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PhD Thesis

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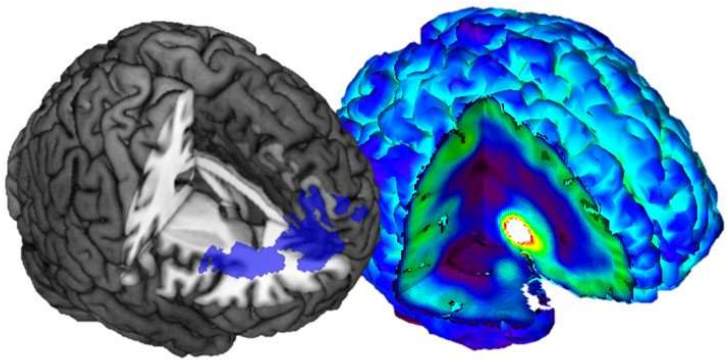
# Multimodal Neuroimaging of Aggression in Violent Offenders



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**Title:** Multimodal Neuroimaging of Aggression in Violent Offenders

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# Dansk Resumé

Personer med impulsiv-aggressiv adfærd bliver ofte involveret i voldelige, kriminelle handlinger med personskade og udgør en betydelig samfundsmæssig belastning. Man har kun delvist afdækket hvilke forhold i hjernen, der er af betydning for aggressiv adfærd; denne viden kommer fortrinsvis fra dyreforsøg, der tyder på, at serotonin 1B ($5\text{-}HT_{1B}$) receptoren er involveret.

Formålet med PhD-afhandlingen er at identificere de neurobiologiske mekanismer ved aggression. Fra fængsler rekrutteredes 19 mænd dømt og fængslet for grov, personfarlig kriminalitet og de blev sammenlignet med 24 lav-aggressive mænd. Alle blev undersøgt neuropsykologisk og med funktionelle (fMRI) og molekylære (PET) hjerneskanninger.

Under fMRI-skanningen blev forsøgspersonerne sat til at spille et spil, hvor en modstander fra tid til anden stjal penge fra dem (provokationer). Vi så her, at de voldsdømte mænd havde øget hjerneaktivitet i amygdala og striatum, når de blev provokeret, og denne aktivitet var højest hos de, der var mest aggressive. Under provokationerne havde de voldsdømte også en ringere korrespondance mellem amygdala/striatum og præfrontal cortex i forhold til kontrollerne. Ved PET-skanningen så vi, at der i striatum var en positiv association mellem $5\text{-}HT_{1B}$ receptorbinding og aggressionsniveau, men denne sammenhæng var langt mere udtalt hos de voldsdømte end hos kontrollerne. Ved at kombinere de to metoder kunne vi i den samlede gruppe påvise, at hjernens $5\text{-}HT_{1B}$ receptorbinding var positivt korreleret med såvel aggressionsniveau som reaktivitet i amygdala og striatum under provokationer.

I afhandlingen påviser vi for første gang, at $5\text{-}HT_{1B}$ receptoren er forbundet både med graden af aggression og med amygdala og striatums reaktion på provokationer. De voldsdømte mænd adskilte sig således ved at være særligt sensitive for provokationer. Vores fund tyder på, at $5\text{-}HT_{1B}$ receptoren repræsenterer en interessant mulighed for ad farmakologisk vej at reducere den overdrevne reaktion i amygdala og striatum under provokationer.

# Thesis summary

Aggression underlies many criminal and antisocial behaviours and is thus a serious concern for society. An understanding of the neurobiological mechanisms involved in aggression is essential for developing effective treatments and thereby preventing societal violence. Accumulating evidence from human neuroimaging studies has identified neural circuits and neurotransmitters involved in human aggression. In spite of this progress, the specific molecular mechanisms in humans remain unknown. Preclinical research strongly suggests that serotonin 1B (5-$HT_{1B}$) receptors are involved in impulsive and aggressive behaviour.

The overall aim of the work presented in this thesis was to uncover neurobiological mechanisms involved in human aggression by the use of functional neuroimaging. Specifically, we aimed to examine the role of 5-$HT_{1B}$ receptors and the neural correlates of reactive aggression using a laboratory measure of aggressive behaviour. The thesis is based on three functional neuroimaging studies that have been undertaken in a cohort of 19 incarcerated violent offenders and 24 healthy control subjects.

We used positron emission tomography (PET) for quantification of 5-$HT_{1B}$ receptors and functional magnetic resonance imaging (fMRI) for evaluation of brain activity when the participants were provoked (monetary subtractions by a fictitious opponent) in the scanner. Self-report questionnaires were used for assessment of trait aggression, trait anger, trait psychopathy and impulsivity. We also evaluated level of psychopathy clinically.

The main findings of the studies were:

1) Violent offenders had heightened brain activity within the amygdala and striatum when they were provoked (compared with controls).
2) Violent offenders had reduced functional connectivity between the amygdala/striatum and the prefrontal cortex during provocations (compared with controls).
3) In violent offenders, striatal 5-$HT_{1B}$ receptor binding was positively correlated with trait anger, trait psychopathy and level of psychopathy according to clinical evaluation.
4) 5-$HT_{1B}$ receptor binding was positively associated with amygdala and striatal reactivity to provocations and with trait anger (across all participants).

These findings support that impulsive aggression may arise from combination of heightened “limbic” reactivity or reduced frontolimbic connectivity in the context of provocative stimuli. The results also show for the first time that 5-$HT_{1B}$ receptor availability is involved in human anger and psychopathy.

Given that violent offenders had increased amygdala and striatal reactivity to provocations, reduction of aggressive and violent behaviours may be achieved by interventions that can reduce this heightened neural responsiveness. 5-$HT_{1B}$ receptors may represent a molecular target for reducing excessive amygdala and striatal reactivity to provocative stimuli.

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*Sofi da Cunha-Bang*, Copenhagen, April 2016

# List of manuscripts

1. **da Cunha-Bang S,** Fisher PM, Perfalk E, Skibsted AP, Hjordt LV, Bock C, Baandrup AO, Thomsen C, Sestoft D, Knudsen GM. Violent offenders respond to provocations with high amygdala and striatal reactivity. *Human Brain Mapping*, under review.

2. **da Cunha-Bang S**, Hjordt LV, Perfalk E, Beliveau V, Bock C, Lehel S, Thomsen C, Sestoft D, Svarer C, Knudsen GM. Serotonin 1B receptor binding is associated with trait anger and level of psychopathy in violent offenders. *Biological Psychiatry*, in press.

3. **da Cunha-Bang S,** Fisher PM, Hjordt LV, Perfalk E, Knudsen GM. Men with high serotonin 1B receptor binding have high trait anger and respond to provocations with heightened amygdala and striatal reactivity.

**Related papers:**

**da Cunha-Bang S,** Mc Mahon B, Fisher PM, Jensen P, Svarer C, Knudsen GM. High trait aggression in men is associated with low 5-HT levels, as indexed by 5-HT4 receptor binding. *Soc Cogn Affect Neurosci*. 2016 Jan 15.

**da Cunha-Bang S**, Stenbæk DS, Holst K, Licht CL, Jensen PS, Frokjaer VG, Mortensen EL, Knudsen GM. Trait aggression and trait impulsivity are not related to frontal cortex 5-HT2A receptor binding in healthy individuals. *Psychiatry Res.* 2013 May 30;212(2):125-31.

Skibsted A, **da Cunha-Bang S**, Carré J, Hansen AH, Beliveau V, Knudsen GM, Fisher PM. Aggression-related brain function assessed with the point subtraction aggression paradigm in functional magnetic resonance imaging. *Submitted.*

# Abbreviations

| | |
|---|---|
| **5-HT** | 5-hydroxytryptamine, Serotonin |
| **BOLD** | Blood Oxygen Level Dependent |
| **DSM-4** | Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition |
| **DSM-5** | Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition |
| **fMRI** | Functional magnetic resonance imaging |
| **GABA** | Gamma-Aminobutyric Acid |
| **HRRT** | High Resolution Research Tomograph |
| **ICD-10** | International Classification of Diseases |
| **IQ** | Intelligence Quotient |
| **MAO-A** | Monoamine Oxidase A |
| **MNI** | Montreal Neurological Institute |
| **MRI** | Magnetic Resonance Imaging |
| **MRTM2** | Multilinear Reference Tissue Model |
| **PET** | Positron Emission Tomography |
| **PSAP** | Point Subtraction Aggression Paradigm |
| **SPM8** | Statistical Parametric Mapping for fMRI |

# Introduction

Aggression is present across species and evolutionary explanations for human aggression include resource competition, survival and reproduction (Archer 2009). So although aggression is a deeply grounded part of humanity, certain expressions of aggression are not considered socially acceptable. In a scientific context aggression has been defined as "behaviour directed toward another individual with the immediate intent to cause harm, while the target is motivated to avoid such behaviour" (Bushman and Anderson 2001). This form of aggression is considered illegal in most countries. Aggression underlies costly criminal and antisocial behaviours and is thus a serious concern for society. Equally important, acts of aggression have detrimental consequences for the victims, relatives and often also for the perpetrators.

Accumulating evidence from human neuroimaging studies have identified neural circuits and neurotransmitters involved in human aggression. In spite of this progress, the specific molecular mechanisms remain unknown. Improved knowledge of the neurobiological mechanisms underlying aggressive behaviour will aid our understanding of the human brain in general and is of central importance in the development of specific pharmacological treatment, in strategies for effective prevention of violence and may potentially also complement psychological assessments in forensic risk assessments.

## Aggression and Related Disorders

Aggression is traditionally divided into two major subtypes, impulsive (reactive) and premeditated (instrumental) aggression. Impulsive aggression is triggered by a provocative or threatening stimulus whereas premeditated aggression is goaloriented and deliberate. It has been proposed that distinct neural mechanisms underlie these types of aggression; impulsive aggression engages "limbic" structures whereas premeditated aggression is less dependent on limbic regions and more regulated by higher cortical regions (Nelson and Trainor 2007). Impulsive aggression is often accompanied by anger, which is an emotion that can be triggered by a variety of factors. The experience of anger is subject to interindividual variability (Gilam and Hendler 2015).

Aggression can be conceptualised as a personality trait within a normal spectrum, but can also be defined as pathological when it is "exaggerated, persistent or expressed out of context" (Nelson and Trainor 2007). Pathological aggression is present across several psychopathologies including intermittent explosive disorder, borderline personality disorder, antisocial personality disorder and psychopathy. The presence of the aggressive subtypes varies across these psychopathologies. Patients with intermittent explosive disorder and borderline personality disorder display mainly impulsive aggression (Coccaro, Sripada et al. 2011) whereas individuals with psychopathy are at risk for both impulsive and premeditated aggression (Blair 2010).

## Psychopathy and Antisocial Personality Disorder

Psychopathy is characterized by antisocial behavior along with deficiencies in interpersonal functioning and affect, including for example manipulation, deception, lack of empathy, guilt and remorse, shallow affect, impulsivity, aggression, stimulation seeking and irresponsibility (table 1). Although psychopaths often cover up these deficiencies with superficial charm, it prevents them from forming stable relationships, learning from their mistakes and emphasizing with others (Kiehl 2010). "Their brains process information differently from those of other people. It's as if they have a learning disability that impairs emotional development" (Kiehl 2010). Psychopaths represent approximately 1-2% of the general population and approximately 15-35% of prisoners in the United States (Kiehl 2010). As opposed to antisocial personality disorder, psychopathy is not specifically recognized in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) or in the International Classification of Diseases (ICD-10). Antisocial personality disorder and psychopathy are closely related. Criteria for antisocial personality disorder focus more on the antisocial behaviours and less so on the interpersonal/affective domains that characterize psychopathy. A personality disorder is an "enduring pattern of inner experience and behavior that deviates markedly from the expectations of the individual's culture, is pervasive and inflexible, has an onset in adolescence or early adulthood, is stable over time, and leads to distress or impairment" (DSM-5 2013). The essential feature of antisocial personality disorder is disregard for, and violation of, the rights of others, and is defined by the following criteria (according to DSM-5):

A) A pervasive pattern of disregard for and violation of the rights of others, occurring since age 15 years, as indicated by three (or more) of the following:

1. Failure to conform to social norms with respect to lawful behaviors, as indicated by repeatedly performing acts that are grounds for arrest.
2. Deceitfulness, as indicated by repeated lying, use of aliases, or conning others for personal profit or pleasure.
3. Impulsivity or failure to plan ahead.
4. Irritability and aggressiveness, as indicated by repeated physical fights or assaults.
5. Reckless disregard for safety of self or others.
6. Consistent irresponsibility, as indicated by repeated failure to sustain consistent work behavior or honor financial obligations.
7. Lack of remorse, as indicated by being indifferent to or rationalizing having hurt, mistreated, or stolen from another.

B) The individual is at least age 18 years.

C) There is evidence of conduct disorder with onset before age 15 years.

D) The occurrence of antisocial behavior is not exclusively during the course of schizophrenia or bipolar disorder.

(DSM-5 2013)

## Assessing Aggression and Related Constructs

When conceived as traits (stable personality characteristics), aggression and related constructs (anger, impulsivity and psychopathy) can be measured by self-report questionnaires. Numerous questionnaires are available for assessment of trait aggression, anger, psychopathy and impulsivity (see methods section). However, self-report measurements have disadvantages such as subjectivity and social desirability that can influence response style. There is a risk of measuring the individual's self-perception of the construct instead of measuring the actual trait. Therefore, it is beneficial to also use objective measures of aggression, for example clinical instruments or laboratory tests designed to elicit a measurable aggressive response. The goldstandard clinical instrument assessing level of psychopathy is the Psychopathy Checklist-Revised (PCL-R). The PCL-R is based on a semistructured interview and collateral information from criminal files. It consists of 20 items that are scored on three-point scale from 0 to 2. A score of 2 indicates presence of the item, 1 indicates that the item may be present and 0 indicates that the item does not apply, yielding a maximum of 40 (table 1). The scale can be used dimensionally indicating the level of psychopathy, or categorically in which a score of 30 or above classifies a psychopath (Hare 2003).

***Table 1.*** *PCL-R Items.*

| **Factor 1: Interpersonal/Affective** | **Factor 2: Social deviance** |
|---|---|
| • Glibness/superficial charm | • Need for stimulation/proneness to boredom |
| • Grandiose sense of self-worth | • Parasitic lifestyle |
| • Pathological lying | • Poor behavioural controls |
| • Conning/manipulative | • Early behavioural problems |
| • Lack of remorse or guilt | • Lack of realistic long-term goals |
| • Shallow affect | • Impulsivity |
| • Callous/lack of empathy | • Irresponsibility |
| • Failure to accept responsibility of own action | • Juvenile delinquency |
| | • Criminal versatility |
| ***Additional items*** | |
| Promiscuous sexual behavior | |
| Many short-term marital relationships | |

The two most commonly used laboratory measures of aggression are the Taylor Aggression Paradigm and the Point Subtraction Aggression Paradigm (PSAP). In both paradigms the participants play with a fictitious opponent. The Taylor Aggression Paradigm is a competitive reaction time task in which the winner gets to punish the loser with an aversive stimulus, for example an electric shock or a loud noise. Aggression in the Taylor Aggression Paradigm is defined by the severity of pain the participant inflict on the opponent. The PSAP is a game in which the participants can pursue monetary rewards by

continuously pressing a button. Throughout the game the opponent repeatedly steals money with the aim to provoke the participant. The participant can choose to remove money from the opponent, but is not allowed to keep this money. Thus, this action reflects an aggressive behaviour, void of any monetary incentive. The PSAP has been validated in behavioural studies, where aggressive individuals respond with aggression at a higher frequency than control subjects (Cherek, Moeller et al. 1997). Recently, both paradigms have been adapted for use in the context of functional magnetic resonance imaging (fMRI) (Kramer, Jansma et al. 2007; Lotze, Veit et al. 2007; Skibsted 2015).

Other tasks sometimes used to study aspects of aggression include the Ultimatum Game and Go/NoGo tasks. In the Ultimatum Game, a sum of money is given to two participants and one of the participants proposes how to split the money between them. The responder can choose to either accept the offer, whereby both will get their share of money, or reject the offer, whereby non of them will get any money. Rejecting unfair offers is sometimes interpreted as a form of provocation, but the paradigm is mainly used to index social decision making and perceived fairness (Gabay, Radua et al. 2014). Inhibitory control describes the cognitive ability to withhold prepotent and automatic actions and is often used as an index of impulsivity. Response inhibition can be measured with Go/NoGo paradigms in which participants respond to a frequent "Go" stimulus and restrain their response to an infrequent "NoGo" stimulus.

## Neural Circuits of Aggression

In parallel with the two distinct aggression subtypes in humans, animal aggression is often categorized as 'defensive rage' or 'predatory attack' behaviour (Gregg and Siegel 2001). Animal studies reveal a neural circuit comprising the hypothalamus, amygdala and periaqueductal grey in the defence response (Gregg and Siegel 2001; Nelson and Trainor 2007; Blair 2015). This was initially shown in electric stimulation studies in cats, where activation of neurons in the medial hypothalamus and midbrain periaqueductal grey mediated defensive rage, whereas activation of neurons in the lateral hypothalamus caused predatory attack (Gregg and Siegel 2001). Whereas classical electrical stimulation methods activate passing axons, the recent introduction of optogenetic techniques allows for activation of only the neuronal cell bodies. Optogenetic experiments confirm the role of hypothalamus, amygdala and prefrontal cortex in aggression, identifying the location more precisely. Stimulation (referring to optogenetic stimulation) of the ventrolateral subdivision of the ventromedial nucleus of hypothalamus causes male mice to attack both female mice and non-animal objects (Lin, Boyle et al. 2011). Stimulation of gamma-aminobutyric acid (GABA) neurons in the posterior dorsal subdivision of the medial amygdala promotes aggression in mice (Hong, Kim et al. 2014). Also, stimulation of the medial prefrontal cortex (prelimbic and medial orbital cortex) in mice reduces aggression, whereas optogenetic silencing of the medial prefrontal cortex increases aggression (Takahashi, Nagayasu et al. 2014).

One theory argues that the neural circuit involved in the "acute threat response" in animals also mediates impulsive aggression in humans, not only to threat but also to frustration and social provocation (Blair 2004; Blair 2015). Human brain lesion studies recognize the involvement of the amygdala and prefrontal cortex in aggression; amygdalotomy has been used for treatment of refractory aggressive behaviours and patients with traumatic brain injury in the prefrontal cortex are more aggressive than patients with injuries in nonprefrontal regions (Heimburger, Whitlock et al. 1966; Mpakopoulou, Gatos et al. 2008; Pardini, Krueger et al. 2011). In addition to the amygdala and prefrontal cortex, human structural and functional MRI studies also suggest involvement of other brain regions in aggression, including the striatum (Glenn and Yang 2012; Kolla, Matthews et al. 2015), anterior cingulate cortex (Beyer, Munte et al. 2015) and the insula (Kramer, Jansma et al. 2007). The role of the hypothalamus and periaqueductal grey is less characterized in humans. An explanation for this may be that these regions are very small or difficult to visualise using existing neuroimaging techniques.

To examine neural circuits of human aggression in vivo, functional neuroimaging studies primarily use paradigms with threatrelated facial expressions, social exchange paradigms (e.g. the ultimatum game) and the Taylor Aggression Paradigm. Angry and fearful facial expressions signal presence of threat in the environment and robustly activate the amygdala (Fusar-Poli, Placentino et al. 2009). Impulsive aggressive patients diagnosed with intermittent explosive disorder show heightened amygdala reactivity to angry faces (Coccaro, McCloskey et al. 2007). In contrast, individuals with psychopathy show reduced autonomic responsiveness to distress cues (fearful or sad faces) (Blair, Jones et al. 1997) and reduced amygdala activity when processing affective words (Kiehl, Smith et al. 2001), indicating that psychopaths are hyporesponsive to emotional stimuli. Indeed, one theory holds that amygdala responding can serve to distinguish instrumental forms of aggression associated with antisocial personality disorder and psychopathy from reactive aggression, which is more characteristic of borderline personality disorder and intermittent explosive disorder (Coccaro, Sripada et al. 2011). It is also of importance to consider the distinction between threat- and frustration-related reactive aggression in the context of the neural mechanisms mediating aggressive responses; in contrast to frustrations, threats can trigger both fear and anger (Blair 2012). As such, it is argued that reactive aggression in psychopaths is based on frustration based as opposed to threatrelated (Blair 2012).

In the context of fMRI, the only paradigm to date directly tapping reactive aggression is the Taylor Aggression Paradigm. In healthy controls, provocations (high level of punishment by the opponent) in the Taylor Aggression Paradigm are associated with activity in the dorsal anterior cingulate cortex and dorsal striatum (Kramer, Jansma et al. 2007). Also, when healthy controls watch an opponent suffering while receiving punishment the medial orbitofrontal cortex is activated (Lotze, Veit et al. 2007). In a modified version of the Taylor Aggression Paradigm, the participants viewed their opponent with either neutral or angry facial expressions during punishment selection (Beyer et al. (2014). When the opponent was angry (versus neutral), activity in the medial orbitofrontal cortex correlated negatively with taskrelated aggressive behaviour (level of punishment), whereas activity in the dorsal anterior cingulate cortex was positively

correlated with punishment selection (Beyer, Munte et al. 2014). The study did not show any significant correlations between amygdala reactivity and aggression.

Getting unfair offers in the Ultimatum Game is sometimes interpreted as provocative stimuli and rejecting unfair offers a form of retaliation. Receiving unfair offers in the Ultimatum Game is associated with activity in the anterior insula, dorsomedial prefrontal cortex and the anterior cingulate cortex (Sanfey, Rilling et al. 2003), whereas punishing unfair offers activates the striatum (Strobel, Zimmermann et al. 2011). Moreover, periaqueductal grey activity is associated with both rejecting (Corradi-Dell'Acqua, Civai et al. 2013) and punishing unfair offers (White, Brislin et al. 2014).

Thus, existing neuroimaging studies implicate a network of brain regions responsive to social threatrelated stimuli, provocations or frustrations that includes the amygdala, striatum, periaqueductal grey, anterior cingulate and orbitofrontal cortex. These regions overlap considerably with regions involved in emotion regulation. Thus, it is suggested that impulsive aggression can arise because of faulty emotion regulation (Davidson, Putnam et al. 2000). A crucial issue is to understand how the implicated regions interact functionally. The dominant conceptual framework of how neural circuits regulate impulsive aggression is that the prefrontal cortex modulates, inhibits or ”puts brakes on” subcortical activity mediating the aggressive response (Nelson and Trainor 2007; Siever 2008; Blair 2015; Rosell and Siever 2015). That is, reduced prefrontal activity combined with heightened subcortical activity in the context of threatrelated or provocative stimuli poses an increased risk for impulsive aggression. Studies showing reduced functional connectivity between the amygdala and prefrontal regions in aggressive individuals putatively reflect such framework, although connectivity only indicates correlations between separate brain regions. For example, inmates fulfilling criteria for psychopathy have reduced connectivity between the amygdala and ventromedial prefrontal cortex during resting state fMRI compared with nonpsychopathic inmates (Motzkin, Newman et al. 2011). Also, youths with disruptive behaviour disorders have reduced amygdala-prefrontal connectivity during high provocation trials (unfair offers) in the Ultimatum Game (White, VanTieghem et al. 2015). As opposed to merely putting “brakes” on the acute threat response, Blair (2004, 2005) argues that the ventromedial prefrontal cortex rather allows representation of expected rewards and punishments associated with an action. Other brain regions then utilize this information to either initiate or inhibit aggression (Blair 2004; Blair 2015). According to this view, the costs and benefits of engaging in impulsive aggression are poorly represented in individuals with dysfunctions of the medial prefrontal cortex, and therefore they are at risk for aggressive behaviour.

## Neurobiology of the serotonin system

The majority of serotonin is produced in neurons located in the brainstem raphe nuclei. From here, serotonergic neurons project to most parts of the brain where its actions are mediated by 15 distinct receptors, grouped into seven receptor families (Bockaert, Claeysen

et al. 2006). Serotonin is synthesised from the amino acid tryptophan within the nerve cell and is transported in synaptic vesicles to the presynaptic terminal. Upon an action potential and calcium influx into the cell, the synaptic vesicle fuses with the cell membrane and serotonin is released to the synaptic cleft. Here it binds to available receptors located postsynaptically on other neurons or presynaptically as autoreceptors. Serotonin is then transported back into the neuron by proteins located presynaptically, the serotonin transporters. Autoreceptors include the 5-$HT_{1A}$ and 5-$HT_{1B}$, which in conjunction with serotonin transporters regulate serotonin levels in the synaptic cleft. After being transported back into the neuron by serotonin transporters, serotonin is degraded by the enzyme monoamine oxidase A (MAO-A) to 5-hydroxyindoleacetic acid.

## 5-$HT_{1B}$ receptors

5-$HT_{1B}$ autoreceptors provide negative feedback control of serotonin levels by regulating serotonin synthesis and release, as well as regulating serotonin transporter activity (Hagan, McDevitt et al. 2012). In addition to its function as an autoreceptor, 5-$HT_{1B}$ receptors are also located postsynaptically as heteroreceptors on axon terminals of nonserotonergic neurons. 5-$HT_{1B}$ auto- and heteroreceptors coexist in both the raphe nuclei and projection areas. 5-$HT_{1B}$ receptors are present at much lower levels than serotonin transporters and 5-$HT_{1A}$ receptors in the dorsal raphe, according examination of to human postmortem brain tissue (Varnas, Halldin et al. 2004; Varnas, Hurd et al. 2005). In the raphe nuclei of rats, 5-$HT_{1B}$ heteroreceptors are located on GABA interneurons (Bagdy, Kiraly et al. 2000). Here, serotonergic and GABAergic neurons synapse and form a reciprocal system regulating serotonin release locally and in projection areas (Bagdy, Kiraly et al. 2000). Activation of 5-$HT_{1B}$ heteroreceptors located on GABAergic interneurons exert a direct inhibition on GABA release, which in turn increase serotonin release in projection areas (Bagdy, Kiraly et al. 2000). Thus, 5-$HT_{1B}$ auto- and heteroreceptors in raphe nuclei jointly regulate serotonin release, whereas 5-$HT_{1B}$ heteroreceptors in other brain regions regulate release of other neurotransmitters (Sari 2004; Hu, Wang et al. 2007). However, the functional role of 5-$HT_{1B}$ heteroreceptors located on cholinergic, glutaminergic and GABAergic neurons is not fully elucidated. Microdialysis after manipulation of 5-$HT_{1B}$ receptors suggests distinct regional effects on specific transmitter levels. Administration of a 5-$HT_{1B}$ receptor antagonist increases acetylcholine levels in the frontal cortex and hippocampus, presumably mediated by cholinergic 5-$HT_{1B}$ receptors, but no effect on glutamate or GABA are reported in these regions (Hu, Wang et al. 2007). In the striatum, knockdown of 5-$HT_{1B}$ heteroreceptors in mice increases dopamine levels in the nucleus accumbens but not in the dorsal striatum (Nautiyal, Tanaka et al. 2015). These studies indicate that the serotonergic effects on other neurotransmitter systems via 5-$HT_{1B}$ heteroreceptors are regional.

Examination of post-mortem tissue along with *in vivo* PET imaging with the newly developed selective 5-$HT_{1B}$ receptor radioligands [$^{11}$C]AZ10419369 and [$^{11}$C]P943 has established the distribution of 5-$HT_{1B}$ receptors in the human brain. However, these radioligands bind to both receptor populations and distinctions between auto- and heteroreceptors cannot be made. High binding regions in PET include the occipital cortex,

pallidum and ventral striatum followed by intermediate binding in the dorsal striatum, prefrontal and temporal cortex and very low binding in the cerebellum, which is in line with human postmortem data (Varnas, Halldin et al. 2004).

## Role of serotonergic neurotransmission in aggression

Prevailing views postulate an inverse relationship between cerebral serotonin levels and impulsive aggression. This theory initially emerged from early studies demonstrating lower concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid in the cerebrospinal fluid of impulsive violent offenders compared with violent offenders acting instrumentally (Linnoila, Virkkunen et al. 1983). Also, 5-hydroxyindoleacetic acid in cerebrospinal fluid correlated negatively with and aggression scores in personality disordered patients (Brown, Goodwin et al. 1979). This inverse correlation was subsequently supported by numerous studies and is sometimes referred to as the "serotonin deficiency hypothesis" (Duke, Begue et al. 2013). Whereas the initial study by Brown et al. (1979) found that 80% of the variance in aggression scores was explained by 5-hydroxyindoleacetic acid (Brown, Goodwin et al. 1979), a recent meta-analysis find that the combined variance explained across several methodologies assessing serotonin levels is only 1.2 % (Duke, Begue et al. 2013). The authors criticize the serotonin deficiency hypothesis for being oversimplified and suggest that the relation may be specific to certain aspects of serotonin functioning. However, the meta-analysis did not include receptor binding studies. Also, there are currently no methods available for direct measurements of cerebral serotonin levels in humans; assessing serotonin levels rely on indirect measures or interventions that theoretically alter serotonin levels in the brain. Neuroreceptor PET is state-of-the-art in assaying components of the serotonin system *in vivo*. Neuroreceptor studies evaluating the relationship between serotonin and aggression are few in numbers, but give the opportunity to examine specific components of serotonergic neurotransmission. Other neuroimaging studies investigating the involvement of serotonin in aggression include fMRI studies combined with interventions that alter serotonin levels.

### PET studies

PET studies of both healthy controls and aggressive individuals identify several serotonin receptor subtypes and serotonergic components implicated in aggression, including 5-$HT_{1A}$, 5-$TH_{1B}$, 5-$HT_{2A}$ and 5-$HT_4$ receptors, the serotonin transporter and the MAO-A (Alia-Klein, Goldstein et al. 2008; Meyer, Wilson et al. 2008; Witte, Floel et al. 2009; Rosell, Thompson et al. 2010; Rylands, Hinz et al. 2012; Kolla, Matthews et al. 2015). To my knowledge, seventeen PET studies link aspects of serotonin signalling to aggression (including the study 2 in this thesis). These are summarized in table 2.

***Table 2.*** *PET studies of aggression.*

| Study | Participants | Radio-ligand | Outcome | Aggression assessment | Findings |
|---|---|---|---|---|---|
| **Parsey et al. 2002** | 25 healthy controls (12F/13M) | [carbonyl-$C^{11}$]-WAY-100635 | 5-$HT_{1A}$ receptors | BLA | Negative association between 5-$HT_{1A}$ receptor binding and lifetime aggression score in raphe, amygdala, cingulate and prefrontal cortex. |
| **Frankle et al. 2005** | 10 patients (5F/5M) with IED and 10 age and gender matched healthy controls | $^{11}$C-McN 5652 | SERT | Patients were diagnosed using the SCID-II. | Lower anterior cingulate SERT availability in patients relative to controls |
| **Brown et al. 2007** | 30 patients with alcoholism and 18 healthy controls | $^{11}$C-DASB | SERT | BHI | No group differences in SERT or association with measures of aggression. |
| **Soloff et al. 2007** | Fourteen female BPD subjects and 11 healthy female controls | $^{18}$F-altanserin | 5-$HT_{2A}$ receptors | BLA<br>BIS | Higher hippocampal 5-$HT_{2A}$ receptor binding in BPD subjects, not related to measures of impulsivity or aggression |
| **Meyer et al. 2008** | 16 individuals with ASPD and/or conduct disorder (6F/10M) and 16 healthy controls (7F/9M) | $^{18}$F-setoperone | 5-$HT_{2A}$ receptors | BHI<br>BIS | BIS scores in ASPD-subjects were negatively correlated with frontal, temporal and anterior cingulate 5-$HT_{2A}$. |
| **Alia-Klein et al. 2008** | 27 healthy male subjects | $^{11}$C-clorgyline | MAO-A | MPQ | Brain MAO A correlated inversely with the MPQ trait measure of aggression |
| **Witte et al. 2009** | 33 healthy subjects (16F/17M) | [carbonyl-$^{11}$C]-WAY-100635 | 5-$HT_{1A}$ receptors | Questionnaire for Measuring Factors of Aggression | Positive correlation between anterior cingulate and frontal 5-$HT_{1A}$ receptor binding and total aggression score |
| **Rosell et al. 2010** | Fourteen IED subjects (4F/10M) with current physical aggression, 15 IED subjects (3F/12M) without current physical aggression and 25 healthy controls (10F/15M) | $^{11}$C-MDL100907 | 5-$HT_{2A}$ receptors | BPAQ<br>BHI | Orbitofrontal 5-$HT_{2A}$ receptor availability was increased in patients with current physical aggression compared with patients without current physical aggression and healthy control subjects |
| **Booij et al. 2010** | 8 men with history of physical aggression in childhood and 18 lifetime non-aggressive men | $^{11}$C-AMT | Serotonin synthesis | BLA<br>BIS | Men with history of aggression had significantly lower trapping of $^{11}$C-AMT bilaterally in the orbitofrontal cortex. Not associated with measures of aggression or impulsivity. |
| **Soliman et al. 2011** | 37 healthy subjects (17F/20M) | $^{11}$C-harmine | MAO-A | NEO-PI-R | Prefrontal MAO-A binding correlated negatively with |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | angry-hostility and positively with deliberation. |
| **Rylands et al. 2012** | 14 men with high impulsive aggression, diagnosed with ASPD or BPD. 13 men with low levels of impulsive aggression. | $^{11}$C-DASB and $^{11}$C-MDL100907 | 5-$HT_{2A}$ receptors and SERT | EXPAGG<br>PPI<br>IVE<br>BIS<br>STAXI<br>BHI | Men with impulsive aggression had lower 5-$HT_{2A}$ receptor binding across regions and higher SERT in brainstem/midbrain. Across all subjects, brainstem SERT was positively correlated with impulsivity, aggression and anger scores. |
| **da Cunha-Bang et al. 2013** | 94 healthy subjects (34F/60M) | $^{18}$F-altanserin | 5-$HT_{2A}$ receptors | BPAQ<br>BIS | No significant association between frontal 5-$HT_{2A}$ receptor binding and aggression or impulsivity scores |
| **Soloff et al. 2014** | 33 BPD patients (20F) and 27 healthy controls (12F) | $^{18}$F-altanserin | 5-$HT_{2A}$ receptors | BLA<br>BIS | 5-$HT_{2A}$ receptor binding predicted impulsivity and aggression only in BPD females and in healthy control males |
| **van de Giessen et al. 2014** | 29 IED patients and 30 healthy controls | $^{11}$C-DASB | SERT | BPAQ<br>OASM<br>LHA<br>BIS | No group differences in SERT.<br>Positive correlation between anterior cingulate SERT and trait callousness |
| **Kolla et al. 2015** | 18 male violent offenders with ASPD, 18 age and sex matched healthy controls | $^{11}$C-harmine | MAO-A | The Iowa Gambling Task<br>NEO-PI-R<br>PCL-R | Orbitofrontal and ventral striatum MAO-A VT were lower in ASPD compared to controls. In ASPD, ventral striatum MAO-A VT was negatively correlated with self-report and behavioural measures of impulsivity |
| **da Cunha-Bang et al. 2016** | 61 healthy controls (14F/47M) | $^{11}$C-SB207145 | 5-$HT_4$ receptors | BPAQ<br>BIS | Among male subjects, there was a positive correlation between global 5-$HT_4$ receptor binding and BPAQ total score as well as BPAQ physical aggression. |
| **da Cunha-Bang et al. 2016** | 19 male violent offenders with personality disorders and 24 healthy males. | | 5-$HT_{1B}$ receptors | BPAQ<br>BIS<br>PCL-R<br>STAXI<br>PPI | Striatal 5-$HT_{1B}$ receptor binding predicted trait anger and level of psychopathy among violent offenders. |

**F:** Female, **M:** Male, **IED:** Intermittent Explosive Disorder, **BPD:** Borderline Personality Disorder, **ASPD:** Antisocial Personality Disorder, **BLA:** Brown-Goodwin Lifetime Aggression, **BHI:** Buss-Durkee Hostility Index, **BPAQ**: Buss-Perry Aggression Questionnaire, **BIS:** Barratt Impulsiveness Scale, **PCL-R**: Psychopathy Checklist Revised, **STAXI:** State-Trait Anger Expression Inventory, **MPQ**: Multidimensional Personality Questionnaire, **OASM**: Overt Aggression Scale-Modified, **PPI:** Psychopathic Personality Inventory, **IVE**: Impulsiveness-Venturesomeness-Empathy questionnaire, **EXPAGG:** Expression Aggression Questionnaire, **NEO-PI-R**: NEO Personality Inventory Revised, **SERT**: serotonin transporters

Most of these studies (n=7) evaluate 5-$HT_{2A}$ receptors; two studies demonstrate higher 5-$HT_{2A}$ receptor binding (Soloff, Price et al. 2007; Rosell, Thompson et al. 2010), two studies find no significant differences in 5-$HT_{2A}$ receptor binding between aggressive individuals and healthy controls (Rylands, Hinz et al. 2012; Soloff, Chiappetta et al. 2014), one study finds an inverse correlation between trait impulsivity and cortical 5-$HT_{2A}$ receptor binding in antisocial individuals (Meyer, Wilson et al. 2008) and one study finds no correlation between 5-$HT_{2A}$ receptor binding, trait aggression and trait impulsivity in healthy subjects (da Cunha-Bang, Stenbaek et al. 2013). Studies examining serotonin transporters and 5-$HT_{1A}$ receptors are also inconsistent. Four studies examine serotonin transporter availability in aggressive individuals, of which two studies find no group differences (Brown, George et al. 2007; van de Giessen, Rosell et al. 2014), one study demonstrates lower anterior cingulate serotonin transporter binding (Frankle, Lombardo et al. 2005) and one study finds increased brainstem/midbrain serotonin transporter binding, which also correlated positively with measures of aggression and impulsivity (Rylands, Hinz et al. 2012). The two 5-$HT_{1A}$ receptor studies have included only healthy controls. One finds that lifetime aggression correlates inversely with 5-$HT_{1A}$ receptor binding across regions (Parsey, Oquendo et al. 2002), whereas the other one shows a positive correlation with trait aggression in the frontal and anterior cingulate cortex (Witte, Floel et al. 2009).

