

Medizinische Hochschule Hannover

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Hochschule Hannover

Neuronale Veränderungen nach Gabe von Botulinumtoxin A bei Patientinnen mit Borderline-Persönlichkeitsstörung

INAUGURALDISSERTATION

zur Erlangung des Grades einer Doktorin
der Humanbiologie
-Doctor rerum biologicarum humanarum-
(Dr. rer. biol. hum.)

vorgelegt von

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aus Hannover

Hannover 2023

Angenommen vom Senat der Medizinischen Hochschule am: 27.02.2024

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für Willi

Abstract

Introduction

Several years after the initial use of botulinum toxin (BTX) to treat physical conditions, the assumption persists that it also influences emotions and is effective in the treatment of depression, a mental disorder with difficulties in emotionality (Schulze et al., 2021). A group of researchers found less negative mood in patients treated with BTX for glabellar wrinkles in comparison to people with other cosmetic interventions (Lewis & Bowler, 2009). Additionally, glabellar BTX injection can attenuate the activity of the amygdala, a structure within the brain believed to be involved with emotion processing in response to emotional stimuli (Hennenlotter et al., 2009; Kim et al., 2014). As BTX induces paralysis at the injection site, the mechanisms behind the potential influence on emotion, specifically depression, do not seem obvious. The most discussed and believed mode of action is the facial feedback hypothesis (FFH; Strack et al., 1988). Patients with mental disorders such as depression show apparent emotionality and the FFH formulates that the subjective feeling of an emotion is influenced by the facial emotional expressions that are sent back to the brain. The paralysis of facial muscles through BTX is thought to interrupt this feedback loop and thus influences emotions (Adelmann & Zajonc, 1989). In addition to depression, borderline personality disorder (BPD) is a disorder with a high excess of negative emotions. As there is no approved medication for the treatment of BPD and a high burden emerging from this disorder, this thesis tries to look at underlying mechanisms of the alternative treatment with BTX.

Objectives

The aims of this thesis are to examine the effectiveness and underlying mechanisms of BTX in BPD. Therefore, this thesis investigates potential neuronal changes in addition to clinical variances after BTX injections in comparison to a control condition in patients with BPD.

Methods

In **Publication 1**, in addition to psychometric tests, 45 women with BPD underwent an emotional go/no-go paradigm during functional magnetic resonance imaging (fMRI) to test for interference with emotion processing and impulsivity. The subjects received either BTX or serial acupuncture (ACU) and were scanned prior to treatment and four weeks after.

In **Publication 2**, 45 women underwent fMRI to collect resting-state functional connectivity data. The women were also allocated to the BTX or ACU condition and were scanned prior to treatment and four weeks after.

Results

Four weeks after treatment, patients from the BTX and ACU groups showed a reduction of borderline symptoms. In **Publication 1**, however, the BTX injection was

specifically linked to improved inhibition and increased activity in the motor cortex. Furthermore, the processing of negative emotional faces was complemented by a reduction in right amygdala activity. In **Publication 2** we found specific effects of BTX in the motor face region and the anterior cingulate cortex.

General discussion

From a clinical standpoint, the fact that there was no difference in improvement under the two conditions warrants further investigations. Choosing ACU as a control condition has led to many more questions arising. However, as this minimal ACU treatment does not seem comparable to traditional ACU, it is unlikely that the ACU itself is responsible for the improvement of symptoms in this group. It is more likely that a combination of general efficacy factors, such as the relationship to the practitioner, the placebo effect and the effect of expectations, led to the clinical improvement in both groups.

Publication 1 can be integrated into a neurobiopsychological model of emotions. Following this model, the starting conditions of emotion generation could be impaired. The motor system is physiologically and neuronally impaired and the missing output of the motor system leads to a reduced emotional body state. This reduced emotional body state is fed back to the limbic system, which could result in reduced feeling. Neuronally, the findings of **Publication 2** seem to be unrelated to emotionality. The main findings pointed towards an influence of areas within the brain that are connected to motor control. Therefore, it could be assumed that BTX changes the general connectivity of motor-related areas.

Conclusion

Although we did find specific neuronal effects of BTX in **Publications 1** and **2**, it could be questioned if the neurotoxin leads to clinical symptom improvement in patients with BPD. From the clinical and neuronal findings, the idea emerges that the neuronal effects of BTX seem rather emotion-related and non-specific for BPD.

Kurzzusammenfassung

Einleitung

Einige Jahrzehnte nach dem erstmaligen Einsatz von Botulinumtoxin (BTX) zur Behandlung körperlicher Erkrankungen hält sich die Annahme, dass BTX ebenso Emotionen beeinflussen kann. So konnte gezeigt werden, dass BTX bei der Behandlung von Depressionen wirksam sein kann (Schulze et al., 2021). Eine Forschergruppe stellte zudem fest, dass Patienten, die BTX zur Behandlung von Glabellafalten injiziert bekamen, im Vergleich zu Personen, die andere kosmetische Eingriffe erhielten, weniger negativ gestimmt waren (Lewis & Bowler, 2009). Darüber hinaus gibt es Befunde, die darauf hindeuten, dass BTX injiziert in die Glabellafalte die Aktivität der Amygdala dämpft, einer Struktur im Gehirn, von der angenommen wird, dass sie an der Verarbeitung von Emotionen als Reaktion auf emotionale Reize beteiligt ist (Hennenlotter et al., 2009; Kim et al., 2014). BTX ruft an der Injektionsstelle eine Lähmung hervor. Die Mechanismen der Wirkweise, insbesondere auf Depressionen, sind dabei bisher nicht eindeutig erklärt. Der am meisten diskutierte und vermutete Wirkmechanismus ist die Facial Feedback-Hypothese (FFH; Strack et al., 1988). Patienten mit psychischen Störungen wie Depressionen zeigen eine in der Mimik sichtbare Emotionalität. Die FFH formuliert, dass das subjektive Gefühl einer Emotion durch den emotionalen Gesichtsausdruck beeinflusst wird, der an das Gehirn zurückgemeldet wird. Es wird angenommen, dass die Lähmung der Gesichtsmuskeln durch BTX diese Rückkopplungsschleife unterbricht und somit die Emotionen beeinflusst (Adelman & Zajonc, 1989). Neben der Depression ist die Borderline-Persönlichkeitsstörung (BPD) eine Störung mit einer starken Ausprägung an negativen Emotionen. Da es keine zugelassenen Medikamente für die Behandlung der BPD gibt und diese Störung eine hohe Belastung für die Betroffenen darstellt, wird in dieser Arbeit versucht, die zugrunde liegenden Mechanismen der alternativen Behandlung der BPD mit BTX zu untersuchen.

Ziele

Ziel dieser Arbeit ist es, die Wirksamkeit und die zugrunde liegenden Mechanismen von BTX bei BPD zu untersuchen. Daher werden in dieser Arbeit neben den klinischen Veränderungen vor allem mögliche neuronale Veränderungen nach BTX-Injektionen im Vergleich zu einer Kontrollbedingung bei Patientinnen mit BPD untersucht.

Methoden

In Publikation 1 wurden 45 Frauen mit BPD untersucht. Die Frauen unterzogen sich zusätzlich zu psychometrischen Tests einem emotionalen Go/No-Go-Paradigma während einer funktionellen Magnetresonanztomographie (fMRT), um Abweichungen der Emotionsverarbeitung und Impulsivität zu untersuchen. Die Probandinnen erhielten entweder BTX oder Akupunktur (ACU) und wurden vor und vier Wochen nach der Behandlung gescannt.

In Publikation 2 unterzogen sich 45 Frauen einem fMRI, um Daten zur funktionellen Konnektivität im Ruhezustand zu sammeln. Die Frauen wurden ebenfalls der BTX- oder ACU-Bedingung zugeordnet und vor und vier Wochen nach der Behandlung gescannt.

Ergebnisse

Vier Wochen nach der Behandlung zeigten die Patientinnen der BTX- und ACU-Gruppen einen Rückgang der Borderline-Symptome. In der Publikation 1 war die BTX-Injektion zusätzlich behavioral mit einer verbesserten Hemmung und einer erhöhten Aktivität im motorischen Kortex verbunden. Außerdem wurde die Verarbeitung negativer emotionaler Gesichter durch eine Verringerung der Aktivität der rechten Amygdala ergänzt. In der Publikation 2 fanden sich spezifische Effekte von BTX in der motorischen Gesichtsregion und im anterioren zingulären Kortex.

Allgemeine Diskussion

Da sich klinisch kein Unterschied in der Verbesserung unter den beiden Bedingungen ergab, ist weitere Forschung indiziert. Da sich die beiden Gruppen klinisch nicht unterschieden, ergab sich die Frage, ob ACU bei BPD ebenso eine Wirkung hat. Allerdings ist dies fraglich, da die minimale ACU-Behandlung in dieser Studie nicht mit der traditionellen ACU-Behandlung vergleichbar ist. Deshalb ist es unwahrscheinlich, dass diese selbst für die Verbesserung der Symptome in dieser Gruppe verantwortlich ist. Es ist wahrscheinlicher, dass eine Kombination allgemeiner Wirksamkeitsfaktoren, wie die Beziehung zum Behandelnden, der Placebo-Effekt und die Wirkung der Erwartungen, zu der klinischen Verbesserung in beiden Gruppen geführt hat.

Die Publikation 1 kann in ein neurobiopsychologisches Modell der Emotionen integriert werden. Diesem Modell folgend, könnten die Ausgangsbedingungen der Emotionsentstehung durch BTX beeinträchtigt sein. Das motorische System ist physiologisch und neuronal beeinträchtigt und der fehlende Output des motorischen Systems führt zu einem reduzierten emotionalen Körperzustand. Dieser reduzierte emotionale Körperzustand wird an das limbische System zurückgemeldet, was zu einem reduzierten Gefühl führen könnte. Neuronal scheinen die Befunde der Publikation 2 eher nicht mit Emotionalität in Verbindung zu stehen. Die Ergebnisse deuten auf eine Beeinflussung von Arealen im Gehirn hin, die mit der motorischen Kontrolle verbunden sind. Es könnte daher angenommen werden, dass BTX die allgemeine Konnektivität motorisch relevanter Areale verändert.

Schlussfolgerung

Obwohl wir in den Publikationen 1 und 2 spezifische neuronale Effekte von BTX gefunden haben, ist es fraglich, ob das Neurotoxin zu einer klinischen Symptomverbesserung bei Patientinnen mit BPD führt. Aus den klinischen und neuronalen Befunden ergibt sich der Gedanke, dass die neuronalen Wirkungen von BTX eher emotionsbezogen und nicht spezifisch für die BPD zu sein scheinen.

1. Introduction

Treatment of the facial glabellar region with botulinum toxin (BTX) was initially developed and approved by local authorities for treating wrinkles. Its use has been prolific since its introduction over 30 years ago and it is now the most applied non-surgical cosmetic intervention globally (Sundaram et al., 2016). In addition to its known paralysis at the injection site, for some years BTX has been thought to influence emotions because the connected facial expressions are initiated through muscle contractions and thereby are influenceable by paralysis. The theory behind this is called the facial feedback hypothesis (FFH; Strack et al., 1988). According to the FFH, the subjective feeling of an emotion is influenced by the proprioceptive feedback, the facial emotional expressions sent back to the brain. Thus, the injection of BTX is thought to interrupt this feedback loop via the paralysis of facial muscles and thus influences emotional states (Adelmann & Zajonc, 1989). Several studies show empirical evidence of the influence of BTX on the perception and experience of emotions (Baumeister et al., 2016; Bulnes et al., 2019; Davis et al., 2010; Havas et al., 2010; Sommer et al., 2003). This led to the idea to test BTX in psychiatric disorders with emotional problems, such as depression (Finzi & Wasserman, 2006).

Therefore as a preparatory work for this thesis, I conducted a meta-analysis to evaluate the effects of BTX on depressive symptoms (Schulze et al., 2021), as various randomized clinical trials (RCTs) pointed in this direction and new research had been published (Brin et al., 2020; Finzi & Rosenthal, 2014; Magid et al., 2014; Wollmer et al., 2012; Zamanian et al., 2017). The results of my analysis showed that treatment with BTX was indeed superior compared to placebo saline injections in the same region ($d = 1.47$ vs. $d = 0.63$). Additionally, a case series showed a reduction of symptomatology in patients with borderline personality disorder (BPD), another disorder with an excess and a bias of negative emotions (Kruger et al., 2016).

In all psychiatric studies, BTX had been injected into the corrugator supercilii and procerus muscles, both of which are key muscles for the expression of negative emotions (Wingenbach, 2023). Interestingly, patients with BPD show an overactivity of these muscles (Matzke et al., 2014). Despite the known involvement of the muscles in emotional experience (Strack et al., 1988), the precise mode of action behind this phenomenon stays underexplored. Thus, besides the effects of BTX on psychiatric

symptomatology, research on the underlying mechanisms is also necessary to provide better understanding and consequently make it possible to use BTX in psychiatric conditions.

The focal point of this thesis is to explore the potential of BTX injections in muscles involved in displaying emotions in patients with BPD, mainly from a neuronal perspective. For this purpose, I will start with an introduction to BTX, followed by the relation between BTX and emotions and a description of previous research regarding BTX and psychiatric disorders.

1.1 Botulinum toxin

In 1897 the microbiologist Emile Pierre Marie van Ermengem was the first to describe the correlation between certain cases of food poisoning and an anaerobic microorganism known today as the bacterium *Clostridium botulinum* (Erbguth, 2008). Over the years it was discovered that *C. botulinum* produces a neurotoxin, which has been named botulinum toxin (BTX), which that inhibits the release of acetylcholine at the neuromuscular junction, as first described by Burgen et al. (1949). In the 1970s Alan Scott was the first person to use BTX for therapeutic purposes. First in monkeys and later in humans, he used the neurotoxin to treat strabismus (Scott, 1980). After extensive research in the following years, the United States Food and Drug Administration (FDA) approved BTX-A in 1989 for the treatment of strabismus, blepharospasm and cranial nerve disorders, and in 2000 for cervical dystonia (Lew, 2002). Following the observations of Carruthers and Carruthers (1992), which described the improvement in glabellar lines in patients treated with BTX for blepharospasm, the FDA approved the neurotoxin in 2002 for glabellar wrinkles.

1.1.1 Structure

Depending on the subtype of the drug, BTX is a composition of a complex mixture of ingredients. BTX can be differentiated by its serotypes, but only types A and B are available for cosmetic and therapeutic use (Truong et al., 2009). The drug BTX consists of the BTX complex and excipients, which are substances such as lactose, sucrose, serum albumin and buffer systems. The BTX component consists of the botulinum

neurotoxin and complexing non-toxic proteins. The botulinum neurotoxin is further formed by heavy and light amino acid chains that are connected via a single disulfide bridge (Truong et al., 2009).

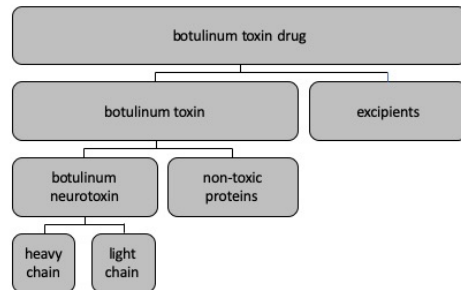


Figure 1: Chemical composition of the botulinum toxin drug.

1.1.2 Mode of action

Muscle contractions are initiated by the release of acetylcholine at the neuromuscular junction. After choline and acetyl coenzyme A react enzymatically and form acetylcholine, the neurotransmitter is stored in synaptic vesicles at the pre-synapses of lower motor neurons (Huang et al., 2000). Normally an influx of calcium in the nerve terminal leads to docking and fusion of the vesicles with the cell membrane. Several proteins (SNAP-25, VAMP) then lead to the release of acetylcholine into the synaptic cleft, where it diffuses through the synaptic cleft to the post-synaptic membrane. There, the neurotransmitter binds onto nicotinic receptors in the post-synapse. This initiates a second messenger cascade that triggers muscle contraction (Huang et al., 2000).

As already described, the botulinum neurotoxin comprises a heavy chain (H-chain) and a light chain (L-chain) connected via a disulfide bond (Sharma & Singh, 1998). When injected near a cholinergic synapse, the H-chain binds onto receptors of the pre-synapse and the toxin is internalized via endocytosis (Huang et al., 2000). Hereafter, the disulfide bond is destroyed by an unknown mechanism and the H-chain translocates the L-chain from the endosome into the cytoplasm. The L-chain then cleaves different kinds of proteins, depending on the type of BTX used. For BTX-A, SNAP-25 is split and acetylcholine cannot be released into the synaptic cleft. The L-chain survives several weeks in the cytoplasm and therefore also cleaves newly built proteins (Huang et al., 2000).

The effect of BTX can be observed, at the earliest, 24–48 hours after injection (Sommer et al., 2006). Usually, however, clinically relevant paralysis is observed no sooner than 7–10 days after treatment. The maximum effect is reached after 4–6 weeks and the remission period is completed 10–12 weeks after injection (Sommer et al., 2006).

1.1.3 The use of BTX in upper facial lines

The discovery that BTX can be used for cosmetic purposes was by coincidence. The ophthalmologist Jean Carruthers treated a patient for blepharospasm using BTX, reporting an improvement of facial lines, especially in the glabellar region. The clinical success of BTX in the glabellar region led to rapid spread of this method, making it possible to approve the compound for upper facial lines. The number of side effects are small in comparison to surgical interventions and long-term adverse reactions have not been reported, as discussed further in the following section (Sommer et al., 2006). The locations for cosmetic use of BTX in the facial area are the glabellar region, forehead, eyebrows, crow's feet, the suborbital region, nose, mouth and chin.

1.1.4 Adverse events

The type and duration of adverse events (AEs) following BTX injection differ according to injection site and dose. Generally, they can be classified as short-term and long-term AEs. Short-term AEs occur roughly 1 week after injection and last for 1–2 weeks. AEs can also be sub-grouped into obligate, local and systemic AEs (Dressler & Bigalke, 2009): obligate AEs are caused by the treatment itself; local AEs arise from diffusion of BTX from the target tissue into nearby tissue; and systemic AEs occur through transport of the substance via the bloodstream to areas distant from the injection site (Dressler & Bigalke, 2009).

Local short-term AEs described in the context of glabellar BTX injections are eye disorders, eyelid ptosis and heavy eyelids (Jia et al., 2016). Also, headaches seem to occur more often than in people with placebo treatment (Jia et al., 2016). Long-term AEs have not been reported since the introduction and start of BTX monitoring in the 1980s (Dressler & Bigalke, 2009).

