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Complete List of Authors:	Eckstein, Monika; UniversitatsKlinikum Heidelberg, Zietlow, Anna-Lena; UniversitatsKlinikum Heidelberg, Institute for Medical Psychology Gerchen, Martin; Central Institute for Mental Health, Department of Clinical Psychology Schmitgen, Mike; UniversitatsKlinikum Heidelberg, Department of General Psychiatry Ashcroft-Jones, Sarah; UniversitatsKlinikum Heidelberg Kirsch, Peter; Central Institute for Mental Health, Department of Clinical Psychology; Bernstein Center for Computational Neuroscience Ditzen, Beate; UniversitatsKlinikum Heidelberg, Institute of Medical Psychology
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The NeMo Real-Time fMRI Neurofeedback Study: Protocol of a Randomised Controlled Clinical Intervention Trial in the Neural Foundations of Mother-Infant-Bonding

*Monika Eckstein[1], *Anna-Lena Zietlow[1], Martin Fungisai Gerchen[2,3], Mike Michael Schmitgen[4], Sarah Ashcroft-Jones[1], Peter Kirsch[2,3], *Beate Ditzen[1]

[1]Institute of Medical Psychology, Centre of Psychosocial Medicine, University Hospital, Heidelberg University, Germany.

[2]Department of Clinical Psychology, Central Institute of Mental Health, Medical Faculty

Mannheim, Heidelberg University, Germany.

[3]Bernstein Center for Computational Neuroscience Heidelberg/Mannheim, Mannheim, Germany.

[4]Department of General Psychiatry, Centre of Psychosocial Medicine, University Hospital, Heidelberg University, Germany.

* contributed equally to this paper

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*Corresponding Authors:

Monika Eckstein, Anna-Lena Zietlow and Beate Ditzen

Institute of Medical Psychology,

Centre for Psychosocial Medicine,

Heidelberg University Hospital, Heidelberg University,

Bergheimer Str. 20,

69115 Heidelberg,

Germany

Email: monika.eckstein@med.uni-heidelberg.de

anna-lena.zietlow@med.uni-heidelberg.de

beate.ditzen@med.uni-heidelberg.de

Tel: +49 (0) 2221 56-7871

ABSTRACT:

Objectives:

Despite the challenges of motherhood, most mothers feel an immediate, strong emotional bond with their new-born. Maternal bonding is expressed in care-giving behaviours and sensitivity to the needs of the infant,[1]. On a neurobiological level, this is accompanied with the activation of the brain's reward system, specifically the striatal dopaminergic region,[2, 3]. However, approximately 10% of all mothers report difficulties to bond emotionally with their infant and display impaired reward responses to the interaction with their infant,[4] which might have long-term negative effects for the child's development,[5].
Functional Magnetic Resonance Imaging (fMRI) based Neurofeedback (NFB) is a new intervention technique with which participants gain direct feedback on their brain activity and can learn to control activation of specific areas. As previous studies suggest that activation of the striatal reward system can be regulated through fMRI-NFB,[6] we conceptualized and offer fMRI-NBF training to treat maternal bonding difficulties.

Setting:
In the planned trial, mothers will be presented pictures of their infant and real-time fMRI, peripheral measures, neural, endocrine, psychophysiological and behavioural measures will be assessed.

Participants:

Mothers with bonding difficulties (N= 68) will then be randomised to one of two double blind intervention groups at 4-6 months postpartum.

Interventions:

They will participate in three repeated neurofeedback training sessions with rtfMRI NFB training to increase activation of a) the ventral striatum or b) the anterior cingulate.

Outcome measures:

Interview data and real-time mother-infant interaction behaviour pre-, post-intervention and at follow-up will serve as clinical outcome measures.

Conclusions:

Rather than constituting a new standalone intervention, fMRI-NFB has the potential to serve as non-pharmacological and targeted treatment option to add to the current portfolio of therapeutic interventions in the field of maternal bonding in the postpartum period.

Trial registration:

The study is registered in the German Register for Clinical Trials (DRKS) DRKS00014570.

ARTICLE SUMMARY:

Strengths and Limitations of this Study:

- The proposed study design allows for the effect of real-time Functional Magnetic Imaging (rtfMRI) neurofeedback (NFB) to be understood in context and over time, establishing a novel therapy form that re-construes the mother-infant interaction as rewarding for the mother, in turn improving mother-infant bonding in real-time.

- The simultaneous investigation of two brain regions as well as the presence of a control group enables a comparative analysis of the benefits of the rtfMRI training, as well as helping to increase clarity regarding the role of differential brain regions in social reward.

- The proposed method, through its' longitudinal design with follow-up measures, will help to evidence how neurofeedback training brings about behavioural change, in turn allowing for better understanding of the potential applications, predictive neural signatures and participant criteria crucial for the success of rtfMRI.

- The proposed intervention aims to actively increase the response of the reward system in the mother. However, given the statistical co-occurrence of maternal bonding difficulties and postpartum depression and the similar pattern of muted reward responses in both instances, the explanatory power of the results may be confounded and therefore limited.

- The results of the planned study and increased understanding of the effects of the rtfMRI NFB method are highly relevant topics and hold clear potential for the treatment of postpartum illnesses and the long-term positive development of the infant.

INTRODUCTION:

The Neurobiological, Emotional and Behavioural Aspects of Maternal Bonding:

The parental bond is the first social bond that infants experience in life. Usually, it is the mother who serves as the child's immediate and most central social interaction partner. Maternal bonding is reflective of the first emotional bond that a mother gradually develops with her infant, within the first weeks postpartum, and is characterized by positive feelings, emotional warmth and affection towards the infant,[7]. In the eyes of some researchers, maternal bonding represents one of the most important psychological processes that gradually enfold after birth,[8] and is a significant and central aspect of the emerging relationship between mother and infant,[9].

The initial period after birth is also known as the "Baby-Honeymoon", a state of euphoria and happiness fuelled by the desire to constantly be near and to "fall in love" with the infant, which allows the mother to take on the challenges of the adaption to parenthood. In this way, maternal bonding also fulfils a biological function by ensuring the care for and subsequent survival of the new-born,[10]. On the behavioural level maternal bonding emerges through touch, eye-contact, use of "motherese", and the smiling at or caressing of the infant,[11]. Maternal bonding can therefore said to be expressed in the caregiving behaviours of the parent, in their focus on and high level of sensitivity to the needs of the infant,[1]. On the other hand, maternal bonding is also formed in the mother's emotions and thoughts about the infant and in the mental representations of motherhood that the mother holds,[12].

The strength of maternal bonding can also be demonstrated on a neurobiological level,[13]. One finding of central relevance to this topic is that mothers who report a good emotional bonding to their infant show increased reward-related activation in dopaminergic brain regions (the nucleus accumbens in the ventral striatum) in response to infant stimuli,[14]. These effects are particularly evident in the mother, in comparison to other caregivers, and have been assumed to be reinforced through the hormonal changes that accompany both birth and breast-feeding behaviours,[15, 16].

However, the bonding process is not always successful,[4, 17] and impaired maternal bonding can lead to avoidance or ambivalent emotions or behaviour in the mother. Impaired bonding can result not only in a lack of affection but also, in extreme cases, in the immediate rejection or neglect of the infant, as well as the possible presence of hostility or aggressive impulses,[8, 12]. Although postpartum bonding difficulties also occur in psychologically healthy mothers, they are observed particularly in the context of postpartum depression,[18, 19]. In a representative German sample 6.4% of psychologically healthy mothers reported difficulties in establishing emotional bonding with their infant. In depressed mothers the rate of prevalence is significantly higher at 17-29%,[20]. Furthermore, it has been shown that even subclinical depressive symptoms may negatively affect the developing bonding during the first months postpartum,[21].

Depression during the postpartum period itself is generally characterised by loss of interest or joylessness, insomnia, feelings of guilt, difficulties concentrating and, in severe cases, suicidal tendencies. Intense feelings and self-blame about being a bad mother are also often reported,[22]. On a neurobiological level, postpartum depression relates to a reduced level of reactivity in the striatal reward system, for example in the putamen,[23]. Studies show that in particular the mother-infant relationship,[24, 25], as well as the actual mother-infant interaction,[26], both short and long-term can be impaired by peri- and postpartum depression,[24, 27]. Results of a recent study suggest that the link between maternal depressive symptoms at 2 months postpartum and maternal report about interaction with the infant at 6 months was mediated by maternal feelings of attachment,[28]. The present study focuses on bonding impairments, rather than postpartum depression, although there will be inevitable co-occurrence in the participant sample.

Research indicates that experiences and interaction in the “sensitive” postpartum period have long term consequences for the cognitive as well as socio-emotional development in children,[24, 29]. Animal studies have also demonstrated that positive caring behaviours of the mother positively influence offspring brain development and long-term stress-coping behaviours even in the next generation via epigenetic changes,[30]. Conversely, in humans, decreased maternal bonding can result in higher rates of self-directed affect regulation strategies and increased stress-reactivity [31] of the infant, as well as variable self-comfort behaviours, moderated by the infant’s gender and age,[31]. Research also indicates that restrained (distant) maternal behaviour can have far-reaching negative consequences for self-regulation, social behaviour and stress management in the infant [24, 29], even into adulthood with potential influences on own parenting behaviour,[32]. Furthermore, the association between maternal depression, anxiety disorders and stress-reactivity with changes in the hypothalamic-pituitary-adrenocortical axis (HPA axis) is well-documented,[33]. Indeed, recent studies indicate that infant exposure to maternal mental disorders can increase infant baseline cortisol levels, as the main outcome of the HPA axis, in the case of maternal depression and increase infant cortisol reactivity in cases of maternal anxiety,[34]. In a lower income setting, exposure to variable intensities of maternal depression demonstrated a U-shaped response curve in infant cortisol reactivity levels in the context of immunisation. Those with exposure to moderate-maternal depression levels demonstrated the lowest cortisol reactivity during immunisation,[35]. The importance of the influence of maternal interaction behaviours themselves is also clear. One particular study demonstrates, for example, that maternal sensitivity during the interaction with their infant has a moderating influence between maternal mental health during pregnancy and infant salivary cortisol levels,[36]. Given data on the transgenerational transmission of maternal care via epigenetics,[37], both in maternal-infant bonding,[38-40] and care-giving behaviours,[41] the importance of effective treatment and intervention plans are evident.

Current inpatient and outpatient mother-infant therapies, established in the context of postpartum mental disorders, focus not only on the disorder-specific treatment but also on specific elements for the promotion of the mother-infant relationship (e.g. baby massage and video feedback methods). Video feedback interventions have been shown to have a positive influence on mother-infant interaction,[42, 43] as well as the maternal self-efficacy in interacting and coping with the infant,[44, 45]. To our knowledge, maternal bonding has thus far only been recorded as an outcome variable in one study using video feedback invention,[46]. This intervention demonstrated improvements in maternal bonding; however, the participant pool only included mentally healthy women without postpartum bonding problems. Thus, interventions focussing on maternal bonding in women experiencing difficulties forming an emotional bond to their infant are urgently needed. Presently, such interventions or findings, directly relating maternal bonding difficulties and their effects on the infant’s stress reactivity in either biological or behavioural domains, are still lacking and in particular non-pharmacological treatment concepts would substantially broaden the treatment options for affected mothers and their infants.

Reward Learning in Depression and its Relation to Postpartum Bonding Difficulties:

On a neural level the dopamine-mediated reward system (specifically the ventral striatum and the nucleus accumbens) is crucial to the rewarding aspects of social interaction,[47, 48]. The ventral striatum also appears to be specifically involved in learning rewarding stimuli, where high neural activation, correlated with the "BOLD response" in the fMRI, is accompanied by an increased dopaminergic response,[47]. As part of the learning process this reward activation is first triggered by primary enhancers, such as glucose, and over the course of the learning process transfers to secondary enhancers, such as food or money. In humans, from early in life, repeated positive social experiences can be seen as very strong reward and provided the basis for the much discussed “social brain hypothesis”, in brain research [49, 50].

Images of happy children are generally perceived as positive by healthy individuals and activate the striatal areas of the brain,[51]. Psychologically healthy mothers also show a differential neural activation response to visual stimuli of happy children, as compared with stimuli of more ambiguous facial expressions of children. Expressions of joy activated the right limbic and paralimbic areas, by comparison ambiguous expressions correlated to activity in the left higher cognitive and motor areas that are often linked to cognitive effort,[52]. Consequently, positively reinforcing activities that lead to such activation, i.e. positive social interaction, are likely to be subsequently performed more frequently. In the case of the presence of depressive symptoms, however, such neural activation (i.e. activation in striatal and limbic networks) seems to be diminished during positive social interactions, these activities are therefore perceived as less rewarding,[53, 54] and diminish the motivation for social contact. Mothers with depressive symptoms demonstrated reduced activation in subcortical and cortical limbic regions (amygdala, cingulate) and cortical regions involved in emotion regulation (including the frontal cortex, insula, anterior cingulate); in line with symptomology of diminished capacities to experience joy and to down-regulate anger, anxiety, or worry,[23, 55, 56]. Notably mothers with postpartum depression show a reduced activation in striatal reward areas in response to stimuli of their own children,[23, 55, 56].

On a neuroendocrine level, basic research suggests that in rodents social-interaction related reward is associated with receptor-activation for the neuropeptide oxytocin in the nucleus accumbens,[57]. Initial data in humans indicates, that methylation of the oxytocin receptor gene is associated with attachment in different phases of the individual's lifespan,[58] and higher methylation is associated with postpartum depression,[59]. Antidepressant treatment, in turn, is assumed to lower methylation,[60]. This may explain, on a neurobiological level. the correlation between postpartum depression and maternal bonding difficulties,[61]. However, no contemporary intervention has yet focussed on the perception of the mother-infant interaction in terms of reward and involved oxytocin-related outcomes as possible evaluation criteria.

rtfMRI NFB as a Treatment Method:

Neurofeedback (NFB) is a novel method which, through the visualization of real-time brain activation, allows an individual to consciously regulate one's own brain activation. While it is well-established that mental strategies modulate brain activation as measured by fMRI blood oxygen level dependent signal (BOLD), NFB asks participants to modulate activation in prescribed regions of their brain by their own volition and in response to the immediate feedback/ visualization of the related brain activation patterns,[62]. Thus, volunteers can learn to regulate the activation of a previously defined brain region (see,[63] for a review). NFB interventions have previously often used electroencephalography (EEG) and other electrophysiological methods to visualize and modulate activation in higher-cortical areas. However, smaller and deeper areas of the limbic and reward system can be imaged primarily with high-resolution magnetic resonance imaging (MRI). From a therapeutic point of view, the targeted modulation of these brain areas and associated circuits via neurofeedback should be associated with the improvement of mental symptoms.

In initial pilot studies, for example, patients with borderline personality disorder, anxiety or obsessive-compulsive disorders have shown the ability to down regulate activation in the insula or the hyperactive amygdala in response to stress-related stimuli,[64-66]. Similarly, high risk alcohol consumers learned to down regulate the reward related activation of alcohol related stimuli,[6]. In PTSD patients, the changes in brain connectivity after fMRI neurofeedback correlate with the reduction of symptoms,[67]. Based on the previously articulated and evidenced notion of the psychobiological mechanisms underlying attachment and bonding as goal-oriented and reward-drive,[68], the proposed study looks to investigate whether specific activation of the ventral striatum can be voluntarily increased through training in the context of mother-infant bonding. Indeed in previous research,

patients with major depressive disorders or schizophrenia were successfully trained to increase the activation of the hypoactive amygdala and the anterior cingulum in conjunction with positive stimuli,[69, 70]. Therefore, in line with the current understanding and treatment of mental illnesses, rtfMRI NFB represents a promising new intervention method for complex mental processes. Particularly important for understanding the potential of rtfMRI interventions is the transfer of the modulation of the BOLD signal towards behavioural changes,[62] which allows the value of this intervention form in a clinical setting to be distilled.

The Planned Trial:

In the planned double-blind randomized intervention trial (the "Neurofeedback for Mothers with Postpartum Bonding Difficulties Study", "NeMo-Study"), three central points will be addressed.

1. Women with postpartum bonding difficulties (including women with postpartum depression) will be compared to healthy unaffected women in terms of reward-related brain activation to pictures showing their own infant and control stimuli. It is hypothesized that women with bonding difficulties show less brain response in the reward-related areas (ventral striatum) compared to the control group.

