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**Regular Research Article**

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# Subanesthetic Ketamine Suppresses Locus Coeruleus–Mediated Alertness Effects: A 7T fMRI Study

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## Abstract

**Background:** The NMDA antagonist S-ketamine is gaining increasing use as a rapid-acting antidepressant, although its exact mechanisms of action are still unknown. In this study, we investigated ketamine in respect to its properties toward central noradrenergic mechanisms and how they influence alertness behavior.

**Methods:** We investigated the influence of S-ketamine on the locus coeruleus (LC) brain network in a placebo-controlled, cross-over, 7T functional, pharmacological MRI study in 35 healthy male participants (25.1±4.2 years) in conjunction with the attention network task to measure LC-related alertness behavioral changes.

**Results:** We could show that acute disruption of the LC alertness network to the thalamus by ketamine is related to a behavioral alertness reduction.

**Conclusion:** The results shed new light on the neural correlates of ketamine beyond the glutamatergic system and underpin a new concept of how it may unfold its antidepressant effects.

**Keywords:** Ketamine, locus coeruleus, LC functional connectivity, alertness, ultra-high field MRI

### Significance Statement

As a rapid-acting antidepressant, the NMDA antagonist S-ketamine is becoming increasingly used in psychiatric therapy, but its precise modes of action are still unclear. In this placebo-controlled functional MRI study, we could show that ketamine affects a specific brain network associated with noradrenaline, which is known to play a prominent role in depression, and that those effects influence noradrenaline-related behavior. Specifically, we could show that a brainstem nucleus, the locus coeruleus, which regulates most noradrenaline spillover in the brain, changes its functional connectivity in our healthy young study population during ketamine administration. This drug-induced change of central noradrenergic regulation was related to an alertness reduction in the same participants in a behavioral attention task. Our results show how ketamine influences central noradrenergic mechanisms and support a novel theory regarding how it might reveal its antidepressant effects.

## INTRODUCTION

Ketamine is a commonly used anesthetic drug in emergency medicine and surgery. Recently, subanesthetic ketamine was discovered to act as a rapid-acting antidepressant, and numerous studies show promising effects in the treatment of major depressive disorder (MDD), which are corroborated by meta-analyses ( Berman et al., 2000a; Zarate et al., 2006; Caddy et al., 2014; Fond and Boyer, 2014; McGirr et al., 2015; Wan et al., 2015; Bahji et al., 2020). Based on these findings, ketamine is used as a therapeutic option in treatment-resistant depression (Nugent et al., 2020; Park et al., 2020; Zarate, 2020). Frequently, it is infused i.v. at a low dose, such as 0.5 mg/kg over 40 minutes (Schwartz et al., 2016). Furthermore, ketamine received FDA approval as a nasal spray for the treatment of MDD in 2019 (FDA, 2019). The different pharmacological properties of ketamine (Short et al., 2018), including the antidepressant effect (Berman et al., 2000;