Nonetheless, indications for the use of BTX should be evaluated carefully before treatment. Pregnancy and pre-existing paralyses can be contraindications for application. If conditions such as amyotrophic lateral sclerosis, myopathies, motor polyneuropathies or myasthenia gravis exist, then the advantages and disadvantages should be discussed with an experienced practitioner (Dressler & Bigalke, 2009). After reports of an interaction of botulism with aminoglycoside antibiotics, simultaneous use is not recommended (Dressler & Bigalke, 2009).

1.1.5 Injection technique and sites

For the injection of BTX there are two possible methods: intramuscular injection directly into the target muscle and subcutaneous or intracutaneous injection. With the latter, the substance diffuses into nearby tissue (Sommer et al., 2006).

1.2 Botulinum toxin and emotions

After the discovery of the potential of BTX for the treatment of glabellar facial lines, the idea soon emerged that it might also have an influence on emotional states. The thought behind this was that subjective experiences of emotions seem to be influenced by the facial expression, as facial muscles are used to signal/display emotions. As treatment with BTX includes the possibility of manipulating the facial expression, multiple research groups investigated its possible effect on emotions. In the following section, I will thus review this pre-existing literature on the impact of BTX on emotions.

1.2.1 Research regarding behavioral and neuronal effects of BTX

In the 2000s, Lewis and Bowler (2009) found less negative mood in patients treated with BTX for glabellar wrinkles in comparison to people with other cosmetic interventions. In the following years more research was conducted and showed that BTX does indeed have an impact on the rating of emotional video clips and the processing of emotional language (Baumeister et al., 2016; Bulnes et al., 2019), in that mildly emotional video clips were judged less positive and emotional sentences were

read slower under BTX treatment when the content needed frowning muscles to express the congruent emotion. Furthermore, with gradually changing emotions in video clips (from neutral to full-blown happy or angry emotions), BTX treatment had an impact on the recognition rates (Bulnes et al., 2019).

To look deeper into the topic, BTX was also used in regions associated with positive facial expressions, such as crow's feet (wrinkles created by laughter). The results found that BTX treatment of laughter lines was associated with increased depression scores and also with reduced emotion recognition rates and sexual function (Lewis, 2018).

Adding to the behavioral results of facial BTX, there have also been neurobiological findings. During the imitation of angry facial expressions, people treated with BTX showed diminished amygdala activation and reduced functional connectivity with the brainstem (Hennenlotter et al., 2009), which returned to its starting point after the effects of BTX receded (Kim et al., 2014).

For better understanding of emotions, in the following section a short overview of emotion theories is displayed. The focus is set on neurophysiological theories or pioneers of neurophysiological theories.

1.2.2 Digression: Emotion theories

The foundation of biological emotion theories was set by Charles Darwin, who published his book "The expression of the emotions in man and animals" in 1872 (Darwin, 1872). Darwin suggested that emotions in humans and animals share common principles and thus that the results from animal experiments can be transferred to humans. Following this, animal experiments were used widely to gain information about the functioning of an emotional brain.

These early findings were the basis for the pathophysiological theory of emotions, which was independently formed by the American psychologist William James and the Danish psychologist Carl Lange. They simultaneously stated that physiological or motor reactions such as trembling, fleeing and crying are not the *consequence* of emotions but their *origin*. Thus, they state that the conscious sensation of physical

emotional states is identical to the subjective feeling of an emotion. The bodily/physical state is sufficient and necessary for the emotional experience (James, 1884).

In the following years, many other psychophysiological theories of emotions emerged from criticism of the James–Lange theory. Cannon, a former student of James, questioned the sufficiency and need of physiological states, based on the observation that disruptions of the connection between brain and periphery did not yield a change in emotional reactions (Cannon, 1927). The subsequent postulated emotion theory of Cannon and Bard is displayed in Fig. 2.

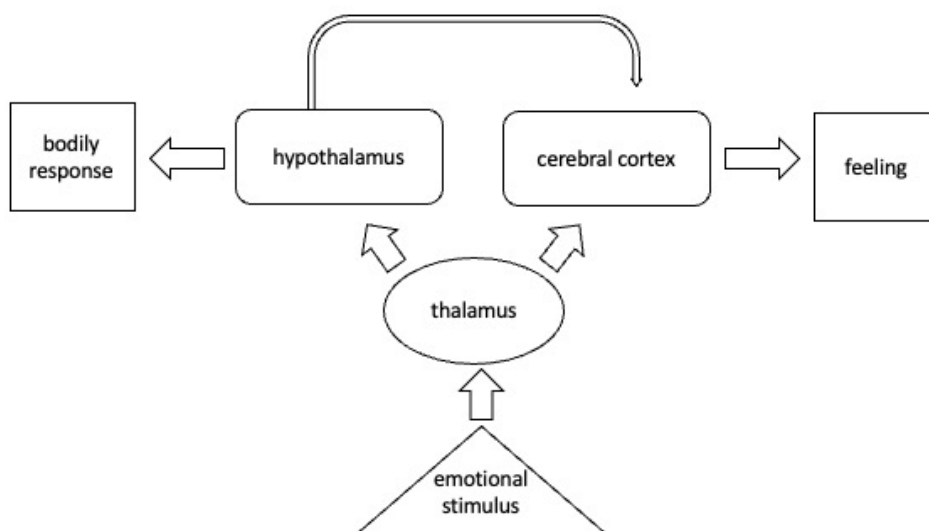


Figure 2: Diagram of the Cannon–Bard theory of emotions (figure modified from LeDoux, 1998, p. 112).

Differing from the James–Lange theory, Cannon and Bard stated that the processing of emotions was exclusively in areas within the brain, with the hypothalamus as a key player (LeDoux, 1998). Its task is to initiate a bodily response and transmit information to the cerebral cortex to produce the emotional experience. Using this approach, the emotional experience and the bodily response occur simultaneously (LeDoux, 1998).

Different approaches followed until the 1940s, whereupon emotional theories faded into the background, mainly due to the rise of behaviorism and its decline in interest in subjective phenomena. This recess lasted until the early 1960s, when Schachter and Singer conceptualized their two-factor theory of emotions (Schachter & Singer, 1962). With their theory, they added a new viewpoint to the James–Lange and Cannon–Bard debate. Agreeing with James and Lange, they proposed that the bodily response is

necessary for the etiology of emotions. And following Cannon and Bard, Schachter and Singer believed that the physiological feedback is rather non-specific. Following the trend of cognitive theories that were omnipresent by this time, they assumed cognitions to be the puzzle key that links the non-specific physiological states to the emotional experiences. Thus, when someone experiences an emotionally ambiguous body reaction (e.g., sweaty palms, muscle tension), they start to interpret these and attribute them to external or internal causes. This results in the experience of a specific emotion. The authors further tested whether the experience of the emotion could be biased by the context in which a person experiences bodily arousal. In fact, they found that mood varied with the context (Schachter & Singer, 1962).

LeDoux (1998) postulated a theory for the processing, expression and conditioning of the basic emotion “fear” (Fig. 3). In this theory, the central neuronal structure involved in fear conditioning and fear expression is the amygdala. Sensory information is projected onto the sensory thalamus and from there, via the low road (also the direct road), information is passed directly from the thalamus to the amygdala. This is also described as the “fast” subcortical pathway. Additionally, the sensory thalamus forwards the information via the high road (also the indirect road) onto primary sensory cortices for an in-depth evaluation. After a “slow” and more detailed processing of the information, a signal is also sent to the amygdala to potentially correct the fast and unconscious processing of the low road.

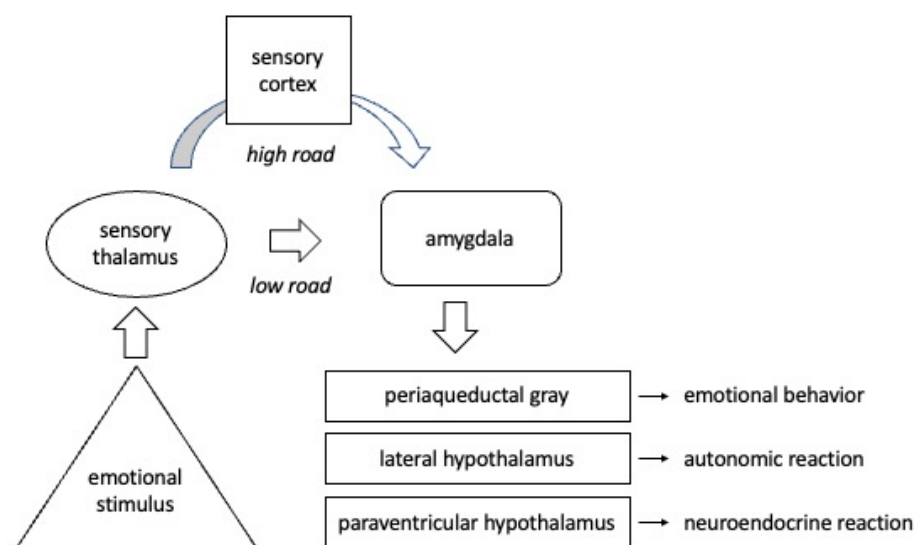


Figure 3: Diagram of the LeDoux theory of fear conditioning (figure modified from LeDoux, 1998, p. 200).

Another neuronal theory of emotions was formulated by the Portuguese neurologist Antonio Damasio. Damasio differentiates between primary, secondary and background emotions. Primary emotions are happiness, sadness, fear, anger, surprise and disgust. These emotions are largely processed in the limbic system circuitry, such as the amygdala, the anterior cingulate cortex, the hypothalamus, the brainstem and the basal forebrain (Damasio, 1995). For primary emotions, Damasio hypothesizes that emotional stimuli activate the amygdala. The amygdala in turn sends a signal towards the hypothalamus, which launches an endocrine and autonomic bodily reaction. Furthermore, the amygdala triggers a chemical reaction via the neurotransmitter system and communicates with the ventral striatum in the basal ganglia, which turns into a motor reaction (Damasio, 1995).

Secondary emotions, also called social emotions, are states such as embarrassment, jealousy, guilt and pride. The processing of these emotions, which are formed by associations between objects and situations and primary emotions and feelings, cannot be sufficiently explained by structures of the limbic system. For secondary emotions, Damasio believes the input is at first also processed by the amygdala but is further forwarded onto cortical networks, including the anterior cingulate cortex, ventromedial prefrontal cortex and somatosensory cortex, where the processing also incorporates previous experience (Damasio, 2018).

After giving a short overview of neurophysiological emotion theories, in the following section the FFH will be introduced, which is discussed as a theory that could explain the mode of action of BTX on emotional states.

1.2.3 The facial feedback hypothesis

The interplay between body and mind is called embodiment (Storch, 2015). With regard to an embodied approach of emotions, there is the question of which is the cause and which is the effect: Am I happy because I smile or do I smile because I am happy? Since the emotion theories of Charles Darwin and William James, a reoccurring approach focusing on embodiment is the FFH, as it was later called. The

FFH was proposed in differing implementations from different research groups throughout the 20th century. All of these approaches had in common that facial expressions do somehow have an impact on emotional experience. Darwin (1872) stated that changing the emotional expression also changes the intensity of the emotion. James (1884), however, proposed that physiological changes such as facial movements are the emotion. In the following years this has been further debated by different researchers (Adelmann & Zajonc, 1989; Cannon, 1927; Izard, 1971; Laird, 1974; Tomkins, 1992).

So far there has been no uniform definition of the FFH. However, most research agrees on the fact that emotional experience and emotional expression influence each other via feedback loops. Facial emotional expression can be presented by the activation of different muscles (McIntosh, 1996). The corrugator muscles and procerus muscle are essential for expression of negative emotions (Schwartz et al., 1976). Expression of emotions (e.g., smiling and frowning) thus create proprioceptive feedback from the face to the brain. This feedback then influences the experience of happiness or anger. Emerging from the FFH, the hypothesis was formulated that the paralysis of certain facial muscles can interrupt the proprioceptive feedback loop from the face to the brain and thereby influence emotional states (Adelmann & Zajonc, 1989).

Over the years, there have been four different questions regarding facial feedback: (1) Are facial expressions associated with positive or negative affect? (2) Do facial expressions modulate emotional experience? (3) Can facial expressions initiate emotions? (4) Are facial expressions necessary for the experience of emotions?

The association of facial actions and emotional experience (1) (Buck et al., 1972; Cacioppo et al., 1986; Ekman et al., 1980), as well as the modulation of effect (2) (Adelmann & Zajonc, 1989; Manstead, 1988), could be affirmed empirically. Regarding the initiation of emotions from mimicking facial expressions (3), there are ambiguous results from the research (Adelmann & Zajonc, 1989; Levenson et al., 1990; McIntosh, 1996). Also, the question of whether facial motor action is necessary for the experience of emotion (4) needs further research because previous studies did not yield a clear result (McIntosh, 1996).

Empirical evidence for and against the FFH has been collected in many experimental studies. Recently, a comprehensive multi-laboratory study showed that happiness can

be amplified and initiated by mimicking facial expressions or voluntarily activating muscles involved in the expression of happiness (Coles et al., 2022). Furthermore, a meta-analysis of preexisting literature confirmed the effects of facial manipulations on emotional experience and emotional judgement (Coles et al., 2019). Treatment with BTX even showed larger effect sizes in comparison to less invasive techniques (e.g., active muscle manipulation). However, the overall effect sizes of the FFH studies were rather small and methodological difficulties with blinding were criticized (Coles et al., 2019).

1.2.4 BTX in psychiatry

As outlined in Section 1.2, the application of facial BTX seems to have an impact on emotional states. Thus, it seems obvious to explore the possible effects of BTX in psychiatric disorders with emotional/affective problems.

In 2006, a case series was the first to describe the potential effect of BTX on patients with depressive symptoms (Finzi & Wasserman, 2006). Depressive disorders are often severe and prolonged, regularly with recurring episodes of relapse (Murray et al., 2012). The World Health Organization (WHO) considers depression to be one of the central reasons for disease-related disability worldwide (Murray et al., 2012). Depressive disorders are characterized by sadness, emptiness and/or irritable mood, depending on the type of depressive disorder (American Psychiatric Association, 2013). The most classic condition among depressive disorders is major depressive disorder (MDD). MDD features distinct episodes that last at least 2 weeks, with changes in affect, cognition and neurovegetative functions (American Psychiatric Association, 2013).

In the above-mentioned case series, nine out of ten treated patients did not meet the criteria for MDD 8 weeks after the injection. Even meta-analyses found a reduction of depressive symptomatology (Qian et al., 2020; Schulze et al., 2021) and these were based on various RCTs (Brin et al., 2020; Finzi & Rosenthal, 2014; Magid et al., 2014; Wollmer et al., 2012; Zamanian et al., 2017).

Further research into treatment options for depression is necessary because estimates suggest that 53% of those with MDD show no response to first-line anti-depressive

medication and 67% do not go into remission (Warden et al., 2007). Furthermore, one-third of patients with depressive disorders are considered to be treatment resistant (Rush et al., 2006). Thus, innovative approaches for the treatment of depression are needed and BTX might be an option in this field.

Borderline personality disorder (BPD). A psychiatric disorder with an excess of negative emotions is BPD. A lot of patients with BPD are treated in inpatient or outpatient settings, which underpins the severe burden of this disease. In fact, patients with BPD make up 10% of all outpatients and 20% of all psychiatric inpatients (American Psychiatric Association, 2013). BPD is characterized by a pervasive pattern of instability in different domains: interpersonal relationships, self-image, affects and impulsivity (American Psychiatric Association, 2013). A core feature of BPD is emotional instability, which is characterized by general impairment of emotions and cognition, specifically by the intensive perception of negative emotions. BPD is often accompanied by depressive symptoms. Negative emotional conditions seem to be triggered at a low threshold, possess high intensity and are distinguished by delayed regression of affectivity (American Psychiatric Association, 2013). Increased vigilance in the form of increased attention and alertness and enhanced sensitivity towards social emotional stimuli (Donegan et al., 2003), particularly towards human facial expressions, can also be observed. This can be underlined by the study of Domes et al. (2008), which showed a tendency of patients with BPD to perceive neutral and ambiguous faces as negative.

Neuroimaging studies in BPD have found abnormalities of brain regions involved in the processing and regulation of negative emotions. Some studies detected an increased activation of the amygdala in response to negative emotional stimuli in BPD compared to healthy controls (Donegan et al., 2003; Hazlett et al., 2012; Koenigsberg, Siever, et al., 2009; Schulze et al., 2011). However, there is also evidence that limbic regions do not show altered activity in BPD (Beblo et al., 2006; Guitart-Masip et al., 2009) or even the contrasting finding of hypoactivation of the amygdala in patients with BPD (Koenigsberg, Fan, et al., 2009; Smoski et al., 2011). In addition to these amygdala findings, the medial and posterior parts of the insula, the anterior cingulate cortex (ACC), the dorsolateral prefrontal cortex and the medial and orbitofrontal regions are found to show abnormal functioning in patients with BPD (Driessen et al., 2009;

Koenigsberg, Siever, et al., 2009; Kraus et al., 2010; Krause-Utz et al., 2012; Niedtfeld et al., 2010; Schulze et al., 2011; Silbersweig et al., 2007; Smoski et al., 2011).

When looking into the functional connectivity research of patients with BPD, there seems to be an increased connectivity between the amygdala, the insula and the orbitofrontal cortex, and an aberrant connectivity between the ACC, the posterior cingulate cortex (PCC), the dorsomedial prefrontal cortex and the medial occipital regions (Krause-Utz et al., 2014).

Concepts such as behavioral therapy and pharmacological treatment have been considered to ease the suffering of patients with BPD. However, so far no authorized drug exists for the disorder itself, only for sub-symptoms. Thus, there is a need for further research into the treatment options to improve the short- and long-term outcome of patients with BPD. This thesis was conducted to test BTX as a novel treatment approach to BPD, with a focus on neuronal changes after BTX application to account for underlying mechanisms.

2. Aims, research questions and hypotheses

The main aim of this research was to look further into the neuronal changes following BTX treatment in patients with BPD.

The neuroscientific approach was chosen to better understand the functioning of BTX in psychiatric disorders and thus provide a solid basis for its potential use in psychiatry.

Publication 1 focused on a task-based fMRI approach that used an emotional go/no-go paradigm to look further into the emotional processing and inhibition performance. The hypotheses in this publication were that glabellar injection of BTX in comparison to minimal acupuncture treatment leads to increased activation in prefrontal areas during inhibition performance and decreases amygdala activity during the processing of emotional stimuli in general.