2. The clinical group will then undergo a regiment of NFB training. Participants will learn to consciously increase the activation of reward-associated brain areas (specifically the ventral striatum), or a control region (anterior cingulate cortex, ACC), during the presentation of images of their own infant (as opposed to unknown pictures of babies). The activation of the central nervous dopamine system is hypothesized to improve bonding motivation and social interaction behaviour. The coupling of infant stimuli to the central nervous reward activation should make it easier for these women to feel more joy in the real interaction,[62] with their infant post-training and to be more attentive and more sensitive in their interactions. Training of the ventral/rostral ACC with a rather unspecific role in emotion regulation, such as in cognitive reappraisal,[71], may have general beneficial effects on parental affect regulation,[69] as well as in the more specific context of maternal bonding as based on the rewarding aspects of the mother-child interaction.

3. Effects of the NFB training will be related to the changes in behaviourally coded mother-infant interaction, maternal bonding quality and diagnostics, and epigenetic oxytocin receptor alterations, as assessed from peripheral blood. It is hypothesized that following the ventral striatum intervention, interaction, maternal bonding, and epigenetic markers will approximate the values of the healthy control group.

Thus, the study will address reward-related processes in impaired maternal bonding, observable changes on a behavioural and neuroendocrine level post-training - important steps in illustrating the validity of rtfMRI NFB as an intervention method,[62]. The proposed study is therefore well placed to produce a wealth of valuable and informative data, both regarding the potential scope of rtfMRI as a non-pharmacological intervention to young mothers.

METHODS AND ANALYSIS:

Design Overview:

To investigate points 1) – 3) (above) a controlled longitudinal study with randomised allocation of the two intervention groups to one of two anatomically defined regionally targeted areas in the NFB training will be employed. The two intervention groups will consist of mothers with identified maternal-bonding difficulties, while the control group will consist of psychologically-healthy mothers without bonding difficulties. The intervention groups will receive three NFB training sessions at intervals of approximately 14 days. Changes in maternal behaviours during mother-infant-interaction, measured using standardised coding of behavioural observations, will be assessed as the primary outcome measure. Possible alterations in oxytocin receptor methylation and gene expression will serve as secondary outcome measures. See Figure 1 for overview of study design and timeline (Figure 1).

[Figure 1: Overview of Study Design around here]

Participant Eligibility and Recruitment:

Eligible Participants:

Intervention Group: Mothers who report postpartum bonding difficulties, may present with a wide range of depressive symptoms.

Control Group: Psychologically healthy mothers with intact bonding to child.

Exclusion Criteria for all Groups:

Mothers: acute suicidality, bipolar or schizophrenic disorders, diagnosed dementia or substance abuse or substance dependency. Necessary fMRI exclusion criteria also apply.

Infants: multiple birth infants, preterm birth, confirmed physical or developmental disorders, which make participation impossible or unwise.

Patient and Public Involvement

Participants will be recruited both online and by flyers disseminated through midwives, gynaecologists, paediatricians, in pharmacies and in maternity hospitals as well as mothering forums or self-help groups (e.g. “Shadow and Light “) and registration offices. They receive thorough information about the study procedures both orally and in written form. The volunteer recruitment structure of the study makes it possible that given the intense feelings of self-blame symptomatic of the often co-occurring postpartum depression,[19] those most severely affected may not volunteer for the study. This limitation will be addressed via carefully worded and emphatic recruitment materials and processes.

Screening assessment and group allocation:

Screening Assessment:

For inclusion in the study all potential participants will be screened (T0) prior to inclusion.

Random Group Assignment Procedure:

True random assignment to the two clinical intervention groups or the control group cannot occur due to the quasi-experimental nature of the design: The mothers are assigned to being clinical or control based on the quality of their postpartum bonding to their infant. Inclusion as part of the clinical intervention group is based on the categorical assessment of impaired maternal bonding using an in-depth interview based on the proposed criteria by,[20] and asked during the clinical interview. This approach additionally allows for the dimensional assessment of maternal-bonding impairments. Bonding assessment is further elaborated by the use of additional questionnaires exploring bonding impairment, such as the Postpartum Bonding Questionnaire 16 (PBQ-16), which holds an internal consistency of Cronbach’s α=0.085,[11].

Within the intervention group participants will be completely randomly and automatically assigned to one of the following two conditions: NFB of the right ventral striatum (clinical intervention group I) or NFB of the right anterior cingulate cortex (ACC) (clinical intervention group II). This randomisation is based on the order of inclusion into the study and pre-assigned lists that are double-blind in nature. During all three intervention sessions, participants will be trained to modulate activation of the same pre-defined brain region (right ventral striatum or right ACC) based on the functional tasks during T1.

Power Analysis and Size Estimation:

The program G-Power v.3.1.9.2,[72] was used, in order to calculate the required sample size. To test the hypotheses mentioned above, a total of approximately N = 100 cases is estimated.

To perform a simple group comparison of maternal sensitivity between two treatment groups with a middle to large effect size (d = 0.78),[43] with a power of 90% and an alpha-error threshold of p = .05, a minimum group size of N = 30 for each clinical intervention group is required (I, NFB of the ventral striatum, II, NFB of the ACC). Assuming a drop-out rate of approx. 10%, an experimental sample of N = 100 will be aimed for in the projected study. With a repeated measures analyses and the T3 (follow-up) measurement the power is improved, which makes it possible to observe even medium effects with this planned sample.

N = 68 mothers with postpartum bonding difficulties will be included in the intervention (N = 34 to NFB region I ventral striatum, N = 34 to NFB region II ACC). Mental disorders, and especially postpartum depressive symptomatology, are measured in a multi-dimensional fashion through a structured clinical interview and a validated peri-partum period questionnaire. Variability in depressive symptoms, ranging from none to moderate symptom-load is sought, thus allowing for appropriate and adequate statistical analysis of this covariate factor. As a control group N = 32 psychologically healthy women with a good bond with their infant will be recruited.

Pre- Post and Follow-Up Assessments (T1, T2 and T3):

Before (T1) and after the training (T2), participants will perform an extensive battery of diagnostic assessments characterizing the clinical aspects of bonding, including the instructed mother-child-interaction in the Face-To-Face-Still-Face-Paradigm,[73, 74]. A baseline fMRI session will assess the sensitivity of both the reward system and the limbic system using established fMRI tasks and blood samples will be collected for analysing hormonal and (epi-)genetic markers. After the end of the last neurofeedback training a post-intervention measurement (T2) and with 12 month a follow up (T3) will be performed using the same methods as at T1. At T3, mothers and infants will be instructed to freely play,[75] and videotaped. Age-specific markers of the infant's development will be coded using a standardized developmental test,[76]. (Refer to Figure 1 for timeline clarification).

This combination of assessments allows for a comprehensive understanding of maternal bonding and behaviour on a neural, neuroendocrine, epigenetic and behavioural level.

See Table 1 for further details on the assessments and scheduling of measure collection.

Survey instruments and Coding Systems used in the Study:

Measure	Citation	M.	F.	I.	T0	T1	N	T2	T3
Mother and infant:									
Mother-Infant Interaction (FFSF)	[74, 77]	X		X		X		X	
Free-Play Situation	[75]	X		X					X
Interviews:									
Diagnostic Interview for Psychiatric Disorders	Adapted for DSM-5, unpublished manual	X							
Interview for Postpartum Bonding Disorders	[78]	X	X						
Attachment Style Interview	[79]	X	X						
Questionnaires:									
Postpartum Bonding Questionnaire 16-R	[11]	X	X			X		X	X
Edinburgh Postnatal Depression Scale	[80]	X	X			X		X	X
Agoraphobic Cognitions Questionnaire, Body Sensations Questionnaire, and Mobility Inventory	[81]	X	X			X		X	X
Maternal Self-Confidence Scale	[82]	X	X			X			X
Prenatal Emotional Stress Index	[18]	X	X						
Parental Bonding Instrument	[83]	X	X			X			
German version of the EMBU questionnaire regarding remembered parenting behaviours	[84]	X	X			X			
Experiences in Close Relationships- Revised	[85]	X	X			X			
Social Support Questionnaire	[86]	X	X			X		X	X
Personality Inventory- DSM 5 Short Form	[87]	X	X			X			
Childhood Trauma Questionnaire	[88]	X	X			X			
Partnership Questionnaire	[89]	X	X			X		X	X
Dyadic Coping Inventory	[90]					X		X	X
Parenting Stress Inventory	[91]	X	X					X	X
Vulnerable Attachment Style Questionnaire	[92]	X	X			X			
Infant:									
Infant Behaviour Questionnaire	[93]	X	X			X			X
Development Assessment: Infant									
Bayley's Infant Development Scale III	[76]			X					X
Physiological Measures									
Infant Saliva Sample				X		X		X	X
Mother Blood Sample		X				X		X	X
Neurofeedback Training		X					X		
Reward Task	[94]	X				X		X	X
Emotional Go-No-Go	[95]	X				X		X	X
Passive Viewing Task	[96]	X				X		X	X

Table 1: Schedule of measures used the in study. M. Mother Response, F. Father response, I. Infant response, T0 screening assessment, T1 baseline assessment, N-neurofeedback sessions, T2 post assessment, T3 follow-up.

Detailed Description of Measures, Methods and Instruments used:

Initial Assessments:

Baseline Assessment:

For the baseline testing period (T1) the mothers will be invited to our video-laboratory together with their infants. A structured clinical interview according to DIPS as well as an interview regarding postpartum bonding impairment will be carried out. Subsequently, parent-infant interaction will be assessed and videotaped during the Face-to-Face-Still-Face (FFSF) paradigm, a widely used paradigm for evaluating the quality of early parent-infant interaction,[73]. To determine infant stress-reactivity, cortisol and alpha-amylase will be extracted from infant saliva according to standard protocols,[97], which is collected before (C1), immediately after (C2), 20 minutes (C3) and 30 minutes after the FFSF (C4).

Instructed Mother-infant interaction:

Mother-infant-interaction pre- post-intervention (T1 and T3): The standardised mother-infant-interaction before and after the intervention follows the established FFSF paradigm,[74, 77]. It consists of three consecutive instructed two-minute episodes in which the mother, with the infant seated in the baby chair, interacts in accordance with a fixed pattern: First, an initial face-to-face interaction in which the mothers are instructed to play with their infant as usual (without the aid of toys and pacifiers). Next, the still-face episode in which the mothers have to turn their head aside while silently counting to ten and then turn back to the infant but not engage in any gestures, facial expressions, or vocalizations. Finally, the procedure ends with the reunion episode in which the mother is required to resume face-to-face play with her infant.

Mother-infant-interaction Follow-Up (T4): As the Still-face paradigm can be conducted up to the age of 9 months, parent-infant interaction will be assessed at T4 during a 15-minute free-play situation and a subsequent limit setting task. All interaction episodes will be videotaped and coded according to the Coding Infant Behaviour Scales (CIB),[98]. The CIB scales assess parental sensitivity and responsiveness as well as intrusiveness and withdrawal via composite scores,[99]).

Psychobiological Measurements:

Epigenetic Information on the Oxytocin-System and Cortisol/Alpha Amylase in Saliva:

Maternal blood samples are taken to examine the endogenous oxytocin level, gonadal hormones and epigenetic parameters of the oxytocin gene and oxytocin receptor gene. For the endocrine investigation of the stress hormone cortisol painless saliva samples are taken from the infants directly before, immediately after 20 and 30 minutes after the mother-infant interaction using a saliva probe. An elevation of infant stress-reactivity is expected during the interaction, from these elevations the Peak and Recovery be ascertained. The area under the curve is therefore analysed as a reactivity index,[100] as is standard practice,[101].

MRI-tasks:

Reward Task:

The task, (adapted from,[94]), requires participants to perform a spatial working memory task with two levels of cognitive load, differentiated by the number of circles to be remembered. Subjects first see a cue informing them of the potential monetary reward value – high or low. After presentation of the fixation cross, an array of yellow circles (3 or 7 circles), is displayed followed the target, a green circle, that is then presented at any position on the screen. The participants must decide whether this circle is in the same position as one of the circles presented previously. In the rewarded condition, a feedback about the win (followed by the cumulated amount of earned money appears. Correct responses are reinforced by two different amounts of monetary reward that are counterbalanced with the levels of cognitive

load. Incorrect responses on rewarded trials result in no monetary gain. While performing the task, participants rate their mood and stress levels a quarter of the trials.

Emotional Go/NoGo:
The participants are presented with positive and negative expressions of unknown babies, unknown adults as well as un-social control stimuli (geometric figures; a circle, a cross, a diamond and a triangle) over three presentation blocks.

The following factors are systematically manipulated: child vs adult and emotionality of facial expression (positive vs negative). In two blocks, the participants receive instructions to respond by pressing a button as fast as possible to all facial expression except the negative (one block babies, one block adults). In two other blocks they are instructed to respond as fast as possible to all except the positive (one block babies, one block adults). In the two non-social blocks the participants should react as fast as possible to all shapes but not to a circle or a diamond,[95].

Passive Viewing Task:
The participants view previously collected neutral-positive images of partners and their babies in positive and negative affect, with instructions to observe carefully. Unfamiliar men and babies will serve as control stimuli. Viewing images of partners and children leads to the activation of a broad socio-emotional neural network, that is involved with empathy and socio-emotional cognition,[23, 96].

Coding Systems Used in the Study:
Coding Interactive Behaviour:
For the evaluation of the mother-child interaction over all measurement points the Coding Interactive Behaviour System was used (CIB:[75]). The CIB is a widely used, global rating system for analysing mother-infant-interaction. The system uses multiple codes for the infants, parents and dyadic codes that aggregate into meaningful theoretically-based constructs (e.g., sensitivity, intrusiveness, reciprocity, social engagement, withdrawal). The psychometric characteristics are all well described,[102].

Infant Development Diagnostics used in the Study:
Bayley Scales of Infant and Toddler Development-III:
The Bayley Scales of Infant and Toddler Development-III (Bayley-III;[103, 104]) assess the development of infants and toddlers between one and 42 months of age. The test battery covers the domains of cognition, language, motor, social-emotional and adaptive development using I-Q-scaled composite scores. Whereas the first three aspects are assessed by behavioural observation and the latter two utilize questionnaires, with duration ranging between 50 and 90 min,[105]. The Cognitive Scale assesses sensory-motor development, exploration and manipulation, object relatedness, concept formation and memory. The Language Scale is composed of the two subscales receptive and expressive communication. Testing pre-verbal behaviour, vocabulary development, understanding of morphological markers, social referencing and verbal comprehension and pre-verbal communication, vocabulary development and morpho-syntactic development respectively. In addition, the Bayley Scales include a Gross- and Fine-Motor Scale, a Social-Emotional Scale, and an Adaptive Behaviour Scale. This study focuses on the language and cognitive composite scores due to the rather small proposed sample size.

The Bayley-III indices and subscales demonstrate good internal consistency and good split-half-consistency according to the Spearman-Brown formula,[105]. Regarding construct validity, confirmatory factor analysis of the subtests of the Cognitive, Language, and Motor scales supported a three-factor model across all ages. The Bayley-III scales have

been normed for German infants and children,[106]. This method is taken standard internationally, particularly in terms of reviewing developmental delays and planning targeted early interventions. The order of the subtests can be adapted to the needs of the child.

Neurofeedback Setup:
The mothers with bonding difficulties will be randomised to receive one of the two following interventions: I) NFB for activation of the ventral striatum or II) neurofeedback for activation of the anterior cingulate cortex (ACC). Participants will partake in NFB training over three sessions at intervals of approximately 14 days (refer to Figure 1). At baseline, a high-resolution structural MRI scan and the activation pattern based on the infant-like stimuli will be recorded (using a Tim Trio 3T MRI scanner, Siemens, Erlangen, Germany).

Each intervention session will last approximately 60 minutes and will begin with a 10minute preparatory structural MRI scan. A 6minute resting state fMRI scan is then conducted to allow a resting baseline to be established and to prepare the neurofeedback set-up. Afterwards, three rtfMRI NFB runs of 9:29 minutes are conducted. During neurofeedback training positive and neutral pictures of the participants' own child taken from the recorded mother-infant interaction session will be presented together with an on-screen "thermometer" which represents the current intensity of activation in the striatum or ACC and must be upregulated. In each run, six alternating phases of up-regulation (~41 seconds, including a ~10 second initial period without thermometer display) and of rest (observation of a fixation cross, ~41 seconds) will be performed. (See Figure 2 for details). The women are instructed to try several strategies and use the one that works best. Afterwards, they are asked to report which strategy they used. In the first and third scanning session the third run is implemented as a "transfer" block without the visible "thermometer" display,[6, 65, 70]. For more technical details regarding the neurofeedback setup please refer to [107] which uses an identical neurofeedback procedure in the context of alcohol addiction.

