Nx4 attenuated stress-induced activity of the anterior cingulate cortex—A *post-hoc* analysis of a randomized placebo-controlled crossover trial

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**Abstract**

**Objective:** Stress-related symptoms are associated with significant health and economic burden. Several studies suggest Nx4 for the pharmacological management of the stress response and investigated the underlying neural processes. Here we hypothesized that Nx4 can directly affect the stress response in a predefined stress network, including the anterior cingulate cortex (ACC), which is linked to various stress-related symptoms in patients.

**Methods:** In a randomized, placebo-controlled, double-blind, crossover trial, 39 healthy males took a single dose of placebo or Nx4. Psychosocial stress was induced by the ScanSTRESS paradigm inside an MRI scanner, and stress network activation was analyzed in brain regions defined a priori.

**Results:** Using the placebo data only, we could validate the activation of a distinct neural stress pattern by the ScanSTRESS paradigm. For Nx4, we provide evidence of an attenuating effect on this stress response. A statistically significant reduction in differential stress-induced activation in the right supracallosal ACC was observed for the rotation stress task of the ScanSTRESS paradigm. The results add to previously published results of Nx4 effects on emotion regulation.

**Conclusions:** Our results strengthen the hypothesis that Nx4 modulates the stress response by reducing the activation in parts of the neural stress network, particularly in the ACC.

**Trial registration:** NCT02602275; ClinicalTrials.gov

**Keywords**

acute psychosocial stress, anterior cingulate cortex, functional magnetic resonance imaging, neural stress network, Nx4
1 | INTRODUCTION

Stress is associated with significant health and economic burden. Stressed individuals experience impaired physical and mental functioning, more workdays lost, increased impairment at work, and a high use of health care services (Kalia, 2002). Stressors have a significant influence on our mood, sense of well-being, behavior, and health (Schneiderman et al., 2005), and they have been described to trigger physiological stress responses. An unpleasant surprise, performing under observation, or a social conflict are acute stressors, whereas poverty, suffering from a severe chronic disease, taking care of a relative or living in a ‘broken’ family are examples of chronic stressors (Selye, 1973).

Psychosocial interventions proved to be useful for treating stress-related disorders. For pharmacological management, antidepressants, anxiolytics, and beta-blockers are widely used. These medications are often associated with side effects and are not recommended for long-term use. Neurexan (Nx4) is a natural medicinal product composed of herbal extracts of oat (Avena sativa), coffee (Coffea arabica), passionflower (Passiflora incarnata), and a mineral salt (Zincum isovalerianicum). Nx4 has been shown to have comparable sleep-inducing effects as valerian, significantly reducing sleep latency and increasing sleep duration with less daytime fatigue (Waldschütz & Klein, 2008). In an observational study in 49 German general practices, customized doses of Nx4 for 2 weeks led to significantly reduced nervousness/restlessness (Hubner et al., 2009). Modulated electroencephalography (EEG) signatures of several brain regions comparable to antidepressants suggested a calming effect of Nx4 (Dimpfel, 2019). The efficacy of Nx4 on acute stress in humans was investigated in a clinical trial analyzing the physiological stress response to the Trier Social Stress Test (Doering et al., 2016). Nx4 modulated the physiological stress response, particularly by significantly reducing salivary cortisol and plasma adrenaline. The underlying neural processes and the mode of action of Nx4 have been partly described previously.

Stress-related changes in the brain can be tracked by inducing a stress response in an experimental condition and measuring the brain’s responses using functional magnetic resonance imaging (fMRI). In the context of stress, the hippocampus has an inhibitory function on the hypothalamus, which initiates the secretion of stress hormones via the hypothalamic-pituitary-adrenal (HPA) axis (Smith & Vale, 2006). While increased blood flow in the hippocampus may indicate enhancement of emotional memory formation (Tillfors et al., 2002), deactivation of the hippocampus may disturb the inhibition of the HPA axis and lead to an enhanced hormonal response (Pruessner et al., 2008). Depending on the stressor type, different brain regions seem to be involved in the stress response, although the subjective and hormonal response is similar. Physiological stressors activate brain regions associated with pain processing like the insula, striatum and middle cingulate cortex, while psychosocial stressors activate the right superior temporal gyrus and deactivate the striatum. The inferior frontal gyrus and the anterior insula are activated independent of the type of stressor (Kogler et al., 2015).