Publication 2 used a resting-state fMRI to detect potential changes in the functional connectivity of structurally distinct brain regions. For this purpose, we pre-selected seed regions of interest and calculated their functional connectivity throughout the brain. We hypothesized: (1) a changed functional connectivity in regions that are discussed to be affected through BTX treatment, specifically in the primary motor cortex and brainstem nuclei; and (2) a normalization of the functional connectivity of areas that show abnormal activation in BPD and are related to the emotional imbalance of the disorder after BTX treatment, specifically the amygdala, ACC and PCC.

3. Methods (incl. study design)

To assess the above-mentioned questions, an RCT was conducted with additional acquisition of functional imaging data. This clinical trial was carried out at Hannover Medical School and Asklepios Clinic North, Ochsenzoll, in Germany (ClinicalTrials.gov Identifier: NCT02728778) from August 2016 through to February 2019.

Recruitment took place via public advertisements, social media and in the outpatient units of the two study sites. A total of 54 women were included and randomly assigned to either BTX or minimal acupuncture treatment (ACU) as a control condition (BTX: $n = 27$; ACU: $n = 27$).

The 54 women fulfilled the following inclusion criteria: (1) a current diagnosis of BPD according to the 10th edition of the International Classification of Diseases (ICD-10; Dilling et al., 2015) and the Structured Clinical Interview for Axis II DSM-IV disorders (SCID-II; (Fydrich et al., 1997), conducted by trained study personnel; (2) age between 18 and 40 years; and (3) stable treatment for at least 8 weeks prior to and after enrolment (i.e., no qualitative or quantitative changes in medication or psychotherapy). Exclusion criteria were any comorbid psychiatric disorders dominating the clinical impression, apart from psychoactive substance abuse (ICD-10 F1x.1) and mild depressive episodes (ICD-10 F32.0 or F33.0).

ASKLEPIOS proresearch as a third party was responsible for the randomization procedure, which was done using variable block length. Blinding of the participants was difficult due to the obvious and perceivable paralytic effects of the BTX injections, therefore the study design was single blinded. The participants and physicians were aware of the allocated intervention but the psychometric raters (trained psychologists and doctors) remained blinded throughout the study. For unblinding purposes, participants wore a cap during all visits and were instructed to maintain confidentiality about their treatment.

The BTX condition comprised a single BTX treatment of the glabellar region. The injection contained 34 U of incobotulinum toxin A (Bocouture®, Merz, Germany) dissolved in 0.9% NaCl solution (100 U/2.5 ml), which was applied using a 30G needle after baseline assessments at time T0 from a trained physician. The scheme for injection included five intramuscular points in the glabellar region: 8 U applied to the

procerus muscle, 7 U bilaterally to the medial parts and 6 U to the lateral parts of the corrugator supercilii muscle.

The choice of an accurate control condition is rather complicated because, for example, saline injection might lead to unfulfilled expectations affecting the outcome ratings (Magid et al., 2015). Therefore, this study used ACU as an active control treatment. It was rationalized that ACU would avoid disappointment by the participants and thereby decrease dropout rates and prevent nocebo effects.

Patients in the ACU group were treated with acupuncture needles (B-type No. 3, 0.20 × 15 mm, Seirin) applied by a trained physician or a study nurse with special expertise in acupuncture. The treatment took place after baseline assessment (T0) and 2, 4 and 6 weeks later. An acupuncture expert designed the injection scheme, which comprised Yintang, BI2 paired, GB14 and Du20 at the forehead and apex of the head.

As the BTX and ACU treatments had different durations (BTX injection around 5 min; ACU around 30 min), each visit for each patient included 30 min with the clinician before the clinical rating, so that the rater was not able to estimate group allocation based on the treatment time.

Clinical outcomes were assessed at baseline and followed up at 2, 4, 6, 8, 12 and 16 weeks, whereas MRI data were collected only at baseline and 4 weeks after baseline assessment. Therefore, for this thesis the baseline assessment (T0) and the visit 4 weeks after baseline (T1) were used. This resulted in a different endpoint and differing participant numbers (BTX = 24; ACU = 21) because nine women did not agree with the MRI acquisition. An outline of the time points for this thesis is displayed in Fig. 4.

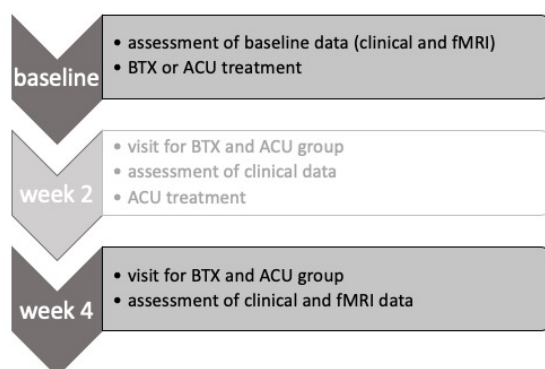


Figure 4: Timeline for this thesis.

The BPD symptomology was assessed on the Zanarini Rating Scale for Borderline Personality Disorder (ZAN-BPD; Zanarini, 2003). For self-evaluation of BPD symptoms, the Borderline Symptom List 23 (BSL-23; Wolf et al., 2009) was used. Secondary outcomes were measured using the Montgomery-Åsberg Depression Rating Scale (MADRS; Montgomery & Asberg, 1979), Clinical Global Impression-Severity Scale (CGI-S; Guy, 1976), Beck Depression Inventory II (BDI-II; Beck et al., 1996), Symptom Checklist 90-R (SCL-90-R; Derogatis, 1992), a modified version of the Credibility/Expectancy Questionnaire (CEQ; Borkovec & Nau, 1972) and a four-point Clinical Severity Score for Glabellar Frown Lines (CSS-GFL; Honeck et al., 2003).

Functional magnetic resonance imaging (fMRI)

The fMRI data used for this thesis were collected after baseline assessment but prior to the first treatment at T0 and 4 weeks after baseline assessment at T1.

The method of MRI was discovered in 1973 by Lauterbur (Lauterbur, 1973). Revolutionizing modern medicine, MRI is used nowadays as a standard instrument in hospitals. The principles of MRI lie in the display of tissue contrasts. Because of its electromagnetic properties, hydrogen nuclei in the tissue can be affected by a magnetic field, the core mechanism behind MRI. This effect is measured and translated into an image, thus displaying structure. In the brain it is therefore possible to display different brain structures. Advancements of MRI in later years led to the development of fMRI. Activity in a certain brain area results in an increased oxygen metabolism, leading to an increase of oxygen supply. Thus, through the activity of certain brain areas, the ratio of high and low oxygenated blood changes. Hemoglobin is responsible for the transport of oxygen and its electromagnetic properties change with the oxygenic state (oxygenated hemoglobin is diamagnetic; deoxygenated hemoglobin is paramagnetic). This change in signal strength can be measured using fMRI (the blood-oxygenation-level-dependent or BOLD signal) and displays brain activity.

There are several different concepts for analyzing fMRI data. In the publications for this thesis, both a task-based and a resting-state fMRI were used. When using task-based fMRI, the activity following a stimulus or task is measured. Thus, task-based fMRI displays the relative BOLD changes from baseline during a response to a task or

stimulus (Lee et al., 2013). Resting-state fMRI looks for correlations between time series of different brain regions and illustrates the functional architecture of the brain by evaluating synchronous activity between structurally distinct regions (Lee et al., 2013).

4. Publications

Because of the cumulative character of this dissertation, subsequently the two publications in peer-reviewed journals are listed:

Tillmann H. C. Kruger*, **Jara Schulze***, Agnès Bechinie, Insa Neumann, Stefanie Jung, Christian Sperling, Jannis Engel, Antje Müller, Jonas Kneer, Kai G. Kahl, Matthias Karst, Julian Herrmann, Larissa Fournier-Kaiser, Liza Peters, Frank Jürgensen, Matthias Nagel, Welf Prager, Birger Dulz, Peter Wohlmuth, Volker Heßelmann, Christopher Sinke & M. Axel Wollmer (2022). Neuronal effects of glabellar botulinum toxin injections using a valenced inhibition task in borderline personality disorder. *Scientific Reports* (12).

Jara Schulze, Christopher Sinke, Insa Neumann, M. Axel Wollmer, Tillmann H.C. Kruger (2023). Effects of glabellar botulinum toxin injections on resting state functional connectivity in borderline personality disorder. *European Archives of Psychiatry and Clinical Neuroscience*

*shared first authorship

In addition, the following meta-analysis was published:

Jara Schulze, Insa Neumann, Michelle Magid, Eric Finzi, Christopher Sinke, M. Axel Wollmer, Tillmann H.C.Krüger (2021). Botulinum toxin for the management of depression: An updated review of the evidence and meta-analysis. *Journal of Psychiatric Research* (135). Pp. 332-340.



OPEN

Neuronal effects of glabellar botulinum toxin injections using a valenced inhibition task in borderline personality disorder

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Previous studies have indicated that glabellar botulinum toxin (BTX) injections may lead to a sustained alleviation of depression. This may be accomplished by the disruption of a facial feedback loop, which potentially mitigates the experience of negative emotions. Accordingly, glabellar BTX injection can attenuate amygdala activity in response to emotional stimuli. A prototypic condition with an excess of negative emotionality and impulsivity accompanied by elevated amygdala reactivity to emotional stimuli is borderline personality disorder (BPD). In order to improve the understanding of how glabellar BTX may affect the processing of emotional stimuli and impulsivity, we conducted a functional magnetic resonance imaging (fMRI) study. Our hypotheses were (1) glabellar BTX leads to increased activation in prefrontal areas during inhibition performance and (2) BTX decreases amygdala activity during the processing of emotional stimuli in general. Using an emotional go-/no-go paradigm during fMRI, the interference of emotion processing and impulsivity in a sample of $n = 45$ women with BPD was assessed. Subjects were randomly assigned to BTX treatment or serial acupuncture (ACU) of the head. After 4 weeks, both treatments led to a reduction in the symptoms of BPD. However, BTX treatment was specifically associated with improved inhibition performance and increased activity in the motor cortex. In addition, the processing of negative emotional faces was accompanied by a reduction in right amygdala activity. This study provides the first evidence that glabellar BTX injections may modify central neurobiological and behavioural aspects of BPD. Since the control treatment produced similar clinical effects, these neurobiological findings may be specific to BTX and not a general correlate of symptomatic improvement.

There is accumulating evidence that botulinum toxin injections (BTX) into the glabellar region may alleviate the symptoms of major depressive disorders^{1–5}. A meta-analysis has demonstrated that botox decreases depressive symptoms ($d = 1.47$) and is more efficient than placebo ($d = 0.63$)⁶, although they may be inflated by methodological issues such as blinding procedures. Earlier studies had already indicated a modulation of emotion processing and experiencing after aesthetically motivated glabellar BTX injections^{7,8}.

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The mechanism of action of glabellar or facial BTX injection on mood states has not yet been elucidated. A favoured explanation of the emotion modulating effects of facial BTX is the facial feedback hypothesis (FFH), which originates from early observations in the nineteenth century by Charles Darwin⁹. According to the FFH, the facial expression of an emotion causes a proprioceptive feedback to the brain, potentially facilitating a change from an initially faint, cool or semi-cognitive sensation to a powerful and sensually warm experience^{10–12}. However, experimental evidence for the impact of facial expressions on subjectively perceived emotions, especially on the original ‘pen in the mouth’ condition is weak^{13,14} and the influence is smaller than initially thought¹⁵. Glabellar injections of BTX paralyse the corrugator supercilii and procerus muscles, which are key muscles in the expression of negative emotions, and thereby interrupt the feedback loop that would maintain and reinforce these emotions⁷. Patients with depression may have a relative overactivity of these “grief” muscles that might be corrected by BTX injections¹⁶. On a neuronal level, there is initial evidence of a reduction of amygdala reactivity in response to negative emotional stimuli after glabellar BTX injections in healthy subjects^{17,18}.

A prototypic disease with an excess of negative emotions and impulsivity is borderline personality disorder (BPD¹⁹). BPD patients show enhanced activity of the corrugator muscles in response to the presentation of faces with negative emotional expressions²⁰. The basic processing of emotions in BPD is usually biased towards the perception of negative emotions²¹ and patients show a decreased detection threshold for negative facial expressions²². Etiologically, the altered emotional processing and problems with emotion regulation may be associated with decreased behavioural inhibition^{23,24}. The deficit in generating behavioural inhibition may manifest clinically as impulsivity, e.g. self-harming or suicidal behaviour and substance abuse²⁵. Altered processing of emotional cues is consistently associated with an over-responsiveness of the amygdala in BPD^{26–28}. Neuronal correlates of impulsivity are less consistent and may include the prefrontal and parietal areas²⁴, with inhibition processes being primarily located in prefrontal areas²⁵.

Preliminary data indicated that glabellar BTX injections may successfully treat the symptoms of BPD, including excessive negative emotionality and impulsivity²⁹. Consequently, a randomised controlled trial (RCT) on the clinical effects of BTX in BPD was initiated (NCT02728778). As an experimental part of the RCT using a previously established emotional go/no-go paradigm²³, we investigated the neurobiological mechanisms of glabellar BTX injections on the processing of emotional stimuli and impulsivity. Using functional magnetic resonance imaging (fMRI), we assessed two separate hypotheses: (1) glabellar BTX can lead to increased activation in prefrontal areas during inhibition performance and (2) the neurotoxin decreases amygdala activity during the processing of emotional stimuli in general, both in comparison to minimal acupuncture treatment.

Results

Clinical data. BPD symptoms significantly decreased over time by 6.11 points (BTX 5.67, ACU 6.62) in both groups as assessed by expert rating using the ZAN-BPD scale ($F(1,42) = 44.71$, $p < 0.001$, $\eta^2 = 0.51$). There were no significant differences between the interventions ($F(1,42) = 0.30$, $p = \text{n.s.}$) and no interaction effect ($F(1,42) = 0.25$, $p = \text{n.s.}$). BSL-23 scores depicted a similar pattern over time with an average decrease of 0.58 points (BTX 0.58, ACU 0.58) ($F(1,41) = 20.24$, $p < 0.001$, $\eta^2 = 0.33$), but no significant differences between the groups ($F(1,41) = 0.86$, $p = \text{n.s.}$) and no interaction effects ($F(1,41) = 0.000$, $p = \text{n.s.}$) (see Table 1). Finally, the H2A also showed a decrease over time of 7.58 points (BTX 9.68, ACU 5.38) ($F(1,41) = 15.17$, $p < 0.001$, $\eta^2 = 0.27$), with neither group differences ($F(1,41) = 0.06$, $p = \text{n.s.}$) nor interaction effects ($F(1,41) = 1.24$, $p = \text{n.s.}$).

Results of inhibitory performance hypothesis. *Behavioural data.* At a behavioural level, there were no differences between the groups regarding their average sensitivity [d'] for angry and happy pictures at baseline (angry: $U = 239.000$, $Z = -0.315$, $p = \text{n.s.}$; happy: $U = 235.500$, $Z = -0.387$, $p = \text{n.s.}$, Mann–Whitney U test). For the BTX condition, sensitivity for angry pictures increased from T0 to T1 ($Z = -2.384$, $p = 0.017$, $\eta_p^2 = 12.16$, Wilcoxon signed-rank test), whereas in the ACU condition this effect did not occur ($Z = -0.334$, $p = \text{n.s.}$). For the happy picture condition, no time effects were seen in either groups.

Further analyses of group differences at T1 using Mann–Whitney U tests revealed higher sensitivity in the BTX group compared to the ACU group, again for the angry picture ($U = 164.500$, $Z = -2.250$, $p = 0.024$, $\eta^2 = 0.088$) but not for the happy picture condition ($U = 231.500$, $Z = -0.503$, $p = \text{n.s.}$; see Fig. 1A).

ROI and whole brain data. To assess the impact of BTX on behavioural inhibition, the no-go > go contrasts as an indicator of change in inhibitory performance over time ($T1[\text{no-go} > \text{go}] > T0[\text{no-go} > \text{go}]$) was evaluated for the inferior frontal gyrus and the pre-SMA as ROIs and additionally on the whole brain level. For the ROI analysis, the angry picture contrast revealed higher activation of the inferior frontal gyrus over time in the BTX group in comparison to the ACU group. No results were found for the pre-SMA. On the whole brain level over time, the precentral gyrus (primary motor cortex) of the BTX group showed higher activation in comparison to the ACU group for the angry picture condition (see Fig. 1B and Table 2). Both analyses were also performed for the happy picture condition, which did not yield any significant results.