[Figure 2: Two Trials of the Neurofeedback Intervention within a Run around here]

Data Analysis Plan:

Statistical analyses, namely the main comparison of the two groups (mothers with bonding difficulties vs. the control group) and the longitudinal analysis of positive relationships changes after the NFB intervention (interaction behaviour, attachment data, as well as psychophysiological, neuroendocrine and epigenetic markers (see Table 1) will be done using IBM SPSS Statics and R (r-project.org). The MRI data will be analysed with general linear models using statistical parametric mapping with SPM (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) implemented (Wellcome Department of Cognitive Neurology, London, UK) in MATLAB (MathWorks, Natick, MA) MATLAB . To calculate pre-, post- and follow-up group differences between the intervention and control groups, repeated measures ANOVAs will be calculated, while multi-level models will be used to model changes over the time course.

Figure Legends:

Figure 1:

Overview of Study Design

Figure 2:

Two Trials of the Neurofeedback Intervention within a Run

ETHICS AND DISSEMINATION:

Ethics, consent to participate and Dissemination:
Study procedures are in line with the recommendations of the World Medical Association (revised Declaration of Helsinki) and were approved by the Ethics Committee of the Medical Faculty, s-450/2017, Heidelberg University. The study is registered as clinical trial in the German Register for Clinical Trials (DRKS) DRKS00014570. All participants will provide written informed consent after receiving a detailed oral and written explanation of all procedures and can withdraw their consent at any time without negative consequence.

Results will be internationally published and disseminated, to further the discussion on non-pharmacological treatment options in complex mental disorders.

Consent for Publication:
Not applicable.

Availability of Data and Material
For protection of personal rights and due to the sensitivity of the clinical information, raw data will not be available in the public domain. The medical confidentiality and the provisions of the EU-DSGV (Data Protection Act) are therefore complied with; the collected medical findings and / or personal information are recorded in the examination centre or stored electronically.

Furthermore, important data for the study are stored in an anonymised format, evaluated and, if necessary, passed on to the necessary persons. Third parties gain no insight into the original data with the database remaining restricted for scientific use only. If results are published, it will not be possible to draw any conclusions to an individual as the confidentiality of personal data remains guaranteed at all time

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AUTHORS' CONTRIBUTION STATEMENT:
B.D., P.K., A.L.Z. and M.E. designed the study; M.E. and A.L.Z. lead the study; S.A.J. collected data; P.K., M.F.G. and M.M.S. established the experimental set-up; S.A.J., A.L.Z and ME wrote the manuscript; All authors provided comments on the manuscript.

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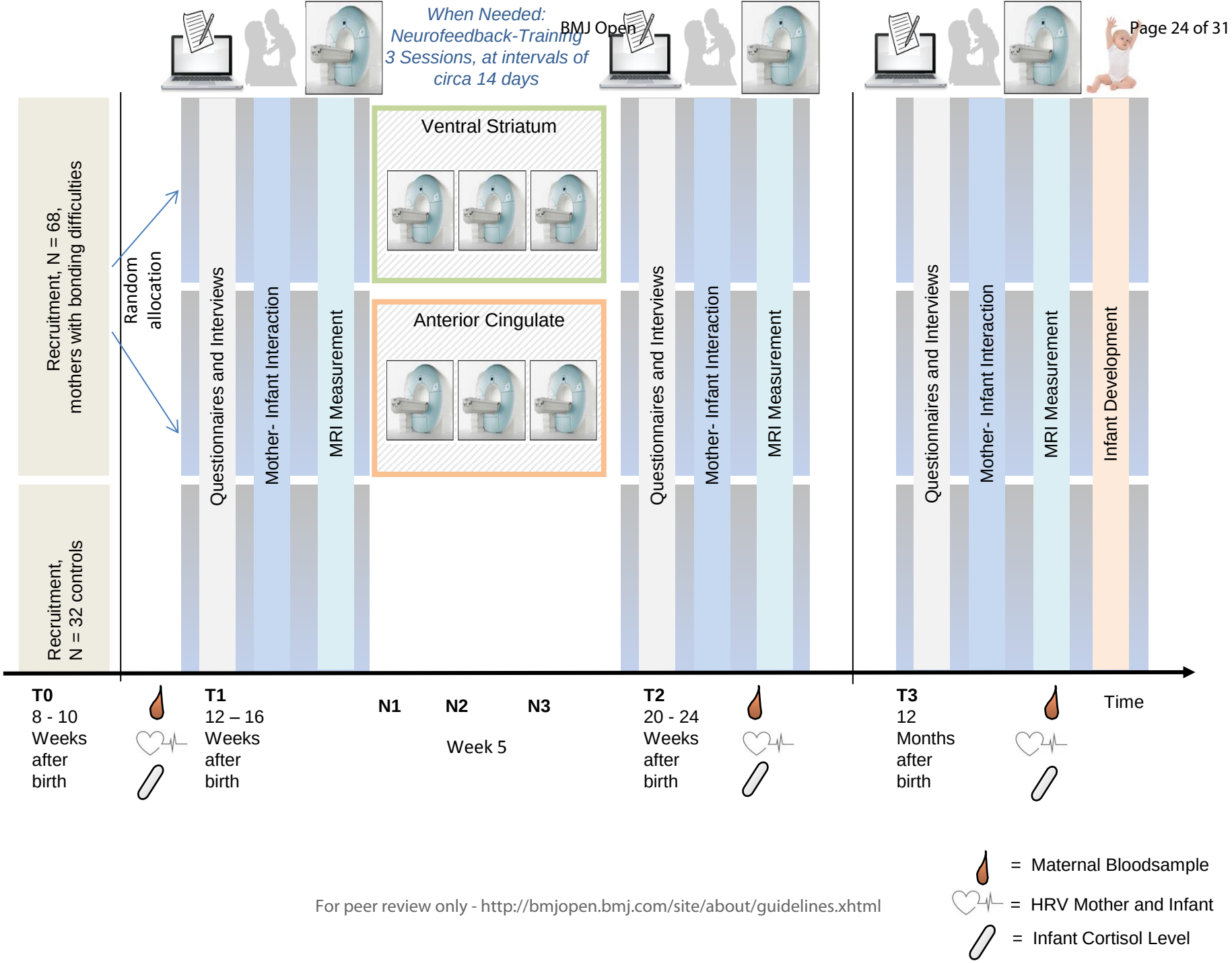
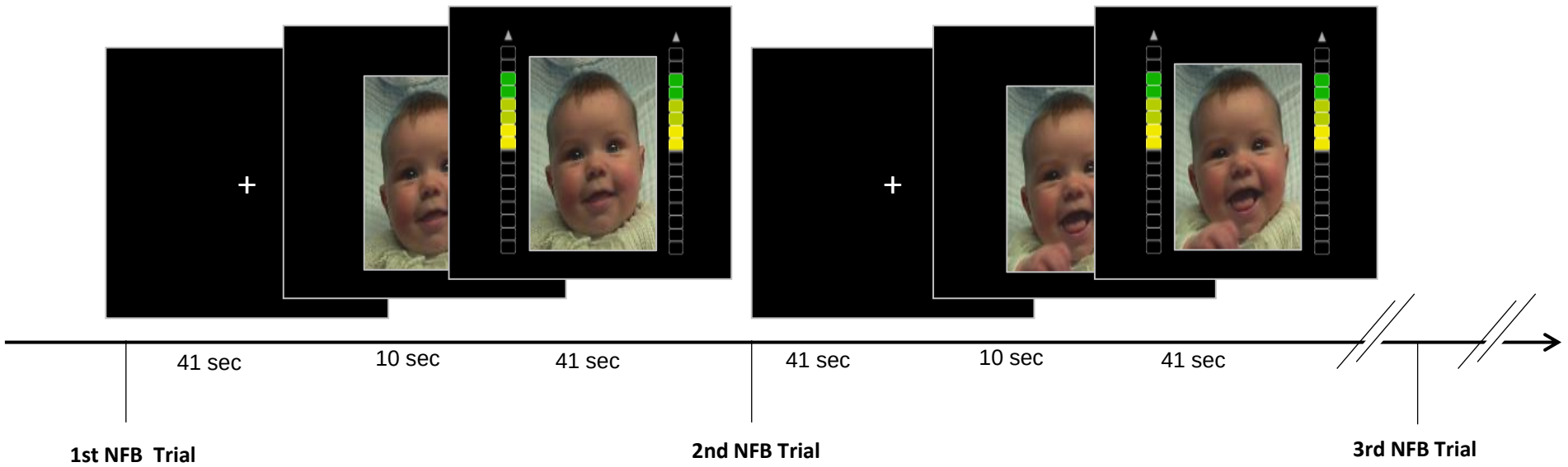
The authors declare that they have no competing interests.

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1st NFB Trial
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		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	23
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	23
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	23

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-7
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	7
Objectives	#7	Specific objectives or hypotheses	7
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	14
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-10

Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12-14
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed	

mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	15
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	17
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Data access	#29	Statement of who will have access to the final trial dataset,	17

		and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

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The NeMo Real-Time fMRI Neurofeedback Study: Protocol of a Randomised Controlled Clinical Intervention Trial in the Neural Foundations of Mother-Infant-Bonding

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The NeMo Real-Time fMRI Neurofeedback Study: Protocol of a Randomised Controlled Clinical Intervention Trial in the Neural Foundations of Mother-Infant-Bonding

*Monika Eckstein[1], *Anna-Lena Zietlow[1], Martin Fungisai Gerchen[2,3], Mike Michael Schmitgen[4], Sarah Ashcroft-Jones[1], Peter Kirsch[2,3], *Beate Ditzen[1]

[1]Institute of Medical Psychology, Centre of Psychosocial Medicine, University Hospital, Heidelberg University, Germany.

[2]Department of Clinical Psychology, Central Institute of Mental Health, Medical Faculty

Mannheim, Heidelberg University, Germany.

[3]Bernstein Center for Computational Neuroscience Heidelberg/Mannheim, Mannheim, Germany.

[4]Department of General Psychiatry, Centre of Psychosocial Medicine, University Hospital, Heidelberg University, Germany.

* contributed equally to this paper

Key words: mother-infant bonding, maternal bonding difficulties, ventral striatum, functional magnetic resonance imaging (fMRI) neurofeedback, oxytocin, dopamine

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*Corresponding Authors:

Monika Eckstein, Anna-Lena Zietlow and Beate Ditzen

Institute of Medical Psychology,

Centre for Psychosocial Medicine,

Heidelberg University Hospital, Heidelberg University,

Bergheimer Str. 20,

69115 Heidelberg,

Germany

Email: monika.eckstein@med.uni-heidelberg.de

anna-lena.zietlow@med.uni-heidelberg.de

beate.ditzen@med.uni-heidelberg.de

Tel: +49 (0) 2221 56-7871

ABSTRACT:

Introduction:

Most mothers feel an immediate, strong emotional bond with their new-born. On a neurobiological level, this is accompanied with the activation of the brain's reward system, specifically the striatal dopaminergic region. However, approximately 10% of all mothers report difficulties to bond emotionally with their infant and display impaired reward responses to the interaction with their infant which might have long-term negative effects for the child's development.
As previous studies suggest that activation of the striatal reward system can be regulated through Functional Magnetic Resonance Imaging (fMRI) based Neurofeedback (NFB), we conceptualized and offer fMRI-NBF training to treat maternal bonding difficulties.

Methods and analysis:

In the planned trial, mothers will be presented pictures of their infant and real-time fMRI, peripheral measures, neural, endocrine, psychophysiological and behavioural measures will be assessed. Mothers with bonding difficulties (N= 68) will be randomised to one of two double blind intervention groups at 4-6 months postpartum. They will participate in three repeated neurofeedback training sessions with rtfMRI NFB training to increase activation of a) the ventral striatum or b) the anterior cingulate. Interview data and real-time mother-infant interaction behaviour pre-, post-intervention and at follow-up will serve as clinical outcome measures.

Ethics and Dissemination:

Study procedures are in line with the recommendations of the World Medical Association (revised Declaration of Helsinki) and were approved by the Ethics Committee of the Medical Faculty, s-450/2017, Heidelberg University. All participants will provide written informed consent after receiving a detailed oral and written explanation of all procedures and can withdraw their consent at any time without negative consequence.

Results will be internationally published and disseminated, to further the discussion on non-pharmacological treatment options in complex mental disorders.

Trial registration details:

The study is registered in the German Register for Clinical Trials (DRKS) DRKS00014570.

ARTICLE SUMMARY:

Strengths and Limitations of this Study:

- The novelty of used methods allow the investigation of the effect of real-time Functional Magnetic Imaging (rtfMRI) neurofeedback (NFB), on maternal bonding.
- The study investigates the ability of rtfMRI NFB to upregulate brain activity in correlation with behavioural and biopsychological markers.
- The simultaneous investigation of two brain regions as well as the presence of a control group proposed in the methods of this protocol enables a comparative analysis of the benefits of the rtfMRI training, as well as increasing clarity regarding the role of differential brain regions in social reward.
- The proposed method, through its' longitudinal design with follow-up measures, will help to evidence how neurofeedback training brings about behavioural change, in turn allowing for better understanding of the potential applications, predictive neural signatures and participant criteria crucial for the success of rtfMRI.
- Due to the statistical co-occurrence of maternal bonding difficulties and postpartum depression and the similar pattern of muted reward responses in both instances, the explanatory power of the results of the rtfMRI NFB intervention may be confounded.s

INTRODUCTION:

The Neurobiological, Emotional and Behavioural Aspects of Maternal Bonding:

The parental bond is the first social bond that infants experience in life. Usually, it is the mother who serves as the child's immediate and most central social interaction partner. Maternal bonding is reflective of the first emotional bond that a mother gradually develops with her infant, within the first weeks postpartum, and is characterized by positive feelings, emotional warmth and affection towards the infant,[1]. In the eyes of some researchers, maternal bonding represents one of the most important psychological processes that gradually enfold after birth,[2] and is a significant and central aspect of the emerging relationship between mother and infant,[3].

The initial period after birth is also known as the "Baby-Honeymoon", a state of euphoria and happiness fuelled by the desire to constantly be near and to "fall in love" with the infant, which allows the mother to take on the challenges of the adaption to parenthood. In this way, maternal bonding also fulfils a biological function by ensuring the care for and subsequent survival of the new-born,[4]. On the behavioural level maternal bonding emerges through touch, eye-contact, use of "motherese", and the smiling at or caressing of the infant,[5]. Maternal bonding can therefore said to be expressed in the caregiving behaviours of the parent, in their focus on and high level of sensitivity to the needs of the infant,[6]. On the other hand, maternal bonding is also formed in the mother's emotions and thoughts about the infant and in the mental representations of motherhood that the mother holds,[7].

The strength of maternal bonding can also be demonstrated on a neurobiological level,[8]. Central structures such as the striatum, ventral tegmental area, amygdala, septum and hypothalamus are involved in affiliative behaviour, together with a combination of neurotransmitters and modulators such as dopamine and oxytocin[9, 10]. For affiliative traits, it has been proposed that those are mediated by dopaminergic projections from the ventral tegmental area and endogenous opioids in the hypothalamus with projections to the septum, especially during physical contact [11], that underlie rewarding feelings of affiliation.

One finding of central relevance to maternal bonding in particular is that mothers who report a good emotional bonding to their infant show increased reward-related activation in dopaminergic brain regions (the nucleus accumbens in the ventral striatum) in response to infant stimuli,[12]. These effects are particularly evident in the mother, in comparison to other caregivers, and have been assumed to be reinforced through the hormonal changes that accompany both birth and breast-feeding behaviours,[13, 14].

However, the bonding process is not always successful,[15, 16] and impaired maternal bonding can lead to avoidance or ambivalent emotions or behaviour in the mother. Impaired bonding can result not only in a lack of affection but also, in extreme cases, in the immediate rejection or neglect of the infant, as well as the possible presence of hostility or aggressive impulses,[2, 7]. Although postpartum bonding difficulties also occur in psychologically healthy mothers, they are observed particularly in the context of postpartum depression,[17, 18]. In a representative German sample 6.4% of psychologically healthy mothers reported difficulties in establishing emotional bonding with their infant. In depressed mothers the rate of prevalence is significantly higher at 17-29%,[19]. Furthermore, it has been shown that even subclinical depressive symptoms may negatively affect the developing bonding during the first months postpartum,[20].