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Murrough et al., 2013), are likely to arise from its influence on multiple receptor types: although the inhibition of the NMDA receptor is widely assumed as the primary mechanism of antidepressant action (Krystal et al., 1994; Boyer, 1998; Zanos et al., 2016), other receptors may also play an important role in the action of the drug (Zanos et al., 2016). Based on the known effects of ketamine on the autonomous nervous system and its inhibitory component on the norepinephrine transporter (NET) (Baraka et al., 1973; Miletich et al., 1973; Doenicke et al., 1992; Hara et al., 1998, 2002; Nishimura et al., 1998; Zhao and Sun, 2008), we previously assessed both ketamine cardiovascular side effects and their influence on resting-state functional connectivity (Biswal et al., 1995; Van Den Heuvel and Pol, 2010) brain networks by focusing on effects related to the sympathetic nervous system (Liebe et al., 2017, 2018). We found that the cardiovascular adverse effects of ketamine differ depending on the NET genotype (Liebe et al., 2017). Moreover, we uncovered a NET-dependent influence of ketamine on the locus coeruleus (LC)—a brainstem nucleus on which we focused due to its regulatory role on the sympathetic nervous system and its high NET density—in 7T resting-state functional magnetic resonance imaging (rs fMRI) (Liebe et al., 2018). More specifically, we found that ketamine rapidly disrupts the functional connectivity (fc) from the LC to the thalamus in the resting state following drug infusion. A more complex relationship of noradrenergic and glutamatergic antidepressant mechanisms is also reflected in the pathophysiological situation in MDD. While subunits of the NMDAR are cortically decreased, subcortical NR2B subunits were found to be increased, including amygdala and locus coeruleus. Subcortical NMDAR hyperactivity was thus discussed as a target for diminishing glutamatergic inputs and subsequently modifying ascending brain stem innervation (Chandley et al., 2014; Hillhouse and Porter, 2015; Liu et al., 2017). We proposed the relevance of those findings with respect to the functional importance of the sympathetic key region LC in general brain function due to its regulation of norepinephrine (NE) spillover, its anatomical properties in terms of effective connections, and the involvement of the region in antidepressant mechanisms (Liebe et al., 2018): the LC has widespread connections to the whole brain, including the thalamus, cerebellum, prefrontal cortex, and limbic structures (Samuels and Szabadi, 2008a, 2008b). Functionally, the LC is involved in the control of autonomic functions, arousal, and, within the concepts of orienting, conflict, and alertness (Corbetta and Shulman, 2002; Fan et al., 2005, 2009; Corbetta et al., 2008; Petersen and Posner, 2012), in attention regulation. Specifically, the LC-thalamic connectivity was attributed to the alertness properties of LC function (Périn et al., 2010; Petersen and Posner, 2012; Murphy et al., 2014). The alerting effect forms a key element in sensory signal perception by switching from rest to a preparation of reaction to behaviorally relevant incoming information (Petersen and Posner, 2012). This specific mechanism can be uncovered in behavioral tasks by presenting warning signals before an action is required, which decreases the reaction times of the subject due to increasing awareness of the requested operation (Fan et al., 2002, 2005; Xuan et al., 2016). Interestingly, ketamine administration was found to have a rapid impact on attention functions in task studies (Oranje et al., 2000; Umbricht et al., 2000; Musso et al., 2011; Nikiforuk and Popik, 2014), but these effects were assigned to neither alertness nor the LC NE system so far. Since in animal models the LC was also shown to be involved in the regulation of several depression- and alertness-related functions like stress vulnerability and social defeat (Curtis et al., 2012; Isingrini et al., 2016; Szot et al., 2016), we thought to investigate ketamine effects on LC connectivity also regarding the drug's antidepressant properties. Furthermore, next to affective appraisal, biased and impaired attention is another key diagnostic feature of MDD whose neural correlates, however, have been somewhat less investigated than those related to positive and negative affect.

Based on these considerations in conjunction with our former physiological, genetic, and fMRI results, we proposed a ketamine-induced suppression of the alerting effect by disruption of LC noradrenergic control over the thalamus, which could induce reduced acute response sensitivity to sensory stimuli with respect to alertness (Liebe et al., 2017, 2018, 2020). In the following study, we aimed to examine this proposed hypothesis by investigating the effects of ketamine on alertness in the attention network task (Fan et al., 2002, 2005; Xuan et al., 2016). On the basis of our constrained hypothesis of LC networks, we aimed to replicate the results of our previous ketamine study in rs fMRI and extend our former view by examining possible relations of alertness reduction effects on the LC functional brain network.

# METHODS

## Study Design and Participants

The study was conducted with a randomized, placebo-controlled, double-blind, cross-over design applying a single subanesthetic S-ketamine infusion or placebo in 35 healthy male participants (mean age± SD=25.1±4.2 years; for participant characteristics, see also supplementary Table 1). The main inclusion criteria were no current or lifetime major psychiatric disorder, including substance or alcohol dependence or abuse, according to DSM-IV (American Psychiatric Association, 2005) as assessed by the Structured Clinical Interview for DSM-IV (First and Gibbon, 2004); no family history of psychiatric disorders as assessed by a demographic questionnaire; no neurological or physical constraints or severe illnesses as evaluated by a study physician during screening; right-handedness; and the absence of MRI contraindications. All participants gave informed written consent. The study was reviewed and approved by the institutional review board of the Otto-von-Guericke-University Magdeburg and was performed in accordance with the recommendations made in the Declaration of Helsinki and local legal requirements.