MAO-A seems to be a more promising marker for aggression. Three studies consistently show inverse associations between MAO-A and measures of aggression in both healthy controls (Alia-Klein, Goldstein et al. 2008; Soliman, Bagby et al. 2011) and in antisocial personality disordered subjects (Kolla, Matthews et al. 2015). Also, MAO-A within the orbitofrontal cortex and ventral striatum is lower in antisocial personality disordered subjects compared with controls (Kolla, Matthews et al. 2015). Since MAO-A degrades serotonin, dopamine and norepinephrine, the mechanisms cannot be attributed to serotonin alone. The MAO-A findings are of particular interest in light of the longitudinal study by Caspi et al. showing that the MAO-A genotype moderates the association between childhood maltreatment antisocial behaviour in adulthood (Caspi, McClay et al. 2002). That is, male children having the low-activity MAO-A genotype and who are severely maltreated develop some form of antisocial behaviour. This MAO-A-gene-by-environment interaction has subsequently been replicated in several studies (Kim-Cohen, Caspi et al. 2006).

In line with the serotonin deficiency hypothesis, one study shows that high 5-$HT_4$ receptor binding is associated with high trait aggression in healthy men (da Cunha-Bang, Mc Mahon et al. 2016). Given that chronic treatment with fluoxetine (a selective serotonin receptor inhibitor) reduces 5-$HT_4$ receptor binding in healthy subjects (Haahr, Fisher et al. 2013), we interpret 5-$HT_4$ receptor binding as a biomarker for central serotonergic tonus. Therefore, this study suggests that men with high trait aggression have low serotonergic tonus. This is the first in vivo neuroimaging study to actually support the inverse relationship between serotonin levels and aggression.

So what have the serotonin receptor binding studies uncovered in terms specific serotonergic components involved in aggression? The mechanisms regarding 5-$HT_{1A}$, 5-$HT_{2A}$ and serotonin transporters in human aggression are unclear because of the many

conflicting findings. The most consistent finding is the negative association between MAO-A levels and aggression. The association between aggression and serotonin levels indexed by 5-$HT_4$ receptor binding makes sense but it would be reassuring to see the data replicated.

## Pharmaco-fMRI studies

A number of fMRI studies have investigated the effects of serotonergic interventions on neural activity during different tasks assessing constructs linked to aggression; inhibitory control in Go/No-Go paradigms and viewing angry or fearful faces. Only one study has evaluated the effects of serotonergic modulation in reactive aggression using the Taylor Aggression Paradigm. In this study, acute tryptophan depletion (transiently lowering cerebral serotonin levels) reduced anterior insula reactivity during a phase where the participants decide the punishment level for the opponent, but there was no effect on taskrelated behaviour (Kramer, Riba et al. 2011). Other effects of acute tryptophan depletion in healthy subjects include reduced orbitoinferior prefrontal activation and increased activation in the temporal cortex during behavioural inhibition (No-Go conditions) (Rubia, Lee et al. 2005) as well as reduced functional connectivity between the amygdala and prefrontal cortex while viewing angry faces (versus neutral) (Passamonti, Crockett et al. 2012). Consistent with this, administration of escitalopram to healthy controls (for 10 days) was associated with stronger inhibition of amygdala reactivity to emotional facial expressions (versus objects) via the orbitofrontal cortex (Sladky, Spies et al. 2015). Escitalopram (for seven days) also reduced amygdala reactivity to fearful, but not to happy faces in 15 healthy volunteers (Maron, Wall et al. 2016). In contrast, a case-control study with intermittent explosive disordered patients has shown that a single dose of escitalopram (2-3 hours before scanning) was associated with increased amygdala activation to emotional faces in healthy controls but not in the patients (Cremers, Lee et al. 2016). In a combined fMRI and PET study, acute blocking of 5-$HT_{2A}$ receptors (with ketanserin) reduced orbitofrontal reactivity to fearful faces, although independently of 5-$HT_{2A}$ receptor occupancy or neocortical 5-$HT_{2A}$ receptor binding (Hornboll, Macoveanu et al. 2013).

The studies reviewed above are not fully comprehensive of existing pharmaco-fMRI studies with serotonergic interventions, and are not directly comparable given the different use of paradigms and interventions. The overall interpretation is that serotonergic interventions modulate brain function in neural circuits relevant for aggression and emotion processing. Lowering serotonin levels (acute tryptophan depletion) reduce frontal activation during behavioural inhibition and amygdala-prefrontal connectivity in the context of angry faces whereas augmenting serotonin levels (serotonin reuptake inhibitors) increase the prefrontal inhibition of amygdala reactivity and attenuate amygdala reactivity to fearful faces, at least in healthy volunteers.

### 5-$HT_{1B}$ receptors and aggression

The involvement of 5-$HT_{1B}$ receptors in animal aggression is well-established (Olivier and van Oorschot 2005). Knockout of the 5-$HT_{1B}$ receptor in mice increases aggression and impulsivity (Saudou, Amara et al. 1994; Nautiyal, Tanaka et al. 2015) and administration of 5-$HT_{1B}$ receptor agonists inhibits aggressive behaviours (De Almeida, Rosa et al. 2006; Faccidomo, Quadros et al. 2012). Wholebrain knockdown of both 5-$HT_{1B}$ auto- and heteroreceptors in transgenic mice increases aggression *and* impulsivity, but specific knockdown of only forebrain heteroreceptors increases only aggression and not impulsivity (Nautiyal, Tanaka et al. 2015). Interestingly, early postnatal rescue of receptor expression reverses both aggressive and impulsive behaviour, whereas rescue of receptor expression in adulthood reverses the impulsive, but not the aggressive phenotype. The authors suggest that the effects of serotonin on aggression and impulsivity are mediated by 5-$HT_{1B}$ receptors through distinct circuits and during different time periods in life (Nautiyal, Tanaka et al. 2015).

Until now, evidence for the involvement of 5-$TH_{1B}$ receptors in human aggression has been limited. In the nineties, a class of mixed 5-$HT_{1A/1B}$ receptor agonists called "serenics" was tested in humans (Tiihonen, Hakola et al. 1993; de Koning, Mak et al. 1994), but did not have convincing anti-aggressive effects and further development was terminated after two patients became psychotic (Moriarty J 1994; Verhoeven 2007). Various polymorphisms of the gene coding for 5-$HT_{1B}$ receptors are associated with aggressive and antisocial behaviours (Conner, Jensen et al. 2010; Hakulinen, Jokela et al. 2013), but the functional role of these polymorphisms is unknown. Radioligands that bind to 5-$HT_{1B}$ receptors have just recently been developed and has been used in individuals with impulse control disorders, including pathological gamblers (Potenza, Walderhaug et al. 2013), cocaine addiction (Matuskey, Bhagwagar et al. 2014) and alcohol dependence (Hu, Henry et al. 2010), but never in cohorts with a high levels of aggression and a documented history of violent crime.

# Background summary, aims and hypotheses

* The involvement of serotonin in aggression is well replicated in both animal and human studies.
* Serotonin levels have long been thought to correlate inversely with level of aggression. This theory is based on indirect measures of serotonin function, as serotonin levels cannot be measured in vivo in humans.
* Preclinical research strongly implicates 5-$HT_{1B}$ receptors in impulsive and aggressive behaviours, but this has never been examined in humans.
* Brain regions and neural circuitries involved in aggression include the amygdala, prefrontal cortex (orbitofrontal cortex and medial prefrontal cortex), the anterior cingulate cortex, striatum and periaqueductal grey.

* fMRI studies examining neural correlates of aggression have predominantly been undertaken in healthy volunteers.

Important gaps in our current knowledge of the serotonergic mechanisms in aggression remain, and this generates a set of questions. For example, is the involvement of serotonin in aggression a global phenomenon or is it attributable to specific serotonin receptors or brain regions? Can the involvement of 5-$HT_{1B}$ receptors in animal aggression be corroborated to human aggression? Are the neural circuits of aggression observed in healthy controls dysfunctional in individuals with pathological levels of aggression, and are these circuits influenced by serotonin signalling?

To address some of these questions, we undertook functional neuroimaging examinations in a cohort of incarcerated violent offenders and healthy control subjects. The aims were to examine the neural correlates of reactive aggression using a laboratory measure of aggressive behaviour and to examine whether 5-$HT_{1B}$ receptors are involved in human aggression. This thesis is based on three studies, with the following specific aims and hypotheses:

## Study 1

In study 1 we aimed to evaluate neural correlates of reactive aggression using the PSAP during fMRI. We hypothesised that relative to control subjects, the violent offenders would have 1) increased reactivity to provocations within the amygdala, striatum, periaqueductal grey and anterior cingulate cortex, 2) reduced reactivity to provocations within the orbitofrontal/prefrontal cortex and 3) reduced amygdala-prefrontal connectivity as a function of provocations.

## Study 2

In study 2 we aimed to compare 5-$HT_{1B}$ receptor binding in violent offenders and in control subjects. We hypothesised that violent offenders would have reduced 5-$HT_{1B}$ receptor binding and that 5-$HT_{1B}$ receptor binding would be inversely correlated with level of aggression, anger, impulsivity and psychopathy. Our regions of interests included the anterior cingulate cortex, orbitofrontal cortex and the striatum. These were selected based on our prior expectations regarding which brain regions are involved in aggression given the existing literature.

## Study 3

In study 3 we integrated PET and fMRI data from study 1 and 2 with the aim to investigate whether 5-$TH_{1B}$ receptor binding was associated with neural reactivity to provocations. Based on our observations from study 1 and 2, we hypothesised that 5-$HT_{1B}$ receptor

binding across regions (raphe, striatum, amygdala, anterior cingulate and orbitofrontal cortex) would be positively associated with trait anger, amygdala and striatal reactivity to provocations.

# Methods

## Study design

The studies had cross-sectional case control designs, in which we selected participants based on high and low levels of aggression: a group of inmates with a history of violent offending and a group of men from the community who reported low trait aggression.

## Participants

### Violent offenders

We recruited violent offenders from closed state prisons in the Copenhagen area. Prison staff asked inmates who had been convicted of violent crimes (murder, attempt to kill, aggravated assault, rape or attempt to rape) if they would be interested in participating in a study investigating aggression and brain function. Inmates who were interested were then interviewed for assessment of eligibility according to the following inclusion and exclusion criteria:

Inclusion criteria:

- Above 18 years old.
- History of violent offending that had been impulsive in character.

Exclusion criteria:

- History of major depressive disorder, bipolar disease, schizophrenia or psychotic symptomatology.
- Contraindications for MRI.
- IQ below 70.
- Severe hearing or visual impairments.
- Current use of psychotropic medications.
- Current use of illicit drugs.

A total of 51 inmates were interviewed, after which we gave them oral as well as written information about the study. Seventeen inmates were deemed not eligible for the study due to the following; contraindications for MRI (n=1), previous or current diagnosis of depression (n=5), severe head trauma (n=3), previous intravenous drug abuse (n=2), current

pharmacological treatment for attention deficit hyperactive disorder (n=5) and violent offending not impulsive in character (n=1). Eight inmates did not want to participate after receiving information. Twenty-six inmates were included, of whom one was moved to another prison before scanning and two did not get final approval from the Prison and Probation Service for transport to the scanning facilities. Out of the remaining 23 inmates, one got claustrophobic in the PET scanner and one got claustrophobic in the MRI scanner and could not proceed after acquisition of the T1 protocol necessary for PET analysis. Three participants retrospectively reported taking psychotropic medications at the time of scan (tramadol, seroquel, varenicline) and were therefore excluded. Thus, 19 PET and 18 PSAP fMRI datasets were eligible for full data analysis.

After informed consent was obtained, the Danish Prison and Probation Service approved the transport to the scanning facilities for each inmate. Inmates were transported to the hospital accompanied by prison staff. Due to the high prevalence of men in the prisons we recruited from, no women were included.

### Healthy control non-offenders

We recruited healthy control participants in parallel with recruiting inmates, matching the groups on age and sex. The study was announced on a Danish website where researchers can recruit volunteers for biomedical studies. We also recruited control subjects using paper ads on bulletin boards in vocational schools. People that were interested in participating were invited to complete a questionnaire on their demographic information as well as the Buss-Perry Aggression Questionnaire for assessment of their trait aggression. This was done to ensure not recruiting individuals with high levels of aggression from the community.

Out of the volunteers that could be matched on age and sex, we contacted people with as low education and as low trait aggression scores as possible. Candidates were interviewed on the phone to assess inclusion and exclusion criteria, which for healthy controls were the same as for inmates except for having a history of violent offending.

Twenty-six control participants were included, of whom one had a pathological MRI of the brain and one got claustrophobic after one hour in the PET scanner and could not proceed with further scans. Two subjects were excluded from the PSAP experiment due to not believing in the paradigm or because of a behaviour (aggressive and monetary options) of above 4 standard deviations from the group mean. Moreover, two participants were included 6 months after fMRI analysis and manuscript preparation for Study 1 had been done. These two participants were therefore not included in Study 1. Thus, 24 PET and 22 PSAP fMRI datasets were eligible for full data analysis in study 2, however only 20 fMRI datasets were included in study 1.

## Clinical assessment of the participants

For clinical characterization of the violent offenders, we evaluated presence of personality disorders using the Structured Clinical Interview for DSM-4 (SCID-II) and level of

psychopathy using the PCL-R. The SCID-II was administered by a psychiatrist or myself (course-certified). We ensured diagnostic consistency by co-rating a subset of the subjects (n=4). For assessment of PCL-R score, we conducted semi-structured interview and collected collateral information from criminal files. The PCL-R scorings for each violent offender were consensus decisions by the project group (medical doctor, medical student and psychologist), all trained in the administration of the PCL-R. During the screening interview and the semi-structured PCL-R interview we also obtained detailed information about previous drug and alcohol abuse. All violent offenders reported no current use of drugs, and were regularly screened with urine tests in the prisons. Also, all participants tested negative on urine screen (Rapid Response Multi-Drug Screen Test Panel: amphetamine, metamphetamine, barbiturates, buprenorphine, benzodiazepines, cocaine, cannabis, methadone, methylenediocymetamphetamine, morphine, opiate, oxycodone, phencyclidine, propoxyphene and tricyclic antidepressants) on the day of scanning.

## Aggression and personality measures

A number of self-report trait and state questionnaires were administered to all participants, assessing their trait aggression (Buss-Perry Aggression Questionnaire), trait and state anger (State-Trait Anger Expression Inventory), trait impulsivity (Barratt Impulsiveness Scale), trait psychopathy (Psychopathic Personality Inventory), childhood trauma (Childhood Trauma Questionnaire), state mood disturbances (Profile of Mood States) and state depression (Major Depression Inventory).

The Buss-Perry Aggression Questionnaire 29-item scale that consists of four aggression subscales; physical aggression, verbal aggression, anger and hostility, providing a total score that can be used as a measure of trait aggressiveness (Buss and Perry 1992).

The Barratt Impulsiveness Scale is a 30-item scale that consists of three subscales; attentional impulsivity, motor impulsivity and non-planning impulsivity (Patton, Stanford et al. 1995). The total score provides a measure of global impulsivity.

The State-Trait Anger Expression Inventory is a 57-item inventory that consists of a Trait Anger scale with a total score that describes a general propensity to experience angry feelings, Anger Expression and Anger Control scales that measures the outward expression of anger and the ability to control this expression, and a State Anger scale that assesses the intensity of anger at a given time (Moeller, Novaco et al. 2015). Examples of items in the Trait Anger scale include:

- I am a hotheaded person
- It makes me furious when I am criticized in front of others
- When I get frustrated, I feel like hitting someone
- I am quick tempered

For each item the participant rates on a 4-point Likert scale how often they generally feel or react this way: almost never, sometimes, often, almost always.

The Psychopathic Personality Inventory consists of 154 items describing psychopathy as a dimensional trait (Lilienfeld 2005). It consists of eight content subscales grouped into three factor scores. The total score reflects variations in global psychopathy. The higher the total score, the more psychopathic is the individual on average. The factor score 'Self-Centered Impulsivity' comprises the content scales 'Machiavellian Egocentricity', 'Rebellious Nonconformity', 'Blame Externalization' and 'Carefree Nonplanfulness', reflecting a willingness to manipulate others, a reckless defiance of societal norms, proneness to boredom and a tendency to act before thinking. The factor score 'Fearless Dominance' consists of the content scales 'Social Influence', 'Fearlessness' and 'Stress Immunity', reflecting a propensity to be charming and skilled at influencing others, a lack of anxiety regarding threats and a tendency to remain calm under pressure. 'Coldheartedness' is both a factor score and a content scale, describing a lack of empathy and feelings guilt. The Inventory also contains a score for inconsistent responding to identify individuals completing the scale in a random or haphazard manner. Examples of items in the Psychopathic Personality Inventory include:

- To be honest, I believe that I am more important than most people (Machiavellian Egocentricity)
- I get restless when my life gets to predictable (Rebellious Nonconformity)
- If I'd had fewer bad breaks in life, I'd be more successful (Blame Externalization)
- I like to act first and think later (Carefree Nonplanfulness)
- If I really want to, I can persuade most people of almost anything (Social Influence)
- When my life gets boring, I like to take chances (Fearlessness)
- When I'm in a frightening situation, I can "turn off" my fear almost at will (Stress Immunity)
- I look out for myself before I look out for anyone else (Coldheartedness)

For each item the participant rates how false or true each statement is for them: false, mostly false, mostly true, true.

The Childhood Trauma Questionnaire consists of five main subscales; Emotional Abuse, Physical Abuse, Sexual Abuse, Emotional Neglect and Physical Neglect, along with a subscale assessing minimalization/denial.

Translation procedures for the Buss-Perry Aggression Questionnaire, Barratt Impulsiveness Scale and the State-Trait Anger Expression Inventory are described elsewhere (da Cunha-Bang, Stenbaek et al. 2013; Moeller 2015). The translation into Danish and back-translation of the Psychopathic Personality Inventory has been approved by the distributors of the scale (Par.Inc). The internal consistencies of the Danish versions of the scales are provided in table 3. For the Psychopathic Personality Inventory, the score for inconsistent responding indicated that only two participants (violent offenders) replied in an atypical manner.

***Table 3.*** *Internal consistencies (Cronbach's alpha) for the aggression, impulsivity and anger scales.*

| Scale | Cronbach's alpha |
|---|---|
| Buss-Perry Aggression Questionnaire | 0.95 |
| Barratt Impulsiveness Scale | 0.81 |
| Trait Anger Scale | 0.91 |
| Psychopathic Personality Inventory | 0.91 |

# Positron Emission Tomography

PET is an imaging technique allowing for *in vivo* mapping of neuroreceptors. A radioisotope, e.g. $^{11}C$ or $^{18}F$ is attached to a tracer, which for neuroimaging is typically an agonist or antagonist to a receptor of interest. The radioligand [$^{11}C$]AZ10419369 (8(5-methyl-8-(4-[11C]methyl-piperazin-1-yl) - 4- oxo - 4*H* - chromene - 2- carboxylic acid (4-morpholin-4-yl-phenyl)-amide), a partial agonist of 5-$HT_{1B}$ receptors, was used for the PET examinations. A tracer should be administered in very low doses as to not elicit pharmacological effects. The radioligand is injected into the bloodstream and is distributed throughout the body, where it binds to the target of interest. When the positron emitting isotope decays, a positron is emitted and will annihilate when encountering an electron. During annihilation, a pair of gammarays (photons) is emitted at 180 degrees from each other. The PET camera can detect these gammarays and thus locate the annihilation. Images can then be obtained after reconstruction and correction for absorption and random events. PET scans in this thesis were all conducted with a high-resolution research tomograph (HRRT) at the PET and cyclotron unit at Rigshospitalet in Copenhagen.

## PET image analysis

The dynamic PET examination provides an estimation of the PET tracer in a given volume of interest over time and can be visualized with a time-activity curve (TAC). The volumes of interests are defined anatomically using an MRI, which is coregistered to the PET image (figure 1). We adopted an automated method for this, thus avoiding potential bias from manually defining the volumes of interests (Svarer, Madsen et al. 2005).

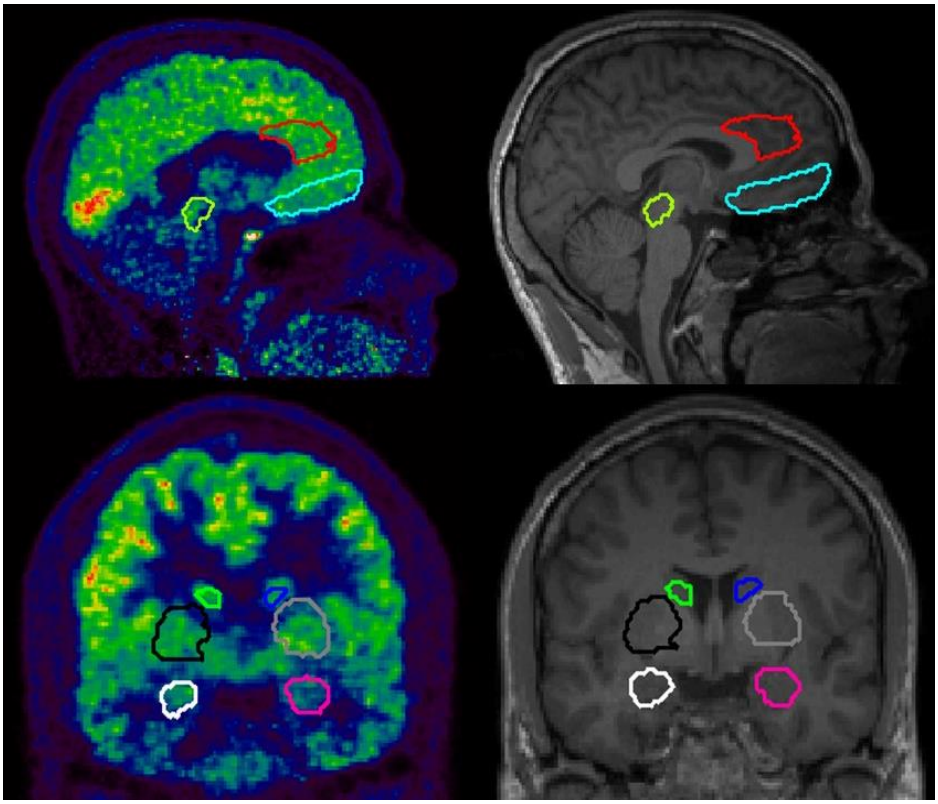

***Figure 1**. Volume of interests (in study 2 and 3) delineated on a [$^{11}$C]AZ10419369 PET image (left) and on an MRI (right). Striatum: black/grey/green/dark blue, Amygdala: pink/white, Raphe nuclei: yellow, Orbitofrontal cortex: light blue, Anterior cingulate cortex: red.*

The quantification of the receptor is estimated by means of kinetic modelling. In this process, the radioactive concentrations (Bq/ml) in a given volume of interest is extracted from the PET scanning and is fitted to a function to estimate the specific binding of radiotracer, binding potential (BP). The binding potential refers to the amount of radioactive uptake in the brain, and is defined for *in vitro* radioligand binding as the ratio of receptor density ($B_{max}$) to the radioligand equilibrium dissociation constant (Kd). In vivo the binding potential can be determined by specific binding relative to a reference concentration, which can either be free or total plasma concentration or a reference region. Several kinetic models for quantifying PET data exist. Reference tissue models require a region devoid of the receptor of interest, but the advantage is that an arterial cannulation is unnecessary.

We analysed the [$^{11}$C]AZ10419369 PET data using the simplified reference tissue model (SRTM) with the cerebellum (excluding vermis) as a reference region. This approach shows comparable results relative to models using arterial blood as input function (Varnas, Nyberg et al. 2011). The outcome of a reference tissue model is the $BP_{ND}$, referring to the ratio of specifically bound radioligand to that of "nondisplaceable" (ND) radioligand in

tissue: $BP_{ND} = f_{nd} * B_{avail}/K_d$, where $f_{nd}$ is the free fraction of tracer in the non-displaceable tissue compartment, $B_{avail}$ is the number of receptors available for binding and $K_d$ is the equilibrium dissociation constant for the tracer. The non-displaceable uptake refers to non-specifically bound and free ligand in tissue. The total concentration of radioligand in the tissue is the sum of radioligand specifically bound to receptors and the non-displaceable uptake.

The use of [$^{11}$C]AZ10419369 for quantification of 5-$HT_{1B}$ receptor binding is well validated (Varnas, Nyberg et al. 2011; Nord, Finnema et al. 2014). Regional binding of [$^{11}$C]AZ10419369 is in agreement of 5-$HT_{1B}$ receptor distribution observed in human post-mortem studies (Varnas, Halldin et al. 2004). Also, the use of reference models for the quantification of $BP_{ND}$ values has been validated and shows good correlation with quantifications using arterial input functions (Varnas, Nyberg et al. 2011). The absolute variability in [$^{11}$C]AZ10419369 $BP_{ND}$ within subjects are 5-7 % in cortical regions, 7-14 % in subcortical regions and higher (20%) in the raphe nuclei (Nord, Finnema et al. 2014). However, the anatomical boundaries of the raphe are not directly visible on an MRI, and the region is a relatively small structure, resulting in lower signal-to-noise ratio than larger regions. These factors may explain the high variability in raphe $BP_{ND}$.

### Voxel-based analysis

In voxel-based analyses, a parametric image containing a binding potential in each voxel is constructed. This is obtained by applying kinetic modelling of the TACs in each voxel. In study 2, we generated parametric $BP_{ND}$ maps by first normalizing the co-registered PET image into standard (Montreal Neurological Institute, MNI) space. This is necessary for analysing the data across a group. The PET images were then smoothed with a 6 mm full-width half-maximum Gaussian kernel. To generate parametric images, we used the PXMOD tool of the PMOD software (3.0, https://www.pmod.com/) using the multilinear reference tissue model (MRTM2) (Ichise, Liow et al. 2003). Visual inspection of images created using MRTM2 revealed fewer voxels that did not fit and were thereby limited by the threshold ($BP_{ND}$ <0 or >10) compared with images computed using SRTM. We used SPM8 for group analyses of the parametric $BP_{ND}$ maps. In voxelbased analysis the statistical analyses are performed on each voxel, resulting in a high number of multiple tests. The method used for correcting multiple comparisons is described in the section ‘statistical modelling for fMRI’ below.

## Functional Magnetic Resonance Imaging

FMRI is a neuroimaging technique that indirectly measures neural activity during performance of a task in the MRI scanner. The principle of fMRI is based on the

phenomenon that neuronal activity results in increased blood flow to that area of the brain, also referred to as the hemodynamic response. The most common method to assess neural activity is the blood oxygenlevel dependent (BOLD) signal, which is based on altered MR signal due to changes in the oxygenation of haemoglobin in blood associated with neural activity. The BOLD signal is typically measured during an active condition in a task and contrasted with a baseline condition, and is then evaluated across a cohort to examine task-related brain function at a group level. Additionally, fMRI allows for evaluation of functional connectivity, which is a measure of correlation between the BOLD signals in two brain regions over time (Friston, Buechel et al. 1997). In fMRI "activation" or "reactivity" in a brain region is associated with an event, stimulus or emotional state, not causing or imply the presence of the state.

MRI raw data require several preprocessing steps to be prepared for fMRI analysis. We preprocessed all MRI data in one batch using Statistical Parametric Mapping for fMRI 8 (SPM8, http://www.fil.ion.ucl.ac.uk/spm), thus avoiding risk of manual errors. Prepocessing operations included realignment (motion correction), co-registration (fMRI data is co-registered to the anatomical T1 images), alignment of images to the anterior commissure (using acpcdetect (https://www.nitrc.org/projects/art)), segmentation (the brain is segmented into grey matter, white matter and cerebrospinal fluid), normalization (transformation of images so they align with each other) and smoothing. In normalization, we re-sampled the images to a voxel size of 2x2x2 $mm^3$. In spatial smoothing a filter is applied to the image, essentially blurring the image by replacing the value of each individual voxel with a weighted average of itself and its neighbours. The width of distribution of the smoothing is described by the full width halfmaximum, which was 8 mm in our studies. Spatial smoothing is applied to account for real uncertainty in the normalization procedure. It also accounts for interindividual variability in brain anatomy (e.g. gyral folding). We also removed low frequency information by applying a "high-pass" filter to our data. Low frequency fluctuations in the BOLD are artifacts of the scanner ("drift" effects). Thus, we applied a high pass filter to remove those lowfrequency oscillations in our BOLD time series. We used a high-pass filter of 128 seconds (SPM default). In addition, we used the toolbox ART that includes the time-courses for six motion parameters (translation and rotation) as nuisance regressors in the statistical models to reduce motion-related noise in the data. ART attempts to identify individual frames in the BOLD time series where the image quality is excessively problematic. Either the participant moved "too much" during one frame or the variability of that particular frame deviated from the variability of all the frames in the time series. The withinframe movement threshold that we used was 2 mm and the withinframe variability threshold was 4 standard deviations. Those frames that were "flagged" as exceeding these thresholds were censored by adding a frame-specific regressor to the single-subject design matrix. This effectively removes those frames when estimating the effect of task. As quality control, all data were visually inspected after each preprocessing step.

## Statistical modelling in fMRI

In fMRI the time series of each voxel is examined to evaluate whether the BOLD-signal changes in response to an experimental manipulation. The general linear model can be used to detect this variation, in which the BOLD time series is the dependent variable and the predicted stimulus time course is the independent variable. The hemodynamic response function describes the changes in blood flow associated the neuronal activity. The general linear model approach is based on the assumption that the hemodynamic response function is a linear transformation of an underlying neuronal signal. The stimulus onset time series is convolved (blending of two functions) with the hemodynamic response function to create the shape of the BOLD response. The BOLD signal is first modeled in each voxel of the brain separately for each individual. These single-subject effects are then combined across subjects for group analyses.

To determine areas of the brain that respond to the task more strongly than the baseline, the mean value for a voxel is tested to determine whether this value is significantly different than zero (null hypothesis). This is done for each individual statistical image, and is then combined across individuals for grouplevel analyses. However, given the large number of voxels in a brain, inference at a voxel-level is problematic due to inflation of the type I error (false positives) that results from multiple comparisons. To account for this problem, the statistical analyses can be corrected for multiple tests using various methods. The method used in this thesis is based on the familywise error rate, which is the chance of one or more false positives anywhere in the image. Methods for controlling the familywise error rate include Bonferroni, random fields theory and Monte Carlo simulation, the latter applied for fMRI data in this thesis. In the Monte Carlo cluster-level inference method, random data are simulated and smoothed based on the estimated intrinsic smoothness of the observed data to create alternate statistic images and estimate the distribution of observed clusters under the null hypothesis (Forman, Cohen et al. 1995) A threshold is applied to these surrogate images to determine cluster extent thresholds for wholebrain or specific regions of interests unlikely to have occurred by chance (alpha < 0.05) at a given voxel-level statistical threshold (e.g. $p<0.01$ or $p<0.001$).

## The Point Subtraction Aggression Paradigm in fMRI

Prior to using the PSAP in study 1, we evaluated an fMRI adapted version of the PSAP in a cohort of healthy subjects (Skibsted 2015).

### *Procedure*

The instructions were read out loud to the participants before the scanning (appendix 1). We told the participants that they would be paired with another person that they were not allowed to meet, and that the main goal of the game was to earn as many points as possible. The points would then be exchangeable for real money. The three options were explained to the participants: pressing Option 1 100 consecutive times earns 1 point, pressing Option 2 10 consecutive time removes a point from the opponent and pressing Option 3 10

consecutive times protects the participant from the opponent stealing a point. Responses were made with a five-finger button-box on the right hand and responses were made with the index (Option 1), middle (Option 2) and ring (Option 3) finger. After starting an option, it was required that this option was finished before the participant could choose a new option. We told the participants that they had been assigned to the group that did not get to keep the points stolen from their opponent. In this way, Option 2 represents an aggressive behaviour, void of any monetary incentive. Participants were also told that their opponent was given alternate instructions and could, among other things, keep the points they stole. Comprehension of the instructions was secured by asking the participants a set of questions (see appendix 1). The participants completed a one minute training session in the scanner, after which they were given a short break while we "called the other research group to hear whether the other player was ready".

The participants could follow the status of the game (figure 2A) that was projected onto a screen in the scanner room. The paradigm was programmed in E-prime version 2.0. In the absence of Option 2 and 3 selections, the program was design to subtract points from the participants every 6 to 60 seconds. If Option 2 or 3 was selected, a provocation free interval was initiated (0-60 sec). The participants were not aware that Option 2 initiated provocation free interval. This was done to mimic that a real opponent would "back off". We defined aggressive behavior as: (Number of option 2 presses / Number of provocations) / Number of button presses. This adjusts aggressive behavior both to the number of provocations and to individual differences in buttonpress rate. Other common definitions of aggressive behavior in the PSAP is the number of Option 2 presses (Carre and McCormick 2008), or the number of Option 2 presses divided by the number of provocations (Kose, Steinberg et al. 2015) and the number of Option 2 presses divided by the number of total presses (Geniole, Cunningham et al. 2015). After the scan, we administered a questionnaire in which the participants were asked to describe their opponent. Participants who clearly indicated not believing they played against a real person were excluded.

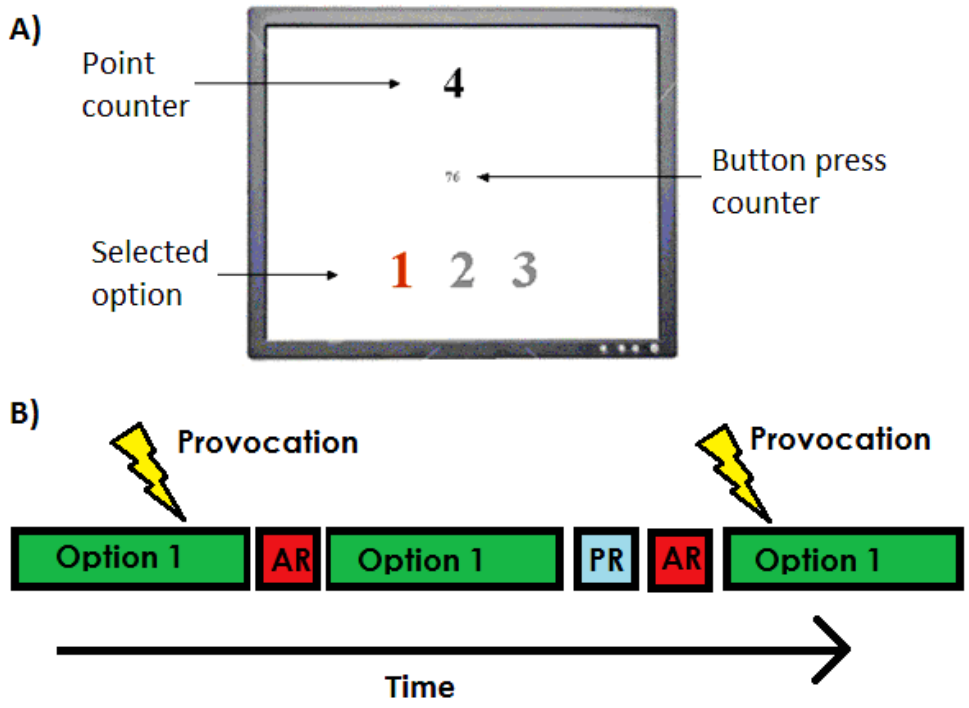


***Figure 2***. *(A) Screen displaying what the participant viewed during the PSAP. The red-coloured digit denotes that the participant is currently in Option 1. (B) Timeline with schematic representation of the task conditions. In this example, the player receives provocations while using Option 1, but has to finish this option before choosing either aggressive response (Option 2), protective response (Option 3) or Option 1 again. AR: aggressive response, PR: protective response.*

## fMRI design

We defined a baseline condition and two conditions of interests; provocations and aggressive response (figure 2B). We used the first 10 seconds of Option1 as the "baseline condition" to avoid any confounding reward effects. When a provocation occurred during the first 10 seconds of Option 1, the time from beginning of Option 1 until the provocation occurred was used. Provocations were modeled as events and aggressive responses were modeled as blocks (figure 2B). The following contrasts were then estimated: provocations > Option 1 and aggressive responses > Option 1.