Depression during the postpartum period itself is generally characterised by loss of interest or joylessness, insomnia, feelings of guilt, difficulties concentrating and, in severe cases, suicidal tendencies. Intense feelings and self-blame about being a bad mother are also often reported,[21]. On a neurobiological level, postpartum depression relates to a reduced level of reactivity in the striatal reward system, for example in the putamen,[22], but also the amygdala network [23] together with endocrine changes in the serotonin and steroid systems [24]. Studies show that in particular the mother-infant relationship,[25, 26], as well

as the actual mother-infant interaction,[27], both short and long-term can be impaired by peri- and postpartum depression,[25, 28]. Results of a recent study suggest that the link between maternal depressive symptoms at 2 months postpartum and maternal report about interaction with the infant at 6 months was mediated by maternal feelings of attachment,[29]. The present study focuses on bonding impairments, rather than postpartum depression, although there will be inevitable co-occurrence in the participant sample.

Research indicates that experiences and interaction in the "sensitive" postpartum period have long term consequences for the cognitive as well as socio-emotional development in children,[25, 30]. Animal studies have also demonstrated that positive caring behaviours of the mother positively influence offspring brain development and long-term stress-coping behaviours even in the next generation via epigenetic changes,[31]. Conversely, in humans, decreased maternal bonding can result in higher rates of self-directed affect regulation strategies and increased stress-reactivity [32] of the infant, as well as variable self-comfort behaviours, moderated by the infant's gender and age,[32]. Research also indicates that restrained (distant) maternal behaviour can have far-reaching negative consequences for self-regulation, social behaviour and stress management in the infant [25, 30], even into adulthood with potential influences on own parenting behaviour,[33]. Furthermore, the association between maternal depression, anxiety disorders and stress-reactivity with changes in the hypothalamic-pituitary-adrenocortical axis (HPA axis) is well-documented,[34]. Indeed, recent studies indicate that infant exposure to maternal mental disorders can increase infant baseline cortisol levels, as the main outcome of the HPA axis, in the case of maternal depression and increase infant cortisol reactivity in cases of maternal anxiety,[35]. In a lower income setting, exposure to variable intensities of maternal depression demonstrated a U-shaped response curve in infant cortisol reactivity levels in the context of immunisation. Those with exposure to moderate-maternal depression levels demonstrated the lowest cortisol reactivity during immunisation,[36]. The importance of the influence of maternal interaction behaviours themselves is also clear. One particular study demonstrates, for example, that maternal sensitivity during the interaction with their infant has a moderating influence between maternal mental health during pregnancy and infant salivary cortisol levels,[37]. Given data on the transgenerational transmission of maternal care via epigenetics,[38], both in maternal-infant bonding,[39-41] and care-giving behaviours,[42] the importance of effective treatment and intervention plans are evident.

Current inpatient and outpatient mother-infant therapies, established in the context of postpartum mental disorders, focus not only on the disorder-specific treatment but also on specific elements for the promotion of the mother-infant relationship (e.g. baby massage and video feedback methods). Video feedback interventions have been shown to have a positive influence on mother-infant interaction,[43, 44] as well as the maternal self-efficacy in interacting and coping with the infant,[45, 46]. To our knowledge, maternal bonding has thus far only been recorded as an outcome variable in one study using video feedback invention,[47]. This intervention demonstrated improvements in maternal bonding; however, the participant pool only included mentally healthy women without postpartum bonding problems. Thus, interventions focussing on maternal bonding in women experiencing difficulties forming an emotional bond to their infant are urgently needed. Presently, such interventions or findings, directly relating maternal bonding difficulties and their effects on the infant's stress reactivity in either biological or behavioural domains, are still lacking and in particular non-pharmacological treatment concepts would substantially broaden the treatment options for affected mothers and their infants.

Reward Learning in Depression and its Relation to Postpartum Bonding Difficulties:
On a neural level the dopamine-mediated reward system (specifically the ventral striatum and the nucleus accumbens) is crucial to the rewarding aspects of social interaction,[48, 49]. The ventral striatum also appears to be specifically involved in learning rewarding stimuli,

where high neural activation, correlated with the "BOLD response" in the fMRI, is accompanied by an increased dopaminergic response,[48]. As part of the learning process this reward activation is first triggered by primary enhancers, such as glucose, and over the course of the learning process transfers to secondary enhancers, such as food or money. In humans, from early in life, repeated positive social experiences can be seen as very strong reward and provided the basis for the much discussed “social brain hypothesis”, in brain research [50, 51].

Images of happy children are generally perceived as positive by healthy individuals and activate the striatal areas of the brain,[52]. Psychologically healthy mothers also show a differential neural activation response to visual stimuli of happy children, as compared with stimuli of more ambiguous facial expressions of children. Expressions of joy activated the right limbic and paralimbic areas, by comparison ambiguous expressions correlated to activity in the left higher cognitive and motor areas that are often linked to cognitive effort,[53]. Consequently, positively reinforcing activities that lead to such activation, i.e. positive social interaction, are likely to be subsequently performed more frequently. In the case of the presence of depressive symptoms, however, such neural activation (i.e. activation in striatal and limbic networks) seems to be diminished during positive social interactions, these activities are therefore perceived as less rewarding,[54, 55] and diminish the motivation for social contact. Mothers with depressive symptoms demonstrated reduced activation in subcortical and cortical limbic regions (amygdala, cingulate) and cortical regions involved in emotion regulation (including the frontal cortex, insula, anterior cingulate); in line with symptomology of diminished capacities to experience joy and to down-regulate anger, anxiety, or worry,[22, 23, 56]. Notably mothers with postpartum depression show a reduced activation in striatal reward areas in response to stimuli of their own children,[22, 23, 56].

On a neuroendocrine level, basic research suggests that in rodents social-interaction related reward is associated with receptor-activation for the neuropeptide oxytocin in the nucleus accumbens,[57]. Initial data in humans indicates, that methylation of the oxytocin receptor gene is associated with attachment in different phases of the individual’s lifespan,[58] and higher methylation is associated with postpartum depression,[59]. Antidepressant treatment, in turn, is assumed to lower methylation,[60]. This may explain, on a neurobiological level. the correlation between postpartum depression and maternal bonding difficulties,[61]. However, no contemporary intervention has yet focussed on the perception of the mother-infant interaction in terms of reward and involved oxytocin-related outcomes as possible evaluation criteria.

rtfMRI NFB as a Treatment Method:

Neurofeedback (NFB) is a novel method which, through the visualization of real-time brain activation, allows an individual to consciously regulate one's own brain activation. While it is well-established that mental strategies modulate brain activation as measured by fMRI blood oxygen level dependent signal (BOLD), NFB asks participants to modulate activation in prescribed regions or even networks of their brain by their own volition and in response to the immediate feedback/ visualization of the related brain activation patterns,[62]. Thus, volunteers can learn to regulate the activation of a previously defined brain region (see,[63] for a review). NFB interventions have previously often used electroencephalography (EEG) and other electrophysiological methods to visualize and modulate activation in higher-cortical areas. However, smaller and deeper areas of the limbic and reward system can be imaged primarily with high-resolution magnetic resonance imaging (MRI). One previous study has proven that healthy volunteers can be trained to voluntarily increase their brain activation patterns that have been classified for affiliative emotions in the same individual subject using voxel pattern analyses [64]. From a therapeutic point of view, the targeted modulation of

specific brain areas and associated circuits via neurofeedback should be associated with the improvement of mental symptoms.

In initial clinical pilot studies, for example, patients with borderline personality disorder, anxiety or obsessive-compulsive disorders have shown the ability to down regulate activation in the insula or the hyperactive amygdala in response to stress-related stimuli,[65-67]. Similarly, high risk alcohol consumers learned to down regulate the reward related activation of alcohol related stimuli,[68]. In PTSD patients, the changes in brain connectivity after fMRI neurofeedback correlate with the reduction of symptoms,[69]. Based on the previously articulated and evidenced notion of the psychobiological mechanisms underlying attachment and bonding as goal-oriented and reward-drive,[70], the proposed study looks to investigate whether specific activation of the ventral striatum can be voluntarily increased through training in the context of mother-infant bonding. Indeed in previous research, patients with major depressive disorders or schizophrenia were successfully trained to increase the activation of the hypoactive amygdala and the anterior cingulum in conjunction with positive stimuli,[71, 72]. Therefore, in line with the current understanding and treatment of mental illnesses, rtfMRI NFB represents a promising new intervention method for complex mental processes. Particularly important for understanding the potential of rtfMRI interventions is the transfer of the modulation of the BOLD signal towards behavioural changes,[62] which allows the value of this intervention form in a clinical setting to be distilled.

The Planned Trial:

In the planned double-blind randomized intervention trial (the “Neurofeedback for Mothers with Postpartum Bonding Difficulties Study”, “NeMo-Study”), three central points will be addressed.

1. Women with postpartum bonding difficulties (including women with postpartum depression) will be compared to healthy unaffected women in terms of reward-related brain activation to pictures showing their own infant and control stimuli. It is hypothesized that women with bonding difficulties show less brain response in the reward-related areas (ventral striatum) compared to the control group.

2. The clinical group will then undergo a regiment of NFB training. Participants will learn to consciously increase the activation of reward-associated brain areas (specifically the ventral striatum), or a control region (anterior cingulate cortex, ACC), during the presentation of images of their own infant. For ethical reasons, an active control treatment was chosen rather than joke or non-feedback. The activation of the central nervous dopamine system via the striatum is hypothesized to improve bonding motivation and social interaction behaviour. The coupling of infant stimuli to the central nervous reward activation should make it easier for these women to feel more joy in the real interaction,[62] with their infant post-training and to be more attentive and more sensitive in their interactions. Training of the dorsal/rostral ACC with a rather unspecific role in emotion regulation, such as e.g. in cognitive reappraisal,[73] or response inhibition [74], may have general beneficial effects as shown in another NFB study [71] but also indirectly influence parental affect regulation,. However, the more specific striatal feedback based on the rewarding aspects of the mother-child interaction are assumed to have stronger effects.

3. Effects of the NFB training will be related to the changes in behaviourally coded mother-infant interaction, maternal bonding quality and diagnostics, and epigenetic oxytocin receptor alterations, as assessed from peripheral blood. It is hypothesized that following the ventral striatum intervention, interaction, maternal bonding, and epigenetic markers will approximate the values of the healthy control group.

Thus, the study will address reward-related processes in impaired maternal bonding, observable changes on a behavioural and neuroendocrine level post-training - important steps in illustrating the validity of rtfMRI NFB as an intervention method,[62]. The proposed study is therefore well placed to produce a wealth of valuable and informative data, both regarding the potential scope of rtfMRI as a non-pharmacological intervention to young mothers.

METHODS AND ANALYSIS:

Design Overview:

To investigate points 1) – 3) (above) a controlled longitudinal study with randomised allocation of the two intervention groups to one of two anatomically defined regionally targeted areas in the NFB training will be employed. The two intervention groups will consist of mothers with identified maternal-bonding difficulties, while the control group will consist of psychologically-healthy mothers without bonding difficulties. The intervention groups will receive three NFB training sessions at intervals of approximately 14 days. Changes in maternal behaviours during mother-infant-interaction, measured using standardised coding of behavioural observations, will be assessed as the primary outcome measure. Possible alterations in oxytocin receptor methylation and gene expression will serve as secondary outcome measures. See Figure 1 for overview of study design and timeline (Figure 1).

[Figure 1: Overview of Study Design around here]

Participant Eligibility and Recruitment:

Eligible Participants:

Intervention Group: Mothers who report postpartum bonding difficulties, may present with a wide range of depressive symptoms.

Control Group: Psychologically healthy mothers with intact bonding to child.

Exclusion Criteria for all Groups:

Mothers: acute suicidality, bipolar or schizophrenic disorders, diagnosed dementia or substance abuse or substance dependency. Necessary fMRI exclusion criteria also apply.

Infants: multiple birth infants, preterm birth, confirmed physical or developmental disorders, which make participation impossible or unwise.

Participant Recruitment:

Participants will be recruited both online and by flyers disseminated through midwives, gynaecologists, paediatricians, in pharmacies and in maternity hospitals as well as mothering forums or self-help groups (e.g. “Shadow and Light “) and registration offices. They receive thorough information about the study procedures both orally and in written form. The volunteer recruitment structure of the study makes it possible that given the intense feelings of self-blame symptomatic of the often co-occurring postpartum depression,[18] those most severely affected may not volunteer for the study. This limitation will be addressed via carefully worded and emphatic recruitment materials and processes.

Patient and Public Involvement

Patients and the general public were not involved in the design or implementation of this study. However, the participants are asked to report on the cognitive strategies they adopted during their rtfMRI NFB sessions and their subjective success with those strategies. These findings may inform future formulations of patient based NFB interventions.

The results of this study will not be disseminated to the participants directly but will be openly accessible online.

Screening assessment and group allocation:

Screening Assessment:

For inclusion in the study all potential participants will be screened (T0) prior to inclusion.

Random Group Assignment Procedure:

True random assignment to the two clinical intervention groups or the control group cannot occur due to the quasi-experimental nature of the design: The mothers are assigned to being clinical or control based on the quality of their postpartum bonding to their infant. Inclusion as part of the clinical intervention group is based on the categorical assessment of impaired maternal bonding using an in-depth interview based on the proposed criteria by,[19] and asked during the clinical interview. This approach additionally allows for the dimensional assessment of maternal-bonding impairments. Bonding assessment is further elaborated by the use of additional questionnaires exploring bonding impairment, such as the Postpartum Bonding Questionnaire 16 (PBQ-16), which holds an internal consistency of Cronbach's α=0.085,[5].

Within the intervention group participants will be completely randomly and automatically assigned to one of the following two conditions: NFB of the right ventral striatum (clinical intervention group I) or NFB of the right anterior cingulate cortex (ACC) (clinical intervention group II). This randomisation is based on the order of inclusion into the study and pre-assigned lists that are double-blind in nature using nonpublic lists made by a colleague not involved in assessments and analyses. During all three intervention sessions, participants will be trained to modulate activation of the same pre-defined brain region (right ventral striatum or right ACC) based on the functional tasks during T1. Blinding of the treatment group will be revealed after the analyses at group level.

Power Analysis and Size Estimation:

The program G-Power v.3.1.9.2,[75] was used, in order to calculate the required sample size. To test the hypotheses mentioned above, a total of approximately N = 100 cases is estimated.

To perform a simple group comparison of maternal sensitivity between two treatment groups with a middle to large effect size (d = 0.78),[44] with a power of 90% and an alpha-error threshold of p = .05, a minimum group size of N = 30 for each clinical intervention group is required (I, NFB of the ventral striatum, II, NFB of the ACC). Assuming a drop-out rate of approx. 10%, an experimental sample of N = 100 will be aimed for in the projected study. With a repeated measures analyses and the T3 (follow-up) measurement the power is improved, which makes it possible to observe even medium effects with this planned sample.

N = 68 mothers with postpartum bonding difficulties will be included in the intervention (N = 34 to NFB region I ventral striatum, N = 34 to NFB region II ACC). Mental disorders, and especially postpartum depressive symptomatology, are measured in a multi-dimensional fashion through a structured clinical interview and a validated peri-partum

period questionnaire. Variability in depressive symptoms, ranging from none to moderate symptom-load is sought, thus allowing for appropriate and adequate statistical analysis of this covariate factor. As a control group N = 32 psychologically healthy women with a good bond with their infant will be recruited.

Pre- Post and Follow-Up Assessments (T1, T2 and T3):

Before (T1) and after the training (T2), participants will perform an extensive battery of diagnostic assessments characterizing the clinical aspects of bonding, including the instructed mother-child-interaction in the Face-To-Face-Still-Face-Paradigm,[76, 77]. A baseline fMRI session will assess the sensitivity of both the reward system and the limbic system using established fMRI tasks and blood samples will be collected for analysing hormonal and (epi-)genetic markers. After the end of the last neurofeedback training a post-intervention measurement (T2) and with 12 month a follow up (T3) will be performed using the same methods as at T1. At T3, mothers and infants will be instructed to freely play,[78] and videotaped. Age-specific markers of the infant's development will be coded using a standardized developmental test,[79]. (Refer to Figure 1 for timeline clarification).
This combination of assessments allows for a comprehensive understanding of maternal bonding and behaviour on a neural, neuroendocrine, epigenetic and behavioural level.

The primary behavioural outcome measures includes the quality of maternal-infant interaction behavior (composite scores of maternal and dyadic behavior) while on the on the neuronal level, we assess the BOLD response to positive stimuli of the child as primary outcome measure. Physiological, endocrine and genetic markers serve as secondary outcomes.

See Table 1 for further details on the assessments and scheduling of measure collection.