Several studies consistently found functional changes in the anterior cingulate cortex (ACC) and hippocampus, as well as orbitofrontal cortex during or in the immediate aftermath of the stress condition (Dedovic, D’Aguiar, & Pruessner, 2009). The ACC is known for its role in pain processing and emotion regulation (Vogt, 2005) and monitors cognitive and motor responses during conflict and decision-making (Haber & Knutson, 2010). Together with the amygdala, the dorsal portion of the ACC is affiliated to the salience network, which plays a crucial role in integrating salient emotional stimuli and, therefore, mediates a state of hypervigilance and increased attention towards environmental stimuli and internal states (van Oort et al., 2017). The orbitofrontal cortex shows reduced activation in response to stress (Dedovic, D’Aguiar, & Pruessner, 2009). It is involved in collecting and integrating sensory information (Kringelbach, 2004), and monitors the reward value of possible future outcomes to guide appropriate adaptive behavior (Elliott, 2000; O’Doherty et al., 2001). Several networks and brain regions are involved in stress processing. We hypothesized that the stress-relieving properties of Nx4 are based on a direct effect on neural processing linked to these stress networks and regions, particularly in the context of acute stress.

In the NEURIM study, healthy male participants experiencing mild to moderate chronic stress were exposed to acute psychosocial stress to examine the efficacy of Nx4 in reducing reactivity of brain areas associated with stress processing. Previously reported results of this trial showed that Nx4 reduced activation in a region associated with social stress, the left amygdala, in response to negative emotional stimuli in a face-matching task (Herrmann et al., 2020). In the analyses reported here, we were particularly interested in brain regions that are consistently referred to in the context of stress. Our a priori regions of interest (ROIs) included ACC, hippocampus, amygdala, medial orbitofrontal cortex, and hypothalamus. To induce acute psychosocial stress, we employed the ScanSTRESS paradigm, an established and validated fMRI compatible psychosocial stress task (Streit et al., 2014). We hypothesized that Nx4 would significantly dampen the effect of acute stress on the neural stress network, including ACC, medial orbitofrontal cortex, hippocampus, amygdala, and hypothalamus compared to placebo.

2 | METHODS

2.1 | Clinical trial procedures

The data reported here originate from the NEURIM study (ClinicalTrials.gov identifier: NCT02602275; registered 2015-10-28), a clinical trial that has been published previously (Herrmann et al., 2020). It was conducted as a randomized, placebo-controlled, double-blind, two-period, two-treatment crossover trial with 1:1 randomization of the two treatment sequences, Nx4-Placebo and Placebo-Nx4. Eligible participants were healthy males, aged 31–59 years, with mild to moderate chronic stress defined by a Trier Inventory for Chronic Stress - Screening Scale for Chronic Stress...
(TICS-SSCS) Score $\geq 9$ and $\leq 36$ as well as a Perceived Stress Scale (PSS) $> 9$. A total of 40 participants were planned to be included at a single site at the Clinical Affective Neuroimaging Laboratory (CANLAB), Magdeburg, Germany.