Results of emotional processing hypothesis. *ROI and whole brain data.* For the conducted ROI analysis of both amygdalae during the presentation of affective stimuli, a direct comparison between groups and times ($\text{BTX } [T1 > T0] > \text{ACU } [T1 > T0]$) did not yield any significant results, neither for angry nor for happy pictures. Regardless, examining the angry pictures over time for each group separately indicated a decrease in the activation of the right amygdala within the BTX but not the ACU group (see Fig. 2A). Moreover, extracted parameter estimates of the cluster in the right amygdala showed a positive correlation with the change in symptomatology as assessed by ZAN-BPD ($r = 0.51$, $p = 0.015$) and BSL-23 ($r = 0.55$, $p = 0.011$) in the BTX group only (for ZAN-BPD see Fig. 2B, for BSL-23 see Fig. 2C). Thus, a decrease in right amygdala activation was associated

(A)					
Demographic variables	BTX group, n = 24	ACU group, n = 21	p-value		
	M ± SD or n (%)	M ± SD or n (%)			
Age, in years					
	28.75 ± 5.93	26.62 ± 4.61	0.19 ^a		
Highest level of education					
Primary school	0 (0.0)	2 (9.5)			
Lower secondary school	2 (8.3)	2 (9.5)			
Intermediate secondary school	8 (33.3)	10 (47.6)			
Advanced secondary school	14 (58.3)	7 (33.3)	0.22 ^b		
Handedness, n (%)					
Right	20 (83.3)	18 (85.7)			
Left	4 (16.7)	3 (14.3)	1.00 ^b		
Comorbidity, n (%)					
Yes	24 (100.0)	20 (95.2)	0.47 ^b		
Affective disorder	9 (37.5)	6 (28.6)	0.75 ^b		
Anxiety disorder or obsessive compulsive disorder	12 (50.0)	14 (66.7)	0.37 ^b		
Alcohol abuse	7 (29.2)	6 (28.6)	1.00 ^b		
Current psychotropic medication, n (%)					
Yes	20 (83.3)	13 (61.9)	0.18 ^b		
(B)					
Psychometric variables	BTX group, n = 24		ACU group, n = 21		p-value (group / time / group × time)
	T0 M ± SD	T1 M ± SD	T0 M ± SD	T1 M ± SD	
ZAN-BPD	16.04 ± 6.37	10.35 ± 4.75	15.76 ± 5.38	9.14 ± 5.10	0.59 / <0.001 / 0.62 ^c
Affect	6.13 ± 2.32	4.13 ± 2.36	5.90 ± 2.43	3.95 ± 2.60	0.73 / <0.001 / 0.96 ^c
Cognition	4.13 ± 2.07	2.74 ± 1.86	3.67 ± 2.46	2.48 ± 1.84	0.50 / <0.001 / 0.76 ^c
Impulsivity	2.70 ± 1.69	2.00 ± 1.73	2.14 ± 1.11	1.00 ± 1.00	0.04 / <0.001 / 0.33 ^c
Relationship	3.09 ± 2.19	1.48 ± 1.28	4.05 ± 1.50	1.71 ± 1.35	0.15 / <0.001 / 0.20 ^c
BSL-23	2.16 ± 0.66	1.58 ± 0.91	2.36 ± 0.77	1.78 ± 0.89	0.35 / <0.001 / 0.99 ^c
H2A	34.68 ± 14.31	25.00 ± 15.72	33.38 ± 12.88	28 ± 9.19	0.81 / <0.001 / 0.27 ^c

Table 1. Demographic and psychometric characteristics: (A) Demographic characteristics and the test statistics at baseline separate for BTX and ACU (total $N = 45$). (B) The scores of the ZAN-BPD, BSL-23 and H2A at baseline (T0) and at 4-week follow-up (T1) separate for BTX and ACU and their test statistics (total $N = 45$). BTX incobotulinumtoxinA, ACU acupuncture, M mean, SD standard deviation, T0 baseline, T1 4-week follow-up, ZAN-BPD Zanarini Rating Scale for Borderline Personality Disorder, BSL-23 Borderline Symptom List—23, H2A Hamburg-Hannover Agitation Scale. ^astatistical analysis: t -test, ^bstatistical analysis: Fishers exact test, ^cstatistical analysis: repeated measures analysis of variance.

with a decrease in symptomatology after BTX treatment. A correlation analysis of d' change of angry pictures did not show any significant results with the fMRI data ($r = -0.41$ ($p = n.s.$)). For the happy picture condition, ROI analysis of the bilateral amygdalae showed a decrease of left and right amygdala activity over time, again only within the BTX group (for all results, see Table 2). On the whole brain level, no interaction effects could be detected.

Discussion

This study investigated the neuronal effects of a single glabellar BTX injection in patients with BPD compared to an acupuncture control condition. After 4 weeks, both groups showed similar clinical improvement as assessed by standard expert (ZAN-BPD) and patient ratings (BSL-23). This finding is in line with a previous trial that also reported a relatively high placebo response compared to a psychotropic drug in BPD³⁰. In contrast, behavioural and neuronal assessments of emotion processing and inhibition performance of this study revealed an indication for differences between the two treatments.

For our first hypothesis on the effect of BTX on inhibitory performance in BPD, we examined the behavioural data of the go/no-go paradigm and the fMRI data of the group (BTX vs. ACU) × time (T1 vs. T0) × condition (go vs. no-go) comparison on specific ROIs and on the whole brain level.

On the behavioural level, the increase in sensitivity and thus the decrease in wrong answers during no-go trials in the angry condition after BTX may be interpreted as an improvement of inhibition performance, which in turn may be due to lowered impact of negative emotions on inhibition processes. According to previous research, patients with BPD showed reduced sensitivity towards angry and happy faces compared to controls²³

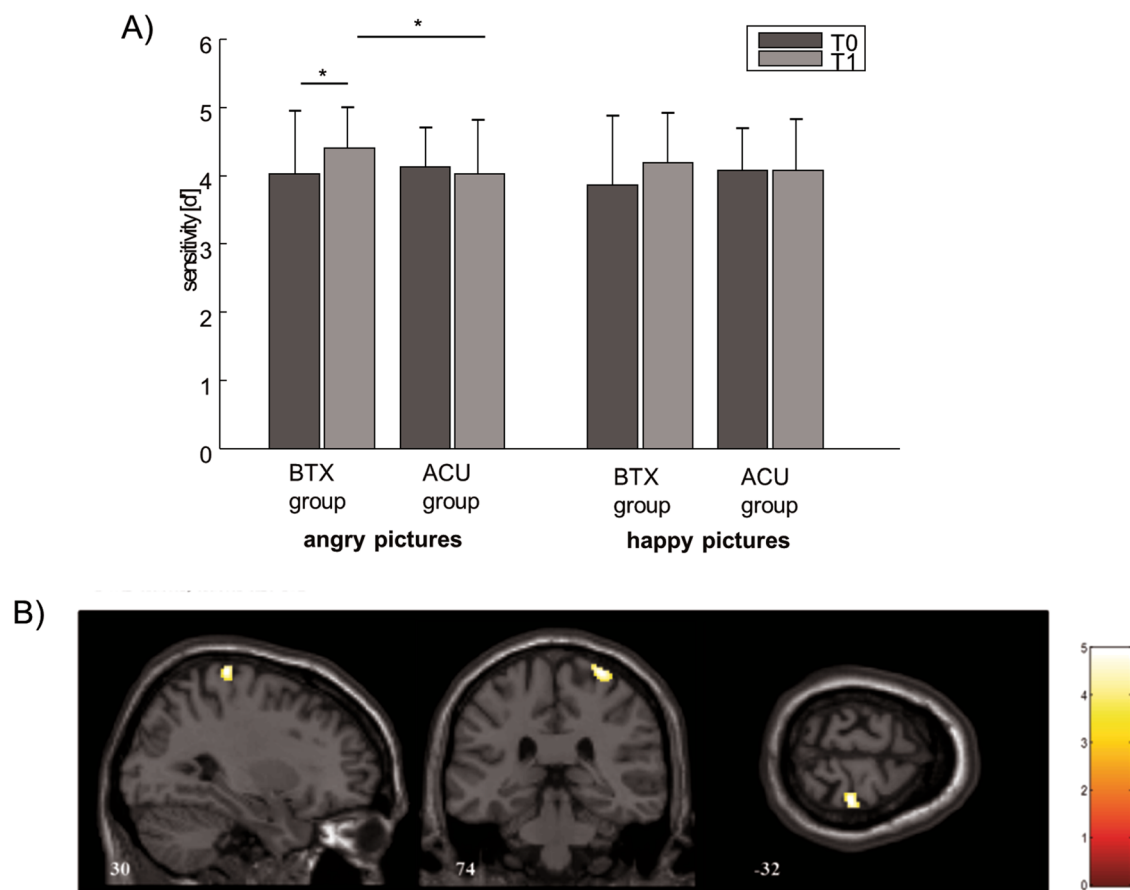


Figure 1. Behavioral effects of BTX and ACU in the emotional go/no-go task and brain activation of the GO/NO-GO $\times$ TIME $\times$ GROUP interaction for angry pictures: **(A)** Shown are the pre- and post-scores of sensitivity [d'], separately for angry and happy pictures. **(B)** BOLD activations (FWE corrected on a cluster level) of the primary motor cortex (no-go > go) over time (T1 > T0) in the BTX group compared to the ACU group. For visualization results are overlaid on a T1-weighted image.

(A)			
Region	BTX > ACU		
	T	CS	Peak coord
Inhibitory-related neuronal activation (angry [T1 nogo > go] > [T0 nogo > go])			
Inferior frontal gyrus, left	5.01*	65	-40 30 -10
Precentral gyrus, right	4.96	172	30 -32 74
(B)			
Region	BTX		
	T	CS	Peak coord
Processing-related neuronal activation (happy T0 > T1)			
Amygdala, right	5.08*	36	26 -4 -20
Amygdala, left	4.69*	46	-26 -8 -18
Processing-related neuronal activation (angry T0 > T1)			
Amygdala, right	3.81*	8	28 -8 -20

Table 2. BOLD activation peaks: shown are the peak activations, cluster size, t-value and corresponding AAL labels for the following contrasts. **(A)** Peaks in the direct contrast BTX > ACU. **(B)** Peaks for the BTX group only. BTX incobotulinumtoxinA, ACU acupuncture, CS cluster size, Peak coordinates of clusters are according to the MNI-space (Montreal Neurological Institute), T t-score of peak voxel, FWE correction with $p < 0.05$ at whole-brain level, T-values marked with * are corrected using small volume correction (SVC) in a-priori regions of interest (see “Methods”) at an FWE corrected level of $p < 0.05$.

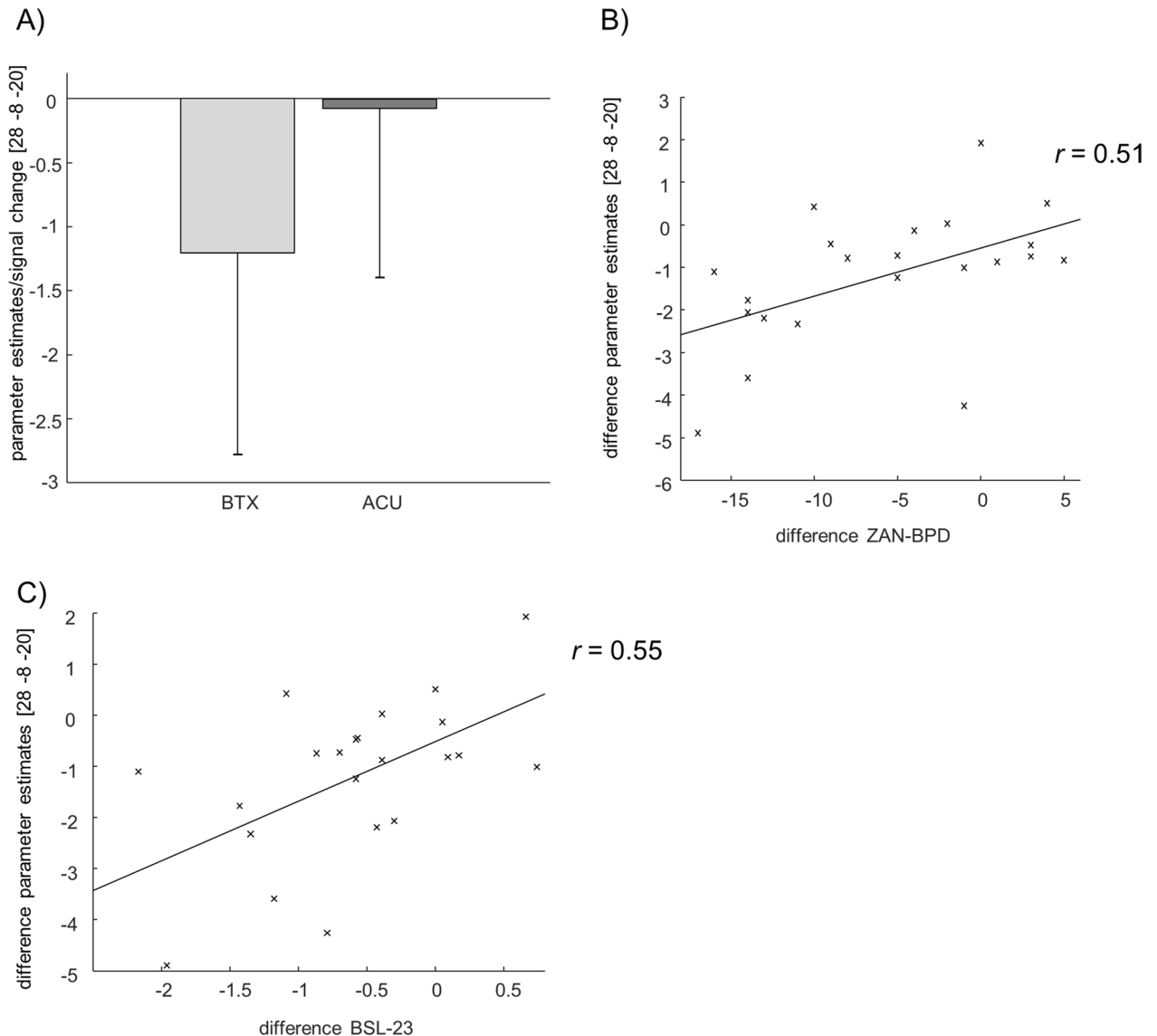


Figure 2. Brain activation of the amygdala at angry pictures: (A) Change of parameter estimates in the right amygdala for the BTX and ACU group. (B) Correlation between the difference of the parameter estimates in the amygdala and the difference of ZAN-BPD for BTX. (C) Correlation between the difference of the parameter estimates in the amygdala and the difference of BSL-23 for BTX.

and the effects were even more pronounced for the happy picture condition. Effects of increased alertness during presentation of affective facial expressions may be a reason for this difference. In the current study, a certain normalisation or improvement of inhibitory performance after BTX treatment occurred. Based on the FFH, we argue that diminished facial expressions and/or reduced perception of the muscle tone eventually lead to blunted emotions elicited by the perception of emotional images, which in turn lead to a reduction of interference with inhibitory performance.

For the ROI analysis of the inferior frontal gyrus and the pre-SMA, the analysis revealed increased activity of the inferior frontal gyrus over time in the BTX group in comparison with the ACU group. These results are in line with previous literature, which suggests the involvement of the left and right inferior frontal gyrus in response inhibition^{31,32}.

With respect to the interaction effects of emotion processing and inhibition performance in BPD^{23,24}, the whole brain analysis detected an increased activity of parts of the right precentral gyrus during the angry picture condition after BTX treatment. The activation occurred in the area of representation of the upper extremities on the primary motor cortex (M1). Activation in this area was unexpected, as we had hypothesised prefrontal activation. Contrary to the usual assumption that the prevention of planned movement engages parts of the prefrontal cortex and the basal ganglia, some research suggests that M1 also shows an intracortex inhibitory network that seems to be important particularly in the final stage of response inhibition³³. Specifically, movement decisions, made in pre-motor areas, can still be prevented in M1. The go/no-go paradigm used in this study was designed to trigger a sustained specific movement pattern, where the movement intention in some trials should be suppressed

on short notice. A lack of intracortex inhibition of M1 and its connection to impaired movement prevention has been described in psychiatric disorders with difficulties in behavioural inhibition such as Tourette's syndrome and attention deficit hyperactivity disorder^{34,35}. Therefore, it can be assumed that the higher activation of M1 after BTX in the angry picture condition could be a sign of enhanced inhibitory control on movement patterns immediately before a respective action potential is generated by motor neurons and is sent to the spinal cord.

For our second hypothesis that BTX might have an impact on emotion processing in BPD we conducted a ROI analysis of the group (BTX vs. ACU) $\times$ time (T1 vs. T0) contrasts, regardless of the condition (go vs. no-go). The ROI analysis showed a reduction of right amygdala activation during the processing of angry faces, that occurred within the BTX group but not the ACU group, although a direct effect of the between group and time comparison was not significant. A reduction of right and left amygdala activity was also seen for the happy picture condition, when comparing the two time points within the BTX group, but not when contrasting time within the ACU group.

A wealth of studies have highlighted the role of the amygdala in processing highly salient emotions such as anger and fear, and a functional connection between amygdala and striatum has been described as important for generating coping behaviour in response to negative emotions (see Ref.³⁶ for review). For BPD and other psychiatric disorders, this interrelationship is assumed to be intensified compared to healthy controls and is seen as a general marker for psychopathology³⁷. Interestingly, some studies showed an aberrant connectivity^{37–39} and higher activation of right amygdala during processing of fearful stimuli⁴⁰ in BPD, thus one could speculate that the reduction in the right amygdala after treatment with BTX as observed here may represent a kind of normalization.

The deactivation of the right and left amygdala during the happy face condition after BTX treatment seems counterintuitive at first glance because we did not address muscles of positive emotional expression such as the orbicularis oculi, risorius or major zygomatic muscles. However, it is possible that small amounts of the incobotulinumtoxinA paralyzed parts of the orbicularis oculi by diffusion and thereby affected the processing of positive emotions. Moreover, facial EMG studies in BPD patients did not show increased activity of corrugator supercilli and procerus muscles in response to positive facial expressions²⁰. However, there are findings of general negative affectivity in patients with BPD²¹, a bias toward interpreting neutral faces as negative²¹, a general intensified activation of the amygdala in response to happy faces^{26,41}, and a general reduction of sensitivity measures in an emotional go/no-go paradigm irrespective of valence²³. Therefore, the current findings may indicate that a reduced expression and/or perception of negative emotions may lead to a normalisation of altered emotion processing, which includes those of positive valence.

Strengths and weaknesses. The study was only conducted in female participants. A generalisation to male patients with BPD is therefore limited and poses an opportunity for future research. Furthermore, the usage of psychotropic medication could not be controlled in the sample, since the participants varied greatly in terms of the prescribed compound and dose. It should be additionally noted that although comorbid disorders dominating the clinical appearance were an exclusion criteria, usually a high number of patients with BPD show symptoms of axis-I disorders⁴². Because of the variety and differing severity of secondary comorbid disorders in this study, we could not control for them, thus the results are limited. As we did not exclude participants with attention deficit hyperactivity disorder (ADHD) nor assessed ADHD with psychometric tools, we could not control for its influence on our effects. During the screening, two women in the ACU and one woman in the BTX indicated a lifetime diagnosis of ADHD and one of them disclosed the use of typical ADHD medication.

For the second hypothesis, we were only able to show a time effect for the BTX group. Thus, it is not clear if the effects show a therapeutic effect of BTX in BPD or if it is a general BTX finding. For further research, a non-patient control group would be meaningful.

Conclusion

This is the first study to investigate a novel approach to the peripheral manipulation of emotional expression in an undertreated disorder (BPD), combining clinical data with neuroimaging. The findings contribute to the highlighted role of the amygdala in emotional processing and its possible connection to impulsivity in BPD and support the idea of embodied emotions and their modulation by glabellar BTX injections. Despite the absence of a clear clinical benefit of BTX on BPD symptomatology compared to ACU in this study, these results may encourage further research into the use of BTX in the treatment of BPD and other mental disorders involving an excess of negative emotionality.

