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Fostering participation: Including animals in therapy for patients in a minimally conscious state

A cumulative dissertation submitted to the Faculty of Psychology, University of Basel, in partial fulfillment of the requirements for the degree of Doctor of Philosophy by

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Declaration of scientific fairness

I, **Rahel Marti**, hereby declare that the present work was written independently without the help of third parties and without the use of any means other than those indicated. Sources used for help are marked as such. The manuscripts published or submitted for publication in journals were prepared in cooperation with the coauthors and were not published elsewhere by any of the participants, submitted for publication, or submitted to any other examination authority as a qualification paper. These are the following manuscripts:

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Abbreviations

AAI	Animal-assisted intervention
AAT	Animal-assisted therapy
BAVESTA	Basler Vegetative State Assessment
DGNR	Deutsche Gesellschaft für Neurorehabilitation (German Association for Neurorehabilitation)
EAN	European Academy of Neurology
fNIRS	Functional near-infrared spectroscopy
HF	High frequency
HRV	Heart-rate variability
HR	Heart rate
IAHAIO	International Association of Human–Animal Interaction Organizations
LF	Low frequency
MCS	Minimally conscious state
NN interval	Distance between two N peaks (normalized HR)
RMSSD	Square root of the mean of the sum of the squares of the differences between adjacent NN intervals
RR interval	Distance between two R peaks (unfiltered HR)
WHO	World Health Organization

Abstract

Minimally conscious states, which occur after severe brain injuries, represent a significant burden and can lead to long-term disability. Patients in minimally conscious states are a vulnerable patient group that needs early and effective treatments. Animal-assisted therapy is a possible treatment for minimally conscious patients and is applied for various reasons. The stimulation provided by animals is multisensory and emotional. Interactions with animals function nonverbally, and these situations are easy to understand. First studies have shown that animal-assisted therapy can increase active movements, awareness, and brain activity. However, the evidence base for animal-assisted therapy in treatments of minimally conscious states is minimal.

We conducted three studies to better understand the effect of animal interaction on minimally conscious patients. First, we wanted to investigate how animal-assisted therapy affects behavior, physiological parameters, and the level of consciousness of minimally conscious patients. For this purpose, we conducted a randomized two-treatment multiperiod crossover study that measured patients during eight animal-assisted and eight conventional therapies (study I). Second, we were interested in the mechanisms involved in the interaction between minimally conscious patients and animals. For this purpose, we measured brain activity in two experimental studies with healthy adults and minimally conscious patients (studies II and III). We compared the responses to different forms of contact with a dog and a plush animal. We also analyzed patients' heart rates and heart-rate variability in study III.

The crossover study revealed that the minimally conscious patients showed more behavioral responses, more awareness, and higher physiological arousal in the animal-assisted therapy sessions compared to conventional sessions (study I). Healthy participants in the experimental study showed higher brain activity when interacting with a dog than with a plush animal. The closer the interaction with the dog or plush animal was, the higher the brain activity became. Minimally conscious patients also had increased brain activity with increased proximity to a dog or a plush animal. But the patients reacted equally strongly to the dog and the plush animal. However, the patients' heart rates were higher during interaction with the dog than with the plush animal.

The three studies indicate that interactions with animals have the potential to arouse minimally conscious patients physiologically and emotionally. This arousal allows these patients to participate more fully in therapy through a higher level of consciousness. The three studies make an important contribution to better understanding the influence of animals on minimally conscious patients. However, one of many new questions is how animal-assisted therapy should be delivered and which patients can benefit most from this therapy approach. More studies will be needed to enable a safe, evidence-based application of animal-assisted therapy in minimally conscious patients.

1 Introduction

Minimally conscious state (MCS), a condition occurring after a severe brain injury, represents a significant burden, required medical and nursing care, and can lead to long-term disability (Boitier et al., 2020; Katz et al., 2009). Treatment of MCS patients is a challenge for all parties involved, from the medical staff in intensive care to the patients' families (Bender et al., 2015; Covelli et al., 2016).

After a severe brain injury, patients emerging from a coma often transit through altered states of consciousness (Bodien et al., 2016). A vegetative state or unresponsive wakefulness syndrome defines the first remission state, which is usually followed by MCS (Perrin et al., 2015). These two states belong to the disorders of consciousness (Giacino et al., 2002). Patients in MCS show phases of wakefulness and minimal reaction to their environment. MCS is distinguished from a vegetative state by the partial preservation of consciousness (Fins et al., 2007; Giacino et al., 2002). Minimal signs of preserved consciousness include object fixation with the eyes and turning one's gaze or head toward a stimulus, localization of noxious stimuli, and movements or affective behaviors that occur appropriately in relation to relevant environmental stimuli (Bruno, Vanhaudenhuyse, et al., 2011).

Early treatment should reduce long-term hospitalization and disability, and support spontaneous remission (Fins et al., 2007; Lippert Grüner & Terhaag, 2000; Pistarini & Maggioni, 2018). Therapy aims to prolong periods of wakefulness, increase awareness, and promote complex behaviors (Seel et al., 2013). Promoting neuronal plasticity through diverse learning opportunities fosters neuronal reorganization (Bach-Y-Rita, 2003; Lichtensztejn et al., 2014). Protection from sensory deprivation is another goal in treating MCS patients (Di & Schnakers, 2018; Perrin et al., 2015).

Therapy concepts in the early rehabilitation of MCS patients are mostly activity-oriented and strongly related to everyday life (Boitier et al., 2020; Rietz & Hagel, 2000; Zieger, 2016). An integrative part of such concepts is early onset stimulation in an enriched environment with individualized stimuli (La Gattuta et al., 2018; Pistarini & Maggioni, 2018). Studies have shown greater behavioral and neuronal changes if these stimuli are sensorily multimodal, personally relevant, familiar, and emotional (Di Stefano et al., 2012; Perrin et al., 2015; Wilson et al., 1996). Examples of such emotional stimuli are children's cries (Laureys et al., 2004), one's own name (Cheng et al., 2013; Laureys et al., 2004), family pictures (Zhu et al., 2009), familiar persons or voices (Bekinschtein et al., 2004; Eickhoff et al., 2008), and interaction with an animal (Bardl et al., 2013; Borgi & Cirulli, 2016; Kurdek, 2009b). Further, studies by Di Stefano et al. (2012) and advocates of the Affolter® approach (Affolter et al., 2009) have argued that it is essential to present stimuli in a context (semantic, emotional, or situational). However, these sensory stimulation programs are not without criticism. One concern is that overstimulation leads to fatigue (Di & Schnakers, 2018; Seel et al., 2013). A second is that patients become habituated to the stimuli and lose interest in them (Di & Schnakers, 2018; Seel et al., 2013). Some researchers question the evidence on the effectiveness of sensory stimulation programs because the studies on them have methodological biases, and some studies have found no effect (German Association for Neurorehabilitation [DGNR], 2022; Di & Schnakers, 2018; Johnson et al., 1993).

In recent years, animal-assisted therapy (AAT) has been used more often in treating MCS patients (Arnskötter et al., 2022; Blankenburg et al., 2011; Hediger, 2019). In AAT sessions, a trained animal is actively integrated into therapeutic activities to support a therapeutic goal. According to the

International Association of Human–Animal Interaction Organizations (IAHAIO; 2018), AAT is a goal-oriented, structured therapeutic intervention delivered by formally trained professionals. AAT is implemented with various groups of patients, including patients with brain injuries (Gocheva et al., 2018; Hediger, Thommen, et al., 2019).

There are multiple reasons for including animals in therapy with MCS patients. Animals are emotionally relevant to many people (Aragunde-Kohl et al., 2020; Borgi & Cirulli, 2016; R. Hawkins & Williams, 2017; Kurdek, 2009a, 2009b). Also, according to the biophilia hypothesis, animals are thought to receive greater attention than inanimate objects (Wilson, 1984). Further, it is in the nature of AAT that different senses are involved: besides visible events, there are tactile stimuli through the living animal body (body contact, warmth, breathing movements, heart action), auditory stimuli through vocalizations (breathing, sniffing, rustling), and olfactory stimuli (Bardl et al., 2013). Interactions with animals are situations that are easy for patients to understand. They enable patients to perceive the stimulation in a context. In addition, AAT often takes place in a particular room or outside the clinic, which further contributes to a unique context. By feeding or brushing an animal, patients can take over a caring role that is otherwise impossible in their condition (Bardl et al., 2013). Caring for others is a basic human need (Bowlby, 1982). Moreover, animals communicate nonverbally, as is the case with MCS patients. Animals do not judge by human standards (Geist, 2011), and they can provide a kind of closeness and tenderness that human caregivers cannot. These aspects can lead to relationships between animals and patients that function unconsciously without language. The inclusion of an animal creates a relevant form of social interaction (Kurdek, 2009a). An affective response to the intervention in the form of bonding may occur (Hart, 2014; Kurdek, 2009a). These factors lead to the fact that AAT goes beyond basal stimulation and represents a change from the daily routine in the clinic (Bardl et al., 2013). Furthermore, the problem of habituation is minimal with AAT, and the calming effects of the animal interaction could prevent overstimulation (Beetz, 2017).

Only a few studies have investigated the effect of AAT in the treatment of MCS patients. In a case study, a patient in a persistent vegetative state showed increasing vegetative, emotional, and motor responses as well as minor signs of cognitive functioning over the long-term course of dog-assisted therapy (Bardl et al., 2013). In a second case study, an MCS patient exhibited broader variability of different behavioral reactions with more consistency, higher frequency, and higher quality during AAT sessions compared to during control therapy sessions (Boitier et al., 2020). The reactions of this patient were interpreted as signs of higher arousal and increased awareness, communication, and stimulation-discrimination abilities. A pilot study examined the brain activity in how two MCS patients and two healthy participants responded to animals compared to a robotic plush toy. The brain activation patterns of these two patients were inconclusive. One patient had greater brain activation with the animal, while the other had greater activation with the robotic plush toy (Arnskötter et al., 2022).

Research with MCS patients is particularly challenging because they cannot provide information about their feelings and well-being during therapy. It is therefore essential to investigate the effect of AAT on MCS patients with different approaches (Bruno, Vanhaudenhuyse, et al., 2011; Monti et al., 2009). This thesis examined how integrating an animal into therapy affects MCS patients. In the first study, we investigated how the behavior, level of consciousness, heart rate (HR), and heart-rate variability (HRV) of MCS patients differ between AAT and conventional therapy (study I). Behavior was measured using video coding. We assessed consciousness with the Basler Vegetative State Assessment (BAVESTA; Huber, 2014). In the second and third studies, we investigated specific aspects of AAT. We looked at the effect of the presence of a dog compared to a plush animal and at the effect of the proximity to these stimuli on frontal brain activity. In one study, we conducted this experiment with healthy participants to understand the healthy response to dogs and their proximity (study II). We conducted the same experiment again with MCS patients (study III). In these two studies, we used functional near-infrared spectroscopy (fNIRS), which is particularly suited for studying the human–animal relationship (Arnskötter et al., 2022; Calcaterra et al., 2015; Kobayashi et al., 2017; Matsuura et al., 2020).

The second chapter provides theoretical background on the MCS diagnosis, AAT, and the applied measurement methods (video coding, BAVESTA, HR, HRV, and fNIRS). The aims of this thesis are outlined in chapter three. The fourth chapter contains the three original studies that form the core of the thesis. In the fifth chapter, the study results are summarized, discussed, and implications are elaborated. The sixth chapter presents a final summary.

2 Theoretical background

2.1 Minimally conscious states (MCS)

2.1.1 Diagnosis

Disorders of consciousness include comas, vegetative states (unresponsive wakefulness syndrome), MCS, and posttraumatic confusional states (emergence from MCS; Giacino et al., 2014). These states can occur after acquired brain injuries and usually follow each other in the natural course of recovery (see Figure 1; Katz et al., 2009). In vegetative states, patients have a sleep-wake rhythm but show no signs of awareness of themselves or their environment (World Health Organsiation [WHO], 2022). Patients have transitioned to a state of minimal consciousness when they show purposeful behavior, including movements or affective behaviors, that occur in contingent relation to relevant environmental stimuli and are not due to reflexive activity. Bruno, Vanhaudenhuyse, et al. (2011) proposed to divide MCS into two subcategories, MCS- and MCS+, based on the complexity of patients' behaviors. Patients in MCS- show signs of nonreflex behaviors such as eye tracking, orientation to pain, or contingent response to relevant environmental stimuli. Patients who exhibit command following qualify for MCS+ (WHO, 2022). In addition to the WHO criteria, Bruno et al. (2011) proposed that patients should qualify for MCS+ when they are able to show comprehensible verbalization or provide yes/no responses (gestural or verbal). Patients emerge from MCS and enter a confusional state when they are able to reply correctly to yes/no questions repeatedly or use objects functionally (Giacino et al., 2002), which means that they can use an object according to its function—or example, bring a comb to the head or use a washcloth.

Figure 1

Disorders of consciousness in relation to sleep stages

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The figure can be viewed in the printed version at the University Library of Basel.

Note. Locked-in syndrome is not a disorder of consciousness. REM = rapid eye movement, NREM = non-REM. This graph has been adapted from Zieger, A. (2016) Neurologische Frühreha und Teilhabe von Komapatienten *Intensiv, 24*(01), 32–39. doi: 10.1055/s-0041-107574. and Bender, A. *et al.* (2015) Persistent Vegetative State and mininally consious steat: A systematic review and meta-analysis of diagnostic procedures, *Deutsches Ärzteblatt International*, *I*(14), 235–242. doi: 10.3238/arztebl.2015.0235.

The guideline from the DGNR and the European Academy of Neurology (EAN) suggests basing differential diagnosis on behavioral evidence (DGNR, 2022; Di Stefano et al., 2012; Kondziella et al., 2020). Today, imaging techniques increasingly play a role in the diagnostics of disorders of consciousness since sensory deficits, motor dysfunction, or diminished drive may lead to an underestimation of cognitive capacity (Bruno, Gosseries, et al., 2011; Giacino et al., 2002; Pistarini & Maggioni, 2021). Since the evidence for diagnostics with imaging techniques is still scarce, these methods are not recommended by the EAN guidelines (Kondziella et al., 2020). Correct diagnosis is central to designing an appropriate care plan, establishing an accurate prognosis, and providing detailed information to caregivers (Bender et al., 2015; Giacino et al., 2014; Pistarini & Maggioni, 2021). The high misdiagnosis rate is still a significant problem in the area of disorders of consciousness (Bender et al., 2015; Pistarini & Maggioni, 2021; Produturi et al., 2022). On the one hand, studies have thus evaluated new methods to better differentiate between MCS and vegetative states and have validated behavioral rating tools (Produturi et al., 2022). On the other, some researchers are reevaluating diagnostic criteria (Golden et al., 2022). A result of this is the relatively new and still dynamically changing diagnostic category of cognitive motor dissociation (DGNR, 2022; Schiff, 2015). These patients do not show any behavioral signs of consciousness, but they do exhibit detectable reactions to their environment in assessments with imaging techniques.

2.1.2 Treatment

Treating MCS patients is challenging (Giacino et al., 2014; Puggina et al., 2012) and involves many professionals from different fields of expertise (DGNR, 2022; Hodelín-Tablada, 2016). General supportive care is crucial for patients' recovery (Hodelín-Tablada, 2016). This care includes pain assessment and treatment, prevention of pressure ulcers and other secondary damages, nutrition, respiration, hygiene, and activities of daily living (DGNR, 2022; Hodelín-Tablada, 2016; Puggina et al., 2012). Information from nursing staffs is of utmost importance for the diagnostic, progress evaluation and for pain assessment due to their prolonged and intense contact with patients (Puggina et al., 2012). Further, physiotherapy and occupational therapy primarily focus on body functions such as passive-assistive movement for contracture prophylaxis, endurance training, and stretching (Giacino et al., 2014; Hellweg, 2012). Speech therapists evaluate and treat swallowing, respiration, and communication (Sautet et al., 2022).

The DGNR guidelines mention electrical and magnetic stimulation techniques. These invasive and noninvasive techniques aim to foster consciousness. Based on the literature, the DGNR (2022) only recommends transcranial direct-current stimulation to the left dorsolateral prefrontal cortex in treating MCS (Feng et al., 2020). The evidence for other electrical and magnetic stimulation techniques is too limited, so they are not recommended (DGNR, 2022; see Feng et al., 2020 for more). Pharmacological interventions are used to treat the source of the brain injuries or subsequent damages and in pain management (Puggina et al., 2012). Physicians also try to influence MCS patients' consciousness level with pharmacological interventions (e.g., with zolpidem and amantadine; DGNR, 2022; Georgiopoulos et al., 2010; Giacino et al., 2014; Hodelín-Tablada, 2016; Produturi et al., 2022).

Occupation, physio, and speech therapists and nursing staff are involved in multisensory-stimulation programs to protect patients from stimulation deprivation (DGNR, 2022; Hellweg, 2012; Latchem et al., 2016; Weaver et al., 2022b). The DGNR guidelines (2022) suggest that multisensory stimulation is an integral part of treating MCS patients. These programs are based on the assumption that an enriched environment promotes brain plasticity and supports recovery from injured brains (Di & Schnakers, 2018). Therapists provide patients with multisensory stimulation (e.g., auditory, verbal, visual, olfactory, tactile, and gustatory) to potentially stimulate affected neuronal networks and promote their reactivity to the environment (DGNR, 2022). The stimulation focuses on one single sense (unimodal stimulation) or is directed to all the senses using various stimuli (multimodal stimulation; Di & Schnakers, 2018). Music therapy is one form of multisensory stimulation (DGNR, 2022; Di & Schnakers, 2018; Lichtensztejn et al., 2014). In recent years, AAT has been increasingly used as another form of multisensory stimulation in treating MCS patients (Arnskötter et al., 2022; Blankenburg et al., 2011; Hediger, 2019).

2.2 Animal-assisted therapy (AAT)

2.2.1 Definition

AAT falls under the umbrella of animal-assisted interventions (AAI), which also include animal-assisted education, animal-assisted activities, and animal-assisted coaching (IAHAIO, 2018). AAT is a goal-oriented, planned, and structured therapeutic intervention directed or delivered by health professionals. The IAHAIO white paper states (2018) that these professionals need to be formally trained within the scope of the practice of their professions and to have adequate knowledge about the animals involved. AAT aims to enhance the physical, cognitive, behavioral, or socioemotional functioning of the particular human recipient. AAT can be conducted in either a group or an individual setting.

2.2.2 Animals in AAT

The key element of AAT is the presence of an animal. It is therefore of particular importance that animal welfare is guaranteed (IAHAIO, 2018). Animals should be selected for AAT based on whether they enjoy this type of activity and whether they have a suited personality for therapy (IAHAIO, 2018; Tierärztliche Vereinigung für Tierschutz e.V., 2021). Interactions should be fashioned to allow animals to interact with patients voluntarily and to be able to withdraw if needed (Gut et al., 2018; Hediger, Meisser, et al., 2019).

Working with patients can be stressful for the animals involved. The impact of AAT on the involved animals has been studied in dogs (Corsetti et al., 2019; L. Glenk, 2017; L. M. Glenk & Foltin, 2021), horses (De Santis et al., 2017), and guinea pigs (Gut et al., 2018; Wirth et al., 2020). For example, studies have measured increased behavioral stress, salivary cortisol, heart rate, and respiratory rate associated with AAI in dogs (L. M. Glenk & Foltin, 2021). But studies have also shown that interaction with humans in the context of AAI does not only have to have adverse effects. d’Angelo et al. (2021) found a decrease in salivary cortisol in shelter dogs after an AAI. Two studies on guinea pigs have also concluded that AAI could lead to enrichment and thus contribute to animal welfare if guinea pigs can interact voluntarily and withdraw from the situation (Gut et al., 2018; Wirth et al., 2020). This relationship is still, however, too poorly understood, and more research is needed to better understand how best to protect animal welfare during AAI.

2.2.3 Application and evidence of AAT

AAT has found application in a variety of settings. In the area of mental disorders, reviews and meta-analyses have shown medium to large effects for depression (Borgi et al., 2018; Souter & Miller, 2007) and posttraumatic stress disorder (Hediger et al., 2021; Leighton et al., 2022). Systematic reviews have shown an unclear picture of the efficacy of AAT for schizophrenia (Hawkins et al., 2019) and eating disorders (Fennig et al., 2022). AAT has shown promising effects for treating substance use disorder (Klemetsen & Lindstrøm, 2017). Further, it has exhibited positive effects in treating children with autism-spectrum disorders (Droboniku & Mychailyszyn, 2021; Nieforth et al., 2021; O’Haire, 2013). Much research exists on AAT for persons with dementia and older adults. Studies have shown psychological, psychosocial, cognitive, and behavioral benefits in this area (Babka et al., 2021; Chang et al., 2021; Chen et al., 2022; Franklin et al., 2022; Park et al., 2020). Animal-assisted approaches are also used in occupational and physical therapy to treat physical conditions (Şahin et al., 2018; Wood & Fields, 2021).

Studies on AAT have found a variety of positive effects. For example, studies with various patient groups have indicated that AAT reduces feelings of loneliness (Banks & Banks, 2002; Virués-Ortega et al., 2012), anxiety (Barker et al., 2003; Cole et al., 2007) and agitation (Filan & Llewellyn-Jones, 2006; Perkins et al., 2008). AAT can promote quality of life (Bachi et al., 2012; Beetz & Grebe, 2012; Davis et al., 2009), increase motivation (Hediger, Thommen, et al., 2019; Jones et al., 2019; Künzi et al., 2022), and improve pain management (Braun et al., 2009; Calcaterra et al., 2015; Ichitani

& Cunha, 2016; Stensland & McGeary, 2022). Further, AAT can stimulate social behavior (Filan & Llewellyn-Jones, 2006; Perkins et al., 2008; Villalta-Gil et al., 2009) and increase patients' willingness to trust psychotherapists with personal information (Schneider & Harley, 2006). The stress-reducing effects of AAT have been demonstrated by measuring physiological correlates (Barker et al., 2005; Beetz et al., 2011; Calvo et al., 2016; Cole et al., 2007). However, the quality of a lot of these studies is low, and more well-designed studies are needed to prove the efficacy of AAT and to replicate the found effects (López-Cepero, 2020; Serpell et al., 2017).

AAT is also increasingly used in neurorehabilitation (Muñoz Lasa et al., 2015). The use of an animal-assisted approach has been studied in poststroke patients (An & Park, 2021; Bunketorp-Käll et al., 2017; Lee et al., 2014), patients with aphasia (Macauley, 2006), patients with traumatic brain injuries (Gocheva et al., 2018; Hediger, Thommen, et al., 2019; Künzi et al., 2022; Theis et al., 2020), MCS patients (Bardl et al., 2013; Boitier et al., 2020; Hediger, Petignat, et al., 2019), and children with severe neurological impairments (Hediger et al., 2020). These studies have shown that the inclusion of animals affects attention, motivation, social behavior, therapy adherence, episodic memory, and positive emotions. Moreover, hippotherapy positively affects gait and balance in poststroke patients (Lee et al., 2014; Sunwoo et al., 2012). Still, studies on AAT in neurorehabilitation, especially for treating MCS, are sparse, and the trials have often not had a control group (Hediger, Thommen, et al., 2019; Weaver et al., 2022a). Further studies are needed. This thesis therefore aims to contribute to more high-quality research in the field of AAT in treating MCS.

Many theories have been advanced about how the effects of AAT might be produced (Beetz, 2017). But only a few of these theories have been sufficiently studied empirically. Many researchers have recently requested more studies on the mechanisms and theories of AAT (Kazdin, 2017; López-Cepero, 2020; Marino, 2012; Rodriguez et al., 2021; Serpell et al., 2017). Future research needs to examine what characteristics make AAT effective and how interventions must be designed and implemented to be effective. In addition, the quality of the studies on AAT has been criticized. Future studies must strive for better quality by following existing guidelines (Kazdin, 2017; Serpell et al., 2017). For this reason, this thesis includes two experimental control studies investigating the mechanisms of AAT in MCS patients by measuring brain activity and heart rate.

2.3 Methods

In our studies, we used several methods to investigate the effects of AAT and animal interaction on MCS patients. In study I, we used video coding, HR, HRV, and BAVESTA to measure the effects of AAT. In studies II and III, we used fNIRS to measure brain activity during contact with a dog or plush animal. In study III, we additionally analyzed HR and HRV and evaluated the level of consciousness with BAVESTA. The following sections describe these methods and provide more information about their application.

2.3.1 Video analysis

Various research disciplines have used video analysis for qualitative and quantitative analyses (Knoblauch et al., 2009). This umbrella term also includes interpretative procedures and standardized coding where data collection can be automated or done manually (Knoblauch et al., 2009, 2014). Especially in sociology, these interpretative procedures are applied in research on "natural" situations (Knoblauch et al., 2009). Other fields of application include medicine, law enforcement, sports, anthropology, behavioral biology, human–computer interaction, and education (Knoblauch et al., 2014; Leng et al., 2019). These fields mostly use standardized situations and coding procedures.