## Experimental Procedure

The experimental procedure was designed as 2 consecutive days per treatment arm, including MR scanning before infusion (baseline measures), during infusion (immediate effects), and 24 hours after infusion. The interval between the 2 treatment arms was 3 weeks on average. In a randomized order, participants received a single i.v. infusion (Injectomat MC Agilia; Fresenius Kabi GmbH, Bad Homburg, Germany) as a bolus of 0.11 mg/kg body weight S-ketamine or 0.9% saline followed by a maintenance dose of 0.22 mg/kg body weight S-ketamine or 0.9 % saline administered over a period of 40 minutes. Because of the administration of S-ketamine, a reduced dosage compared with the standard infusion regime of racemic ketamine (Sinner and Graf, 2008) was used.

For a minimum of 4 hours after infusion, the participants were observed to ensure medical safety.

For the investigation of alertness effects, the attention network task (ANT; Fan et al., 2005) was conducted 4 hours after the infusion and 24 hours after infusion. A training session was performed by all participants prior to the experimental fMRI procedure.

## MRI Image Acquisition

Image acquisition was performed using a Siemens MAGNETOM 7 T MRI scanner with syngo MR E11 software and a 32-channel head coil with the following parameters for the anatomical images: 3D-MPRAGE sequence, echo time (TE) = 2.54 milliseconds, repetition time (TR) = 1700 milliseconds, inversion time (T1) = 1050 milliseconds, flip angle = 5°, bandwidth = 160 Hz/pixel, acquisition volume = 256 × 256 × 176 $mm^3$, isometric voxel size = 1.0 $mm^3$, and scan duration = 3 minutes 39 seconds. Blood oxygenation level dependent (BOLD) signals were acquired using a multi-band accelerated T2*-weighted echo-planar imaging sequence.

We acquired eyes-closed rs fMRI data with the following parameters: 60 axial slices parallel to the anterior–posterior commissure plane covering the whole brain acquired in an interleaved order, acquisition volume = 212 × 212 × 132 $mm^3$, slice thickness = 2.0 mm, leading to a high resolution of 2.0 mm isotropic voxels and no gap, TR = 1500 milliseconds, TE = 25.0 milliseconds, flip angle = 70°, 400 volumes in total, scan duration = 10:23 minutes. Visual inspection of raw data for each participant and each time point was performed.

To optimally locate LC individually, a neuromelanin-sensitive T1 MRI sequence using a Siemens MAGNETOM Prisma 3 T MRI scanner with syngo MR E11 software and a 64-channel head coil was applied in 25 participants according to the original work of Sasaki et al. (Sasaki et al., 2006), with the following imaging parameters: 14 axial slices, acquisition volume = 192 × 192 × 42 $mm^3$, slice thickness = 2.50 mm, inter-slice gap 0.5 mm, leading to an effective slice thickness of 3 mm, TR = 634.0 milliseconds, TE = 10.0 milliseconds, bandwidth = 165 Hz/Pixel, flip angle = 80°, scan duration = 10 minutes and 50 seconds.

### *Data Preprocessing*

Echo-planar imaging series preprocessing was performed with CONN (Whitfield-Gabrieli and Nieto-Castanon, 2012), and statistical analysis of extracted MRI beta-values and behavioral scores was performed with SPSS (Statistics, 2011). We previously validated our MNI space mask–based preprocessing pipeline with respect to the exact individual localization of the LC (Liebe et al., 2020). The SPM12 (https://www.fil.ion.ucl.ac.uk/spm/) based preprocessing steps included simultaneous realignment, unwarping, and field map correction of functional data, slice time correction (with slice timings of the multi-band sequence provided to SPM), ART-based outlier detection (conservative settings with nuisance regression of time points exceeding the 95th percentile of movement compared with a normative sample, www.nitrc.org/projects/artifact detect/), structural and functional direct segmentation and normalization to MNI space (structural target resolution 1 mm, functional target resolution 2 mm), and smoothing with a 4-mm kernel. Denoising included regression of white matter, CSF, and physiological noise (CompCor; Behzadi et al., 2007), removal of linear/quadratic trends, regression of subject motion (3-rotation and 3-translation parameters as well as their first-order temporal derivatives), removal of motion outliers (scrubbing), and band-pass filtering at 0.008–0.09 Hz.