The overall study procedures were as follows: After informed consent and psychometric data collection, participants were randomized 1:1 to two treatment sequences, Nx4-Placebo and Placebo-Nx4. The procedures on day 1 (Figure 1) included two MRI sessions, including EEG data acquisition. First, a structural MRI scan and a resting state EEG measurement were conducted during a simultaneous EEG/fMRI scan session. After administering a single dose (three pills) of Nx4 or placebo, two computerized tests, the Attention Modulation by Salience Task (AMST) and an auditory oddball task, were performed while EEG data were acquired. A second EEG/fMRI scan session was conducted, starting 40–60 min after dosing. This session including a second resting-state measurement followed by the Hariri emotional face-matching task, an expectancy task, and the ScanSTRESS paradigm as well as another resting-state measurement. Subjective ratings of anxiety, nervousness, and mood were recorded at different time points before and after dosing as well as before and after the induction of stress. After a washout period of 7–35 days, the crossover session took place on day 2 essentially as on day 1. To minimize any confounding effects of circadian rhythm, the procedures were performed at almost the same time of the day, in the afternoon.

### 2.2 ScanSTRESS paradigm

The analysis reported here focused on the ScanSTRESS paradigm (Streit et al., 2014), an fMRI compatible adaptation of the Trier Social Stress Test, used to induce acute psychosocial stress. It was composed of demanding serial subtraction tasks and mental rotations. Simple matching versions of the two tasks (no rotation of figures and no subtractions, just numbers) were used as a control condition. The experiment was set up in a blockwise design with alternating 40-s blocks of stress performance and control condition, presented in 2 runs. In the stress blocks, participants were pushed for time, and two experimenters in professional attire explicitly showed their dissatisfaction with the correctness and speed of the answers via video stream. In the control condition, participants did not receive any feedback. Task speed and difficulty were automatically adapted to the individual performance, ensuring that the participants were unable to meet the expectations. Between the two runs, participants were interrupted and given extensive, disapproving verbal feedback stating that the data would be unusable if the participant did not put more effort into the task. The ScanSTRESS task was conducted approximately 73–93 min after dosing.

### 2.3 fMRI data acquisition

fMRI data were acquired with a Philips 3T scanner. Structural T1-weighted images for spatial normalization were measured using a turbo field echo sequence with the following parameters: 274 sagittal slices covering the whole brain, flip angle $= 8^\circ$, $256 \times 256$ matrix, voxel size $0.7 \times 0.7 \times 0.7$ mm$^3$. T2*-weighted echo-planar images consisting of 225 volumes were acquired for the ScanSTRESS task. The following scanner settings were used: 34 axial slices covering the whole brain, repetition time $= 2000$ ms, echo time $= 30$ ms, flip angle $= 90^\circ$, $96 \times 94$ matrix, field of view $= 240 \times 240$ mm$^2$, voxel size $= 2.5 \times 2.5 \times 3$ mm$^3$.

### 2.4 Data preprocessing

Data were preprocessed using Matlab 2019a (The Mathworks Inc., Natick, MA, USA) and SPM12 (Statistical parametric mapping software, SPM: Wellcome Department of Imaging Neuroscience, London, UK). Data were corrected for different slice timing within one volume, and head motion correction was performed by realigning all images to the first image of the task. Six head motion parameters (translation: x, y, z; and rotation: pitch, roll, yaw) were extracted during this step and later used as regressors of no interest in the first level design. Co-registration of functional images to the anatomical T1 image was done to shift the images into the same space before spatial normalizing to Montreal Neurological Institute (MNI) space. Anatomical T1 images were segmented using the unified

![Figure 1](image-url)  
**Figure 1** Detailed study procedures on each day, 1 and 2. During the first functional magnetic resonance imaging (fMRI) scan an anatomical scan and a baseline resting-state measurement were acquired, followed by drug administration. Afterwards, two electroencephalography (EEG) paradigms (Attention Modulation by Salience Task (AMST) and Oddball) were recorded. The second fMRI scan consisted of three task measurements, the Hariri paradigm, the Expectancy paradigm, and the ScanSTRESS paradigm, and two resting-state sequences.
segmentation procedure that also estimates deformation fields used on all functional images for spatial normalization. Functional images were resampled with a resolution of $3 \times 3 \times 3$ mm$^3$. The smoothing of the data was done with a Full-Width-Half-Maximum kernel of $8 \times 8 \times 8$ mm$^3$. Participants with movement $>3$ mm for more than one volume were excluded for further analyses. In case of exclusion, the measurements of both days were excluded.