## Functional Connectivity

Connectivity analyses can be used for evaluation of brain networks. Functional connectivity refers to correlations between the signals in two separate regions over time. Psychophysiological interactions refer to correlations between two regions that change as a function of task state, e.g. a condition in the paradigm, and is the method used in study 1. In psychophysiological interaction analyses two regressors are added to the general linear

model; the seed time series and the interaction term (product of the task condition (provocations) and the seed time series). Thus, it is evaluated whether the correlation between the BOLD activity in a seed region and the BOLD activity in a target region is dependent on the task stimulus of interest. We assessed the provocation-associated functional connectivity with seed regions in either amygdala or striatum, using the contrast Provocations > Option 1. The generalized PPI toolbox v. 7.12 (McLaren, Ries et al. 2012) was used to 1) extract the mean seed time series for each subject, 2) create the phychophysiological interaction regressor (interaction term) and 3) create/estimate new single-subject design matrices including these terms. The beta image associated with the interaction term was then entered into group level analyses to evaluate psychophysiological interaction effects across the cohort.

## Statistical analyses

In study 1 the statistical analyses were performed at voxel-level within SPM8. Group comparisons were evaluated with unpaired t-tests and brain-behaviour correlations across the groups were examined with multiple linear regression analyses, adjusting for age and group status. We used the Monte Carlo simulation method to correct for multiple comparisons, establishing cluster extent thresholds (number of voxels) for the whole brain and for each region of interest at an uncorrected p=0.001 (voxel-level) and an $\alpha<0.05$ (cluster-level).

In study 2 the data were analysed using R studio and SAS. Group comparisons were evaluated with unpaired t-tests. Associations between 5-$HT_{1B}$ receptor binding and scale measures of aggression, anger, psychopathy and impulsivity were evaluated in multiple linear regression analyses, adjusting for age, injected mass/kg bodyweight and intelligence quotient (IQ). Group status was included as a covariate in analyses where all participants were pooled. We corrected for multiple comparisons (four trait measures and three brain regions) using the false discovery rate (Hochberg and Benjamini 1990; Benjamini 1995).

In study 3 we analysed the associations between 5-$HT_{1B}$ receptor binding, BOLD responses to provocations and trait anger in latent variable framework across all participants. We included age, group status, injected mass/kg bodyweight and IQ as covariates. A latent variable model is a structural equation model that consists of regression-like relationships among variables. A latent variable can be conceptualised as something that is not directly observable and is assumed to affect manifest (observed) variables. It can also be seen as a scheme for data reduction where the latent variable summarizes a number of variables in many fewer factors. Using a latent variable model instead of evaluating associations in separate univariate analyses has the advantage of avoiding multiple testing.

Age was motivated as a covariate because 5-$HT_{1B}$ receptor binding declines with age (Nord, Cselenyi et al. 2014). Injected mass/kg bodyweight was included as a covariate because of theoretical effects of injected mass of [$^{11}$C]AZ10419369 on $BP_{ND}$. The statistical

models were also adjusted for IQ since previous studies have shown associations between serotonin and cognitive function (Madsen, Erritzoe et al. 2011; Haahr, Fisher et al. 2013; Penttila, Hirvonen et al. 2015). These covariates are further discussed in the section covering methodological considerations below.

# Results and discussion

The outcomes of the personality assessments and the neuroimaging experiments are described and discussed below. Detailed neuroimaging results and discussion of the findings in the context of existing literature are further provided in the papers covering study 1-3.

## Personality and clinical characteristics

Personality disorders, previous substance/alcohol abuse and PCL-R scores are given for each violent offender in table 4. According to the cut-off score for psychopathy of 30 or above, nine violent offenders meet criteria for psychopathy. However, some studies suggest that due to cultural differences, the cut-off score for psychopathy in Europe is 25 (Cooke, Michie et al. 2005) and in that instance, 17 violent offenders meet criteria for psychopathy.

***Table 4.*** *Clinical characteristics of the violent offenders.*

| Subject Number | Personality Disorder | Previous Substance /Alcohol abuse |
|---|---|---|
| **PCL-R score below 25** | | |
| 1 | Unspecified | None |
| 2 | Antisocial | Cannabis |
| **PCL-R score 25-29.9** | | |
| 3 | Antisocial | Cannabis, cocaine |
| 4 | Schizoid | None |
| 5 | Paranoid | Cannabis |
| 6 | Unspecified | None |
| 7 | Borderline, Dependent, Other Specified | Amphetamine |
| 8 | Antisocial, Borderline | Cannabis, cocaine |
| 9 | Antisocial | Cannabis |
| 10 | Unspecified | None |
| **PCL-R score 30 or above** | | |
| 11 | Antisocial | None |
| 12 | Antisocial, Unspecified | None |
| 13 | Antisocial | Cocaine, cannabis, alcohol, anabolic steroids |
| 14 | Antisocial | Cocaine, anabolic steroids |
| 15 | Antisocial | Cocaine, cannabis |
| 16 | Antisocial, Unspecified | Cannabis |
| 17 | Antisocial | Cannabis, amphetamine, cocaine, opioids |
| 18 | Antisocial, Unspecified | Cocaine, opioids |
| 19 | Antisocial | Cannabis, opioids |

The violent offenders scored significantly higher on trait aggression, trait anger, anger expression, trait impulsivity and trait psychopathy (table 5). Unexpectedly, there were no group differences in reported childhood trauma (table 5). We suspect that this might be due to underreporting in the violent offender group.

***Table 5.*** *Group differences in personality measures.*

| Personality Questionnaire | Violent Offenders N=19 | Healthy Controls N=24 | p-value |
|---|---|---|---|
| **Buss-Perry Aggression Questionnaire** | | | |
| Total Score | 87.7 ± 21.7 | 51.6 ±10.2 | <0.0001 |
| Physical Aggression | 28.3 ± 10.9 | 14.8 ± 3.9 | <0.0001 |
| Verbal Aggression | 16.8 ± 3.6 | 12.8 ± 4.1 | <0.0001 |
| Anger | 21.2 ± 7.6 | 11.6 ± 3.2 | <0.0001 |
| Hostility | 21.3 ± 5.6 | 13.9 ± 4.4 | <0.0001 |
| **Barratt Impulsiveness Scale** | | | |
| Total Score | 67.0 ± 9.9 | 58.5 ± 9.5 | 0.007 |
| Attentional Impulsivity | 15.6 ± 3.9 | 13.9 ± 3.7 | 0.16 |
| Motor Impulsivity | 24.1 ± 4.2 | 20.6 ± 2.5 | 0.003 |
| Nonplanning Impulsivity | 27.3 ± 4.6 | 23.9 ± 5.6 | 0.04 |
| **State-Trait Anger Expression Inventory** | | | |
| Trait Anger Scale | 21.4 ± 6.8 | 14.4 ± 2.4 | 0.0003 |
| Anger Expression Index | 38.4 ± 14.0 | 26.5 ± 7.6 | 0.003 |
| State Anger | 15.9 ± 2.5 | 15.0 ± 0.0 | 0.14 |
| **Psychopathic Personality Inventory** | | | |
| Total Score | 313.4 ± 35.7 | 278.1 ± 22.7 | 0.0009 |
| Self-centered Impulsivity | 153.4 ± 19.5 | 123.6 ± 13.5 | <0.0001 |
| Fearless Dominance | 124.0 ± 15.6 | 119.5 ± 17.4 | 0.39 |
| Coldheartedness | 36.0 ± 10.1 | 35.1 ± 7.4 | 0.75 |
| Inconsistent Responding | 34.6 ± 8.9 | 27.1 ± 7.0 | 0.006 |
| **Childhood Trauma Questionnaire** | | | |
| Total Score | 37.3 ± 12.2 | 33.2 ± 8.6 | 0.2 |
| Emotional Abuse | 8.1 ± 2.9 | 6.6 ± 2.8 | 0.13 |
| Physical Abuse | 6.8 ± 5.1 | 5.6 ± 2.1 | 0.32 |
| Sexual Abuse | 5.3 ± 0.8 | 5.1 ± 0.4 | 0.41 |
| Emotional Neglect | 10.5 ± 3.8 | 9.8 ± 3.8 | 0.56 |
| Physical Neglect | 6.6 ± 1.9 | 6.1 ± 1.8 | 0.41 |

## Violent offenders respond to provocations with high amygdala and striatal reactivity

This study shows that violent offenders had higher reactivity to provocations within the amygdala, striatum and periaqueductal grey compared with controls (figure 3).

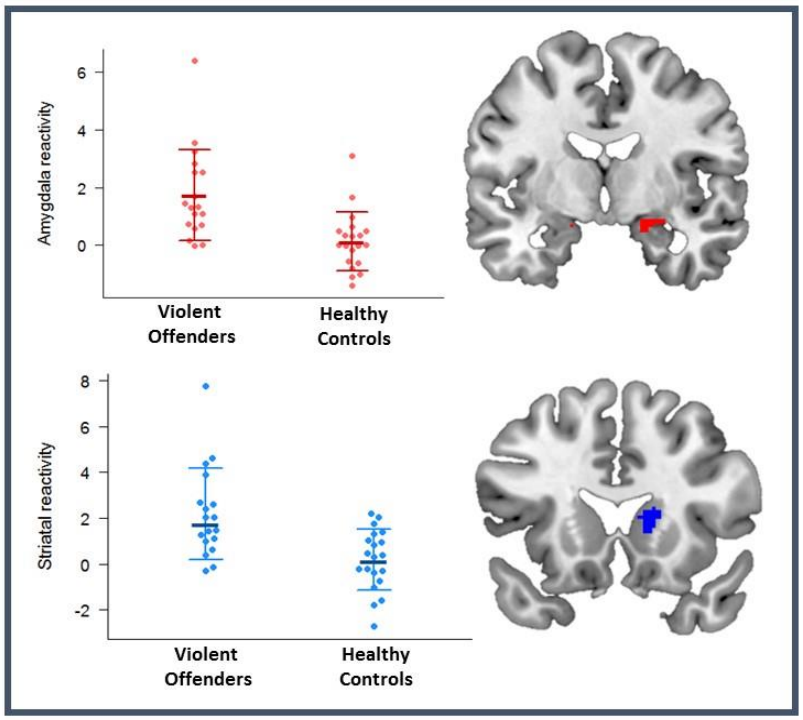


***Figure 3***. *Violent offenders had significantly higher reactivity to provocations within the right (40 voxels, $p_{corr}<0.05$) and left amygdala (14 voxels, $p_{corr}<0.05$) shown in red, and within the right striatum (157 voxels, $p_{corr}<0.05$), shown in blue. Amygdala reactivity reflects extracted mean signal values from amygdala clusters bilaterally and striatal reactivity reflects extracted mean signal values from the right striatal cluster.*

The violent offenders had also reduced amygdala-prefrontal and striato-prefrontal functional connectivity as a function of provocations (figure 4).

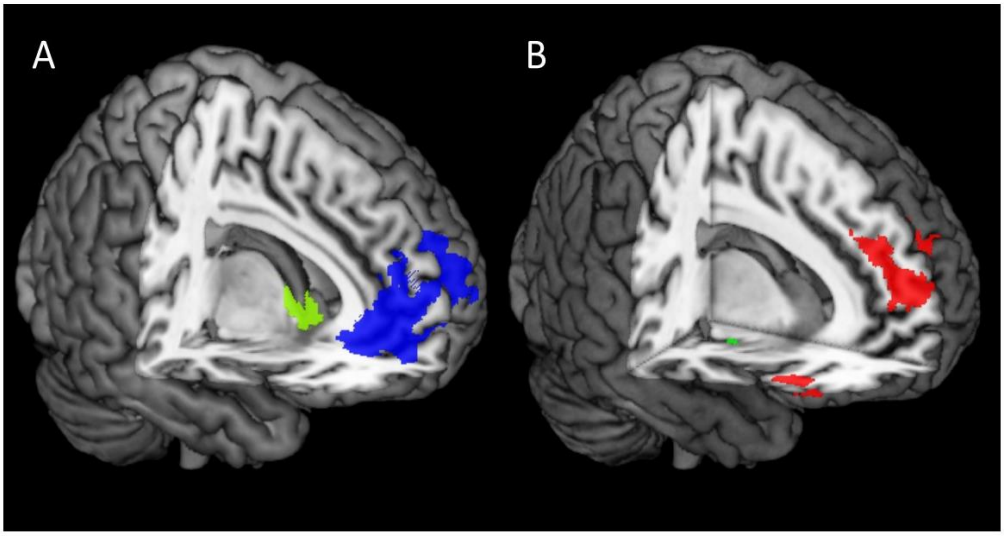


***Figure 4.*** *Group differences in functional connectivity as a function of provocations. (A) Blue cluster (3338 voxels, $p_{corr}<0.05$) in which the right striatum (green seed) functional connectivity was significantly greater in control subjects relative to violent offenders. (B) Red cluster (3094 voxels, $p_{corr}<0.05$) in which the right amygdala (green seed) functional connectivity was significantly greater in control subjects relative to violent offenders.*

Across all participants, striatal and amygdala reactivity to provocations was positively correlated with both taskrelated aggressive behaviour and trait anger (figure 5). Moreover, level of psychopathy (PCL-R score) correlated positively with left amygdala reactivity to provocations in the violent offenders (figure 5).

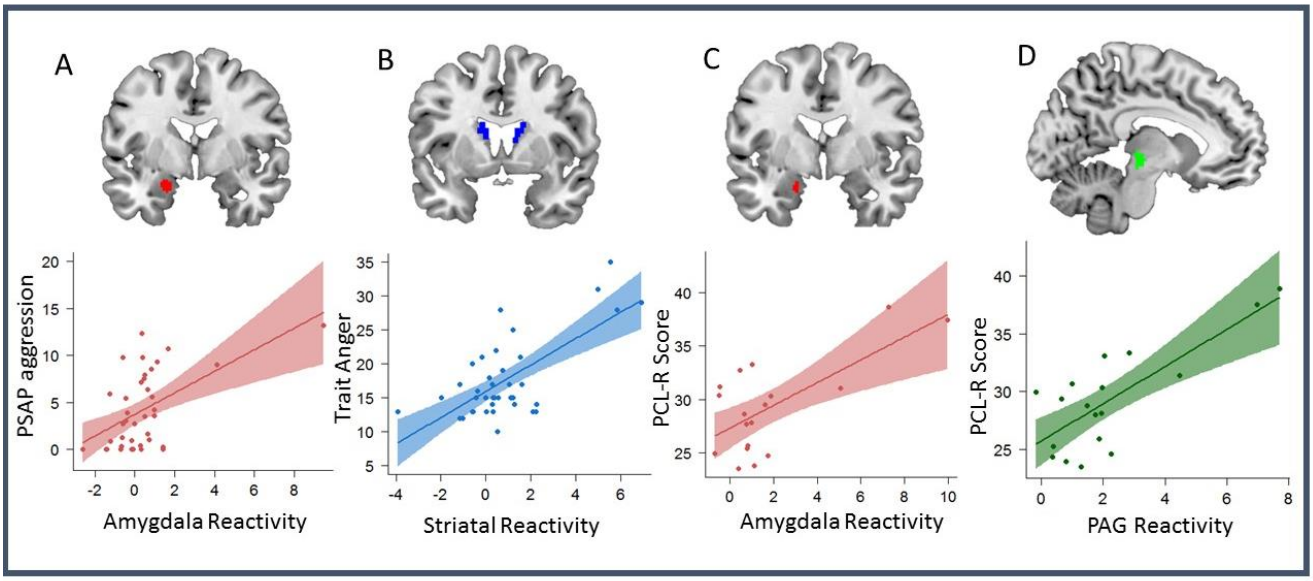


***Figure 5****: Brain-behaviour correlations. Clusters where reactivity to provocations was positively correlated with (A) task-related aggressive behaviour (PSAP aggression), (B) trait anger, (C) and (D) Psychopathy Checklist-Revised Score (PCL-R). Mean signal values for the significant clusters were extracted and plotted for illustrative purposes. Red clusters: amygdala, blue clusters: striatum, green cluster: periaqueductal grey (PAG). Plots are shown given a mean age.*

These findings support a model where impulsive aggression may arise from a combination of heightened "limbic" reactivity or reduced frontolimbic connectivity in the context of provocative stimuli. Assuming that the reduced connectivity reflects reduced prefrontal regulation of subcortical regions, this interpretation is consistent with the theory that the prefrontal cortex inhibits subcortical activity mediating anger and aggression. This interpretation is further substantiated by the heightened reactivity to provocations being associated with taskrelated aggressive behaviour, as well as with self-report measures of trait aggression and trait anger.

In line with the a priori evidence implicating amygdala hyper-reactivity in impulsive aggression, we hypothesized that the violent offenders would have increased amygdala activity when provoked. Interestingly, level of psychopathy was positively associated with amygdala reactivity to provocations, indicating amygdala hyper-responsiveness also in psychopathy. At first glance, this conflicts with evidence suggesting the amygdala is hyporesponsive to threats in psychopaths. Here the distinction between threatrelated and

frustration based reactive aggression is relevant to consider. It has been argued that reactive aggression in psychopaths is frustration based as opposed to threat-related (Blair 2012). We argue that reactive aggression in the PSAP is not threat-related, but rather elicits anger, frustration or irritation (although we have not explicitly asked the participants about this). We speculate that hyporesponsivity of the amygdala in the context of threats and hyper-responsiveness of the amygdala in the context of provocations might explain that psychopaths display both instrumental and reactive aggression.

# Study 2

## Serotonin 1B receptor binding is associated with trait anger and level of psychopathy in violent offenders

This study shows that the associations between 1) striatal 5-$HT_{1B}$ receptor binding and trait anger, and 2) anterior cingulate 5-$HT_{1B}$ receptor binding and trait psychopathy were moderated by group, i.e. significant interaction effects (figure 6).

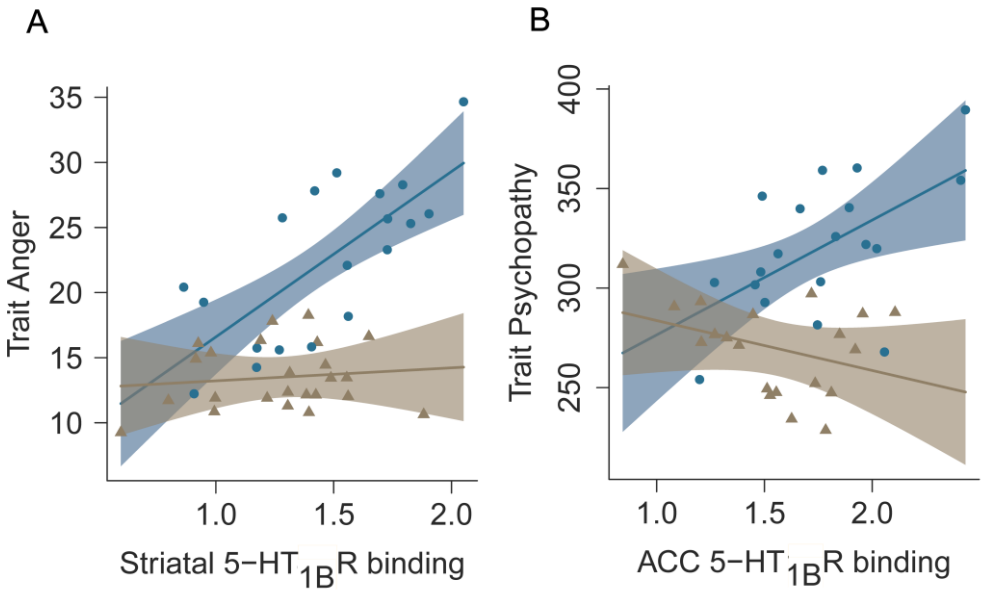


***Figure 6:*** *Plots showing group by 5-$HT_{1B}$ receptor binding interaction effects. (A) The association between striatal 5-$HT_{1B}$ receptor binding and trait anger was moderated by group (difference in slopes, $p_{corrected}$=0.04). (B) The association between anterior cingulate cortex (ACC) 5-$HT_{1B}$ receptor binding and trait psychopathy was moderated by group (difference in slopes, $p_{corrected}$=0.08). Blue circles: violent offenders, brown triangles: healthy controls. Plots are shown given a mean age, injected mass/kg and IQ.*

In the violent offenders, striatal 5-$HT_{1B}$ receptor binding was positively correlated with trait anger (p=0.002), trait psychopathy (p=0.01) and PCL-R score (p=0.03) (figure 7).

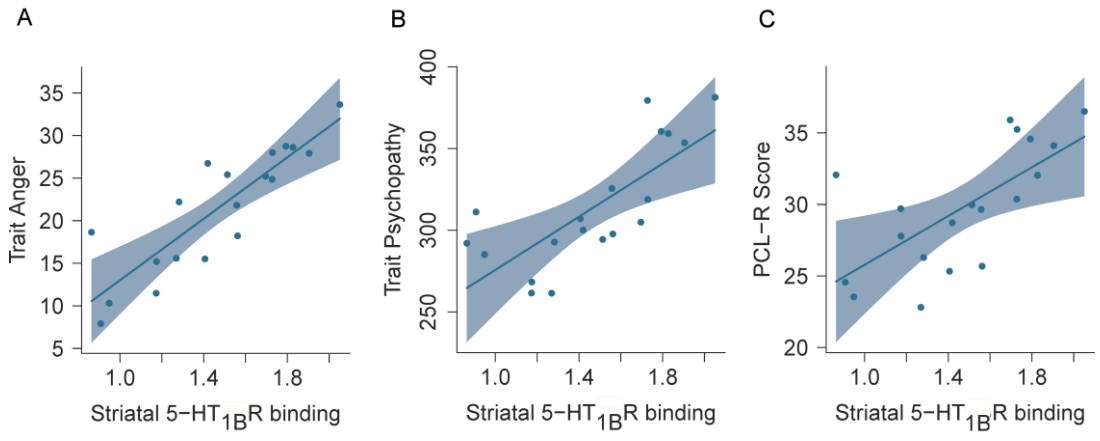


***Figure 7**: Associations between striatal 5-HT$_{1B}$ receptor binding and A) trait anger, B) trait psychopathy and C) Psychopathy Checklist-Revised (PCL-R) score in violent offenders. Plots are shown given a mean age, injected mass/kg and IQ.*

The association between 5-HT$_{1B}$ receptor binding and trait anger in violent offenders was confirmed in a wholebrain voxelbased analysis (figure 8).

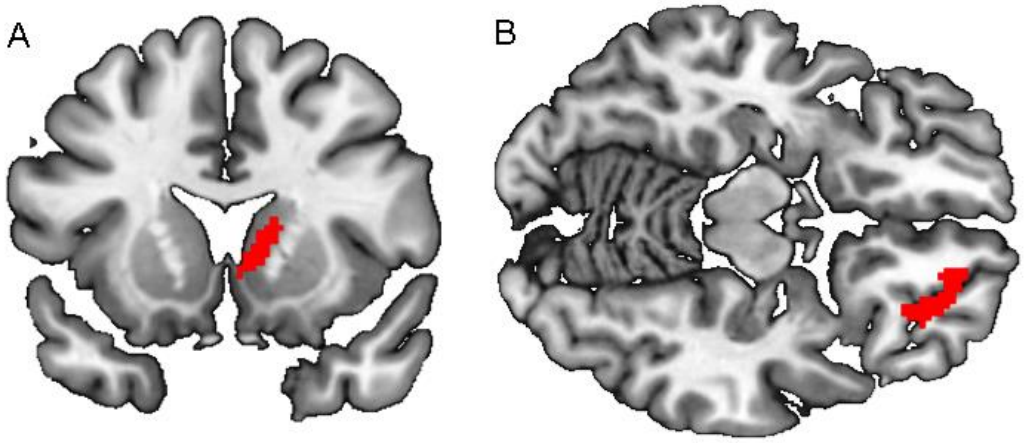


***Figure 8**. Whole brain voxelwise analysis showing two clusters (in red) mapped onto a magnetic resonance template image, where trait anger is positively correlated with 5-HT$_{1B}$ receptor binding. (A) Cluster of k=183 voxels covering the right orbitofrontal cortex ($p_{corrected}$<0.05). (B) Cluster of k=178 voxels covering the right caudate ($p_{corrected}$<0.05).*

We did not find evidence for group differences in 5-HT$_{1B}$ receptor binding, suggesting that 5-HT$_{1B}$ receptor availability is not a marker for violent offending in itself, but rather reflects symptom severity (level of anger and psychopathy) in the violent offenders. We further

speculate that the association between 5-$HT_{1B}$ receptor binding and trait anger is present also in healthy controls, but we cannot exclude that the low variability of trait anger in the healthy control group (given the inclusion criteria) might have generated significant interaction effects.

We selected the participants based on a hypothesis that differences in aggression-related neurobiology would be detectable in individuals from the two extremes of the aggression spectrum. Therefore, we selected aggressive individuals that were incarcerated due to violent offending and control participants based on low self-report trait aggression. Thus, our study sample was dichotomized into either being highly aggressive or placid. However, aggression is a dimensional construct and interindividual differences in aggression reflect a spectrum of different combinations of aggression and constructs closely linked to aggression; anger, callous-unemotional traits, impulsivity and so on. For evaluation of how the neuroimaging parameters are related to aggression and anger as dimensional constructs, it would be optimal to also include nonoffenders with medium to high levels of aggression or anger. The interaction effect may represent a true interaction but may also be a result of the low variance in trait anger within the control group. As show in figure 11, the variance in trait anger and trait aggression was larger in the violent offender group than in the control group. Also, when evaluating the associations across groups (in a model with group status as an additional covariate), we found significant positive correlations between trait anger and 5-$HT_{1B}$ receptor binding within the striatum (slope estimate: 6.3, 95% confidence interval: [2.0 ; 10.6], p=0.006), anterior cingulate cortex (slope estimate: 4.8, 95% confidence interval: [0.04 ; 9.5], p=0.05) and orbitofrontal cortex (slope estimate: 5.8, 95% confidence interval: [0.9 ; 10.7], p=0.02).

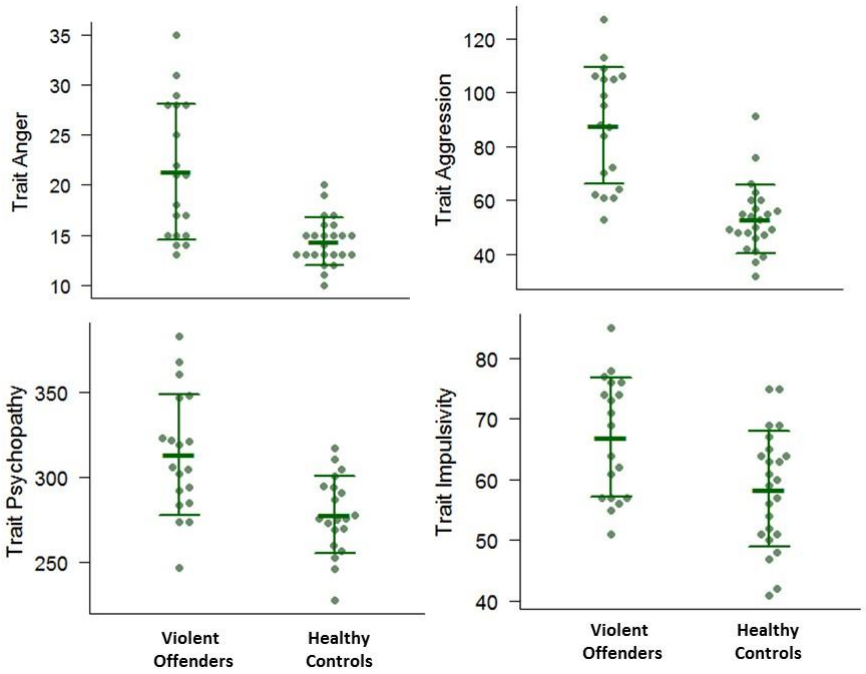

***Figure 11**. Group differences in trait anger, trait aggression, trait psychopathy and trait impulsivity.*

The finding that *high* 5-$HT_{1B}$ receptor binding was related to high trait anger was unanticipated and contradicts our hypothesis stating that high aggression would be associated with *low* 5-$HT_{1B}$ receptor binding. We based this hypothesis on numerous replicated preclinical observations of aggression in 5-$HT_{1B}$ receptor knockout mice and aggression-reducing effects of 5-$HT_{1B}$ receptor agonists. We therefore hypothesized that this would translate to low levels of 5-$HT_{1B}$ receptors in aggressive humans. Speculative explanations for these seemingly divergent findings include:

1) In animal 5-$HT_{1B}$ receptor knockout models it is possible that receptor upregulation or other compensatory mechanisms could be involved in the observed behavioural changes.
2) The association between 5-$HT_{1B}$ receptor binding and trait anger/psychopathy in humans may be specific to either heteroreceptor or autoreceptor function, a distinction that cannot be evaluated given that [$^{11}$C]AZ10419369 binds to both receptor populations. In mice, it is possible to specifically knock down only auto- or heteroreceptors. Indeed, specific knockout of the autoreceptors did not change impulsivity or aggression whereas specific knockout of heteroreceptors increased aggression only, indicating specific involvement of only heteroreceptors in animal aggression (Nautiyal, Tanaka et al. 2015).
3) In humans, the emotional experience of feeling angry is most likely subject to interindividual variability. It is not known whether aggression is accompanied by such emotional experience in mice. Psychopathy is a personality dimension consisting of a combination of traits. I am unaware of any animal models of psychopathy, and doubt that this construct is possible to model in animals.

The discrepant findings emphasize the importance of evaluating the role of 5-$HT_{1B}$ receptors in aggression also in humans.

An issue with neuroreceptor PET is that receptor binding data are indirect measures, subject to several interpretations. The PET measure of 5-$HT_{1B}$ receptor binding depends on receptor density, affinity of the radioligand for the receptor and can also be affected by synaptic serotonin levels. Thus, high 5-$HT_{1B}$ receptor binding in individuals with high anger and psychopathy may therefore reflect different aspects of serotonin signalling; low synaptic serotonin levels or high 5-$HT_{1B}$ receptor density. In non-human primates, 5-$HT_{1B}$ receptor binding is sensitive to acute changes in serotonin levels. Finnema et al. demonstrate that administration of fenfluramine (a potent serotonin releaser) to monkeys reduces 5-$HT_{1B}$ receptor binding (Finnema, Varrone et al. 2010). However, in humans a single dose of serotonin reuptake inhibitors, which is assumed to elevate serotonin levels, increased 5-$HT_{1B}$ receptor binding in projection areas (Nord, Finnema et al. 2013). The authors speculate that the acute administration of serotonin reuptake inhibitors in this study led to activation of serotonin autoreceptors, thereby reducing synaptic serotonin levels

which might have resulted in this unexpected increase. A study evaluating 5-$HT_{1B}$ receptor binding after chronic exposure to serotonin reuptake inhibitors would be needed to evaluate whether 5-$HT_{1B}$ receptor binding is inversely related to stable serotonin levels. 5-$HT_{1B}$ receptor binding may also reflect a genetic or neurodevelopmentally determined 5-$HT_{1B}$ receptor density. In this way, serotonin putatively affects anger and psychopathy through 5-$HT_{1B}$ heteroreceptors via other neurotransmitter systems.

## Study 3

### Men with high serotonin 1B receptor binding have high trait anger and respond to provocations with heightened amygdala and striatal reactivity

In this study we show that an underlying latent construct captures shared variance between trait anger, reactivity to provocations and 5-$HT_{1B}$ receptor binding across regions (figure 9). That is, individuals with high 5-$HT_{1B}$ receptor binding across regions have high trait anger *and* high amygdala and striatal reactivity to provocations, independent of group status.

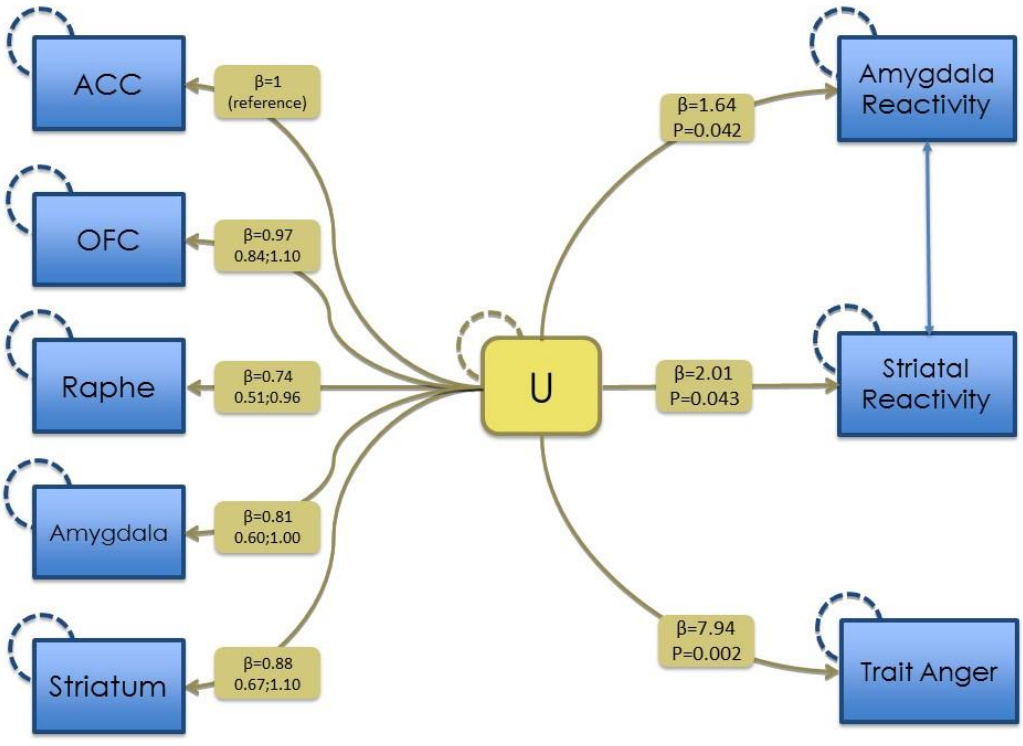


***Figure 9.*** *Latent variable model of 5-$HT_{1B}$ receptor binding, trait anger, amygdala and striatal reactivity to provocations. The yellow oval box represents the latent variable (u). Blue boxes represent measured variables including regional 5-$HT_{1B}$ receptor binding, trait anger, amygdala and striatal reactivity to provocations. Blue line between amygdala and*

*striatal reactivity and between amygdala and raphe denotes additional shared correlation. Hatched circles indicate error estimates included in the model. Parameter estimates (β) for each model path are shown in the corresponding box. Group status was included as a predictor for trait anger, striatal and amygdala reactivity, and age, IQ and injected mass/kg were included as predictors for regional 5-HT$_{1B}$ receptor binding, but omitted from the figure for clarity.*

Whereas we show that trait anger is associated with amygdala and striatal reactivity to provocations (study 1) and with 5-HT$_{1B}$ receptor binding (study 2), we find in study 3 shared correlations between these measures. Even though we cannot infer causality from the latent variable model, we speculate that the association between 5-HT$_{1B}$ receptor binding and trait anger is mediated by amygdala and striatal reactivity to provocations. A theoretical framework with assumed directionalities for such theory is shown in figure 10. However, in mediation analyses larger sample sizes are required, even for small effect sizes (MacKinnon, Lockwood et al. 2002).