Video analysis is also often used in research on human–animal relationships. This method can lead to a better understanding of human–animal interactions (Beetz et al., 2011; Horowitz & Bekoff, 2007; Lethlean et al., 2017; Nagasawa et al., 2017) or study the influence of AAI on humans (Schretzmayer et al., 2017; Wesenberg et al., 2019) or animals (Cavalli et al., 2020; Grandgeorge et al., 2019; Gut et al., 2018). In research with patients with acquired brain injuries or MCS patients, video analysis is used to capture subtle behavioral changes (Affolter et al., 2009; Boitier et al., 2020; Gocheva et al., 2018; Lichtensztejn et al., 2014; O'Kelly et al., 2013)

Video analysis makes it possible to observe various situations and analyze specific variables. Compared with observations by the naked human eye, observations via video have the advantage that they are very detailed, complete, and accurate (Knoblauch et al., 2009). They allow interpretations of the data independent of the person collecting them (Knoblauch et al., 2009). Video analysis can therefore contribute to multifaceted evidence in the field of animal-assisted interventions (Kazdin, 2017). However, this type of analysis is particularly time-consuming and personnel intensive. In video analysis in which the rater cannot be blinded for the condition, possible bias is a potential problem. It is also important to consider that introducing a video camera into a situation can change the behavior because participants can feel observed or be distracted (Knoblauch et al., 2009).

The first step in analyzing videos involves creating a coding scheme based on theoretical assumptions. Creating the coding scheme directs focus to aspects relevant to the situation and allows raters to analyze the video. Usually, raters practice this scheme with a test video, and raters can be validated through interrater reliability (Knoblauch et al., 2014). Computer software facilitates coding and comparisons between raters. In study I, our coding scheme included the dimensions "eyes open/closed," "eye movement," "movement," "phonation," and "facial expression." Moreover, we coded the amount of each patient's physical contact with the animal and the amount of verbal and tactile stimuli offered by the therapist.

2.3.2 Basler Vegetative State Assessment (BAVESTA)

BAVESTA is an observation instrument in the German language designed to observe patients with impaired consciousness in daily clinical care. BAVESTA systematically maps the basal reactions and abilities of MCS patients in different everyday situations (Huber, Koch, Hund-Georgiadis, et al., 2014). An interdisciplinary team developed BAVESTA due to the lack of a sensitive observational instrument that can measure changes in different ability domains (Huber et al., 2012; Huber, Koch, Hund-Georgiadis, et al., 2014). The development team included the approaches of action and activity-oriented therapies in their considerations (Huber, 2014). BAVESTA is intended to facilitate holistic, interprofessional observation during the course of treatment (Huber, 2014).

BAVESTA consists of a physiological and functional status (Huber, 2014). In the physiological-status section, the patient's medical factors and basic abilities are recorded. The functional status is indispensable for monitoring the patient's progress and differential diagnosis. The functional status focuses on describing and assessing changing or recurring abilities, for example, participation, communication, and food management. Furthermore, BAVESTA also takes into account emotional response. The scales of BAVESTA are "vegetative control," "attention," "perception and orientation," “emotional responsiveness,” “nonverbal communication,” “verbal communication,” “motor skills,” “swallowing,” “information processing,” and “food management” (Hediger, Petignat, et al., 2019). BAVESTA was proven to be valid and reliable in past studies (Huber, Koch, Hund-Georgiadis, et al., 2014; Huber, Koch, Mäder, et al., 2014).

BAVESTA was primarily developed to evaluate patients’ progress in clinics (Huber, Koch, Mäder, et al., 2014). However, this assessment tool is also suited for research due to its sensitivity (e.g., Schaub et al., 2020). In order to perform an assessment, BAVESTA does not require that specific questions are asked or tasks are performed, unlike other behavioral assessment tools (Giacino et al., 2004). We therefore assessed consciousness levels with this instrument in our first and third studies.

2.3.3 Heart rate (HR) and heart-rate variability (HRV)

HR and HRV measurements reveal a lot about a person’s health. Not only does health status of the heart influence HRV, so too do poor sleep, smoking, physical overstrain, and mental health (Kemp & Quintana, 2013; Lohninger, 2017; Stein & Pu, 2012; Taralov et al., 2016). The autonomic nervous system controls all primary body functions, and HRV reflects its reactions (Lohninger, 2017). It is important to know that “a healthy heart is not a metronome” (Shaffer et al., 2014, p. 1). A healthy system shows flexibility and rapid adaptation (Shaffer & Ginsberg, 2017). HRV decreases during physiological or mental stress and increases during recovery. HRV is thus a marker of the physiological adaptability of the autonomic nervous system (Shaffer et al., 2014). The autonomic nervous system consists of the sympathetic and parasympathetic nervous systems (Lohninger, 2017; Taralov et al., 2016). Usually there is a dynamic balance between them, but extreme and long-acting stress may dysregulate the parasympathetic nervous system (Taralov et al., 2016).

The measurement of HRV is a noninvasive method for assessing the reactivity of the autonomic nervous system. HRV is the variation in the time between two adjacent heartbeats. This time interval from peak to peak is called the RR interval in original data and the NN interval in filtered data (Lohninger, 2017; Shaffer et al., 2014). A lot of different parameters exist in HRV analysis. For short-term measurements, the following parameters are reliable: the square root of the mean of the sum of the squares of the differences between adjacent NN intervals (RMSSD), low frequency (LF), and high frequency (HF; Heathers, 2014; Shaffer & Ginsberg, 2017). RMSSD and HF indicate parasympathetic activation, while LF can indicate sympathetic and parasympathetic activation (Taralov et al., 2016). High values in HF and RMSSD thus signify that a person is currently relaxed. Further, RMSSD can provide information about how quickly a person can recover after exercise (Goldberger et al., 2006).

In research on human–animal relationships, HR and HRV are physiological parameters that provide an indication of how a person or animal perceives the study situation. The few studies that have measured HR and HRV in people during AAT have had various designs, groups, and settings. It is therefore impossible to draw general conclusions about how AAT affects HR and HRV (Ein et al., 2018; Hediger, Petignat, et al., 2019; Kaminski et al., 2019; Motooka et al., 2006; Schretzmayer et al., 2017). The measurement of HR and HRV is also a powerful tool for obtaining additional information on MCS patients’ reactions to emotional and affective stimuli (Dolce et al., 2008; Gutiérrez et al., 2010; Keller et al., 2007; Lee et al., 2011; Machado et al., 2011; Riganello et al., 2011).

2.3.4 Functional near-infrared spectroscopy (fNIRS)

To design effective animal-assisted interventions, it is crucial to better understand the physiological mechanism behind human–animal interactions (Beetz, 2017; Borgi & Cirulli, 2016; López-Cepero, 2020). Brain activity is thus a crucial physiological correlate. Measuring brain activity in human–animal interactions is not that simple because neuroimaging devices such as functional magnetic-resonance imaging or positron-emission tomography confine participants during the measurement. It is not possible to sit or stand during interaction with an animal. Further, these technologies make disturbing sounds that might scare animals, and the devices are expensive and require expertise to operate. In our studies, we chose fNIRS to measure the neuronal response in the prefrontal cortex. Noninvasive fNIRS technology is particularly suited to measuring brain activity during human–animal interactions. It has already been used in several studies on human–animal interactions (Aoki et al., 2012; Arnskötter et al., 2022; Calcaterra et al., 2015; Kobayashi et al., 2017; Matsuura et al., 2020). This technique does not restrict subjects' movements, does not produce noise, is easy to handle, and is relatively inexpensive.

MCS patients have a limited understanding of their environment, so measuring brain activity can be an irritation to them. Because it can be applied without annoying noises and heavy devices, fNIRS is also advantageous for measurements with MCS patients. In MCS diagnostics research, efforts are being made to support and improve diagnostics using fNIRS technology (Rupawala et al., 2018). Further, researchers are also trying to improve therapy by measuring reactions to stimuli with fNIRS (Kempny et al., 2016; Zhang et al., 2018).

Functional near-infrared spectroscopy is a vascular-based neuroimaging technology. It makes use of tight neurovascular coupling. An increased blood flow follows changes in neuronal activity in the region of an activated cortical area (Ferrari & Quaresima, 2012). Oxygen transported to the activated region typically exceeds the oxygen utilization of neurons, leading to an increase in oxygen saturation in this region (Ferrari & Quaresima, 2012). An increase in oxygenated hemoglobin or oxygen saturation therefore suggests increased brain activity in the measured area.

Based on the characteristic hemoglobin-absorption spectra in the near-infrared range, it is possible to detect changes in the concentration of oxygenated and deoxygenated hemoglobin molecules in blood. Biological tissue is effectively transparent in the near-infrared light spectrum, with wavelengths between ~600 and 900 nm. In contrast to lipids, oxygenated and deoxygenated hemoglobin absorb near-infrared light maximally. An fNIRS device needs a set of light-emitting diodes on the scalp and an equal or larger set of detectors to measure changes in blood oxygen saturation. The emitted light passes through the tissue and gets absorbed and scattered by the molecules in the tissue. Scattered near-infrared light follows a trajectory back toward the surface of the scalp in a characteristic "banana" shape (Figure 2). The fNIRS device measures the amount of total hemoglobin and oxygen saturation of the blood from the intensity of the incoming light (Rupawala et al., 2018). Skin, scalp, skull, cerebrospinal fluid, gray matter, and white matter attenuate near-infrared light (Rupawala et al., 2018). Because of that, this technology uses multiple-distance optodes (i.e., a short-separation channel and a long-separation channel) to improve deep-tissue spatial resolution. Short-separation detectors are more sensitive to activity in the superficial layers. The long-separation detectors are sensitive to both the brain and superficial layers (Figure 2, Rupawala et al., 2018). Then superficial components can be effectively filtered out by regressing out the short-separation signals from the long-separation signals (Rupawala et al., 2018).

Figure 2

Illustration of banana-shaped profile of the sampled fNIRS signal at multiple source-detection distances

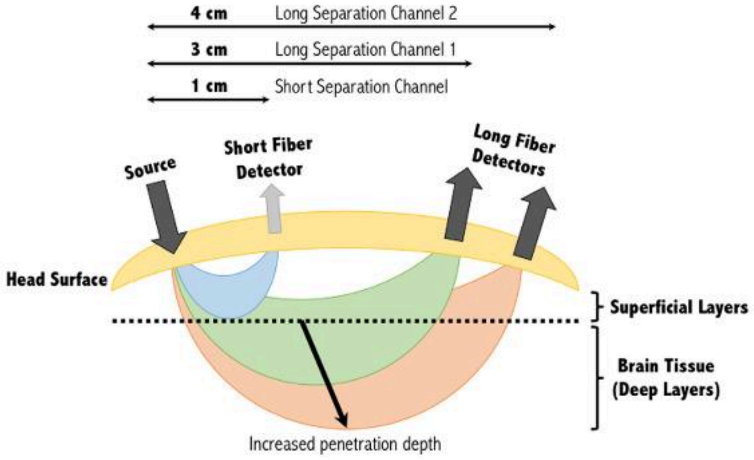

FIGURE 1 | Banana shape profiles of the sampled functional near-infrared spectroscopy signal at multiple source-detector distances. A single source and detector constitute the simplest NIRS channel. Depending on the source-detector separation distance, and the subjects' skull and scalp thicknesses, the light may or may not sufficiently penetrate the superficial layers to sample the deeper layers. A separation of 3 cm is commonly used, however, increasing this to 4 cm can increase the penetration depth of the light sampled tissues. Short separation channels are located within 1 cm of the source and can provide physiological (noise) data within the superficial layers. This activity can then be regressed from the long separation channel, resulting in a signal corresponding to activity solely within deep brain tissues. Figure adapted from Ref. (34). No permissions were required.

Note. From Rupawala, M., Dehghani, H., Lucas, S. J. E., Tino, P., & Cruse, D. (2018). Shining a light on awareness: A review of functional near-infrared spectroscopy for prolonged disorders of consciousness. *Frontiers in Neurology, 9*. https://doi.org/10.3389/fneur.2018.00350, licensed under CC BY 4.0

3 Aims of the thesis

AAT is being increasingly used in the treatment of MCS (Arnskötter et al., 2022; Blankenburg et al., 2011; Hediger, 2019). There are many reasons for applying AAT, but there is a lack of evidence (Boitier et al., 2020; Weaver et al., 2022a). Because of this, this thesis aimed to investigate the effectiveness and mechanisms of the integration of animals into MCS treatments.

1. *What are the effects of AAT on behavior, physiological responses, and the level of consciousness in MCS patients during therapy?*

Study I: We conducted a two-treatment multiple-period crossover study. MCS patients received eight AAT sessions and eight conventional sessions over four weeks. MCS patients are not able to give information about themselves during therapy. We were therefore interested in the influence of AAT on physiological and behavioral responses as well as on the level of consciousness. By using different measurement methods, we hoped to obtain a differentiated picture of the influence of AAT on the patients.

Mechanisms are still poorly understood in AAT research. We therefore aimed to conduct studies to better understand how AAT works.

2. *What is the effect of contact with a dog and different levels of proximity to the dog on the frontal brain activity of healthy adults?*

Study II: In a first step, we investigated the frontal brain activity of healthy adults during interaction with a dog. The frontal cortex is involved in social and emotional processes, executive functions, attentional control, working memory, and problem-solving (Grossmann, 2013; Kuo & Nitsche, 2015). This area also plays a role in approach motivation (Harmon-Jones & Allen, 1998) and positive affect (Burgdorf & Panksepp, 2006). We compared the contact with the dog to contact with a plush animal. In therapy, the dog is not always close enough to be petted. Sometimes, patients just observe it at a distance. We therefore also studied different levels of closeness. This study aimed to understand healthy frontal brain activation to then better understand the brain activation pattern in MCS patients.

3. *What is the effect of contact with a dog and different levels of proximity to the dog on frontal brain activity, HR, HRV, and the level of consciousness in MCS patients?*

Study III: We performed the same study design with MCS patients. In addition, we examined HR, HRV, and consciousness level in the MCS patients. The aim was to better understand the active mechanisms during AAT through different measurement methods.

4 Studies

4.1 Animal-assisted therapy for patients in a minimally conscious state: A randomized two-treatment multiperiod crossover trial

RESEARCH ARTICLE

Animal-assisted therapy for patients in a minimally conscious state: A randomized two treatment multi-period crossover trial

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Abstract

Objective

To investigate if animal-assisted therapy (AAT) leads to higher consciousness in patients in a minimally conscious state during a therapy session, measured via behavioral reactions, heart rate and heart rate variability.

Methods

In a randomized two treatment multi-period crossover trial, 10 patients in a minimally conscious state participated in eight AAT sessions and eight paralleled conventional therapy sessions, leading to 78 AAT and 73 analyzed control sessions. Patients' responses during sessions were assessed via behavioral video coding and the Basler Vegetative State Assessment (BAVESTA), heart rate and heart rate variability (SDNN, RMSSD, HF and LF). Data were analyzed with generalized linear mixed models.

Results

Patients showed more eye movements (IRR = 1.31, 95% CI: 1.23 to 1.40, $p < 0.001$) and active movements per tactile input during AAT compared to control sessions (IRR = 1.13, 95% CI: 1.02 to 1.25, $p = 0.018$). No difference was found for positive emotions. With BAVESTA, patients' overall behavioral reactions were rated higher during AAT (b = 0.11, 95% CI: 0.01 to 0.22, $p = 0.038$). AAT led to significantly higher LF (b = 5.82, 95% CI: 0.55 to 11.08, $p = 0.031$) and lower HF (b = -5.80, 95% CI: -11.06 to -0.57, $p = 0.030$), while heart rate, SDNN, RMSSD did not differ.

Conclusions

Patients in a minimally conscious state showed more behavioral reactions and increased physiological arousal during AAT compared to control sessions. This might indicate increased consciousness during therapeutic sessions in the presence of an animal.

OPEN ACCESS

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Trial registration

ClinicalTrials.gov NCT02629302.

Introduction

Acquired brain injuries can result in severe disorders of consciousness, such as minimally conscious state (MCS), with often serious lifelong consequences for patients and their families [1–3]. Early onset of rehabilitation is a crucial factor with the goal of enhancing the patient's consciousness by creating learning possibilities [4,5]. Current treatment concepts focus on stimuli which are activity-oriented and relevant for the individual patients, because personally and emotionally relevant stimuli induce higher-level activation in patients with disorders of consciousness [6,7]. Since animals are highly emotionally relevant [8], animal-assisted therapy (AAT) is an increasingly utilized approach in neurorehabilitation. AAT is a goal-directed intervention, in which a trained animal is an integral part of therapeutic activities [9]. Although there is anecdotal practical evidence [10], and AAT is becoming increasingly common in treatment of disorders of consciousness, empirical evidence from randomized controlled studies is lacking. Therefore, the aim of this study was to examine the effect of AAT on consciousness in patients in a minimally conscious state compared to conventional standard therapy in a randomized controlled trial. To investigate effects on patients' consciousness, we assessed patients' behavior and measured physiological arousal via heart rate and heart rate variability.

Methods

Participants

Subjects were 10 inpatients in a minimally conscious state. All patients were in stationary neurorehabilitation in a Swiss rehabilitation clinic, aged 17 to 71 years ($M = 47.20$, $SD = 19.36$) and diagnosed with acquired brain injury with either traumatic (N = 4) or non-traumatic causes (N = 6). Secondary diagnoses were not considered. Severity of the disorder of consciousness was assessed via the original JFK Coma Recovery Scale (CRS) [11,12] that does not include all of the behavioral criteria necessary to diagnose the minimally conscious state. The diagnosis was therefore based on clinical assessment by the responsible physician according to the Aspen diagnostic criteria [13] and to Bruno and colleagues [14] for the division of MCS+ and MCS-. MCS+ is characterized by the presence of command following, intelligible verbalization or gestural or verbal yes/no responses. MCS- patients in contrast only show minimal levels of behavioral interaction characterized by the presence of non-reflex movements. Patients were eligible for participation in the study if the scores and clinical assessment indicated a minimally conscious state following an acquired brain injury. Exclusion criteria were medical contraindications, such as phobias and allergies, assessed via interviews with relatives. The data was collected from May 2015 until April 2017. Fig 1 shows the CONSORT flowchart.

Standard protocol approvals, registrations, and participant consents

The screening process involved the family members of the patients as well as the responsible physicians and therapists. The legal representative of the patients provided written informed consent. The human-related protocols were approved by the Ethics Committee for Northwest and Central Switzerland and the animal-related protocols were approved by the Veterinary Office of the Canton Basel-Stadt, Switzerland. AAT was performed according to the guidelines

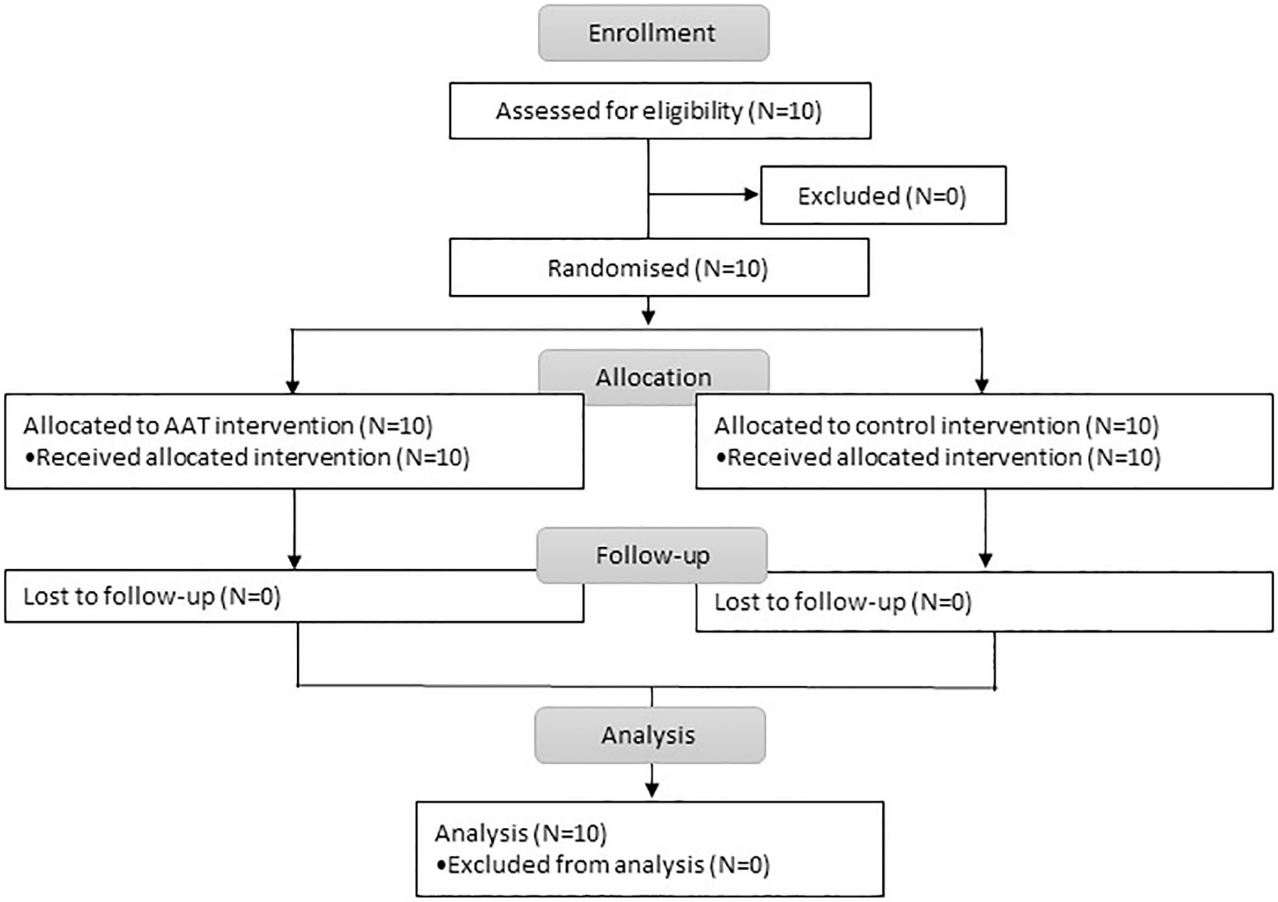

Fig 1. CONSORT flowchart.

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of the International Association of Human Animal Interaction Organizations (IAHAIO) to ensure patient safety and animal welfare [9]. No therapy session had to be ended early and no adverse incidents occurred. After participating in the study, all patients had the possibility to continue with AAT. The study was registered at ClinicalTrials.gov (Identifier: NCT02629302).

Study design and procedure

The study was designed as a randomized two treatment multi-period crossover design to evaluate the immediate effects of the different interventions on patient reactions. Standardized therapy sessions that integrated an animal served as experimental condition and are referred to as AAT sessions. In the control condition, paralleled, comparable standardized therapy sessions without the presence of an animal (treatment as usual) were used. Each patient participated in 16 therapy sessions over a period of 4 weeks (*N* AAT = 8, *N* control = 8). Sessions lasted for approximately 15 minutes and were held four times a week, twice with an animal and twice without an animal. Each control session was paralleled with an AAT session such that two sessions in two consecutive weeks were as similar as possible regarding the involved therapist, day of the week, time of day and therapeutic activity. All participants were allocated randomly to start with either AAT or a control session. Allocation sequence was generated via a random number generator by the principal investigator who also enrolled and assigned participants to interventions. Some of the originally planned 160 sessions were cancelled due to illness of patient or therapist, and for some sessions data was lost due to technical problems. In total, we coded the behavior of 151 sessions (*N* AAT = 78, *N* control = 73) and analyzed assessment data of 136 sessions (*N* AAT = 69, *N* control = 67) and heart rate data of 115 sessions (*N* AAT = 61, *N* control = 54). All AAT and control sessions were held in a therapy room within the therapy animal facility at the rehabilitation center. The patients were transported to the therapy room by wheelchair. Patients wore a heart rate monitor belt on their chest which continuously measured heart rate and heart rate variability during the session. All sessions were videotaped and at the end of each session, the behavior of the patients was assessed via the Basler Vegetative State Assessment by the therapists. Prior to the study start, a suitable animal was selected for each patient according to preference and abilities. Included species were dogs, guinea pigs and rabbits. All animals were trained for AAT, had experience working with patients in a minimally conscious state, and were kept and handled according to the IAHAIO standards [9]. Guinea pigs and rabbits were put into a table cage where they could interact with patients or retreat at will. During the AAT sessions, therapeutic activities were performed by physically guiding the patient's hands according to the Affolter concept [15]. Examples of therapeutic activities were: brushing a dog, cutting vegetables and feeding them to the rabbits or guinea pigs, or opening a box with herbs and feeding them to the rabbits or guinea pigs. Paralleled control sessions consisted of therapeutic interventions with basic activities selected from a range of occupational therapy assignments. These activities were also performed according to the Affolter concept. Corresponding examples of control activities were: brushing a fake fur, preparing food by cutting vegetables and putting them in a bowl, or opening an empty box and filling it.