2.5 | Evaluation of task effect

Ascertaining the reproduction of previously shown brain activation by the ScanSTRESS paradigm was done on a whole-brain level using a second level one sample t-test in SPM on the first level general linear models (GLM) in the placebo condition. In general, a first level GLM was modeled assuming a block design for the ScanSTRESS task with identical onset times of the different blocks for each participant. The task was split into two runs, before and after the verbal video-feedback, which were modeled as separate sessions in the GLM. The task was structured into four conditions of interest: control subtraction, control mental rotation, stress subtraction, stress mental rotation. Five separate regressors were constructed, one for each of the four conditions of interest and the instruction. Regressors were convolved with the canonical hemodynamic response function provided by SPM12. The six head motion parameters obtained during preprocessing were used as additional regressors of nuisance. For each participant, the contrasts of interest, (stress-control), (mental rotation stress-mental rotation control) and (subtraction stress-subtraction control), were calculated, and these resulting contrast images were then used for all further group analyses in the second level. In the second-level analysis for a main effect of task, a one-sample t-test on whole-brain level for the placebo condition only was performed. The sequence of study medication was added as a covariate of no interest. Significance was assessed after correcting for multiple comparisons on peak-level, for a Family-Wise Error (FWE) corrected $p < 0.05$.

2.6 | Evaluation of Nx4 effect

The effect of Nx4 on the stress network activation was analyzed in a priori defined ROIs merged into a stress network. For the ROIs ACC, medial-orbitofrontal cortex, hippocampus and amygdala search volumes were anatomically defined by the Automated Anatomical Labeling (AAL) Atlas 3. For the hypothalamus, a search volume of a sphere with a radius of 5 mm around the coordinates $\pm 8, -4, -4$ has been used (Kroemer et al., 2013). To test the drug effect on the contrasts (stress-control), (mental rotation stress-mental rotation control) and (subtraction stress-subtraction control), the respective first level contrasts for each participant were taken to a second level analysis of all voxels comprised by the stress network mask or individual masks for the single ROIs (e.g. ACC mask). A voxelwise paired t-test, comparing placebo and verum conditions, was controlled for multiple comparison across all voxels via a search volume (small volume correction [SVC]) implemented in SPM. This correction applies a peak-level FWE correction (Worsley et al., 1996). The sequence of study medication was added as a covariate of no interest to the respective second level model.

The efficacy of Nx4 on reducing the stress network activation was defined as the sixth primary outcome in the a priori ordered chain of primary outcomes in the NEURIM clinical trial. Since a preceding outcome (primary outcome 2: reduced resting state global functional connectivity density [gFCD] of amygdala after Nx4 compared to placebo) missed statistical significance, this analysis is considered as exploratory.

3 | RESULTS

3.1 | Baseline data, numbers analyzed, and harms

In total, 40 healthy males participated. 20 participants were randomly assigned to each of two treatment sequences (Figure 2). Participants were in the age range of 31–59 years and had a mild to moderate level of stress. There were no substantial differences between the two sequences in terms of demographics and baseline characteristics (See Table 1). One participant of the placebo first sequence dropped out of the study due to an incidental baseline MRI finding before drug administration. Out of the 39 participants completing the study, 36 were included in the ScanSTRESS task analysis (20 participants receiving verum first and 16 participants receiving placebo first). Three participants had to be excluded due to motion inside the scanner. None of the participants experienced any adverse events during the trial. Vital signs were within expected ranges and showed no abnormalities.