As a secondary analysis, we investigated the LC fc based on subject-specific segmentation of the individual LC location in every subject, with CONN-based preprocessing steps as previously described (Liebe et al., 2020). In short, a radiologist experienced in neuroradiology manually segmented the location of the LC in the individual brainstems based on the neuromelanin contrast using FSLview (https://fsl.fmrib.ox.ac.uk/fsl/). The bilateral, participant-specific LC masks were then registered to the anatomical and functional brain images using FSL FLIRT and used for extracting the BOLD signal from the individual location of the LC (supplementary Figure 1).

For seed-based correlation, Fisher-transformed bivariate correlation coefficients of the extracted averaged time series within a validated MNI space LC mask (Keren et al., 2015) (or of individual segmented LC masks in 25 participants) and time series of MNI space whole-brain voxels were calculated. For region of interest (ROI)-to-ROI fc analysis, a matrix of Fisher-transformed bivariate correlation coefficients between the MNI LC masks' time series and MNI space whole-brain ROIs were calculated. For obtaining target ROIs, the default atlas implemented in CONN covering the whole brain was used (based on the FSL Havard-Oxford Atlas (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) and the cerebellar parcellation from the AAL Atlas (Tzourio-Mazoyer et al., 2002). The complete list of ROIs included in the analysis is provided in supplementary Table 2.

The cluster defining threshold was determined as $P < .001$ at voxel level and only clusters below a threshold of FDR $P < .05$ were reported in the seed-to-voxel analysis. In the ROI-to-ROI analysis, the connection-level threshold was set to $P < .05$ and seed-level threshold to an FDR $P < .05$, as suggested by the standard CONN settings.

### *ANT Task*

The ANT was initially published in 2002 (Fan et al., 2002), applied in the fMRI environment (Fan et al., 2005), and slightly modified with an extended focus on the alerting effect as applied here (Rueda et al., 2004; Togo et al., 2015). In short, the ANT could be seen as a modified version of the classical Erikson flanker task (Eriksen and Eriksen, 1974). Participants are requested to respond immediately to a target that can be accompanied by distraction flankers (conflict effect) and that is sometimes cued for the stimulus onset (spatial cue for an orienting and nonspatial cue for an alertness effect). More detailed information about stimulus onset times and task procedure is provided in the supplementary Text and supplementary Figure 2. A training of the task was implemented before the first infusion day to account for potential ceiling effects. The main task results (alerting, orienting, and conflict effects) were analyzed by a linear mixed-model design with the fixed factor "time" and the items ketamine, 24-hour ketamine, placebo, and 24-hour placebo. Additionally, the raw reaction times of the task were investigated by a repeated-measures ANOVA (factors "cue" [nocue, doublecue, centercue]; "flanker" [congruent, incongruent, neutral]; "group" [ketamine, placebo]; "time" [4 hours after infusion (acute), 24 hours after infusion (follow-up)]. All posthoc tests were Bonferroni corrected for multiple comparisons.

# RESULTS

## ANT Task

We found a significant overall effect of time in the linear mixed model of the alerting effect (ketamine 4 hours acute, ketamine 24 hours, placebo 4 hours acute, placebo 24 hours) during the attention network task. Posthoc tests revealed a diminished alertness in the ketamine condition compared with the placebo condition ($P = .04$, $F = 63.003$, mean difference: −10.817 s; Figure 4A). Neither orienting nor executive control showed any significant effects. We additionally investigated the single items of the ANT and found comparable results with previously published studies (Fan et al., 2002, 2005). We found significant effects of time $P < .001$ (RT

4 hours acute=541.15 milliseconds, RT 24 hours after=520.12 milliseconds), cue $P<.001$ (RT centercue=519.65 milliseconds, RT double-cue=516.29 milliseconds, RT no-cue=555.96 milliseconds) with faster reaction in the center cue compared with the double-cue condition ($P=.044$) and slowest reaction in the no-cue condition compared with center cue and double-cue conditions ($P<.001$) and of flanker (RT neutral=472.63 milliseconds, RT congruent=509.17 milliseconds, RT incongruent=610.09 milliseconds, all $P<.001$). Of note, we found no significant effect of time*cue, so no ceiling effects could potentially have influenced the results of alertness calculation.

## fMRI

### *Immediate Ketamine Effects on LC Functional Connectivity*

We first assessed the baseline fc of the LC and found patterns comparable with previous studies with a prominent LC fc to the thalamus, cerebellum, and anterior cingulate cortex (Zhang et al., 2015; Jacobs et al., 2018; Liebe et al., 2018, 2020, 2022).