Methods and materials

This functional MRI study is part of a larger randomised clinical trial (RCT) which was conducted from August 2016 through February 2019 at Hannover Medical School and Asklepios Clinic North—Ochsenszoll in Germany (ClinicalTrials.gov Identifier: NCT02728778). Primary and secondary clinical outcomes are described elsewhere (Ref.⁴³). The subjects were randomly allocated to a single BTX treatment of the glabellar region or a control intervention, which consisted of acupuncture treatment (ACU) of the head for about 20 min at week 0 and 2. The control condition diverged from previously used saline injections because prior studies have hypothesised the smaller placebo effects in comparison to BTX might be due to unfulfilled expectations due to unblinding. We implemented a minimal acupuncture treatment of the head as a control condition with respect to good comparability in terms of (a) treatment type (needles) and (b) treatment site (head). Although considered a treatment option for psychiatric disorders, as some authors have reported significant psychotropic effects, the support for acupuncture for psychiatric indications remains insufficient due to the low quality of the data high risk of bias^{44–48}, it was therefore chosen as a control condition in this study.

The MRI data were collected at baseline (T0) and four weeks after baseline (T1) to evaluate the immediate paralytic effects of BTX injections rather than the mid-term impact. Additionally, clinical data was also assessed at one visit between T0 and T1. The study was conducted in accordance with the Declaration of Helsinki and was approved by the leading ethics committee at the Hannover Medical School. After patients were informed about the random allocation to either the BTX or ACU group, they gave written informed consent to participate. They were free to withdraw from the study at any time and received monetary compensation for each MRI visit. To control for the effects of disappointment, all subjects were offered to receive the other treatment after completing the clinical trial.

Participants. Participants were recruited via public advertisements, social media and in outpatient units of the two study sites. In total, 45 women were deemed eligible according to the inclusion and exclusion criteria and gave consent for the MRI assessment ($n = 24$ in BTX group and $n = 21$ in ACU group). The groups did not show any significant differences regarding age, education years, handedness, number of comorbidities or current medication (Table 1). A detailed description of the sample and clinical effects can be found in Wollmer et al.⁴³.

Inclusion criteria were a current diagnosis of BPD according to the International Classification of Diseases (10th ed.; ICD10⁴⁹) and the Structured Clinical Interview for Axis I DSM-IV disorders (SCID-I⁵⁰), conducted by trained study personnel; age between 18 and 40 years and a stable treatment for at least 8 weeks prior to and after enrolment (i.e. no qualitative or quantitative changes in medication or psychotherapy). Women with a manifest comorbid psychiatric disorder dominating the clinical impression except psychoactive substance abuse (ICD-10 F1x.1) and mild depressive episodes (ICD-10 F32.0 or F33.0) were excluded. Especially in case of major depression this strict exclusion was necessary in order to restrain confounding effects of the treatment on the comorbid disorders, as it was previously shown that BTX injection in the glabellar region has a beneficial effect on depressive symptoms^{6,51}.

Clinical interventions, randomisation and blinding. The BTX group received an injection of 34 U incobotulinumtoxinA (Bocouture[®], Merz, Germany), dissolved in 0.9% NaCl solution (100 U/2.5 ml) using a 30G needle by a trained physician after baseline assessments at T0. The injection sites consisted of five intramuscular points in the glabellar region: 8U were applied to the procerus muscle, 7U bilaterally to the medial parts and 6 U the lateral parts of the corrugator supercilii muscle. For the ACU group, acupuncture needles (B-type needle, acupuncture needle, No. 3, 0.20 × 15 mm, Seirin) were placed by either a trained physician or a study nurse with special expertise in acupuncture after baseline assessment (T0) and 2 weeks after. The acupuncture scheme was designed by an acupuncture expert and comprised Yintang, BI2 paired, GB14 and Du20 at the forehead and apex of the head. Essentially, because of only two dates of administration with a long interval in between (2 weeks), short needle retention time (20 min), an intensity at the lower limit for any conceivable specific acupuncture effect can be assumed and thus ACU treatment may be regarded as a “minimal acupuncture” intervention. Acupuncture was chosen as control condition in order to avoid disappointment of the participants, thereby decrease dropout rates, and prevent nocebo effects that may arise from discovering allocation to the placebo group.

Regardless of the allocated group, the participants spent around 30 min with the clinician at each visit before the assessment of the clinical data to avoid debinding the rater. This was necessary as the different treatments had different durations (BTX injection around 5 min acupuncture around 30 min) and otherwise the rater could guess the treatment based on the treatment time. The randomisation procedure was conducted by a third party (ASKLEPIOS proresearch) that was not involved in the practical execution of the study, using variable block length. Blinding of the participants was difficult due to the obvious and perceivable paralytic effects of BTX injections. The study design was single blinded; participants and the treatment administrators were aware of the allocated intervention, while the psychometric raters remained blind throughout the study. To prevent their unblinding, participants wore a cap covering their forehead during all visits and were instructed to keep confidentiality about their treatment.

Psychometric assessments. BPD symptomology was assessed on the Zanarini Rating Scale for Borderline Personality Disorder (ZAN-BPD⁵²). For the self-evaluation of BPD symptoms, the Borderline Symptom List 23 (BSL-23⁵³) was used. In addition, agitation as an important clinical feature of BPD was measured using the Hamburg-Hannover Agitation Scale (H2A⁵⁴).

Experimental procedure, paradigm and stimuli. We were specifically interested in the influence of glabellar BTX injections on emotion processing in general and the interaction between inhibitory performance and emotional processing. Therefore, we used an established valenced go/no-go paradigm²³. Participants were asked to respond as fast as possible to geometric figures (squares or circles) overlaid on faces displaying a happy or angry emotion⁵⁵. Presentation[®] was used for the stimuli display and the recording of behavioural data (Presentation 16.3, Neurobehavioral Systems Inc, Berkeley, CA, USA; www.neurobs.com). Stimulus material was shown on a 32" monitor from NordicNeuroLab (NNL; Bergen, Norway; www.nordicneurolab.com) placed at the foot of the subjects which was visible via a mirror. Behavioural data was collected using response grips from NNL in the right hand of the participants. The pictures span a visual angle of 6.6° × 9°, while the geometrical figures had a visual angle of 0.5° overlaid on the nose region of the faces.

The visual stimuli were extracted from the NimStim Set of Facial Expressions⁵⁵. The facial expressions implied open emotions (mouth open) to facilitate recognition and decrease the scope for interpretation. Per condition (angry/happy), pictures of 15 female and 15 male faces were shown. Each condition was displayed three times, resulting in a total of 90 angry faces and 90 happy faces. The faces were displayed for 2 s, followed by a variable

break between four and eight seconds (6 s on average). Participants were instructed to press a button as fast as possible whenever a black circle appeared (65 trials/condition, 72% of pictures) and suppress a reaction whenever a black square appeared instead (25 trials/condition, 28% of pictures). The experiment started with a training phase where only go cues were presented in 30 trials using faces with closed expressions (i.e. mouth closed). Overall, the experiment took 24 min to complete.

fMRI data acquisition and processing. For MRI data acquisition, a Siemens 3 T Skyra running Syngo VE11 with a standard 64 channel head coil was utilised. Using a gradient simultaneous multislice EPI T2* sensitive sequences, a total of 84 axial slices (resolution $2 \times 2 \times 2$ mm) per volume were acquired in interleaved ascending order. The following parameters were applied: repetition time (TR) = 1.55 s, echo time (TE) = 32 ms, flip angle = 90° , field of view = 256×256 mm, acceleration factor = 4. Using a T1 weighted magnetisation prepared rapid acquisition gradient echo sequence, an individual high resolution anatomical image was acquired for each participant prior to functional scans (resolution $0.9 \times 0.9 \times 0.9$ mm, TR = 2.3 s, TE = 3 ms, flip angle = 9° , 255×270 mm). Using dcm2nii (McCauley Center for Brain Imaging, University of South Carolina), DICOM images were converted to NIFTI format. Functional scans were realigned after removing the first five scans to compensate for T1 saturation effects. Afterwards, the mean echo planar image was co-registered to the individual T1 images. Using SPM12 (Functional Imaging Laboratory, Wellcome Trust Centre for Neuroimaging Institute of Neurology, UCL), structural and functional images were normalised to MNI space with a voxel size of $2 \times 2 \times 2$ mm and smoothed with a $4 \times 4 \times 4$ mm FWHM Gaussian kernel.

Statistical analyses. *Analysis of clinical data.* Clinical data (ZAN-BPD, BSL-23 and H2A) were analysed using SPSS® (Version 26, IBM Inc.). Two-tailed testing and a p -value ≤ 0.05 was chosen for statistical significance. According to Kolmogorov–Smirnov test the data was normally distributed and thus parametric testing was chosen. A 2×2 repeated measures analysis (rmANOVA) was conducted to test for intervention and time differences as well as time by intervention interactions on the outcomes of ZAN-BPD, BSL-23 and H2A. INTERVENTION (BTX/ACU) was entered as the between-subject factor and TIME (T0/T1) as the within subject factor. To determine correlations between functional and clinical data, the Pearson correlation coefficient was chosen and Bonferroni corrected because of multiple comparisons. Consequently, a p -value ≤ 0.017 was considered as statistically significant.

Analysis of behavioural data. Behavioural data was analysed with SPSS® (Version 26, IBM Inc.). Statistical analyses were performed using two-tailed testing. For dependent variables, the correct go responses and the incorrect no-go responses were calculated. To account for possible individual answer strategies and decrease response bias, we chose to report sensitivity (d') instead of commission errors; d' is calculated by subtracting the z -transformed false alarm rate (incorrect no go responses) from the z -transformed hit rate (correct go responses). Values of 0 were transformed into 0.01 and values of 1 into 0.99. As the Kolmogorov–Smirnov test revealed deviations from the normal distribution, non-parametric testing was chosen. Wilcoxon signed-rank tests were performed and Bonferroni corrected because of multiple testing. Thus a p -value ≤ 0.025 was considered as statistically significant.

Analysis of fMRI data. Data analysis was performed using the general linear model (GLM). For the final analysis, three subjects (2 BTX, 1 ACU) had to be excluded due to head movement > 3 mm, resulting in a sample of $n = 42$ for the final analysis. The variables age and H2A were entered into the GLM as covariates. Age is a parameter often included in models, due to its known effect on fMRI analysis⁵⁶. Since research suggests that agitation potentially modulates the relationship between BTX treatment and clinical outcomes⁵⁷ and agitation is a known symptom of BPD, we included the H2A total score as a covariate.

On a subject level, the two time points (T0 and T1) were modelled using four regressors of interest (angry/go, angry/no-go, happy/go and happy/no-go) as well as six regressors of no interest (movement parameters in the scanner) for each time point. Each boxcar stimulus function was convolved with a canonical hemodynamic response function. The data was then high-pass filtered with a cut-off period of 128 s. To set up a random effects analysis, the contrast images of main and interaction effects were used at the group level. Afterwards, a two-sided t -test was computed to identify group differences. On the cluster level, a threshold for all analyses was set to $p \leq 0.05$ family wise error (FWE), corrected for multiple comparisons. Further, an extent threshold of five voxels was applied. The peak voxel of significant clusters was localised using automatic anatomical labelling (AAL⁵⁸).

For our first hypothesis, which tested the effects of BTX on inhibitory performance, a region-of-interest (ROI) analysis and a whole-brain analysis for the happy and angry picture contrasts of time (T1 vs. T0) $\times$ condition (no vs. go) (e.g. (angry no-go T1 $>$ angry go T1) $>$ (angry no-go T0 $>$ angry go T0)) was performed. As the inferior frontal gyrus and the pre-supplementary motor area (pre-SMA) are thought to be involved in the inhibition of responses³², they were defined as ROIs, applying a small volume correction^{59,60}.

Regarding our second hypothesis on the effect of BTX on emotional processing, we conducted a ROI analysis on the group (BTX vs. ACU) $\times$ time (T1 vs. T0) contrasts for the emotional pictures regardless of the condition (e.g. angry go + angry no-go for T1 $>$ angry go + angry no-go for T0). Based on the a priori hypothesis, the amygdala was defined as an ROI. This structure was chosen due to evidence of increased activation in patients with BPD²⁴ and the reported inhibitory effect of glabellar botulinum toxin injections on amygdala function^{17,18}. The anatomical definition was used from the AAL atlas⁵⁸. Parameter estimates were extracted using the eigenvariate function in SPM.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Received: 11 October 2021; Accepted: 26 July 2022

Published online: 20 August 2022

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Acknowledgements

We thank Silke Malecha who played a pivotal role in the study's execution and sadly passed away- we dedicate this paper to her.

Author contributions

W.A. and T.H.C.K. were the principal investigators (PI) in Hamburg and Hannover, respectively. M.N. acted as deputy PI to A.M.W., T.H.C.K., B.D., and S.J. designed the study and wrote the study protocol. A.M.W., I.N., L.F.-K., A.M., L.P., A.B., C.S. and T.H.C.K. collected the data. A.M.W. analyzed the clinical data of the RCT and helped with its interpretation. J.S. and C.S. analyzed the fMRI data. J.S. wrote the initial draft of the manuscript in constant consultation with T.H.C.K. A.M.W., M.N., A.B., J.S., C.S., and T.H.C.K. provided interpretation of the results. A.M.W., T.H.C.K., W.P., A.M., F.J. acted as study physicians. I.N., T.H.C.K., and A.M.W. contributed to the discussion throughout the writing of the manuscript and helped with the proof-reading. All authors have contributed to, read, and approved the final version of the manuscript.

Funding

Open Access funding enabled and organized by Projekt DEAL. This research was funded by grants of the German Research Foundation (KR 3706/3-1, TK & MAW) and the Asklepios Kliniken Hamburg GmbH Forschungsförderung (MAW). The Hannover Medical School represented by Hannover Clinical Trial Center GmbH (HCTC) acted as the study sponsor. Neither the funding sources nor the sponsor had any role in the study design, the collection, analysis and interpretation of the data, in the writing of the publication, and in the decision to submit the paper for publication.

Competing interests

Kruger has received honoraria for talks and/or advisory board activities from Allergan, Lilly, Lundbeck, Otsuka, Schwabe, Servier, and Trommsdorf. Authors Kruger and Wollmer were members of an advisory board of Allergan. All other authors have no conflict of interest to declare.

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Effects of glabellar botulinum toxin injections on resting-state functional connectivity in borderline personality disorder

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Received: 11 October 2022 / Accepted: 23 January 2023
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Abstract

Meta-analyses suggest a sustained alleviation of depressive symptoms through glabellar botulinum toxin (BTX) injections. This can be explained by the disruption of facial feedback loops, which may moderate and reinforce the experience of negative emotions. Borderline personality disorder (BPD) is characterized by excessive negative emotions. Here, a seed-based resting-state functional connectivity (rsFC) analysis following BTX ($N=24$) or acupuncture (ACU, $N=21$) treatment in BPD is presented on areas related to the motor system and emotion processing. RsFC in BPD using a seed-based approach was analyzed. MRI data were measured before and 4 weeks after treatment. Based on previous research, the rsFC focus was on limbic and motor areas as well as the salience and default mode network. Clinically, after 4 weeks both groups showed a reduction of borderline symptoms. However, the anterior cingulate cortex (ACC) and the face area in the primary motor cortex (M1) displayed aberrant rsFC after BTX compared to ACU treatment. The M1 showed higher rsFC to the ACC after BTX treatment compared to ACU treatment. In addition, the ACC displayed an increased connectivity to the M1 as well as a decrease to the right cerebellum. This study shows first evidence for BTX-specific effects in the motor face region and the ACC. The observed effects of BTX on rsFC to areas are related to motor behavior. Since symptom improvement did not differ between the two groups, a BTX-specific effect seems plausible rather than a general therapeutic effect.

Keywords Neuroimaging · Resting state · Botulinum toxin · Borderline personality disorder

Introduction

Since its introduction in the 1990s, the use of botulinum toxin (BTX) in aesthetic medicine has risen steadily. Being the most popular nonsurgical cosmetic intervention worldwide [1], the neurotoxin is approved for the use in the

glabellar region to treat wrinkles [2]. These wrinkles are shaped by the tensioning of the corrugator supercilii muscles and the procerus muscle.

The paralytic effect of BTX can be explained by the inhibition of release of acetylcholine (ACh), which triggers muscle contractions in the peripheral nervous system. When the intramuscular-injected BTX enters the adjoining nerve terminal, the protein SNAP-25 is cleaved, and as a consequence, the vesicles of the synapse are no longer able to release ACh into the synaptic cleft [3]. But the effects of lacking muscle contractions seem not only limited to the muscle itself. Studies focusing on facial paralysis as an output of certain medical conditions show, for example, a change of motor cortex functioning [4, 5]. Likewise the effect of BTX is thought to go beyond the paralytic effect at the injection side and to influence the central nervous system (CNS) via an indirect pathway [6]. The assumption behind this is that parts of the CNS are modulated and reorganized by the denervation and changes of the afferent input.

The neurotoxin, when injected into the face, is described as influencing the expression and further the perception of

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emotions [7]. Healthy individuals who are injected with BTX show a blunted perception of emotional stimuli, specified by lower recognition rates of facial expressions [8, 9]. The paralysis of the corrugator supercilii muscles and the procerus muscle is also associated with a reduction in negative emotions [10].

This effect is accompanied by a reduction in the amygdala activity when subjects mimic negative emotions after having received BTX injections into the glabellar region [11, 12]. A psychophysiological analysis also revealed a coupling between the reduced amygdala activity and the dorsolateral pons [11].

Nevertheless, the mode of action of glabellar BTX injections on mood states has yet to be enlightened. The most discussed theory assumes an indirect pathway to structures of the CNS: the facial feedback hypothesis (FFH) states that a proprioceptive feedback to the brain is caused by the facial expression of an emotion. This embodiment results in a change of an initially faint cool or semicognitive perception into a powerful and warm experience [13–15]. The paralysis of the corrugator supercilii and procerus muscles by glabellar BTX injections lead to an interruption in a feedback loop that would maintain and reinforce the negative emotions [10]. The corrugator supercilii is innervated by the temporal [16] and the procerus muscles by the temporal, lower zygomatic and buccal branches of the cranial nerve VII (facial nerve) [17].

Clinically, cumulating data suggests that BTX injections into the glabellar region might ease depressive symptoms [18–22], which was speculated to be connected to the overactivity of the glabellar muscles in patients showing those symptoms. Another disorder with an unusual extent of negative emotions and an enhanced activity of the corrugator muscles [23] is borderline personality disorder (BPD). Originating from this finding, a case series was conducted on the effects of BTX in BPD, which showed promising clinical outcomes [24].

BPD is a serious mental disorder, which is present in around 1% of the general population [25, 26]. BPD is characterized by a pervasive pattern of affective instability, uncertainty in interpersonal relationships and self-image, as well as heightened impulsivity (DSM-IV-TR) [27]. Specifically, the intensive basic processing of negative emotions is common in BPD [28]. Usually there is a heightened sensitivity for negative emotions [29], and thus patients may indicate a decreased detection threshold for negative facial expressions [30]. Etiologically, the perceived enhanced emotion perception and the difficulties with the regulation of those emotions are discussed in the context of behavioral inhibition [31, 32]. Because of the high burden of disease originating from BPD [33, 34] and the deficits in pharmacological treatment approaches, it seems legitimized to investigate further the potential of BTX for the treatment of BPD.

