control of behavioral impulses and may thereby increase risky behavior (Sabine Peters et al., 2015). In turn, testosterone was found to increase the connectivity between the ventromedial prefrontal cortex, anterior insula, and temporo-parietal cortex during an auction task in another study and the stronger connectivity between these brain regions resulted in an increased status-seeking behavior (Van Den Bos et al., 2013). These two studies demonstrate representatively that possible neurophysiological modes of action influenced by hormonal parameters need further investigations.

Comparing results regarding risk-taking in adolescents and adults revealed ambiguous findings. Some studies described increased risk-taking in adolescents compared to adults, while others did not find any differences between age groups (Defoe et al., 2015; Shulman et al., 2016). Meta-analytic results regarding the willingness to take risks in various laboratory tasks indicate that during adolescence immediate outcome feedback may lead to more risk-taking compared to

adulthood. This behavior may probably be enforced by a greater reward drive, when assuming a hypersensitive dopamine system during adolescence (Defoe et al., 2015; Galván, 2014). For tasks with temporally delayed feedback no differences in risk-taking were observed when comparing adolescents and adults (Defoe et al., 2015). In addition to that, ongoing brain maturation of the prefrontal cortex circuitry and limbic structures may affect developmental differences in risk-taking behavior, inter alia by affecting cognitive control and reward-drive (Li, 2017; Walker et al., 2017). A developmental mismatch of the fine tuning of the connection between prefrontal cortex and subcortical structures, also due to the heterochronous maturation of the human brain, is additionally considered as a possible reason for riskier behavior (Casey et al., 2008).

The results of Study IV supply initial evidence that already during adolescence the interaction of salivary testosterone and cortisol concentration seemed to affect risk-taking behavior in boys. In adult men and women this has been observed previously with larger effect sizes for men than women (Dekkers et al., 2019). A subsequent study should also assess brain activity during risky decisions, reward anticipation, and reward receipt to examine the impact of dopamine-related neurophysiological structures. Moreover, also girls and pre-adolescents should be included as participants due to previously reported differences of reward processing in children, adolescents and adults. The measurement of hormonal concentrations and neurophysiological activity during the transition from childhood to adolescence may contribute to a better understanding of steroid hormone effects on reward-related risk-taking.

Conclusion and future perspectives

The thesis explored on the association between steroid hormones and reward-related behavior in adolescents and young adults. For this, various decision-making tasks were used to investigate aspects of reinforcement learning and risk-taking. It was observed that endogenous hormone concentrations affect reward-related behavior already during adolescence. Not only an influence of individual sex hormones on behavior was demonstrated, but also the interaction of the steroid hormones, testosterone and cortisol.

However, the observed relations between behavior and basal endogenous hormone concentrations, which were mostly assessed from morning samples, should, if possible, be complemented in subsequent studies by samples during the test. In addition, to gain better insight into the interactions between hormones and neurophysiological processes during

reward-related decision-making, it may be useful to use further neuroimaging techniques such as fMRI that could reach a deeper understanding of brain structure and function.

Unfortunately, data collection of Study III could not be finished due to the onset of the coronavirus pandemic in early 2020. Therefore, the female control group was too small and potentially under-powered, while impeded an appropriate comparison of the control and stress group.

Because of the difficulty in recruiting adolescents, more transnational collaborative projects should be strived, in which larger subject groups of adolescents, but also children and older adults, can be evaluated. Thereby, the statistical power and informative value of the analyses could probably be enhanced. Moreover, the phases of pubertal development (early, middle, and late adolescence) supposedly all represent important stages of cognitive, neurophysiological, and hormonal maturation and the associated developmental trajectories should be examined in more detail. Especially the stages of psychological and cognitive development (e.g. measured by the pubertal development), which demonstrated even more importance than age, should be addressed in the future (Herting et al., 2014; Wierenga et al., 2018).

Despite the few significant findings regarding the influences of stress and cortisol on cognition in the present thesis, which could be a result of the slightly under-powered sample of Study III, this topic should be investigated in more detail in particular with adolescents. A number of lifetime mental disorders (e.g., anxiety, mood disorders) begin at the time of puberty. Stress-mediated changes in mood and cognition during early adolescence may thereby play a central role, especially for girls (Marceau et al., 2014; S. Smith, 2013). Many aspects of later life are determined during adolescence, which will suffer from the detrimental effect of stress on social interactions or the tendency to resort to unhealthy coping mechanisms like increased consumption of alcohol or other drugs. The increased social interest in peers and the enhanced sensitivity for social isolation, as it occurred during the state-imposed “lockdown” as an anti-coronavirus-pandemic measure, represent a dangerous mixture, through which future development can be negatively primed. Social stress induced by negative peer interactions (e.g., bullying) or failure at school can trigger maladaptive social avoidance, behavioral inhibition, higher vulnerability to addiction, anxiety- and depression-like symptoms, and may even affect immune regulation (Snyder-Mackler et al., 2016). Therefore, besides reinforcement learning the impact of stress on reward-related behavior also during risky decision-making should further be investigated during the lifespan from childhood, through adolescence into adulthood.

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Declaration

Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resources and aids.

Hanstorf, 10.04.2022

Date and Place

Signature
Sina Kohne

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