Survey instruments and Coding Systems used in the Study:

Measure	Citation	M.	F.	I.	T0	T1	N	T2	T3
Mother and infant:									
Mother-Infant Interaction (FFSF)	[76, 77]	X		X		X		X	
Free-Play Situation	[78]	X		X					X
Interviews:									
Diagnostic Interview for Mental Disorders	[80]	X							
Interview for Postpartum Bonding Difficulties		X	X						
Attachment Style Interview	[81]	X	X						
Questionnaires:									
Postpartum Bonding Questionnaire 16-R	[5]	X	X			X		X	X
Edinburgh Postnatal Depression Scale	[82]	X	X			X		X	X
Agoraphobic Cognitions Questionnaire, Body Sensations Questionnaire, and Mobility Inventory	[83]	X	X			X		X	X
Maternal Self-Confidence Scale	[84]	X	X			X			X
Prenatal Emotional Stress Index	[17]	X	X						
Parental Bonding Instrument	[85]	X	X			X			

German version of the EMBU questionnaire regarding remembered parenting behaviours	[86]		X	X			X		
Experiences in Close Relationships- Revised	[87]		X	X			X		
Social Support Questionnaire	[88]		X	X			X	X	X
Personality Inventory- DSM 5 Short Form	[89]		X	X			X		
Childhood Trauma Questionnaire	[90]		X	X			X		
Partnership Questionnaire	[91]		X	X			X	X	X
Dyadic Coping Inventory	[92]						X	X	X
Parenting Stress Inventory	[93]		X	X				X	X
Vulnerable Attachment Style Questionnaire	[94]		X	X			X		
Edinburgh Handedness Questionnaire	[95]								X
Infant:									
Infant Behaviour Questionnaire	[96]		X	X			X		X
Development Assessment: Infant									
Bayley's Infant Development Scale III	[79]				X				X
Physiological Measures									
Infant Saliva Sample					X		X	X	X
Mother Blood Sample			X				X	X	X
Neurofeedback Training			X			X			
Reward Task	[97]		X				X	X	X
Emotional Go-No-Go	[74]		X				X	X	X
Passive Viewing Task	[98]		X				X	X	X

Table 1: Schedule of measures used the in study. M. Mother Response, F. Father response, I. Infant response, T0 screening assessment, T1 baseline assessment, N-neurofeedback sessions, T2 post assessment, T3 follow-up.

Detailed Description of Measures, Methods and Instruments used:

Initial Assessments:

Baseline Assessment:

For the baseline testing period (T1) the mothers will be invited to our video-laboratory together with their infants. A structured clinical interview according to DSM-5 as well as an interview regarding postpartum bonding difficulties will be carried out. Subsequently, parent-infant interaction will be assessed and videotaped during the Face-to-Face-Still-Face (FFSF) paradigm, a widely used paradigm for evaluating the quality of early parent-infant interaction,[76]. To determine infant stress-reactivity, cortisol and alpha-amylase will be extracted from infant saliva according to standard protocols,[99], which is collected before (C1), immediately after (C2), 20 minutes (C3) and 30 minutes after the FFSF (C4).

Instructed Mother-infant interaction:

Mother-infant-interaction pre- post-intervention (T1 and T3): The standardised mother-infant-interaction before and after the intervention follows the established FFSF paradigm,[76, 77]. It consists of three consecutive instructed two-minute episodes in which the mother, with the infant seated in the baby chair, interacts in accordance with a fixed pattern: First, an initial face-to-face interaction in which the mothers are instructed to play with their infant as usual (without the aid of toys and pacifiers). Next, the still-face episode in which the mothers have to turn their head aside while silently counting to ten and then turn back to the infant but not engage in any gestures, facial expressions, or vocalizations. Finally, the procedure ends with

the reunion episode in which the mother is required to resume face-to-face play with her infant.

Mother-infant-interaction Follow-Up (T4): As the Still-face paradigm can be conducted up to the age of 9 months, parent-infant interaction will be assessed at T4 during a 15-minute free-play situation and a subsequent limit setting task. All interaction episodes will be videotaped and coded according to the Coding Infant Behaviour Scales (CIB),[100]. The CIB scales assess parental sensitivity and responsiveness as well as intrusiveness and withdrawal via composite scores,[101]).

Psychobiological Measurements:

Epigenetic Information on the Oxytocin-System and Cortisol/Alpha Amylase in Saliva:

Maternal blood samples are taken to examine the endogenous oxytocin level, gonadal hormones and epigenetic parameters of the oxytocin gene and oxytocin receptor gene. For the endocrine investigation of the stress hormone cortisol painless saliva samples are taken from the infants directly before, immediately after 20 and 30 minutes after the mother-infant interaction using a saliva probe. An elevation of infant stress-reactivity is expected during the interaction, from these elevations the Peak and Recovery be ascertained. The area under the curve is therefore analysed as a reactivity index,[102] as is standard practice,[103].

MRI-tasks:

Reward Task:

The task, (adapted from,[97]), requires participants to perform a spatial working memory task with two levels of cognitive load, differentiated by the number of circles to be remembered. Subjects first see a cue informing them of the potential monetary reward value – high or low. After presentation of the fixation cross, an array of yellow circles (3 or 7 circles), is displayed followed the target, a green circle, that is then presented at any position on the screen. The participants must decide whether this circle is in the same position as one of the circles presented previously. In the rewarded condition, a feedback about the win (followed by the cumulated amount of earned money appears. Correct responses are reinforced by two different amounts of monetary reward that are counterbalanced with the levels of cognitive load. Incorrect responses on rewarded trials result in no monetary gain. While performing the task, participants rate their mood and stress levels a quarter of the trials.

Emotional Go/NoGo:

The participants are presented with positive and negative expressions of unknown babies, unknown adults as well as un-social control stimuli (geometric figures; a circle, a cross, a diamond and a triangle) over three presentation blocks.

The following factors are systematically manipulated: child vs adult and emotionality of facial expression (positive vs negative). In two blocks, the participants receive instructions to respond by pressing a button as fast as possible to all facial expression except the negative (one block babies, one block adults). In two other blocks they are instructed to respond as fast as possible to all except the positive (one block babies, one block adults). In the two non-social blocks the participants should react as fast as possible to all shapes but not to a circle or a diamond,[74].

Passive Viewing Task:

The participants view previously collected neutral-positive images of partners and their babies in positive and negative affect, with instructions to observe carefully. Unfamiliar men and babies will serve as control stimuli. Viewing images of partners and children leads to the activation of a broad socio-emotional neural network, that is involved with empathy and socio-emotional cognition,[22, 98].

Coding Systems Used in the Study:

Coding Interactive Behaviour:

For the evaluation of the mother-child interaction over all measurement points the Coding Interactive Behaviour System will be used (CIB:[78]). The CIB is a widely used, global rating system for analysing mother-infant-interaction. The system uses multiple codes for the infants, parents and dyadic codes that aggregate into meaningful theoretically-based constructs (e.g., sensitivity, intrusiveness, reciprocity, social engagement, withdrawal). The psychometric characteristics are all well described,[104]. The mother-infant interaction will be coded by blind and reliable coders who are independent of the current study, 10-20% of the videos will be double coded for inter-rater reliability.

Infant Development Diagnostics used in the Study:

Bayley Scales of Infant and Toddler Development-III:

The Bayley Scales of Infant and Toddler Development-III (Bayley-III;[105, 106]) assess the development of infants and toddlers between one and 42 months of age. The test battery covers the domains of cognition, language, motor, social-emotional and adaptive development using I-Q-scaled composite scores. Whereas the first three aspects are assessed by behavioural observation and the latter two utilize questionnaires, with duration ranging between 50 and 90 min,[107]. The Cognitive Scale assesses sensory-motor development, exploration and manipulation, object relatedness, concept formation and memory. The Language Scale is composed of the two subscales receptive and expressive communication. Testing pre-verbal behaviour, vocabulary development, understanding of morphological markers, social referencing and verbal comprehension and pre-verbal communication, vocabulary development and morpho-syntactic development respectively. In addition, the Bayley Scales include a Gross- and Fine-Motor Scale, a Social-Emotional Scale, and an Adaptive Behaviour Scale. This study focuses on the language and cognitive composite scores due to the rather small proposed sample size.

The Bayley-III indices and subscales demonstrate good internal consistency and good split-half-consistency according to the Spearman-Brown formula,[107]. Regarding construct validity, confirmatory factor analysis of the subtests of the Cognitive, Language, and Motor scales supported a three-factor model across all ages. The Bayley-III scales have been normed for German infants and children,[108]. This method is taken standard internationally, particularly in terms of reviewing developmental delays and planning targeted early interventions. The order of the subtests can be adapted to the needs of the child.

Neurofeedback Setup:

The mothers with bonding difficulties will be randomised to receive one of the two following interventions: I) NFB for activation of the ventral striatum or II) neurofeedback for activation of the anterior cingulate cortex (ACC).

The individual masks for extraction of the neurofeedback value are built after the 1st level analyses of the reward task and the emotional go/nogo task submitting the peak voxel coordinates in the right ventral striatum and ACC based on the contrasts [reward > baseline] and [faces nogo > faces go] to build a 12mm-sphere for the striatum ROI and ACC using the marsbar toolbox,[109].

Participants will partake in NFB training over three sessions at intervals of approximately 14 days (refer to Figure 1). At baseline, a high-resolution structural MRI scan and the activation pattern based on the infant-like stimuli will be recorded (using a Tim Trio 3T MRI scanner, Siemens, Erlangen, Germany).

Each intervention session will last approximately 60 minutes and will begin with a 10minute preparatory structural MRI scan. A 6minute resting state fMRI scan is then conducted to allow a resting baseline to be established and to prepare the neurofeedback

set-up. Afterwards, three rtfMRI NFB runs of 9:29 minutes are conducted. During neurofeedback training positive and neutral pictures of the participants' own child taken from the recorded mother-infant interaction session will be presented together with an on-screen "thermometer" which represents the current intensity of activation in the striatum or ACC and must be upregulated. In each run, six alternating phases of up-regulation (~41 seconds, including a ~10 second initial period without thermometer display) and of rest (observation of a fixation cross, ~41 seconds) will be performed. (See Figure 2 for details). The women are instructed to try and explore different self-chosen strategies (of up-regulation) and find the one that works best for them. Afterwards, they are asked to report which strategy they used and about their subjective success experience. In the first and third scanning session the third run is implemented as a "transfer" block without the visible "thermometer" display,[66, 68, 72].

Functional images are acquired with a Siemens Tim Trio using a T2*weighted echoplanar sequence (TR = 1.64s, TE = 30ms, 30 slices, 3mm slice thickness, FoV = 192mm, flip angle 73°, voxel size 3x3x3 mm, 343 volumes per trainings run, distance factor of 33% and GRAPPA with iPat = 2) and a 32 channel head coil. Control for cardiovascular parameters is conducted using the built-in pulse clip. In-house Matlab software based on SPM12 functions is used to conduct rtfMRI NFB and Presentation software (Neurobehavioral Systems, Inc., Albany, CA, USA) is used to present pictures and the feedback signal. At the beginning of each NFB training session, the anatomical image is segmented and normalized to MNI standard space. The inverse deformations of the normalization are then applied to warp the masks of the target regions into subject space.

To correct for movement, each acquired volume is realigned to the first image of the run. Then, volumes with more than 0.5 mm scan-to-scan movements are identified and marked in dummy regressors. Afterwards, the average intensity values from the target region and a cerebrospinal fluid (CSF) mask are extracted and the signal of the target region is corrected for the estimated motion parameters, high-motion dummy regressors, and the CSF signal. For calculation of the feedback signal the ROI intensity value of the last 3 volumes is averaged and compared to the average intensity of the baseline condition. For further technical details regarding the neurofeedback setup please refer to Gerchen et al [110] which uses an identical neurofeedback procedure in the context of alcohol addiction.

[Figure 2: Two Trials of the Neurofeedback Intervention within a Run around here]

Data Analysis Plan:

Statistical analyses, namely the main comparison of the two groups (mothers with bonding difficulties vs. the control group) and the longitudinal analysis of positive relationships changes after the NFB intervention (interaction behaviour, attachment data, as well as psychophysiological, neuroendocrine and epigenetic markers (see Table 1) will be done using IBM SPSS Statics and R (r-project.org). The MRI data will be analysed with general linear models using statistical parametric mapping with SPM (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) implemented (Wellcome Department of Cognitive Neurology, London, UK) in MATLAB (MathWorks, Natick, MA) MATLAB.

For all MRI tasks, all events of the paradigms are modelled by means of general linear model. The relevant contrasts for the go/nogo task are [faces nogo > faces go],[child faces nogo > adult faces nogo] and [negative faces nogo > positive faces nogo]. The relevant contrasts for the reward task are [reward high > reward low], [reward high cognitive load > reward low cognitive load] and [anticipation of reward > baseline]. The relevant contrasts for the passive viewing task are [own infant > unfamiliar child], [positive infant > negative infant], [own partner > unfamiliar man] and [own infant > own partner].

To calculate pre-, post- and follow-up group differences between the intervention and control groups, repeated measures ANOVAs will be calculated, while multi-level models will be used to model changes over the time course.

Figure Legends:

Figure 1:
Overview of Study Design

Figure 2:
Two Trials of the Neurofeedback Intervention within a Run

ETHICS AND DISSEMINATION:

Ethics, consent to participate and Dissemination:
Study procedures are in line with the recommendations of the World Medical Association (revised Declaration of Helsinki) and were approved by the Ethics Committee of the Medical Faculty, s-450/2017, Heidelberg University. The study is registered as clinical trial in the German Register for Clinical Trials (DRKS) DRKS00014570. All participants will provide written informed consent after receiving a detailed oral and written explanation of all procedures and can withdraw their consent at any time without negative consequence.

Results will be internationally published and disseminated, to further the discussion on non-pharmacological treatment options in complex mental disorders.

Consent for Publication:
Not applicable.

Availability of Data and Material
For protection of personal rights and due to the sensitivity of the clinical information, raw data will not be available in the public domain. The medical confidentiality and the provisions of the EU-DSGV (Data Protection Act) are therefore complied with; the collected medical findings and / or personal information are recorded in the examination centre or stored electronically.

Furthermore, important data for the study are stored in an anonymised format, evaluated and, if necessary, passed on to the necessary persons. Third parties gain no insight into the original data with the database remaining restricted for scientific use only. If results are published, it will not be possible to draw any conclusions to an individual as the confidentiality of personal data remains guaranteed at all time

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B.D., P.K., A.-L.Z. and M.E. designed the study; M.E. and A.-L.Z. lead the study; S.A.J. collected data; P.K., M.F.G. and M.M.S. established the experimental set-up; S.A.J., A.-L.Z and ME wrote the manuscript; All authors provided comments on the manuscript.