Behavioral analysis via video coding

As primary outcome, the patient's reactions was assessed via behavioral video coding. Therapy sessions (*N* = 151) were videotaped with a handheld camera (Sony HDR-CX240) and analyzed with a behavioral coding system software (Observer XT 12, Noldus). Analyses were done continuously, defining each second of the video with the different variables as present or not for state behavior variables. We calculated the percentage of the duration of each state variable in relation to the observed time period of a therapy session. Count variables were coded only if they occurred, and the total occurrence within a therapy session was calculated. All videos were coded according to a strict ethogram defined by detailed descriptions of the behaviors with inclusion and exclusion examples. The coding scheme was developed for the purpose of this study. As basis, 11 existing paper-pencil behavioral assessment tools in German and English for patients with disorders of consciousness were screened. Items were pooled and reduced to behaviors that could be observed during video analysis and that occur according to a stimulus during a therapeutic situation. Our coding scheme included the dimensions "eyes open/closed", "eye movement", "movement", "phonation" and "emotion" (operationalized via facial expression). Moreover, we coded the amount of verbal and tactile stimuli offered by the therapist as well as the amount of the patient's physical contact with the animal. Inter-rater reliability was measured by Cohen's kappa for all coded variables. Before coding the actual data, each rater achieved an inter-rater reliability of $k > 0.80$. Inter-rater reliability ranged between 0.83 and 0.99 indicating excellent agreement among coders.

Basler Vegetative State Assessment

The Basler Vegetative State Assessment (BAVESTA) [16], a behavioral assessment tool for patients with disorders of consciousness, was used as an additional tool to measure behavioral reactions of patients during each therapy session and served as secondary outcome. This study used 22 of the original 33 items, targeting behaviors that are observable during a short period of time, and adjusted the calculations of total short-term mean score and short-term subscores accordingly, with a range from 0 (behavior is not shown) to 5 (behavior is consistently shown). After each therapy session, the therapist assessed the patient with this short-term BAVESTA.

Heart rate and heart rate variability recording

Heart rate (HR) and heart rate variability (HRV) were measured using non-invasive HR monitoring belts (Polar® RS800CX, Polar® Electro Oy) as further secondary outcomes. The recorded inter-beat intervals were analyzed with Kubios HRV analysis software version 3.0.2 (Biosignal Analysis and Medical Imaging Group, University of Kuopio, Finland). In each therapy session, a 5-minute recording was selected. In control sessions, the 5-minute sequence was taken from the middle of the whole session. For AAT sessions, the duration of interaction between the patient and the animal was identified via the videos and the 5-minute sequence was taken from the middle of the interaction phase. Before processing, all RR-series were visually checked and, when necessary, artifacts were corrected. If the number of corrected beats was higher than 5%, the data was excluded from analysis (*N* AAT = 1, *N* control = 2). We also excluded data if the total recording or the interaction between the patient and the animal was shorter than 5 minutes (*N* = 1). We calculated the following HRV parameters: time domain: the standard deviation of all normal-to-normal RR intervals (SDNN, ms) and root-mean square differences of successive RR intervals (RMSSD, ms); and frequency domain: relative power of the low frequency (LF) and high frequency (HF) band in normal units.

Statistical analysis

Behavior analysis was performed using generalized linear mixed models. Count data were modeled as rates using a Poisson distribution and the logarithm of the duration of the sessions as an offset variable. The primary models included only the outcome variable and the treatment type as single predictor. Participant IDs were included as random effect to account for multiple observations within each subject. The Incident Rate Ratio (IRR) was used as effect size. The model holds under the assumption that there is no time effect which might be violated. Therefore, we checked the robustness of the model by fitting a second model equivalent to the previous one but including session number as a categorical fixed effect. During data inspection we noticed that the therapists behave differently in AAT and control sessions, primarily with respect to the number of tactile inputs. Because those inputs trigger most of the patients' reactions, we fitted a third model that includes time as well as the log of tactile inputs. For descriptive statistics, the number of observed count behaviors (count variables) was transformed into rate per time ((n/time)*100 sec) and rate per tactile inputs ((n/tactile inputs)* 100). To analyze the effect of AAT on BAVESTA scores and HR/HRV parameters as secondary outcomes, generalized linear mixed models with condition as fixed effect and the individual patient as random effect with the mean difference (b) as effect size was used. All variables were visually checked to detect extreme values (histogram and Q-Q-plot). Model diagnostics of linear mixed models included visual checks for normality of residuals and homogeneity of residuals. All residuals were approximately normally distributed with the exception of RMSSD, which was therefore log-normal transformed. No data were excluded except for HR/HRV data with corrected beats greater than 5% and recordings where patient and animal interacted for less than 5 minutes. Sample size was estimated based on clinical experience and on a pre-analysis of an ongoing study. The significance level was set at the 5% level and all statistical analyses were performed using SPSS, Version 24, and R, Version 3.5.1.

Table 1. Sample characteristics.

Subject	Gender	Age	Etiology	Main pathology	Days since event	Admission	CRS	Diagnosis*
1	Male	71	TBI	Polytrauma	265	Initial rehabilitation	22	MCS+
2	Female	60	nonTBI	Subarachnoid hemorrhage	114	Initial rehabilitation	22	MCS+
3	Male	61	nonTBI	Cerebrovascular ischemia	103	Initial rehabilitation	21	MCS+
4	Female	27	nonTBI	Cerebrovascular ischemia	102	Initial rehabilitation	15	MCS-
5	Male	27	TBI	Polytrauma	2654	Readmission	17	MCS-
6	Male	17	TBI	Polytrauma	120	Initial rehabilitation	17	MCS-
7	Male	70	TBI	Subarachnoid hemorrhage	83	Initial rehabilitation	17	MCS+
8	Male	57	nonTBI	Subarachnoid hemorrhage	138	Initial rehabilitation	16	MCS+
9	Male	37	nonTBI	Hypoxic and metabolic encephalopathy	105	Initial rehabilitation	14	MCS-
10	Male	45	nonTBI	Hypoxic-ischemic encephalopathy	4979	Readmission	17	MCS-

TBI: traumatic brain injury, CRS: JFK Coma Recovery Scale total score at study start, MCS: minimally conscious state, refers to the original, not the revised instrument with a maximum total score of 25,

*diagnosis according to the Aspen Workgroup criteria and the criteria of Bruno et al., 2011.

https://doi.org/10.1371/journal.pone.0222846.t001

Results

Two female and eight male participants, between age 17 to 71 with an average age of 47 years ($M = 47.20$, $SD = 19.36$), participated in this study. CRS values at study start ranged between 14 and 22. All patients were diagnosed with MCS in a clinical assessment by the responsible physician according to the Aspen diagnostic criteria [13] and the criteria of Bruno and colleagues [14]. Table 1 summarizes the principal clinical and demographic characteristics of participants. Table 2, S1 and S2 Tables provide an overview of the intervention characteristics.

Behavior analysis

There were more tactile inputs from therapists during control sessions than during AAT sessions (control: M = 148.04, SD = 71.51, AAT: M = 114.19, SD = 57.35; IRR = 0.74, 95% CI: 0.68 to 0.81, $p < 0.001$), while verbal inputs from therapists did not differ significantly between conditions (control: M = 33.86, SD = 21.45, AAT: M = 28.66, SD = 18.86; b = -0.05, 95% CI: -0.11 to 0.01, $p = 0.074$).

Patients showed a significantly higher rate of eye movement of 5 movements per 100 seconds during AAT compared to control therapy sessions with a rate of 4 (IRR = 1.17, 95% CI: 1.11 to 1.24, $p < 0.001$). This effect was also present for the models that include time or time

Table 2. Intervention characteristics.

Variable		AAT	Control	AAT (%)	Control (%)
Therapy time	Morning	28	25	52.83	47.17
	Afternoon	50	47	51.55	48.45
Variable		**AAT M**	**Control M**	**AAT SD**	**Control SD**
Video length*		887.50	855.79	199.51	189.24
Total number of tactile input		114.19	148.04	57.35	71.51
Total amount of verbal input*		251.77	287.00	184.36	209.35

AAT: animal-assisted therapy, M: mean, SD: standard deviation,

* in seconds

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and tactile input. The rate of eye movement per tactile input even increased by a factor of 1.7 during AAT compared to control therapy sessions. The rate of total movements per 100 seconds was higher during control therapy sessions and decreased from 3.5 to 3 during AAT (IRR = 0.85, 95% CI: 0.80 to 0.91, $p < 0.001$). However, this effect reversed when time and tactile inputs were added to the model (IRR = 1.08, 95% CI: 1.00 to 1.16, $p = 0.048$) with a rate of 23 during AAT and a rate of 20 movements per 100 seconds during control therapy sessions. While there was no difference in self-initiated (active) movements in model one or two, patients showed significantly more self-initiated movements per tactile input during AAT compared to control therapy sessions (IRR = 1.13, 95% CI: 1.02 to 1.25, $p = 0.018$). The opposite effect was found for reactive movements of the patients. The rate of reactive movements per time was lower during AAT sessions (IRR = 0.74, 95% CI:0.67 to 0.80, $p < 0.001$) but this difference disappeared when looking at the rate per tactile input. Patients showed a higher amount of phonation during AAT compared to control therapy sessions (IRR = 1.92, 95% CI: 1.32 to 2.78, $p < 0.001$) but again, this effect disappeared when time and tactile inputs were included in the model. There was no difference regarding positive emotions, operationalized via positive facial expressions. Negative emotions were reduced during AAT compared to control therapy sessions but this difference was only statistically significant when the amount of tactile inputs were taken into account (IRR = 0.35, 95% CI: 0.20 to 0.59, $p < 0.001$, see Table 3).

Basler Vegetative State Assessment

In the BAVESTA, the patients overall behavioral reactions were rated higher during AAT sessions compared to control sessions (b = 0.11, 95% CI: 0.01 to 0.22, $p = 0.038$). While there was no difference regarding the subscales "attention", "verbal communication" "emotional reactions" or "motor reactions", we found significantly higher perception and information processing scores (perception: b = 0.21, 95% CI: 0.01 to 0.41, $p = 0.041$; information processing: b = 0.19, 95% CI: 0.03 to 0.34, $p = 0.023$) as well as significantly more nonverbal communication (b = 0.19, 95% CI: 0.05 to 0.33, $p = 0.010$) during AAT compared to standard therapy sessions (see Table 4).

Heart rate / heart rate variability

Heart rate as well as heart rate variability parameters SDNN and RMSSD did not differ significantly between AAT and control sessions (see Table 5). In contrast, patients showed significantly higher LF (b = 5.82, 95% CI: 0.55 to 11.08, $p = 0.031$) and lower HF values (b = -5.80, 95% CI: -11.06 to -0.57, $p = 0.030$) during AAT compared to control sessions.

Discussion

We present the first randomized controlled trial of patients in a minimally conscious state assessing behavioral reactions and arousal during AAT and control therapy sessions. AAT led to significantly more eye movements, self-initiated movements as well as movements in total compared to control therapy sessions in the systematic behavior analysis. This is in line with results of Bardl and Bardl's case-study [10] that documented improvements in visual exploration, spontaneous reactions and target-oriented movements in a patient in a persistent vegetative state during the presence of a dog, as well as Jones, Rice and Cottons' review who showed increased engagement during therapy due to AAT in adolescents with mental health disorders [17]. We did not find differences in positive emotional reactions which somewhat contrasts to previously published results. In the BAVESTA, patients had a higher total score during AAT indicating higher consciousness, and they showed more nonverbal communication and higher

Table 3. Analyzed behaviors during AAT and control therapy sessions.

							Model 1			**Model 2**			**Model 3**		
Behavior	**Setting**	**N**	**M**	**SD**	**Rate time**	**Rate input**	**IRR**	**95% CI**	**p-value**	**IRR**	**95% CI**	**p-value**	**IRR**	**95% CI**	**p-value**
Eye movement[+]	Control	73	26.71	29.68	3.99	18.04	1.17	1.11 to 1.24	<0.001*	1.17	1.10 to 1.25	<0.001*	1.31	1.23 to 1.40	<0.001*
	AAT	78	35.47	37.46	5.05	31.07									
Movement total	Control	73	30.03	31.79	3.51	20.28	0.85	0.80 to 0.91	<0.001*	0.87	0.82 to 0.93	<0.001*	1.08	1.00 to 1.16	0.048*
	AAT	78	26.06	29.17	2.94	22.82									
Movement active	Control	73	14.95	14.68	1.75	10.10	0.97	0.89 to 1.05	0.441	0.95	0.86 to 1.04	0.240	1.13	1.02 to 1.25	0.018*
	AAT	78	14.97	17.55	1.69	13.11									
Movement reactive	Control	73	15.08	24.68	1.76	10.19	0.74	0.67 to 0.80	<0.001*	0.78	0.71 to 0.87	<0.001*	0.98	0.88 to 1.10	0.756
	AAT	78	11.09	17.65	1.25	0.71									
Phonation	Control	73	0.60	1.61	0.07	0.41	1.92	1.32 to 2.78	<0.001*	1.38	0.87 to 2.18	0.173	1.23	0.74 to 2.07	0.423
	AAT	78	0.96	4.51	0.11	0.84									
Positive facial expression	Control	73	1.19	2.65	0.14	0.81	1.14	0.85 to 1.52	0.382	1.10	0.79 to 1.54	0.567	1.05	0.72 to 1.55	0.795
	AAT	78	1.23	32.48	0.14	1.09									
Negative facial expression	Control	73	2.10	6.57	0.24	1.42	0.86	0.68 to 1.08	0.200	0.71	0.48 to 1.06	0.096	0.35	0.20 to 0.59	<0.001*
	AAT	78	1.78	6.11	0.20	1.54									

AAT: animal-assisted therapy, N: number of analyzed sessions, M: mean (absolute), SD: standard deviation, rate time: rate per 100 seconds, rate input: rate per 100 tactile inputs, IRR: Incident Rate Ratio, CI: confidence interval, Model 1: therapy type as fixed effect, Model 2: therapy type and time as fixed effect, Model 3: therapy type, time and log tactile input as fixed effect,

*statistically significant,

[+] log of the time when eyes were observable was used as offset to analyze eye movement.

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Table 4. Basler Vegetative State Assessment after AAT and control therapy sessions.

Scale	**Setting**	**N**	**M**	**SD**	**b**	**95% CI**	**p-value**
BAVESTA total	Control	67	1.91	0.30	0.11	0.01 to 0.22	0.038*
	AAT	69	2.03	0.49			
Attention	Control	67	3.28	0.64	0.12	-0.10 to 0.34	0.289
	AAT	69	3.39	0.84			
Perception	Control	67	2.60	0.62	0.21	0.01 to 0.41	0.041*
	AAT	69	2.82	0.83			
Emotional reactions	Control	67	1.75	0.93	0.27	-0.01 to 0.56	0.061
	AAT	69	2.01	1.18			
Nonverbal communication	Control	67	1.78	0.44	0.19	0.05 to 0.33	0.010*
	AAT	69	1.97	0.66			
Verbal communication	Control	67	0.63	0.27	-0.04	-0.13 to 0.04	0.321
	AAT	69	0.60	0.28			
Motor reactions	Control	67	1.09	0.36	0.07	-0.04 to 0.19	0.219
	AAT	71	1.15	0.46			
Information processing	Control	67	1.90	0.52	0.19	0.03 to 0.34	0.023*
	AAT	69	2.08	0.68			

Scales are adapted and only include items targeting short-term behavior. AAT: animal-assisted therapy session, N: number of analyzed sessions, M: mean, SD: standard deviation, b: mean difference, CI: confidence interval,

*statistically significant

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Table 5. Heart rate and heart rate variability.

Parameter	Setting	N	M	SD	b	95% CI	p-value
HR, bpm	Control	54	80.22	17.22	0.898	-1.143 to 3.23	0.446
	AAT	61	80.81	16.75			
SDNN, ms	Control	54	22.82	18.79	-1.37	-5.41 to 2.67	0.503
	AAT	61	20.34	15.66			
RMSSD, ms+	Control	54	22.20	29.12	-0.06	-0.26 to 0.15	0.601
	AAT	61	17.90	22.67			
LFnu	Control	54	64.77	27.22	5.82	0.55 to 11.08	0.031*
	AAT	61	68.87	24.00			
HFnu	Control	54	35.12	27.16	-5.80	-11.06 to -0.57	0.030*
	AAT	61	31.04	23.94			

HR: mean heart rate; bpm: beats per minute; SDNN: the standard deviation of all normal-to-normal RR intervals; RMSSD: root-mean square differences of successive RR intervals; pNN50: percentage of successive normal RR intervals exceeding 50 ms; LF: low frequency; HF: high frequency; nu: normalized units; PA: physical activity, AAT: animal-assisted therapy session, N: number of analyzed sessions, M: mean, SD: standard deviation, b: coefficient, CI: confidence interval,

+absolute data is presented, while the model was run with ln transformed data;

*statistically significant

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perception and information processing scores. No verbal communication was shown by most of the patients, so it is not surprising that we found no difference. The BAVESTA subscales "attention", "emotional reactions" and "motor reactions" did not differ between the conditions. The observed effects of higher behavior reactions in the presence of an animal, measured using two different approaches, indicate a higher level of awareness [18], one of the two components of consciousness [19]. While the previous study involved a dog [10], our study documents that guinea-pigs and rabbits might have same beneficial effects as dogs.

We observed differences in the frequency domain heart rate variability parameters. During AAT sessions, HF values were significantly lower and values of LF were significantly higher compared to conventional standard therapy. The decrease in HF values reflects decreased activity of the parasympathetic nervous system, while the increase in LF values is associated with increased activity of the sympathetic nervous system [20,21], so both outcomes indicate higher physiological activity and an increase in arousal in the presence of an animal. Along with awareness, arousal is the other component of consciousness [19]. Increased arousal could therefore reflect a higher level of consciousness and indicate an underlying process that might explain the observed behavioral effects of the patients in the presence of an animal. Lowered values of HF have also been associated with mental activity and mental stress [22,23]. But the observed HF in the AAT condition was within the range of normal values [20] and the reduction might also indicate an increase in arousal associated with positive emotions, excitement and emotional involvement [24,25] rather than distress. However, since patients in a minimally conscious state are highly vulnerable, further research is needed to clarify these effects. Our findings are in line with a previous investigation documenting lower values of HF in autistic children following interaction with a live dog compared to a robotic dog [26]. However, there are mixed outcomes from studies, documenting no effects [27] or even higher heart rate variability as a result of an interaction with an animal [28]. We found no statistically significant difference in heart rate between AAT and treatment as usual. This is in contrast to studies documenting decreases in heart rate during animal-assisted interventions for a broad range of populations [29] or an increased heart rate in hospitalized children with chronic disorders prior to and following dog assisted therapy as compared to control therapy sessions [30].

Neither participants nor raters responsible for video coding could be blinded to the conditions. The crossover design of this study only allowed for detecting short-term effects of AAT on behavioral and heart rate measurements during therapy sessions, and the small sample size limits the study outcomes and warrants further trials with more patients. Strengths of this study are inclusion of a paralleled control condition, behavior measured with different approaches and inclusion of a physiological parameter to identify underlying mechanisms. Moreover, our results showed that the presence of an animal can also influence the behavior of the involved therapists and that patients' reactions should be interpreted in relation to the behavior of the therapists. This is a relevant aspect that should be taken into account in further study designs.

Conclusion

Our results indicate that AAT is a feasible approach to increase behavioral reactions and arousal in patients in a minimally conscious state. Integration of animals could be used to increase consciousness of these patients and lead to achieving a relevant therapeutic goal. Although this result is promising, the data are preliminary and it is necessary to further investigate whether AAT might be an effective approach to improve therapeutic effects of neurorehabilitation for patients in a minimally conscious state.

Supporting information

S1 Fig. Rate of eye movement during AAT and control therapy sessions over the course of the time.
(TIF)

S2 Fig. Rate of eye movement for each patient over the course of time.
(TIF)

S3 Fig. Rate of eye movement in the presence of different animals.
(TIF)

S4 Fig. Rate of total movement during AAT and control therapy sessions over the course of the time.
(TIF)

S5 Fig. Rate of total movement for each patient over the course of time.
(TIF)

S6 Fig. Rate of active movement during AAT and control therapy sessions over the course of the time.
(TIF)

S7 Fig. Rate of active movement for each patient over the course of time.
(TIF)

S8 Fig. Correlation matrix of the analyzed behaviors.
(TIF)

S1 Table. Sessions held by the different therapists.
(DOCX)

S2 Table. Allocation of therapy sessions to different days of the week.
(DOCX)

S3 Table. Correlations of the analyzed behaviors.
(DOCX)

S1 File. CONSORT checklist.
(DOC)

S2 File. Trial protocol.
(PDF)

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4.2 Effects of contact with a dog on prefrontal brain activity: A controlled trial

PLOS ONE

RESEARCH ARTICLE

Effects of contact with a dog on prefrontal brain activity: A controlled trial

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Abstract

Background

There is a broad range of known effects of animal contact on human mental and physical health. Neurological correlates of human interaction with animals have been sparsely investigated. We investigated changes in frontal brain activity in the presence of and during contact with a dog.

Methods

Twenty-one healthy individuals each participated in six sessions. In three sessions, participants had contact with a dog, and in three control sessions they interacted with a plush animal. Each session had five two-minute phases with increasing intensity of contact to the dog or plush animal from the first to the fourth phase. We measured oxygenated, deoxygenated, and total hemoglobin and oxygen saturation of the blood in the frontal lobe/frontopolar area with functional near-infrared spectroscopy (SenSmart Model X-100) to assess brain activity.

Findings

In both conditions, the concentration of oxygenated hemoglobin increased significantly from the first to the fourth phase by 2.78 μmol/l (CI = 2.03–3.53, $p < .001$). Oxygenated hemoglobin concentration was 0.80 μmol/l higher in the dog condition compared to in the control condition (CI = 0.27–1.33, $p = .004$). Deoxygenated-hemoglobin concentration, total hemoglobin concentration, and oxygen saturation showed similar patterns.

Conclusion

Prefrontal brain activation in healthy subjects increased with the rise in interaction closeness with a dog or a plush animal. Moreover, interaction with a dog stimulated more brain activity compared to the control condition, suggesting that interactions with a dog can activate stronger attentional processes and elicit more emotional arousal than interacting with a nonliving stimulus.

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1 Introduction

Although the effects of contact with animals on human mental and physical health have received increasing attention [1–5], the neurophysiological correlates of these effects are not yet fully understood [6, 7]. These correlates are, however, highly relevant to understanding the mechanisms underlying human–animal relationship [8–11] and to designing effective animal-assisted interventions. Authors of several studies have reported that positive interaction with a dog reduces stress parameters, such as blood pressure, heart rate, or cortisol level [12–14], and leads to an increase of neurochemicals associated with bonding or affiliation, such as β-endorphin, oxytocin, and prolactin [15–17]. However, the results for these parameters remain inconclusive [13, 18, 19].

Investigations into neurological correlates in the context of human–animal interaction are scarce. Initial studies have investigated neurological reactions to interactions with animals using neuroimaging techniques [20–27]. Most of these studies presented images of animals, whereas only a small number of investigations have addressed the effects of real animals. A positron-emission-tomography (PET) study observed that brain areas associated with stress and sympathetic arousal were less activated in the presence of a familiar dog than in a relaxing condition [23]. Other investigators have observed lateralization with greater activity in the right frontopolar area while petting a horse compared to petting a plush animal, seeing a horse, or seeing a plush animal [24]. Another study measuring hemodynamic response found that participants reacted with activation in the left inferior frontal gyrus while petting a cat [25]. Moreover, children showed higher activity in the prefrontal cortex in an attention task after interacting with a dog than after interacting with a robot dog [26]. Similarly, in a small pilot study, participants had a stronger brain reaction to a live animal than to a mechanical toy animal [27]. While these studies provide first insights into neurological correlates of the human–animal interaction, additional research is needed to understand what happens in different forms of human–animal interactions. The knowledge gained will be crucial for conducting effective animal-assisted interventions [28]. Dogs are the most common animals used in animal-assisted interventions [4, 29, 30]. The aim of this study was to investigate neurological correlates of different forms of human–dog contact in an animal-assisted intervention setting using a strong study design. To ensure that the results would be as valuable as possible for practical application, we investigated the reactions of the participants in an animal-assisted intervention setting in a clinic and involving direct contact and interaction with a dog. This also enabled us to control for different amounts of contact with the dog.

Interacting with an animal is a social situation that is emotionally relevant to most people [7, 31–34]. Several reviews have identified the prefrontal cortex as the key region for different aspects of social cognitive processing, such as theory of mind/mentalizing [35] and understanding self and others [36]. Activity in the prefrontal cortex is thus important for investigating the underlying mechanisms of human–animal interactions.