When testing for differences within the 4 conditions in a voxel-to-voxel analysis (mixed-design ANOVA, factors baseline ketamine, during ketamine infusion, baseline placebo, during placebo infusion), we found a connectivity decrease of the LC to thalamus (left thalamus cluster size=279, FDR $P<.001$; right thalamus cluster size=163, FDR $P<.001$), cerebellum (cluster size=279, FDR $P<.001$), and brainstem regions (cluster size=122, FDR $P<.001$; Figure 1). In concordance with our previous study, a posthoc paired $t$ test showed that this effect was driven by a connectivity decrease during the ketamine infusion condition compared with the corresponding baseline session (left thalamus cluster size=260, FDR $P<.001$; right thalamus cluster size=84, FDR $P<.001$; cerebellum (cluster size=305, $P<.001$); brainstem regions (cluster size=134, $P<.001$; Figure 2).

We also investigated the ROI-to-ROI fc of the LC to whole brain comparing baseline ketamine and during ketamine infusion. We accordingly found a LC-thalamic connectivity decrease, specifically to the ventral lateral nucleus (VLN), ventral posterolateral nucleus (VPL), mediodorsal nuclei (MD) bilateral, and the right subthalamic nucleus (VLNl, FDR $P=.005$; VLNr, FDR $P=.03$; VPLl, FDR $P=.005$; VPLr, FDR $P=.007$; MDNl, FDR $P=.02$; MDNr, FDR $P=.04$; SThNr, FDR $P=.04$).

In a secondary confirmatory analysis, in which we extracted the LC signal at the exact individual position based on neuromelanin-sensitive imaging in 25 of the same participants (as in Liebe et al., 2020), we could also verify the reduction of LC fc to the thalamus (supplementary Text, supplementary Figures 3 and 4).

### *Short-Term Effect on Brain Activity Assessed by ALFF*

By investigating the effects of ketamine on ALFF in the mixed design analysis, we found a significant change in brain activity in the thalamus (left thalamus cluster size=88, FDR $P<.001$; right thalamus cluster size=34, FDR $P=.002$) and cerebellum (multiple clusters, $P<.001$), interestingly, regions that also show a connectivity decrease to the LC. Posthoc tests revealed a significant decrease of ALFF within the left thalamus (cluster size=55, FDR $P<.001$; Figure 3A) and the cerebellum (cluster size=68, FDR $P<.001$) in the ketamine infusion condition compared with the placebo infusion and comparable ALFF decrease in the left