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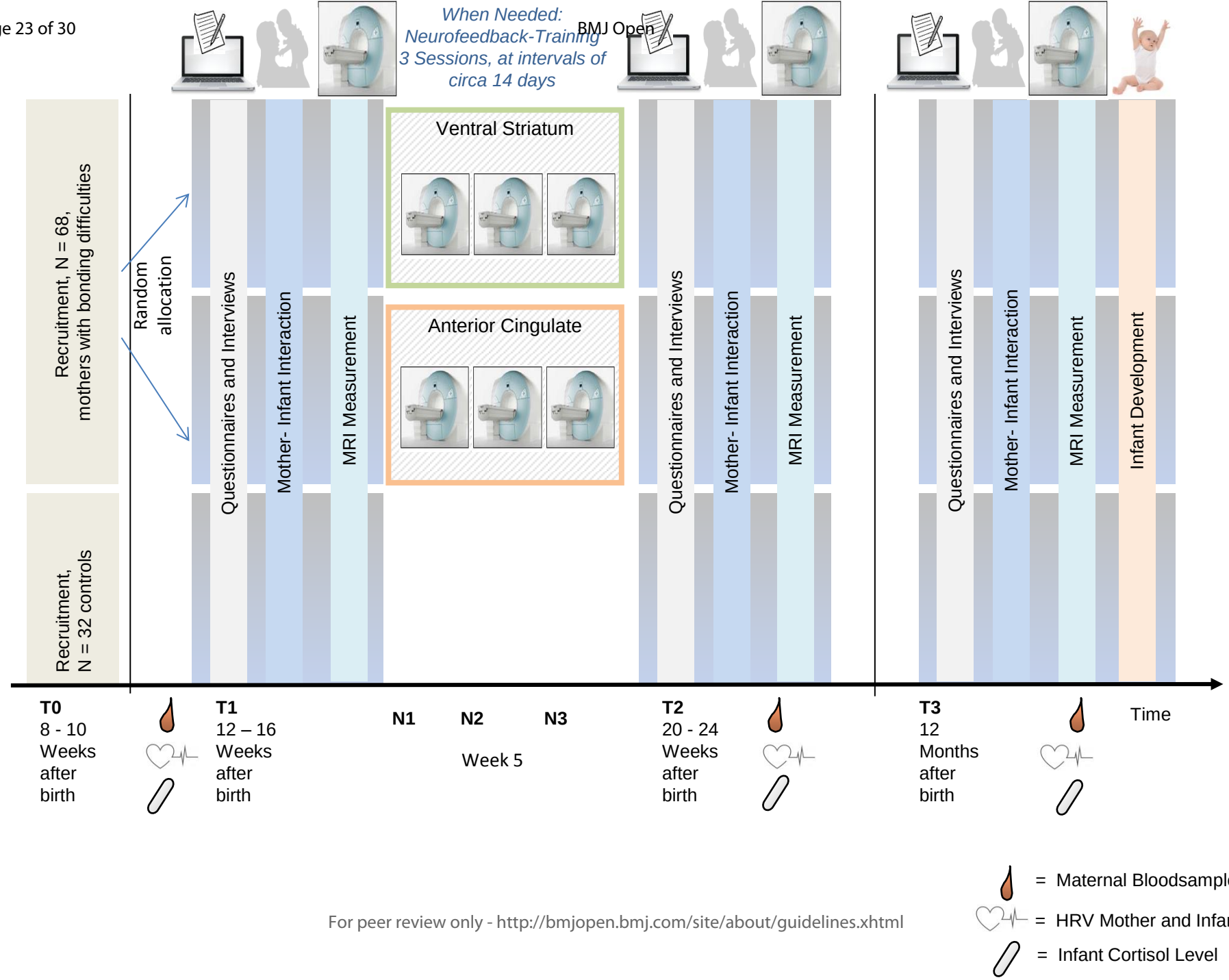

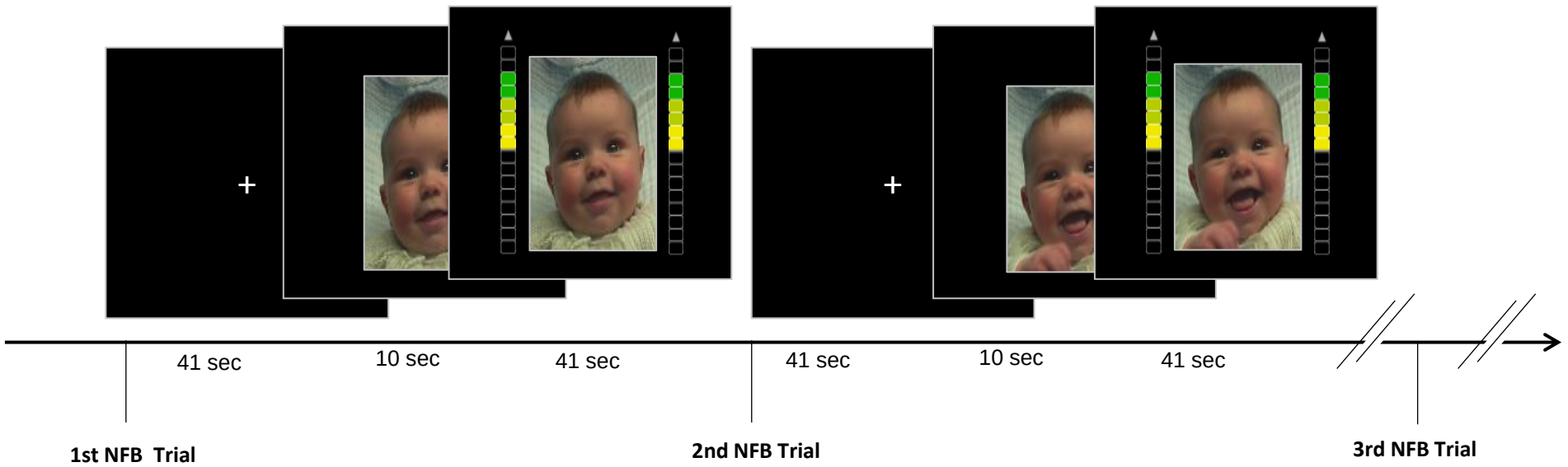
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		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	23
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	23
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	23

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-7
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	7
Objectives	#7	Specific objectives or hypotheses	7
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	14
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-10

Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12-14
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed	

mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	15
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	17
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Data access	#29	Statement of who will have access to the final trial dataset,	17

		and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

BMJ Open

The NeMo Real-Time fMRI Neurofeedback Study: Protocol of a Randomised Controlled Clinical Intervention Trial in the Neural Foundations of Mother-Infant-Bonding

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The NeMo Real-Time fMRI Neurofeedback Study: Protocol of a Randomised Controlled Clinical Intervention Trial in the Neural Foundations of Mother-Infant-Bonding

*Monika Eckstein[1], *Anna-Lena Zietlow[1], Martin Fungisai Gerchen[2,3], Mike Michael Schmitgen[4], Sarah Ashcroft-Jones[1], Peter Kirsch[2,3], Beate Ditzen[1]

[1]Institute of Medical Psychology, Centre of Psychosocial Medicine, University Hospital, Heidelberg University, Germany.

[2]Department of Clinical Psychology, Central Institute of Mental Health, Medical Faculty

Mannheim, Heidelberg University, Germany.

[3]Bernstein Center for Computational Neuroscience Heidelberg/Mannheim, Mannheim, Germany.

[4]Department of General Psychiatry, Centre of Psychosocial Medicine, University Hospital, Heidelberg University, Germany.

* contributed equally to this paper

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*Corresponding Authors:

Monika Eckstein and Anna-Lena Zietlow

Institute of Medical Psychology,

Centre for Psychosocial Medicine,

Heidelberg University Hospital, Heidelberg University,

Bergheimer Str. 20,

69115 Heidelberg,

Germany

Email: monika.eckstein@med.uni-heidelberg.de

anna-lena.zietlow@med.uni-heidelberg.de

Tel: +49 (0) 2221 56-7871

ABSTRACT:

Introduction:

Most mothers feel an immediate, strong emotional bond with their new-born. On a neurobiological level, this is accompanied with the activation of the brain reward systems, including the striatum. However, approximately 10% of all mothers report difficulties to bond emotionally with their infant and display impaired reward responses to the interaction with their infant which might have long-term negative effects for the child's development.
As previous studies suggest that activation of the striatal reward system can be regulated through Functional Magnetic Resonance Imaging (fMRI) based Neurofeedback (NFB), we have designed and investigate fMRI-NBF training to treat maternal bonding difficulties.

Methods and analysis:

In the planned trial, mothers will be presented pictures of their infant and real-time fMRI, peripheral measures, neural, endocrine, psychophysiological and behavioural measures will be assessed. Mothers with bonding difficulties (N= 68) will be randomised to one of two double blind intervention groups at 4-6 months postpartum. They will participate in three repeated neurofeedback training sessions with rtfMRI NFB training to increase activation of a) the ventral striatum or b) the anterior cingulate. Interview data and real-time mother-infant interaction behaviour pre-, post-intervention and at follow-up will serve as clinical outcome measures.

Ethics and Dissemination:

Study procedures are in line with the recommendations of the World Medical Association (revised Declaration of Helsinki) and were approved by the Ethics Committee of the Medical Faculty, s-450/2017, Heidelberg University. All participants will provide written informed consent after receiving a detailed oral and written explanation of all procedures and can withdraw their consent at any time without negative consequence.

Results will be internationally published and disseminated, to further the discussion on non-pharmacological treatment options in complex mental disorders.

Trial registration details:

The study is registered in the German Register for Clinical Trials (DRKS) DRKS00014570.

ARTICLE SUMMARY:

Strengths and Limitations of this Study:

- The novelty of used methods allow the investigation of the effect of real-time Functional Magnetic Imaging (rtfMRI) neurofeedback (NFB), on maternal bonding.
- The study investigates the ability of rtfMRI NFB to upregulate brain activity in correlation with behavioural and biopsychological markers.
- The simultaneous investigation of two brain regions as well as the presence of a control group proposed in the methods of this protocol enables a comparative analysis of the benefits of the rtfMRI training, as well as increasing clarity regarding the role of differential brain regions in social reward.
- The proposed method, through its' longitudinal design with follow-up measures, will help to evidence how neurofeedback training brings about behavioural change, in turn allowing for better understanding of the potential applications, predictive neural signatures and participant criteria crucial for the success of rtfMRI.
- Due to the statistical co-occurrence of maternal bonding difficulties and postpartum depression and the similar pattern of muted reward responses in both instances, the explanatory power of the results of the rtfMRI NFB intervention may be confounded.s

INTRODUCTION:

The Neurobiological, Emotional and Behavioural Aspects of Maternal Bonding:

The parental bond is the first social bond that infants experience in life. Usually, it is the mother who serves as the child's immediate and most central social interaction partner. Maternal bonding is reflective of the first emotional bond that a mother gradually develops with her infant, within the first weeks postpartum, and is characterized by positive feelings, emotional warmth and affection towards the infant,[1]. In the eyes of some researchers, maternal bonding represents one of the most important psychological processes that gradually enfold after birth,[2] and is a significant and central aspect of the emerging relationship between mother and infant,[3].

The initial period after birth is also known as the "Baby-Honeymoon", a state of euphoria and happiness fuelled by the desire to constantly be near and to "fall in love" with the infant, which allows the mother to take on the challenges of the adaption to parenthood. In this way, maternal bonding also fulfils a biological function by ensuring the care for and subsequent survival of the new-born,[4]. On the behavioural level maternal bonding emerges through touch, eye-contact, use of "motherese", and the smiling at or caressing of the infant,[5]. Maternal bonding can therefore said to be expressed in the caregiving behaviours of the parent, in their focus on and high level of sensitivity to the needs of the infant,[6]. On the other hand, maternal bonding is also formed in the mother's emotions and thoughts about the infant and in the mental representations of motherhood that the mother holds,[7].

The strength of maternal bonding can also be demonstrated on a neurobiological level,[8]. Central structures such as the striatum, ventral tegmental area, amygdala, septum and hypothalamus are involved in affiliative behaviour, together with a combination of neurotransmitters and modulators such as dopamine and oxytocin[9, 10]. For affiliative traits, it has been proposed that those are mediated by dopaminergic projections from the ventral tegmental area and endogenous opioids in the hypothalamus with projections to the septum, especially during physical contact [11], that underlie rewarding feelings of affiliation.

One finding of central relevance to maternal bonding in particular is that mothers who report a good emotional bonding to their infant show increased reward-related activation in dopaminergic brain regions (the nucleus accumbens in the ventral striatum) in response to infant stimuli,[12]. These effects are particularly evident in the mother, in comparison to other caregivers, and have been assumed to be reinforced through the hormonal changes that accompany both birth and breast-feeding behaviours,[13, 14].

However, the bonding process is not always successful,[15, 16] and impaired maternal bonding can lead to avoidance or ambivalent emotions or behaviour in the mother. Impaired bonding can result not only in a lack of affection but also, in extreme cases, in the immediate rejection or neglect of the infant, as well as the possible presence of hostility or aggressive impulses,[2, 7]. Although postpartum bonding difficulties also occur in psychologically healthy mothers, they are observed particularly in the context of postpartum depression,[17, 18]. In a representative German sample 6.4% of psychologically healthy mothers reported difficulties in establishing emotional bonding with their infant. In depressed mothers the rate of prevalence is significantly higher at 17-29%,[19]. Furthermore, it has been shown that even subclinical depressive symptoms may negatively affect the developing bonding during the first months postpartum,[20].

Depression during the postpartum period itself is generally characterised by loss of interest or joylessness, insomnia, feelings of guilt, difficulties concentrating and, in severe cases, suicidal tendencies. Intense feelings and self-blame about being a bad mother are also often reported,[21]. On a neurobiological level, postpartum depression relates to a reduced level of reactivity in the striatal reward system, for example in the putamen,[22], but also the amygdala network [23] together with endocrine changes in the serotonin and steroid systems [24]. Studies show that in particular the mother-infant relationship,[25, 26], as well as the actual

mother-infant interaction,[27], both short and long-term can be impaired by peri- and postpartum depression,[25, 28]. Results of a recent study suggest that the link between maternal depressive symptoms at 2 months postpartum and maternal report about interaction with the infant at 6 months was mediated by maternal feelings of attachment,[29]. The present study focuses on bonding impairments, rather than postpartum depression, although there will be inevitable co-occurrence in the participant sample.

Research indicates that experiences and interaction in the "sensitive" postpartum period have long term consequences for the cognitive as well as socio-emotional development in children,[25, 30]. Animal studies have also demonstrated that positive caring behaviours of the mother positively influence offspring brain development and long-term stress-coping behaviours even in the next generation via epigenetic changes,[31]. Conversely, in humans, decreased maternal bonding can result in higher rates of self-directed affect regulation strategies and increased stress-reactivity [32] of the infant, as well as variable self-comfort behaviours, moderated by the infant's gender and age,[32]. Research also indicates that restrained (distant) maternal behaviour can have far-reaching negative consequences for self-regulation, social behaviour and stress management in the infant [25, 30], even into adulthood with potential influences on own parenting behaviour,[33]. Furthermore, the association between maternal depression, anxiety disorders and stress-reactivity with changes in the hypothalamic-pituitary-adrenocortical axis (HPA axis) is well-documented,[34]. Indeed, recent studies indicate that infant exposure to maternal mental disorders can increase infant baseline cortisol levels, as the main outcome of the HPA axis, in the case of maternal depression and increase infant cortisol reactivity in cases of maternal anxiety,[35]. In a lower income setting, exposure to variable intensities of maternal depression demonstrated a U-shaped response curve in infant cortisol reactivity levels in the context of immunisation. Those with exposure to moderate-maternal depression levels demonstrated the lowest cortisol reactivity during immunisation,[36]. The importance of the influence of maternal interaction behaviours themselves is also clear. One particular study demonstrates, for example, that maternal sensitivity during the interaction with their infant has a moderating influence between maternal mental health during pregnancy and infant salivary cortisol levels,[37]. Given data on the transgenerational transmission of maternal care via epigenetics,[38], both in maternal-infant bonding,[39-41] and care-giving behaviours,[42] the importance of effective treatment and intervention plans are evident.

Current inpatient and outpatient mother-infant therapies, established in the context of postpartum mental disorders, focus not only on the disorder-specific treatment but also on specific elements for the promotion of the mother-infant relationship (e.g. baby massage and video feedback methods). Video feedback interventions have been shown to have a positive influence on mother-infant interaction,[43, 44] as well as the maternal self-efficacy in interacting and coping with the infant,[45, 46]. To our knowledge, maternal bonding has thus far only been recorded as an outcome variable in one study using video feedback invention,[47]. This intervention demonstrated improvements in maternal bonding; however, the participant pool only included mentally healthy women without postpartum bonding problems. Thus, interventions focussing on maternal bonding in women experiencing difficulties forming an emotional bond to their infant are urgently needed. Presently, such interventions or findings, directly relating maternal bonding difficulties and their effects on the infant's stress reactivity in either biological or behavioural domains, are still lacking and in particular non-pharmacological treatment concepts would substantially broaden the treatment options for affected mothers and their infants.

Reward Learning in Depression and its Relation to Postpartum Bonding Difficulties:
On a neural level the dopamine-mediated reward system (specifically the ventral striatum and the nucleus accumbens) is crucial to the rewarding aspects of social interaction,[48, 49]. The ventral striatum also appears to be specifically involved in learning rewarding stimuli, where

high neural activation, correlated with the "BOLD response" in the fMRI, is accompanied by an increased dopaminergic response,[48]. As part of the learning process this reward activation is first triggered by primary enhancers, such as glucose, and over the course of the learning process transfers to secondary enhancers, such as food or money. In humans, from early in life, repeated positive social experiences can be seen as very strong reward and provided the basis for the much discussed “social brain hypothesis”, in brain research [50, 51].