Our study aimed to investigate brain activation in the prefrontal cortex of healthy subjects with functional near-infrared spectroscopy (fNIRS) in a controlled trial. We compared different forms of interaction with a dog and different forms of interaction with a plush animal. We expected, first, that the increase of closeness in contact with a dog or plush animal would correlate with an increased amount of stimulation and therefore also with increased brain activity. Second, we hypothesized that participants would exhibit higher brain activity in the dog condition compared to the control condition with the plush animal.

2 Materials and methods

2.1 Study design

The study had a controlled, within-subject crossover design with repeated measurements. Participants were measured during six standardized sessions (1–6) consisting of three sessions with a live dog and three control sessions with a plush animal. The six sessions took place within 2 weeks. The sequence of the conditions within these six sessions was influenced by the presence of the dog and therefore only partly randomized. The study design was approved by the local ethics committee, Ethics Commission Northwest and Central Switzerland (Project ID 2017–00540), and by the Veterinary Office of the Canton of Basel-Stadt, Switzerland (No. 2713) and was registered at clinicaltrials.gov (NCT03341325). The study design followed the Animals (Scientific Procedure) Act 1986, European Directive EU 2010/63, and the guidelines for handling animals in research as outlined by the Association for Studies on Animal Behavior and the Society for Animal Behavior. All sessions were conducted according to the guidelines of the International Association for Human–Animal Interaction Organizations and the Helsinki guidelines [37, 38]. We planned to compare the results of this study with a study population of patients with severe disorders of consciousness in a future trial, so the study design complied with the requirements for measuring a group of patients with severe disorders of consciousness.

2.2 Participants

Twenty-one healthy subjects (10 women, 11 men) participated in this study. Participants were over 18 years old and without allergies or phobias toward dogs. They were recruited with flyers at the Faculty of Psychology at the University of Basel and via an advertisement on the university's website. We obtained written informed consent from every participant before the study started. The sample size was determined a priori based on data from a previous study [39] and with regard to the pilot character of this study.

2.3 Procedure

The sessions were held in a room at the neurorehabilitation center REHAB Basel in Switzerland from February 2018 until July 2018. During the experiments, the participants sat upright on a Bobath therapy couch. They faced a white wall located at a distance of 1.5 m. The study staff attached two fNIRS sensors to measure oxygen saturation on the participants' foreheads. Three of the six sessions per participant were conducted in the presence of a dog and three with a plush animal (see Fig 1). The participants therefore had a first, second, and third contact with both the dog and the plush animal. All sessions were videotaped, and heart rate and electrodermal activity were recorded. Each session consisted of five 2-minute phases, which were always conducted in a similar way and in the same order in both the dog and plush-animal conditions. Before each phase, the study staff verbally instructed the participant according to a standardized protocol. The first phase served as a baseline where the participant looked straight at the white wall and relaxed (neutral 1). In the next phase, the participant watched a dog or a plush animal from a distance of 1 m (watching). The dog or plush animal was placed or asked to lie on a mat and a blanket on a height-adjustable table. Then the dog lay down next to the participant on the couch or the plush animal was placed on the participant's thigh. The participant could passively feel the animal but was not yet allowed to pet it (feeling). Next, the participant petted the dog or the plush animal (petting). Finally, there was a second neutral phase where the participant again looked at the white wall while the dog or the plush animal was out of sight (neutral 2). Each phase concluded after 2 minutes, and then there was a short

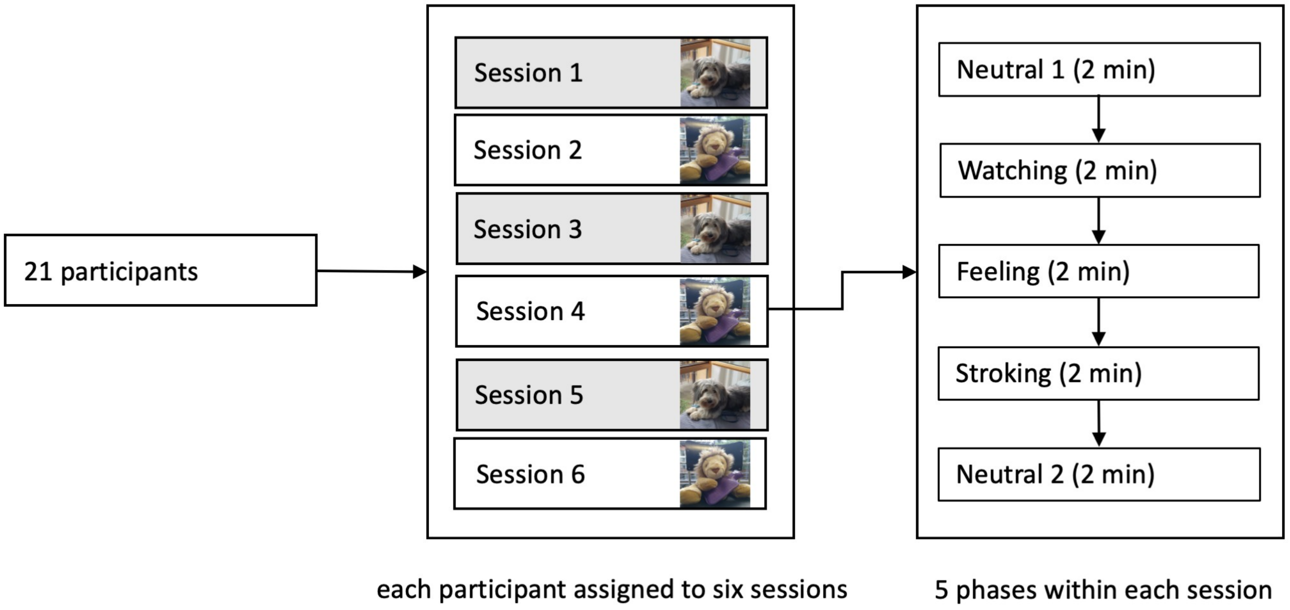

Fig 1. Study procedure.

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break in which the study staff prepared the room for the next phase. Interactions between each participant and the dog or the plush animal were standardized and comparable regarding the amount of contact.

For every participant, we scheduled three of the sessions in the morning and three in the afternoon to control for time of day. The order of the phases was not counterbalanced because the same design was also used for patients with severe disorders of consciousness. These patients need time and a lot of context to understand a situation. A random order with a sudden increase of contact to the animal would not be ethically justifiable. For the same reason, it was not possible to measure a pretask and posttest baseline for each phase.

2.4 Dogs

The dogs participating in the study were used to human contact and trained to work with patients in a hospital setting. The dogs were a female Jack Russel (6 years of age), a female Goldendoodle (4 years of age), and a female Golden Retriever (4 years of age). Each dog participated in a maximum of two sessions in a row. The dogs and their owner were in the room before the sessions started, which enabled them to become acquainted with the room and to feel safe. The dog owner was present throughout the session and was responsible for handling the dog but was instructed not to interact with the participant during the measurements. The dogs were trained to lie silently on the table and beside the participant in contact with the participant's thigh, but they could choose their position themselves. Owners monitored their dogs for signs of stress and predetermined stop criteria. Due to the highly standardized situations and interaction, the behavior of the dog was comparable between the sessions within and between participants.

2.5 Plush animal

For the control sessions, we used a lion plush animal. The plush animal (58 × 40 × 20 cm) contained in its body a hot water bottle that was filled with warm water before the sessions started to control not only for the sensation of soft fur but also for the body temperature and weight of a dog. We introduced the plush animal to participants as "Leo."

2.6 Functional near-infrared spectroscopy

We chose fNIRS to measure the response in the prefrontal cortex as it is particularly suited for investigating the neuronal correlates of such a complex social situation of human–animal interaction. fNIRS has been used as a noninvasive technique to measure brain activity within the context of human–animal interactions [24, 25, 27, 40, 41]. Compared to functional magnetic-resonance imaging (fMRI) or PET, participants are not confined to a scanner but can sit or stand during measurements. This makes the test situation more comparable to clinical situations. fNIRS also has other advantages: there are no disturbing sounds, and the device is easy to handle. fNIRS is a vascular-based neuroimaging technology that measures the oxygen saturation of hemoglobin and changes in total hemoglobin concentration (tHb) based on the characteristic hemoglobin-absorption spectra in the near-infrared range. This technology relies on the well-known tight neurovascular coupling, which induces changes in oxygen saturation and tHb in response to neuronal activity. An increase in oxygenated hemoglobin (O_2Hb) in the region of an activated cortical area mirrors increased brain activity [42].

We recorded percent oxygen saturation (%) and tHb (g/dl) in the prefrontal cortex using a Nonin fNIRS device (SenSmart Model X-100). Two sensors of the device (Model 8004CA Sensors–Adhesive) were placed right and left of the midline on the forehead as close to the hairline as possible and then attached with an adjustable band. This corresponded to locations F1, F3, F2, and F4 on the frontopolar area according to the international 10–20 system and to the Brodmann areas 9, 10, and 46. The wavelength of the infrared light was 730, 760, 810, and 880 nm, and measurements were recorded at a frequency of 0.25 Hz. After recording, data were transferred to a laptop using SenSmart software (version 1.0.1.0).

Within this study, we also measured other physiological endpoints such as heart rate, heart-rate variability, and skin conductance. These data will be published separately.

2.7 Data processing and analysis

We converted the data from g/dl to μmol/l based on the molar mass of hemoglobin of 64458 g/mol. We calculated the concentration of O_2Hb and HHb from raw data. To exclude unreliable data due to measurement errors, two raters independently rated plots of the data for reliability. The raters were blinded for the condition. Conflicts were resolved by a third rater (R. M.).

For all included data, we calculated the mean concentration of O_2Hb, HHb, and tHb and mean oxygen saturation in each phase. To do so, we cut the data from one session into segments of five 2-minute phases at the markings. The data between the phases was not used. We were interested in changes from phase to phase, so we subtracted the mean of the first phase from each following phase within the same session for each participant.

O_2Hb reflects the neuronal-discharge frequency, while HHb reflects the quantity of recruited neurons [43]. We chose O_2Hb as the primary outcome because O_2Hb more directly reflects task-related cortical activation than does HHb [44]. HHb, tHb, and oxygen saturation served as secondary outcomes. For the primary and secondary outcomes, we conducted prespecified linear mixed-effect models and used the mean difference as the effect size. Within the models, condition and phase were used as fixed effects, and an intercept for the participant was used as the random effect. We conducted the same models again with visibility of the dog owner as a fixed effect.

We conducted explorative analyses because repetition of contact with the dog or the plush animal seemed to influence the outcome. Within these nonprespecified linear mixed-effect models, condition and contact (first, second, or third contact between participant and dog or plush animal) were used as fixed effects. Moreover, we included an interaction term and an intercept for the participant as the random effect.

We visually checked the normality (q-q plot, histogram of residuals), linearity, and homoscedasticity (residuals vs. fitted plot), and influential outliers (leverage and Cook's distance). Leverage was checked with the R package influence.ME [45]. The significance level was set at .05. All analyses were conducted with R 4.1.0 [46] and R package lme4 [47].

3 Results

Of the 21 participants measured between January and July 2018, one participant dropped out after one session. We conducted 119 of the 126 planned sessions (Fig 2). Of these 119 sessions, we excluded data from one channel for 55 sessions and from both channels for 10 sessions due to low data quality (Fig 2). Six of these 10 completely removed datasets originated from one participant who dropped out of the analysis, while the other removed datasets were distributed among different participants. We thus analyzed 108 sessions (53 dog conditions, 55 plush-animal conditions) of 19 participants with at least one of the two channels available.

These 19 participants compromised nine women and 10 men. The mean age was 32.4 years (*SD* = 12.8) and did not differ between the sexes (estimate = 2.2, CI = −15.4–11.1, *p* = .732). On average, we analyzed 2.89 control sessions and 2.84 dog sessions per participant. The number of analyzed sessions per participant did not differ between the conditions (*M* = 2.87, *SD* = 0.34; estimate = −0.05, CI = −0.18–0.28, *p* = .642). The first session was significantly more often the dog condition (14/19, *p* = .025), and the second session was significantly more often the control condition (14/18, *p* = .025). In sessions three to six, the number of sessions per condition did not differ significantly. In two-thirds of the sessions in the dog condition, the participant could see the dog owner during the measurement. No adverse or unintended effects in participants or in the involved dogs occurred during data collection.

3.1 Primary analysis

With increased stimulation, oxygenated hemoglobin (O_2Hb) in the prefrontal lobe increased significantly from phase neutral 1 to phase petting by 2.78 μmol/l (CI = 2.03–3.53, $p < .001$). After removal of the stimulation in phase neutral 2, O_2Hb stayed constant and was still significantly higher compared to phase neutral 1 (estimate = 2.91 μmol/l, CI = 2.16–3.65, $p < .001$).

O_2Hb was 0.80 μmol/l higher in the presence of the dog compared to in the presence of the plush animal (CI = 0.27–1.33, *p* = .004). The difference between the conditions was highest in the phase petting (Fig 3A). This result was not influenced by the visibility of the dog owner.

3.2 Secondary analysis

3.2.1 Deoxygenated hemoglobin. When stimulation increased, deoxygenated hemoglobin (HHb) in the prefrontal lobe decreased significantly from phase neutral 1 to the petting phase by 1.23 μmol/l (CI = −1.75 to −0.72, *p* = .003). After removal of the stimulation in phase neutral 2, HHb stayed constant and was still significantly lower compared to phase neutral 1 (estimate = −1.20 μmol/l, CI = −1.72 to −0.69, *p* = .005).

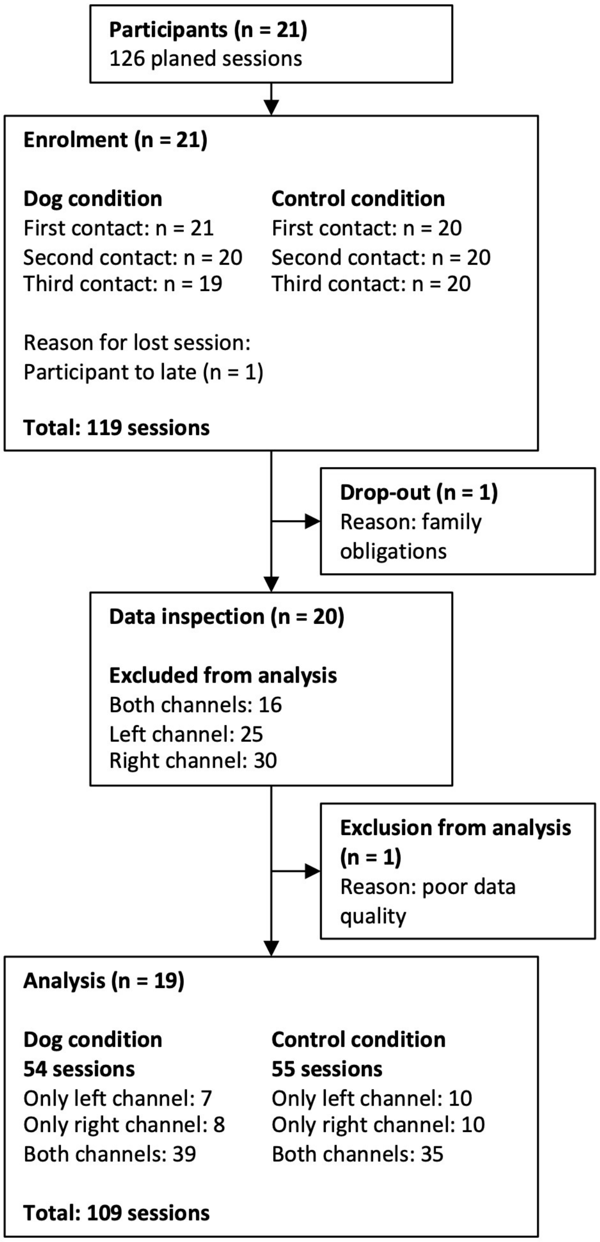

Fig 2. Flow diagram of the study.

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HHb tended to be lower in the presence of the dog compared to in the presence of the plush animal (estimate = -0.35 μmol/l, CI = −0.71–0.02, $p = 0.064$). The difference was highest in phase neutral 2 (Fig 3B). This result was not influenced by the visibility of the dog owner.

3.2.2 Total hemoglobin. When stimulation increased, total hemoglobin (tHb) in the prefrontal lobe increased significantly from phase neutral 1 to the petting phase by 1.54 μmol/l (CI = 1.08–2.01, $p < .001$). After removal of the stimulation in phase neutral 2, tHb stayed constant and was still significantly higher compared to phase neutral 1 (estimate = 1.70 μmol/l, CI = 1.24–2.17, $p < .001$).

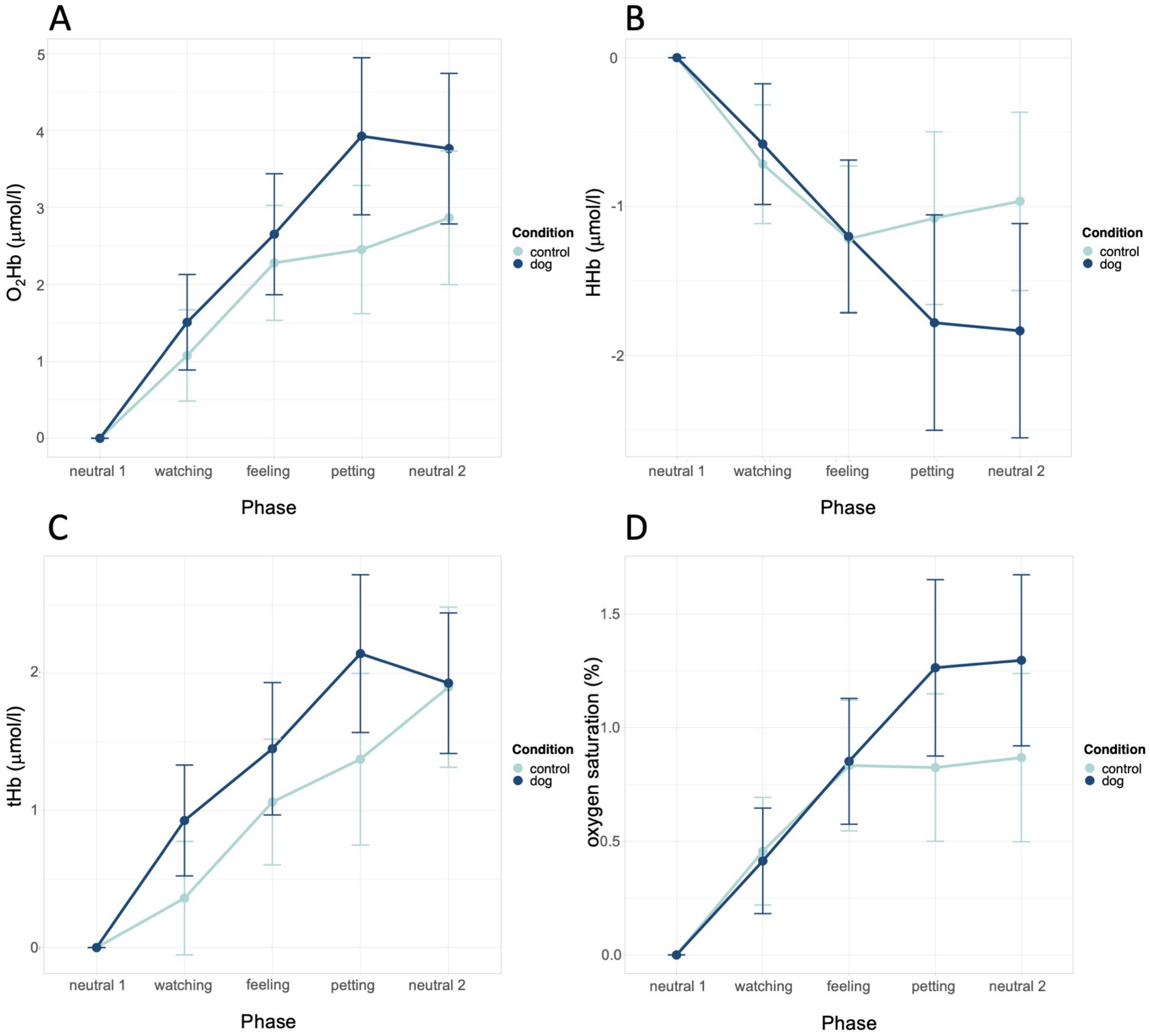

Fig 3. Effects of condition and phase on O_2Hb, HHb, tHb, and oxygen saturation. (A) O_2Hb, (B) HHb, (C) tHb, and (D) oxygen saturation. Error bars denote confidence interval. Data is shown as relative change from phase neutral 1.

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The concentration of tHb was significantly higher by 0.45 μmol/l in the presence of the dog compared to in the presence of the plush animal (CI = 0.12–0.78, $p = .008$). The difference was highest in the petting phase (Fig 3C). In the dog condition, tHb was lower when the participant could see the dog owner than when the dog owner was out of sight (estimate = −0.84, CI = −1.33 to −0.33, $p < .001$). The results of the other factors in the model, including visibility of the dog owner, remained unchanged.

3.2.3 Oxygen saturation. When stimulation increased, oxygen saturation in the prefrontal lobe increased significantly from phase neutral 1 to the petting phase by 0.93% (CI = 0.64–1.22, $p < .001$). After removal of the stimulation in phase neutral 2, saturation stayed constant and was still significantly higher compared to phase neutral 1 (estimate = 0.97%, CI = 0.68–1.27, $p < .001$).

Oxygen saturation was significantly higher by 0.21% in the presence of the dog compared to in the presence of the plush animal (CI = 0.00–0.42, $p = .047$). The difference was highest in phase neutral 2 (Fig 3D). The visibility of the dog owner had no effect.

3.3 Explorative analysis

During the first contact (first session), there was no relevant difference in O_2Hb between the dog condition and the plush-animal condition (estimate dog = 2.15 μmol/l, estimate plush animal = 2.60 μmol/l). We observed a significant interaction, which indicates that with repeated contact over time, there was an increasing difference between the dog condition and the plush-animal condition (second contact: $p = .001$, third contact: $p = .023$, Table 1, Fig 4A).

There was no relevant difference in HHb between the dog condition and the plush-animal condition during the first contact (dog = −0.98 μmol/l, plush animal = −1.16 μmol/l). We observed a significant interaction between the condition and number of contacts with an effect on HHb in the second contact but not in the third (second contact: $p = .002$, third contact: $p = .695$, Table 1, Fig 4B).

During the first contact, there was no relevant difference in tHb between the dog condition and the plush-animal condition (dog = 1.17 μmol/l, plush animal = 1.44 μmol/l). We observed a significant interaction effect on tHb, which indicates that the difference between the dog condition and the plush-animal condition increased with repeated contact over time (second contact: $p = .053$, third contact: $p = .001$, Table 1, Fig 4C).

There was no relevant difference in oxygen saturation between the dog condition and the plush-animal condition during the first contact (dog = 0.77%, plush animal = 0.80%). We observed a significant interaction between the condition and number of contacts with an effect on oxygen saturation in the second contact but not in the third (second contact: $p = .010$, third contact: $p = .823$, Table 1, Fig 4D).

4 Discussion

This study compared the prefrontal brain activity of healthy adults during contact with a dog and contact with a plush animal. Prefrontal activity increased with increased intensity of contact with a dog or a plush animal. This confirms our first hypothesis that more stimulation correlates with higher brain activity. It also corroborates previous studies linking closer contact with animals or control stimuli with increased frontal brain activation [24, 25, 27].

The participants had higher prefrontal brain activity when they interacted with a dog than when they interacted with a plush animal. This confirms our second hypothesis. In the presence of the dog, O_2Hb, tHb, and oxygen saturation were significantly higher while HHb tended to be lower compared to the control condition. This pattern indicates increased oxygen consummation in prefrontal areas and thus higher brain activation in the presence of a dog [48, 49]. This result is in line with previous studies. An fNIRS pilot study with patients in a minimally conscious state and healthy controls found that three of four participants showed a higher hemodynamic response when stroking a live animal (dog, rabbit, or guinea pig) compared to stroking a mechanical toy [27]. Children who underwent a 20-min session with a therapy dog after surgery showed faster electroencephalogram diffuse beta activity, while children in the control group who received standard postoperative care showed no beta activity [41]. The passive infrared hemoencepahlography signal of children who performed an attention test was significantly higher after the interaction with a real dog compared to after the interaction with a robotic dog [26].

Table 1. Marginal effects of condition by number of contacts.