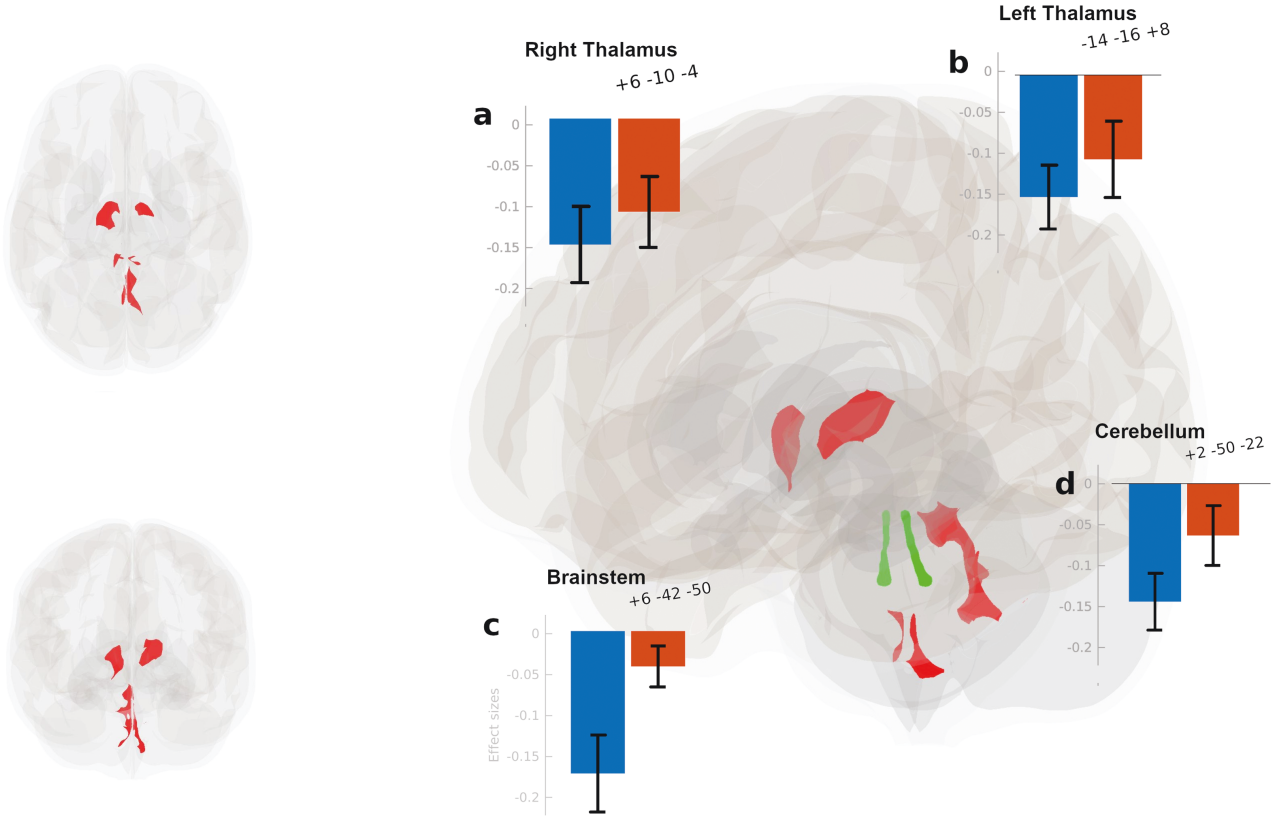


**Figure 1.** The LC functional connectivity mixed design ANOVA comparing sessions (ketamine (blue boxplots), placebo (red boxplots) and time (baseline, administration) reveals changes to bilateral thalamus (left thalamus cluster size=279, FDR $P<.001$; right thalamus cluster size=163, FDR $P<.001$); a, b), brainstem regions (cluster size=122, FDR $P<.001$; c) and cerebellum (d, cluster size=279, FDR $P<.001$).