Images of happy children are generally perceived as positive by healthy individuals and activate the striatal areas of the brain,[52]. Psychologically healthy mothers also show a differential neural activation response to visual stimuli of happy children, as compared with stimuli of more ambiguous facial expressions of children. Expressions of joy activated the right limbic and paralimbic areas, by comparison ambiguous expressions correlated to activity in the left higher cognitive and motor areas that are often linked to cognitive effort,[53]. Consequently, positively reinforcing activities that lead to such activation, i.e. positive social interaction, are likely to be subsequently performed more frequently. In the case of the presence of depressive symptoms, however, such neural activation (i.e. activation in striatal and limbic networks) seems to be diminished during positive social interactions, these activities are therefore perceived as less rewarding,[54, 55] and diminish the motivation for social contact. Mothers with depressive symptoms demonstrated reduced activation in subcortical and cortical limbic regions (amygdala, cingulate) and cortical regions involved in emotion regulation (including the frontal cortex, insula, anterior cingulate); in line with symptomology of diminished capacities to experience joy and to down-regulate anger, anxiety, or worry,[22, 23, 56]. Notably mothers with postpartum depression show a reduced activation in striatal reward areas in response to stimuli of their own children,[22, 23, 56].

On a neuroendocrine level, basic research suggests that in rodents social-interaction related reward is associated with receptor-activation for the neuropeptide oxytocin in the nucleus accumbens,[57]. Initial data in humans indicates, that methylation of the oxytocin receptor gene is associated with attachment in different phases of the individual’s lifespan,[58] and higher methylation is associated with postpartum depression,[59]. Antidepressant treatment, in turn, is assumed to lower methylation,[60]. This may explain, on a neurobiological level. the correlation between postpartum depression and maternal bonding difficulties,[61]. However, no contemporary intervention has yet focussed on the perception of the mother-infant interaction in terms of reward and involved oxytocin-related outcomes as possible evaluation criteria.

rtfMRI NFB as a Treatment Method:

Neurofeedback (NFB) is a novel method which, through the visualization of real-time brain activation, allows an individual to consciously regulate one's own brain activation. While it is well-established that mental strategies modulate brain activation as measured by fMRI blood oxygen level dependent signal (BOLD), NFB asks participants to modulate activation in prescribed regions or even networks of their brain by their own volition and in response to the immediate feedback/ visualization of the related brain activation patterns,[62]. Thus, volunteers can learn to regulate the activation of a previously defined brain region (see,[63] for a review). NFB interventions have previously often used electroencephalography (EEG) and other electrophysiological methods to visualize and modulate activation in higher-cortical areas. However, smaller and deeper areas of the limbic and reward system can be imaged primarily with high-resolution magnetic resonance imaging (MRI). One previous study has proven that healthy volunteers can be trained to voluntarily increase their brain activation patterns that have been classified for affiliative emotions in the same individual subject using voxel pattern analyses [64]. From a therapeutic point of view, the targeted modulation of specific brain areas and associated circuits via neurofeedback should be associated with the improvement of mental symptoms.

In initial clinical pilot studies, for example, patients with borderline personality disorder, anxiety or obsessive-compulsive disorders have shown the ability to down regulate activation in the insula or the hyperactive amygdala in response to stress-related stimuli,[65-67]. Similarly, high risk alcohol consumers learned to down regulate the reward related activation of alcohol related stimuli,[68]. In PTSD patients, the changes in brain connectivity after fMRI neurofeedback correlate with the reduction of symptoms,[69]. Based on the previously articulated and evidenced notion of the psychobiological mechanisms underlying attachment and bonding as goal-oriented and reward-drive,[70], the proposed study looks to investigate whether specific activation of the ventral striatum can be voluntarily increased through training in the context of mother-infant bonding. Indeed in previous research, patients with major depressive disorders or schizophrenia were successfully trained to increase the activation of the hypoactive amygdala and the anterior cingulum in conjunction with positive stimuli,[71, 72]. Therefore, in line with the current understanding and treatment of mental illnesses, rtfMRI NFB represents a promising new intervention method for complex mental processes. Particularly important for understanding the potential of rtfMRI interventions is the transfer of the modulation of the BOLD signal towards behavioural changes,[62] which allows the value of this intervention form in a clinical setting to be distilled.

The Planned Trial:

In the planned double-blind randomized intervention trial (the “Neurofeedback for Mothers with Postpartum Bonding Difficulties Study”, “NeMo-Study”), three central points will be addressed.

1. Women with postpartum bonding difficulties (including women with postpartum depression) will be compared to healthy unaffected women in terms of reward-related brain activation to pictures showing their own infant and control stimuli. It is hypothesized that women with bonding difficulties show less brain response in the reward-related areas (ventral striatum) compared to the control group.

2. The clinical group will then undergo a regiment of NFB training. Participants will learn to consciously increase the activation of reward-associated brain areas (specifically the ventral striatum), or a control region (anterior cingulate cortex, ACC), during the presentation of images of their own infant. For ethical reasons, an active control treatment was chosen rather than joke or non-feedback. The activation of the central nervous dopamine system via the striatum is hypothesized to improve bonding motivation and social interaction behaviour. The coupling of infant stimuli to the central nervous reward activation should make it easier for these women to feel more joy in the real interaction,[62] with their infant post-training and to be more attentive and more

sensitive in their interactions. Training of the dorsal/rostral ACC with a rather unspecific role in emotion regulation, such as e.g. in cognitive reappraisal,[73] or response inhibition [74], may have general beneficial effects as shown in another NFB study [71] but also indirectly influence parental affect regulation,. However, the more specific striatal feedback based on the rewarding aspects of the mother-child interaction are assumed to have stronger effects.

3. Effects of the NFB training will be related to the changes in behaviourally coded mother-infant interaction, maternal bonding quality and diagnostics, and epigenetic oxytocin receptor alterations, as assessed from peripheral blood. It is hypothesized that following the ventral striatum intervention, interaction, maternal bonding, and epigenetic markers will approximate the values of the healthy control group.

Thus, the study will address reward-related processes in impaired maternal bonding, observable changes on a behavioural and neuroendocrine level post-training - important steps in illustrating the validity of rtfMRI NFB as an intervention method,[62]. The proposed study is therefore well placed to produce a wealth of valuable and informative data, both regarding the potential scope of rtfMRI as a non-pharmacological intervention to young mothers.

METHODS AND ANALYSIS:

Design Overview:

To investigate points 1) – 3) (above) a controlled longitudinal study with randomised allocation of the two intervention groups to one of two anatomically defined regionally targeted areas in the NFB training will be employed. The two intervention groups will consist of mothers with identified maternal-bonding difficulties, while the control group will consist of psychologically-healthy mothers without bonding difficulties. The intervention groups will receive three NFB training sessions at intervals of approximately 14 days. Changes in maternal behaviours during mother-infant-interaction, measured using standardised coding of behavioural observations, will be assessed as the primary outcome measure. Possible alterations in oxytocin receptor methylation and gene expression will serve as secondary outcome measures. See Figure 1 for overview of study design and timeline (Figure 1).

[Figure 1: Overview of Study Design around here]

Participant Eligibility and Recruitment:

Eligible Participants:

Intervention Group: Mothers who report postpartum bonding difficulties, may present with a wide range of depressive symptoms.

Control Group: Psychologically healthy mothers with intact bonding to child.

Exclusion Criteria for all Groups:

Mothers: acute suicidality, bipolar or schizophrenic disorders, diagnosed dementia or substance abuse or substance dependency. Necessary fMRI exclusion criteria also apply.

Infants: multiple birth infants, preterm birth, confirmed physical or developmental disorders, which make participation impossible or unwise.

Participant Recruitment:

Participants will be recruited both online and by flyers disseminated through midwives, gynaecologists, paediatricians, in pharmacies and in maternity hospitals as well as mothering forums or self-help groups (e.g. “Shadow and Light “) and registration offices. They receive thorough information about the study procedures both orally and in written form. The volunteer recruitment structure of the study makes it possible that given the intense feelings of self-blame symptomatic of the often co-occurring postpartum depression,[18] those most severely affected may not volunteer for the study. This limitation will be addressed via carefully worded and emphatic recruitment materials and processes.

Patient and Public Involvement

Patients and the general public were not involved in the design or implementation of this study. However, the participants are asked to report on the cognitive strategies they adopted during their rtfMRI NFB sessions and their subjective success with those strategies. These findings may inform future formulations of patient based NFB interventions.

The results of this study will not be disseminated to the participants directly but will be openly accessible online.

Screening assessment and group allocation:

Screening Assessment:

For inclusion in the study all potential participants will be screened (T0) prior to inclusion.

Random Group Assignment Procedure:

True random assignment to the two clinical intervention groups or the control group cannot occur due to the quasi-experimental nature of the design: The mothers are assigned to being clinical or control based on the quality of their postpartum bonding to their infant. Inclusion as part of the clinical intervention group is based on the categorical assessment of impaired maternal bonding using an in-depth interview based on the proposed criteria by,[19] and asked during the clinical interview. This approach additionally allows for the dimensional assessment of maternal-bonding impairments. Bonding assessment is further elaborated by the use of additional questionnaires exploring bonding impairment, such as the Postpartum Bonding Questionnaire 16 (PBQ-16), which holds an internal consistency of Cronbach’s α=0.085,[5].

Within the intervention group participants will be completely randomly and automatically assigned to one of the following two conditions: NFB of the right ventral striatum (clinical intervention group I) or NFB of the right anterior cingulate cortex (ACC) (clinical intervention group II). This randomisation is based on the order of inclusion into the study and pre-assigned lists that are double-blind in nature using nonpublic lists made by a colleague not involved in assessments and analyses. During all three intervention sessions, participants will be trained to modulate activation of the same pre-defined brain region (right ventral striatum or right ACC) based on the functional tasks during T1. Blinding of the treatment group will be revealed after the analyses at group level.

Power Analysis and Size Estimation:

The program G-Power v.3.1.9.2,[75] was used, in order to calculate the required sample size. To test the hypotheses mentioned above, a total of approximately N = 100 cases is estimated.

To perform a simple group comparison of maternal sensitivity between two treatment groups with a middle to large effect size (d = 0.78),[44] with a power of 90% and an alpha-error threshold of p = .05, a minimum group size of N = 30 for each clinical intervention group

is required (I, NFB of the ventral striatum, II, NFB of the ACC). Assuming a drop-out rate of approx. 10%, an experimental sample of N = 100 will be aimed for in the projected study. With a repeated measures analyses and the T3 (follow-up) measurement the power is improved, which makes it possible to observe even medium effects with this planned sample.

N = 68 mothers with postpartum bonding difficulties will be included in the intervention (N = 34 to NFB region I ventral striatum, N = 34 to NFB region II ACC). Mental disorders, and especially postpartum depressive symptomatology, are measured in a multi-dimensional fashion through a structured clinical interview and a validated peri-partum period questionnaire. Variability in depressive symptoms, ranging from none to moderate symptom-load is sought, thus allowing for appropriate and adequate statistical analysis of this covariate factor. As a control group N = 32 psychologically healthy women with a good bond with their infant will be recruited.

Pre- Post and Follow-Up Assessments (T1, T2 and T3):

Before (T1) and after the training (T2), participants will perform an extensive battery of diagnostic assessments characterizing the clinical aspects of bonding, including the instructed mother-child-interaction in the Face-To-Face-Still-Face-Paradigm,[76, 77]. A baseline fMRI session will assess the sensitivity of both the reward system and the limbic system using established fMRI tasks and blood samples will be collected for analysing hormonal and (epi-)genetic markers. After the end of the last neurofeedback training a post-intervention measurement (T2) and with 12 month a follow up (T3) will be performed using the same methods as at T1. At T3, mothers and infants will be instructed to freely play,[78] and videotaped. Age-specific markers of the infant's development will be coded using a standardized developmental test,[79]. (Refer to Figure 1 for timeline clarification).
This combination of assessments allows for a comprehensive understanding of maternal bonding and behaviour on a neural, neuroendocrine, epigenetic and behavioural level.

The primary behavioural outcome measures includes the quality of maternal-infant interaction behavior (composite scores of maternal and dyadic behavior) while on the on the neuronal level, we assess the BOLD response to positive stimuli of the child as primary outcome measure. Physiological, endocrine and genetic markers serve as secondary outcomes.

See Table 1 for further details on the assessments and scheduling of measure collection.

Survey instruments and Coding Systems used in the Study:

Measure	Citation	M.	F.	I.	T0	T1	N	T2	T3
Mother and infant:									
Mother-Infant Interaction (FFSF)	[76, 77]	X		X		X		X	
Free-Play Situation	[78]	X		X					X
Interviews:									
Diagnostic Interview for Mental Disorders	[80]	X							
Interview for Postpartum Bonding Difficulties		X	X						
Attachment Style Interview	[81]	X	X						
Questionnaires:									
Postpartum Bonding Questionnaire 16-R	[5]	X	X			X		X	X

Edinburgh Postnatal Depression Scale	[82]	X	X		X	X	X	
Agoraphobic Cognitions Questionnaire, Body Sensations Questionnaire, and Mobility Inventory	[83]	X	X		X	X	X	
Maternal Self-Confidence Scale	[84]	X	X		X		X	
Prenatal Emotional Stress Index	[17]	X	X					
Parental Bonding Instrument	[85]	X	X		X			
German version of the EMBU questionnaire regarding remembered parenting behaviours	[86]	X	X		X			
Experiences in Close Relationships- Revised	[87]	X	X		X			
Social Support Questionnaire	[88]	X	X		X	X	X	
Personality Inventory- DSM 5 Short Form	[89]	X	X		X			
Childhood Trauma Questionnaire	[90]	X	X		X			
Partnership Questionnaire	[91]	X	X		X	X	X	
Dyadic Coping Inventory	[92]				X	X	X	
Parenting Stress Inventory	[93]	X	X			X	X	
Vulnerable Attachment Style Questionnaire	[94]	X	X		X			
Edinburgh Handedness Questionnaire	[95]						X	
Infant:								
Infant Behaviour Questionnaire	[96]	X	X		X		X	
Development Assessment: Infant								
Bayley's Infant Development Scale III	[79]			X			X	
Physiological Measures								
Infant Saliva Sample				X	X	X	X	
Mother Blood Sample		X			X	X	X	
Neurofeedback Training		X				X		
Reward Task	[97]	X			X	X	X	
Emotional Go-No-Go	[74]	X			X	X	X	
Passive Viewing Task	[98]	X			X	X	X	

Table 1: Schedule of measures used the in study. M. Mother Response, F. Father response, I. Infant response, T0 screening assessment, T1 baseline assessment, N-neurofeedback sessions, T2 post assessment, T3 follow-up.

Detailed Description of Measures, Methods and Instruments used:

Initial Assessments:

Baseline Assessment:

For the baseline testing period (T1) the mothers will be invited to our video-laboratory together with their infants. A structured clinical interview according to DSM-5 as well as an interview regarding postpartum bonding difficulties will be carried out. Subsequently, parent-infant interaction will be assessed and videotaped during the Face-to-Face-Still-Face (FFSF) paradigm, a widely used paradigm for evaluating the quality of early parent-infant interaction,[76]. To determine infant stress-reactivity, cortisol and alpha-amylase will be extracted from infant saliva according to standard protocols,[99], which is collected before (C1), immediately after (C2), 20 minutes (C3) and 30 minutes after the FFSF (C4).

Instructed Mother-infant interaction:
Mother-infant-interaction pre- post-intervention (T1 and T3): The standardised mother-infant-interaction before and after the intervention follows the established FFSF paradigm,[76, 77]. It consists of three consecutive instructed two-minute episodes in which the mother, with the infant seated in the baby chair, interacts in accordance with a fixed pattern: First, an initial face-to-face interaction in which the mothers are instructed to play with their infant as usual (without the aid of toys and pacifiers). Next, the still-face episode in which the mothers have to turn their head aside while silently counting to ten and then turn back to the infant but not engage in any gestures, facial expressions, or vocalizations. Finally, the procedure ends with the reunion episode in which the mother is required to resume face-to-face play with her infant.

Mother-infant-interaction Follow-Up (T4): As the Still-face paradigm can be conducted up to the age of 9 months, parent-infant interaction will be assessed at T4 during a 15-minute free-play situation and a subsequent limit setting task. All interaction episodes will be videotaped and coded according to the Coding Infant Behaviour Scales (CIB),[100]. The CIB scales assess parental sensitivity and responsiveness as well as intrusiveness and withdrawal via composite scores,[101]).

Psychobiological Measurements:
Epigenetic Information on the Oxytocin-System and Cortisol/Alpha Amylase in Saliva:
Maternal blood samples are taken to examine the endogenous oxytocin level, gonadal hormones and epigenetic parameters of the oxytocin gene and oxytocin receptor gene. For the endocrine investigation of the stress hormone cortisol painless saliva samples are taken from the infants directly before, immediately after 20 and 30 minutes after the mother-infant interaction using a saliva probe. An elevation of infant stress-reactivity is expected during the interaction, from these elevations the Peak and Recovery be ascertained. The area under the curve is therefore analysed as a reactivity index,[102] as is standard practice,[103].