		95% CI	
	Estimate	**Lower limit**	**Upper limit**
O_2Hb			
Dog condition			
First contact	2.15	1.35	2.95
Second contact	3.43	2.62	4.25
Third contact	3.36	2.52	4.19
Plush-animal condition			
First contact	2.60	1.78	3.41
Second contact	1.62	0.81	2.44
Third contact	2.25	1.45	3.05
HHb			
Dog condition			
First contact	−0.98	−1.53	−0.43
Second contact	−1.70	−2.26	−1.14
Third contact	−1.31	−1.89	−0.74
Plush-animal condition			
First contact	−1.16	−1.72	−0.60
Second contact	−0.44	−1.00	0.12
Third contact	−1.32	−1.87	−0.76
tHb			
Dog condition			
First contact	1.17	0.66	1.68
Second contact	1.74	1.22	2.26
Third contact	2.04	1.50	2.57
Plush-animal condition			
First contact	1.44	0.92	1.96
Second contact	1.18	0.66	1.70
Third contact	0.94	0.42	1.45
Oxygen saturation			
Dog condition			
First contact	0.77	0.47	1.08
Second contact	1.10	0.78	1.41
Third contact	0.98	0.66	1.30
Plush-animal condition			
First contact	0.80	0.49	1.11
Second contact	0.45	0.13	0.76
Third contact	0.95	0.64	1.26

Marginal effects were estimated by condition and contact number, and an intercept for participant as random effect.

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4.1 Comparison with other studies

We found that prefrontal brain activity increased with a rise in the intensity of contact with a dog or a plush animal. From watching the animal to feeling it passively to actively petting the animal, the interactional closeness increased and, with it the intensity of stimulation as well as the number of senses involved. This led to an increase in brain activation. We detected the same pattern in a pilot study with a similar study design and comparable forms of contact to an animal [27]. In line with this, another study revealed higher frontopolar activity when

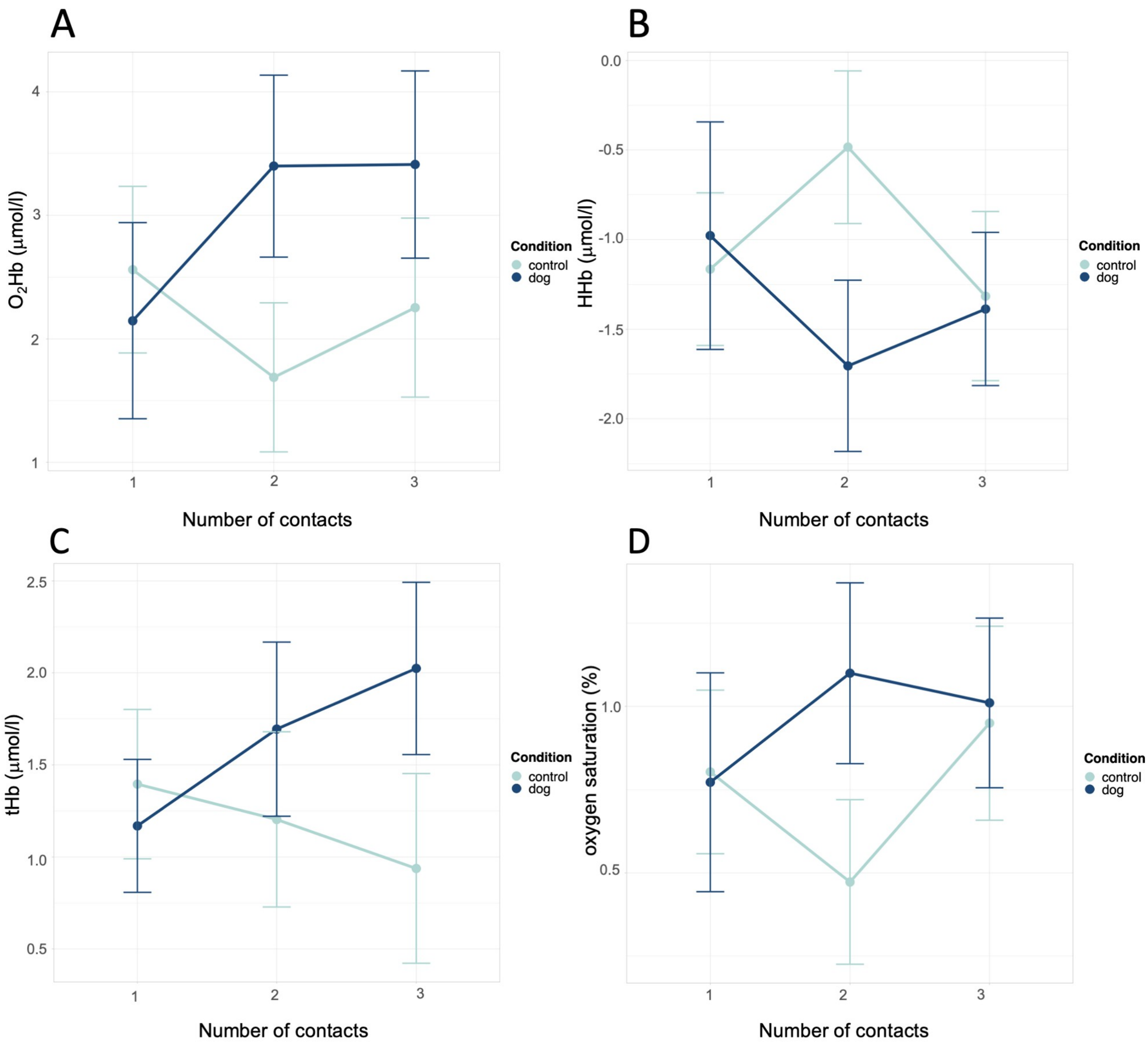

Fig 4. Effects of condition and number of contacts on O_2Hb, HHb, tHb, and oxygen saturation. (A) O_2Hb, (B) HHb, (C) tHb, and (D) oxygen saturation. Error bars denote confidence intervals. Data are shown as relative change from phase neutral 1. The data for phase neutral 1 are not included in the presented means.

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participants stroked a plush animal or a miniature horse compared to just seeing them [24]. Moreover, stroking a cat stimulated higher activation of the inferior frontal gyrus compared to just touching a cat [25].

We observed clear differences in brain activity in the presence of the dog compared to the plush animal. This contrasts with a study reporting that healthy participants had similar activation patterns of the inferior frontal gyrus when petting a cat or a plush animal [25]. That study also noted that female and male participants showed different activation patterns. A PET study observed deactivation in the left middle frontal gyrus, the right fusiform gyrus, the left putamen, and the thalamus in healthy participants during the presence of a familiar dog compared to a resting condition [23]. The authors suggested that this deactivation signaled a reduction in emotional stress induced by the presence of the familiar dog. These results cannot be directly compared with our results, because fNIRS cannot reach areas like the putamen or the thalamus. Nevertheless, the tasks in our design might have been more activating and our imaging technology less stressful.

Other studies identified lateralized activation patterns in frontal areas during petting a horse or a cat compared to a plush animal [24, 25]. For example, participants exhibited lateralization in the right frontopolar cortex while petting a real horse compared to no lateralization while petting a plush horse[24]. The authors attributed the lateralized activity to differences in function of the left and right frontal regions. We did not test for lateralization in the present study, but visual inspection of our data does not suggest lateralization. However, future studies should address the possibility of lateralization.

Summing up, the current literature indicates that frontal brain activation patterns in humans correlate with the level of interaction with animals. Our results show that this is also the case with a live dog compared to a plush animal and that the intensity of interaction is relevant. Looking at a dog correlates with the lowest frontal activity, while passive contact with more and active stroking correlates with the highest frontal activity.

4.2 Brain activity across sessions

In the second neutral phase, brain activation did not return to the level of the first neutral phase. We assume that activation persisted in both conditions and did not decline as quickly as expected. We therefore assume that the subjects were basically more activated in the second neutral phase than in the first.

We also found a pattern in O_2Hb and tHb levels indicating that prefrontal brain activity increased with repeated contact to the dog while it did not increase with repeated contact in the plush-animal condition. There seems to be a difference, especially between the first and the second contact with the dog suggesting that familiarity might play a different role in interactions with live and plush animals. However, the other two outcomes (HHb and oxygen saturation) did not show an increase with repeated contact and do not support this hypothesis. This result of this explorative analysis therefore needs to be further investigated in future studies.

4.3 Hypothesis about underlying mechanisms

We have different hypotheses explaining our result of higher activation in the dog condition compared to the plush-animal condition. The prefrontal cortex is known to be involved not only in executive functions such as attention control, working memory, and problem-solving but also in social and emotional processes [50, 51]. It has reciprocal connections with brain regions that are involved in emotional processing such as the amygdala and higher-order sensory regions within the temporal cortex [51].

Social interactions with animals are highly emotionally relevant for a majority of people [7, 31–34]. We thus hypothesize that interacting with the dog led to higher emotional involvement in the participants compared to interacting with the plush animal. This higher emotional involvement correlates with higher frontal activity. Previous studies using neuroimaging or behavioral outcomes support this hypothesis of higher emotional arousal by live animals [21, 39, 52–55].

Potential higher emotional involvement might in parallel also lead to more attention for and a stronger focus on the dog compared to the plush animal. Several authors have shown that interactions with animals can promote attention and activate attention networks [20, 21, 26, 56, 57]. Attentional processes such as attentional set-shifting or attention monitoring are located in the frontal cortex [50, 58, 59].

Another consequence of higher emotional arousal or of touching a live dog can be increased physiological arousal [60]. This arousal can be related to a positive state, but interacting with a dog could also cause higher stress than interacting with a plush animal. Further parameters such as heart rate or skin conductance are needed to distinguish physiological arousal from other processes such as emotional involvement and attention. Further, the increase in activation might also have been caused by a greater cognitive load as a dog is a more complex stimulus than a plush animal [61, 62]. A last hypothesis might be that motor control played a role [63, 64] as stroking a live dog might demand different motor adaption in the participants.

In sum, there are several possible explanations for our results that would benefit from being investigated in the future. Based on the recent literature, we hypothesize that emotional involvement might be a central underlying mechanism of the neurological frontal brain correlates of human–animal interaction. We therefore suppose that the increase in brain activity in the dog condition over the three contacts might be explained based on a developing relationship between the participant and the dog. Familiarity and a relationship with the dog could have raised the salience of the dog, kept the participant's attention on the dog's behavior, and increased emotional arousal during the experiment. An fMRI study on pet attachment found a correlation between pet attachment and brain activity in areas involved in increasing attention and attentional load [21].

4.4 Implications for clinical practice

It is important that future research tries to replicate our findings because they could have important implications for clinical practice such as animal-assisted therapy. Our results indicate that interactions with a dog might activate more attentional processes and elicit stronger emotional arousal than comparable nonliving stimuli. Moreover, it seems that especially close and active physical contact to a familiar dog might promote social attention in humans. This is especially relevant for patients with deficits in motivation, attention, and socioemotional functioning. High involvement is a crucial factor for learning, as has been shown in several studies [65, 66]. For example, it has been shown that emotional relevance is central [67].

If patients with deficits in motivation, attention, and socioemotional functioning show higher emotional involvement in activities connected to a dog, then such activities could increase the chance of learning and of achieving therapeutic aims. These hypotheses should be investigated in future studies, as they suggest that integrating animals into therapeutic interventions might be a promising approach for improving emotional involvement and attention.

4.5 Limitations and strengths

Blinding was not possible due to the nature of the study. Moreover, randomizing the sequence of the conditions was not completely possible because of the irregular presence of the dogs. It should also be noted that there was an additional person present during the presence of the dog. The dog owners did not interact with the participants during the measurements, but participants could see the dog owners in two-thirds of the sessions. For most of the outcomes, visibility of the dog owner had no effect, but this factor should be controlled in future studies. Moreover, we did not assess attitudes toward animals. The sample size reflects the pilot character of the study. The results thus must be interpreted carefully.

While fNIRS technology has several advantages, measurements of regional cerebral oxygen saturation can be affected by skull thickness, gyration, hemoglobin concentration, or extracranial blood flow [68, 69]. We decided to use fNIRS because it allowed the study to take place in a natural environment and did not produce any sounds that could irritate the participants or the dogs. Since we repeatedly measured the outcomes for each condition and had a within-subject design where each participant served as their own control, these issues are limited. In addition, the probe design with a multidistance approach naturally reduces sensitivity to extracranial effects [70]. Drifts are also not likely because the fNIRS device corrects for that. Further, O_2Hb concentration and oxygen saturation show the same pattern, which would not be the case if there was a drift. It could be argued that we should have detrended for the difference from the first to the second neutral phase. But the carry-over effect in the second neutral phase is not the same in the dog condition and the plush-animal condition. Detrending could thus have covered up effects that we assume reflect real changes.

The strengths of the study are that we investigated the effects of live dogs on neuronal activation instead of dogs presented via photos or videos and that we controlled for different levels of closeness and physical contact between the participant and the dog or the plush animal. We also carefully controlled the environmental factors in the room, the wording of the instructions, and the time of day of the sessions. Interactions between participants and the dog or the plush animal were standardized and kept as similar as possible. With regard to the plush animal used in the control condition, we controlled for tactile inputs such as its fur, warmth, and weight, and it was named and called by a name just as the dogs were called by a name in the study.

4.6 Future research

Future studies should take into account participants' characteristics like gender, pet ownership, and attitude toward animals. It has been shown that participants who loved horses exhibited lateralization while petting a horse. In contrast, participants who only "kind of liked" horses did not exhibit lateralization [24]. A study on brain activity during cat petting indicated a gender difference [25], and in an fMRI study, pet owners showed greater activation than non-pet owners while looking at images of unfamiliar pets [21]. Future research should replicate our findings with larger sample sizes and different participants. Moreover, the effects of direct interaction with a live dog could be investigated with other neuroimaging techniques that can measure brain activity in different brain areas simultaneously. It is important to further understand the effect of familiarity and relationship as well as of the type of interaction with the dog. To do so, future studies could use different interactions such as speaking to the dog or include reciprocal interactions such as playing with the dog. Familiarity and relationship should be systematically controlled by involving unfamiliar dogs, unfamiliar dogs with repeated contact, and participants' own pet dogs. It would be interesting to compare the effects of different animal species or of different features of dogs' appearances and to use different control conditions. Obtaining subjective ratings of the different interactions such as perceived pleasantness, stress, and relationship with the dog or plush animal should be introduced in the future. Moreover, imposing a concurrent cognitive task might be useful to see if the presence of a real dog has facilitating effects on behavioral performance. Moreover, it is important to test our hypotheses regarding clinical relevance. Future studies should involve patients with deficits in motivation, attention, and socioemotional functioning and investigate if the same results can be found regarding brain activity and also look at therapeutic outcomes such as achieving rehabilitation goals.

With regard to standardization, we recommend implementing a manipulation test to check for motor functions, to randomize the phases, and to control for the number of people in the room, the position of the dog owner, and the handedness of the participants. If it is possible, we would recommend implementing a pretask and posttask baseline. The length of the neutral phase should be longer to avoid carry-over effects.

5 Conclusion

The present study demonstrates that prefrontal brain activity in healthy subjects increased with a rise in interactional closeness with a dog or a plush animal. Moreover, participants had higher brain activation in the presence of a dog compared to in the presence of a plush animal. This indicates that interactions with a dog might activate more attentional processes and elicit stronger emotional arousal than comparable nonliving stimuli. Our results also suggest that a relationship with the dog might be a crucial factor. The results are clinically relevant for patients with deficits in motivation, attention, and socioemotional functioning. Integrating animals into therapeutic interventions might therefore be a promising approach for improving emotional involvement and attention.

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4.3 Effects of contact with a dog on prefrontal brain activity in patients in a minimally conscious state: A controlled crossover trial

Effects of contact with a dog on prefrontal brain activity in patients in a minimally conscious state: A controlled crossover trial

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Abstract

Background: First studies indicate that animal-assisted therapy benefit patients in a minimally conscious state (MCS), but evidence is still scarce. It is thus crucial to understand how these patients react to contact with an animal.

Methods: This study aimed to measure the prefrontal brain activity in MCS patients to contact with a dog compared with a plush animal using functional near-infrared spectroscopy (fNIRS). We conducted a randomized controlled crossover trial (NCT03341325), enrolling 22 MCS patients, each participating in six sessions. Patients interacted with a dog in three sessions and with a plush animal in three control sessions. Each session was divided into five two-minute phases. The intensity of contact with the dog or plush animal increased from the first to the fourth phase.

Results: FNIRS parameters did not differ between the two conditions. Mean heart rate (HR) was significantly higher in the dog condition compared to the control condition. In both conditions, the concentration of fNIRS parameters, mean HR, and one heart rate variability parameter increased from the first to the fourth phase.

Conclusions: The MCS patients show the same frontal activation with the dog and the plush animal. The increased mean HR in contact with the dog indicates physiological arousal. Overall, this study indicates that the implementation of animals in individual therapy of MCS has the potential to activate patients and thus enable greater participation. More research is needed to understand the effects of animals on MCS patients.

Keywords

Minimally conscious state, human–animal interaction, functional near-infrared spectroscopy, heart rate, heart-rate variability,

1 Introduction

Patients in a state of minimal conscious (MCS) following severe, acquired brain injury need effective and early therapy to support spontaneous remission and prevent long-term hospitalization and disability (Lippert Grüner & Terhaag, 2000; Seel et al., 2013). Exposure to an enriching environment is an integrative part of early rehabilitation programs of MCS patients (La Gattuta et al., 2018; Pistarini & Maggioni, 2018). Such rehabilitation programs should be individualized and activity-oriented to promote inner perception and emotional sensation (Zieger, 2002). Studies showed that personally salient, familiar, sensory multimodal, and emotionally relevant stimuli elicit widely-distributed cortical activation and higher-level behavioral activation in MCS patients (Di Stefano et al., 2012; Perrin et al., 2015; Sun et al., 2018). Further, Di Stefano et al. (2012) showed that the context of stimulation plays a role. Animals are emotionally relevant to many people (Borgi & Cirulli, 2016; Hawkins & Williams, 2017; Kurdek, 2009a, 2009b), and interactions with the animal add context to stimulation. Animal-assisted therapy (AAT) is increasingly being applied in treatment of MCS patients. Besides anecdotal practical evidence (Bardl et al., 2013; Boitier et al., 2020), only a few studies have systematically investigated the use of AAT in MCS patients so far. In a randomized controlled study, patients showed more behavioral responses and higher physiological arousal measured via heart rate variability (HRV) during AAT sessions compared to standard therapy sessions (Hediger, Petignat, et al., 2019). A pilot study investigated the response in the frontal cortex to contact with an animal compared to contact with a plush toy in two MCS patients and found individually different reactions to the two conditions and an increase in brain activity with increased stimulation (Arnskötter et al., 2022). Such neurophysiological correlates of contact with animals are essential to understand the mechanisms of AAT and are crucial for MCS patients as these patients cannot communicate whether they perceive treatment as helpful (Bruno, Gosseries, et al., 2011).

Evidence base on AAT for MCS patients is scarce, and more studies are needed. AAT is often more personnel-intensive and expensive than standard therapy such as physiotherapy, speech, occupational, or music therapy (Charry-Sánchez et al., 2018). It is therefore important to clarify whether MCS patients benefit from the presence of an animal. Current research claims that it is especially relevant to investigate whether animals elicit the observed higher arousal (Hediger, Thommen, et al., 2019) or whether plush toy animals might elicit the same effects (López-Cepero, 2020; Marino, 2012; Marti et al., 2022; Wagner et al., 2022). In a previous study we found that interactions with a dog stimulated more prefrontal activity than a plush animal. Further, participants showed increasing prefrontal activation with increased interaction closeness with the dog or the plush animal (Marti et al., 2022).

This study investigated the response in the prefrontal cortex in MCS patients in the presence of and contact with a dog compared to a plush animal using functional near-infrared spectroscopy (fNIRS) in a controlled trial. As in the previous study, we compared different forms of interaction with a dog and a plush animal.

2 Materials and methods

2.1 Study design

We used a controlled, within-subject crossover design with repeated measurements. The study design followed our previous design (Marti et al., 2022). We measured participants' brain activity and heart rate (HR) in six standardized sessions (1–6). These consisted of three sessions with a dog and three control sessions with a plush animal and took place within 2 weeks. Compliance with the crossover-allocation sequence was not always possible because the presence of the dogs influenced the sequence of the conditions within these six sessions.

The local ethics committee, Ethics Commission Northwest and Central Switzerland (Project ID 2017-00540), and the Veterinary Office of the Canton of Basel-Stadt, Switzerland (No. 2713) approved the study design. The study was registered at clinicaltrials.gov (NCT03341325). We followed the Animals (Scientific Procedure) Act 1986, European Directive EU 2010/63, and the guidelines for handling animals in research outlined by the Association for Studies on Animal Behavior and the Society for Animal Behavior. All sessions were conducted following the International Association for Human–Animal Interaction Organizations guidelines and the Helsinki guidelines (IAHAIO, 2018; World Medical Association, 2013).

2.2 Participants

Participants were over 16 years old, inpatients at the neurorehabilitation clinic REHAB Basel, and diagnosed with acquired brain injury from either traumatic or non-traumatic causes. Secondary diagnoses were not considered. Severity of the disorder of consciousness was assessed via the original JFK Coma Recovery Scale (CRS; Giacino et al., 1993) and later via the Coma Remission Scale revised (CRS-r; Giacino et al., 2004; table 1). The diagnosis for MCS was additionally gaged with a clinical assessment by the responsible physician according to the Aspen diagnostic criteria (Giacino et al., 2002) and to Bruno and colleagues (Bruno, Vanhaudenhuyse, et al., 2011) for the division of MCS+ and MCS–. Patients were included in the study if assessment with the CRS or CRS-R and clinical assessment led to the diagnosis of MCS. Exclusion criteria were: Allergies or reported fears of dogs and medical contraindications for contact with a live dog (i.e., infections). For the screening process, we consulted physicians, therapists, relatives, and legal representatives. All involved parties helped assess whether a patient was included or excluded. For those included it also allowed careful evaluation if contact with a dog would be a positive situation. Written, informed consent was obtained from legal representative. We aimed for a sample size of 20 people based on a previous study (Hediger, Thommen, et al., 2019) and on feasibility of working with patients with severe disorders of consciousness.

Table 1: Demographic information on MCS patients.

Number	sex	age	GCS	CRS-R	BAVESTA	days since brain injury	etiology	frontal lesion
ID1	m	23	6	19[a]	n.a.	390	traumatic	postconcussion subdural hematoma bifrontal, left frontobasal

ID2	m	30	13	22[a]	3.27	233	traumatic	post-ischemic gray matter defect frontobasal bilateral
ID3	f	25	12	20[a]	2.76	139	traumatic	posttraumatic hypodense alteration in superior frontal gyrus left, frontolateral. Prominent calcifications frontal right.
ID4	f	16	11	13[a]	2.64	311	traumatic	hydrodense fluid collection subdural along the left convexity with splintering frontoparietally. Partially displaced left frontal sinus
ID5	m	47	12	14[a]	1.85	233	traumatic	gray matter hemorrhage frontobasal right
ID6	m	34	12	19[a]	1.78	130	traumatic	dural contrast image over left hemisphere and in frontal interhemispheric cleft, muliple old substance defects left frontobasally
ID7	f	57	9	13[a]	1.52	326	traumatic	original, posttraumatic, encephaloic alteration in right superior frontal gyrus and left pedunculus cerebri, residual canal from biopsy frontal right
ID8	m	28	10	11[a]	1.61	106	traumatic	no lesions, but EEG identified a retarding focus in left fronto-temporal area
ID9	f	54	10	10[a]	2.64	180	non-traumatic	focal hypodense subcortical white matter lesion frontal right
ID10	m	31	11	17[b]	2.09	146	traumatic	left hemispheric, multiple, small gray matter contusion hemorrhages fronto-temporal with accompanying intracortical and traumatic

								subarachnoid hemorrhage bilaterally, posttraumatic lesions frontal and temporal left
ID11	m	23	9	9[b]	1.82	171	traumatic	extensive gray matter defects bifrontal and left temporal
ID12	m	31	12	16[b]	2.48	387	traumatic	epidural hemorrhage fronto-temporoparietal right, low subdural collection frontotemporal left, effect especially frontal right with asymmetric, right flattened contour of frontal lobe, extensive defect areas fronto-pariento-temporo-occipital right.
ID13	f	67	10	10[b]	2.64	102	non-traumatic	gray matter hemorrhage right frontal, extended defect areas frontotemporal right, frontoparietal right
ID14	m	55	7	5[b]	2	82	traumatic	acute, narrow subdural hematoma left frontal
ID15	m	26	12	19[b]	1.76	112	traumatic	large subdural hematoma left-hemispheric and frontal right.
ID16	m	63	7	8[b]	1.33	135	traumatic	shearing injuries frontal bilateral
ID17	m	24	11	13[b]	1.91	263	traumatic	subarachnoid hemorrhage frontal bilateral, contusion hemorrhages frontal right
ID18	m	41	10	8[b]	1.21	76.00	traumatic	subarachnoid hemorrhage right high frontal.
ID19	f	52	6	7[b]	1.97	108.00	non-traumatic	rupture of aneurysma with haemarroage of gay matter in left cortex

ID20	m	75	8	9[b]	1.36	67.00	non-traumatic	no lesions, EEG identified a retarding focus in left fronto-temporal cortex bilateral
ID21	f	48	6	6[b]	0.85	106.00	non-traumatic	hypoxic encephalopathy. Ischemic brain damage involving cortex, hippocampus and caudate nuclei.