3.2 | ScanSTRESS task effect

To validate the induction of the stress activation pattern described for the ScanSTRESS paradigm during the task, we contrasted the stress and control blocks within the placebo condition. On a whole-brain level, the comparison revealed that psychosocial stress led to significantly higher activation in the following brain areas (Figure 3): anterior insula (bilaterally), premotor area (left), angular gyrus (bilaterally), inferior frontal gyrus (bilaterally), posterior medial frontal gyrus (left), occipital lobe, cerebellum, and supplementary motor area. Significant deactivations were found in the default mode network regions, namely the ventromedial prefrontal cortex, posterior cingulate cortex, and temporal pole/hippocampus. These findings were in line with the original description of this stress paradigm. All activations are illustrated in Figure 3a. Similar activation and deactivation patterns were seen for the overall contrast stress > control, the contrast subtraction stress > subtraction control (Figure 3b), as well as in a more specific way for the more cognitive demanding rotation task in the contrast rotation stress > rotation control (Figure 3c).
The drug effect was investigated in the contrast stress > control using an SVC for the predefined stress network. For the overall stress condition (subtraction stress and rotation stress), no significant differential activation was found for Nx4 versus placebo. The two types of tasks, subtraction and rotation, were then investigated separately since they showed slightly different task activation and deactivation patterns. For the cognitively more demanding rotation task, we observed a significant effect of Nx4 on stress-induced activity within the ACC (as defined by the AAL Atlas 3). We found a significantly lower peak \( p = 0.020 \), peak-level FWE-corrected after SVC; MNI coordinates 15 32 20) in the contrast rotation stress > rotation control under Nx4 compared to placebo. That demonstrates reduced stress-induced ACC activation under Nx4. A significant peak was seen in the supracallosal part of the right ACC (Figure 4). For the other ROIs, we have not observed differential activation patterns under Nx4 versus placebo. Comparing the placebo and Nx4 conditions in the less challenging subtraction task, we have not observed significant peaks in any of the ROIs.

The drug effect was observed during the fMRI scan session conducted 73-93 min after a single oral dose of Nx4.

### 4 | DISCUSSION

#### 4.1 | ScanSTRESS task effect

Our study could validate the induction of a distinct neural stress activation pattern by the ScanSTRESS paradigm. The acute stress activation pattern observed in 36 placebo-treated, generally healthy but mildly to moderately stressed participants followed essentially the previously described pattern (Streit et al., 2014). The effect was observed for both the subtraction and the mental rotation task. This supports the validity of the ScanSTRESS paradigm to evaluate drug effects on psychosocial stress activation.
4.2 | Nx4 effect in subtraction versus rotation task

The drug effect was only observed in the mental rotation task and not in the subtraction task. Performance in mental rotation as well as in arithmetic tasks, is impaired by stress (Hou et al., 2015; Qi et al., 2016). Under stress, attention is partly taken away from the task itself, and the working memory capacity is shared between task performance and thoughts caused by the stressor (Beilock, 2008). Although there is some evidence that mental rotation ability and mathematical skills are related (Kyttälä & Lehto, 2008), mental rotations seem to be more demanding than arithmetic due to the complexity of the stimuli (Ruchkin et al., 1991). The complexity of our rotation stress task with a rotation angle up to 180° likely increased time pressure (Searle & Hamm, 2017). Therefore, participants may have perceived the mental rotation condition as more challenging, thus, more stressful than the subtraction condition. Indeed, comparison of mental rotation task and subtraction task within the placebo condition revealed a significantly higher differential activation within the ACC in the mental rotation task ($p = 0.044$, peak-level FWE-corrected after SVC; MNI coordinates $6 50 14$; supporting information S1). That indicates increased stress-induced ACC activation during the mental rotation task. It can be speculated that a relatively high stress induction is

For Nx4, we found some evidence of an attenuating effect on neural stress network activation, driven by significantly reduced activation in the right ACC.
required to demonstrate a drug effect in this setting. However, the proposed higher stress burden for rotation compared to the math was not supported by differentially changed cortisol levels or subjective stress ratings by the participants (data not shown).