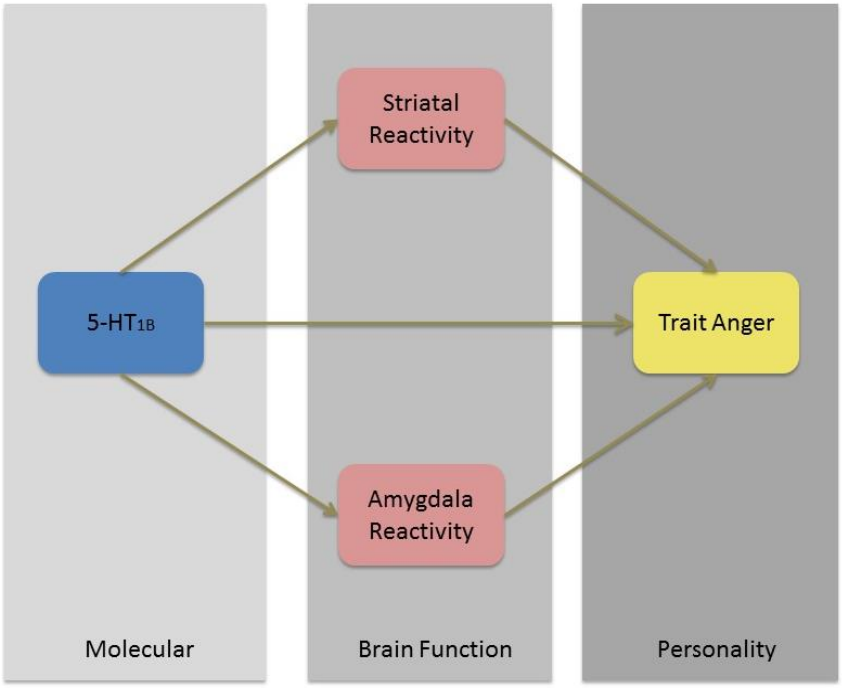


***Figure 10.*** *Hypothesized directionalities in the associations between 5-HT$_{1B}$ receptor binding, reactivity to provocations and trait anger.*

# Methodological Considerations

## The Point-Subtraction Aggression Paradigm

Whereas normal aggression is a defence mechanism to a perceived or real threat, pathological aggression can be regarded as a reaction to an exaggerated emotional experience (e.g. anger) associated with minor provocations or frustrations. Unfair provocations in the PSAP and other retaliatory versions of social exchange paradigms probably initiate anger (Blair 2015). Given that the participants lose only 1.34 euro/1.5$ during each provocation in the PSAP, we consider these provocations as minor. Thus, with the distinction between normal and pathological aggression in mind, the PSAP can be considered as targeting aspects of pathological aggression. In addition to impulsive aggression and anger, the PSAP probably entails some aspect of competition. Even though the instructions state that that the participants will receive the amount of money corresponding to the points they earn regardless of who gets most points, some of the participants seemed to interpret the game as a competition. For example, they would ask us "who won?" after they came out of the scanner. The element of competition might for people that are "bad losers" result in especially heightened irritation. On the other hand, if the PSAP is perceived as a competition, this might be a more socially acceptable setting to treat the other participant unfairly by removing money from them.

Another limitation with the PSAP is the user driven number of aggressive responses and the variable amounts of provocations. However, this also makes the paradigm more realistic.

## Cold mass

Injection of high mass of unlabelled compound can result in an underestimation of the binding potential due to competitive binding between the unlabelled and radiolabelled ligand. Because of this, we included injected mass/kg bodyweight as a covariate in the regression analyses in study 2 and 3. However, the upper cold dose of [$^{11}$C]AZ10419369 required to yield a receptor occupancy of 5 % (which is commonly described as the limit for tracer doses) has not been established. This could for example be estimated by measurements with different mass doses in the same person. Two participants received a particularly high dose of cold mass (5.5 and 6 µg). Therefore, we conducted a sensitivity analysis (supplementary data) in study 2, in which the results are presented after exclusion of these participants.

## Intelligence Quotient

Because of previously reported associations between domains of cognitive function and serotonin signalling (Madsen, Erritzoe et al. 2011; Haahr, Fisher et al. 2013; Penttila,

Hirvonen et al. 2015), we included an estimate of IQ in the statistical analyses of the PET data. For estimation of IQ, we used the Reynolds Intellectual Screening Test (RIST), which has shown good reliability for screening of intelligence. The RIST index is based on two subtests within a full IQ assessment and takes shorter time to administer (approximately 25 minutes). The correlation with another well-known IQ test, the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III) is 0.67 (Reynolds).

The violent offenders had a significantly lower RIST index compared with controls (violent offenders: mean 98.3 ± 8.6, range 84 – 120, healthy controls: mean 108.0 ± 7.1, range 95 – 121). This group difference in IQ may partly reflect the significant group differences in education. Indeed, the number of years in school was significantly positively correlated with RIST index (slope estimate: 1.4, CI: [0.17;2.72], p=0.02 in a multiple linear regression model adjusting for age and group status). Most of the violent offenders had conduct disorder during childhood, which is part of antisocial personality disorder according to the DSM-4. The syndrome of conduct disorder involve rulebreaking behaviours, aggression and disregard for others (Blair, Leibenluft et al. 2015). These types of behaviours will most often interfere with education. Therefore, a low IQ (in some of the violent offenders) can partly be regarded as inherent to the disorder itself. That being said, the mean IQ of the violent offender group was close to normal population, it was rather the control subjects who performed above average in the RIST. This is in line with healthy control subjects participating in other studies at the Neurobiology Research Unit.

# Perspectives

An understanding of the neurobiological mechanisms involved in aggression will ideally result in improved antiaggressive treatments. Eventually, it will hopefully also be possible to use neuroimaging or other biological markers as a supplement for psychiatric assessment techniques; in differentiating patient groups with similar clinical presentations, in predicting treatment sensitivity and in predicting risk for recidivism or reoffending. These scenarios will probably require many years of research.

## 5-$HT_{1B}$ receptors as a pharmacological target for anti-aggressive treatment

Existing pharmacological drugs acting on 5-$HT_{1B}$ receptors include the triptans, which are 5-$HT_{1B/1D}$ receptor agonists and are used for the treatment of migraine pain. The mechanism of these effects are thought include vasoconstriction of dilated extracranial arteries and inhibition of nociception of the trigeminocervical complex (Tepper, Rapoport et al. 2002). One small study of 11 healthy subjects tested the putative antiaggressive effects of zolmitriptan in conjunction with alcohol intake, reporting that zolmitriptan decreased aggressive responses in the PSAP but only after intake of alcohol (Gowin, Swann et al.

2010). Zolmitriptan is a triptan that crosses the bloodbrain barrier, but the receptor occupancy at pharmacological doses (10 mg) is very low (4-5%), whereas lower doses (5 mg) do not affect 5-$HT_{1B}$ receptor binding (Varnas, Jucaite et al. 2013). The 5 mg dose was used in the Gowin study, questioning the reported effects of zolmitriptan on aggression (Gowin, Swann et al. 2010). Given that administration of 5-$HT_{1B}$ receptor agonists reduce aggression in rodents, 5-$HT_{1B}$ agonism can be assumed also to have antiaggressive effects in humans. As mentioned in the introduction, this was tested in the nineties with serenics, which are 5-$HT_{1A/1B}$ agnoists. Serenics did not have convincing antiaggressive effects, and further development was terminated (Verhoeven 2007).

However, given the discrepant findings of 5-$HT_{1B}$ receptor function in human and animal aggression, the antiaggressive effects of 5-$HT_{1B}$ receptor agonists in animals may not be directly translatable to humans. The findings presented in this thesis may suggest that 5-$HT_{1B}$ antagonists can have antiaggressive effects. The effects of 5-$HT_{1B}$ receptor antagonists on autoreceptors would in theory reduce the negative feedback of serotonin, thereby increasing serotonin release into the synaptic cleft. Given that the 5-$HT_{1B}$ receptor is an inhibitory receptor (Sari 2004), the antagonistic effects on heteroreceptors would theoretically also increase the firing of nonserotonergic neurons. Nonetheless, infusion of a 5-$HT_{1B}$ agonists into the rat nucleus accumbens increases dopamine levels (Yan and Yan 2001), so the effects are not always theoretically predictable. The 5-$HT_{1B}$ receptor antagonist AZD3783 has been given to humans in a PET study evaluating the receptor occupancy of the drug (Varnas, Nyberg et al. 2011). Although showing high receptor occupancy and no serious adverse side effects during the PET study, further development of the drug was terminated due to safety concerns (Varnas, Nyberg et al. 2011; Chang, Ciaccio et al. 2014). Thus, there are currently no selective 5-$HT_{1B}$ receptor antagonists approved for use in humans.

## Neuroethics, neuroscience and the law

Neurobiological studies of aggression can putatively have impact on legal culpability and criminal responsibility. For example, lawyers in the United States, Britain and Italy have used the low activity MAO-A gene in murderers to advocate for a mitigation of the sentence due to a genetic predisposition for violent behaviour. Theoretically, deficits shown in brain regions involved in impulsive aggression could be used to argue that a defendant did not act purposefully when committing the crime. On the other hand, this kind of argument could probably also be used to support an opposing argument; that the perpetrator has a neurobiological predisposition for aggression and is therefore more likely to reoffend. Even though definitive causal links between brain and behaviour are too premature to be used for such arguments, PET and MRI have been used in American courtrooms to advocate for frontal lobe dysfunction or structural abnormalities (Glenn and Raine 2014).

An intriguing example considering both criminal responsibility and causal relationships between brain dysfunctions and behavioural disinhibtion is the case of a 40-year old male schoolteacher, who over the course of a year began collecting child pornography and

solicited prostitutes (Burns and Swerdlow 2003). He had no history of criminal behaviour, and had lived happily with his wife and stepdaughter. He eventually took sexual advances towards his prepubescent stepdaughter and was found guilty for child molestation. He was expelled from a rehabilitation center because he could not restrain himself from seeking sexual favours from the staff and other clients. The night before his prison sentencing, he sought medical help because of a bad headache. The MRI of the brain revealed a large tumour in the orbitofrontal cortex. After resection of this tumour, his behaviour returned to normal. A year later, he again developed headache and had started collecting new child pornography. MRI showed tumour regrowth and was again resected, after which his behaviour stayed normal for at least six years (Glenn and Raine 2014). Since this patient had a pre-existing strong interest in pornography, the authors of this case argue that the tumour caused his behavioural inhibition of the strong sexual impulses. The one major issue in terms of cause and responsibility is that behavioural genetics and neuroimaging generally cannot explain behaviour at an individual level yet; in the fMRI and PET experiments used this thesis it is not established whether a given activity is within a normal range and there are no defined pathological levels of binding potentials. Therefore, heightened activity of e.g. the amygdala during provocations in fMRI, or high levels of serotonin receptor availability may represent neurobiological predispositions but cannot be used to establish the cause of the behaviour. Moreover, multiple brain circuits and functions are most likely involved in violent behaviour.

## Research perspectives

Replications are warranted given the observational nature of the studies in this thesis and that the PET examinations are the first of 5-$HT_{1B}$ receptors in aggressive individuals. It would be interesting to use the fMRI-version of the PSAP to evaluate effects on brain reactivity to provocations of interventions targeting serotonin functions, preferably acting on 5-$HT_{1B}$ receptors. It would also be relevant to evaluate 5-$HT_{1B}$ receptor binding or brain reactivity to provocations as biomarkers for reoffending. This would require longitudinal followup of the violent offenders. However, such study is not feasible in the current sample because many of the violent offenders still had many years left of their prison sentences. An interesting study of "neuroprediction" used a study design in which inhibitory control was evaluated (using a Go/NoGo task in fMRI) in male offenders just before release (Aharoni, Vincent et al. 2013). Male offenders with low anterior cingulate activity during behavioural inhibition were at higher risk for being rearrested within four years from release. In the future, neuroimaging might supplement current risk assessments for recidivism.

# Conclusion

The studies included in this thesis have uncovered novel aspects of human aggression by the use of multimodal neuroimaging. We have for the first time used an fMRI version of the PSAP and 5-$HT_{1B}$ receptor PET imaging in a cohort of pathologically aggressive individuals. The results demonstrate that in humans, 5-$HT_{1B}$ receptors are involved in trait anger, level of psychopathy and neural reactivity to provocations.

Violent offenders showed heightened amygdala and striatal reactivity to provocations and reduced functional connectivity between these regions and the prefrontal cortex when provoked. Assuming that the reduced connectivity reflects prefrontal control of the amygdala and striatum, this supports that impulsive aggression may arise from combination of heightened "limbic" reactivity and reduced frontolimbic connectivity in the context of provocative stimuli. Whereas there were no group differences in 5-$HT_{1B}$ receptor binding, striatal 5-$HT_{1B}$ receptor binding was positively associated with trait anger and level of psychopathy in the violent offenders. When combining PET and fMRI data across all participants, we found that a common construct (an estimated latent variable) predicted variability in 5-$HT_{1B}$ receptor binding across regions, trait anger and brain reactivity to provocations. Thus, men with high 5-$HT_{1B}$ receptor binding within a neural circuitry relevant for aggression react to provocations with heightened amygdala and striatal responsiveness and report a general disposition to experience angry feelings.

The current findings substantially support that serotonin signalling is involved constructs closely linked to aggression and accentuate 5-$HT_{1B}$ receptors to specifically influence responses to provocations in the context of a social interaction. Given that violent offenders had increased amygdala and striatal reactivity to provocations, reduction of aggressive and violent behaviours might be achieved by interventions that can reduce this heightened neural responsiveness. 5-$HT_{1B}$ receptors may represent a molecular target for reducing excessive amygdala and striatal reactivity to provocative stimuli.

# References


Aharoni, E., G. M. Vincent, et al. (2013). "Neuroprediction of future rearrest." Proc Natl Acad Sci U S A **110**(15): 6223-6228.

Alia-Klein, N., R. Z. Goldstein, et al. (2008). "Brain monoamine oxidase A activity predicts trait aggression." J Neurosci **28**(19): 5099-5104.

Archer, J. (2009). "The nature of human aggression." Int J Law Psychiatry **32**(4): 202-208.

Bagdy, E., I. Kiraly, et al. (2000). "Reciprocal innervation between serotonergic and GABAergic neurons in raphe nuclei of the rat." Neurochem Res **25**(11): 1465-1473.

Benjamini, Y., Hochberg Y. (1995). "Controlling the false discovery rate: a practical and powerful approach to multiple testing." Journal of the Royal Statistical Society B **57**: 289-300.

Beyer, F., T. F. Munte, et al. (2014). "Orbitofrontal Cortex Reactivity to Angry Facial Expression in a Social Interaction Correlates with Aggressive Behavior." Cereb Cortex.

Beyer, F., T. F. Munte, et al. (2015). "Orbitofrontal Cortex Reactivity to Angry Facial Expression in a Social Interaction Correlates with Aggressive Behavior." Cereb Cortex **25**(9): 3057-3063.

Blair, R. J. (2004). "The roles of orbital frontal cortex in the modulation of antisocial behavior." Brain Cogn **55**(1): 198-208.

Blair, R. J. (2010). "Psychopathy, frustration, and reactive aggression: the role of ventromedial prefrontal cortex." Br J Psychol **101**(Pt 3): 383-399.

Blair, R. J. (2012). "Considering anger from a cognitive neuroscience perspective." Wiley Interdiscip Rev Cogn Sci **3**(1): 65-74.

Blair, R. J. (2015). "The Neurobiology of Impulsive Aggression." J Child Adolesc Psychopharmacol.

Blair, R. J., L. Jones, et al. (1997). "The psychopathic individual: a lack of responsiveness to distress cues?" Psychophysiology **34**(2): 192-198.

Blair, R. J., E. Leibenluft, et al. (2015). "Conduct disorder and callous-unemotional traits in youth." N Engl J Med **372**(8): 784.

Bockaert, J., S. Claeysen, et al. (2006). "Neuronal 5-HT metabotropic receptors: fine-tuning of their structure, signaling, and roles in synaptic modulation." Cell Tissue Res **326**(2): 553-572.

Brown, A. K., D. T. George, et al. (2007). "PET [11C]DASB imaging of serotonin transporters in patients with alcoholism." Alcohol Clin Exp Res **31**(1): 28-32.

Brown, G. L., F. K. Goodwin, et al. (1979). "Aggression in humans correlates with cerebrospinal fluid amine metabolites." Psychiatry Res **1**(2): 131-139.

Burns, J. M. and R. H. Swerdlow (2003). "Right orbitofrontal tumor with pedophilia symptom and constructional apraxia sign." Arch Neurol **60**(3): 437-440.

Bushman, B. J. and C. A. Anderson (2001). "Is it time to pull the plug on the hostile versus instrumental aggression dichotomy?" Psychol Rev **108**(1): 273-279.

Buss, A. H. and M. Perry (1992). "The aggression questionnaire." J Pers Soc Psychol **63**(3): 452-459.

Carre, J. M. and C. M. McCormick (2008). "Aggressive behavior and change in salivary testosterone concentrations predict willingness to engage in a competitive task." Horm Behav **54**(3): 403-409.

Caspi, A., J. McClay, et al. (2002). "Role of genotype in the cycle of violence in maltreated children." Science **297**(5582): 851-854.

Chang, J. C., P. Ciaccio, et al. (2014). "Pathology and Neurotoxicity in Dogs after Repeat Dose Exposure to a Serotonin 5-HT1B Inhibitor." J Toxicol Pathol **27**(1): 31-42.
Cherek, D. R., F. G. Moeller, et al. (1997). "Studies of violent and nonviolent male parolees: I. Laboratory and psychometric measurements of aggression." Biol Psychiatry **41**(5): 514-522.
Coccaro, E. F., M. S. McCloskey, et al. (2007). "Amygdala and orbitofrontal reactivity to social threat in individuals with impulsive aggression." Biol Psychiatry **62**(2): 168-178.
Coccaro, E. F., C. S. Sripada, et al. (2011). "Corticolimbic function in impulsive aggressive behavior." Biol Psychiatry **69**(12): 1153-1159.
Conner, T. S., K. P. Jensen, et al. (2010). "Functional polymorphisms in the serotonin 1B receptor gene (HTR1B) predict self-reported anger and hostility among young men." Am J Med Genet B Neuropsychiatr Genet **153B**(1): 67-78.
Cooke, D. J., C. Michie, et al. (2005). "Assessing psychopathy in the UK: concerns about cross-cultural generalisability." Br J Psychiatry **186**: 335-341.
Corradi-Dell'Acqua, C., C. Civai, et al. (2013). "Disentangling self- and fairness-related neural mechanisms involved in the ultimatum game: an fMRI study." Soc Cogn Affect Neurosci **8**(4): 424-431.
Cremers, H., R. Lee, et al. (2016). "Effects of Escitalopram Administration on Face Processing in Intermittent Explosive Disorder: An fMRI Study." Neuropsychopharmacology **41**(2): 590-597.
da Cunha-Bang, S., B. Mc Mahon, et al. (2016). "High trait aggression in men is associated with low 5-HT levels, as indexed by 5-HT4 receptor binding." Soc Cogn Affect Neurosci **11**(4): 548-555.
da Cunha-Bang, S., D. S. Stenbaek, et al. (2013). "Trait aggression and trait impulsivity are not related to frontal cortex 5-HT2A receptor binding in healthy individuals." Psychiatry Res **212**(2): 125-131.
Davidson, R. J., K. M. Putnam, et al. (2000). "Dysfunction in the neural circuitry of emotion regulation--a possible prelude to violence." Science **289**(5479): 591-594.
De Almeida, R. M., M. M. Rosa, et al. (2006). "5-HT(1B) receptors, ventral orbitofrontal cortex, and aggressive behavior in mice." Psychopharmacology (Berl) **185**(4): 441-450.
de Koning, P., M. Mak, et al. (1994). "Eltoprazine in aggressive mentally handicapped patients: a double-blind, placebo- and baseline-controlled multi-centre study. The Eltoprazine Aggression Research Group." Int Clin Psychopharmacol **9**(3): 187-194.
DSM-5 (2013). Diagnostic and Statistical Manual of Mental Disorders DSM-5 Fifth Edition, American Psychiatric Association.
Duke, A. A., L. Begue, et al. (2013). "Revisiting the serotonin-aggression relation in humans: a meta-analysis." Psychol Bull **139**(5): 1148-1172.
Faccidomo, S., I. M. Quadros, et al. (2012). "Infralimbic and dorsal raphe microinjection of the 5-HT(1B) receptor agonist CP-93,129: attenuation of aggressive behavior in CFW male mice." Psychopharmacology (Berl) **222**(1): 117-128.
Finnema, S. J., A. Varrone, et al. (2010). "Fenfluramine-induced serotonin release decreases [11C]AZ10419369 binding to 5-HT1B-receptors in the primate brain." Synapse **64**(7): 573-577.
Forman, S. D., J. D. Cohen, et al. (1995). "Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold." Magn Reson Med **33**(5): 636-647.

Frankle, W. G., I. Lombardo, et al. (2005). "Brain serotonin transporter distribution in subjects with impulsive aggressivity: a positron emission study with [11C]McN 5652." Am J Psychiatry **162**(5): 915-923.
Friston, K. J., C. Buechel, et al. (1997). "Psychophysiological and modulatory interactions in neuroimaging." Neuroimage **6**(3): 218-229.
Fusar-Poli, P., A. Placentino, et al. (2009). "Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies." J Psychiatry Neurosci **34**(6): 418-432.
Gabay, A. S., J. Radua, et al. (2014). "The Ultimatum Game and the brain: a meta-analysis of neuroimaging studies." Neurosci Biobehav Rev **47**: 549-558.
Geniole, S. N., C. E. Cunningham, et al. (2015). "Costly retaliation is promoted by threats to resources in women and threats to status in men." Aggress Behav **41**(6): 515-525.
Gilam, G. and T. Hendler (2015). "Deconstructing Anger in the Human Brain." Curr Top Behav Neurosci.
Glenn, A. L. and A. Raine (2014). "Neurocriminology: implications for the punishment, prediction and prevention of criminal behaviour." Nat Rev Neurosci **15**(1): 54-63.
Glenn, A. L. and Y. Yang (2012). "The potential role of the striatum in antisocial behavior and psychopathy." Biol Psychiatry **72**(10): 817-822.
Gowin, J. L., A. C. Swann, et al. (2010). "Zolmitriptan and human aggression: interaction with alcohol." Psychopharmacology (Berl) **210**(4): 521-531.
Gregg, T. R. and A. Siegel (2001). "Brain structures and neurotransmitters regulating aggression in cats: implications for human aggression." Prog Neuropsychopharmacol Biol Psychiatry **25**(1): 91-140.
Hagan, C. E., R. A. McDevitt, et al. (2012). "5-HT(1B) autoreceptor regulation of serotonin transporter activity in synaptosomes." Synapse **66**(12): 1024-1034.
Hakulinen, C., M. Jokela, et al. (2013). "Serotonin receptor 1B genotype and hostility, anger and aggressive behavior through the lifespan: the Young Finns study." J Behav Med **36**(6): 583-590.
Hare, R. (2003). Hare Psychopathy Checklist-Revised (PCL-R), 2nd edition. Toronto, Multi-Health Systems Inc.
Heimburger, R. F., C. C. Whitlock, et al. (1966). "Stereotaxic amygdalotomy for epilepsy with aggressive behavior." JAMA **198**(7): 741-745.
Hochberg, Y. and Y. Benjamini (1990). "More powerful procedures for multiple significance testing." Stat Med **9**(7): 811-818.
Hong, W., D. W. Kim, et al. (2014). "Antagonistic control of social versus repetitive self-grooming behaviors by separable amygdala neuronal subsets." Cell **158**(6): 1348-1361.
Hornboll, B., J. Macoveanu, et al. (2013). "Acute serotonin 2A receptor blocking alters the processing of fearful faces in the orbitofrontal cortex and amygdala." J Psychopharmacol **27**(10): 903-914.
Hu, J., S. Henry, et al. (2010). "Serotonin 1B receptor imaging in alcohol dependence." Biol Psychiatry **67**(9): 800-803.
Hu, X. J., F. H. Wang, et al. (2007). "Effects of the 5-HT1B receptor antagonist NAS-181 on extracellular levels of acetylcholine, glutamate and GABA in the frontal cortex and ventral hippocampus of awake rats: a microdialysis study." Eur Neuropsychopharmacol **17**(9): 580-586.
Haahr, M. E., P. Fisher, et al. (2013). "The 5-HT4 receptor levels in hippocampus correlates inversely with memory test performance in humans." Hum Brain Mapp **34**(11): 3066-3074.
Ichise, M., J. S. Liow, et al. (2003). "Linearized reference tissue parametric imaging methods: application to [11C]DASB positron emission tomography studies

of the serotonin transporter in human brain." J Cereb Blood Flow Metab **23**(9): 1096-1112.
Kiehl, K. A., A. M. Smith, et al. (2001). "Limbic abnormalities in affective processing by criminal psychopaths as revealed by functional magnetic resonance imaging." Biol Psychiatry **50**(9): 677-684.
Kiehl, K. B., JW (2010). "Inside the mind of a psychopath." Scientific American Mind **21**(4): 22-29.
Kim-Cohen, J., A. Caspi, et al. (2006). "MAOA, maltreatment, and gene-environment interaction predicting children's mental health: new evidence and a meta-analysis." Mol Psychiatry **11**(10): 903-913.
Kolla, N. J., B. Matthews, et al. (2015). "Lower Monoamine Oxidase-A Total Distribution Volume in Impulsive and Violent Male Offenders with Antisocial Personality Disorder and High Psychopathic Traits: An [(11)C] Harmine Positron Emission Tomography Study." Neuropsychopharmacology **40**(11): 2596-2603.
Kose, S., J. L. Steinberg, et al. (2015). "Neural correlates of impulsive aggressive behavior in subjects with a history of alcohol dependence." Behav Neurosci **129**(2): 183-196.
Kramer, U. M., H. Jansma, et al. (2007). "Tit-for-tat: the neural basis of reactive aggression." Neuroimage **38**(1): 203-211.
Kramer, U. M., J. Riba, et al. (2011). "An fMRI study on the role of serotonin in reactive aggression." PLoS One **6**(11): e27668.
Lilienfeld, S. O. W. M. R. (2005). Psychopathic Personality Inventory™–Revised (PPI™-R).
Lin, D., M. P. Boyle, et al. (2011). "Functional identification of an aggression locus in the mouse hypothalamus." Nature **470**(7333): 221-226.
Linnoila, M., M. Virkkunen, et al. (1983). "Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentration differentiates impulsive from nonimpulsive violent behavior." Life Sci **33**(26): 2609-2614.
Lotze, M., R. Veit, et al. (2007). "Evidence for a different role of the ventral and dorsal medial prefrontal cortex for social reactive aggression: An interactive fMRI study." Neuroimage **34**(1): 470-478.
MacKinnon, D. P., C. M. Lockwood, et al. (2002). "A comparison of methods to test mediation and other intervening variable effects." Psychol Methods **7**(1): 83-104.
Madsen, K., D. Erritzoe, et al. (2011). "Cognitive function is related to fronto-striatal serotonin transporter levels--a brain PET study in young healthy subjects." Psychopharmacology (Berl) **213**(2-3): 573-581.
Maron, E., M. Wall, et al. (2016). "Effect of short-term escitalopram treatment on neural activation during emotional processing." J Psychopharmacol **30**(1): 33-39.
Matuskey, D., Z. Bhagwagar, et al. (2014). "Reductions in brain 5-HT1B receptor availability in primarily cocaine-dependent humans." Biol Psychiatry **76**(10): 816-822.
McLaren, D. G., M. L. Ries, et al. (2012). "A generalized form of context-dependent psychophysiological interactions (gPPI): a comparison to standard approaches." Neuroimage **61**(4): 1277-1286.
Meyer, J. H., A. A. Wilson, et al. (2008). "Serotonin2A receptor binding potential in people with aggressive and violent behaviour." J Psychiatry Neurosci **33**(6): 499-508.
Moeller, S. B., R. W. Novaco, et al. (2015). "Validation of the Novaco Anger Scale-Provocation Inventory (Danish) With Nonclinical, Clinical, and Offender Samples." Assessment.

Moeller, S. B. N., R.W.; Heinola-Nielsen, V. ; Hougaard, H. (2015). "Validation of the Novaco Anger Scale-Provocation Inventory (Danish) With Nonclinical, Clinical, and Offender Samples " Assessment: 1-13.
Moriarty J, S. B., Trimble MR, De Koning P (1994). "A trial of eltoprazine in the treatment of aggressive behaviours in two populations: patients with epilepsy or Gilles de la Tourette's syndrome. ." Human Psychopharmacology **9**: 253-258.
Motzkin, J. C., J. P. Newman, et al. (2011). "Reduced prefrontal connectivity in psychopathy." J Neurosci **31**(48): 17348-17357.
Mpakopoulou, M., H. Gatos, et al. (2008). "Stereotactic amygdalotomy in the management of severe aggressive behavioral disorders." Neurosurg Focus **25**(1): E6.
Nautiyal, K. M., K. F. Tanaka, et al. (2015). "Distinct Circuits Underlie the Effects of 5-HT1B Receptors on Aggression and Impulsivity." Neuron **86**(3): 813-826.
Nelson, R. J. and B. C. Trainor (2007). "Neural mechanisms of aggression." Nat Rev Neurosci **8**(7): 536-546.
Nord, M., Z. Cselenyi, et al. (2014). "Distinct regional age effects on [11C]AZ10419369 binding to 5-HT1B receptors in the human brain." Neuroimage **103**: 303-308.
Nord, M., S. J. Finnema, et al. (2013). "Effect of a single dose of escitalopram on serotonin concentration in the non-human and human primate brain." Int J Neuropsychopharmacol **16**(7): 1577-1586.
Nord, M., S. J. Finnema, et al. (2014). "Test-retest reliability of [11C]AZ10419369 binding to 5-HT(1B) receptors in human brain." Eur J Nucl Med Mol Imaging **41**(2): 301-307.
Olivier, B. and R. van Oorschot (2005). "5-HT1B receptors and aggression: a review." Eur J Pharmacol **526**(1-3): 207-217.
Pardini, M., F. Krueger, et al. (2011). "Prefrontal cortex lesions and MAO-A modulate aggression in penetrating traumatic brain injury." Neurology **76**(12): 1038-1045.
Parsey, R. V., M. A. Oquendo, et al. (2002). "Effects of sex, age, and aggressive traits in man on brain serotonin 5-HT1A receptor binding potential measured by PET using [C-11]WAY-100635." Brain Res **954**(2): 173-182.
Passamonti, L., M. J. Crockett, et al. (2012). "Effects of acute tryptophan depletion on prefrontal-amygdala connectivity while viewing facial signals of aggression." Biol Psychiatry **71**(1): 36-43.
Patton, J. H., M. S. Stanford, et al. (1995). "Factor structure of the Barratt impulsiveness scale." J Clin Psychol **51**(6): 768-774.
Penttila, J., J. Hirvonen, et al. (2015). "Verbal memory and 5-HT receptors in healthy volunteers - A PET study with [carbonyl-C]WAY-100635." Eur Neuropsychopharmacol.
Potenza, M. N., E. Walderhaug, et al. (2013). "Serotonin 1B receptor imaging in pathological gambling." World J Biol Psychiatry **14**(2): 139-145.
Reynolds, C. R. K., R.W. Reynolds Intellectual Screening Test™ (RIST™) Professional Manual, Hogrefe Psykologisk Forlag A/S, 2011.
Rosell, D. R. and L. J. Siever (2015). "The neurobiology of aggression and violence." CNS Spectr **20**(3): 254-279.
Rosell, D. R., J. L. Thompson, et al. (2010). "Increased serotonin 2A receptor availability in the orbitofrontal cortex of physically aggressive personality disordered patients." Biol Psychiatry **67**(12): 1154-1162.
Rubia, K., F. Lee, et al. (2005). "Tryptophan depletion reduces right inferior prefrontal activation during response inhibition in fast, event-related fMRI." Psychopharmacology (Berl) **179**(4): 791-803.

Rylands, A. J., R. Hinz, et al. (2012). "Pre- and postsynaptic serotonergic differences in males with extreme levels of impulsive aggression without callous unemotional traits: a positron emission tomography study using (11)C-DASB and (11)C-MDL100907." Biol Psychiatry **72**(12): 1004-1011.
Sanfey, A. G., J. K. Rilling, et al. (2003). "The neural basis of economic decision-making in the Ultimatum Game." Science **300**(5626): 1755-1758.
Sari, Y. (2004). "Serotonin1B receptors: from protein to physiological function and behavior." Neurosci Biobehav Rev **28**(6): 565-582.
Saudou, F., D. A. Amara, et al. (1994). "Enhanced aggressive behavior in mice lacking 5-HT1B receptor." Science **265**(5180): 1875-1878.
Siever, L. J. (2008). "Neurobiology of aggression and violence." Am J Psychiatry **165**(4): 429-442.
Skibsted, A. d. C.-B., S.; Carré, J.; Hansen, A.H; Beliveau, V.; Knudsen, G.M.; Fisher, P.M.; (2015). Aggression-related brain function assessed with the point subtraction aggression paradigm in functional magnetic resonance imaging European Neuropsychopharmacology 2015. Amsterdam. **25(Suppl2):S191**.
Sladky, R., M. Spies, et al. (2015). "(S)-citalopram influences amygdala modulation in healthy subjects: a randomized placebo-controlled double-blind fMRI study using dynamic causal modeling." Neuroimage **108**: 243-250.
Soliman, A., R. M. Bagby, et al. (2011). "Relationship of monoamine oxidase A binding to adaptive and maladaptive personality traits." Psychol Med **41**(5): 1051-1060.
Soloff, P. H., L. Chiappetta, et al. (2014). "Effects of serotonin-2A receptor binding and gender on personality traits and suicidal behavior in borderline personality disorder." Psychiatry Res **222**(3): 140-148.
Soloff, P. H., J. C. Price, et al. (2007). "5HT2A receptor binding is increased in borderline personality disorder." Biol Psychiatry **62**(6): 580-587.
Strobel, A., J. Zimmermann, et al. (2011). "Beyond revenge: neural and genetic bases of altruistic punishment." Neuroimage **54**(1): 671-680.
Svarer, C., K. Madsen, et al. (2005). "MR-based automatic delineation of volumes of interest in human brain PET images using probability maps." Neuroimage **24**(4): 969-979.
Takahashi, A., K. Nagayasu, et al. (2014). "Control of intermale aggression by medial prefrontal cortex activation in the mouse." PLoS One **9**(4): e94657.
Tepper, S. J., A. M. Rapoport, et al. (2002). "Mechanisms of action of the 5-HT1B/1D receptor agonists." Arch Neurol **59**(7): 1084-1088.
Tiihonen, J., P. Hakola, et al. (1993). "Eltoprazine for aggression in schizophrenia and mental retardation." Lancet **341**(8840): 307.
van de Giessen, E., D. R. Rosell, et al. (2014). "Serotonin transporter availability in impulsive aggressive personality disordered patients: a PET study with [11C]DASB." J Psychiatr Res **58**: 147-154.
Varnas, K., C. Halldin, et al. (2004). "Autoradiographic distribution of serotonin transporters and receptor subtypes in human brain." Hum Brain Mapp **22**(3): 246-260.
Varnas, K., Y. L. Hurd, et al. (2005). "Regional expression of 5-HT1B receptor mRNA in the human brain." Synapse **56**(1): 21-28.
Varnas, K., A. Jucaite, et al. (2013). "A PET study with [11C]AZ10419369 to determine brain 5-HT1B receptor occupancy of zolmitriptan in healthy male volunteers." Cephalalgia **33**(10): 853-860.
Varnas, K., S. Nyberg, et al. (2011). "Quantitative analysis of [11C]AZ10419369 binding to 5-HT1B receptors in human brain." J Cereb Blood Flow Metab **31**(1): 113-123.

Varnas, K., S. Nyberg, et al. (2011). "Dose-dependent binding of AZD3783 to brain 5-HT1B receptors in non-human primates and human subjects: a positron emission tomography study with [11C]AZ10419369." Psychopharmacology (Berl) **213**(2-3): 533-545.
Verhoeven, W., Tuinier, S. (2007). "Serenics: Anti-aggression drugs throughout history." Clinical Neuropsychiatry **4**(4): 135-143.
White, S. F., S. J. Brislin, et al. (2014). "Punishing unfairness: rewarding or the organization of a reactively aggressive response?" Hum Brain Mapp **35**(5): 2137-2147.
White, S. F., M. VanTieghem, et al. (2015). "Neural Correlates of the Propensity for Retaliatory Behavior in Youths With Disruptive Behavior Disorders." Am J Psychiatry: appiajp201515020250.
Witte, A. V., A. Floel, et al. (2009). "Aggression is related to frontal serotonin-1A receptor distribution as revealed by PET in healthy subjects." Hum Brain Mapp **30**(8): 2558-2570.
Yan, Q. S. and S. E. Yan (2001). "Activation of 5-HT(1B/1D) receptors in the mesolimbic dopamine system increases dopamine release from the nucleus accumbens: a microdialysis study." Eur J Pharmacol **418**(1-2): 55-64.