Note. a: Scores from JFK Coma Recovery Scale, b: Scores from Coma Remission Scale revised.

2.3 Procedure

Study sessions were held at the neurorehabilitation center REHAB Basel in Switzerland in a dedicated room. All procedures followed the processes described in the previous publication involving healthy participants (Marti et al., 2022). During measurements, MCS patients were placed in an upright sitting position in their bed and faced a white wall at a distance of 1.5 m. To measure oxygen saturation in the blood of the prefrontal brain, the study staff attached two fNIRS sensors to the patients' foreheads. In three of six sessions, patients had contact with a dog and in three with a plush animal. All sessions were videotaped, and patients' HR and electrodermal activity were recorded. In both conditions, each session consisted of five 2-minute phases, which were always conducted similarly and in the same order. In contrast to the previous study (Marti et al., 2022), an additional person was present in this trial to assist patients with petting the dog or the plush animal and constantly monitor patient's well-being. The study staff verbally instructed patients according to a standardized protocol, explained the procedures and informed them about what will happen before each phase. Patients were asked to look at the white wall and relax in the first phase (neutral 1). This phase served as a baseline. In the second phase, patients watched the dog or the plush animal from a distance of 1 m (watching). The dog or plush animal lay on a mat and a blanket on a height-adjustable table. In the third phase, the dog lay next to the patient on the table or bed while the plush animal was placed on the patient's thigh in the control condition. In this phase, the patients did not pet the animal. They were instructed only to feel the animal passively (feeling). In the fourth phase, the study staff helped the MCS patients pet the dog or the plush animal (petting) by guiding their hands according to the Affolter® concept (Affolter et al., 2009). Finally, in the second neutral phase, the patients were again asked to look at the white wall while the dog or the plush animal was out of sight (neutral 2). Each phase lasted 2 minutes followed by a short break, in which the study staff prepared the room for the next phase. Interactions between each patient and the dog or the plush animal were conducted after a standardized protocol to compare the amount of contact between the conditions. After the session, the person who assisted the patient during the task assessed the patient's behavioral reactions with the Baser Vegetative State Assessment (BAVESTA).

To control for the time of day, we scheduled three sessions in the morning and three in the afternoon for every patient. We could not counterbalance the order of the phases because MCS patients need time and a lot of context to understand a situation. Sudden contact with the animal without an acclimatization period as a consequence of the randomization would not be ethically justifiable. For this reason and to ensure that the experiment took not too long, which would have been challenging for these patients, we also did not measure a pre- and post-task baseline for each phase.

2.4 Dogs

The dogs participating in the study were used and specifically trained to work with patients in a hospital setting within an AAT program. The dogs were a female Jack Russel (6 years of age), a female Goldendoodle (4 years of age), a female Golden Retriever (4 years of age), and a male Corgi and Bearded Colly mix (4 years of age). Each dog participated in a maximum of two sessions in a row. The dogs and their owner entered the room before the sessions started to get accustomed to the room and to feel safe. For the exact procedure of the sessions with the dogs see Marti et al. (2022).

2.5 Plush animal

For the control condition, we used a plush lion (58 × 40 × 20 cm) that contained a hot water bottle in its body to control not only for the sensation of soft fur but also for the body temperature and weight of a dog. The water bottle was filled with warm water before the sessions started. We called the plush animal by the name “Leo,” as we called all the dogs by their names during the study.

2.6 Functional near-infrared spectroscopy

To measure frontal brain activity, we used fNIRS, a vascular-based neuroimaging technology. FNIRS measures the oxygen saturation of hemoglobin and changes in total hemoglobin concentration (tHb) in the blood based on the characteristic hemoglobin-absorption spectra in the near-infrared range. In response to neuronal activity, oxygen saturation and tHb change respectively, known as neurovascular coupling. An increase in oxygenated hemoglobin (O_2Hb) thus indicates increased brain activity (Quaresima & Ferrari, 2016).

We recorded oxygen saturation (%) and tHb (g/dl) in the prefrontal cortex using a Nonin fNIRS device (SenSmart Model X-100), following the same procedure described in Marti et al. (2022). One patient was measured with a different fNIRS device and therefore excluded from the analysis of the fNIRS data. We placed two sensors of the device (Model 8004CA Sensors – Adhesive) right and left of the midline on the forehead as close to the hairline as possible and then attached them with an adjustable band. This location covers Brodmann areas 9, 10, and 46 and locations F1, F3, F2, and F4 on the frontopolar area according to the international 10–20 system (Jasper, 1958). The wavelength of the infrared light was 730, 760, 810, and 880 nm. Measurements were recorded at a frequency of 0.25 Hz. After recording, we transferred the data to a laptop using SenSmart software (version 1.0.1.0).

2.7 Heart rate and heart rate variability

HR and HRV were recorded with the Combi sensor biofeedback device connected to the NeuroAmpII device from Bee Medic GmbH (Burkhard et al., 2018). Data were directly transferred to the Erprec-EEG/ERP Recording Software (version 2.0.7.1). The skin was disinfected before the sensor was put on. We used Kubios HRV Standard (version 3.1.0 – 3.5.0; Biosignal Analysis and Medical Imaging Group University of Kuopio, Finland) to correct artifacts and calculate HRV parameters. We cut the data into five 2-min segments according to the phases. The data between the segments was not used. Each data segment was individually corrected according to visual inspection to correct the artifacts. We used the time-domain parameters mean HR (beats/min), the square root of the mean of the sum of squares of differences between adjacent NN-intervals (RMSSD; ms), the frequency-domain parameters (using fast Fourier transform) power in high frequency (HF; nu) and the power in low frequency (LF; nu; Task Force, 1996).

2.8 Basler Vegetative State Assessment

An adjusted version of the BAVESTA (Huber et al., 2014) was used to assess the consciousness of the patients based on observable behaviors during the sessions. Items that were not observable during the study were removed and not considered in the calculation of the scales (removed items: 7, 8, 9, 12, 13, 14, 23, 25, 27, 28, 31, 32, 33). The total short-term scale ranges from 0 (behavior is not shown) to 5 (behavior is consistently shown) and assesses level of consciousness. The subscales “attention,” “verbal communication,” “emotional reactions,” and “motor reactions” also range from 0 (behavior is not shown) to 5 (behavior is consistently shown). The study staff was trained to assess the patients with this inventory.

2.9 Data processing and analysis

In line with our previous study (Marti et al., 2022), we hypothesized that O_2Hb (primary outcome) is higher in contact with a dog than in contact with a push animal. Our second hypothesis was that increased closeness in contact with a dog or plush animal leads to increased stimulation, resulting in increased O_2Hb. HHb, tHb, and oxygen saturation from the fNIRS data, and mean HR, RMSSD, LF, and HF from the HR data served as secondary outcomes.

The fNIRS data was processed the same way as reported in Marti et al. (2022). We calculated the mean concentration of O_2Hb, HHb, and tHb and mean oxygen saturation in each phase of the included data. For that, we cut the data from each session into segments of five 2-minute phases at the markings. The data between the phases was not used.

Since we were interested in changes from phase to phase, we subtracted the mean of phase neutral 1 from each following phase within the same session for each patient. For every primary and secondary outcome, we conducted prespecified linear mixed-effect models with random intercepts. The mean difference served as the effect size. Within the prespecified models, condition and phase were inserted as fixed effects and participant as the random effect. In these models, we excluded data from phase neutral 1 because of the calculation of the change scores. Further, we plotted each participant’s data to descriptively investigate individual reactions to account for the individual neuropathological heterogeneity (O’Kelly et al., 2013).

In the analysis of the fNIRS data of the healthy participants (Marti et al., 2022), we found a pattern in O_2Hb and tHb levels indicating that prefrontal brain activity increased with repeated contact with the dog while it did not increase with repeated contact in the plush-animal condition. We therefore conducted the same explorative analyses for fNIRS and HR data also in this study. Within these non-prespecified linear mixed-effect models, condition and contact (first, second, or third contact between participant and dog or plush animal) were used as fixed effects. Moreover, we included an interaction term and an intercept for the participant as a random effect.

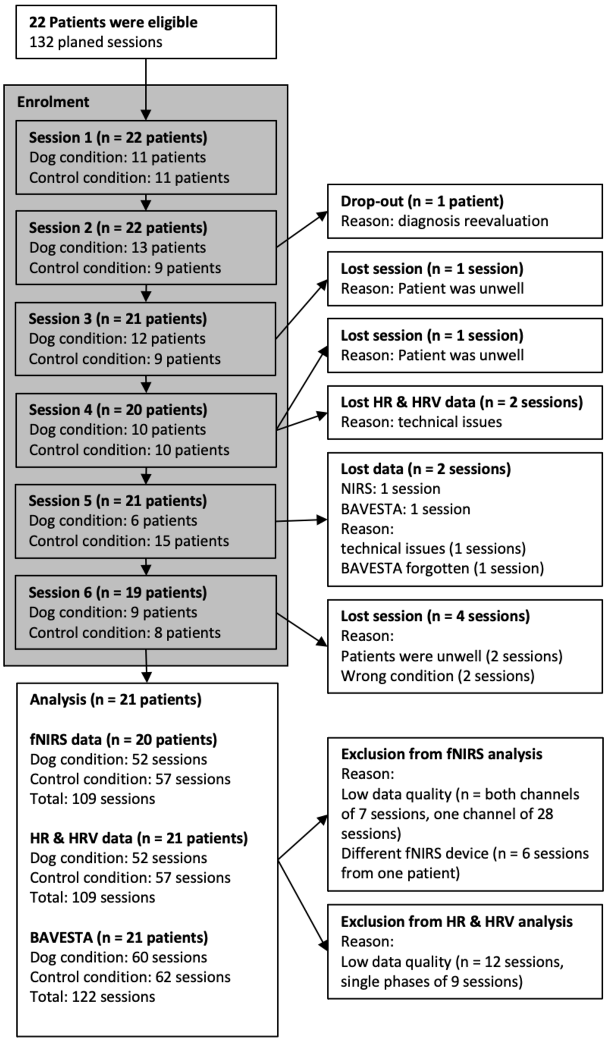
To analyze the behavioral reactions of the patients measured with the BAVESTA, we compared the two conditions using a linear mixed-effect models with condition as fixed effect and an intercept for the participant as the random effect with mean difference used as effect size.

We visually checked the linearity, normality (q-q plot, histogram of residuals), homoscedasticity (residuals vs. fitted plot), and influential outliers (leverage and Cook’s distance) of the fNIRS and the HR data. We checked leverage with the R package influence.ME (Nieuwenhuis et al., 2012). For HR and HRV parameters, we excluded data with low quality due to measuring problems. We also conducted sensitivity analyses where we excluded outliers (mean ±2.5 SD) for fNIRS and HR analysis. For the HR sensitivity analysis, we also excluded data where the percentage of corrected beats was higher than 5% in one phase. We set the significance level at .05. All analyses were conducted with R 4.1.0 (R Core Team, 2018) and R package lme4 (Bates et al., 2015).

3 Results

Twenty-two MCS patients (7 women, 15 men) participated in this study between 4th February 2018 and 28th June 2021 (table 1). One of 22 patients dropped out after two sessions because level of consciousness increased between inclusion and the first session, and we therefore reevaluated the diagnosis (figure 1). The flow diagram shows when sessions were canceled, or we lost data due to technical issues (figure 1). The analyzed 21 patients comprised seven women (M = 45 years) and 14 men (M = 38 years). The mean age was 41 years (*SD* = 16.86). No adverse or unintended effects on patients or the involved dogs occurred during data collection.

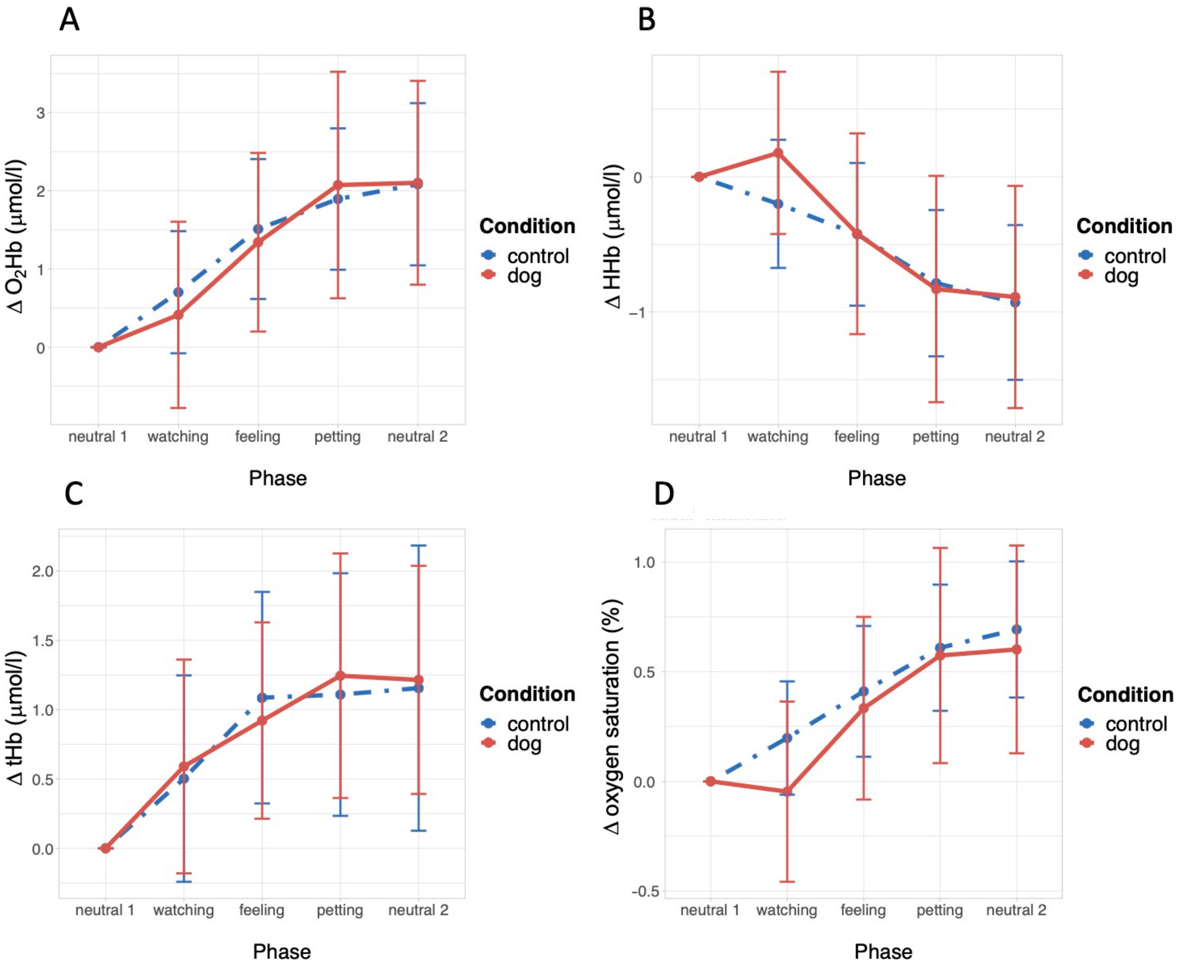
Figure 1: Flow diagram of the study.



3.1 Primary analysis oxygenated hemoglobin

O_2Hb was not significantly different in the presence of the dog compared to in the presence of the plush animal (estimate = 0.07, CI = −0.62–0.75, *p* = .848; figure 2A). With increased stimulation, O_2Hb increased significantly from phase neutral 1 to phase petting by 1.97 mmol/l (CI = 1.00–2.94, *p* = .004). After removal of the stimulation in phase neutral 2, O_2Hb stayed constant and was still significantly higher compared to phase neutral 1 (estimate = 2.08 mmol/l, CI = 1.11–3.04, *p* = .002). The sensitivity analysis did not change the conclusion.

Figure 2: Effects of condition and phase on O_2Hb, HHb, tHb, and oxygen saturation.



Note. (A) O_2Hb, (B) HHb, (C) tHb, and (D) oxygen saturation. Error bars denote confidence interval. Data are shown as relative change from phase neutral 1.

3.2 Secondary analysis

3.2.1 Deoxygenated hemoglobin

HHb did not differ in the presence of the dog compared to in the presence of the plush animal (estimate = 0.00 mmol/l, CI = −0.39–0.40, *p* = .992; figure 2B). When stimulation increased, HHb decreased significantly from phase neutral 1 to the petting phase by 0.83 mmol/l (CI = −1.38 to −0.27, *p* = .005). After removal of the stimulation in phase neutral 2, HHb stayed constant and was still significantly lower compared to phase neutral 1 (estimate = −0.92 mmol/l, CI = −1.47 to −0.37, *p* = .002). The sensitivity analysis revealed no noteworthy differences.

3.2.2 Total hemoglobin

The concentration of tHb was not different in the presence of the dog compared to in the presence of the plush animal (estimate = 0.08, CI = −0.44–0.59, *p* = .771; figure 2C). When stimulation increased, tHb tended to increase from phase neutral 1 to the petting phase by 1.14 mmol/l (CI = 0.42–1.86, *p* = .090). After removal of the stimulation in phase neutral 2, tHb stayed constant and still tended to be higher compared to phase neutral 1 (estimate = 1.16 mmol/l, CI = 0.44–1.87, *p* = .083). In the sensitivity analysis, the increase with stimulation became significant.

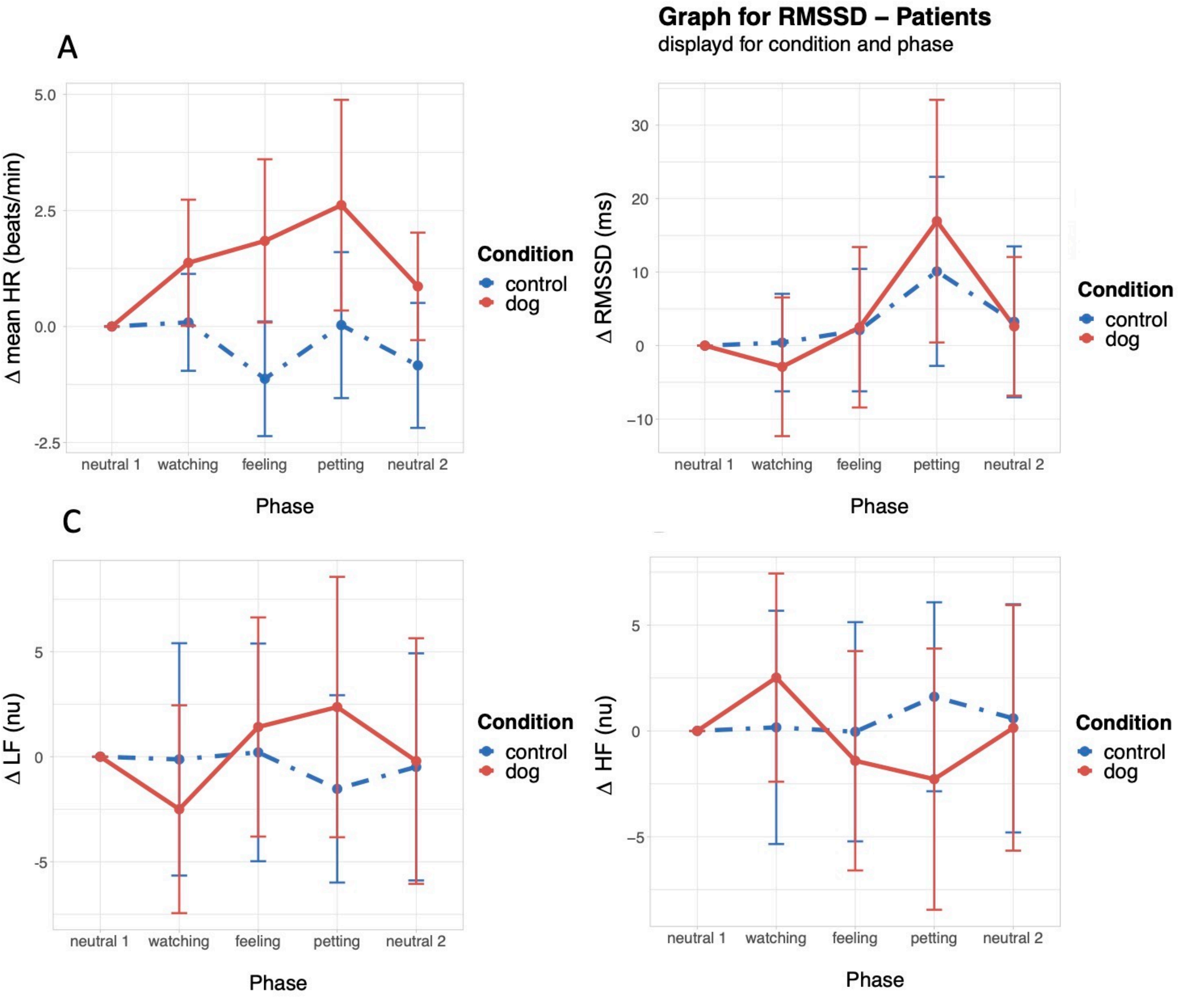
3.2.3 Oxygen saturation

Oxygen saturation did not differ in the presence of the dog compared to in the presence of the plush animal (estimate = –0.07, CI = –0.30–0.16, *p* = .577; figure 2D). When stimulation increased, oxygen saturation increased significantly from phase neutral 1 to the petting phase by 0.63% (CI = 0.31–0.96, *p* = .002). After removal of the stimulation in phase neutral 2, saturation stayed constant and was still significantly higher compared to phase neutral 1 (estimate = 0.69%, CI = 0.36–1.01, *p* = .001). The sensitivity analysis revealed no noteworthy differences.

3.2.4 Mean HR

Mean HR was significantly increased by 2.08 beats/min in the presence of the dog compared to in the presence of the plush animal (CI = 1.07–3.10, *p* < 0.001, figure 3A). Phase had no significant effect (estimate = 0.22, CI = –1.21–1.64, *p* = .503). In the sensitivity analysis, mean HR increased significantly from phase neutral 1 to the petting phase by 0.72 beats/min (CI = –0.23–1.67, *p* = .026).

Figure 3: Effects of condition and phase on mean HR, RMSSD, LF, and HF.



Note. (A) Mean HR, (B) RMSSD, (C) LF, and (D) HF. Error bars denote confidence interval. Data are shown as relative change from phase neutral 1.

3.2.5 RMSSD

RMSSD was not different in the presence of the dog compared to in the presence of the plush animal (estimate = 1.49, CI = −5.53–8.49, *p* = .678) (figure 3B). When stimulation increased, RMSSD increased from phase neutral 1 to the petting phase by 13.01 ms (CI = 3.20–22.81, *p* = .004). The sensitivity analysis revealed no noteworthy differences.

3.2.6 LF and HF

For LF and HF, neither the effect of condition nor the effect of phase were significant (figure 3C and 3D). The sensitivity analysis revealed no noteworthy differences.

3.5 BAVESTA

Mean total score for the BAVESTA was 2.03 (SD = 0.77) over all patients and both conditions. For the BAVESTA data, we found no difference between the dog and plush animal conditions (estimate = 0.06, CI = −0.12–0.24, *p* = .485. We neither found an effect of condition in one of the subscales.

3.3 Explorative analysis

We did not find the same patterns in brain activation of MCS patients as we did in the healthy participants (figure 4, table 2): The patterns for O_2Hb, and tHb show an increase in the second contact and then a decrease in the third contact in the control condition while the levels in the dog condition decrease from the first to the third session. In HHb and oxygen saturation, we found no differences between the contacts and the conditions. A sensitivity analysis had no impact on the results of our statistical analysis. In mean HR, LF, and HF, we found similar patterns as in healthy brain activation (figure 5, table 3): Mean HR increased in the second session and then stayed at the same level in the third session in the dog condition. In the control condition, mean HR decreased and stayed at the same level. RMSSD rose in both conditions from the first to the third session. LF showed an increase from the first to the third contact in the dog condition, while in the control condition, it decreased in the second session and increased again in the third session. In HF, we found the inversed pattern.