### 4.3 | Relevance of the ACC

Concerning the various ROIs, the reduced stress-induced activity with Nx4 treatment was found particularly in the right supracallosal ACC. The ACC is described to process cognitive and emotional information separately via its two main subdivisions (Bush et al., 2000). The dorsal ACC (dACC) is the cognitive division, which is activated during tasks with high mental effort and by social rejection or negative evaluation (Dedovic et al., 2016) as used in the ScanSTRESS paradigm. In line with the finding of reduced reward processing during stress (Kogler et al., 2015), a role in reward-based decision making has been proposed for the dACC (Bush et al., 2002). The supracallosal portion thought to be activated by non-rewards, punishers or unpleasant stimuli (Rolls et al., 2019) and is located in the dorsal part of the ACC, which may also be referred to as the anterior midcingulate cortex (Vogt, 2016). Previous studies have shown activity increases in the anterior midcingulate cortex (Gianaros & Wager, 2015) and increased cerebral blood flow in the ACC (Dedovic, Rexroth, et al., 2009) under demanding cognitive tasks (like subtraction tasks) and psychosocial stress (like negative social evaluation or feedback). It has been suggested that the distinct sub-regions have differential inputs on the HPA axis (Ulrich-Lai & Herman, 2009). For example, the rostral-ventral prelimbic region may inhibit the HPA axis, while the dorsal infralimbic region may have a stimulatory role (Diorio et al., 1993; Radley et al., 2006; Ulrich-Lai & Herman, 2009). Also, the dACC can be involved in fear expression or play a crucial role in detecting salience (Milad et al., 2007). The dACC is the part of the salience network (Kohn et al., 2017; Seeley et al., 2007) upregulated by catecholamine increase in response to acute stress. The salience network has been proposed to play a crucial role in the integration of salient environmental stimuli. That specifically holds true for the ability to reorient attention to potential threats (Hermans et al., 2014; Kohn et al., 2017; Menon & Uddin, 2010) and prepare for the next stress stimulus. Vigilant states are characterized by autonomic arousal mediated by the dACC, amygdala, and the anterior insula (van Marle et al., 2010). Those regions were found to be functionally more coupled in a resting-state measurement following moderate psychological stress (van Marle et al., 2010). It has also been suggested that dACC and ventral ACC collaborate in the top-down regulation of amygdala responses by a mechanism in which dACC stimulates the inhibitory function of the ventral ACC (Etkin et al., 2011). The dACC is also involved in encoding effort valuation for effort-based decisions (Hogan et al., 2020). Previous research in animals showed that inactivation of the ACC led to a reduced readiness for mental exertion (Hosking et al., 2014).

### 4.4 | Relevance of lateralization to right hemisphere

The finding of a lateralized response in the right supracallosal ACC is in line with previous findings about lateralization of stress and emotion processing. It has been postulated that predominantly the right hemisphere is processing emotions (Sackeim et al., 1978). The lateralization of emotions depends on their valence (positive vs. negative) with the right hemisphere processing negative emotions (Davidson, 1992). That has also been shown for the right ACC, which processes negative emotions and pain (Watanabe et al., 2015). Accordingly, functional and structural lateralization are affected by different types of stress (Ocklenburg et al., 2016). Acute stress has been shown to increase activity in the right hemisphere in various studies (Berretz et al., 2020), which led to the idea that the right hemisphere controls the HPA axis and the stress response (Lueken et al., 2009). For the ACC there have been stress-related findings in the left hemisphere. The left ACC showed activation and volume differences associated with stress (MacLullich et al., 2006; Pruessner et al., 2008). Considering the mental rotation task in our study, the right lateralization of the ACC is supported by literature on cognitive control. The right ACC was shown to be involved in visuospatial decisions and to mediate the influence of cognitive control (Stephan, 2003).