# APPENDIX 1
# PSAP instructions

**English Translation:**

**Bold writing is for the experimenter, and should not be read to the participants**

*Italics is what should be read to the participants*

*OK, now I'm going to have you play the game. The main goal of this task is to earn as many points as possible. The more points you earn the more money you will receive at the end of the experiment. For this task, you will be paired with another participant, who is at another room at this hospital. You are not allowed to meet, because this might influence the way you play the game.*

*You have three response options available to you; 1, 2, and 3, which correspond to the buttons you press on the response device unit with your index, middle and ring finger,* **[At this point, the experimenter points out the options on the figure provided… this facilitates the understanding of the task].**

*I will now explain to you what the response options do: Hitting Option 1 a hundred consecutive times will cause your point counter to enlarge, flash several times with positive signs around it, causing your point counter to increase by one point* **[while giving this instruction, the experimenter should point to the 'point counter'].**

*Hitting Option 2 ten consecutive times will steal a point from the participant paired with you. However, although this person will lose a point, you have been randomly assigned to the experimental condition in which you do not get to keep the points that you steal from the other player.*

*Hitting Option 3 ten consecutive times will cause your point counter to be protected from steals for a variable amount to time.*

*Once you select an option, it will remain red, while the two other options will turn grey, and be temporarily unavailable* **[the experimenter should point to these grayed-out options on the figure provided].** *For instance, if you were to select Option 1, you would be unable to choose Option 2 until you have pressed '1' a hundred consecutive times. Once you have hit an option the set number of times in a row, all response options will again be available to select.*

*Depending on the other players's strategy, it may occur that your point counter turns red, flashes several times with negative signs around it, and that your point counter decreases by 1 point. If this occurs, this means that the other player paired with you stole a point from you. The other player got alternative instructions and is, in contrast to you, allowed to keep all the points that he or she steals from you.*

*At the end of the experiment, you will be paid based on how many points you earn during the task, and the guy (or girl) paired with you will be paid based on how many points they earned during the task.*

*Do you have any questions? OK, now I'm going to ask you a few questions to make sure that you understand the task. 1) What does option 1 do?* **[their answer should be… hit 100 times and earn a point].** *2a) What does option 2 do?* **[their answer should be… hit 10 times and steal a point from partner]** *2b) Do you get to keep the points that you steal?* **[answer should be 'no'].** *3) Will this person lose a point if you steal from them?* **[answer should be 'yes'].** *4) What if the other person steals a point, does he (or she) get to keep them?* **[answer should be 'yes'].** *5) What does option 3 do?* **[answer should be… hit 10 times and protect points].**

*OK, so you understand the task. In the scanner you will have the opportunity to do one minute practice trial to get you familiar with the task. You'll notice that if you hit the response options too quickly, your 'button press' counter* **[experimenter should point to the counter at the bottom of the screen]** *will freeze, indicating that these button presses are not being recorded. The practice session will enable you to find the optimal button press rate. During the practice trial, you are not paired with anyone, and the points that you earn during this half minute practice trial will not count towards your point total (in other words, you won't make money in this round). After the practice trial, you will play the game for 12 minutes paired with the other person, at which all points earned will be converted to money at the end of the session. Also, during this session, you will be paired with another guy (or girl). Do you have any questions?*

**[When the trial session is done].**

*"OK, now we're gonna start the real game. I need to call the other group and check if they are ready".* **After some time:** *"You might have to wait a couple of minutes, the other group is not ready yet".* **After another short break:** *"Ok we're ready to go, good luck".*

# Study 1

# Titlepage

## Title: Violent offenders respond to provocations with high amygdala and striatal reactivity

**Short title:** ***Brain responses to provocations in violent offenders***

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**Keywords:** Amygdala, Striatum, Connectivity, fMRI, Point Subtraction Aggression Paradigm, PSAP, Psychopathy

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## Abstract

The ability to successfully suppress impulses and angry affect is fundamental to control aggressive reactions following provocations. Poor behavioral control may stem from deficiencies in fronto-limbic function. We aimed to examine neural responses to provocations in violent offenders using a laboratory model of reactive aggression. We used a novel functional magnetic resonance imaging (fMRI) point-subtraction aggression paradigm in 38 men, of whom 18 were incarcerated violent offenders and 20 were control non-offenders. We measured brain activation following provocations (monetary subtractions), while the subjects had the possibility to behave aggressively or pursue monetary rewards. Behaviorally, violent offenders responded aggressively twice more frequently than controls (aggression frequency per provocation: 5.7 vs. 2.8, p=0.02). Relative to controls, violent offenders showed significantly higher brain reactivity to provocations within the amygdala and striatum, as well as reduced amygdala-prefrontal and striato-prefrontal connectivity when provoked. Amygdala and striatal reactivity to provocations was associated with task-related aggressive behaviour and trait anger. These results suggest that impulsive aggressive individuals with intermediate to high levels of psychopathy display abnormally high neural sensitivity to minor provocations and that this sensitivity is related to aggressive behavior. The identification of neural pathways involved in impulsive aggression may represent a means to more specifically shape interventions that aim to reduce violent behavior.

## Introduction

Aggression and impulsivity play a critical role in the manifestation of violent and criminal behaviors, thereby posing large costs to the victims and the society. A traditional perspective on aggression is the distinction between reactive and instrumental aggression (Vicario, 2014). Reactive aggression is triggered by a provocative, frustrating or threatening stimulus and involves unplanned aggressive behavior, while instrumental aggression is deliberate and goal-oriented. Poor behavioral control and reactive aggression are core features in personality disorders such as antisocial personality disorder (ASPD), borderline personality disorder (BPD) and psychopathy. Delineating relevant neural pathways represents a critical step toward developing more effective preventative approaches and therapeutic strategies for curtailing clinically aggressive behaviour.

In animals, the neuroanatomy of reactive aggression involves a basic threat circuitry comprising the amygdala, hypothalamus and periaqueductal gray (PAG) (Gregg and Siegel, 2001). This circuitry has also been linked to reactive aggression in humans, where the amygdala facilitates the responsiveness of the subcortical threat system, depending on whether the stimulus is perceived as aversive or reinforcing (Blair, 2004). Both animal and human research provides evidence that the prefrontal cortex (PFC) modulates this subcortical circuit mediating reactive aggression (Nelson and Trainor, 2007). In mice, optogenetic activation of excitatory neurons in the medial PFC inhibits aggression while optogenetic silencing of medial PFC neurons increases aggression (Takahashi, et al., 2014). Human functional neuroimaging studies have shown that several prefrontal regions are engaged by angry faces, including the amygdala, OFC, anterior cingulate cortex (ACC) and medial PFC (Beyer, et al., 2014; Coccaro, et al., 2007). These regions are thought to exert top-down regulation of limbic subcortical regions and it has been suggested that the functional coupling between the amygdala and PFC is impaired in aggressive individuals (Rosell and Siever, 2015). Amygdala-OFC coupling was reduced in IED patients relative to controls when presented to angry faces (Coccaro, et al., 2007), and resting-state amygdala-

ventromedial PFC connectivity was reduced in psychopathic inmates compared to inmates with low psychopathic traits (Motzkin, et al., 2011).With respect to the involvement of the hypothalamus, striatum and PAG in human aggression, less data is available. Receiving unfair offers in social exchange paradigms has been associated with increased activity within the anterior insula, dorsomedial PFC and ACC (Sanfey, et al., 2003), whereas striatal reactivity to punishing unfair offers has been reported (Strobel, et al., 2011). Moreover, PAG responses have been observed in social exchange paradigms, both to rejecting (Corradi-Dell'Acqua, et al., 2013) and punishing unfair offers (White, et al., 2014). Thus, accumulating evidence suggests that the neural circuits of reactive aggression involve the PFC, ACC, amygdala, striatum and PAG. However, this theory is based on either structural magnetic resonance imaging (MRI) or functional MRI (fMRI) studies using paradigms that do not directly target reactive aggression, such as watching emotional faces or pictures with negative emotional content or rejecting unfair offers in social exchange paradigms.

We recently implemented a laboratory model of reactive aggression for use in fMRI; the point subtraction aggression paradigm (PSAP). The PSAP is a paradigm wherein a fictitious opponent periodically steals money with the aim to provoke the participant, who can choose to act aggressively or pursue monetary rewards (Cherek, et al., 1997). In healthy controls, we found that receiving provocations (monetary subtractions from a fictitious opponent) activated several key brain regions implicated in aggressive behavior including the amygdala, ACC, insula, striatum and PFC (Skibsted, 2015). The PSAP in fMRI thus constitutes a useful instrument for investigating neural pathways underlying aggression in humans but has until now never been employed in individuals with extreme levels of aggression or a documented history of impulsive violent behavior.

Here we examine the neural responses to provocations and aggressive behavior in impulsive violent offenders and in healthy control non-offenders, testing the following hypotheses: 1) violent offenders respond aggressively with a higher

frequency than control subjects, 2) when provoked, violent offenders show higher reactivity in the amygdala, striatum and PAG, and decreased PFC reactivity, and 3) violent offenders have reduced functional connectivity between the amygdala and prefrontal regions in the context of provocations.

## Materials and methods

### Participants

The final study sample consisted of 18 incarcerated violent offenders with a documented history of severe violent crimes and 20 healthy control subjects recruited from the community. The participants were all male and groups were age-matched. Violent offenders were recruited from closed state prisons within the National Prison and Probation Service in Denmark. Inmates who had a documented history of convictions for violent crimes (murder, rape, attempted murder, aggravated assault) and who were probable to get permission to leave the prison accompanied by prison staff for the duration of the study were invited to an initial screening interview conducted by a medical doctor and a psychologist. Healthy control non-offenders were recruited via community websites and bulletin boards in vocational schools, where people that were interested in participating were invited to complete one questionnaire with demographic information and one questionnaire assessing their trait aggression (Buss-Perry Aggression Questionnaire, BPAQ). To ensure not including a group of individuals with high trait aggression from the community, we also selected control participants based on low scores on the BPAQ in addition to the other inclusion criteria. Only men were included because only 1-2% of inmates in the prisons we recruited from were women.

Exclusion criteria for all participants were: current or lifetime history of major psychiatric disorders (major depressive disorder, bipolar disorder or psychotic symptomatology), symptomatic medical or neurological illness, severe head

trauma, severe visual or hearing impairment, contraindications for MRI, use of psychotropic medications, current substance or alcohol abuse. Fourteen violent offenders had a history of substance abuse including cannabis (n=10), cocaine (n=7), alcohol (n=5), stimulants (n=2), opioids (n=3) and anabolic steroids (n=2), but all had been in remission for at least 6 months up to several years. All participants tested negative on urine drug screen (Rapid Response Multi-Drug; BTNX Inc., Toronto, Ontario, Canada) on the day of scanning and had an unremarkable MRI. As evaluated on the day of scanning, none of the participants had any significant medical or neurological illness according to the Schedules for Clinical Assessment in Neuropsychiatry version 2.1, physical examination and blood biochemistry.

Seven subjects were excluded: one did not believe the deception of the paradigm, one with a task behavioral outcome > 4 SD above the mean, one with a pathological MRI, two who became claustrophobic and two who reported taking psychotropic medications at the time of scanning, leaving a final sample size consisting of of 18 violent offenders and 20 control subjects. All participants have been reported in a previous positron emission tomography study (da Cunha-Bang, 2016).

The study was approved by the National Prison and Probation Service and the local ethical committee (Copenhagen, Denmark, reference H-3-2013-100). All participants provided written informed consent following full description of the procedures, which for the violent offenders included access to criminal files, and received monetary compensation for their participation.

## Personality assessment

The Structured Clinical Interview for DSM-IV II (SCID-II) was administered by two medical doctors for assessment of personality disorders (PD). All violent offenders were diagnosed with one or multiple PD; ASPD (n=14), BPD (n=2), Schizoid (n=1), Dependent (n=1), Paranoid (n=1), Obsessive-compulsive (n=1) and PD not otherwise specified (n=7). Two medical doctors administered SCID-II

and co-rating was conducted on a subset of the violent offenders, which yielded full final diagnostic consistency. Level of psychopathy was assessed using the Psychopathy Checklist-Revised (PCL-R) (Hare, 2003), which consists of 20 items scored from 0 to 2 based on presence of each trait. PCL-R interviews were conducted by course-certified health professionals with a medical or psychology background, and all ratings were consensus decisions based on notes from each interview and collateral information from criminal files. Intelligence quotient (IQ) was evaluated by a trained neuropsychologist using the Reynolds Intellectual Screening Test (RIST).

To assess trait aggression, trait impulsivity and psychopathic traits, the following self-report personality measures were administered to all participants: the Buss-Perry Aggression Questionnaire (BPAQ) (Buss and Perry, 1992; da Cunha-Bang, et al., 2013) , State-Trait Anger Expression Inventory 2 (STAXI-2) (Moeller, et al., 2015), Barratt Impulsiveness Scale version 11 (BIS) (da Cunha-Bang, et al., 2013; Patton, et al., 1995) and Psychopathic Personality Inventory (PPI-R) (Lilienfeld, et al., 2005). State measures administered on the day of scanning included the Profile of Mood States (POMS) (McNair, 1971) and STAXI-2 State (Moeller, et al., 2015).The STAXI-2 State was administered before and after the MRI scan.

## fMRI paradigm

Prior to scanning, participants were instructed that they would play a game with another study participant and they could earn points, which could be exchanged for money. Participants completed one 12-minute session of the PSAP and the monetary reward was 1.34 Euro (10 DKK) per point won. Three response options were available: pressing the button for ‘Option 1’ 100 times resulted in the participant earning 1 point (1.34 Euro), pressing the button for ‘Option 2’ 10 times resulted in removal of a point from the opponent and pressing for ‘Option 3’ 10 times briefly protected the participant’s point total from the opponent stealing. Participants were required to complete an option before choosing a new option. Participants were informed that they were assigned to the experimental condition

in which they did not keep the points they stole from the opponent. However, participants were told that the opponent received alternative instructions and was allowed to keep the points he stole from the participant. In this way, choosing 'Option 2' reflects an aggressive behavior, void of any monetary incentive.

While in the scanner, participants completed a one-minute trial session immediately before playing one 12-minute session of the PSAP. The status of the game was projected onto a screen viewed by the participant while lying in the scanner (Supplemetary Figure SF1). We defined task-related aggressive behavior as:

$$PSAPaggression = \frac{AR}{PR} / TBP * 1000$$

where AR=Number of Aggressive Responses, PR=Number of Provocations and TBP=Number of Total Button Presses. This adjusts aggressive behavior with respect to individual differences in button-press rate and received provocations. Upon completion of the PSAP, participants completed a questionnaire, providing their impression of the opponent. Participants who indicated that they did not play with a real person were excluded. A more detailed description of the fMRI-adapted version of the PSAP and parameter settings is provided in Skibsted et al. (submitted).

## Imaging acquisition

MRI scans were acquired on a 3T Prisma scanner (Siemens, Erlangen, Germany) with a 64-channel head coil. For blood oxygen level dependent (BOLD) fMRI, a T2*-weighted gradient echo-planar imaging (EPI) sequence was used with a repetition time of 2000 ms, echo time of 30 ms, flip angle of 90$^{o}$, and 32 slices with a slice thickness of 3.0 mm (0.75 mm gap). A total of 360 whole-brain fMRI volumes were acquired. We acquired a T1-weighted, TurboFLASH sequence, high-resolution whole-brain three-dimensional structural magnetic resonance scan with an inversion time of 900 ms, echo time of 2.58 ms, repetition time of 1900 ms, flip angle of 9°, in-plane matrix of 256 × 256, in-plane resolution of 0.9 × 0.9

mm, 224 slices and a slice thickness of 0.9 mm, no gap. To reduce motion, an in-house made head fixation system was used.

## fMRI data analysis

Functional neuroimaging data were analyzed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm). Single subject functional images were spatially realigned to the first image. The T1-weighted structural image was co-registered to the first functional image and the origin reset to the anterior commissure (AC) using using acpcdetect (https://www.nitrc.org/projects/art). The co-registered T1-weighted image was normalized into Montreal Neurological Institute (MNI) stereotactic space and the normalization parameters were applied to the functional images. Normalized functional images were smoothed using an 8 mm FWHM Gaussian filter.

PSAP conditions were modeled as blocks or events (Figure 1). The first 10 seconds of Option1 was used as the "baseline condition". If a provocation occurred during the first 10 seconds of Option1, the time from beginning of Option1 until the provocation occurred was used. The following contrasts of interest were estimated: Provocations > Option1 and Aggressive response > Option1. Single-subject design matrices were estimated using the general linear model to determine condition-specific BOLD responses. Individual contrast images (i.e., weighted sum of beta images) were included in group-level analyses to determine task-related brain responses using one-sample *t*-tests. Group differences in brain responses were determined using two-sample t-tests. To investigate whether brain responses were reflected in the behavioral data, linear regression analyses were employed within SPM to evaluate the association between task-related aggressive behavior, trait anger and aggression and neural responses to task, adjusting for age and group status. In the violent offenders, we also evaluated associations between psychopathy score (PCL-R) and reactivity to provocations. Mean signal values for significant clusters were extracted for each subject and used for illustrative purposes where appropriate. We used SAS

version 9.4 and R version 3.2.1 for statistical analyses that were performed outside SPM.

Given the strong a priori evidence, analyses focused on the following regions of interest (ROIs) defined within WFU Pickatlas: bilateral amygdala, striatum (i.e., caudate and putamen) and PFC (i.e., including Brodmann Area's 10, 11 and 47, dilation 2D = 1 and ACC, no dilation). The PAG was defined by a 10mm radius sphere at [-3,-25,-5] (White, et al., 2014). To address the issue of multiple comparisons we used 3dClustSim, a software program within AFNI (http://afni.nimh.nih/gov/afni) that uses a Monte Carlo simulation method to establish family-wise-error-corrected cluster extent thresholds unlikely to have occurred by chance ($\alpha < 0.05$)(Forman, et al., 1995). We used a voxel-level statistical threshold of $p < 0.005$, uncorrected. Based on this, amygdala, PAG, striatum and PFC clusters of $k \geq 6$, 9, 59 and 153 voxels, respectively, were considered statistically significant. We used a whole-brain cluster threshold of $k \geq 495$ voxels for exploratory analyses. Coordinates are reported in Montreal Neurological Institute space as [x,y,z] and k denotes cluster sizes.

## Psychophysiological interaction analyses

We used psychophysiological interaction (PPI) analyses to assess the provocation-associated functional connectivity with seed regions in either amygdala or striatum, using the contrast Provocations>Option1 (Friston, et al., 1997; McLaren, et al., 2012). Amygdala and striatum seeds were defined based on clusters significantly responsive to provocations across subjects (supplementary table 1). Right amygdala and right striatum demonstrated the most pronounced response (cluster size of 55 and 288 voxels, respectively) and were therefore used as seed regions for PPI analyses. Although our *a priori* hypothesis included only amygdala connectivity, we also included the striatum as a seed because of the observed association between trait anger and striatal reactivity to provocations. The mean seed time series for each subject was extracted using the generalized PPI toolbox v7.12 (McLaren, et al., 2012). Single-subject design matrices estimated to determine PPI effects were identical to design matrices used to

estimate main effects of task except including the seed time series and psychophysiological interaction terms as additional regressors. Individual contrast images were included in group-level analyses to determine group differences and associations with behavioural measures as described in the main text. Seeds were analyzed independently. To evaluate associations between connectivity and behaviour, we used extracted functional connectivity estimates (from significant clusters shown in Figure 4) as outcome variables in linear regression models, adjusting for age.

PPI analyses were performed as described elsewhere (Madsen, et al., 2015).

# Results

## Participant characteristics and task behavior

Demographic, clinical and behavioral characteristics of the participants are provided in table 1. Two violent offenders scored < 25 on the PCL-R, the cut-off score for psychopathy in Europe (Cooke, et al., 2005) and 9 violent offenders scored ≥ 30, the cut-off score according to Hare (Hare, 2003). Behaviorally, violent offenders used the aggressive response in the PSAP twice more frequently than controls (mean PSAP aggression 5.7 vs. 2.8, p=0.02). Seven control subjects and only one violent offender did not use the aggressive response. Thus, results shown on the aggressive response are based on 30 participants (17 violent offenders and 13 healthy controls).

## Functional imaging data

### *Main task effects*

ROI analysis across all participants revealed significant activation within amygdala, striatum, PAG and PFC/ACC (supplementary table 1). We found no significant main effects of task in aggressive response. Whole-brain effects of task are described in supplemental results.

***Group comparisons***

ROI analyses revealed that violent offenders had significantly higher reactivity to provocations within the amygdala (right amygdala: k=40, [22,-8,-14], z=3.18, $p_{corr}$<0.05, left amygdala: k=14, [-18,-2,-20], z=2.83, $p_{corr}$<0.05, figure 2), the right striatum (k=157, [8,0,12], z=3.56, $p_{corr}$<0.05, figure 2) and the PAG (k=15, [-2,-18,-8], z=3.18, $p_{corr}$<0.05). The groups did not differ significantly in PFC/ACC response to provocations.

***Brain responses to provocations and behavior***

Across all participants, we found that task-related aggressive behavior was positively correlated with reactivity to provocations within the left amygdala (k=72, [-30,-4,-18], z=3.88, $p_{corr}$<0.05, figure 2) and right striatum (k=404, [-30,-10,6], z=3.64, $p_{corr}$<0.05). In the association between PSAP aggression and amygdala reactivity to provocations, an influential observation (Cook's D: 1.05) was observed. When removing this observation, the left amygdala cluster remained significant, albeit smaller (k=14, [-30,-4,-18], z=3.63, $p_{corr}$<0.05).

Trait anger was positively correlated with reactivity to provocations within the left amygdala (k=43, [-18,-2,-20], z=4.13, $p_{corr}$<0.05) and bilateral striatum (left: k=97, [-14,6,18], z=4.69 and k=91, [-4,10,2], z=3.95, right: k=91, [16,12,20], $p_{corr}$<0.05, figure 2). Trait aggression was positively correlated with reactivity to provocations within the striatum (left: k=199, [-14,8,16], z=4.45, right: k=123, [30,-6,6], z=3.37 and k=80, [16,8,18], z=4.05, $p_{corr}$<0.05) and left ACC (k=328, [8,36,28], z=3.87, $p_{corr}$<0.05). In violent offenders, PCL-R score was positively correlated with left amygdala (k=14, [-18,-2,-22], z=3.26, $p_{corr}$<0.05) and PAG (k=104, [-2,-26,4], z=3.43, $p_{corr}$<0.05) reactivity to provocations.

***Functional Connectivity***

A whole-brain PPI analysis of functional connectivity with a seed in the amygdala revealed that when provoked, violent offenders had reduced connectivity between amygdala and a cluster including the right superior, medial and inferior frontal

gyri (k=3094, [46, 32, -14], z=3.77, $p_{corr}$<0.05, figure 3). Violent offenders also had reduced connectivity between the striatum seed and a cluster including the left and right ACC and superior and medial frontal gyri (k=3338, [8,48.-2], z=4.08, $p_{corr}$<0.05, figure 3).

## Discussion

This study is the first to investigate brain reactivity to provocations in impulsive aggressive violent offenders using the PSAP. The most striking findings are the heightened neural response to provocations in violent offenders, particularly within the amygdala and striatum, and the reduced functional connectivity between these regions and the PFC/ACC in the context of provocations. Our data support a model where impulsive aggression can be attributed to heightened reactivity and reduced fronto-limbic connectivity in the context of provocations. This interpretation is further substantiated by the finding of heightened response to provocations being associated with the violent offenders' excessive adverse behavior, as well as with self-report measures of trait aggression and trait anger. Taken together, these findings highlight critical neural pathways underlying variability in aggression-related behavioural phenotypes.

Our finding that psychopathic violent offenders show increased amygdala reactivity to provocations contrasts with data showing that individuals with psychopathy are hypo-responsive to indices of threat (Blair, 2010). Even though not all violent offenders in this study met criteria for psychopathy (>30 on the PCL-R) we found that PCL-R score was positively correlated with left amygdala reactivity to provocations, indicating hyper-responsiveness of the amygdala in psychopathy. However, reactive aggression in psychopathy is thought to be frustration-based, as opposed to threat-related (Blair, 2010; Blair, 2012). Frustrations can occur when another person's behavior undermines achieving an expected reward/goal (Blair, 2012). Having points/money stolen while working for a monetary reward in the PSAP can evoke similar frustrations and anger. Indeed, when rewards were blocked in an fMRI monetary reward paradigm,

amygdala reactivity correlated positively with frustration (Yu, et al., 2014), and in an economic exchange paradigm, amygdala reactivity to unfair offers correlated positively with offer rejection (Gospic, et al., 2011). The present study provides novel insight into the functional role of the amygdala in pathological aggression, particularly in terms of a putative differential amygdala function in reactive and instrumental aggression. It has been proposed that amygdala responding can serve to distinguish instrumental forms of aggression associated with ASPD and psychopathy from reactive aggression, which is more characteristic of BPD and IED (Coccaro, et al., 2011). However, individuals with psychopathy are at increased risk for both instrumental and reactive aggression (Blair, 2007; Blair, 2010). Amygdala hyper-responsiveness to negative emotional stimuli and social threats has been observed in several studies of patients with BPD (Schulze, et al., 2015) and IED (Coccaro, et al., 2007) but reactive aggression in psychopathy has received less attention. In the present study, violent offenders were selected based on a history of impulsive violent crimes, but most violent offenders had also committed numerous crimes more instrumental in character (e.g., robbery and fraud), emphasizing that these two types of aggression often coexist. It is possible that the neural circuits involved in reactive and instrumental aggression are different and that psychopaths have dysfunctions in both. However, more data are needed for a sufficient evaluation of this distinction, for example using paradigms targeting both forms of aggression in the same cohort.

In addition to the amygdala, violent offenders also responded to provocations with higher activity in the striatum. The striatum is implicated in a variety of functions, including motor function, motivated behaviors and reward (Zink, et al., 2004). However, several types of non-rewarding stimuli activate the striatum and it is suggested that the striatum encodes all salient stimuli (Zink, et al., 2003). Striatal reactivity to provocations might reflect the saliency of this stimulus, but we also speculate that striatal reactivity to provocations might reflect motor vigilance. Importantly, the observation that striatal reactivity to provocations correlated positively with trait aggression and trait anger substantiates the involvement of the

striatum in aggression. Recent evidence implicates a role for the striatum in antisocial behavior and psychopathy (Glenn and Yang, 2012; Yang, et al., 2015). Increased striatal volume is reported in adults with psychopathy (Glenn, et al., 2010), ASPD (Barkataki, et al., 2006) and in adolescents with psychopathic traits (Yang, et al., 2015). In human functional neuroimaging studies, the impulsive-antisocial factor scores of self-reported psychopathic traits in healthy subjects correlated positively with amphetamine-induced dopamine release using positron emission tomography (PET) and with the BOLD response to reward anticipation in the nucleus accumbens (Buckholtz, et al., 2010). A dysfunction of the striatum is speculated to contribute to the increased sensation-seeking, reward-driven and impulsive behavior in ASPD and psychopathy (Yang, et al., 2015). Our finding that violent offenders show heightened striatal reactivity to provocations supports an aberrant striatal function in individuals with high levels of aggression in response to aversive salient stimuli.

It has been suggested that amygdala activity is controlled by prefrontal inhibitory projections and that this process is disrupted in aggressive individuals (Rosell and Siever, 2015). In resting-state fMRI it was shown that inmates fulfilling criteria for psychopathy (n=20) had reduced amygdala-ventromedial PFC connectivity compared to non-psychopathic inmates (Motzkin, et al., 2011), and an fMRI study using the ultimatum game in 30 youths with disruptive behavior disorders (DBD) reported reduced amygdala-prefrontal connectivity during high provocation trials in DBD youths compared to controls (White, et al., 2015). Our findings support that violent offenders have reduced amygdala and striatal connectivity to the PFC in the context of provocations, possibly reflecting reduced prefrontal regulation of subcortical regions. These findings strongly corroborate the observation in DBD-youths and are consistent with the model of impaired PFC regulation of emotional reactions to provocations in antisocial individuals. Collectively, our data show that the combination of heightened amygdala and striatal reactivity to provocations and reduced prefrontal control of this excessive subcortical activity can result in high levels of aggressive behaviors.

An important strength of this study was that the activation patterns in response to provocations could be related to behavior within the paradigm as well as to trait aggression and anger. However, with respect to the amygdala, the group differences in reactivity to provocations were most pronounced in the right amygdala, whereas the correlations with behaviour were most pronounced in the left amygdala. Another limitation include that the groups differed in several aspects that we were unable to match for, for example IQ and education, previous drug abuse, smoking and incarceration. On the other hand, some of these aspects are partly attributable to their personality pathology. With respect to diagnoses, the violent offenders presented with mixed personality disorders, so the results cannot be attributed to specific personality pathology but rather to aggressive and violent behavior. Lastly, it should be noted that the PSAP is a paradigm that is user driven, i.e. the number of provocations are not fixed and the participants are not forced to use the aggressive response. On the other hand, this makes the paradigm more translatable to a realistic setting. With concerns that the variable number of provocations (ranging from 8-16) would influence the activation patterns, we entered the number of provocations as a nuisance covariate, which did not change the outcomes (results not shown).

In conclusion we find that violent offenders display abnormally high neural reactivity to provocations within the amygdala and striatum, and that this sensitivity is related to aggressive behavior. We also demonstrate that violent offenders show reduced amygdala-prefrontal and striato-prefrontal connectivity in the context of provocations. These data provide novel evidence of aberrant brain function in a unique cohort of individuals with a history of extremely violent behavior. The findings suggest that an exaggerated neurobiological sensitivity to provocations or frustrations and lack of prefrontal control are key features of pathological aggression. Prevention and treatment of aggressive behaviors would benefit from interventions targeting this type of vulnerability.

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## Conflicts of interests

GMK has received honoraria as a consultant for H. Lundbeck A/S, as a member of the steering group for Brain Prize. She is also on the advisory board for the Kristian G. Jebsen Foundation and a field editor for Int J Neuropsychopharm. All other authors declare no conflicts of interest and report no financial disclosures.

## Tables

Table 1. Demographic, clinical and behavioral characteristics of participants.

| | Violent Offenders | Healthy Controls | p-value |
|---|---|---|---|
| Number of subjects | 18 | 20 | |
| Age, years | 31.8 ± 8.8 | 30.2 ± 9.8 | 0.61 |
| Duration of education, years | 8.9± 2.6 | 11.5± 0.9 | 0.0007 |
| IQ, RIST score | 99.1 ± 8.1 | 108.3 ± 6.8 | 0.0007 |
| **PCL-R score** | | | |
| Total | 29.3 ± 4.3 | n/a | |
| Factor 1 | 12.6 ± 2.1 | n/a | |
| Factor 2 | 14.0 ± 3.5 | n/a | |
| | | None | |
| Number of violent convictions | 3.7 ± 2.5 | | |
| Number of violent charges against | 16.4 ± 30.2 | None | |
| Age at first violent conviction, years | 19.5 ± 4.3 | n/a | |
| Tobacco use frequency, cigarettes/day | 9.9 ± 10.9 | 1.2 ± 4.1 | 0.004 |
| Alcohol use frequency, days/month | 0 ± 0 | 5.5 ± 4.3 | 0.0001 |
| **Personality Traits** | | | |
| Trait aggression[a] | 87.1 ± 22.2 | 53.1 ± 13.8 | 0.0001 |
| Trait anger[b] | 21.0 ± 6.8 | 14.5± 2.4 | 0.001 |
| Trait impulsivity[c] | 66.7 ± 10.0 | 59.1 ± 9.3 | 0.02 |
| Trait psychopathy [d] | 311.5 ± 35.8 | 280.1 ± 6.8 | 0.006 |
| **State measures** | | | |
| State aggression before PSAP[e] | 15.1 ± 0.3 | 15.0 ± 0.0 | 0.16 |
| State aggression after PSAP[e] | 16.1 ± 1.8 | 15.0 ± 0.0 | 0.026 |
| State anger hostility[g] | 8.72 ± 9.22 | 2.6 ± 2.2 | 0.013 |
| **PSAP behaviour** | | | |
| Option 1 | 2283.6 ± 378.9 | 2479.9 ± 288.3 | 0.09 |
| Option 2 | 150.0 ± 98.0 | 76.5 ± 100.5 | 0.029 |
| Option 3 | 175.0 ± 11.5 | 262.0 ± 104.2 | 0.018 |
| Total button presses | 2608.6 ± 304.8 | 2818.3 ± 174.5 | 0.016 |
| Provocations | 10.6 ± 1.6 | 10.3 ± 1.9 | 0.53 |
| Points earned | 12.1 ± 3.4 | 14.3 ± 3.1 | 0.048 |
| Option 2 per provocation | 14.5 ± 9.5 | 7.7 ± 10.4 | 0.044 |
| PSAP aggression | 5.7 ± 3.8 | 2.8 ± 3.8 | 0.023 |

Group data are mean ± standard deviation unless stated otherwise. P-value represents two-sample t-test, [d] n=15 controls and 18 inmates. [e] n= 15 inmates, 9 controls. [a] Buss-Perry Aggression Questionnaire total score, [b] State-Trait Anger Expression Inventory, [c] Barratt Impulsiveness Scale version 11 total score, [d] Psychopathic Personality Inventory Revised total score, [e] State-Trait Anger Expression Inventory, [g, f] Profile of Mood States. Abbreviations: PCL-R = Psychopathy Checklist-Revised. IQ=intelligence quotient. RIST= Reynolds Intellectual Screening Test. n/a= not applicable.

## Figures

**Figure 1.** (A) Screen displaying what participants viewed in the scanner. The red-colored digit denotes that the participant is currently in Option 1. (B) Timeline with schematic representation of the task conditions. Conditions modeled as blocks or events are indicated as blocks or arrows, respectively. AR = Aggressive Response, PTR = Protective Response.

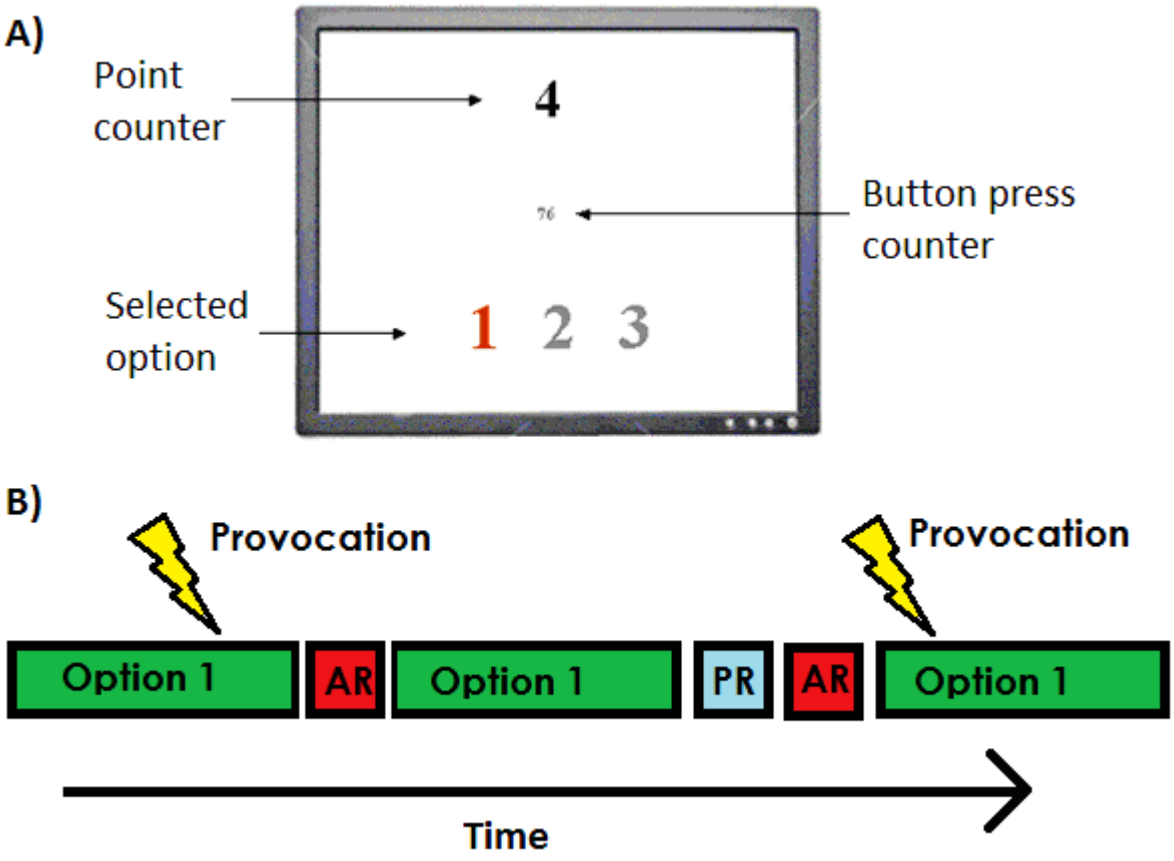


**Figure 2.** Heightened amygdala and striatal reactivity to provocations in violent offenders and associations between brain reactivity, task-related aggressive behavior (PSAP aggression) and trait anger.

A) Left and right amygdala clusters in which violent offenders had increased reactivity to provocations. The plot shows extracted mean values from both clusters for each group.

B) Left amygdala cluster in which amygdala reactivity to provocations was significantly correlated with PSAP aggression across participants, adjusted for group and age. The plot shows PSAP aggression as a function of the extracted mean signal values from this cluster.

C) Cluster within the right striatum where violent offenders had increased reactivity to provocations. The plot shows extracted mean values from this cluster.