MRI-tasks:
Reward Task:
The task, (adapted from,[97]), requires participants to perform a spatial working memory task with two levels of cognitive load, differentiated by the number of circles to be remembered. Subjects first see a cue informing them of the potential monetary reward value – high or low. After presentation of the fixation cross, an array of yellow circles (3 or 7 circles), is displayed followed the target, a green circle, that is then presented at any position on the screen. The participants must decide whether this circle is in the same position as one of the circles presented previously. In the rewarded condition, a feedback about the win (followed by the cumulated amount of earned money appears. Correct responses are reinforced by two different amounts of monetary reward that are counterbalanced with the levels of cognitive load. Incorrect responses on rewarded trials result in no monetary gain. While performing the task, participants rate their mood and stress levels a quarter of the trials.

Emotional Go/NoGo:
The participants are presented with positive and negative expressions of unknown babies, unknown adults as well as un-social control stimuli (geometric figures; a circle, a cross, a diamond and a triangle) over three presentation blocks.

The following factors are systematically manipulated: child vs adult and emotionality of facial expression (positive vs negative). In two blocks, the participants receive instructions to respond by pressing a button as fast as possible to all facial expression except the negative (one block babies, one block adults). In two other blocks they are instructed to respond as fast as possible to all except the positive (one block babies, one block adults). In the two non-social blocks the participants should react as fast as possible to all shapes but not to a circle or a diamond,[74].

Passive Viewing Task:
The participants view previously collected neutral-positive images of partners and their babies in positive and negative affect, with instructions to observe carefully. Unfamiliar men and babies will serve as control stimuli. Viewing images of partners and children leads to the activation of a broad socio-emotional neural network, that is involved with empathy and socio-emotional cognition,[22, 98].

Coding Systems Used in the Study:
Coding Interactive Behaviour:
For the evaluation of the mother-child interaction over all measurement points the Coding Interactive Behaviour System will be used (CIB:[78]). The CIB is a widely used, global rating system for analysing mother-infant-interaction. The system uses multiple codes for the infants, parents and dyadic codes that aggregate into meaningful theoretically-based constructs (e.g., sensitivity, intrusiveness, reciprocity, social engagement, withdrawal). The psychometric characteristics are all well described,[104]. The mother-infant interaction will be coded by blind and reliable coders who are independent of the current study, 10-20% of the videos will be double coded for inter-rater reliability.

Infant Development Diagnostics used in the Study:
Bayley Scales of Infant and Toddler Development-III:
The Bayley Scales of Infant and Toddler Development-III (Bayley-III;[105, 106]) assess the development of infants and toddlers between one and 42 months of age. The test battery covers the domains of cognition, language, motor, social-emotional and adaptive development using I-Q-scaled composite scores. Whereas the first three aspects are assessed by behavioural observation and the latter two utilize questionnaires, with duration ranging between 50 and 90 min,[107]. The Cognitive Scale assesses sensory-motor development, exploration and manipulation, object relatedness, concept formation and memory. The Language Scale is composed of the two subscales receptive and expressive communication. Testing pre-verbal behaviour, vocabulary development, understanding of morphological markers, social referencing and verbal comprehension and pre-verbal communication, vocabulary development and morpho-syntactic development respectively. In addition, the Bayley Scales include a Gross- and Fine-Motor Scale, a Social-Emotional Scale, and an Adaptive Behaviour Scale. This study focuses on the language and cognitive composite scores due to the rather small proposed sample size.

The Bayley-III indices and subscales demonstrate good internal consistency and good split-half-consistency according to the Spearman-Brown formula,[107]. Regarding construct validity, confirmatory factor analysis of the subtests of the Cognitive, Language, and Motor scales supported a three-factor model across all ages. The Bayley-III scales have been normed for German infants and children,[108]. This method is taken standard internationally, particularly in terms of reviewing developmental delays and planning targeted early interventions. The order of the subtests can be adapted to the needs of the child.

Neurofeedback Setup:
The mothers with bonding difficulties will be randomised to receive one of the two following interventions: I) NFB for activation of the ventral striatum or II) neurofeedback for activation of the anterior cingulate cortex (ACC).
The individual masks for extraction of the neurofeedback value are built after the 1st level analyses of the reward task and the emotional go/nogo task submitting the peak voxel coordinates in the right ventral striatum and ACC based on the contrasts [reward > baseline] and [faces nogo > faces go] to build a 12mm-sphere for the striatum ROI and ACC using the marsbar toolbox,[109].
Participants will partake in NFB training over three sessions at intervals of approximately 14 days (refer to Figure 1). At baseline, a high-resolution structural MRI scan and the activation

pattern based on the infant-like stimuli will be recorded (using a Tim Trio 3T MRI scanner, Siemens, Erlangen, Germany).

Each intervention session will last approximately 60 minutes and will begin with a 10minute preparatory structural MRI scan. A 6minute resting state fMRI scan is then conducted to allow a resting baseline to be established and to prepare the neurofeedback set-up. Afterwards, three rtfMRI NFB runs of 9:29 minutes are conducted. During neurofeedback training positive and neutral pictures of the participants' own child taken from the recorded mother-infant interaction session will be presented together with an on-screen "thermometer" which represents the current intensity of activation in the striatum or ACC and must be upregulated. In each run, six alternating phases of up-regulation (~41 seconds, including a ~10 second initial period without thermometer display) and of rest (observation of a fixation cross, ~41 seconds) will be performed. (See Figure 2 for details). The women are instructed to try and explore different self-chosen strategies (of up-regulation) and find the one that works best for them. Afterwards, they are asked to report which strategy they used and about their subjective success experience. In the first and third scanning session the third run is implemented as a "transfer" block without the visible "thermometer" display,[66, 68, 72].

Functional images are acquired with a Siemens Tim Trio using a T2*weighted echoplanar sequence (TR = 1.64s, TE = 30ms, 30 slices, 3mm slice thickness, FoV = 192mm, flip angle 73°, voxel size 3x3x3 mm, 343 volumes per trainings run, distance factor of 33% and GRAPPA with iPat = 2) and a 32 channel head coil. Control for cardiovascular parameters is conducted using the built-in pulse clip. In-house Matlab software based on SPM12 functions is used to conduct rtfMRI NFB and Presentation software (Neurobehavioral Systems, Inc., Albany, CA, USA) is used to present pictures and the feedback signal. At the beginning of each NFB training session, the anatomical image is segmented and normalized to MNI standard space. The inverse deformations of the normalization are then applied to warp the masks of the target regions into subject space.

To correct for movement, each acquired volume is realigned to the first image of the run. Then, volumes with more than 0.5 mm scan-to-scan movements are identified and marked in dummy regressors. Afterwards, the average intensity values from the target region and a cerebrospinal fluid (CSF) mask are extracted and the signal of the target region is corrected for the estimated motion parameters, high-motion dummy regressors, and the CSF signal. For calculation of the feedback signal the ROI intensity value of the last 3 volumes is averaged and compared to the average intensity of the baseline condition. For further technical details regarding the neurofeedback setup please refer to Gerchen et al [110] which uses an identical neurofeedback procedure in the context of alcohol addiction.

[Figure 2: Two Trials of the Neurofeedback Intervention within a Run around here]

Data Analysis Plan:

Statistical analyses, namely the main comparison of the two groups (mothers with bonding difficulties vs. the control group) and the longitudinal analysis of positive relationships changes after the NFB intervention (interaction behaviour, attachment data, as well as psychophysiological, neuroendocrine and epigenetic markers (see Table 1) will be done using IBM SPSS Statics and R (r-project.org). The MRI data will be analysed with general linear models using statistical parametric mapping with SPM (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) implemented (Wellcome Department of Cognitive Neurology, London, UK) in MATLAB (MathWorks, Natick, MA) MATLAB.

For all MRI tasks, all events of the paradigms are modelled by means of general linear model. The relevant contrasts for the go/nogo task are [faces nogo > faces go],[child faces nogo > adult faces nogo] and [negative faces nogo > positive faces nogo]. The relevant contrasts for the reward task are [reward high > reward low], [reward high cognitive load > reward low cognitive load] and [anticipation of reward > baseline]. The relevant contrasts for the passive viewing task are [own infant > unfamiliar child], [positive infant > negative infant], [own partner > unfamiliar man] and [own infant > own partner].

To calculate pre-, post- and follow-up group differences between the intervention and control groups, repeated measures ANOVAs will be calculated, while multi-level models will be used to model changes over the time course.

Figure Legends:

Figure 1:
Overview of Study Design

Figure 2:
Two Trials of the Neurofeedback Intervention within a Run

ETHICS AND DISSEMINATION:

Ethics, consent to participate and Dissemination:

Study procedures are in line with the recommendations of the World Medical Association (revised Declaration of Helsinki) and were approved by the Ethics Committee of the Medical Faculty, s-450/2017, Heidelberg University. The study is registered as clinical trial in the German Register for Clinical Trials (DRKS) DRKS00014570. All participants will provide written informed consent after receiving a detailed oral and written explanation of all procedures and can withdraw their consent at any time without negative consequence.

Results will be internationally published and disseminated, to further the discussion on non-pharmacological treatment options in complex mental disorders.

Consent for Publication:

Not applicable.

Availability of Data and Material

For protection of personal rights and due to the sensitivity of the clinical information, raw data will not be available in the public domain. The medical confidentiality and the provisions of the EU-DSGV (Data Protection Act) are therefore complied with; the collected medical findings and / or personal information are recorded in the examination centre or stored electronically.

Furthermore, important data for the study are stored in an anonymised format, evaluated and, if necessary, passed on to the necessary persons. Third parties gain no insight into the original data with the database remaining restricted for scientific use only. If results are published, it will not be possible to draw any conclusions to an individual as the confidentiality of personal data remains guaranteed at all time

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AUTHORS' CONTRIBUTION STATEMENT:
B.D., P.K., A.-L.Z. and M.E. designed the study; M.E. and A.-L.Z. lead the study; S.A.J. collected data; P.K., M.F.G. and M.M.S. established the experimental set-up; S.A.J., A.-L.Z and ME wrote the manuscript; All authors provided comments on the manuscript.

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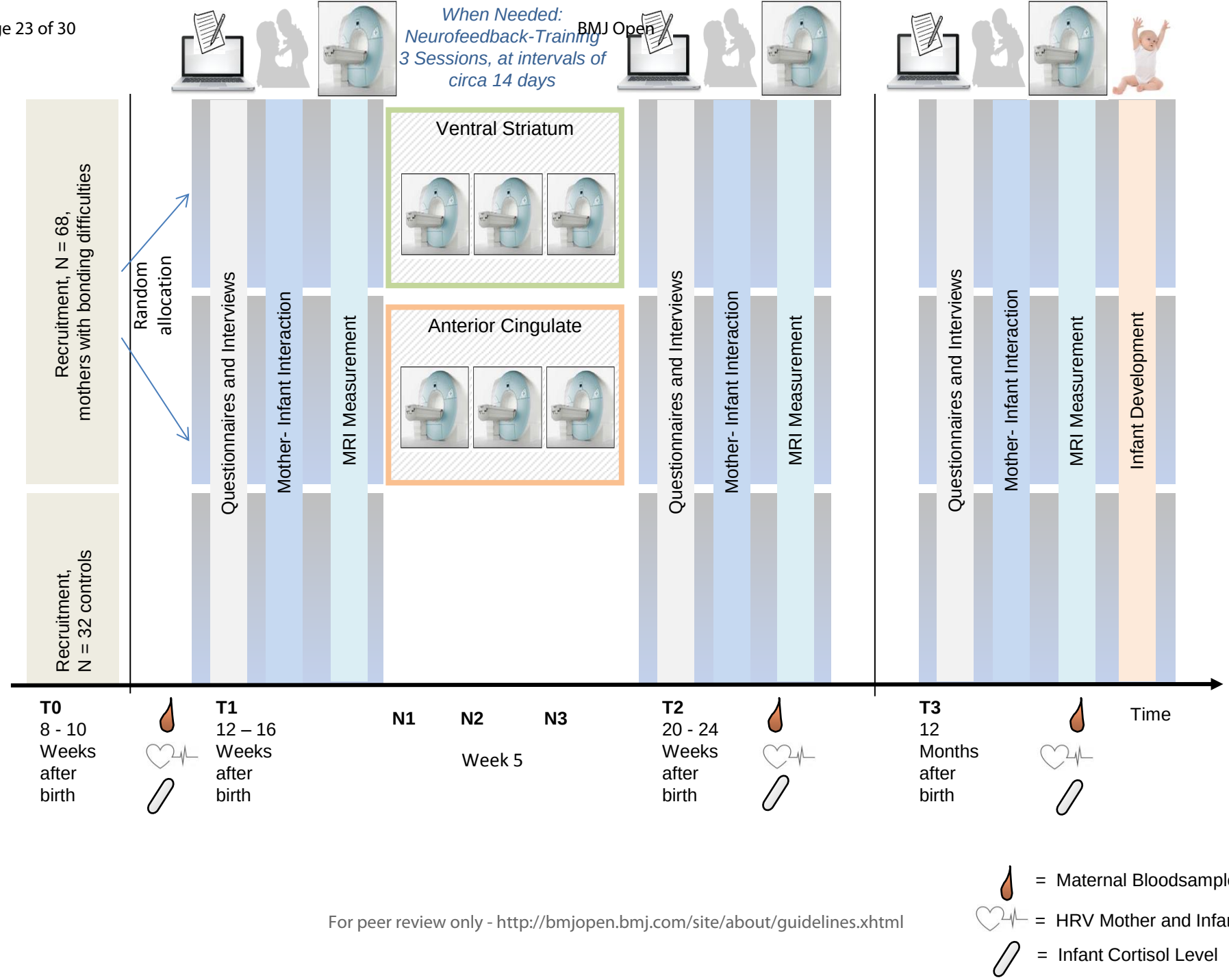

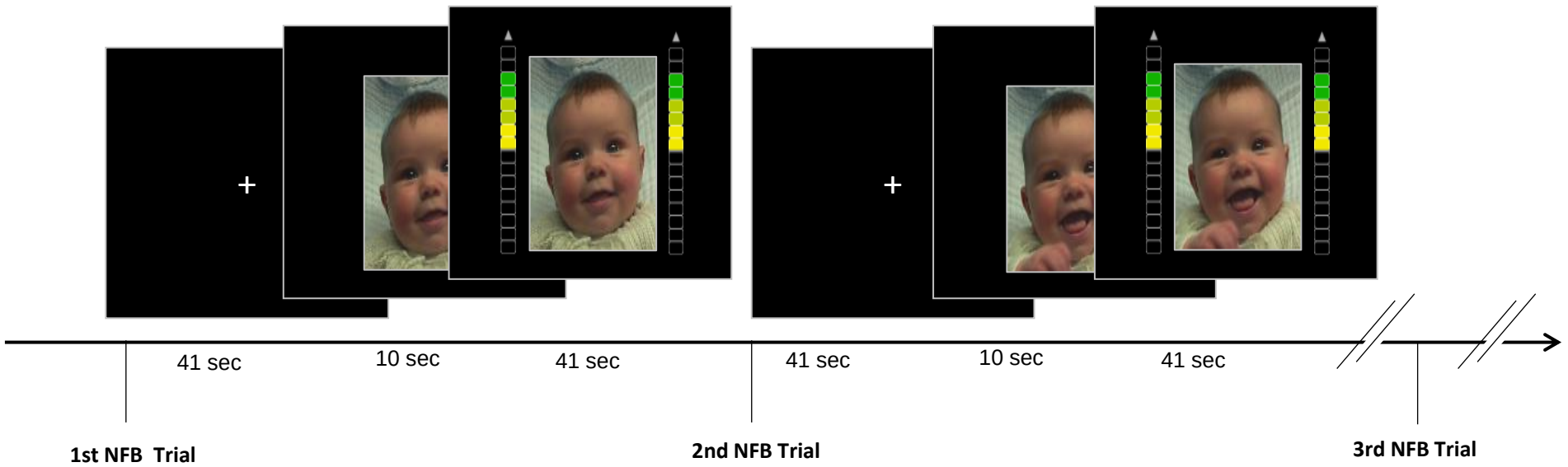
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		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	23
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	23
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	23

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-7
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	7
Objectives	#7	Specific objectives or hypotheses	7
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	14
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-10

Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12-14
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed	

mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	15
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	17
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Data access	#29	Statement of who will have access to the final trial dataset,	17

		and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	2
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	