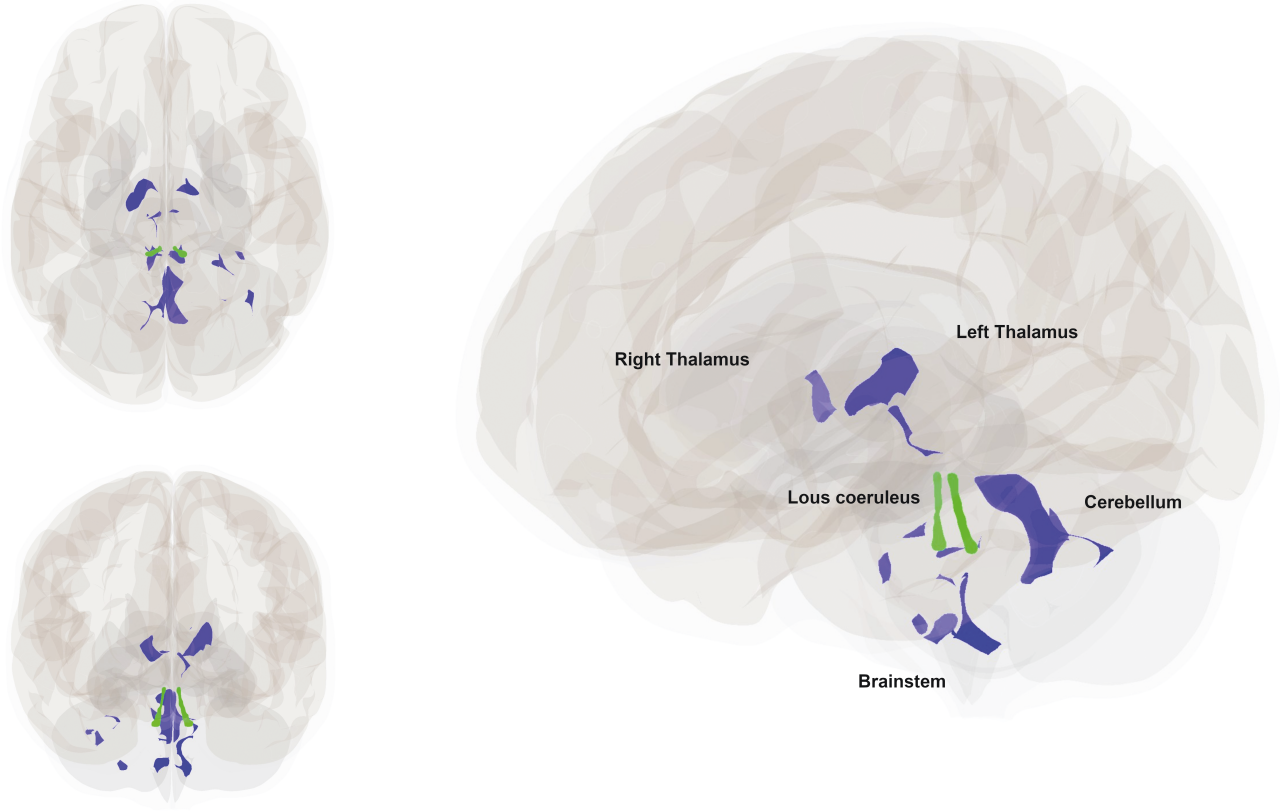


**Figure 2.** LC functional connectivity paired t test reveals decoupling of the LC to the bilateral thalamus, brainstem regions and cerebellum during the ketamine administration condition compared with the corresponding baseline session (left thalamus cluster size = 260, FDR $P < .001$; right thalamus cluster size = 84, FDR $P < .001$; cerebellum (cluster size = 305, $P < .001$); brainstem regions (cluster size = 134, $P < .001$).

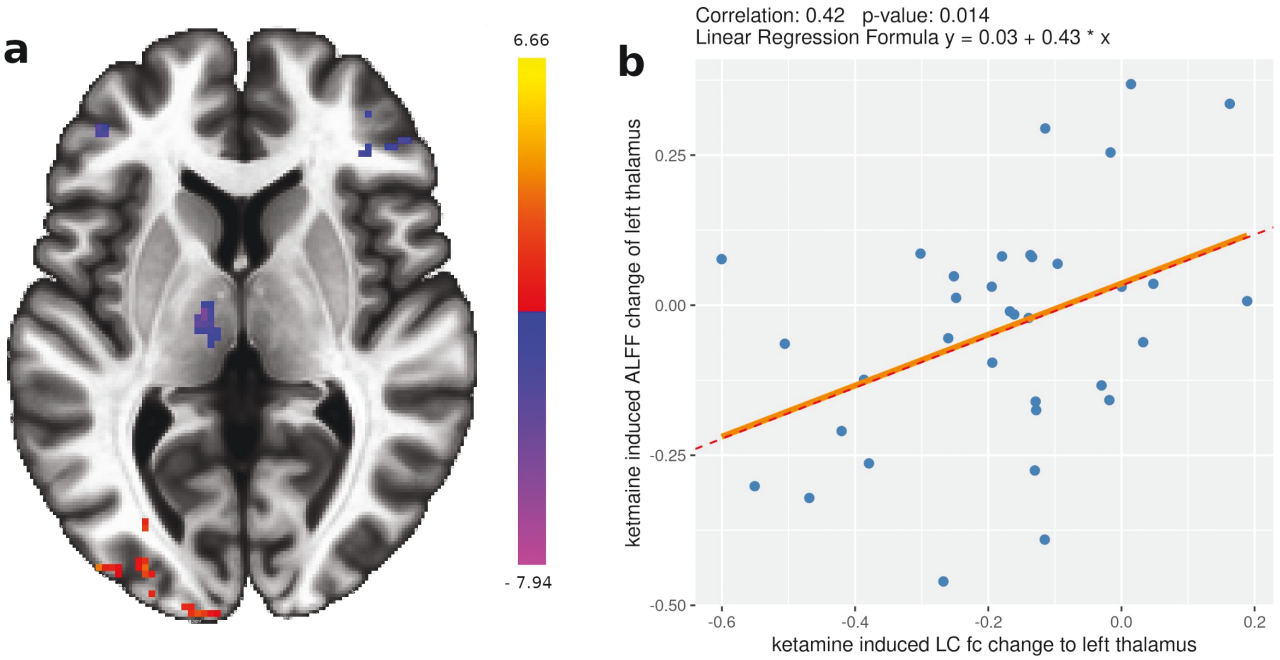


**Figure 3.** (a) A paired t test reveals a significant decrease of ALFF within the left thalamus (cluster size = 55, FDR $P < .001$) in the ketamine infusion condition compared with the placebo infusion. (b) The extracted beta weights from the significant decreased LC fc to the left thalamus are correlated to the ALFF decrease of the left thalamic cluster (mixed design ANOVA; Pearson correlation $P = .014$), with lower LC-thalamic connectivity (measured by fc) relating to lower thalamic activity (measured by ALFF).