D) Clusters in the right and left striatum where striatal reactivity to provocations was significantly correlated with trait anger across participants, adjusted for group and age. The plot shows trait anger as a function of the extracted mean signal values from these clusters.

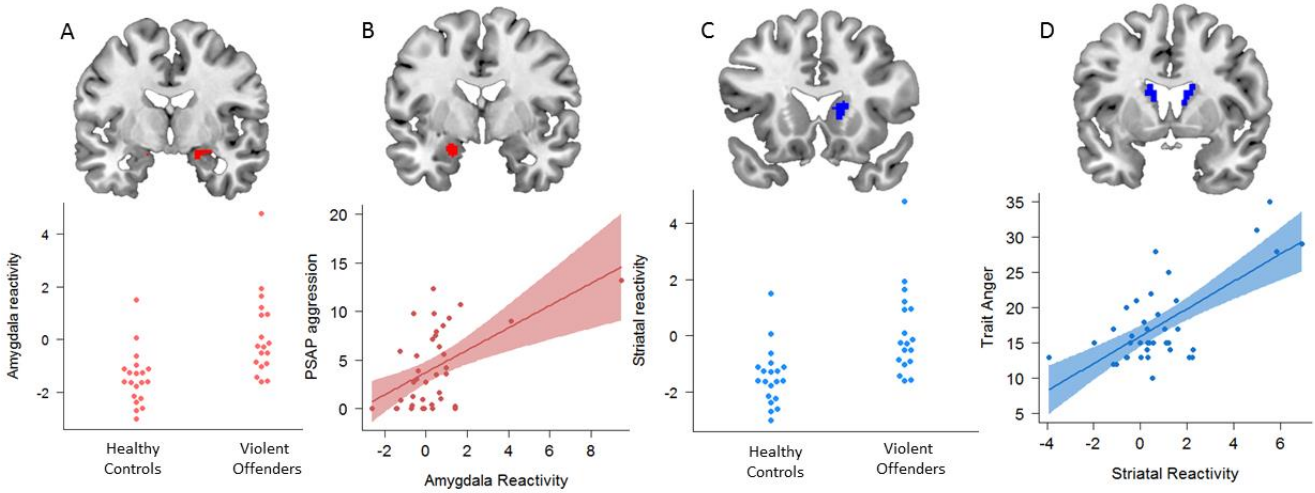
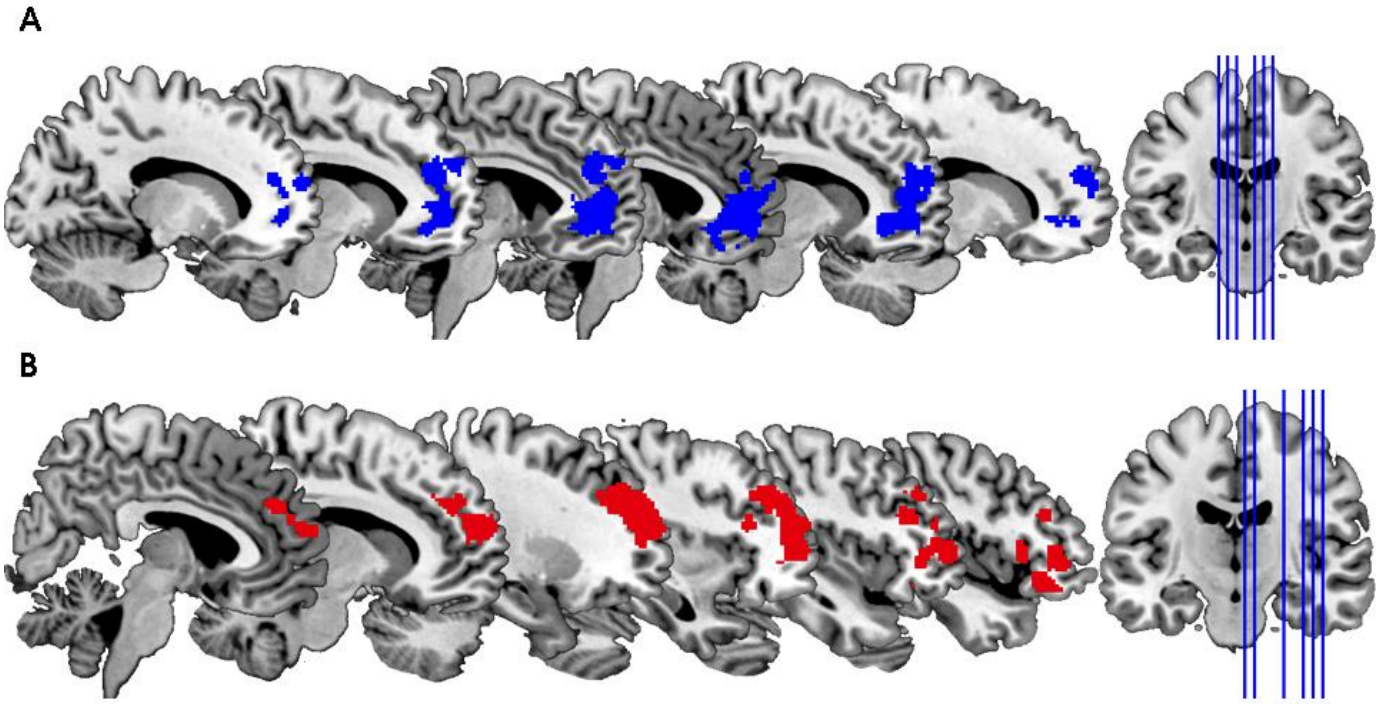


**Figure 3.** Group differences in amygdala and striatal functional connectivity as a function of provocations (A) Cluster in which the right striatum functional connectivity was significantly greater in control subjects relative to violent offenders (k=3338 voxels, [8, 48, -2], z=4.08, $p_{corr}$<0.05). (B) Cluster in which the right amygdala functional connectivity was significantly greater in control subjects relative to violent offenders (k=3094 voxels, [46, 32, -14], z=3.77, $p_{corr}$<0.05).



## REFERENCES


Barkataki, I., Kumari, V., Das, M., Taylor, P., Sharma, T. (2006) Volumetric structural brain abnormalities in men with schizophrenia or antisocial personality disorder. Behavioural brain research, 169:239-47.

Beyer, F., Munte, T.F., Gottlich, M., Kramer, U.M. (2014) Orbitofrontal Cortex Reactivity to Angry Facial Expression in a Social Interaction Correlates with Aggressive Behavior. Cereb Cortex.

Blair, R.J. (2004) The roles of orbital frontal cortex in the modulation of antisocial behavior. Brain and cognition, 55:198-208.

Blair, R.J. (2007) The amygdala and ventromedial prefrontal cortex in morality and psychopathy. Trends in cognitive sciences, 11:387-92.

Blair, R.J. (2010) Psychopathy, frustration, and reactive aggression: the role of ventromedial prefrontal cortex. Br J Psychol, 101:383-99.

Blair, R.J. (2012) Considering anger from a cognitive neuroscience perspective. Wiley interdisciplinary reviews. Cognitive science, 3:65-74.

Buckholtz, J.W., Treadway, M.T., Cowan, R.L., Woodward, N.D., Benning, S.D., Li, R., Ansari, M.S., Baldwin, R.M., Schwartzman, A.N., Shelby, E.S., Smith, C.E., Cole, D., Kessler, R.M., Zald, D.H. (2010) Mesolimbic dopamine reward system hypersensitivity in individuals with psychopathic traits. Nature neuroscience, 13:419-21.

Buss, A.H., Perry, M. (1992) The aggression questionnaire. Journal of personality and social psychology, 63:452-9.

Cherek, D.R., Moeller, F.G., Schnapp, W., Dougherty, D.M. (1997) Studies of violent and nonviolent male parolees: I. Laboratory and psychometric measurements of aggression. Biological psychiatry, 41:514-22.

Coccaro, E.F., McCloskey, M.S., Fitzgerald, D.A., Phan, K.L. (2007) Amygdala and orbitofrontal reactivity to social threat in individuals with impulsive aggression. Biological psychiatry, 62:168-78.

Coccaro, E.F., Sripada, C.S., Yanowitch, R.N., Phan, K.L. (2011) Corticolimbic function in impulsive aggressive behavior. Biological psychiatry, 69:1153-9.

Cooke, D.J., Michie, C., Hart, S.D., Clark, D. (2005) Assessing psychopathy in the UK: concerns about cross-cultural generalisability. The British journal of psychiatry : the journal of mental science, 186:335-41.

Corradi-Dell'Acqua, C., Civai, C., Rumiati, R.I., Fink, G.R. (2013) Disentangling self- and fairness-related neural mechanisms involved in the ultimatum game: an fMRI study. Social cognitive and affective neuroscience, 8:424-31.

da Cunha-Bang, S., Stenbaek, D.S., Holst, K., Licht, C.L., Jensen, P.S., Frokjaer, V.G., Mortensen, E.L., Knudsen, G.M. (2013) Trait aggression and trait impulsivity are not related to frontal cortex 5-HT2A receptor binding in healthy individuals. Psychiatry research, 212:125-31.

da Cunha-Bang, S.H., V.L.;Perfalk, E.;Beliveau, V.; Bock, C.;Lehel, S.;Thomsen, C.;Sestoft, D.;Svarer, C.;Knudsen, G.M. (2016) Serotonin 1B receptor binding is associated with trait anger and level of psychopathy in violent offenders. Biological psychiatry, in press.

Forman, S.D., Cohen, J.D., Fitzgerald, M., Eddy, W.F., Mintun, M.A., Noll, D.C. (1995) Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. Magnetic resonance in medicine, 33:636-47.
Friston, K.J., Buechel, C., Fink, G.R., Morris, J., Rolls, E., Dolan, R.J. (1997) Psychophysiological and modulatory interactions in neuroimaging. NeuroImage, 6:218-29.
Glenn, A.L., Raine, A., Yaralian, P.S., Yang, Y. (2010) Increased volume of the striatum in psychopathic individuals. Biological psychiatry, 67:52-8.
Glenn, A.L., Yang, Y. (2012) The potential role of the striatum in antisocial behavior and psychopathy. Biological psychiatry, 72:817-22.
Gospic, K., Mohlin, E., Fransson, P., Petrovic, P., Johannesson, M., Ingvar, M. (2011) Limbic justice--amygdala involvement in immediate rejection in the Ultimatum Game. PLoS biology, 9:e1001054.
Gregg, T.R., Siegel, A. (2001) Brain structures and neurotransmitters regulating aggression in cats: implications for human aggression. Progress in neuro-psychopharmacology & biological psychiatry, 25:91-140.
Hare, R. (2003) Hare Psychopathy Checklist-Revised (PCL-R), 2nd edition. Toronto. Multi-Health Systems Inc.
Lilienfeld, S.O., Widows, M.R., Staff, P. (2005) Psychopathic Personality InventoryTM-Revised. Social Influence (SOI), 61:97.
Madsen, M.K., Mc Mahon, B., Andersen, S.B., Siebner, H.R., Knudsen, G.M., Fisher, P.M. (2015) Threat-related amygdala functional connectivity is associated with 5-HTTLPR genotype and neuroticism. Social cognitive and affective neuroscience.
McLaren, D.G., Ries, M.L., Xu, G., Johnson, S.C. (2012) A generalized form of context-dependent psychophysiological interactions (gPPI): a comparison to standard approaches. NeuroImage, 61:1277-86.
McNair, D. (1971) Manual profile of mood states. Educational & Industrial testing service.
Moeller, S.B., Novaco, R.W., Heinola-Nielsen, V., Hougaard, H. (2015) Validation of the Novaco Anger Scale-Provocation Inventory (Danish) With Nonclinical, Clinical, and Offender Samples. Assessment.
Motzkin, J.C., Newman, J.P., Kiehl, K.A., Koenigs, M. (2011) Reduced prefrontal connectivity in psychopathy. The Journal of neuroscience : the official journal of the Society for Neuroscience, 31:17348-57.
Nelson, R.J., Trainor, B.C. (2007) Neural mechanisms of aggression. Nature reviews. Neuroscience, 8:536-46.
Patton, J.H., Stanford, M.S., Barratt, E.S. (1995) Factor structure of the Barratt impulsiveness scale. Journal of clinical psychology, 51:768-74.
Rosell, D.R., Siever, L.J. (2015) The neurobiology of aggression and violence. CNS spectrums, 20:254-79.
Sanfey, A.G., Rilling, J.K., Aronson, J.A., Nystrom, L.E., Cohen, J.D. (2003) The neural basis of economic decision-making in the Ultimatum Game. Science, 300:1755-8.

Schulze, L., Schmahl, C., Niedtfeld, I. (2015) Neural Correlates of Disturbed Emotion Processing in Borderline Personality Disorder: A Multimodal Meta-Analysis. Biological psychiatry.

Skibsted, A.d.C.-B., S.; Carré, J.; Hansen, A.H; Beliveau, V.; Knudsen, G.M.; Fisher, P.M.;. (2015) Aggression-related brain function assessed with the point subtraction aggression paradigm in functional magnetic resonance imaging European Neuropsychopharmacology 2015. Amsterdam.

Strobel, A., Zimmermann, J., Schmitz, A., Reuter, M., Lis, S., Windmann, S., Kirsch, P. (2011) Beyond revenge: neural and genetic bases of altruistic punishment. NeuroImage, 54:671-80.

Takahashi, A., Nagayasu, K., Nishitani, N., Kaneko, S., Koide, T. (2014) Control of intermale aggression by medial prefrontal cortex activation in the mouse. PloS one, 9:e94657.

Vicario, C.M. (2014) Aggression traits in youth psychopathy: the key role of serotonin. Frontiers in psychiatry, 5:25.

White, S.F., Brislin, S.J., Sinclair, S., Blair, J.R. (2014) Punishing unfairness: rewarding or the organization of a reactively aggressive response? Human brain mapping, 35:2137-47.

White, S.F., VanTieghem, M., Brislin, S.J., Sypher, I., Sinclair, S., Pine, D.S., Hwang, S., Blair, R.J. (2015) Neural Correlates of the Propensity for Retaliatory Behavior in Youths With Disruptive Behavior Disorders. The American journal of psychiatry:appiajp201515020250.

Yang, Y., Narr, K.L., Baker, L.A., Joshi, S.H., Jahanshad, N., Raine, A., Thompson, P.M. (2015) Frontal and striatal alterations associated with psychopathic traits in adolescents. Psychiatry research, 231:333-40.

Yu, R., Mobbs, D., Seymour, B., Rowe, J.B., Calder, A.J. (2014) The neural signature of escalating frustration in humans. Cortex; a journal devoted to the study of the nervous system and behavior, 54:165-78.

Zink, C.F., Pagnoni, G., Martin-Skurski, M.E., Chappelow, J.C., Berns, G.S. (2004) Human striatal responses to monetary reward depend on saliency. Neuron, 42:509-17.

Zink, C.F., Pagnoni, G., Martin, M.E., Dhamala, M., Berns, G.S. (2003) Human striatal response to salient nonrewarding stimuli. The Journal of neuroscience : the official journal of the Society for Neuroscience, 23:8092-7.

# Supplemental Material

## Violent offenders respond to provocations with high amygdala and striatal reactivity

### RESULTS

### Main task effects

Consistent with our observation in a different cohort on a different MRI scanner, whole-brain analyses showed neural activation for the provocation response bilaterally included two large clusters (41629 and 4117 voxels, respectively) covering the amygdala, PFC, ACC, striatum, insula, precuneus, midbrain, parietal and occipital cortex (Skibsted et al., submitted).

**Supplementary Table 1. Task-related reactivity to provocations (Provocations > Option 1) within regions of interest across all participants.**

Clusters reflect a voxel-level significance threshold of $p < 0.005$, uncorrected, with region specific cluster extent thresholds at $p_{corr}<0.05$. MNI refers to Montreal Neurological Institute coordinate space.

| | | MNI coordinates of peak voxel | | | |
|---|---|---|---|---|---|
| **Anatomical Region** | **Cluster Size** | **X** | **Y** | **Z** | **Z-score** |
| Amygdala | 55 | 22 | -6 | 16 | 4.00 |
| | 17 | -22 | 0 | -18 | 3.00 |
| Prefrontal Cortex | 872 | -2 | 34 | 28 | 5.11 |
| | 388 | -30 | 22 | -6 | 6.95 |
| | 558 | 32 | 22 | -2 | 6.93 |
| | 249 | 46 | 46 | 14 | 4.67 |
| Striatum | 239 | 10 | 8 | 2 | 5.19 |
| | 224 | -8 | 8 | 2 | 5.11 |
| Periaqueductal Gray | 484 | 6 | -28 | -6 | 5.86 |

# Study 2

Archival Report

Biological Psychiatry

# Serotonin 1B Receptor Binding Is Associated With Trait Anger and Level of Psychopathy in Violent Offenders

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## ABSTRACT

**BACKGROUND:** The involvement of serotonin in aggression has traditionally been attributed to impaired prefrontal serotonergic inhibitory control of emotional reactions to provocations in antisocial individuals. However, it is unclear which specific serotonergic receptors are involved in the effects. A large body of preclinical research supports a specific role of serotonin 1B receptors (5-$HT_{1B}$Rs) in aggression and impulsivity, but this has never been evaluated in humans.

**METHODS:** Nineteen incarcerated violent offenders and 24 healthy control nonoffenders were included and examined with positron emission tomography, using the radioligand [$^{11}C$]AZ10419369 for quantification of cerebral 5-$HT_{1B}$R binding in three regions of interest: the anterior cingulate cortex, orbitofrontal cortex, and striatum.

**RESULTS:** Group status significantly moderated the association between striatal 5-$HT_{1B}$Rs and trait anger (difference in slopes, $p_{corrected} = .04$). In the violent offender group, striatal 5-$HT_{1B}$R binding was positively correlated with self-reported trait anger ($p = .0004$), trait psychopathy ($p = .008$), and level of psychopathy according to the Psychopathy Checklist-Revised ($p = .02$). We found no group differences in 5-$HT_{1B}$R binding.

**CONCLUSIONS:** Our data demonstrate for the first time in humans a specific involvement of 5-$HT_{1B}$R binding in anger and psychopathy. 5-$HT_{1B}$Rs putatively represent a molecular target for development of pharmacologic antiaggressive treatments.

*Keywords:* Aggression, 5-$HT_{1B}$, Neuroimaging, PET, Positron emission tomography, Striatum

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Extensive research in both animals and humans strongly supports the involvement of serotonin in impulsive and aggressive behaviors. An inverse relationship between serotonin levels and human aggression was initially supported by studies assessing serotonin function nonspecifically, such as manipulating serotonin levels by acute tryptophan depletion or using endocrine challenges (1). The mechanism by which low levels of serotonin cause aggression has mainly been attributed to impaired serotonergic prefrontal inhibitory control over emotional reactions following provocations (2). It remains to be identified, however, which components of serotonergic neurotransmission are specifically involved.

A large body of preclinical research supports a specific role of serotonin 1B receptors (5-$HT_{1B}$Rs) in aggression and impulsivity. 5-$HT_{1B}$Rs are localized presynaptically as autoreceptors on serotonergic neurons and postsynaptically as heteroreceptors on nonserotonergic neurons, including neurons from other neurotransmitter systems, such as glutamate, gamma-aminobutyric acid, dopamine, and acetylcholine (3). A class of mixed 5-$HT_{1B/1A}$ receptor agonist drugs with the name serenics was developed some 30 years ago with well-established antiaggressive effects in mice [reviewed in (4)]. Also, administration of selective 5-$HT_{1B}$R agonists inhibits aggressive behaviors in rodents (5–7), and 5-$HT_{1B}$R knockout mice are highly aggressive and impulsive (3,8) and exhibit low response inhibition (9). In a recent study, it was shown that specific knockdown of forebrain heteroreceptors in mice resulted in aggressive, but not impulsive, behaviors, while autoreceptor knockdown affected neither aggression nor impulsivity (3). These results suggest that aggression and impulsivity are mediated by 5-$HT_{1B}$ heteroreceptors, as opposed to autoreceptor function.

Although the involvement of 5-$HT_{1B}$Rs in aggression is well established in animals, the evidence in humans is limited to the association with specific polymorphisms of the 5-$HT_{1B}$R genotype (10). The serenic drug eltoprazine has been tested in mentally handicapped and schizophrenic patients (11,12) but with modest antiaggressive effects, and further development of serenic compounds in humans was terminated after one study was stopped due to psychotic reactions in two patients (13,14). To our knowledge, no study has yet investigated in vivo cerebral 5-$HT_{1B}$R distribution in individuals with pathological levels of aggression. To address this, we measured 5-$HT_{1B}$R binding in impulsive violent offenders and



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healthy control nonoffenders with positron emission tomography (PET) using the radioligand [$^{11}$C]AZ10419369, which binds selectively to 5-HT$_{1B}$R (15).

Given that knockdown of 5-HT$_{1B}$R increases aggression and administration of 5-HT$_{1B}$R agonists reduces aggression in rodents, we hypothesized that 1) violent offenders would have reduced 5-HT$_{1B}$R binding compared with healthy control subjects; and 2) 5-HT$_{1B}$R binding would be inversely correlated with behavioral measures. We focused on three brain regions of interest (ROIs)—the anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), and striatum—as they have consistently shown structural and/or functional abnormalities in violent and aggressive individuals (16–20). Post hoc, we also evaluated whether effects of 5-HT$_{1B}$R binding were present in smaller regions within the predefined ROIs, including the nucleus accumbens.

## METHODS AND MATERIALS

### Participants

Violent offenders were recruited from closed state prisons within the National Prison and Probation Service in Denmark. Inmates who had a documented history of convictions for violent crimes were invited to an initial screening interview conducted by a medical doctor and a psychologist. Only men were included, consistent with the small number of female inmates in the prisons we recruited from. Healthy control nonoffenders were recruited via community websites and bulletin boards in vocational schools, where people that were interested in participating were invited to complete a questionnaire on their demographic information and a questionnaire assessing their trait aggression (the Buss-Perry Aggression Questionnaire [BPAQ]) (21,22). All participants were selected according to the following criteria: 1) absence of current or lifetime history of major psychiatric disorders (major depressive disorder, bipolar disorder, or psychotic symptomatology); 2) absence of symptomatic medical or neurological illness, head trauma with loss of consciousness for more than 30 minutes, severe visual or hearing impairment, and contraindications for magnetic resonance imaging (MRI); 3) no use of psychotropic medications within 4 weeks before scanning; and 4) absence of current substance or alcohol abuse. Control participants were also selected based on low scores on the BPAQ (mean BPAQ score for control subjects was 51.6 $\pm$ 10.2, out of maximum score of 145) and matched on age and sex. The final sample consisted of 19 violent offenders with a history of crimes including murder, rape, attempted murder, or aggravated assault and 24 healthy control nonoffenders.

None of the participants had any significant medical or neurological illness according to the Schedules for Clinical Assessment in Neuropsychiatry version 2.1, physical examination, and blood biochemistry, which was evaluated on the day of scanning. Fourteen violent offenders had previously abused one or several substances, cannabis ($n$ = 10), cocaine ($n$ = 7), alcohol ($n$ = 5), stimulants ($n$ = 2), opioids ($n$ = 3), and anabolic steroids ($n$ = 2), but all were currently in remission and had been so for at least 6 months up to several years (median number of months under deprivation of liberty at time of inclusion: 24 months, interquartile range 12–60, range 6–264). All included subjects tested negative on urine drug screen (Rapid Response Multi-Drug; BTNX Inc., Toronto, Ontario, Canada) on the day of scanning and had an unremarkable MRI of the brain. The study was approved by National Prison and Probation Service and the local ethical committee (Copenhagen, Denmark; reference H-3-2013-100). All participants provided written informed consent following full description of the procedures, which for violent offenders included access to criminal files, and similar to the healthy control subjects, they received monetary compensation for their participation.

### Assessment Instruments

The Structured Clinical Interview for DSM-IV Axis II Personality Disorders was administered by two medical doctors for assessment of personality disorders (PDs). All violent offenders were diagnosed with one or multiple PDs: antisocial ($n$ = 14), borderline ($n$ = 2), schizoid ($n$ = 1), dependent ($n$ = 1), paranoid ($n$ = 1), obsessive-compulsive ($n$ = 1), unspecified PD ($n$ = 6), and other specified PD ($n$ = 1). Corating was conducted on a subset of the violent offenders, which yielded full final diagnostic consistency. Level of psychopathy was assessed using the Psychopathy Checklist-Revised (PCL-R) (23), which consists of 20 items scored from 0 to 2 based on presence of each trait. PCL-R interviews were conducted by course-certified health professionals with a medical or psychology background, and all ratings were consensus decisions based on notes from each interviewer and collateral information from criminal files. IQ was evaluated by a trained neuropsychologist using the Reynolds Intellectual Screening Test (24). The following self-report personality trait measures were included: trait aggression, the BPAQ (21,22); trait anger, the State-Trait Anger Expression Inventory 2 (25); trait impulsivity, the Barratt Impulsiveness Scale Version 11 (22,26); and trait psychopathy, the Psychopathic Personality Inventory-Revised (PPI-R) (27). State measures administered on the day of scanning included the Profile of Mood States (28), the Major Depression Inventory (29), and the Symptom Checklist-90 Revised (30).

### Imaging and Quantification of 5-HT$_{1B}$ Receptors

Synthesis of [$^{11}$C]AZ10419369 is described in the Supplement. Participants were scanned in a high-resolution research tomography PET scanner (CTI/Siemens, Knoxville, TN) for 90 minutes after an intravenous bolus injection of [$^{11}$C]AZ10419369 over 20 seconds. MRI scans were acquired on a 3T Prisma scanner (Siemens, Erlangen, Germany) with a 64-channel head coil. Unfiltered PET images were coregistered and aligned to the subject's T1-weighted MRI image. ROIs were automatically delineated using PVElab (Neurobiology Research Unit, Copenhagen, Denmark; https://nru.dk/pveout) and SPM8 (Wellcome Trust Centre for Neuroimaging, London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm), as previously described (31). The nucleus accumbens area was obtained from a whole-brain segmentation performed by FreeSurfer (5.3; Martinos Center for Biomedical Imaging, Boston, MA; http://surfer.nmr.mgh.harvard.edu) (32). Coregistration and ROI placement were visually inspected for each subject. Tissue mean time activity curves were extracted automatically from gray matter in each ROI using PVE lab. The striatum was

defined as volume-weighted means of caudate and putamen. The binding potential ($BP_{ND}$) of [$^{11}$C]AZ10419369 in each region was determined using the simplified reference tissue model, which has been validated for quantification of [$^{11}$C]AZ10419369 binding in the human brain (15). The cerebellum (excluding vermis) was used as a reference region, as it contains negligible levels of 5-$HT_{1B}$R (33). Further details on PET and MR acquisition are available in the Supplement.

## Statistical Analyses

Group differences in demographics, clinical data, personality traits, 5-$HT_{1B}$R binding, and brain volumes were evaluated using two-sample *t* tests. Associations between 5-$HT_{1B}$R binding and scale measures of trait anger, trait aggression, trait impulsivity, and level of psychopathy (PPI-R and PCL-R) were evaluated in multiple linear regression models, with the interaction term regional 5-$HT_{1B}$R binding by group status as the predictor of interest. Covariates included age, IQ, and injected mass per kilogram of body weight. Associations indicating no interaction effects were evaluated across all participants adjusting with group status as an additional covariate.

Age was included in the regression models, as 5-$HT_{1B}$R binding is known to decline with age (34). Injected mass per kilogram of body weight was included as a covariate because of theoretical effects of injected mass of [$^{11}$C]AZ10419369 on $BP_{ND}$. The statistical models were also adjusted for IQ since previous studies have shown associations between serotonin and cognitive function (35–37). Sensitivity analyses are included as Supplemental Tables S1 and S2, showing the results after removing the two participants with very high injected mass (>5 μg) and excluding injected mass as a covariate in models with and without IQ as a covariate.

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC) and RStudio (RStudio Inc., Boston, MA). Model assumptions were tested graphically by examination of the distribution of the residuals, quantile probability plots, and predicted values plotted against residuals, none of which indicated substantial violation of assumptions. Significance level was set at a two-tailed *p* value of .05. The group comparisons and main regression analyses (interactions) were corrected for multiple comparisons according to Benjamini-Hochberg (38). Corrected *p* values are reported as $p_{corrected}$.

## Voxel-Based Analysis

Parametric $BP_{ND}$ maps were generated as follows: normalization to Montreal Neurological Institute space was computed for each individual T1 structural using SPM8 and applied to the coregistered PET images with a 2 mm isotropic resolution. The PET images were subsequently smoothed with a 6-mm full width at half maximum Gaussian kernel. Voxel-level $BP_{ND}$ maps were finally computed using multilinear reference tissue model 2 (39) in PMOD (version 3.0; PMOD Technologies Ltd., Zürich, Switzerland; https://www.pmod.com/).

Whole-brain voxelwise multiple regressions were performed with scale measures of trait anger, trait psychopathy, or PCL-R score as predictive variables, adjusting for age, IQ, and injected mass per kilogram of body weight. Group differences were evaluated using a two-sample *t* test at voxel level. To correct for multiple comparisons, 3dClustSim, a program within AFNI (National Institute of Mental Health, Bethesda, MD; http://afni.nimh.nih.gov/afni) that uses a Monte Carlo simulation method, was used to determine a cluster extent threshold unlikely to have occurred by chance ($\alpha < .05$). The cluster extent threshold for a whole-brain search volume given a voxel level of $p < .001$ uncorrected was $k > 144$. All voxel coordinates are given in Montreal Neurological Institute space.

## RESULTS

Participant characteristics are provided in Table 1. Group comparisons revealed no difference in 5-$HT_{1B}$R binding or gray matter volumes of the striatum, ACC, or OFC (Table 2).

We found significant interactions between group status and striatal 5-$HT_{1B}$R binding in predicting trait anger (difference in slopes: −11.7, confidence interval [CI]: [−19.3; −4.1], $p = .003$, $p_{corrected} = .04$; Figure 1) and between group status and ACC 5-$HT_{1B}$R binding in predicting trait psychopathy at a statistical trend level (difference in slopes: −82.7, CI: [−141.6; −23.9], $p = .007$, $p_{corrected} = .08$; Figure 1). Associations between OFC 5-$HT_{1B}$R binding and trait anger or trait psychopathy or associations across participants did not survive corrections for multiple comparisons.

Given the observed interaction effects, we evaluated the associations in the groups separately in post hoc analyses. In violent offenders, striatal 5-$HT_{1B}$R binding was significantly associated with trait anger (slope estimate 18.0, CI: [9.7; 26.3], $p = .0004$; Figure 2), trait psychopathy (slope estimate 81.4, CI: [25.5; 137.3], $p = .008$; Figure 2), and PCL-R score (slope estimate 8.5, CI: [1.34; 15.7], $p = .02$; Figure 2). Trait anger was also associated with ACC (slope estimate: 12.6, CI: [1.0; 24.2], $p = .04$) and OFC (slope estimate: 12.7, CI: [0.8; 24.5], $p = .04$) 5-$HT_{1B}$R binding. ACC and OFC 5-$HT_{1B}$R binding were not correlated with PCL-R score or trait psychopathy. In the control group, we found no associations between 5-$HT_{1B}$R binding and any traits.

Post hoc, we also evaluated in violent offenders 1) whether specific domains of psychopathy were driving the associations with 5-$HT_{1B}$R binding, and 2) which striatal regions were specifically associated with trait anger and level of psychopathy (referring to both PPI-R and PCL-R). We found that the association between striatal 5-$HT_{1B}$R binding and trait psychopathy in the violent offenders was primarily driven by the self-centered impulsivity factor ($p = .0009$), whereas no significant association was seen with coldheartedness ($p = .30$) or fearless dominance ($p = .10$). Analysis of which striatal regions were specifically associated with trait anger and level of psychopathy in the violent offenders revealed that caudate 5-$HT_{1B}$R binding was most strongly associated with trait anger (slope estimate 17.3, CI: [9.8; 24.7], $p = .0002$), trait psychopathy (slope estimate 76.0, CI: [23.7; 128.3], $p = .008$), and PCL-R score (slope estimate 9.9, CI: [4.1; 15.7], $p = .003$). 5-$HT_{1B}$R binding in the putamen was significantly correlated with trait anger ($p = .02$) but not trait psychopathy ($p = .2$) or PCL-R score ($p = .8$). 5-$HT_{1B}$R binding within the accumbens area was not associated with any of the questionnaire measures or the PCL-R.

A whole-brain voxelwise multiple regression analysis in the violent offender group revealed a significant positive correlation between trait anger and 5-$HT_{1B}$R binding in two clusters

**Table 1. Participant Characteristics**

| | Violent Offenders | Healthy Control Subjects | *p* Value |
|---|---|---|---|
| Number of Participants | 19 | 24 | |
| Age, Years | 31.4 ± 8.7 | 32.4 ± 10.7 | .7 |
| Duration of Education, Years | 8.9 ± 2.5 | 11.3 ± 1.1 | .0008 |
| Education Score | 1.9 ± 1.3 | 3.1 ± 1.4 | .008 |
| Intelligence Quotient[a] | 98.3 ± 8.6 | 108.0 ± 7.1 | .0003 |
| PCL-R Score Total | 29.7 ± 4.6 | | |
| Factor 1 | 12.7 ± 2.1 | | |
| Factor 2 | 14.2 ± 3.6 | | |
| Number of Violent Convictions | 3.5 ± 2.5 | None | |
| Number of Violent Charges Against | 15.6 ± 29.6 | None | |
| Age at First Violent Conviction | 19.6 ± 4.2 | N/A | |
| Childhood Trauma[b] | 37.3 ± 12.2 | 33.2 ± 8.6 | .2 |
| Personality Traits | | | |
| Trait aggression[c] | 87.7 ± 21.7 | 51.6 ±10.2 | .0001 |
| Trait anger[d] | 21.4 ± 6.8 | 14.4 ± 2.4 | .0003 |
| Trait impulsivity[e] | 67.0 ± 9.9 | 58.5 ± 9.5 | .007 |
| Trait psychopathy[f] | 313.4 ± 35.7 | 278.1 ± 22.7 | .0009 |
| State Measures | | | |
| Total mood disturbance[g] | 14.5 ± 25.8 | −1.5 ± 12.5 | .02 |
| State angry hostility[g] | 8.7 ± 8.9 | 2.6 ± 2.4 | .009 |
| State depression[h] | 11.1 ± 7.6 | 4.7 ± 4.9 | .003 |
| Global severity index[i] | 0.4 ± 0.3 | 0.1 ± 0.1 | .0007 |

Group data represent mean ± standard deviation (SD) unless stated otherwise. *p* value represents two-sample *t* test, uncorrected.
N/A, not applicable; PCL-R, Psychopathy Checklist-Revised.
[a]Reynolds Intellectual Screening Test index.
[b]Childhood Trauma Questionnaire.
[c]Buss-Perry Aggression Questionnaire total score.
[d]State-Trait Anger Expression Inventory, Trait Anger total score.
[e]Barratt Impulsiveness Scale version 11 total score.
[f]Psychopathic Personality Inventory-Revised total score; $n = 15$ control subjects and 19 inmates.
[g]Profile of Mood States.
[h]Major Depression Inventory.
[i]Symptom Checklist-90 Revised.

**Table 2. PET Parameters**

| | Violent Offenders Mean ± SD | Control Subjects Mean ± SD | *p* Value |
|---|---|---|---|
| OFC $BP_{ND}$ | 1.5 ± 0.4 | 1.4 ± 0.3 | .3 ($p_{corrected}$ = .3) |
| ACC $BP_{ND}$ | 1.8 ± 0.3 | 1.6 ± 0.3 | .2 ($p_{corrected}$ = .3) |
| Striatum $BP_{ND}$ | 1.5 ± 0.3 | 1.3 ± 0.3 | .06 ($p_{corrected}$ = .2) |
| OFC Volume, mL | 16.1 ± 1.9 (2.2 ± 0.1%) | 17.0 ± 1.2 (2.2 ± 0.1%) | .1 (.4) |
| ACC Volume, mL | 6.3 ± 0.9 (0.9 ± 0.08%) | 6.6 ± 0.5 (0.9 ± 0.05%) | .2 (.8) |
| Striatum Volume, mL | 7.2 ± 1.0 (1.0 ± 0.1%) | 7.1 ± 1.2 (0.9 ± 0.1%) | .8 (.2) |
| Injected Radioactivity, MBq | 562.3 ± 74.1 | 589.6 ± 18.5 | .1 |
| Specific Radioactivity, MBq/μmol | 269.1 ± 197.0 | 293.0 ± 200.1 | .7 |
| Injected Mass, μ per kg Body Weight | 0.02 ± 0.02 | 0.02 ± 0.01 | .4 |
| AZ Cerebellum AUC, Counts | 4158.1 ± 1195.3 | 4680 ± 800.6 | .1 |
| Parent Compound at 90 Minutes, %[a] | 86.7 ± 5.1 | 88.7 ± 4.9 | .2 |

*p* values represent differences between groups. Volumes are shown in absolute values (mL) and as a percent of total brain volume (in parenthesis).
ACC, anterior cingulate cortex; AUC, area under the curve; AZ, [$^{11}$C]AZ10419369; $BP_{ND}$, binding potential; OFC, orbitofrontal cortex; PET, positron emission tomography.
[a]Data available for 18 control subjects and 16 violent offenders.