In this study, we focused on resting-state functional connectivity (rsFC) as an outcome measure. RsFC is a task-free version of functional magnetic resonance imaging (fMRI). A fMRI at rest shows the synchronous activation of spatially distinct regions in the absence of a task or stimulus to determine networks within the brain [35]. Functional connectivity is not directly influenced by context; it illustrates the functional architecture of the brain [36] and is, therefore, useful for identifying underlying basic neuronal functioning. To our knowledge, there have been no previous investigations on the influence of BTX on rsFC in patients with BPD; thus, we shall subsequently present the state of the current research regarding rsFC in BPD and the effects of BTX treatment, separately.

Starting with the literature related to BTX, its effect on neuronal function and structure is frequently discussed as influencing motoric and somatosensory areas within the brain [4, 5, 37, 38]. More precisely, BTX is thought to induce a functional reorganization of the motor cortex similar to irreversible nerve damage [38]. Regarding rsFC, among others, BTX induces altered connectivity within motoric-related brain networks. Specifically, in patients with cervical dystonia, the rsFC increased between the primary motor cortex, the primary visual network, and the secondary visual cortex after BTX injections [39].

Concerning BPD, there is also evidence for an altered motor cortex functioning [40]. Further, the amygdala has been predominant in BPD research. The rsFC in patients with BPD compared to healthy controls seems to be increased between the amygdala and the insula, the orbitofrontal cortex (OFC) and the putamen [41]. The connection is thought to be the reflection of the clinically seen affective hyperarousal and the intense emotional reactions of patients with BPD. The coupling of the amygdala and the putamen, however, is also thought to be important for motor control.

Further an aberrant functioning of the anterior cingulate cortex (ACC) in BPD has been discussed in the context of top-down emotion regulation and impulse control [32]. Here, the ACC shows aberrant rsFC to the posterior cingulate cortex (PCC), dorsomedial prefrontal cortex, and the medial occipital regions. Especially the link between the ACC and the PCC, as part of the default mode network (DMN), is discussed in the context of deficits in switching between brain networks [41]. Abnormal rsFC is also found between the ACC and the frontoparietal executive control network (ECN) [42] and within the salience network, with the ACC as a main hub [43]. In addition, patients with BPD show modified neural activity in the regions of the DMN [44–46]. The altered functioning might also reflect a deficit in switching between baseline and task-related processing [44].

Originating from the previous findings, we hypothesize two things: (1) We hypothesize an aberrant rsFC in regions

that are said to be affected by BTX treatment, specifically primary motor cortex (M1) and brainstem nuclei; and (2) following the FFH, we suggest a normalization of the functional connectivity of areas that show abnormal activation in BPD and are related to the emotional imbalance of the disorder after the BTX treatment, specifically the amygdala, ACC, and PCC.

Methods and materials

As part of a larger randomized clinical trial (RCT), this fMRI study was conducted from August 2016 through February 2019 at Hannover Medical School and Asklepios Clinic North—Ochsenzoll in Germany (ClinicalTrials.gov Identifier: NCT02728778). Primary and secondary clinical outcomes are described elsewhere [47]. The experimental study protocol comprised MRI data acquisition at baseline (T0) and 4 weeks after baseline assessment (T1) as well as clinical assessments to evaluate the immediate paralytic effects of BTX. The allocation of the participants was done randomly, and the intervention was either a single BTX treatment of the glabellar region or, in the control condition, a minimal acupuncture treatment (ACU) for about 20 min at 0 and 2 weeks. We did not refer to the previously used saline injections as a control intervention, as used in previous studies. This was chosen because prior research had hypothesized that the smaller effects of the placebo in comparison to BTX might be due to unblinding. Instead, as a control condition, acupuncture treatment of the head was implemented due to good comparability of this approach in terms of (a) type of treatment (needles) and (b) site of treatment (head).

Although acupuncture has been examined as a possible treatment option for psychiatric disorders, the empirical evidence of it remains poor because of low quality and high risk of bias of the data [48–52]. In addition, acupuncture was chosen as a control condition in a low dosage for a better treatment acceptance by the control subjects compared to saline injection.

The study was approved by the leading ethics committee at the Hannover Medical School and was conducted in accordance with the Declaration of Helsinki. Participants received monetary compensation for each MRI visit, had given written informed consent, and were free to withdraw from the study at any time. All subjects were offered the opportunity to receive the other treatment after the completion of the clinical trial.

Participants

Recruitment was realized via social media, public advertisements, and in outpatient units of the two study sites.

According to the inclusion and exclusion criteria, 45 women were considered eligible and gave consent for the MRI assessment after screening (BTX = 24, ACU = 21). Important inclusion criteria were an age between 18 and 40 years, a present diagnosis of BPD according to the ICD10 [53] and the Structured Clinical Interview for Axis II DSM-IV disorders (SCID-II [54]), evaluated by trained study personnel, and stable psychological or pharmacological treatment for at least 8 weeks before entering the trial. No significant group differences were found regarding age, years of education, handedness, number of comorbidities, or current medication (Table 1).

Randomization, clinical interventions, and blinding

A third party (ASKLEPIOS proresearch), which was not involved in the practical implementation of the study, conducted the randomization procedure, using a variable block length. Due to the perceivable paralytic effects of BTX injections, blinding of the participants was not possible.

Participants who were assigned to BTX treatment received a total of 34 U incobotulinumtoxinA (Bocouture®, Merz), dissolved in 0.9% NaCl solution (100U/2.5 ml). The solution was injected by a trained physician after clinical and MRI baseline assessments at T0, using a 30G needle. Five intramuscular points in the glabellar region were used as injection sites: 8U were applied to the procerus muscle, 7U bilaterally to the medial parts, and 6U to the lateral parts of the corrugator supercilii muscle. For the ACU group after baseline assessment (T0) and at 2 weeks, either a trained physician or a study nurse with special expertise in acupuncture conducted the intervention using acupuncture needles (B-type needle, acupuncture needle, No. 3, 0.20 × 15 mm, Seirin). The applied scheme was designed by an acupuncture expert and included Yintang, BI2 paired, GB14, and Du20 at the forehead and apex of the head.

Due to the methodological problems of blinding, the study was designed as an open trial for participants and the staff who applied the treatment. The psychometric expert rating, however, was conducted by personnel who stayed blinded throughout the entire study. Participants were instructed to wear a hat covering their forehead during the rating process and instructed to not talk about their treatment to prevent unblinding of the staff.

Psychometric assessments and statistical analysis of clinical data

Trained raters assessed the BPD symptomology with the Zanarini Rating Scale for Borderline Personality Disorder (ZAN-BPD [55]), and the Borderline Symptom List 23 was used for self-assessment of BPD symptoms (BSL-23 [56]). Data from the ZAN-BPD and the BSL-23 were analyzed using

Table 1 Demographic and psychometric characteristics: (A) demographic characteristics and the test statistics at baseline separately for BTX and ACU (total $N=45$). (B) The scores of the ZAN-BPD andBSL-23 at baseline ($T0$) and at 4-week follow-up ($T1$) separately for BTX and ACU and their test statistics (total $N=45$)

(A)					
Demographic variables	BTX group, <i>N</i> =24		ACU group, <i>N</i> =21		<i>p</i> value
	<i>M</i> ± <i>SD</i> or <i>n</i> (%)		<i>M</i> ± <i>SD</i> or <i>n</i> (%)		
Age, in years	28.75±5.93		26.62±4.61		0.19 ^a
Highest level of education					
Primary school	0 (0.0)		2 (9.5)		
Lower secondary school	2 (8.3)		2 (9.5)		
Intermediate secondary school	8 (33.3)		10 (47.6)		
Advanced secondary school	14 (58.3)		7 (33.3)		0.22 ^b
Handedness, <i>n</i> (%)					
Right	20 (83.3)		18 (85.7)		
Left	4 (16.7)		3 (14.3)		1.00 ^b
Comorbidity, <i>n</i> (%)					
Yes	24 (100.0)		20 (95.2)		0.47 ^b
Affective disorder	9 (37.5)		6 (28.6)		0.75 ^b
Anxiety disorder or obsessive compulsive disorder	12 (50.0)		14 (66.7)		0.37 ^b
Alcohol abuse	7 (29.2)		6 (28.6)		1.00 ^b
Current psychotropic medication, <i>n</i> (%)					
Yes	20 (83.3)		13 (61.9)		0.18 ^b
(B)					
Psychometric variables	BTX group, <i>N</i> =24		ACU group, <i>N</i> =21		<i>p</i> value (group/ time/group x time)
	T0 <i>M</i> ± <i>SD</i>	T1 <i>M</i> ± <i>SD</i>	T0 <i>M</i> ± <i>SD</i>	T1 <i>M</i> ± <i>SD</i>	
ZAN-BPD	16.04±6.37	10.35±4.75	15.76±5.38	9.14±5.10	0.59/0.00/0.62 ^c
BSL-23	2.16±0.66	1.58±0.91	2.36±0.77	1.78±0.89	0.35/0.00/0.99 ^c

BTX incobotulinumtoxin A, ACU acupuncture, M mean, SD standard deviation, $T0$ baseline, $T1$ 4-week follow-up, ZAN-BPD Zanarini Rating Scale for Borderline Personality Disorder, BSL-23 Borderline Symptom List-23

^aStatistical analysis: t test

^bStatistical analysis: Fishers exact test

^cStatistical analysis: repeated measures analysis of variance

SPSS® (Version 26, IBM Inc.). Because of the normally distributed data, parametric testing was chosen and two-tailed testing with a p value ≤ 0.05 was applied for statistical significance. To test for intervention and time differences as well as time by intervention interactions on the clinical outcomes, a 2×2 repeated measures analysis (rmANOVA) with the between-subject factor GROUP (BTX/ACU) and the between-subject factor TIME ($T0/T1$) was realized.

Neuroimaging

Data acquisition

All rsFC data were acquired using a Siemens 3 T Skyra running Syngo VE11 with a standard 64-channel head coil. The acquisition of the data started with the recording of a structural T1 image, followed by a resting-state fMRI scan with a runtime of 620 s. The participants were

instructed to lay still and focus on a white cross displayed on a black screen, which was visible via a mirror on the head coil.

A total of 400 volumes with 38 axial slices (resolution $2 \times 2 \times 2$ mm) per volume, using a gradient simultaneous multislice EPI T2* sensitive sequence, were acquired in interleaved ascending order (repetition time [TR] = 1.55 s, echo time [TE] = 32 ms, flip angle = 90° , field of view [FOV] = 256×256 mm, acceleration factor [af] = 4, distance factor = 0). An individual high-resolution anatomical image for each participant was acquired using a T1 weighted MPRAGE sequence prior to functional scans (resolution $0.9 \times 0.9 \times 0.9$ mm, TR = 2.3 s, TE = 3 ms, flip angle = 9° , FOV = 255×270 mm).

Data processing and analysis

Data analysis was performed using SPM12 (Wellcome Trust Center for Neuroimaging, London, England) and DPABI 2.3 [57, 58]. Preprocessing was conducted in accordance with the standard protocol described in the literature [57, 58]. The first five scans were removed to compensate for saturation effects. Further slice time correction and motion realignment to a mean template were realized. Using unified segmentation on the T1 image, acquired pictures were normalized to the Montreal Neurological Institute (MNI) space and were resized to $3 \times 3 \times 3$ mm³. Nuisance covariates, like voxel-specific 12-motion parameters, white matter signal, cerebrospinal fluid signal, and global signal, were regressed out. Images were then band-pass filtered (0.01–0.1 Hz). Afterward, a motion-scrubbing procedure was applied by removing scans with a frame-wise displacement (FWD) threshold of 0.4 mm. Finally, all images were smoothed using a $4 \times 4 \times 4$ mm FWHM Gaussian kernel.

Definition of seed regions

For the assessment of between- and within-group effects of BTX on BPD-related brain networks and areas related to the mode of action of BTX, the following seed regions were selected:

The bilateral precentral cortex was selected because of its reactivity to BTX in prior neuroimaging studies [4, 5, 11, 12] and due to the fact that the immobilization of muscles leads to neuroplastic changes in the motor cortex [59, 60]. The MNI coordinates of the precentral gyrus were on the left side $x = -45$, $y = -13$, and $z = 56$, and on the right side $x = 52$, $y = -10$, and $z = 50$. These were selected because they were described as coordinates of the facial area of the motor cortex elsewhere [61].

The bilateral amygdala was also chosen because of its hyperactivity in patients with BPD [32]. The masks of the amygdalae were extracted from automatic anatomical labeling (AAL [62]).

The left and right ACC were defined as part of the salience network and its known abnormal functioning in BPD [32].

Further, we investigated the left and right PCC as part of the DMN.

Because the muscles were paralyzed by BTX (corrugator supercilii and procerus muscles) and because of their innervation by the facial nerve (cranial nerve VII), we additionally selected the brain stem nuclei of the facial nerve as seed regions. For this, we converted their location from the brain stem atlas of Paxinos et al. [63] to the MNI space. Using the Brain Stem Navigator [64, 65], the seeds (ncl. n. facialis, ncl. salvatorius superior, ncl. tractus solitarii) were best displayed by inferior medullary reticular formation (iMRt) and viscerosensory-motor nuclei complex (VSM) in the MNI space. Thus, the iMRt and VSM were used as seed regions.

All ROIs except the motor hand area and the brain stem nuclei of the facial nerve to be defined by the AAL atlas.

Data analysis

Between the predefined seed regions and all other voxels of the brain, Pearson's correlation coefficients were computed between the time series. After applying Fisher z-transformation on the correlation map, a general linear mixed model was generated to identify between- and within-group differences. On a cluster level, a threshold for all analyses was set to $p \leq 0.05$ family wise error (FWE), corrected for multiple comparisons on the cluster level. Peak voxel of significant clusters was localized using AAL.

Results

Demographic and clinical data

BPD symptoms, as assessed by expert rating using the ZAN-BPD scale, decreased significantly over time by 6.11 points (BTX: 5.67, ACU: 6.62) in both groups ($F(1,42) = 44.710$, $p < 0.001$, $\eta^2 = 0.516$). No significant differences between the interventions ($F(1,42) = 0.298$, $p = \text{n.s.}$) nor an interaction effect could be found ($F(1,42) = 0.251$, $p = \text{n.s.}$). A similar pattern was shown for BSL-23. Over time, an average decrease of 0.58 points ($F(1,41) = 20.236$, $p < 0.001$, $\eta^2 = 0.330$) but no differences between the groups ($F(1,41) = 0.860$, $p = \text{n.s.}$) and no interaction effects were observed ($F(1,41) = 0.000$, $p = \text{n.s.}$) (see Table 1).

Functional connectivity

Precentral gyrus

A significant group and time interaction was detected for the right precentral gyrus. Increased rsFC for the BTX group was detected to the left ACC ($t=4.56$, $p=0.041$) and to the left medial superior frontal gyrus ($t=4.17$; $p=0.007$). Decreased rsFC for the BTX group was found to the left precentral gyrus ($t=5.18$, $p=0.022$) (see Table 2 and Fig. 1).

Amygdala

No significant interaction effects were found for the bilateral amygdalae and other voxel across the brain.

ACC

Group and time comparisons revealed a higher functional connectivity of the left ACC to the right precentral gyrus in the BTX group when compared to the ACU group over time ($t=5.05$, $p<0.001$). Additionally, a stronger decrease in rsFC was observed over time for the BTX group, compared to the ACU group between the right ACC and right cerebellum ($t=4.26$, $p=0.004$) (see Table 2 and Fig. 2).

PCC

No significant interaction effects were observed on a cluster level.

Brainstem nuclei

No significant interaction effects were found for either iMRt or VSM.

Correlation between connectivity measures and clinical data

Correlations between the connectivity of the aforementioned results and the clinical data could not be detected.