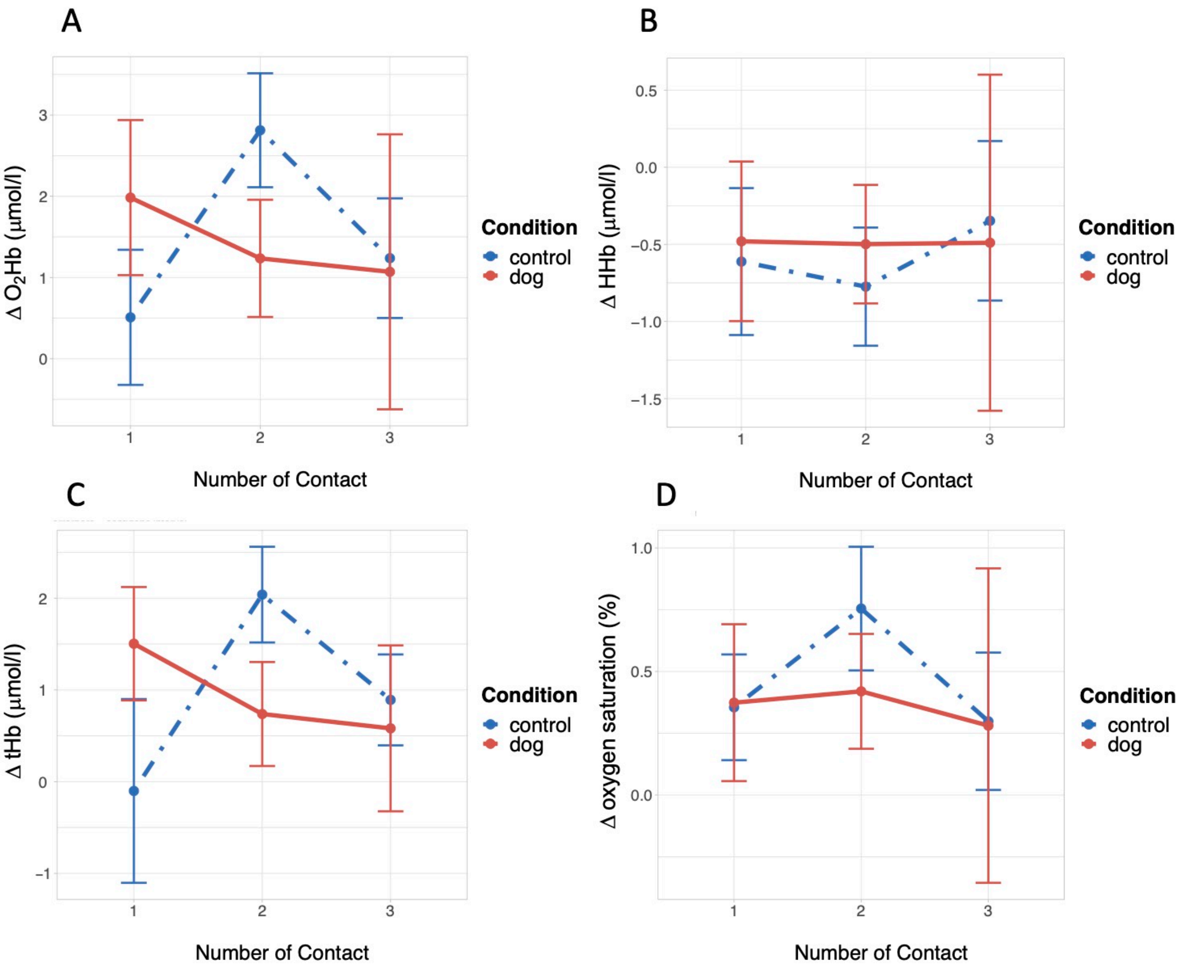
Table 2: Marginal effects in fNIRS of condition by number of contacts.

		95% CI			
	Estimate	**Lower limit**	**Upper limit**	**p-value**	
O2Hb					
Dog condition					
First contact	1.98	0.84	3.12	.014	a
Second contact	1.52	-0.11	3.14	.001	b
Third contact	1.01	-0.70	2.73	.094	c
Plush-animal condition					
First contact	0.55	-0.57	1.66	.336	d
Second contact	2.81	1.67	3.95	<.001	e
Third contact	1.04	-0.14	2.22	.409	f
HHb					
Dog condition					
First contact	-0.48	-1.14	0.18	.633	a
Second contact	-0.64	-1.59	0.31	.954	b
Third contact	-0.53	-1.53	0.47	.339	c
Plush-animal condition					
First contact	-0.64	-1.34	0.05	.070	d
Second contact	-0.77	-1.44	-0.11	.695	e
Third contact	-0.20	-0.89	0.48	.210	f
tHb					
Dog condition					
First contact	1.50	0.67	2.33	<.001	a
Second contact	0.89	-0.30	2.08	<.001	b
Third contact	0.48	-0.77	1.73	.002	c
Plush-animal condition					
First contact	-0.09	-0.97	0.79	.835	d
Second contact	2.04	1.21	2.87	.000	e
Third contact	0.83	-0.03	1.68	.035	f

Oxygen saturation					
Dog condition					
First contact	0.37	-0.01	0.76	.972	a
Second contact	0.50	-0.05	1.06	.387	b
Third contact	0.29	-0.30	0.87	.802	c
Plush-animal condition					
First contact	0.38	-0.01	0.77	.056	d
Second contact	0.75	0.37	1.14	.058	e
Third contact	0.22	-0.18	0.62	.426	f

Note. Marginal effects were estimated by condition and contact number, and an intercept for participant as random effect. a: effect of the condition, b: effect condition x second contact, c: effect condition x third contact, d: effect of the intercept, e: effect of the second condition, f: effect of the third condition

Figure 4. Effects of condition and number of contacts on O_2Hb, HHb, tHb, and oxygen saturation.



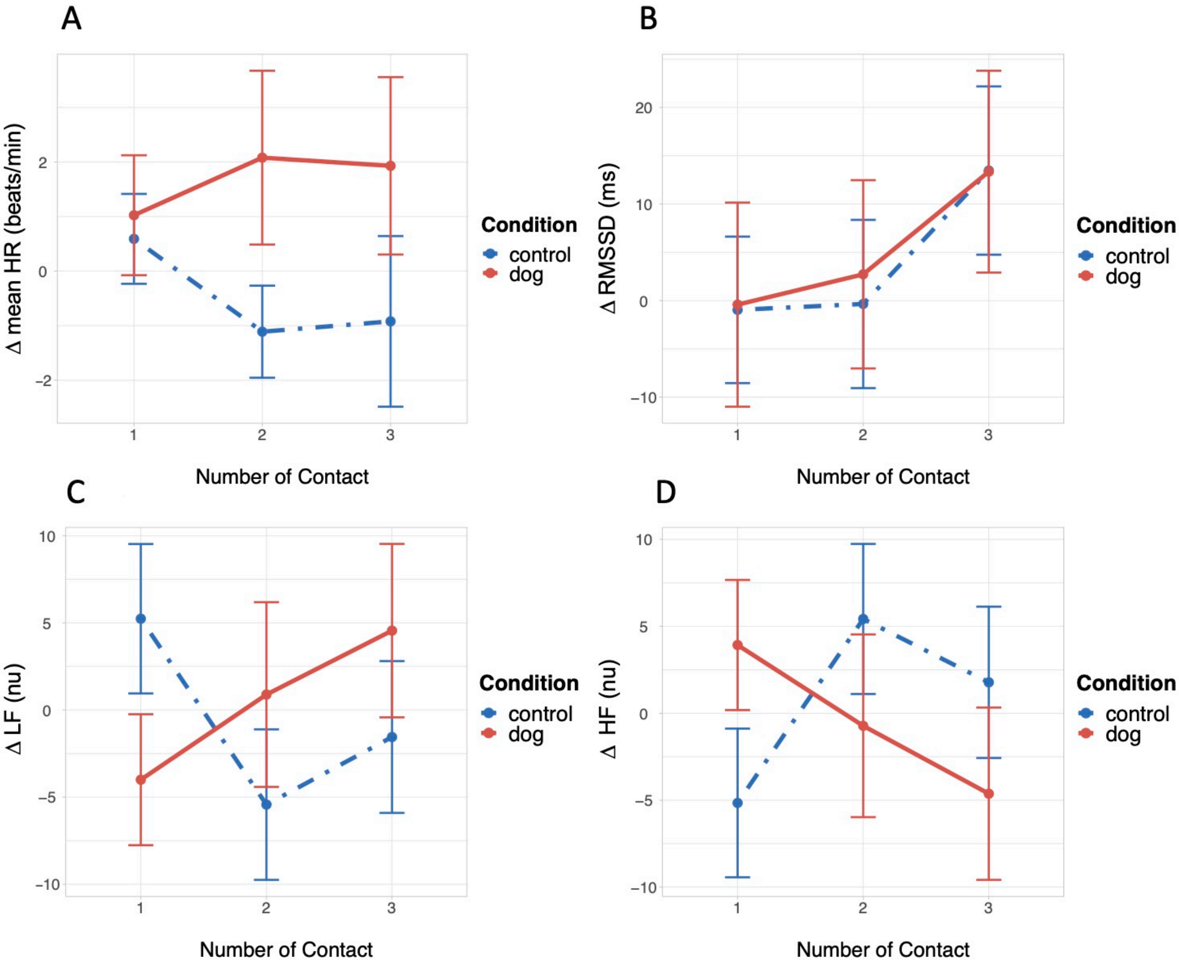
Note. (A) O_2Hb, (B) HHb, (C) tHb, and (D) oxygen saturation. Error bars denote confidence intervals. Data are shown as relative change from phase neutral 1. The data for phase neutral 1 are not included in the presented means.

Table 3: Marginal effects in HR data of condition by number of contacts.

	Estimate	95% CI Lower limit	 Upper limit	p-value	
Mean HR					
Dog condition					
First contact	0.98	-0.74	2.70	.665	a
Second contact	2.28	-0.16	4.73	.015	b
Third contact	1.56	-0.98	4.09	.107	c
Plush-animal condition					
First contact	0.60	-0.77	1.97	.390	d
Second contact	-1.13	-2.86	0.59	.049	e
Third contact	-0.90	-2.64	0.84	.090	f
RMSSD					
Dog condition					
First contact	0.33	-11.50	12.16	.971	a
Second contact	2.87	-13.98	19.71	.747	b
Third contact	15.98	-1.49	33.44	.773	c
Plush-animal condition					
First contact	0.11	-10.90	11.12	.985	d
Second contact	-0.12	-12.02	11.77	.970	e
Third contact	13.19	1.19	25.19	.033	f
LF					
Dog condition					
First contact	-4.13	-10.08	1.83	.004	a
Second contact	0.92	-7.55	9.39	<.001	b
Third contact	4.10	-4.69	12.89	.002	c
Plush-animal condition					
First contact	4.72	-0.25	9.69	.063	d
Second contact	-5.59	-11.57	0.39	.001	e
Third contact	-0.99	-7.02	5.04	.063	f
HF					
Dog condition					
First contact	4.05	-1.87	9.98	.004	a
Second contact	-0.77	-9.20	7.67	.001	b
Third contact	-4.20	-12.94	4.55	.002	c
Plush-animal condition					
First contact	-4.63	-9.58	0.32	.067	d
Second contact	5.57	-0.38	11.53	.001	e
Third contact	1.24	-4.77	7.24	.056	f

Note. Marginal effects were estimated by condition and contact number, and an intercept for participant as random effect. P-values: a: effect of the condition, b: effect condition x second contact, c: effect condition x third contact, d: effect of the intercept, e: effect of the second condition, f: effect of the third condition

Figure 5. Effects of condition and number of contacts on mean HR, RMSSD, LF, and HF.

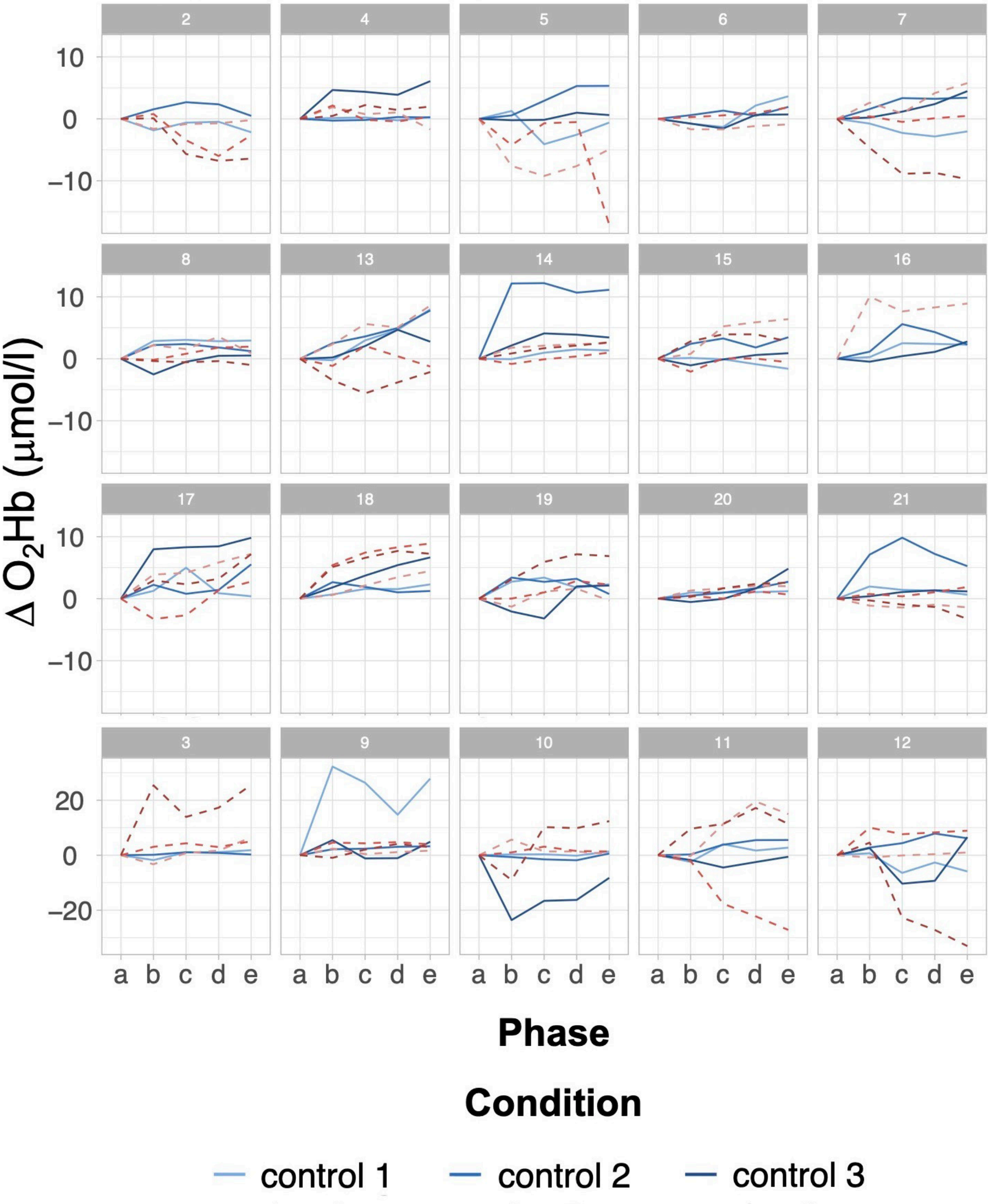


Note. (A) Mean HR, (B) RMSSD, (C) LF, and (D) HF. Error bars denote confidence intervals. Data are shown as relative change from phase neutral 1. The data for phase neutral 1 are not included in the presented means.

3.4 Individual Patterns

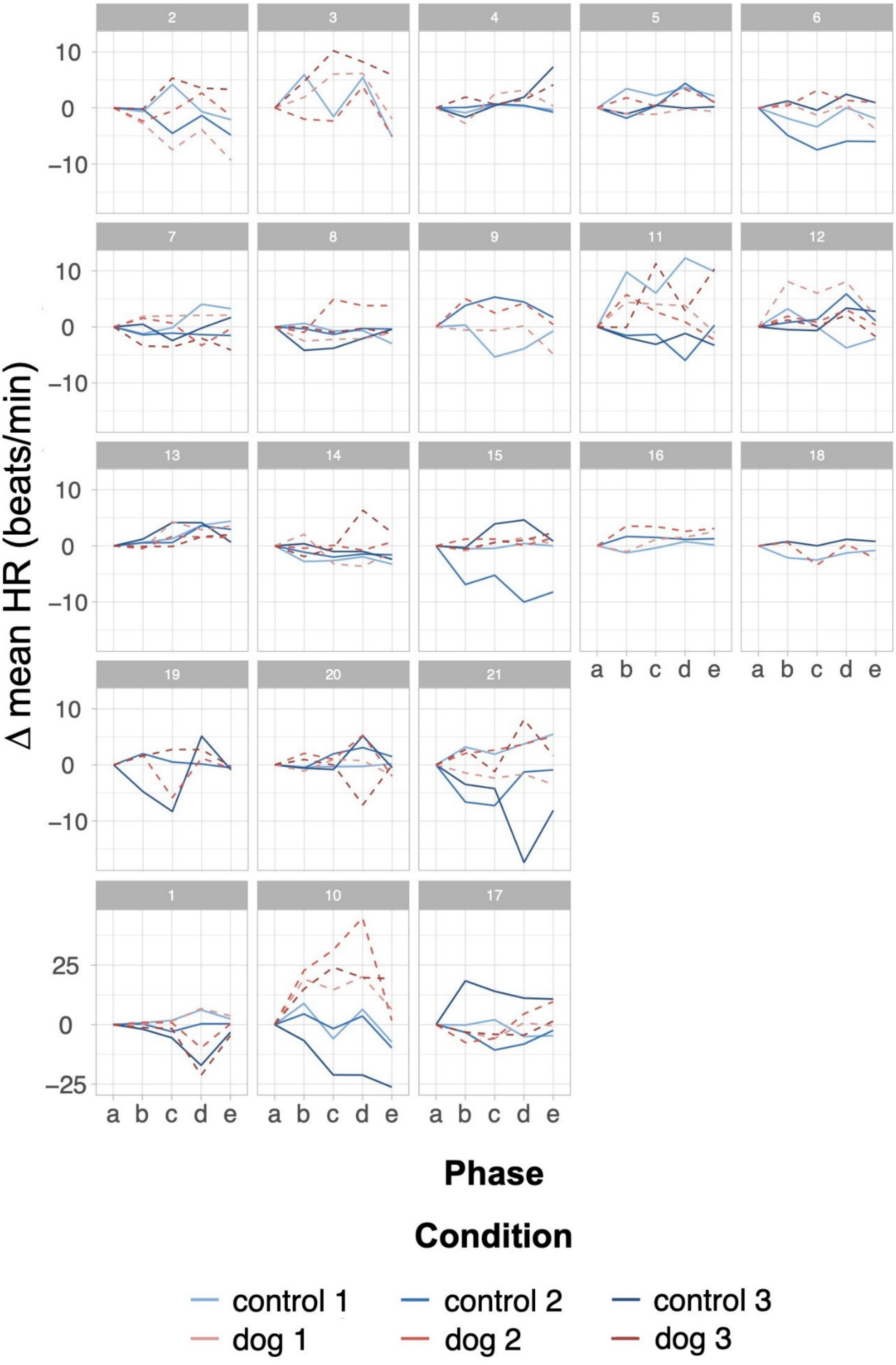
In the individual reaction pattern of the O_2Hb (figure 6), we identified a group of patients (35%) who reacted with higher prefrontal brain activity in the presence of a dog compared to the plush animal: ID9, ID10, ID11, ID15, ID16, ID18, and ID19. Another group of patients (35%) has similar activation when interacting with a dog or a plush animal: ID3, ID4, ID5, ID6, ID7, ID8, and ID20. The third group of patients (30%) shows a higher prefrontal activation in the plush animal's presence than in the dog's presence: ID2, ID12, ID13, ID14, ID17, and ID21. In the individual mean HR graphs (figure 7), slightly more patients (43%) have a higher mean HR during contact with the dog than with the plush animal: ID3, ID6, ID8, ID10, ID11, ID12, ID14, ID18, and ID21. In most patients (52%), the condition does not affect mean HR: ID1, ID2, ID4, ID5, ID7, ID9, ID13, ID15, ID16, ID19, and ID20. Only ID17 (5%) had a slightly higher mean HR in the control condition compared to the dog condition.

Figure 6. Individual reaction patterns in O_2Hb of condition and phase



Note. Data are shown as relative change from phase neutral 1. The data for phase neutral 1 are not included in the presented means. Different axis scales are used to make patterns better visible.

Figure 7. Individual reaction patterns in mean HR of condition and phase



Note. Data are shown as relative change from phase neutral 1. The data for phase neutral 1 are not included in the presented means. Different axis scales are used to make patterns better visible.

4 Discussion

This study investigated MCS patients' prefrontal brain activity, HR, HRV, and behavioral reactions during different forms of contact with a dog and with a plush animal. We found no difference in prefrontal brain activation between the dog and the control condition with a plush animal. Patients showed a higher mean HR in sessions with a dog than the plush animal, but we found no differences in the HRV parameters between the conditions. The prefrontal activity of the MCS patients increased with increased intensity of contact with a dog or a plush animal. Further, we found an increase in RMSSD with an increase in contact with both the dog and the plush animal, indicating an activation of the parasympathetic system. We found no such pattern for the LF and HF. We also found no difference between conditions in BAVESTA scores.

4.1 Dog or plush animal

We found no difference in patients' brain activity between conditions, which runs contrary to the findings of our previous study with healthy participants, where we found that brain activation was higher when a dog was present compared to the presence of the plush animal (Marti et al., 2022). Other studies also found higher neuronal activation in healthy children or children undergoing surgery in the presence of or while interacting with a dog compared to a control condition (Calcaterra et al., 2015; Hediger & Turner, 2014). The only pilot study investigating MCS patients revealed a heterogenous picture (Arnskötter et al., 2022). Our results suggest that MCS patients show a different brain activation pattern than healthy participants during contact with a dog.

The higher mean HR in the dog condition compared to the plush animal condition indicates higher physiological arousal in patients in contact with a dog. Our finding of higher physiological arousal in MCS patients is partly found in the study of Schretzmayer et al. (2017). They found no effect of the dog overall in autistic children, but when the dog condition was first, they found a trend that the children had higher mean HR with a dog. Further, they also found no difference in HRV parameters. Moreover, our findings contradict the study of Hediger, Petignat et al. (2019), where the HR was not different in AAT compared to conventional therapy. However, in this study, HF was lower and LF higher in the AAT sessions, which indicates higher physical arousal. Therefore, both studies point in the same direction. Further, the mean HR pattern matches the healthy participants' brain activation pattern in our first study (Marti et al., 2022). Motooka et al. (2006) found greater parasympathetic activity in senior citizens when they walked with a dog compared to without a dog.

The patient's brain lesions might explain the discrepancy between the elevated mean HR in the presence of a dog and no changes in fNIRS parameters. Most patients had frontal lesions and might process information differently (Gilbert et al., 2018). Another study also found an inconclusive activation pattern in the area of lesions in an MCS patient (Molteni et al., 2013). Damaged structures could impede the detection of activity (Rupawala et al., 2018). Therefore, lesions could alter neurological activities or fNIRS measurements of neuronal activity. Since the brainstem has a role in regulating HR, one hypothesis to interpret our results is that the higher HR represents a higher basal emotional activation of the patients in the presence of a dog compared to a plush animal. This basal emotional activation is processed in the brainstem but does not lead to the expected prefrontal cortical due to the lesions (Venkatraman et al., 2017). It is thus the question if other brain areas would have been more appropriate to investigate the different information processing of dogs and plush animals.

The study of Kempny et al. (2016) showed that it is possible to measure neuronal reactions in patients with prolonged disorders of consciousness with fNIRS. However, the authors measured the premotor and motor cortex. Future studies should therefore consider a broader measurement of brain activity in this group of patients.

Another hypothesis is that the dog was not embedded in a broader context where this interaction was meaningful. The experiment was conducted in a highly standardized manner without a therapeutic context. Previous studies found that MCS patients react to animals and also show higher behavioral reactions and physiological arousal in therapy sessions in the presence of an animal compared to conventional therapy sessions (Boitier et al., 2020; Hediger, Petignat, et al., 2019). Therefore, we speculate that integrating animals into a meaningful and holistic activity might be central. This holistic view has also been proposed by agents of the Affolter approach (Affolter et al., 2009).

However, the patterns of brain activity can also be interpreted to imply that interaction with a dog compared to a plush animal has no significant additional effect on MCS patients. Interactions with a plush animal or robot might be equivalent to interactions with a dog for these patients because of their lack of understanding of the environment. Robots in animal form are already used in patients with dementia and show positive effects on quality of life, affect, agitation, social interaction, neuropsychiatric symptoms, and the use of psychotropic or pain medication (Kang et al., 2020; Thunberg et al., 2020). Further research is needed to address this question.

4.2 Increase of stimulation

Our results show that the patient’s brain activity increased as stimulation increased. This confirms our second hypothesis and is in line with our previous study with healthy patients, where we found the same increase in brain activation with increased stimulation (Marti et al., 2022). Other studies also found similar patterns indicating that higher stimulation leads to higher brain activity in both patients with MCS and healthy participants in contact with cats, horses, dogs, or guinea pigs (Arnskötter et al., 2022; Kobayashi et al., 2017; Matsuura et al., 2020; Perrin et al., 2015). Moreover, in the present study, brain activation increased when stimulation became sensorial multimodal (watching vs. touching, movement, and watching). This pattern is in line with the study of Keller et al. (2007), where patients in a persistent vegetative state showed higher brain activation with tactile stimulation compared to acoustic stimuli.