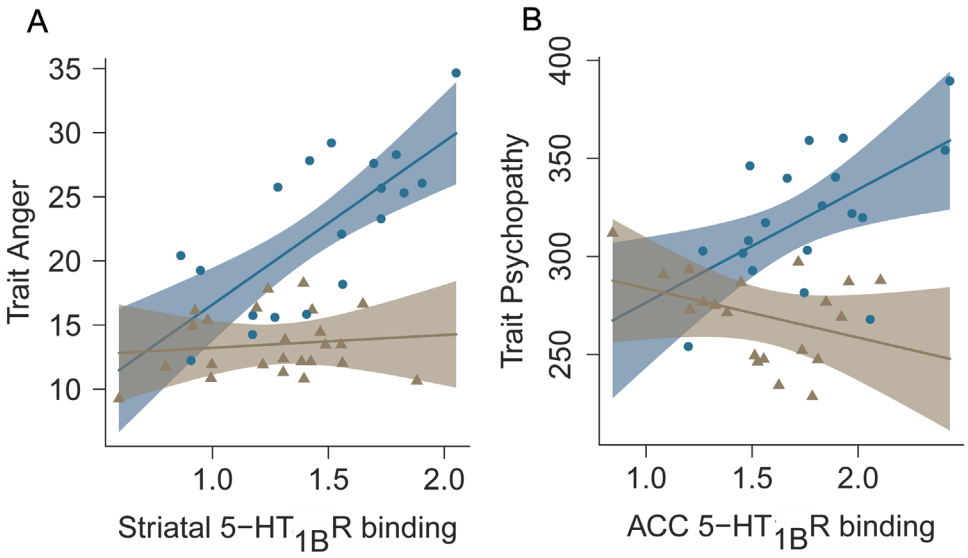


**Figure 1.** Interactions between group status and serotonin 1B receptor (5-$HT_{1B}$R) binding in predicting trait anger and trait psychopathy. **(A)** The association between striatal 5-$HT_{1B}$R binding and trait anger is moderated by group status (test for difference in slopes, $p = .004$, $p_{corrected} = .04$). **(B)** The association between anterior cingulate cortex (ACC) 5-$HT_{1B}$R binding and trait psychopathy is moderated by group (test for difference in slopes, $p = .007$, $p_{corrected} = .08$). Blue circles: violent offenders, brown triangles: healthy control subjects. Shades represent 95% confidence intervals. Plots are shown given a mean age, mean IQ, and mean injected mass per kilogram.

covering the right caudate (k = 178, z = 4.65, $p < .05$ corrected, x = 14, y = 18, z = 4; Figure 3) and the right OFC (k = 183, z = 4.09, $p < .05$ corrected, x = 36, y = 42, z = −8; Figure 3). Whole-brain voxelwise two-sample *t* test revealed no significant group differences in 5-$HT_{1B}$R binding.

## DISCUSSION

The key finding in this study was that high striatal 5-$HT_{1B}$R binding was related to high levels of psychopathy and trait anger in violent offenders. Whole-brain voxelwise analysis in the violent offenders confirmed the positive correlation with trait anger in the caudate and OFC.

The association between 5-$HT_{1B}$R binding and trait anger was moderated by group status, as indicated by significant interaction effects with a particularly prominent association in the violent offender group. Given that control participants were included based on their a priori low trait aggression, it cannot be excluded that the group interaction effect was partly driven by the narrow range of trait anger within the control group (range: 10–20) compared with the violent offender group (range: 13–35). On the other hand, it is reasonable to expect that the effect seen in the violent offenders versus control subjects represents two extremes of a normally distributed personality trait.

We did not find evidence for group differences in 5-$HT_{1B}$R binding. Thus, our data do not support that 5-$HT_{1B}$R binding is a marker for criminal violent behavior in itself but instead appears to reflect symptom severity (level of anger and psychopathy) in individuals with pathological aggression. The involvement of serotonin in aggression has traditionally been attributed to impaired prefrontal serotonergic control of emotional reactions to provocations in antisocial individuals (2). Unexpectedly, we did not find evidence for associations between 5-$HT_{1B}$R binding and aggression per se but rather

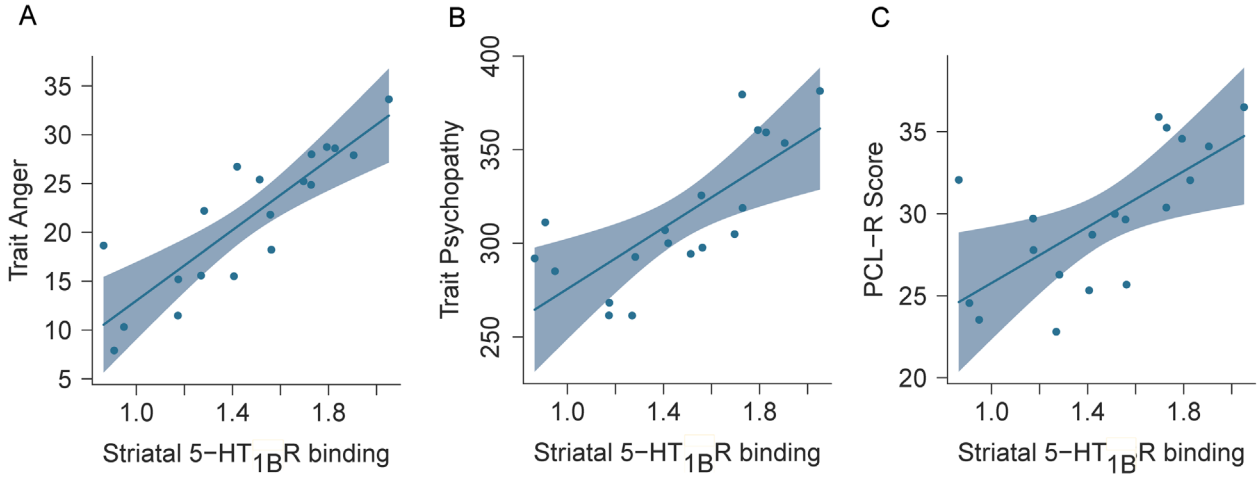


**Figure 2.** Plots showing associations in violent offenders between striatal serotonin 1B receptor (5-$HT_{1B}$R) binding and **(A)** trait anger ($p = .0004$), **(B)** trait psychopathy ($p = .008$), and **(C)** psychopathy score (Psychopathy Checklist-Revised [PCL-R], $p = .02$). Shades represent 95% confidence intervals. Plots are shown given a mean age, mean IQ, and mean injected mass per kilogram.

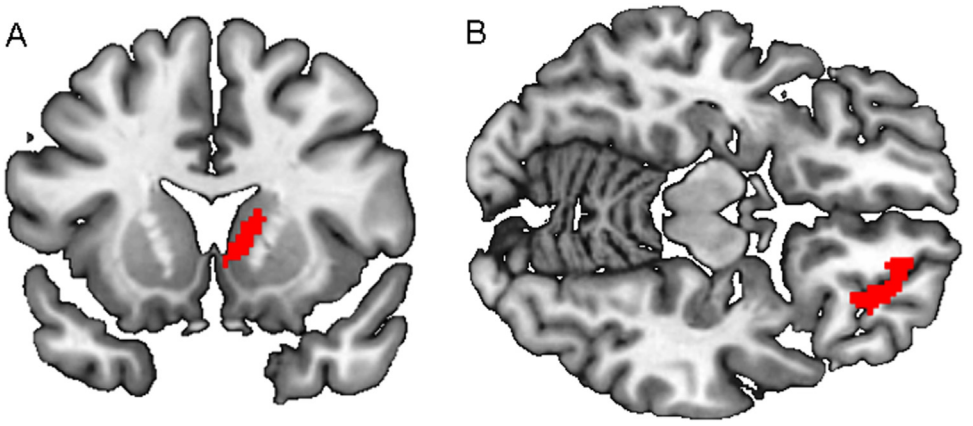


**Figure 3.** Whole-brain voxel-based analysis in the violent offender group showing two clusters (in red) mapped onto a magnetic resonance template image, where trait anger shows a significant positive correlation with serotonin 1B receptor binding. **(A)** Cluster of k = 178 voxels covering the right caudate, z = 4.65, $p < .05$ corrected, x = 14, y = 18, z = 4. Image shown at y = 14. **(B)** Cluster of k = 183 voxels in the right orbitofrontal cortex, z = 4.09, $p < .05$ corrected, x = 36, y = 42, z = −8. Image shown at z = −14.

that 5-$HT_{1B}$R binding was involved in constructs closely related to aggression. Whereas anger is a basic emotion and a common emotional precipitant of impulsive aggression, psychopathy reflects several dimensions, including emotional and interpersonal functioning. The PPI-R specifically reflects psychopathic traits in the absence of antisocial behavior, whereas the PCL-R describes psychopathy in terms of both interpersonal functioning and antisocial behaviors. In the violent offender group, PPI-R and PCL-R scores were positively correlated with trait anger (results not shown). Thus, the collective findings indicate that violent offenders with high striatal 5-$HT_{1B}$R binding get easily angered with a higher degree of anger but also display deficits in interpersonal functioning and thus have overall high levels of psychopathy as evaluated clinically using the PCL-R.

Several alternative interpretations of high 5-$HT_{1B}$R binding in violent offenders with high trait anger and level of psychopathy are plausible. A high 5-$HT_{1B}$R binding might reflect chronically low synaptic serotonin levels, which would be consistent with the longstanding belief that serotonin levels are inversely related to impulsive aggression (1). Even though 5-$HT_{1B}$R binding is sensitive to acute changes in serotonin levels (40,41), a study evaluating 5-$HT_{1B}$R binding after chronic exposure to selective serotonin reuptake inhibitors would be needed to evaluate whether 5-$HT_{1B}$R binding is inversely related to stable serotonin levels and hence whether we can interpret high trait anger and level of psychopathy to be associated with chronically low serotonin levels. Presuming that 5-$HT_{1B}$R binding is sensitive to acute changes in serotonin levels, another possibility is that violent offenders with high trait anger respond with changes in serotonin levels during a day out of prison. However, given that 5-$HT_{1B}$R was related to trait and not state measures, we do not find this plausible. Lastly, high 5-$HT_{1B}$R binding might reflect a genetically and/or neurodevelopmentally determined high 5-$HT_{1B}$R density, in which serotonin affects trait anger and level of psychopathy through 5-$HT_{1B}$ heteroreceptors via other neurotransmitter systems. There are preclinical data to favor this interpretation since heteroreceptor, as opposed to autoreceptor, function seems to be specifically involved in animal aggression (3). In a study using 5-$HT_{1B}$R knockout mice, early postnatal rescue, but not adult rescue, of 5-$HT_{1B}$R expression reduced aggressive behaviors. Impulsivity, on the other hand, was not mediated by forebrain heteroreceptors, and rescue in adult animals reversed the impulsive phenotype. These preclinical data indicate that serotonin can act through 5-$HT_{1B}$R to affect aggression and impulsivity through distinct circuits during different time periods in life. Even though this model seems plausible also in humans, our data suggest that the mechanisms by which 5-$HT_{1B}$R affects aggression and related constructs appear different in humans than in rodents. Future research of human 5-$HT_{1B}$R in aggression will be needed to disentangle these alternating interpretations.

The effect of 5-$HT_{1B}$R binding on both trait anger and level of psychopathy was most pronounced within the striatum, in line with previous studies showing altered structure and function of the striatum in antisocial individuals (20). A number of structural studies have reported increased volumes of the striatum in antisocial individuals (19,42). Both our post hoc ROI analyses of the striatal regions and the voxel-based analyses revealed that the effects of 5-$HT_{1B}$R binding on trait anger and level of psychopathy in violent offenders were especially strong within the caudate but not the putamen or accumbens area. A recent PET study reported 19% lower monoamine oxidase-A binding in the ventral striatum and OFC of 18 male violent offenders with antisocial PD and intermediate to high levels of psychopathy (18). Also, low monoamine oxidase-A in the ventral striatum was associated with risky and impulsive decision making (18). Given these findings, we had expected to find that 5-$HT_{1B}$R binding within the accumbens also would be related to aggression or related constructs in our participants. The dorsal striatum (i.e., the caudate and putamen) is a part of a circuit involved in decision making, learning processes that support goal-directed behavior, and is activated during anticipation of reward (43). The link between 5-$HT_{1B}$Rs in the dorsal striatum and psychopathy can putatively be related to two alternative entities, reward and harm aversion. It has been proposed that caudate impairments result in failure to signal when the behavior is no longer rewarding, which may contribute to persistent maladaptive behaviors such as aggression (20). As striatal 5-$HT_{1B}$Rs in mice regulate dopamine levels in the striatum (3), it seems plausible that altered 5-$HT_{1B}$Rs can result in disturbed reward processing. It has also been shown that activity in the dorsal striatum during retaliation (rejection of unfair offers in the ultimatum game) is increased when serotonin levels are lowered by means of

acute tryptophan depletion, suggesting that impaired serotonin function may reduce harm aversion toward others (44). The involvement of serotonin in harm aversion has also been demonstrated in a placebo-controlled study, where it was shown that participants who were given selective serotonin reuptake inhibitors increased harm aversion for both themselves and others (45). In addition to impulsivity and aggression, antisocial behavior is also associated with impaired aversive responses to the distress of others (46). This might also be a mechanism by which serotonin is involved in psychopathy and thus may explain the association between 5-$HT_{1B}$R binding and level of psychopathy. With respect to trait anger, the very strong correlation with 5-$HT_{1B}$Rs specifically within the caudate was unforeseen. To our knowledge, this is the first study to report that serotonin function in the dorsal striatum is directly related to anger. Taken together, our data support previous studies showing altered structure and function of the striatum in antisocial individuals and provide novel evidence that these effects may be attributed to serotonin signaling.

As supported by both the regional and the voxel-based analyses, 5-$HT_{1B}$Rs within the OFC were positively associated with trait anger in violent offenders. This is in line with previous case-control PET studies showing increased 5-$HT_{2A}$ receptor availability (47) and lower monoamine oxidase-A (18) within the OFC of aggressive individuals, thus supporting the importance of prefrontal serotonergic functioning in aggression. We had no hypothesis regarding laterality of the findings. However, the voxel-based analysis indicated that the right OFC and striatum were particularly associated with trait anger, consistent with the theory that the right hemisphere is specialized in the experience of emotion (48).

A unique feature of this study was the use of in vivo imaging of cerebral 5-$HT_{1B}$Rs in a cohort of individuals with a documented history of extremely violent behavior. However, the study has limitations inherent to studying such a group of individuals. Hence, the groups differed in terms of several parameters, including IQ, past substance abuse, smoking, and being incarcerated. A few recent studies have reported altered 5-$HT_{1B}$R binding in addiction; compared with control subjects, increased 5-$HT_{1B}$R binding was found in the ventral striatum and pallidum in 10 patients with alcohol dependence with 4 weeks abstinence (49), while 14 subjects with cocaine dependence with a mean abstinence of 6 days before scanning had reduced anterior cingulate, hypothalamic, and frontal 5-$HT_{1B}$R binding (50). Even though current substance use was controlled for by urine screening, a potential influence of past substance use in 14 of the violent offenders is possible. With respect to incarceration, a possible effect is that the violent offenders scored higher on state measures of mood disturbance, which, in turn, might have influenced serotonin signaling. However, the inmates were carefully screened for clinical depression and other psychiatric diseases.

In conclusion, we demonstrate for the first time in humans that high striatal 5-$HT_{1B}$R binding is related to high levels of trait anger and psychopathy in violent offenders but not in healthy control nonoffenders. The findings markedly support the involvement of serotonin in aggression and suggest that striatal 5-$HT_{1B}$R binding reflects symptom severity in individuals with pathological aggression. 5-$HT_{1B}$Rs may putatively represent a molecular target for development of pharmacologic antiaggressive treatments.

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## REFERENCES

1. Duke AA, Begue L, Bell R, Eisenlohr-Moul T (2013): Revisiting the serotonin-aggression relation in humans: A meta-analysis. Psychol Bull 139:1148–1172.
2. Siever LJ (2008): Neurobiology of aggression and violence. Am J Psychiatry 165:429–442.
3. Nautiyal KM, Tanaka KF, Barr MM, Tritschler L, Le Dantec Y, David DJ, *et al*. (2015): Distinct circuits underlie the effects of 5-HT1B receptors on aggression and impulsivity. Neuron 86:813–826.
4. Olivier B, van Oorschot R (2005): 5-HT1B receptors and aggression: A review. Eur J Pharmacol 526:207–217.
5. De Almeida RM, Rosa MM, Santos DM, Saft DM, Benini Q, Miczek KA (2006): 5-HT(1B) receptors, ventral orbitofrontal cortex, and aggressive behavior in mice. Psychopharmacology (Berl) 185:441–450.
6. Faccidomo S, Quadros IM, Takahashi A, Fish EW, Miczek KA (2012): Infralimbic and dorsal raphe microinjection of the 5-HT(1B) receptor agonist CP-93,129: Attenuation of aggressive behavior in CFW male mice. Psychopharmacology (Berl) 222:117–128.
7. da Veiga CP, Miczek KA, Lucion AB, de Almeida RM (2011): Social instigation and aggression in postpartum female rats: Role of 5-Ht1A and 5-Ht1B receptors in the dorsal raphe nucleus and prefrontal cortex. Psychopharmacology (Berl) 213:475–487.
8. Saudou F, Amara DA, Dierich A, LeMeur M, Ramboz S, Segu L, *et al*. (1994): Enhanced aggressive behavior in mice lacking 5-HT1B receptor. Science 265:1875–1878.
9. Pattij T, Broersen LM, van der Linde J, Groenink L, van der Gugten J, Maes RA, Olivier B (2003): Operant learning and differential-reinforcement-of-low-rate 36-s responding in 5-HT1A and 5-HT1B receptor knockout mice. Behav Brain Res 141:137–145.

10. Hakulinen C, Jokela M, Hintsanen M, Merjonen P, Pulkki-Raback L, Seppala I, *et al*. (2013): Serotonin receptor 1B genotype and hostility, anger and aggressive behavior through the lifespan: The Young Finns study. J Behav Med 36:583–590.
11. Tiihonen J, Hakola P, Paanila J, Turtiainen M (1993): Eltoprazine for aggression in schizophrenia and mental retardation. Lancet 341:307.
12. de Koning P, Mak M, de Vries MH, Allsopp LF, Stevens RB, Verbruggen R, *et al*. (1994): Eltoprazine in aggressive mentally handicapped patients: A double-blind, placebo- and baseline-controlled multi-centre study. The Eltoprazine Aggression Research Group. Int Clin Psychopharmacol 9:187–194.
13. Moriarty J SB, Trimble MR, De Koning P (1994): A trial of eltoprazine in the treatment of aggressive behaviours in two populations: Patients with epilepsy or Gilles de la Tourette's syndrome. Hum Psychopharmacol 9:253–258.
14. Verhoeven W, Tuinier S (2007): Serenics: Anti-aggression drugs throughout history. Clin Neuropsychiatry 4:135–143.
15. Varnas K, Nyberg S, Halldin C, Varrone A, Takano A, Karlsson P, *et al*. (2011): Quantitative analysis of [11C]AZ10419369 binding to 5-HT1B receptors in human brain. J Cereb Blood Flow Metab 31:113–123.
16. Yang Y, Raine A (2009): Prefrontal structural and functional brain imaging findings in antisocial, violent, and psychopathic individuals: A meta-analysis. Psychiatry Res 174:81–88.
17. Rosell DR, Siever LJ (2015): The neurobiology of aggression and violence. CNS Spectr 20:254–279.
18. Kolla NJ, Matthews B, Wilson AA, Houle S, Michael Bagby R, Links P, *et al*. (2015): Lower monoamine oxidase-a total distribution volume in impulsive and violent male offenders with antisocial personality disorder and high psychopathic traits: An [C] harmine positron emission tomography study. Neuropsychopharmacology 40:2596–2603.
19. Glenn AL, Raine A, Yaralian PS, Yang Y (2010): Increased volume of the striatum in psychopathic individuals. Biol Psychiatry 67:52–58.
20. Glenn AL, Yang Y (2012): The potential role of the striatum in antisocial behavior and psychopathy. Biol Psychiatry 72:817–822.
21. Buss AH, Perry M (1992): The aggression questionnaire. J Pers Soc Psychol 63:452–459.
22. da Cunha-Bang S, Stenbaek DS, Holst K, Licht CL, Jensen PS, Frokjaer VG, *et al*. (2013): Trait aggression and trait impulsivity are not related to frontal cortex 5-HT2A receptor binding in healthy individuals. Psychiatry Res 212:125–131.
23. Hare R (2003): Hare Psychopathy Checklist-Revised (PCLR) 2nd ed. Toronto: Multi-Health Systems Inc.
24. Reynolds CR, Kamphaus RW (2011): Reynolds Intellectual Screening Test (RIST) Professional Manual. Copenhagen: Hogrefe Psykologisk Forlag A/S.
25. Moeller SB, Novaco RW, Heinola-Nielsen V, Hougaard H (2015): Validation of the Novaco Anger Scale-Provocation Inventory (Danish) with nonclinical, clinical, and offender samples [published online ahead of print May 1]. Assessment.
26. Patton JH, Stanford MS, Barratt ES (1995): Factor structure of the Barratt Impulsiveness Scale. J Clin Psychol 51:768–774.
27. Lilienfeld SO, Widows MR (2005): Psychopathic Personality Inventory–Revised (PPI-R) Professional Manual. Lutz, FL: Psychological Assessment Resources.
28. McNair DM, Lorr M, Droppleman L (1992): Manual for the Profile of Mood States (POMS): Revised. San Diego: Educational and Industrial Testing Service.
29. Forsell Y (2005): The Major Depression Inventory versus Schedules for Clinical Assessment in Neuropsychiatry in a population sample. Soc Psychiatry Psychiatr Epidemiol 40:209–213.
30. Derogatis LR (1994): SCL-90-R: Symptom Checklist-90-R. Administration, Scoring and Procedures Manual, *3rd ed.* Minneapolis: National Computer Systems.
31. Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbol S, Frokjaer VG, *et al*. (2005): MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. Neuroimage 24:969–979.
32. Fischl B, Salat DH, Busa E, Albert M, Dieterich M, Haselgrove C, *et al*. (2002): Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. Neuron 33:341–355.
33. Varnas K, Hall H, Bonaventure P, Sedvall G (2001): Autoradiographic mapping of 5-HT(1B) and 5-HT(1D) receptors in the post mortem human brain using [(3)H]GR 125743. Brain Res 915:47–57.
34. Nord M, Cselenyi Z, Forsberg A, Rosenqvist G, Tiger M, Lundberg J, *et al*. (2014): Distinct regional age effects on [11C]AZ10419369 binding to 5-HT1B receptors in the human brain. Neuroimage 103: 303–308.
35. Haahr ME, Fisher P, Holst K, Madsen K, Jensen CG, Marner L, *et al*. (2013): The 5-HT4 receptor levels in hippocampus correlates inversely with memory test performance in humans. Hum Brain Mapp 34: 3066–3074.
36. Penttila J, Hirvonen J, Tuominen L, Lumme V, Ilonen T, Nagren K, Hietala J (2016): Verbal memory and 5-HT receptors in healthy volunteers - A PET study with [carbonyl-C]WAY-100635. Eur Neuropsychopharmacol 26:570–577.
37. Madsen K, Erritzoe D, Mortensen EL, Gade A, Madsen J, Baare W, *et al*. (2011): Cognitive function is related to fronto-striatal serotonin transporter levels–a brain PET study in young healthy subjects. Psychopharmacology (Berl) 213:573–581.
38. Benjamini Y, Hochberg Y (1995): Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol 57:289–300.
39. Ichise M, Liow JS, Lu JQ, Takano A, Model K, Toyama H, *et al*. (2003): Linearized reference tissue parametric imaging methods: Application to [11C]DASB positron emission tomography studies of the serotonin transporter in human brain. J Cereb Blood Flow Metab 23:1096–1112.
40. Finnema SJ, Varrone A, Hwang TJ, Gulyas B, Pierson ME, Halldin C, Farde L (2010): Fenfluramine-induced serotonin release decreases [11C]AZ10419369 binding to 5-HT1B-receptors in the primate brain. Synapse 64:573–577.
41. Nord M, Finnema SJ, Halldin C, Farde L (2013): Effect of a single dose of escitalopram on serotonin concentration in the non-human and human primate brain. Int J Neuropsychopharmacol 16:1577–1586.
42. Schiffer B, Muller BW, Scherbaum N, Hodgins S, Forsting M, Wiltfang J, *et al*. (2011): Disentangling structural brain alterations associated with violent behavior from those associated with substance use disorders. Arch Gen Psychiatry 68:1039–1049.
43. Balleine BW, Delgado MR, Hikosaka O (2007): The role of the dorsal striatum in reward and decision-making. J Neurosci 27:8161–8165.
44. Crockett MJ, Apergis-Schoute A, Herrmann B, Lieberman MD, Muller U, Robbins TW, Clark L (2013): Serotonin modulates striatal responses to fairness and retaliation in humans. J Neurosci 33: 3505–3513.
45. Crockett MJ, Siegel JZ, Kurth-Nelson Z, Ousdal OT, Story G, Frieband C, *et al*. (2015): Dissociable effects of serotonin and dopamine on the valuation of harm in moral decision making. Curr Biol 25:1852–1859.
46. Blair RJ (2007): The amygdala and ventromedial prefrontal cortex in morality and psychopathy. Trends Cogn Sci 11:387–392.
47. Rosell DR, Thompson JL, Slifstein M, Xu X, Frankle WG, New AS, *et al*. (2010): Increased serotonin 2A receptor availability in the orbitofrontal cortex of physically aggressive personality disordered patients. Biol Psychiatry 67:1154–1162.
48. Demaree HA, Everhart DE, Youngstrom EA, Harrison DW (2005): lateralization of emotional processing: Historical roots and a future incorporating "dominance." Behav Cogn Neurosci Rev 4:3–20.
49. Hu J, Henry S, Gallezot JD, Ropchan J, Neumaier JF, Potenza MN, *et al*. (2010): Serotonin 1B receptor imaging in alcohol dependence. Biol Psychiatry 67:800–803.
50. Matuskey D, Bhagwagar Z, Planeta B, Pittman B, Gallezot JD, Chen J, *et al*. (2014): Reductions in brain 5-HT1B receptor availability in primarily cocaine-dependent humans. Biol Psychiatry 76:816–822.

# Supplemental material

**Serotonin 1B receptor binding is associated with trait anger and level of psychopathy in violent offenders**

## METHODS

### PET

[$^{11}$C]AZ10419369 was produced as previously described (1) with minor modifications. Briefly, [$^{11}$C]methyl triflate was transferred in a stream of helium to a 0.9 ml vial containing 0.3 mg of the labelling (desmetyl-) precursor and 2 µl 2M NaOH in 300 µl acetone and the resulting mixture was heated at 40 °C for 30 seconds. After diluting with 4.5 ml 100mM phosphate buffer pH=7, the reaction mixture was purified by HPLC (Phenomenex Onyx Monolithic C18 column, 10 x 100 mm; eluent 25/75 ethanol/25mM citrate buffer pH=5.2; flow rate 6 ml/min).
The fraction corresponding to the labeled product (ca. 6 min) was collected by allowing the HPLC eluent to flow directly through a 0.22 µm sterile filter (Millex GV, Millipore) into a 20 ml glas vial containing 9 ml sterile phosphate buffered saline solution (pH 7), giving a 15 ml sterile solution of [$^{11}$C]AZ10419369 containing around 7 % w/v ethanol.

Mean specific radioactivity of the radioligand at the time of injection was 282.5±196.7 GBq/µmol range: 38.1 – 823.9, and mean injected mass was 1.6±1.4 µg, range: 0.3 – 6.0). PET images were reconstructed using an iterative method into 39 dynamic frames (6x10 seconds, 6x20 seconds, 6x60 seconds, 8x120 seconds, and 13x300 seconds). All PET images were motion corrected using the AIR (Automated Image Registration, v.5.2.5, LONI, UCLA) software where all frames were aligned to the first 5 minute frame.

Venous blood samples were collected at 2.5, 20, 50 and 90 minutes and analyzed for metabolites using radio-HPLC. No significant amounts of radioactive metabolites were formed during analysis (mean percentage of unchanged radioligand in plasma was 87.6±5.0 % at 90 minutes).

One PET scan had to be finalized after 75 minutes because the subject (violent offender) was lying uncomfortably. Based on an evaluation of time-stability of the BPnd from 90 to 60 minutes in a subset of 5 subjects, where the change in BPnd was <2 % in the PFC, striatum and neocortex, this scan was included in the study.

**MRI**

We acquired a T1-weighted, TurboFLASH sequence, high-resolution whole-brain three-dimensional structural magnetic resonance scan with an inversion time of 900 ms, echo time of 2.58 ms, repetition time of 1900 ms, flip angle of 9°, in-plane matrix of 256 × 256, in-plane resolution of 0.9×0.9 mm, 224 slices and a slice thickness of 0.9 mm, no gap. Based on the T1, a segmented magnetic resonance image was produced with VBM8 in SPM8 to mask gray matter.

**RESULTS**

**Supplementary Table 1.** Interactions between regional $BP_{ND}$ and group status in predicting trait anger and trait psychopathy in models with either age, or age and IQ as covariates. Slope estimates represent difference in slopes of healthy controls relative to violent offenders. ACC: anterior cingulate cortex, OFC: orbitofrontal cortex.

| Model | Slope estimate | Standard Error | 95 % Confidence Interval | p-value |
|---|---|---|---|---|
| TRAIT ANGER | | | | |
| ACC $BP_{ND}$-by-group, age | -10.68 | 4.04 | -18.88 ; -2.47 | 0.012 |
| ACC $BP_{ND}$-by-group, age, IQ | -12.93 | 3.83 | -20.71 ; -5.14 | 0.0018 |
| OFC $BP_{ND}$-by-group, age | -8.39 | 3.95 | 4.19 ; 17.48 | 0.041 |
| OFC $BP_{ND}$-by-group, age, IQ | -10.86 | 3.84 | -18.67 ; -3.04 | 0.0078 |
| Striatum $BP_{ND}$-by-group, age | -8.51 | 0.06 | -17.01 ; -0.02 | 0.049 |
| Striatum $BP_{ND}$-by-group, age, IQ | -10.58 | 3.95 | -18.59 ; 2.56 | 0.011 |
| TRAIT PSYCHOPATHY | | | | |
| ACC $BP_{ND}$-by-group, age | -64.77 | 31.42 | -128.77 ; -0.76 | 0.048 |
| ACC $BP_{ND}$-by-group, age, IQ | -82.35 | 32.08 | -147.78 ; -16.91 | 0.015 |
| OFC $BP_{ND}$-by- group, age | -56.74 | 30.78 | -11.09 ; 87.13 | 0.075 |
| OFC $BP_{ND}$-by- group, age, IQ | -76.89 | 32.09 | -142.34 ; -11.45 | 0.023 |
| Striatum $BP_{ND}$-by- group, age | -32.36 | 31.99 | -20.11 ; 73.59 | 0.32 |
| Striatum $BP_{ND}$-by- group, age, IQ | -42.95 | 32.62 | -10.57 ; 91.18 | 0.20 |

**Supplementary Table 2.** Associations between regional binding potential ($BP_{ND}$) and trait anger, trait psychopathy and Psychopathy Checklist Revised (PCL-R) score in violent offenders in models with and without IQ as a covariate. ACC: anterior cingulate cortex, OFC: orbitofrontal cortex.

| Model | Slope Estimate | Standard Error | 95 %Confidence Intervals | p-value |
|---|---|---|---|---|
| TRAIT ANGER | | | | |
| ACC $BP_{ND}$, age | 11.28 | 5.28 | -0.04 ; 22.61 | 0.051 |
| ACC $BP_{ND}$, age, IQ | 14.56 | 5.75 | 2.13 ; 26.99 | 0.025 |
| OFC $BP_{ND}$, age | 10.72 | 5.45 | -0.96 ; 22.41 | 0.069 |
| OFC $BP_{ND}$, age, IQ | 13.56 | 5.95 | 0.69 ; 26.43 | 0.040 |
| Striatum $BP_{ND}$, age | 10.44 | 4.11 | 1.61 ; 19.26 | 0.024 |
| Striatum $BP_{ND}$, age, IQ | 16.42 | 4.39 | 6.93 ; 25.91 | 0.0025 |
| TRAIT PSYCHOPATHY | | | | |
| ACC $BP_{ND}$, age | 38.98 | 33.57 | -33.02 ; 110.99 | 0.26 |
| ACC $BP_{ND}$, age, IQ | 64.08 | 35.52 | -12.67 ; 140.83 | 0.095 |
| OFC $BP_{ND}$, age | 30.14 | 34.66 | -44.20 ; 104.49 | 0.40 |
| OFC $BP_{ND}$, age, IQ | 51.49 | 37.14 | -28.76 ; 131.78 | 0.19 |
| Striatum $BP_{ND}$, age | 30.77 | 27.54 | -28.29 ; 89.84 | 0.28 |
| Striatum $BP_{ND}$, age, IQ | 66.63 | 30.61 | 0.51 ; 132.76 | 0.049 |
| PCL-R SCORE | | | | |
| ACC $BP_{ND}$, age | 9.00 | 3.91 | 0.60 ; 17.39 | 0.037 |
| ACC $BP_{ND}$, age, IQ | 6.44 | 4.23 | -2.71 ; 15.59 | 0.15 |
| OFC $BP_{ND}$, age | 7.61 | 4.18 | -1.37 ; 16.58 | 0.091 |
| OFC $BP_{ND}$, age, IQ | 4.76 | 4.41 | -4.77 ; 14.29 | 0.30 |
| Striatum $BP_{ND}$, age | 9.07 | 2.87 | 2.91 ; 15.22 | 0.0069 |
| Striatum $BP_{ND}$, age, IQ | 7.50 | 3.57 | -0.22 ; 15.23 | 0.056 |

## REFERENCES

1. Pierson ME, Andersson J, Nyberg S, McCarthy DJ, Finnema SJ, Varnas K, et al. (2008): [11C]AZ10419369: a selective 5-HT1B receptor radioligand suitable for positron emission tomography (PET). Characterization in the primate brain. *NeuroImage*. 41:1075-1085.

# Study 3

# Men with high serotonin 1B receptor binding have high trait anger and respond to provocations with heightened amygdala and striatal reactivity

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## Abstract

Serotonin signalling influences amygdala reactivity to threat-related emotional facial expressions in healthy adults, but in vivo serotonin signalling has never been investigated in the context of provocative stimuli in aggressive individuals. Here, we investigate 18 aggressive violent offenders and 22 healthy control subjects with the aim to evaluate the association between brain serotonin 1B receptor (5-$HT_{1B}$R) levels, brain reactivity to provocations and the personality feature trait anger. We quantified regional 5-$HT_{1B}$R binding using [$^{11}$C]AZ10419369 positron emission tomography (PET) and measured brain activation with functional magnetic resonance imaging (fMRI) following provocations in terms of monetary subtractions from a fictive opponent. With this point-subtraction aggression paradigm (PSAP) adapted for fMRI, subjects have the possibility to either behave aggressively or to pursue monetary rewards. Trait anger was measured with the State-Trait Anger Expression Inventory. We used a single latent variable model framework to test for a common association between these three measures. We found that across participants, one latent variable significantly positively predicted 5-$HT_{1B}$R brain binding (all regions $p < 0.001$), trait anger ($p=0.002$), amygdala ($p=0.04$) and striatal ($p=0.04$) reactivity to provocations. These findings provide novel evidence that 5-$HT_{1B}$R brain levels are linked to both brain reactivity to provocations and trait anger in a cohort of men displaying a wide range of aggressive behaviours. The data suggest that 5-$HT_{1B}$R represents an intriguing target for reducing excessive neural reactivity to provocations and thereby aggressive behaviors.

## INTRODUCTION

Serotonin is a key neurotransmitter in emotional processing, and is involved in a range of neuropsychiatric diseases including depression, anxiety and pathological aggression. The majority of serotonergic neurons originate in the brainstem raphe nuclei, projecting to most parts of the brain where it modulates neural responses through different serotonin receptor subtypes (1). Low levels of global cerebral serotonin levels have been associated with high levels of impulsive aggression in both animals and humans (2, 3). Impulsive aggression can arise as a consequence of impaired emotion regulation, involving a neural circuitry that includes the anterior cingulate cortex (ACC), orbitofrontal cortex (OFC) and amygdala (4, 5). The mechanism by which low levels of serotonin causes aggression has mainly been attributed to insufficient serotonergic prefrontal inhibition of emotional reactions (6).