Discussion

In this study, the effects of a single glabellar BTX injection on rsFC in patients with BPD compared to a minimal acupuncture condition for BTX-related and borderline-related seed regions were examined. Four weeks after the initial treatment, the two groups displayed a comparable clinical improvement as assessed by standard expert (ZAN-BPD) and patient ratings (BSL-23). Regarding the time X group interaction for BTX-specific seeds, the conducted analysis revealed a higher functional connectivity over time for the BTX group in comparison to the ACU group between the right precentral gyrus and the left ACC, as well as the left medial superior frontal gyrus and a lower functional connectivity over time for the BTX group between the right precentral gyrus and left precentral gyrus. For the BPD-specific seeds, we found a time X group interaction with higher functional connectivity after treatment for the BTX

Table 2 Differences in rsFC: displayed are the differences in rsFC between the BTX and the ACU group over time in (A) regions associated with BTX and (B) regions associated with BPD

(A)						
BTX regions	BTX ($T0 > T1$) > ACU ($T0 > T1$)			BTX ($T1 > T0$) > ACU ($T1 > T0$)		
	<i>T</i>	CS	Peak coord	<i>T</i>	CS	Peak coord
Seed: right precentral gyrus						
Left precentral gyrus	5.18	99	− 38 0 22			
Left anterior cingulate cortex				4.56	87	− 2 34 10
Left medial superior frontal gyrus				4.17	122	− 12 52 8
(B)						
BPD regions	BTX ($T0 > T1$) > ACU ($T0 > T1$)			BTX ($T1 > T0$) > ACU ($T1 > T0$)		
	<i>T</i>	CS	Peak coord	<i>T</i>	CS	Peak coord
Seed: right anterior cingulate cortex						
Right precentral gyrus				5.05	229	40 − 12 36
Seed: left anterior cingulate cortex						
Right cerebellum	4.26	132	26 − 72 − 22			

BTX incobotulinumtoxin A, ACU acupuncture, peak coordinates of clusters are according to the MNI space (Montreal Neurological Institute), *T* *t* score of peak voxel, CS cluster size

FWE correction with $p < 0.05$ at whole-brain level

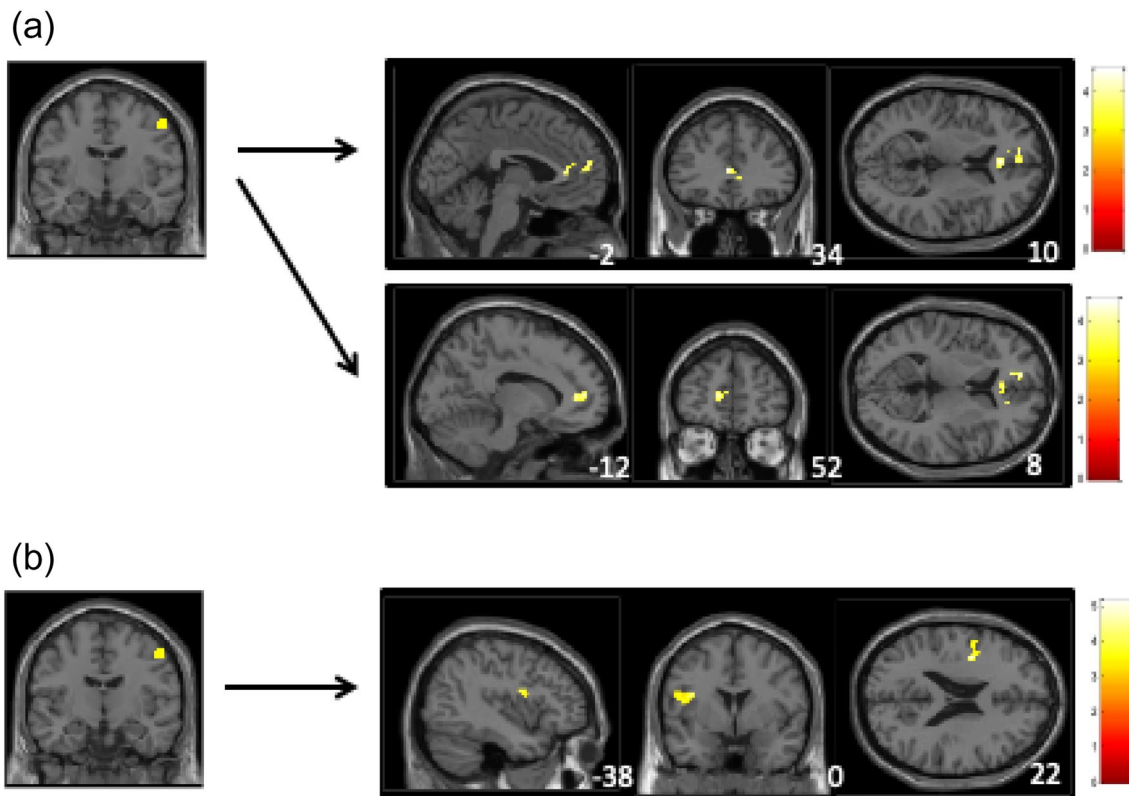


Fig. 1 rsFC of the right precentral gyrus. Displayed is the rsFC of the right precentral gyrus (A) in the contrast (BTX $T1 > T0$) > (ACU $T1 > T0$) to the left ACC and the medial superior frontal gyrus and (B) in the contrast (BTX $T0 > T1$) > (ACU $T0 > T1$) to the left precentral gyrus

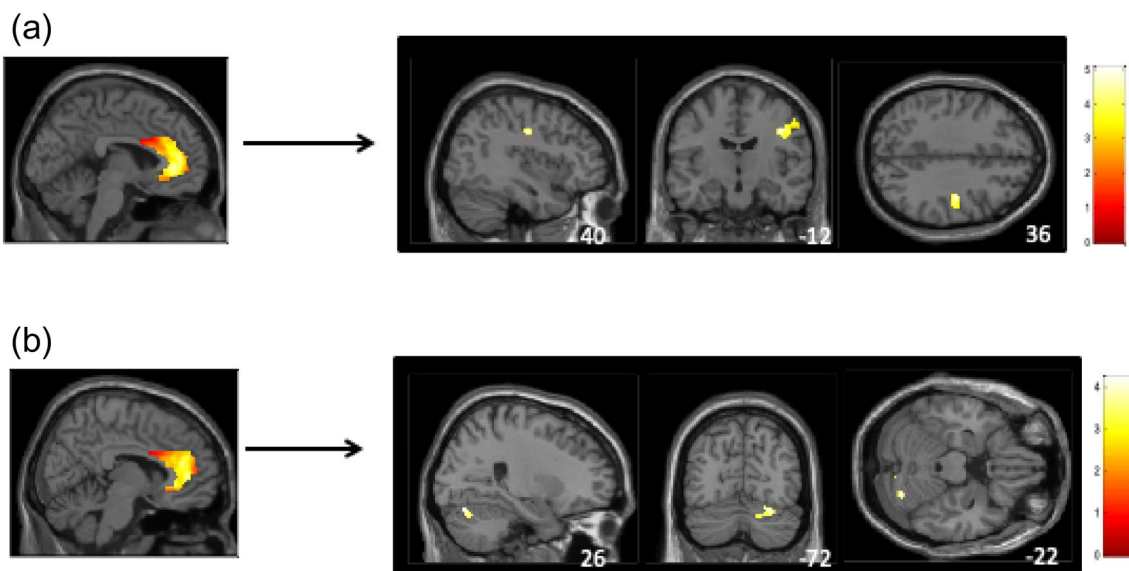


Fig. 2 rsFC of the left and right ACC. Displayed is the rsFC of the (A) left ACC in the contrast (BTX $T1 > T0$) > (ACU $T1 > T0$) to the right precentral gyrus and (B) the right ACC in the contrast (BTX $T0 > T1$) > (ACU $T0 > T1$) to the right cerebellum

group between the left ACC and the right precentral gyrus and a lower functional connectivity of rsFC over time for the BTX group between the right ACC and the right cerebellum.

It is conspicuous that the results point mostly toward areas that are discussed in the context of movements. Whereas the precentral gyrus is responsible in movement execution, it has also been debated in the final stage of motor response inhibition [66]. The ACC is traditionally believed to play a pivotal role in cognition and emotion [67]. With its exceptional position within the human brain, it has been described in terms of the integration of those two entities. However, closer examination reveals that the ACC is also involved in self-referential processing, social cognition, interpersonal transactions, and emotional regulation [68–70] and is essential with respect to this work for the detection of errors, specifically the detection of motor errors [71]. Also, the ACC is structurally described as having extensive connections to the motor cortex and spinal cord [72], and lesions of the ACC are associated with impaired initiation of movements. The increase in ACC functional connectivity to the precentral gyrus after BTX treatment might make sense considering that both structures are involved in motor control [73].

Since BTX blocks the successful execution of the motor commands originating in the primary motor cortex via the blockage of the lower motor neuron, the increased functional connectivity between ACC and the precentral gyrus might be related to this unsuccessful execution. Potentially, the missing execution from the lower motor neurons is detected by the ACC as the expected feedback is not received and thus an error signal is backpropagated to the motor cortex, as suggested by the predictive coding framework [74]. This framework suggests that part of the communication in the brain is not about processing content but is related to the discrepancy between expected signals derived from internal models and perceived signals from sensory organs. Interestingly, the ACC has already been suggested to be a key player in generating error-related signals [75].

The cerebellum has long been viewed as mainly being involved in motor control, especially because of difficulties with movements following lesions in this region. However, it has not been conclusively clarified in what aspects of motor control exactly the cerebellum is involved [76]. Newer research, however, suggests its role, e.g., in error-driven motor learning and thus in the correction of motor errors [71]. Interestingly, the cerebellum is also an ideal candidate for generating internal models of the motor apparatus, which are needed, on the one hand, to generate smooth movements and on the other hand are the basis for calculating prediction errors suggested by the predictive coding theory [77–79].

Integrating previous research with the results of our study indicates a regulatory effect of BTX in areas involved in error detection and correction and execution/inhibition of movements. Since BTX disables the normal movement of

muscles via their paralysis, this may fit into the theory. It is feasible that the ACC compares the motor input with the internal model from the cerebellum and changes communication to both as something is odd. Here, it could be speculated that as the model is obviously wrong (no signal at all, so it is not as useful), communication to the sensory-motor areas is increased, while communication to the model is decreased. However, it has to be noted that functional connectivity has no direction, as it is based on correlations.

In contrast to our hypotheses, this study could not detect differences in the rsFC of the amygdala between groups nor within time. Despite the structure being the one mostly discussed for showing divergent activity in BPD, data from previous connectivity studies have shown heterogeneous results. In one study that suggested apparent differences in connectivity between patients with BPD and healthy controls, the authors used a functional connectivity paradigm, which does not allow conclusions about the resting state [80]. Further, a study that investigated the rsFC between BPD and healthy controls showed a trend toward an altered rsFC, which did not quite reach statistical significance [41]. Also, it is possible that no effects could be detected as both groups profit to the same degree in terms of borderline symptomatology from the treatment. Taking these results into account, it seems not even certain that for the BPD the amygdala shows a difference in rsFC to other brain areas.

Strengths and limitations

The choice of the control condition has been both a strength and weakness of this study. On the one hand, ACU instead of a saline injection has decreased placebo effects as speculated in previous BTX studies [20]. On the other hand, for the clinical outcomes, ACU was equally effective to BTX. The feeling of getting a treatment in both groups might have influenced the clinical results (for a broader discussion see [47]). In addition to this, ACU itself is discussed as having an impact on rsFC [81]. However, as we only applied a minimal ACU treatment by reproducing the BTX injection sites in the glabellar region, we believe our ACU is unlikely to have produced an effect on functional connectivity. Thus, future research should keep this in mind when designing a control condition. Additionally, due to the paralytic effects of BTX, we were not able to design the study as a double-blind trial, a problem that unfortunately all research on BTX has to face. However, we used a single-blind approach, where the psychometric raters stayed blinded throughout the rating process. Further, since we only included psychiatric patients in our sample, an interpretation of the results toward either a BTX effect or a BPD effect seems limited. Future research should consequently include a control condition with healthy adults to investigate further the general effects of BTX.

Only women were eligible for study inclusion, which limits the results genderwise. Since BPD is known to also manifest in men, even if differing in symptoms, further research should comprise a more heterogeneous sample to allow generalization. Also, some baseline characteristics could not be controlled for. We were not able to take the kind and dose of psychotropic medication into account since these were extremely heterogeneous in the sample. Despite depression being the leading clinical diagnosis as an exclusion criterion, a high number of patients with BPD as their leading clinical diagnosis showed comorbid axis-I disorders [82]. But because they were not considered the primary disorder and the type and severity differed, controlling was not possible, and thus results stay limited. In particular, the circumstance that we did not exclude participants with comorbid attention deficit hyperactivity disorder (ADHD) should be noted.

Conclusion

This is the first study to investigate the effect of BTX on rsFC in BPD. The findings suggest an influence of BTX on areas involved in motor behavior. However, the absence of a clear clinical benefit on BPD symptomatology compared to ACU in this study suggests the independence of the BTX treatment from a clinical improvement perspective. The reported outcomes may encourage future research on the use of BTX and its influence on neuronal mechanisms in patients with mental disorders having an excess of negative emotionality and also in healthy subjects.

Acknowledgements The Hannover Medical School represented by Hannover Clinical Trial Center GmbH (HCTC) acted as the study sponsor. Neither the funding sources nor the sponsor had any role in the study design, the collection, analysis, and interpretation of the data, in the writing of the publication, and in the decision to submit the paper for publication. We thank Silke Malecha who played a pivotal role in the study's execution and sadly passed away.

Funding This research was funded by grants of the German Research Foundation (KR 3706/3-1, TK & MAW) and the Asklepios Kliniken Hamburg GmbH Forschungsförderung (MAW).

Data availability The data is available on request.

Declarations

Conflict of interest Author Kruger has received honoraria for talks and/or advisory board activities from Allergan; Lilly, Lundbeck, Otsuka, Schwabe, Servier, and Trommsdorf. Authors Kruger and Wollmer were members of an advisory board of Allergan. All other authors have no conflict of interest to declare.

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5. Discussion

To avoid repetition, in this discussion I will focus on thoughts that have not been discussed previously, to give the reader a broader insight into the potential of BTX in BPD and in psychiatric disorders in general. I will start with a summary of the main results from **Publications 1** and **2**.

5.1 Main results

Four weeks after baseline assessment, both the BTX and ACU groups showed a significant reduction in BPD symptoms. The expert rating (ZAN-BPD) and the self-rating instrument (BSL-23) showed reduced/borderline symptoms after BTX and ACU treatment (i.e., from T0 to T1). There were no differences between interventions and no interaction effects.

The emotional go/no-go paradigm that was analyzed in **Publication 1** revealed an increase in sensitivity and a decrease of wrong answers on no-go trials in the angry picture condition after BTX treatment. This was interpreted as an altered inhibition performance. On a neuronal level, a higher increase in activity of the inferior frontal gyrus over time, as well as a higher increase of activity of the precentral gyrus over time, were found for the BTX group in comparison to the ACU group. With regard to emotional processing, a decrease of amygdala activation could be observed for the BTX group over time when looking at angry and happy pictures, but this was not present in the ACU group.

In the fMRI at rest that was used in **Publication 2**, the right precentral gyrus as a seed region showed a higher increase in functional connectivity to the left ACC and to the left medial superior frontal gyrus and a higher decrease in functional connectivity to the left precentral gyrus in the BTX group compared to the ACU group. Furthermore, the BTX group showed a higher functional connectivity over time between the ACC and the right precentral gyrus and a stronger decrease in connectivity between the ACC and right cerebellum.

5.2 Clinical effectiveness of BTX in BPD

In this section I will discuss the clinical effectiveness of BTX in BPD. This is important for integration with the later discussed neuronal findings and is necessary in order to formulate an overall conclusion.

Clinical improvement of symptoms was found in the BTX as well as the ACU group, with no difference between groups. In the two publications this was discussed as differential effects (i.e., BTX works via inhibition of muscles whereas ACU was effective through the placebo effect) because there are neuronal differences between the groups. However, here I will also discuss the possibility that both interventions are mainly effective via factors other than the actual interventions.

But first I will revisit the problems arising from acupuncture as a control condition, which has been more problematic than expected. Designing an adequate control condition is one of the main challenges in BTX research and, due to the obvious paralytic effect, double blinding is difficult to implement. Therefore, in our study we tried a new approach, as previous research in this area was suspected to be influenced by the expectation of the participants (Magid et al., 2015). Thus, with ACU we opted for a treatment that, from a naive point of view, could potentially be effective and is thus also influenced by (positive) expectations.

However, as ACU is an actual treatment method in traditional Chinese medicine (TCM), the potential specific effect of ACU has to be considered and is discussed in the following paragraph.

ACU has been investigated in the treatment of mental disorders for some years (Lin et al., 2020). As part of TCM, traditional ACU is based on the assumption that a disharmony of energies (e.g., Qi) persists. When the pattern of imbalance is identified, ACU needles can be placed to dissolve the disharmony and therefore ease the present symptoms (Ergil et al., 2009; Flaws & Finney, 1996; Wiseman, 1995). Nevertheless, support for an effect on psychiatric conditions is insufficient because of low quality results and a high risk of bias in the studies (Armour et al., 2019; Hollifield et al., 2007; Pilkington et al., 2007; Shen et al., 2014; Smith et al., 2018). Furthermore, in our study, we applied only a minimal ACU procedure with a low frequency (once after baseline assessment and once between MRI acquisitions), using standardized needle

placement close to five points of the forehead that had also been used for the BTX injections. Normally, for ACU the assessment of disharmonies and the selection of ACU points are highly individualized and do not follow a standardized pattern. Consequently, it seems unlikely that the ACU treatment itself led to the improvement of BPD symptoms in our study.

Other possibilities to explain the improvement of both groups in our sample are factors arising from the treatment situation itself. Grawe et al. (2000) established a set of factors that show efficacy in the treatment of mental disorders, besides and regardless of the underlying psychotherapeutic approach. These factors are the therapeutic relationship, activation of resources, actualization of the patient's problems, motivational clarification and active help for problem solving. Among Grawe's factors, we know by now that the therapeutic relationship is the factor with the highest effect (medium effect sizes) on the therapeutic outcome (Totura et al., 2018). In our study, all patients (BTX and ACU) had repeat visits with a doctor or nurse (for application of the intervention) and with the psychometric raters. Although the intervention period itself differed, the participants stayed with the practitioner or nurse in the treatment room for the same amount of time, mostly chatting, to avoid debinding of the psychometric raters. Consequently, both groups had the opportunity to build a relationship with the practitioner and it can be assumed that this connection and the potential actualization of the patient's problems might have influenced the BPD symptom improvement.

A concept that is often used synonymously with the general efficacy factors is the placebo effect. Nevertheless, the two terms differ as the placebo effect describes the effect of expectation of improvement rather than the pharmacological or physiological changes (Margo, 1999), whereas according to Grawe (2000) specific factors act upon the outcome. The mode of action of the placebo effect is the treatment expectation (Margo, 1999), which can lead to symptom reduction via endogenous mechanisms that are additionally modulated by context factors (such as mode of application, clothes of the practitioner, framing of the explanations). In our study the participants knew that two different treatment approaches were used and both could have a potential influence. This alone could have boosted the placebo effect in both groups and led to the improvement.

When designing the study, we chose BPD as a disorder that, similar to depression, shows an excess of emotionality, mainly of negative emotions. As mentioned previously, the improvement of the BTX and ACU groups could also be due to general treatment factors and/or the placebo effect. With this assumption, the question arises as to how the initially described effect of BTX on depression is achieved, whereas on BPD a clinical effect of BTX might not be seen. It has even been proposed that BPD is a variant of an affective disorder, either depression itself or bipolar disorder (Akiskal, 2002; Akiskal et al., 1985). However, it can also be hypothesized that the two disorders differ more than is assumed. The main symptom of MDD is a pervasive sadness or low mood, accompanied by a loss of interest that persists to an unusual extent for the majority of the day on most days (American Psychiatric Association, 2013). BPD, on the other hand, is characterized by a high extent of impulsivity and problems in affect regulation and interpersonal relationships (American Psychiatric Association, 2013). Thus, it can be argued that MDD and BPD are two disorders with a focus on emotions but that the central problem of each disorder differs. Some decades ago, the research on BPD even suggested that disorders such as MDD might be the primary cause that leads to the development of BPD symptoms as a complication of its own symptoms (Akiskal et al., 1983; Gunderson & Phillips, 1991). The mentioned symptoms of MDD and BPD also point towards a difference in emotionality between the two disorders. It can be hypothesized that MDD with its flat and low mood can be distinguished from BPD, which displays problems with emotions rather than mood. Mood and emotion have long been used alike for aspects of affect (Ekman & Davidson, 1994). Nonetheless, the two do not seem to be the same. One proposition is that mood and emotion differ in their length. Another idea is that mood is not connected to facial expressions (Ekman & Davidson, 1994). Ekman and Davidson (1994), however, propose that the difference lies in the function of the affectiveness: mood is supposed to modulate information processing in the form of cognitions whereas emotions modulate actions because certain situations need adaption. Looking at the difference between mood and emotion and our findings on MDD as a disorder described for having problems with mood and on BPD as a disorder having problems with emotions, it is worth formulating a thesis: it could be possible that BTX has a higher effect on persisting mood than on the rather shortly perceived emotional states.