The increase in RMSSD with increased contact in both conditions is in line with a control study and a case study where RMSSD was increased with increased stimulation with voices or music compared to a resting condition in persistent vegetative state and MCS patients (Gutiérrez et al., 2010; Lee et al., 2011). The patterns of prefrontal brain activation and RMSSD when stimulation increased could indicate that this higher frontal brain activity does not just reflect higher physiological arousal but could be attributed to information processing, attention, or emotional arousal (Grossmann, 2013; Kuo & Nitsche, 2015). Further, this increase in parasympathetic activity could indicate that the procedures with feeling and touching a dog or a plush animal were not perceived as stressful.

We found no difference between the conditions in the assessment of the level of consciousness of the MCS patients with the BAVESTA. Patients showed no visually observable signs of higher consciousness in their behavior while a dog was present, which is in line with the findings from the fNIRS data.

4.3 Reactions across sessions

We investigated the time effect between the sessions because in our first study with healthy participants, we found that their prefrontal brain activity increased with repeated contact with the dog, but this was not the case with the plush animal (Marti et al., 2022). We assumed that the healthy participants established a basal relationship with the dog, which they did not establish with the plush animal. In the present study, MCS patients did not show a brain activation pattern but a physiological activation pattern, which suggests that they developed a basal relationship with the dog. This pattern resembles the brain activation pattern the healthy participants had in our first study (Marti et al., 2022). This is in line with the findings of Boitier et al. (2020). In contrast to our study in this study, the patient was able to show behavioral reactions to the animal: They observed in their case study that an MCS patient reacted to one guinea pig differently than other animals. This led the authors to assume that the patient preferred one guinea pig. But in this study, the patient had contact with the animals twice a week for four weeks. They might therefore need prolonged and repeated contact with shorter time lags in between to be able to react behavioral and with prefrontal brain activation to animals. Possible explanations for the lack of brain activation could be the patients' lesions, their altered consciousness or the lack of context, as suggested above. Thus, we need more research to investigate possible mechanisms such as recognition and building relationships.

4.4 Individual reactions

Our results suggest that interpreting mean results might not reflect the individuality of the patients. In line with the study of Arnskötter et al. (2021), the reactions to the dog and the plush animals are highly individual: some patients' response was higher with the dog (O_2Hb: 35%; HR 43%) while others reacted more to the push animal (O_2Hb: 30%; HR 5%). Furthermore, for some, the condition did not influence their brain activity or mean HR (O_2Hb: 35%; HR 52%). Looking at the individual reactions reveals that some patients may have benefited more from the interaction with a dog than others. At the same time, others seem to benefit from a plush animal's presence. These different reactions align with a previous study investigating effects of AAT in patients with acquired brain injury. They also found that there are individual degrees of added benefit of having an animal in a therapy session (Hediger, Thommen, et al., 2019). This highlights the importance of repeated n-of-1 trials for the future in MCS patients, as future research should aim to find out for whom integrating with an animal might be beneficial and who might profit more from different approaches.

4.5 Limitations and strengths

This study has the same limitations as the similar antecedent study in healthy participants (Marti et al., 2022) concerning blinding, randomization, assessment of attitudes towards animals, sample size, and the fNIRS disadvantages. Due to the study's nature, blinding was not possible. Compliance with the randomization of the conditions' sequence was not completely possible since the dogs were visiting the rehabilitation center on specific days. Another limitation is that an additional person was present in the dog condition which could have increased patients' arousal. The dog owner was instructed to refrain from interacting with patients. Further, we did not collect data on patients' attitudes toward animals. As with most studies on MCS patients, this study has a small sample size, so results must be interpreted carefully.

The advantages of fNIRS allowed us to measure brain activity in a setting where therapy would normally take place for these patients. But the measurement of regional cerebral oxygen saturation can be affected by a variety of factors, e.g. skull thickness, cortical gyration, hemoglobin concentration, or extracranial blood flow (Ostojic et al., 2020; Tanaka et al., 2021). The repetition of the measurement for each condition and the within-subject design where each participant served as their own control limits these issues. The sensitivity to extra-cranial effects is additionally reduced through the probe design with a multi-distance approach (Franceschini et al., 1998). The fNIRS device corrects for drifts, and the multi-distance approach is also insensitive to instrumental drifts. As in the study with healthy participants (Marti et al., 2022), we decided not to detrend for the difference from the first to the second neutral phase to keep the comparability. We controlled for time of the day, but we could not control for the activity the patients had prior to the sessions. Therefore, some patients may have been tired in some sessions but not in others. Another limitation is that the persons who rated the patients with the BAVESTA could not be blinded. The BAVESTA results, therefore, should be interpreted carefully.

An additional limitation in the patient group was that 43% of patients were assessed with the original version of the CRS. In contrast to the revised version, the original version cannot distinguish between vegetative state and MCS (Giacino et al., 2009). Since physicians also assessed the patients using the Aspen diagnostic criteria (Giacino et al., 2002), this limitation should be minimal.

A major strength of this study is the rigorous controlled design to examine the effect of the presence of and contact with a dog in MCS patients. Since this is a core component of AAT, this increases our knowledge about the implementation of animals in the therapy of MCS patients. The combination of fNIRS, HR, HRV, and a behavioral assessment with the BAVESTA made it possible to look at the patients’ response to the dog in a multidimensional way. Since these patients cannot say what they feel, assessing different aspects of their reactions is crucial (Giacino et al., 2009; Monti et al., 2009). Another strength of the study is its strict standardization. We carefully controlled the room’s environmental factors, the wording of the instructions, and the time of day. We also ensured that the interaction with the dog was similar to the interaction with the plush animal. We paid attention to controlling for tactile stimuli such as the fur, the body warmth, and the weight by having a plush animal with a warm hot water bottle inside. In addition, we called the plush animal by its name, just like the dogs. Our study presents insights into individual reactions of MCS patients, thus accounting for individual neuro-pathological heterogeneity (O’Kelly et al., 2013) and leading to hypotheses about differential indications.

4.6 Future research

Our results indicate that it is difficult to make a general statement about the reactions of the different patients. For some, the interaction with the dog seems to be helpful, while others do not respond to the dog. The study by Hediger, Petignat, et al. (2019) indicates that MCS patients may benefit from AAT, whereas the present study is not this clear in its conclusions. AAT, in general, but with this group of patients in particular, it involves much effort. Transportation to the animals can be stressful for the patients. Likewise, transporting the animals to the patients and interacting with MCS patients might be stressful for animals. Future research should therefore investigate if applying AAT for MCS patients is justified. Well-designed randomized controlled trials are thus highly needed. Moreover, it is crucial to understand differential indication and investigate who might profit from contact with dogs and who might profit from other approaches. To understand individual responses to AAT, it will also be curial to conduct repeated n-of-1 trials. Considering different patients characteristics will be important such as attitudes toward animals (Matsuura et al., 2020), gender (Kobayashi et al., 2017) and previous pet ownership (Hayama et al., 2016) as studies have found that these can influence reactions towards animals in healthy individuals.

In the current study, the situation was highly standardized and not adapted to individual patients. In contrast, in the study by Hediger, Petignat, et al. (2019), the animal was embedded in an activity that was individualized to a certain degree as it was a therapeutic situation. Future studies with MCS patients should thus investigate if embedding the animal in a meaningful activity might lead to different reactions. For the implementation of AAT, it will be important to clarify how AAT should be designed for these patients. Different environments (clinic room vs. animal enclosure) could be compared to test this. Future research should also address how to assess and prevent overstimulation in MCS patients during AAT (Perrin et al., 2015). To investigate that aspect, studies may need to also control for activities prior to the AAT sessions. Further, the role of familiarity and relationship with the animal should also be clarified. It will be important to investigate the effect of the duration of contact with an animal and the impact of repetition.

Future studies should use imaging techniques that cover the whole cortex, as certain activations may have been superimposed in this study due to lesions. A combination of electroencephalogram and fNIRS could complement each other by bringing together the advantages of these two imaging techniques and may help to understand the influence of lesions.

5 Conclusion

In the presented study, MCS patients showed no difference in brain activity to the conditions, but mean HR was higher in contact with a dog compared to contact with a plush animal. This discrepancy between brain activity and mean HR leads to the hypothesis that the interaction with a dog caused basal emotional arousal in the brainstem but that this might not be processed frontally. Frontal brain activity increased when the interaction with a dog or a plush animal became more intense. Further, the patients showed increasing physiological arousal from the first to the third session with the dog. Taking all findings together, one can see that patients showed the same activation pattern in mean HR as the healthy participants showed in their brain activation. We found that MCS patients' responses are highly individual. It is therefore important to evaluate exactly how the animal should best be introduced in MCS treatment. This study suggests that the implementation of animals in MCS treatment has potential to activate some patients and thus enable greater participation. But future research should further investigate how to introduce animals and their possible effects in MCS treatment.

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7 Competing interests

M. W. is president of the board and cofounder of OxyPrem AG. R. M., M. P., V. L. M., J. H., M. H.-G., and K. H. declare no potential conflicts of interest.

8 Data availability

Data is are available from the Harvard Dataverse database at (link will be provided after acceptance).

9 Supplementary material

Table X. Values averaged over all sessions, phases and both conditions

	M	SD
O_2Hb	139.42 mmol/l	26.73
HHb	54.24 mmol/l	10.80
tHb	193.66 mmol/l	28.78
oxygen saturation	71.69%	5.43
mean HR	78.78 beats/min	11.79
RMSSD	71.99 ms	64.05
LF	38.82 n.u.	19.72
HF	60.90 n.u.	19.63

Note. The values in this publication represent differences from the first phase to the respective phase. This table shows overall values to give a holistic impression of the effects.

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5 Discussion

There are many challenges to overcome in the treatment of MCS patients. AAT seems to be a suitable addition to therapy programs for them: AAT provides emotional and multisensory stimuli and motivates patients to interact in therapy through purpose, role change, and attention from animals. Further, the problems of habituation and maybe even of overstimulation are minor. However, the application of AAT in treatments for MCS patients is still relatively new, and the evidence is scarce. This thesis aimed to investigate the effect and acting mechanism of integrating animals into therapies for MCS patients.

In **study I**, we conducted the first randomized controlled trial of 10 MCS patients assessing the level of consciousness and behavioral and physiological reactions during both AAT and control therapy sessions. In the AAT sessions, patients showed significantly more eye movement, self-initiated movement, and movement in total compared to the control therapy sessions. HR was not affected, while HRV parameters implied an increase in the sympathetic and a decrease in the parasympathetic nervous system, indicating higher physiological activity and increased arousal in the AAT sessions. BAVESTA revealed a higher level of consciousness in the AAT sessions.

In **study II**, we investigated brain activity in healthy adults during different forms of contact with a dog compared to contact with a plush animal. This study found increased brain activity when the dog or plush animal came closer. Further, brain activity was higher when the healthy participants interacted with the dog than with the plush animal. The explorative analysis revealed that brain activity increased in the dog condition with repeated contact, while the brain activity in the control condition stayed at the same level.

In **study III**, we conducted the same experiment as in study II with MCS patients. We additionally analyzed HR, HRV, and BAVESTA scores. We also found an increase in brain activity when the dog or plush animal came closer. We did not find a difference in brain activity between the dog and the control condition. However, we found a difference in HR between the two conditions: HR was higher in the dog condition compared to the control condition. In HRV, we found a statistical tendency indicating an increase in parasympathetic activation. BAVESTA scores indicated that consciousness was the same in the dog and control conditions. Visual analysis of the individual activation pattern of brain activity and HR showed significant differences between patients.

These three studies are a first step toward better understanding what effect the integration of an animal has on MCS patients and what mechanisms might be at play. While we looked at AAT as a whole in the first study, we examined only the isolated interaction with a dog in the two fNIRS studies.

5.1 Interpretation and comparison of the results

The brain activity of the MCS patients differed from the healthy participants' brain activity. The healthy participants showed higher brain activity in contact with the dog than with the plush animal. The MCS patients did not show this difference. The patients' brain injuries may have made frontal activity unmeasurable due to frontal lesions. Molteni et al. (2013) reported ambiguous signals in the damaged area in their study. Fluid accumulations have an attenuating effect on the fNIRS signal, so lesions and subdural hematomas may mask existing activity (Rupawala et al., 2018). The studies by Arnskötter et al. (2021) and Kempny et al. (2016) did not mention the influence of lesions. Whether lesions masked activity or different processing occurred due to lesions remains questionable. Another explanation could be that the situation lacked context, so the patients could not understand the dog's presence. They processed the two stimuli—dog and plush animal—in the same way. Advocates of the Affolter approach have also emphasized that activities in therapy with brain-injured patients should be in a given semantic, emotional, or situational context (Affolter et al., 2009).

In study III, HR indicated that the MCS patients had greater physiological activation in the dog condition compared with the control condition. We hypothesize that this physiological activation could have been a basal emotional arousal processed only by the brainstem. Since the brainstem also plays a central role in HR regulation, we believe that this is why basal emotional arousal is visible in HR (Venkatraman et al., 2017). Frontal regions could not process this emotional arousal due to lesions or decreased consciousness. In study I, we did not find an increased HR in contact with animals as in study III. However, we did measure increased sympathetic and decreased parasympathetic neuronal activity via the HRV parameters. This pattern, like the increased HR, indicates increased physiological arousal. Both studies thus point in the same direction: the interaction with the animals aroused the MCS patients.

The healthy participants showed increased brain activity during repeated contact with the dog and a consistent activation level during contact with the plush animal. We interpreted this as an indication of a basal relationship with the dog. The MCS patients did not exhibit this pattern in brain activation, but they did show a similar pattern in physiological activation, which suggests that they had developed a basal relationship with the dog. Boitier et al. (2020) also found in their case study that a patient showed signs of a basal relationship: they reported that a patient responded to one guinea pig in particular. There are multiple possible reasons for why our study did not find a behavioral response or brain activation while other studies have. In the study by Boitier et al. (2020), the intervals between contacts were shorter, and the AAT treatment lasted for a longer period of time. Second, the therapy sessions had more context. The MCS patients in study I and in Bardl et al. (2013) also showed behavioral reactions to the animal and had therapy sessions with more context. This explanation would also indicate that MCS patients process the situation differently and thus suggests that we measured genuine brain activation.

However, it must also be considered that the plush animal could have had a greater effect on the MCS patients than we had expected. The data can also be interpreted as showing that the brain-activity response to the dog was not absent but that the MCS patients reacted more strongly than expected to the plush animal. Robots in an animal shape are already used in treatments for people with dementia and show beneficial effects on quality of life, affect, agitation, social interaction, neuropsychiatric symptoms, and the use of psychotropic or pain medication (Kang et al., 2020; Thunberg et al., 2020). It is possible that the MCS patients' lack of understanding of the situation could have caused them to react more strongly to the plush animal. In a pilot study by Arnskötter et al. (2021), one patient reacted stronger to the robot toy. However, this explanation would need further investigation, for example, by testing the response of MCS patients to plush animals compared to nonanimal-like plush objects.

Study III also found that parasympathetic activity increased when contact with the dog or plush animal became closer. This increase could indicate that the patients found the proximity to the dog and plush animal relaxing or at least not frightening. Nevertheless, we found these results in just one of the three HRV parameters, so the relaxing effect of AAT needs more research.

Patients were also assessed with BAVESTA after each session in studies I and III. In study I, the patients were found to have higher consciousness in the AAT sessions. In more detail, they showed higher values in nonverbal communication, perception, information processing, and total score. Study III did not replicate this result, which would again support the explanation that there was a lack of context. The situation in study III provided patients with insufficient information to be motivated to participate. A second explanation is the patients' positioning. Patients sat in their beds during the sessions in study III, while they were mostly sitting in wheelchairs in study I. Studies on tilt tables have shown that upright positioning can increase consciousness in MCS patients (Ng & King, 2021).

The presence of an animal seems to stimulate patients physiologically. Consistent with Bardl et al. (2013) and Boitier et al. (2020), study I indicated that patients showed more motor behavioral responses and higher levels of consciousness in interactions with animals. Greater proximity to the dog or plush animal resulted in greater physiological and neurological arousal. There were significant differences in the response patterns to the stimuli among the MCS patients. These findings are consistent with the study by Arnskötter et al. (2021), who also found differences between patients' reactions and increased brain activity with greater proximity. Two other studies investigating brain activity while patients petted cats and horses have confirmed that brain activity increases with greater proximity to animals (Kobayashi et al., 2017; Matsuura et al., 2020). The patients did not respond with higher brain activity to the presence of a dog, unlike the healthy participants. This result indicates that MCS patients and healthy participants might process interactions with an animal differently.

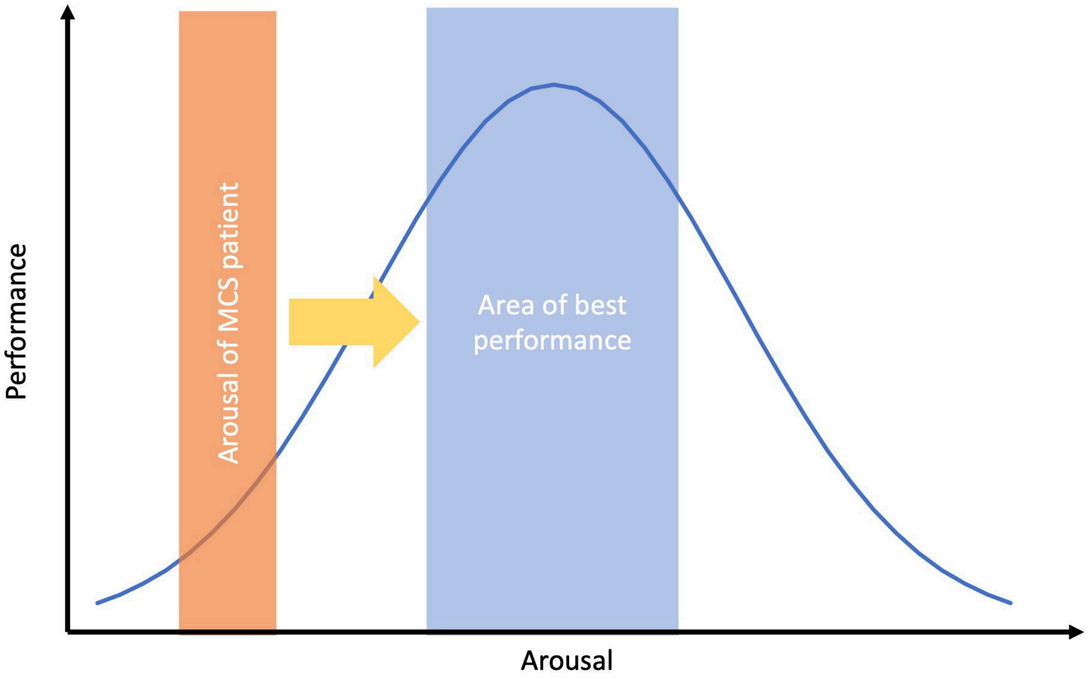
5.1.1 Possible acting mechanism

The Yerkes–Dodson law provides insight into why this activation by an animal is so crucial for therapy (Yerkes & Dodson, 1908). This law predicts that individuals perform poorly when their arousal is too low or too high. Only at an optimal arousal do they show optimal performance. MCS patients tend to be under-aroused due to their brain injuries and its effects on arousal and consciousness (Giacino et al., 2014; Giacino & Whyte, 2005). Studies I and III thus indicate that the patients had higher physiological arousal in the conditions with animal contact (Figure 3). Additionally, study II with healthy adults and the visual analysis of study III indicate that interaction with the animal increased brain activation. This arousal could have helped patients participate in the activity and respond to the stimulation from the animal. In study I, this effect postulated by the Yerkes–Dodson law resulted in observable behavior by the patients.

We attributed the higher neurological response of the healthy participants in the dog condition to more activated attentional processes and emotional processes. We did not find this neurological reaction in the MCS patients, which indicates that these processes did not happen in the frontal cortex in the MCS patients. In this experimental situation, the attention-enhancing effect known from other patient groups did not appear (Böttger et al., 2010; Gee et al., 2012; Gocheva et al., 2018; Hayama et al., 2016; Hediger & Turner, 2014). However, HR gives indications that other emotional networks were possibly addressed.

Figure 3

Optimal performance depending on arousal level according the Yerkes–Dodson law



Note. MCS patients' arousal (orange) is too low for active participation. Interaction with an animal (yellow) promotes physical arousal and therefore raises arousal (light blue). Higher arousal can enable patients to actively participate in therapy.

5.2 Strengths and limitations

The number of studies in the field of AAT for MCS patients is still low. Studies I and III therefore make a significant contribution to investigating AAT in MCS treatments. Study I examined the influence of AAT multidimensionally with the measurement of HR and HRV, the level of consciousness, and behavior analysis. Moreover, study III looked at the interaction of MCS patients and animals in a more structured experimental design. This study provides important information about possible cognitive processes with its measurements of brain activity. Study II also makes it possible to compare the neurological activation of MCS patients with the pattern of healthy adults.

However, our three studies also have limitations. Our studies were designed to measure only short-term effects. The studies had small sample sizes. It was impossible to blind raters for the conditions during video coding and assessment with BAVESTA. It is known from other studies that attitudes toward animals can affect the neurological response of study participants (Hayama et al., 2016). Unfortunately, we did not collect data on participants' attitudes toward animals. In studies II and III, conditions could not be randomized because of the irregular presence of dogs. And another limitation was that there was not an equal number of people present in the room in the dog and plush-animal conditions.

5.3 Future research

Research on AAT in treating MCS is still a young field with many open questions. It is crucial to clarify whether AAT helps patients achieve treatment goals and whether long-term effects also occur. Studies should therefore plan follow-up measurements (O'Brien et al., 2018). Study III indicates that not every patient benefits equally from AAT. It is therefore essential to determine which patient characteristics predict successful therapy with AAT. Further research should be done to determine what effects AAT may have and how these effects could be advantageous for treating MCS in general. For example, Bardl et al. (2013) mentioned that the patient was much more relaxed after AAT, which facilitated physiotherapy.

MCS patients represent a very vulnerable patient group, so an eye should be kept on the risks AAT may have for MCS patients. Especially risks related to tracheostomy, such as pneumonia, need to be looked at more closely. Studies should also clarify if AAT could overstimulate MCS patients.

In contrast to study I, the MCS patients in study III did not show a higher brain reaction or conscious level in the dog condition compared to in the plush-animal condition, which raises the question of how to design an AAT session to most benefit MCS patients. Specifically, it is still too poorly understood how much context needs to be given for patients to understand the presence of animals.

Furthermore, it is essential to investigate the mechanisms involved in more detail. Different measurement methods are needed to answer all these questions. Due to the heterogeneous brain lesions of MCS patients, it is advisable to measure broad areas for neurological measurements. The reason why the neurological response in the healthy participants was absent in the MCS patients should be further investigated.

Therapy with animals is complex, personnel-intensive, expensive, and impossible for certain patients due to allergies or infections. Further, therapy can be stressful for the animals involved. It is therefore crucial to investigate the effects of therapy with MCS patients on animals. Lastly, it is important not only to investigate AAT but also alternatives such as music therapy, aromatherapy, snoezelen, or therapy with robots (Bardl et al., 2013; Kang et al., 2020; Lichtensztejn et al., 2014; Poza et al., 2013).

5.4 Implications for practice

Including an animal is a promising intervention for increasing arousal and consciousness during therapy for MCS patients. Our three studies can provide different clues on how to perform AAT with MCS patients successfully. From our two experimental studies, it is evident that close contact increases stimulation and patients' arousal. Further, it seems important that the interaction with the animal takes place in an understandable context. Additionally, we observed that an upright position could help to increase arousal further. It was also shown that patients reacted quite differently to the dog's presence. Evaluating the benefits of AAT for an individual patient and carefully adjusting the therapy situation and the therapy animal are therefore essential. The studies further indicate that in addition to dogs, guinea pigs and rabbits can also be beneficial for treating MCS.

Regarding animal welfare, it is important to observe how the animals react to the unusual passiveness of MCS patients. The therapists may have the impulse to actively bring the animals closer to the patients because the patients cannot approach the animals themselves. However, this restricts the animal's free will, which can lead to stress. It is therefore essential to have proper concepts for the setting with MCS patients to make voluntary interaction possible.

6 Conclusion

The treatment of MCS patients is challenging, and AAT could be a possible addition to treatment due to its emotional and multisensory character. I aimed in this thesis to investigate the integration of animals into MCS treatments using multiple measuring methods. Our studies indicate that AAT is a promising intervention for MCS patients. Animals reach patients through an emotional and nonverbal pathway and foster patients’ arousal. The increased arousal allows them to participate more actively in therapy. However, further studies need to answer remaining questions regarding how best to design the AAT setting, the positive and negative effects of including animals in treating MCS, and the patient characteristics that predict successful therapy.

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