### 4.5 | Clinical relevance of effect in ACC for patients

By reducing dACC activity, Nx4 might be beneficial for stress-related symptoms linked to dACC activation. In patients with insomnia, a metabolic increase in dACC during non-rapid eye movement sleep has been linked to increased wakefulness after sleep onset (Nofziger et al., 2006). The authors suggested an underlying mechanism of increased activity in arousal systems or activity in cognitive processes related to goal-directed behavior, conflict monitoring, emotional awareness, anxiety, and fear. Similarly, in patients with social anxiety disorder, generalized anxiety disorder, and major depressive disorder, dACC activation is negatively associated with objective sleep efficiency (Klumpp et al., 2017). Anxiety has also been shown to be positively correlated with dACC activation (Straube et al., 2009) and the dACC has been suggested to be a target region for anti-anxiety therapies (Milad et al., 2007). A hyperactivation of the anterior midcingulate cortex has also been linked to post-traumatic stress disorder, which is likely to be affected by chronic stress (Hinojosa et al., 2019).

### 4.6 | Mode of action of Neurexan related to previously reported effects

In fact, Nx4 has been previously shown to reduce sleep latency, increase the duration of sleep, and reduce daytime fatigue (Waldschütz & Klein, 2008), to reduce nervousness/restlessness.
within the stress phase. The validation of the task effect indicated an rotate a figure, leading to a confounding effect of cognitive load regions of the a priori stress network.

The ACC finding was not corrected for testing of different brain related symp- toms linked to this brain region.

4.7 | Outlook

The results encourage further investigations in various indications related to acute and chronic stress, anxiety, and sleep disorders. The excellent safety and tolerability profile of Nx4 allows for testing also higher dosages and prolonged treatment periods to achieve more pronounced effects. According to the patient information leaflet, Nx4 tablets can be taken up to 1 tablet six times a day under acute restlessness, while our participants took a single dose of three tablets.

5 | LIMITATIONS

All results reported in this article are exploratory.

The effect of Nx4 on the neural stress network was limited to a differential activation in the right ACC after SVC in the contrast rotation stress versus rotation control only. For all other ROIs in this contrast as well as all ROIs in the overall stress > control contrast and subtraction task, no significant effect of Nx4 could be observed. The ACC finding was not corrected for testing of different brain regions of the a priori stress network.

Further, the two phases, stress and control phase, of the ScanSTRESS paradigm differ a lot in their level of difficulty. During the control phase the participants neither must subtract any number nor rotate a figure, leading to a confounding effect of cognitive load within the stress phase. The validation of the task effect indicated an activation of brain regions related to stress, though.

6 | CONCLUSION

In this exploratory analysis, we found that a single dose of Nx4 leads to a reduced activation of the neural stress network. We found reduced activation of the right supracallosal anterior cingulate cortex under Nx4 compared to placebo during a mental rotation task with psychosocial stress induction. By reducing the activation of the neural stress network, particularly in the right supracallosal anterior cingulate cortex, Nx4 might be beneficial for stress-related symptoms linked to this brain region.

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CONFLICT OF INTERESTS

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ETHICS STATEMENT

The original study protocol of the data used here has been approved by the local ethics committees of the university hospital Magdeburg and informed consent was obtained by all the participants prior to the examination. The current data were analyzed anonymously.

AUTHOR CONTRIBUTIONS

Martin Walter, Johannes C. Vester, Bernd Seilheimer and Myron Schultz conceived the experiments, Johan Van der Meer conducted the experiments, Luisa Herrmann, Vanessa Kasties, Meng Li,
Yan Fan, and Johannes C. Vester analyzed the results. Luisa Herrmann, Vanessa Kasties, Cindy Boden, and Sarah Alizadeh interpreted the results and wrote the manuscript. All authors reviewed interim drafts and final version of the manuscript and agree to be accountable for all aspects of the work.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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