Another problem that needs to be discussed at this point is the discrepancy between the improvement of self-rated and expert-rated depressive symptomatology. As in

Wollmer et al. (2022), the self-rated depressive symptoms (BDI-II) improved in our study regardless of the allocated group, but with expert rating (MADRS) there were no time, group or interaction effects. This is contrary to the wide-ranging findings of BTX having a physiological impact on depressive disorders via facial feedback (Schulze et al., 2021), independent of self- or expert-rated procedures. A more detailed look into our data revealed that our sample showed lower depression scores than studies investigating BTX for depression: mean MADRS scores in our sample were 21 for BTX versus 23 for ACU; in the samples of Finzi and Rosenthal (2014) and Brin et al. (2021) the respective scores were 32 versus 31. This suggests that BTX might be more effective in more severe manifestations of depressive disorders.

Another approach to explaining why we did not find changes of depressive symptoms is the placebo effect. In our study, the participants were told to participate in a study to treat the symptoms of BPD. Assessment of depressive symptoms was not discussed, so expectations on the treatment of depressive symptoms were not generated by the subjects.

Collectively, in the two included publications we discussed the differential effects of BTX and ACU but it is also necessary to discuss the potential ineffectiveness of BTX in BPD. The improvement of BPD symptoms in both groups could also be driven by general treatment factors and/or placebo effects.

5.3 Neuronal changes after BTX in BPD

The main focus of this thesis is on the neuronal changes after BTX treatment in patients with BPD. For this purpose, the two publications included two different approaches to determine functional connectivity: task-based and resting-state fMRI. A detailed look at the results of **Publications 1** and **2** emphasizes the difference in the two methods used. One could argue that the findings of reduced amygdala activity in **Publication 1** and the lack of findings for the amygdala seed in **Publication 2** seem contradictory. However, it is unlikely that the variations are due to design issues, such as a low number of participants. The functional connectivity assessed by fMRI at rest illustrates the functional architecture of the brain (Lee et al., 2013), whereas changes within this architecture result in an apparent functioning of the brain. When using task-based fMRI

one measures the activity following a stimulus or task. Thus, task-based fMRI displays the relative BOLD changes from baseline during a response to something (task or stimulus) whereas fMRI at rest shows synchronous activity between structurally distinct regions (Lee et al., 2013). The changes in communication of the brain are represented by the results of fMRI at rest and the answering of brain areas during the presentation of certain stimuli (task-based fMRI) can influence each other. Thus, it is possible that a region shows higher activity during a specific task while the underlying functional connectivity does not change (and vice versa), therefore the contrasting finding of amygdala activity is determined through the difference between the two methods.

As described in the Introduction, the FFH had been discussed as a possible mode of action behind the effect of BTX on emotional states. However, the evidence of the FFH stays limited and effect sizes are small (Coles et al., 2019). To my knowledge there is no model describing the basic functioning of BTX on a neuronal level. Nevertheless, the concept of embodied emotions has been integrated into neuronal models, as in the neurobiopsychological approach to explain the initiation of emotions by Antonio Damasio (1995). Here, I will incorporate the findings of my two publications in and around this model, which can also give a potential explanation for the neuronal functioning behind the FFH. I will use an example to explain Damasio's (1995) model: a person who recognizes a friend that he/she has not seen in a very long time. For better understanding, the theory is additionally outlined in Fig. 5a.

When a stimulus emerges, according to Damasio's theory, the early sensory cortices start a cognitive evaluation by reviewing the aspects of the object and reflecting on the current situation. In our example, the person starts by evaluating the aspects of the relationship to the friend, the reflection of the current appearance and the consequences for the person him/herself. Damasio (1995) calls these representations mental images. Some of these images are non-verbal (e.g., the similarity of the friend to yourself) and some are verbal (e.g., the name or the attributes that describe the friend). These images are formed by representations in early sensory cortices. Additionally, representations already acquired and stored in higher order cortices influence these representations in the early sensory cortices.

The processing of the friend in early and higher order cortices creates a signal that is forwarded onto networks within the prefrontal cortex. In the prefrontal cortex a response is generated, which is nourished by individual dispositions towards how the

stimulus, in our case the reappearance of a friend who has not been seen in a long time, had been previously paired with emotions.

This response is further sent to structures of the limbic system (amygdala, ACC, basal ganglia, hypothalamus), where dispositional representations again enrich the generated response. From here the signal is sent to autonomic nuclei, to initiate an autonomic reaction to the motor system to cause facial and body expressions, and also to the endocrine and peptide systems, which trigger the release of chemical signals. All of these three activations are accounted for in the emotional body state, which is fed back to the somatosensory cortex and the limbic system. The signal in the somatosensory cortex is then perceived as an emotion.

For discussion of my publications in the context of this model, I will start with the findings of the resting-state fMRI, which need to be discussed outside of the displayed chart because they focus on motor areas within the brain and seem rather unrelated to emotions.

BTX physiologically interacts with the motor system primarily by paralyzing the muscle (as explained in Section 1.2). To recapitulate, BTX splits SNAP-25, a protein responsible for the release of acetylcholine at the neuromuscular junction, and thus prevents the muscle from contraction. In addition to the physiological effects in the motor system, in **Publication 2** we found impaired connectivity between brain regions, associated with motor execution/inhibition and error detection/correction. We hypothesized that the paralysis of facial muscles in our sample led to the apparent signaling between the ACC and the precentral gyrus (M1) and cerebellum. We suggested that with the application of BTX, the ACC increases its signaling to M1 and decreases its signaling to the cerebellum because the expected signal displayed in the internal model at the cerebellum does not match the motor input of M1. The findings of **Publication 2** consequently seem to influence areas within the brain that are connected to motor control. Thus, it could be assumed that in our sample the injection of BTX did correspond to a change in movement-related circuits, which seems plausible due to the paralysis of the facial muscles.

The physiological changes within the motor system induced by BTX could influence the body state (Fig. 5b). The facial muscles thus cannot be used as usual, due to their paralysis. This brings us to the “motor system” aspect in Damasio’s model (1995). It

could be assumed that the application of BTX changes the starting conditions of emotion generation: the motor system is physiologically and neuronal impaired. When an emotional stimulus is now perceived, it is processed in the early sensory and higher order cortices and sent to the limbic system. The hypothalamus forwards the information to, among others, the motor system. This is thought to be the crucial step because the motor system has been affected by BTX and thus the missing output leads to a reduced emotional body state. This reduced emotional body state is fed back to the limbic system, where a diminished signal is generated in the amygdala due to the reduced feedback. Furthermore, the somatosensory cortices responsible for the generation of feeling receive less feedback from the emotional body state, which could result in reduced feeling. This part of the model calls to mind the FFH, which has been seen to comprise the bodily feedback to the brain, resulting in a change of emotional state.

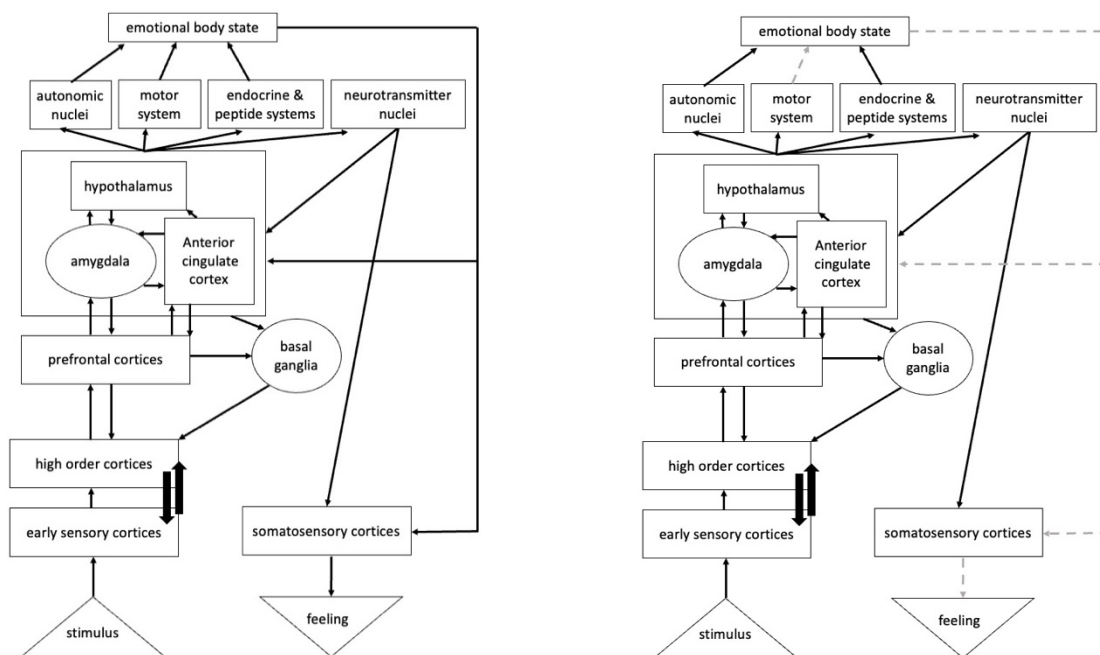


Figure 5: (a) Schematic model of Damasio's neurobiopsychological approach to emotions and (b) modified model under the use of BTX (figure adapted from Damasio, 1995, p. 22).

Looking further into the effects of this changed processing of emotions, a reduction in feeling is associated with a change in executive functioning (Pessoa, 2009). The

impact of emotion on cognition is thought to depend on the intensity level of the emotion, whereas the malfunction of executive processes was higher with highly arousing stimuli, and vice versa (Pessoa, 2009). A possible explanation might be that the neuronal networks involved in cognitive and emotional processing partly overlap, as both are represented in frontal brain areas (Morawetz et al., 2020; Purves, 2018).

The idea of the arousal of an emotion having an impact on executive functioning fits the findings of **Publication 1**. There, we found an improved inhibition performance after BTX treatment. Thus, it can be assumed that the alteration of inhibition performance is an effect of the blunted emotional response. Neuronally, we explained the finding of an intensified M1 performance after BTX injection by the role of M1 in inhibition of movement patterns immediately before execution. Here it could be assumed that the decrease in emotionality also had an effect on the inhibition of motor output, whereas the influence seems to be mediated by cognitive processes.

In summary, the neuronal results of the two publications and their embedding in and around the emotion theory of Damasio can point towards different branches of interpretation. Firstly, it seems that BTX does impact motor-related areas within the brain by changing the functional connectivity within the motor circuit. Consequently, the basic communication seems to be impaired by the use of BTX. Secondly, with emotional stimuli emerging, the results of **Publication 1** fit into Damasio's model of emotion and it could be assumed that the change in M1 induced by BTX leads to an impairment in emotion generation. Finally, the attenuated emotions influence inhibition processes. These were enhanced in our sample, which was represented neuronally by intensified precentral activation.

5.4 Conclusion of clinical and neuronal results

In the previous sections I discussed the clinical and neuronal findings that are further displayed in **Publications 1** and **2** of this thesis. The effectiveness of BTX in the clinical symptom improvement of BPD could be questioned. A closer evaluation of the effects could indicate unspecific placebo effects on borderline symptomatology rather than specific effects of BTX or ACU. These placebo effects could be initiated through contextual factors of the study design, such as the therapeutic relationship between

the participants and the practitioners of the intervention. However, as we did observe neuronal differences for BTX in ACU, the question of specific/unspecific effects needs to be further evaluated in future research.

The effect of BTX goes beyond the physiological local changes due to the neurotoxin. Neuronal circuits underlying motor execution and inhibition, and also motor error detection and correction, seem to be affected. These results point towards the influence of BTX on the functional architecture of the brain in areas where movements are controlled.

Furthermore, **Publication 1** showed an effect of BTX on emotional processing and behavioral inhibition. As the clinical effects in both groups could also be general treatment and/or placebo effects, the changes in neuronal circuits seem specific for BTX and more likely to be independent of the sample of women with BPD. The neuronal effects of BTX include changes in motor areas and in circuits that process emotions. Integration of the results of **Publication 1** into the emotion model of Damasio, therefore, can also give innovative thoughts about the neuronal basis of the FFH. The theoretical beliefs of the FFH fit into the model and for this thesis are enriched with empirical results from a BTX study.

The question arising from the collective view on the clinical and neuronal outcomes of our study is how emotion circuits could be affected by BTX when the BPD symptomatology was more likely induced by general treatment and/or placebo effects? One idea for this could be that, following the model of Damasio, BTX affects the generation of emotions. Patients with BPD, however, show problems with emotion regulation and not generally with their generation. Having less emotions might lead to less situations where emotions need to be regulated. However, emotion regulation itself still needs to happen in case emotions emerge. Consequently, it can be speculated that the potentially unspecific effect of BTX in our sample could also be due to the lack of impact on emotion regulation.

5.5 Outlook

In the last decade especially, the gap between people in need of therapy and the actual availability of psychotherapy has led to the development of alternative approaches to

ease the strain of people with mental disorders. Thus, it is important to test alternative treatment options such as BTX, with easy and fast availability and low costs for its therapeutic usage in the treatment of psychiatric disorders.

This thesis aimed to provide evidence for the use of BTX in patients with BPD. On a clinical level, effectiveness above the control condition could not be proven. However, the neuronal results show that BTX does change the underlying mechanisms, which seem to be rather non-specific to psychiatric disorders.

When looking at the publications underlying this thesis, one might argue that designing an fMRI study for our research question seems premature because some basic problems with the BTX research should have been solved first. For example, difficulties with blinding due to the induced paralysis and an adequate control condition should be focused on in future research. An alternative idea to test the impact of BTX on emotions is the injection of BTX in areas clearly not related to emotional expression in the control condition (e.g., the neck). However, we believe that although our results remain limited due to problems with blinding and the control condition, they do contribute important information about the underlying mechanisms of BTX and its influence on emotions.

In addition to methodical issues, in this thesis the question remains open as to how the change in emotion processing is connected to symptom improvement of disorders with emotional problems. In previously used samples of people with depression, the presumed effect of BTX on emotions co-occurs with an improvement of symptoms. In our sample of patients with BPD, however, this relationship was not so clearcut. Therefore, it is important to focus further research on the basic neuronal mechanisms of BTX (e.g., in people with depression) because, to my knowledge, this research has not existed until now. The results could be compared to my publications, which can contribute essentially to the debate surrounding the basic functioning of BTX on emotions and also contribute to the debate into the functioning of the FFH.

Prospective research on BTX should also try to focus on other psychiatric conditions that display problems with emotions, such as disorders involving the heightened generation of emotions. Another idea would be to test BTX in psychiatry independent of diagnosis and instead on the dimensional displayed values of predominant emotions.

Finally, this thesis could answer some questions in the research field of BTX even though many more have emerged. However, previous research has suggested such promising results of BTX on psychiatric disorders that this approach with its underlying mechanisms needs to be explored further.

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8. List of abbreviations

ACC	Anterior cingulate cortex
ACU	Acupuncture
AE	Adverse event
BDI-II	Beck Depression Inventory-II
BPD	Borderline Personality Disorder
BOLD	Blood-Oxygen-Level-Dependent
BSL-23	Borderline Symptom List-23
BTX	Botulinum toxin
CEQ	Credibility/Expectancy Questionnaire
CGI-S	Clinical Global Impression-Severity Scale
CSS-GFL	Clinical Severity Score for Glabellar Frown Lines
FDA	United States Food and Drug Administration
FFH	Facial Feedback Hypothesis
fMRI	Functional Magnetic Resonance Imaging
H-chain	Heavy Chain
L-chain	Light Chain
MADRS	Montgomery-Åsberg Depression Rating Scale
MD	Major Depression
MRI	Magnetic Resonance Imaging
PCC	Posterior Cingulate Cortex
RCT	Randomized Controlled Trial
SCID-II	Structured Clinical Interview for Axis II DSM-IV disorders
SCL-90-R	Symptom-Checklist- 90-R
TCM	Traditional Chinese Medicine
ZAN-BPD	Zanarini Rating Scale for Borderline Personality Disorder

9. Acknowledgements

This thesis would not have been possible without different important people. First of all, I would like to thank Prof. Tillmann Krüger, for the continuous support and especially for living an open-door policy. I would also like to thank Prof'in Kerstin Schwabe for agreeing to supervise this work and the inspiring thoughts throughout our periodic meetings. In addition, I would like to thank Dr. Christopher Sinke, for always finding the extra time to think ideas through and discuss new approaches. This thesis definitely would not have been possible without him.

I would also like to take the time to thank my family, especially Bettina Schulze for the lifelong guidance, that also contributed to this work. Finally, I would like to thank Leon Gotzmann, for always having my back and being my rock and Kaye Schulze for being my little sunshine boy.

11. Erklärung nach §6 Abs.2 Nr.7 (Anlage 4)

Erklärung nach §6 Abs. 2 Nr. 7 der Promotionsordnung zur Erlangung des Grades eines Doktors der Humanbiologie (Doctor rer. biol. hum.) der Medizinischen Hochschule Hannover.

Hiermit erkläre ich, dass ich die Dissertation

Neuronale Veränderungen nach Gabe von Botulinumtoxin A bei Patientinnen mit Borderline-Persönlichkeitsstörung

selbstständig verfasst habe. Bei der Anfertigung wurden keine Hilfen Dritter in Anspruch genommen: Ich habe keine entgeltliche Hilfe von Vermittlungs- bzw. Beratungsdiensten (Promotionsberater oder anderer Personen) in Anspruch genommen. Niemand hat von mir unmittelbar oder mittelbar entgeltliche Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen. Ich habe die Dissertation an folgenden Institutionen angefertigt:

Arbeitsbereich Klinische Psychologie und Sexualmedizin
Klinik für Psychiatrie, Sozialpsychiatrie und Psychotherapie
der Medizinischen Hochschule Hannover
unter Leitung von Prof. Dr. med. Tillmann Krüger

Die Dissertation wurde bisher nicht für eine Prüfung oder Promotion oder für einen ähnlichen Zweck zur Beurteilung eingereicht.

Ich versichere, dass ich die vorstehenden Angaben nach bestem Wissen vollständig und der Wahrheit entsprechend gemacht habe.

Jara Schulze, 26.04.2023