Preclinical research in animals suggests a specific involvement of serotonin 1B receptors (5-$HT_{1B}$R) in aggression and impulsivity. Dorsal raphe injection of 5-$HT_{1B}$R agonists reduces aggression in mice, indicating a role for presynaptic 5-$HT_{1B}$R in the effects (7). In contrast, specific genetic knockdown of 5-$HT_{1B}$R heteroreceptors results in aggressive mice whereas genetic knockdown of 5-$HT_{1B}$R autoreceptors neither affects aggression nor impulsivity (8). Hence, rodent studies suggest that both pre- and post-synaptic 5-$HT_{1B}$R populations are involved in aggressive responding. However, the involvement of 5-$HT_{1B}$R in aggression in humans is less well known. Given that 5-$HT_{1B}$R agonism reduces aggression and that knockdown of 5-$HT_{1B}$R in mice increases aggression, we have previously hypothesized that low 5-$HT_{1B}$R availability would be related to high levels of aggression in humans. However, in a positron emission tomography (PET) study we instead find that *high* striatal 5-$HT_{1B}$ binding in violent offenders is related to high levels of trait anger and psychopathy (da Cunha-Bang, submitted). This corroborates a role of 5-$HT_{1B}$R function in aspects of aggression also in humans, but in an unexpected direction. These discrepant findings motivate further evaluation of 5-$HT_{1B}$R and aggression in humans.

In addition to PET, a subset of the participants was examined with functional magnetic resonance imaging (fMRI) using the point-subtraction aggression paradigm (PSAP). In this task, the participants have the opportunity to pursue monetary rewards or act aggressively, while a fictive opponent occasionally steals money with the purpose of provoking them (9, 10). This task provides information on both neural reactivity to provocative stimuli and is an objective measure of aggressive behaviour. Consistent with previously reported heightened amygdala reactivity to emotional stimuli in pathologically aggressive individuals (11), we found that the violent offenders had higher reactivity to provocations within the amygdala and striatum compared with non-aggressive healthy controls (da Cunha-Bang, submitted). Multimodal neuroimaging integrating PET and fMRI in humans offers a unique opportunity to directly link brain function and relevant molecular mechanisms in vivo (12). Such approach has been used to show that serotonin signalling modulates amygdala reactivity to threat-related stimuli (13); low reactivity to fearful faces is associated with high dorsal raphe serotonin 1A receptor (5-$HT_{1A}$R) binding (14, 15) and with high medial prefrontal serotonin 2A receptor (5-$HT_{2A}$R) binding (16). Thus, serotonin modulates neural circuits involved in emotional processing such as the amygdala and medial prefrontal cortex.

In this study, we integrate PET and fMRI data in highly aggressive violent offenders and in healthy control subjects, with the aim to evaluate whether the observed amygdala and striatal reactivity to provocations is modulated by 5-$HT_{1B}$R binding. These data provide a unique opportunity to investigate the influence of 5-$HT_{1B}$R signalling on amygdala reactivity in the context of provocations in both controls and in violent offenders. We examine whether 5-$HT_{1B}$R binding across regions rich in serotonin and relevant for aggression (raphe nuclei, amygdala, striatum, ACC and OFC) is associated with neural reactivity to provocations and trait anger, accounting for effects of group status. This is evaluated in a latent variable statistical framework, in which we test whether an

underlying construct can explain a hypothesized shared variance in 5-$HT_{1B}$R binding, trait anger and neural reactivity to provocations, aggregated in one single model. Based on our previous observation that high striatal 5-$HT_{1B}$R binding is associated with high levels of trait anger in violent offenders (da Cunha-Bang, in press), we hypothesized that individuals with high 5-$HT_{1B}$R binding across regions would have high trait anger, amygdala and striatal reactivity to provocations.

## METHODS

Detailed descriptions of the participants, recruitment, acquisition and analysis of fMRI and PET data are available in da Cunha-Bang (in press). For 33 of the participants, PET and MR scans were acquired on the same day. For the remaining seven subjects there was a median interval of eight days between scans (range: 7-61 days).

### Participants

The final study sample consisted of 18 incarcerated violent offenders with a documented history of severe violent crimes (murder, aggravated assault, rape, attempt to rape) and 22 healthy control subjects recruited from the community. All participants were male and the groups were age-matched. Exclusion criteria for all participants were: current or lifetime history of major psychiatric disorders (major depressive disorder, bipolar disorder or psychotic symptomatology), symptomatic medical or neurological illness, severe head trauma, severe visual or hearing impairment, contraindications for MRI, use of psychotropic medications, current substance or alcohol abuse. Fourteen violent offenders had a history of substance abuse including cannabis (n=10), cocaine (n=7), alcohol (n=5), stimulants (n=2), opioids (n=3) and anabolic steroids (n=2), but all were currently in remission. All participants tested negative on urine drug screen (Rapid Response Multi-Drug; BTNX Inc., Toronto, Ontario, Canada) on the day of scanning and had an unremarkable MRI. The study was approved by the National Prison and Probation Service and the local ethical committee (Copenhagen,

Denmark, reference H-3-2013-100). All participants provided written informed consent following full description of the procedures, which for the violent offenders included access to criminal files, and received monetary compensation for their participation. Data from 38 of the participants were reported in two previous studies (da Cunha-Bang, in press, da Cunha-Bang, submitted).

The Structured Clinical Interview for DSM-IV II (SCID-II) was used for assessment of personality disorders. All violent offenders were diagnosed with one or multiple personality disorders; antisocial (n=14), borderline (n=2), schizoid (n=1), dependent (n=1), paranoid (n=1), obsessive-compulsive (n=1) and unspecified personality disorder (n=7). Level of psychopathy was assessed using the Psychopathy Checklist-Revised (PCL-R) (17), which consists of 20 items scored from 0 to 2 based on presence of each trait. Intelligence quotient (IQ) was assessed by a trained neuropsychologist using the Reynolds Intellectual Screening Test.

**The Point Subtraction Aggression Paradigm**

Prior to scanning, participants were instructed that they would play a game with another person and they could earn points, which would be exchanged for money. Participants completed one 12-minute session of the point subtraction aggression paradigm (PSAP) and the monetary reward was 1.34 Euro (10 DKK) per point won. Three response options were available: pressing the button for 'Option 1' 100 times resulted in the participant earning 1 point (1.34 Euro), pressing the button for 'Option 2' 10 times resulted in removal of a point from the opponent and pressing for 'Option 3' 10 times briefly protected the participant's point total from the opponent stealing. Participants were required to complete an option before choosing a new option. Participants were informed that they were assigned to the experimental condition in which they did not keep the points they stole from the opponent. In this way, choosing 'Option 2' reflects an aggressive behavior, void of any monetary incentive.

The status of the game was projected onto a screen viewed by the participant while lying in the scanner. We defined task related aggressive behavior as:

$$PSAPaggression = \frac{AR}{PR} / TBP * 1000$$

where AR=Number of Aggressive Responses, PR=Number of Provocations and TBP=Number of Total Button Presses. This adjusts aggressive behavior with respect to individual differences in button-press rate and received provocations. Upon completion of the PSAP, participants filled in a questionnaire, providing their impression of the opponent. Participants who indicated that they did not play with a real person were excluded (n=1).

**fMRI Data Acquisition and Analysis**

A detailed description of scan parameters and preprocessing of fMRI data is provided da Cunha-Bang et al. (submitted). MRI scans were acquired on a 3T Prisma scanner (Siemens, Erlangen, Germany) with a 64-channel head coil. For blood oxygen level dependent (BOLD) fMRI, a T2*-weighted gradient echo-planar imaging (EPI) sequence was used with a repetition time of 2000 ms, echo time of 30 ms, flip angle of 90$^{o}$, in-plane matrix of 64×64 mm, in-plane resolution of 3.6×3.6 mm and 32 slices with a slice thickness of 3.0 mm (0.75 mm gap). A total of 360 whole-brain fMRI volumes were acquired. We acquired a T1-weighted, TurboFLASH sequence, high-resolution whole-brain three-dimensional structural magnetic resonance scan with an inversion time of 900 ms, echo time of 2.58 ms, repetition time of 1900 ms, flip angle of 9°, in-plane matrix of 256×256, in-plane resolution of 0.9×0.9 mm, 224 slices and a slice thickness of 0.9 mm, no gap. To reduce head motion, an in-house made head fixation system was used.

Functional neuroimaging data were analysed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm). Single subject functional images were spatially realigned to the first image. The T1-weighted structural image was co-registered to the first functional image and the origin reset to the anterior commissure (AC) using acpcdetect (https://www.nitrc.org/projects/art). The co-registered T1-

weighted image was normalized into Montreal Neurological Institute (MNI) stereotactic space and the normalization parameters were applied to the functional images. Normalized functional images were smoothed using an 8 mm FWHM Gaussian filter. The final voxel size was 2 x 2 x 2 mm. After each preprocessing step the images were manually inspected. No dataset required to be excluded due to poor image quality. Artefact Detection Tools (ART) (http://www.nitrc.org/projects/artifact_detect) was used to identify individual functional volumes with excess motion (>2mm) and signal variability (>4 standard deviations), which were censored when estimating task-related effects.

We used the first 10 seconds of Option1 as the "baseline condition" to avoid any confounding reward effects (da Cunha-Bang, submitted). If a provocation occurred during the first 10 seconds of Option1, the time from beginning of Option1 until the provocation occurred was used. Single-subject design matrices were estimated using the general linear model to determine BOLD response to Provocations > Option1. Individual contrast images (i.e., weighted sum of beta images) were included in group-level analyses to determine brain responses to provocations using one-sample *t*-tests. The amygdala and striatum (caudate and putamen) were defined using WFU PickAtlas toolbox (18, 19). To address the issue of multiple comparisons we used 3dClustSim, a software program within AFNI (http://afni.nimh.nih/gov/afni) that uses a Monte Carlo simulation method to establish family-wise-error-corrected extent cluster thresholds unlikely to have occurred by chance ($\alpha < 0.05$)(20). We used a voxel-level statistical threshold of $p < 0.005$, uncorrected. Based on this, amygdala and striatum clusters of $k \geq 6$ and 59 voxels, respectively, were considered statistically significant. Coordinates are reported in Montreal Neurological Institute space.

**PET Data Acquisition**

Participants were scanned in a high-resolution research tomography (HRRT) PET scanner (CTI/Siemens, Knoxville, TN, USA) for 90 minutes after an intravenous bolus injection of [$^{11}$C]AZ10419369 over 20 seconds. Mean specific radioactivity

of the radioligand at the time of injection was 288±202 GBq/μmol, and mean injected mass was 1.61±1.40 μg). PET images were reconstructed using an iterative method into 39 dynamic frames (6x10 seconds, 6x20 seconds, 6x60 seconds, 8x120 seconds, 13x300 seconds). All PET images were motion corrected using the AIR (Automated Image Registration, v.5.2.5, LONI, UCLA) software where all frames were aligned to the first 5-minute frame. Unfiltered PET images were co-registered and aligned to the subject's T1-weighted MRI image. All ROIs were automatically delineated using Pvelab (21), except for the raphe region that was delineated according to Kalbitzer et al. (22). Co-registration and ROI placement were visually inspected for each subject. Based on the T1 described above, a segmented magnetic resonance image was produced with VBM8 in SPM8 to mask gray matter. Tissue time activity curves were extracted automatically from gray matter in each ROI using PVE lab. The binding potential ($BP_{ND}$) of [$^{11}$C]AZ10419369 in each region was determined using the simplified reference tissue model (SRTM), which has been validated for quantification of [$^{11}$C]AZ10419369 binding in the human brain (23). The $BP_{ND}$ is defined as: $BP_{ND} = f_{nd} * B_{avail}/K_d$, where $f_{nd}$ is the free fraction of tracer in the non-displaceable tissue compartment, $B_{avail}$ is the number of receptors available for binding and $K_d$ is the equilibrium dissociation constant for the tracer. The cerebellum (excluding vermis) was used as a reference region, as it contains negligible levels of 5-$HT_{1B}$R (24).

**Statistical analysis**

Group differences in demographics, task behaviour and PET parameters were evaluated using unpaired t-tests. Associations between amygdala and striatal reactivity to provocations and behavioural measures were evaluated in multiple linear regression models, adjusting for age.

*Integration of PET and fMRI data and latent variable model selection*

For amygdala and striatal reactivity to provocations, we extracted the mean contrast estimates across all voxels from clusters within the amygdala and

striatum showing significant task-related activity across all participants. As we had no hypothesis regarding lateralization and left and right amygdala and striatal reactivity estimates were significantly correlated ($r>0.64$), we combined bilateral clusters within each region.

A single latent variable model framework was used to test for an common association between 5-$HT_{1B}$R binding, brain reactivity to provocations and trait anger, applied using the Lava package in R (25). The latent variable model is a linear regression statistical framework for modelling associations where there is shared information across observations, e.g. receptor binding across different brain regions. The latent variable model was based on the assumption that 5-$HT_{1B}$R binding across brain regions are highly intercorrelated. By use of this shared information it is possible to avoid multiple comparisons. Here we estimated a single latent variable modelling the shared correlation between 5-$HT_{1B}$R binding within regions in a neural circuitry implicated in emotional processing and aggression (ACC, OFC raphe nuclei, amygdala and striatum), trait anger and BOLD responses to provocations within the striatum and amygdala. An identifiable model was chosen such that covariate effects can be interpreted in terms of effects on ACC 5-$HT_{1B}$R binding, as a reference. Group status was included as a predictor of BOLD responses and trait anger because of the known group differences in these variables (da Cunha-Bang, submitted). Age, IQ and injected mass per kg bodyweight were included as predictors of regional 5-$HT_{1B}$R binding, because 5-$HT_{1B}$R binding is known to decline with age (26), previous studies have shown associations between serotonin and cognitive function (27-29), and to account for variability in [$^{11}$C]AZ10419369 injected mass across participants (0.3 – 6.0 μg) given its theoretical effects on $BP_{ND}$. Age, IQ and group status were also considered as predictors of the latent variable but excluded from the final latent variable model because they were not significantly associated with the latent variable.

Individual additional model paths were considered based on false-discovery rate corrected Wald tests (30). An additional shared correlation between amygdala and striatal reactivity to provocations ($q<0.004$) and between amygdala and striatal 5-

$HT_{1B}R$ ($q<0.02$) were supported by our data. No subsequent model paths were supported ($q>0.16$). All estimates and significance values were determined simultaneously and p-values <0.05 (two-tailed) were considered statistically significant.

## RESULTS

Participant characteristics are provided in table 1. As previously reported in 38 of the participants (da Cunha-Bang, submitted), significant reactivity to provocations was observed in the amygdala (right amygdala: k=63 voxels, [18, -6, -16], z=4.34, left amygdala: k=25 voxels, [-22,0,-18], z=3.43) and striatum (right striatum: k=266 voxels, [10, 8, 2], z=5.42, left striatum: k=258 voxels, [-8, 8, 2], z=5.41).

Regional 5-$HT_{1B}R$ binding (ACC, OFC raphe, amygdala, striatum) loaded significantly onto the latent variable ($p > 10^{-10}$), so did trait anger (7.94, CI: [2.94;12.93], p=0.002), amygdala (1.64, CI: [0.06;3.21], p=0.042) and striatal reactivity to provocations (1.64, CI: [0.67;1.10), p=0.043). (figure 1). Together, these loadings support that a common construct (i.e., the estimated latent variable) significantly predicts variability in 5-$HT_{1B}R$ binding, brain reactivity to provocations and trait anger.

## DISCUSSION

The key finding in this study was that 5-$HT_{1B}R$ binding, trait anger, amygdala and striatal reactivity to provocations were significantly predicted by an underlying latent construct capturing shared correlation between these variables. In other words, high trait anger and heightened amygdala and striatal responsiveness were associated with high 5-$HT_{1B}R$ binding within the ACC, OFC, raphe nuclei, amygdala and striatum. These associations were present across all participants, independent of group status, IQ and age. As previously reported, the violent offenders had heightened amygdala and striatal reactivity to provocations but the

groups did not differ in 5-$HT_{1B}$ binding (da Cunha-Bang, in press). Thus, our data indicate that rather than being a marker for violent offending in itself, 5-$HT_{1B}$R may represent an underlying molecular mediator modulating amygdala and striatal responsiveness to provocations. The current findings provide intriguing novel evidence specifically linking 5-$HT_{1B}$R availability to both amygdala and striatal reactivity to provocative stimuli and to trait anger.

The current findings demonstrate a molecular feature that is related to both brain function in the context of provocations and a personality characteristic that describes a general predisposition to experience angry feelings and a tendency to respond with anger when a given situation is perceived as criticizing or unfair treatment. Whereas we have previously reported that both 5-$HT_{1B}$R binding and amygdala/striatal reactivity to provocations are related to trait anger, we have in the current study integrated these data in a statistical framework allowing for evaluation of shared correlations between these measures. Even though the data does not allow for causal interpretation, a conceptual framework of the observed data with assumed directionalities comprises high trait anger being a result of heightened striatal and amygdala reactivity, which in turn is determined by high 5-$HT_{1B}$R binding. That is, the association between 5-$HT_{1B}$R and trait anger may be mediated by striatal/amygdala reactivity to provocations. Although it would be intriguing to evaluate these directionally specific effects in our current data, our sample size is underpowered to model such effects (31).

The present data emphasize for the first time involvement of 5-$HT_{1B}$R in the processing of emotional and provocative stimuli. Given the extensive preclinical research demonstrating a specific involvement of 5-$HT_{1B}$R in aggression and impulsivity (8, 32), the current data convincingly implicates 5-$HT_{1B}$R also to be specifically involved in human anger and aggression. Existing animal research indicate that low 5-$HT_{1B}$R availability entails aggression; knockdown of 5-$HT_{1B}$ heteroreceptors results in aggressive rodents and intervention with 5-$HT_{1B}$R agonists reduces aggression (7, 8). These preclinical findings do not directly

translate to the current human data demonstrating that *high* and not low 5-$HT_{1B}$R binding is associated with trait anger and heightened reactivity to provocations. However, in animal knockout models possible receptor upregulation or other compensatory mechanisms could be involved in the observed behavioural changes. It has also been speculated that knockdown of only autoreceptors does not change impulsive or aggressive behaviours because these behaviours may arise from a combination of changes in both 5-$HT_{1B}$ auto- and heteroreceptor function (8). However, since 5-$HT_{1B}$ auto- and heteroreceptors cannot be distinguished with [$^{11}$C]AZ10419369, this is not possible to evaluate in humans at present. It should also be noted that in humans, anger and aggression are closely linked but not equivalent. For example, it is unknown if mice express aggression with or without the emotional experience of feeling angry. This emphasizes the value of directly evaluating these systems with in vivo imaging in humans.

The present findings corroborate previous evidence implicating serotonergic neurotransmission, including 5-$HT_{1A}$R binding, serotonin 4 receptor binding and effects of escitalopram and psilocybin in amygdala reactivity to threat-related facial expressions in healthy subjects (14, 15, 33-35). These previous findings and the current data putatively reflect that serotonin levels modulate amygdala reactivity to threat-related stimuli. 5-$HT_{1B}$R binding may theoretically also reflect a marker for serotonin levels within the examined neural circuitry (36). This would be consistent with the longstanding theory that cerebral serotonin levels are inversely linked to aggression (2). However, our results are not directly comparable with these previous studies as they have all employed emotional faces tasks to elicit amygdala activation. Compared to emotional faces, the PSAP targets aggression in a setting where the participants believe that they are playing against another real person, as opposed to implicit processing of threat-related cues. Even though the neural circuitries involved in the processing of faces expressing negative affect and anger/aggression overlap, it remains speculative whether these two aspects of emotional processing are regulated by different components of the serotonin system. A study evaluating for example 5-$HT_{1A}$R or

5-$HT_4$R binding or effects of interventions manipulating serotonin levels in the context of provocations would be informative.

An important strength of this study is the use of multimodal imaging techniques in which in vivo cerebral 5-$HT_{1B}$ receptor binding could be mapped onto behaviourally relevant amygdala and striatal reactivity. However, the following limitations should be considered when interpreting the results. Only males were included in this study because practically no women served time in the prisons we recruited from. Thus, the cohort closely reflected the gender distribution in the prisons and there is evidence for sex-differences when it comes to aggression (37). Seven participants did not undergo PET and fMRI on the same day. We addressed this by separating the sample into two groups (scanned on same day or not), and evaluated whether the associations between 5-$HT_{1B}$R binding in each region (univariate analyses) and amygdala reactivity to provocations were dependent on group. No such interactions were detected (difference in slopes, $p > 0.85$). Finally, it should be mentioned that in addition to the wide range of aggression in our participants, there was also variation in other aspects that may influence 5-$HT_{1B}$R binding, including smoking (38), previous substance abuse (38), childhood trauma (39) and levels of education. We assessed the influence of these factors by adding them as covariates in the statistical models, none of them contributed significantly to the latent variable model. However, with respect to past substance abuse in 13 of the violent offenders, most of them could not remember exact amounts used, and they had been in remission for a considerable amount of time (minimum 6 months up to several years). Therefore, we could not statistically evaluate effects of previous substance abuse.

In conclusion we demonstrate that individuals with high 5-$HT_{1B}$R binding within a neural circuitry relevant for aggression react to provocations with heightened amygdala and striatal responsiveness and have a general disposition to experience angry feelings. The current findings substantially support that serotonin signalling modulates amygdala reactivity and accentuate 5-$HT_{1B}$R to specifically influence

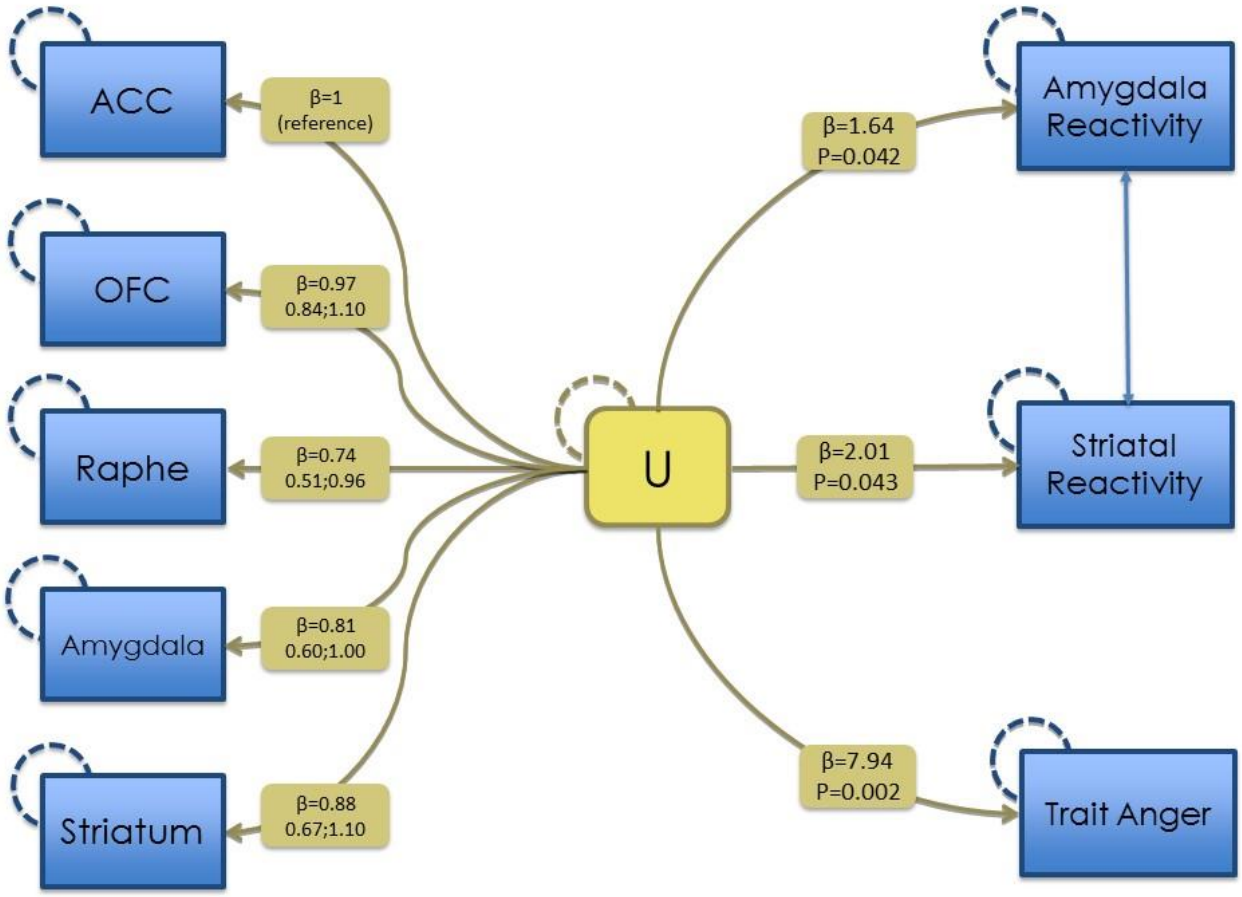
responses to provocations in the context of a social interaction. Given that violent offenders had increased amygdala and striatal reactivity to provocations, reduction of aggressive and violent behaviours might be achieved by interventions that can reduce this heightened neural responsiveness. 5-$HT_{1B}$R may represent a molecular target for reducing excessive amygdala and striatal reactivity to provocative stimuli.

**Table 1.** Participant demographics.

| | Violent Offenders | Control Subjects |
|---|---|---|
| Number of subjects | 18 | 22 |
| Age, years | 31.8 ± 8.8 | 30.21 ± 9.76 |
| Duration of education, years | 8.9 ± 2.6* | 11.55 ± 0.86 |
| IQ, RIST score[a] | 99.1 ± 8.1* | 108.25 ± 6.84 |
| Trait anger[b] | 21.0 ± 6.8* | 14.50 ± 2.44 |
| Trait psychopathy[c] | 311.5 ± 35.8* | 278.61 ± 23.86 |
| PCL-R score[d] | 29.3 ± 4.3 | n/a |
| Cigarettes per day | 9.9 ± 10.9* | 1.1 ± 3.9 |
| Childhood Trauma[e] | 37.7 ± 12.3 | 33.9 ± 8.8 |
| **PSAP behavior** | | |
| Option 1 | 2283.6 ± 378.9 | 2463.8 ± 289.4 |
| Option 2 | 150.0 ± 98.0* | 81.9 ± 99.4 |
| Provocations | 10.6 ± 1.6 | 10.5 ± 2.0 |
| Points earned | 12.1 ± 3.4 | 13.91 ± 3.2 |
| PSAP aggression | 5.7 ± 3.8* | 2.88 ± 3.6 |
| **PET parameters** | | |
| Raphe $BP_{ND}$ | 1.3 ± 0.4 | 1.2 ± 0.4 |
| Orbitofrontal $BP_{ND}$ | 1.5 ± 0.4 | 1.4 ± 0.3 |
| Anterior Cingulate $BP_{ND}$ | 1.8 ± 0.34 | 1.6 ± 0.3 |
| Amygdala $BP_{ND}$ | 1.2 ± 0.37 | 1.1 ± 0.3 |
| Striatum $BP_{ND}$ | 1.4 ± 0.3 | 1.3 ± 0.3 |
| Injected mass / kg bodyweight | 0.02 ± 0.01 | 0.02 ± 0.03 |

* $p<0.01$, group comparisons using two-sample t-test. [a] Reynolds Intellectual Screening Test. [b] State-Trait Anger Expression Inventory. [c] Psychopathic Personality Inventory-Revised. [d] Psychopathy Checklist-Revised, [e] Childhood Trauma Questionnaire. PSAP: point-subtraction aggression paradigm, PET: positron emission tomography, $BP_{ND}$: [$^{11}$C]AZ10419369 binding potential, IQ: intelligence quotient.

**Figure 1.** Latent variable model of 5-$HT_{1B}$ receptor binding on trait anger, amygdala and striatal reactivity to provocations. The yellow oval box represents the latent variable (u). Blue boxes represent measured variables including regional 5-$HT_{1B}$ receptor binding, trait anger, amygdala and striatal reactivity to provocations. Blue line between amygdala and striatal reactivity and between amygdala and raphe denotes additional shared correlation. Hatched blue circles indicate error estimates included in the model. Parameter estimates (β) for each model path are shown in the corresponding box. Group status was included as a predictor for trait anger, striatal and amygdala reactivity, and age, IQ and injected mass/kg were included as predictors for regional 5-$HT_{1B}$ receptor binding, but omitted from the figure for clarity.



## References


1. Hornung JP. The human raphe nuclei and the serotonergic system. Journal of chemical neuroanatomy. 2003;26(4):331-43.
2. Duke AA, Begue L, Bell R, Eisenlohr-Moul T. Revisiting the serotonin-aggression relation in humans: a meta-analysis. Psychological bulletin. 2013;139(5):1148-72.
3. Carrillo M, Ricci LA, Coppersmith GA, Melloni RH, Jr. The effect of increased serotonergic neurotransmission on aggression: a critical meta-analytical review of preclinical studies. Psychopharmacology. 2009;205(3):349-68.
4. Davidson RJ, Putnam KM, Larson CL. Dysfunction in the neural circuitry of emotion regulation--a possible prelude to violence. Science. 2000;289(5479):591-4.
5. Rosell DR, Siever LJ. The neurobiology of aggression and violence. CNS spectrums. 2015;20(3):254-79.
6. Siever LJ. Neurobiology of aggression and violence. The American journal of psychiatry. 2008;165(4):429-42.
7. Bannai M, Fish EW, Faccidomo S, Miczek KA. Anti-aggressive effects of agonists at 5-HT1B receptors in the dorsal raphe nucleus of mice. Psychopharmacology. 2007;193(2):295-304.
8. Nautiyal KM, Tanaka KF, Barr MM, Tritschler L, Le Dantec Y, David DJ, et al. Distinct Circuits Underlie the Effects of 5-HT1B Receptors on Aggression and Impulsivity. Neuron. 2015;86(3):813-26.
9. Skibsted AdC-B, S.; Carré, J.; Hansen, A.H; Beliveau, V.; Knudsen, G.M.; Fisher, P.M.;. Aggression-related brain function assessed with the point subtraction aggression paradigm in functional magnetic resonance imaging European Neuropsychopharmacology 2015; Amsterdam2015.
10. Cherek DR, Moeller FG, Schnapp W, Dougherty DM. Studies of violent and nonviolent male parolees: I. Laboratory and psychometric measurements of aggression. Biological psychiatry. 1997;41(5):514-22.
11. Coccaro EF, McCloskey MS, Fitzgerald DA, Phan KL. Amygdala and orbitofrontal reactivity to social threat in individuals with impulsive aggression. Biological psychiatry. 2007;62(2):168-78.
12. Fisher PM, Hariri AR. Linking variability in brain chemistry and circuit function through multimodal human neuroimaging. Genes, brain, and behavior. 2012;11(6):633-42.
13. Fisher PM, Hariri AR. Identifying serotonergic mechanisms underlying the corticolimbic response to threat in humans. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2013;368(1615):20120192.
14. Selvaraj S, Mouchlianitis E, Faulkner P, Turkheimer F, Cowen PJ, Roiser JP, et al. Presynaptic Serotoninergic Regulation of Emotional Processing: A Multimodal Brain Imaging Study. Biological psychiatry. 2015;78(8):563-71.

15. Fisher PM, Meltzer CC, Ziolko SK, Price JC, Moses-Kolko EL, Berga SL, et al. Capacity for 5-HT1A-mediated autoregulation predicts amygdala reactivity. Nature neuroscience. 2006;9(11):1362-3.
16. Fisher PM, Meltzer CC, Price JC, Coleman RL, Ziolko SK, Becker C, et al. Medial prefrontal cortex 5-HT(2A) density is correlated with amygdala reactivity, response habituation, and functional coupling. Cereb Cortex. 2009;19(11):2499-507.
17. Hare R. Hare Psychopathy Checklist-Revised (PCL-R), 2nd edition. Toronto: Multi-Health Systems Inc.; 2003.
18. Maldjian JA, Laurienti PJ, Burdette JH. Precentral gyrus discrepancy in electronic versions of the Talairach atlas. NeuroImage. 2004;21(1):450-5.
19. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. NeuroImage. 2003;19(3):1233-9.
20. Forman SD, Cohen JD, Fitzgerald M, Eddy WF, Mintun MA, Noll DC. Improved assessment of significant activation in functional magnetic resonance imaging (fMRI): use of a cluster-size threshold. Magnetic resonance in medicine. 1995;33(5):636-47.
21. Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbol S, Frokjaer VG, et al. MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. NeuroImage. 2005;24(4):969-79.
22. Kalbitzer J, Svarer C, Frokjaer VG, Erritzoe D, Baare WF, Madsen J, et al. A probabilistic approach to delineating functional brain regions. Journal of nuclear medicine technology. 2009;37(2):91-5.
23. Varnas K, Nyberg S, Halldin C, Varrone A, Takano A, Karlsson P, et al. Quantitative analysis of [11C]AZ10419369 binding to 5-HT1B receptors in human brain. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2011;31(1):113-23.
24. Varnas K, Hall H, Bonaventure P, Sedvall G. Autoradiographic mapping of 5-HT(1B) and 5-HT(1D) receptors in the post mortem human brain using [(3)H]GR 125743. Brain research. 2001;915(1):47-57.
25. Holst KK, Budtz-Jorgensen E. Linear latent variable models: The lava-package. Comput Stat. 2013;28:1385–452.
26. Nord M, Cselenyi Z, Forsberg A, Rosenqvist G, Tiger M, Lundberg J, et al. Distinct regional age effects on [11C]AZ10419369 binding to 5-HT1B receptors in the human brain. NeuroImage. 2014;103:303-8.
27. Madsen K, Erritzoe D, Mortensen EL, Gade A, Madsen J, Baare W, et al. Cognitive function is related to fronto-striatal serotonin transporter levels--a brain PET study in young healthy subjects. Psychopharmacology. 2011;213(2-3):573-81.
28. Penttila J, Hirvonen J, Tuominen L, Lumme V, Ilonen T, Nagren K, et al. Verbal memory and 5-HT receptors in healthy volunteers - A PET study

with [carbonyl-C]WAY-100635. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology. 2015.
29. Haahr ME, Fisher P, Holst K, Madsen K, Jensen CG, Marner L, et al. The 5-HT4 receptor levels in hippocampus correlates inversely with memory test performance in humans. Human brain mapping. 2013;34(11):3066-74.
30. Fisher PM, Holst KK, Adamsen D, Klein AB, Frokjaer VG, Jensen PS, et al. BDNF Val66met and 5-HTTLPR polymorphisms predict a human in vivo marker for brain serotonin levels. Human brain mapping. 2015;36(1):313-23.
31. MacKinnon DP, Lockwood CM, Hoffman JM, West SG, Sheets V. A comparison of methods to test mediation and other intervening variable effects. Psychological methods. 2002;7(1):83-104.
32. Olivier B, van Oorschot R. 5-HT1B receptors and aggression: a review. European journal of pharmacology. 2005;526(1-3):207-17.
33. Fisher PM, Haahr ME, Jensen CG, Frokjaer VG, Siebner HR, Knudsen GM. Fluctuations in [(1)(1)C]SB207145 PET binding associated with change in threat-related amygdala reactivity in humans. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2015;40(6):1510-8.
34. Windischberger C, Lanzenberger R, Holik A, Spindelegger C, Stein P, Moser U, et al. Area-specific modulation of neural activation comparing escitalopram and citalopram revealed by pharmaco-fMRI: a randomized cross-over study. NeuroImage. 2010;49(2):1161-70.
35. Kraehenmann R, Preller KH, Scheidegger M, Pokorny T, Bosch OG, Seifritz E, et al. Psilocybin-Induced Decrease in Amygdala Reactivity Correlates with Enhanced Positive Mood in Healthy Volunteers. Biological psychiatry. 2015;78(8):572-81.
36. Nord M, Finnema SJ, Halldin C, Farde L. Effect of a single dose of escitalopram on serotonin concentration in the non-human and human primate brain. Int J Neuropsychopharmacol. 2013;16(7):1577-86.
37. da Cunha-Bang S, Mc Mahon B, MacDonald Fisher P, Jensen PS, Svarer C, Moos Knudsen G. High trait aggression in men is associated with low 5-HT levels, as indexed by 5-HT4 receptor binding. Social cognitive and affective neuroscience. 2016.
38. Matuskey D, Bhagwagar Z, Planeta B, Pittman B, Gallezot JD, Chen J, et al. Reductions in brain 5-HT1B receptor availability in primarily cocaine-dependent humans. Biological psychiatry. 2014;76(10):816-22.
39. Murrough JW, Czermak C, Henry S, Nabulsi N, Gallezot JD, Gueorguieva R, et al. The effect of early trauma exposure on serotonin type 1B receptor expression revealed by reduced selective radioligand binding. Archives of general psychiatry. 2011;68(9):892-900.