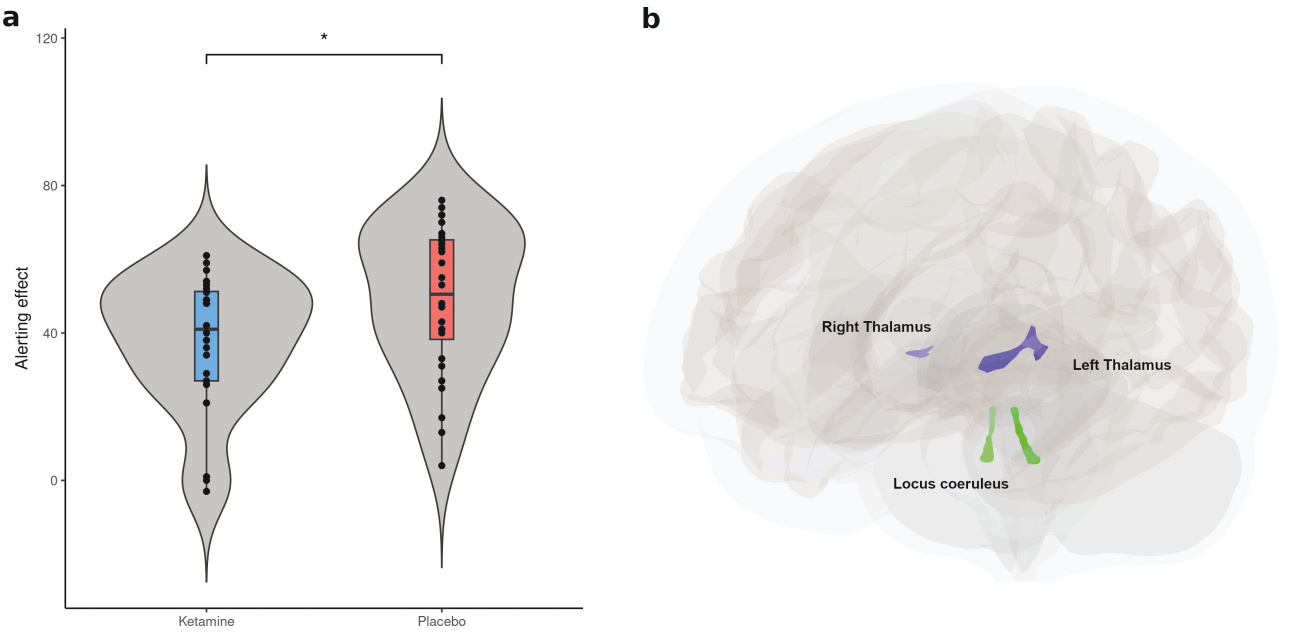


**Figure 4.** (a) In the attention network task we found a significantly diminished alerting effect in the ketamine condition compared with the placebo condition ($P = .04$, $F = 63.003$, mean difference: −10.817s). Neither orienting nor executive control showed any significant effects. (b) Whole brain LC functional connectivity ANCOVA shows correlations of alertness in the placebo administration condition within the bilateral thalamus (left thalamus cluster size = 134, FDR $P < .001$; right thalamus cluster size = 60, FDR $P = .009$).

thalamus and cerebellum when comparing ketamine infusion and ketamine baseline conditions.

We extracted the beta weights from the significantly decreased LC fc to the left thalamus (mixed-design ANOVA) and correlated the weights to the corresponding significant left thalamic ALFF decrease weights (mixed-design ANOVA). We found that the ALFF decrease in the left thalamus was correlated to the decrease of LC fc to the left thalamus (Pearson correlation $P = .014$, $r = 0.417$), with lower LC-thalamic connectivity (measured by fc) relating to the lower thalamic activity (measured by ALFF; Figure 3B).

### *Relation of Attention Network Task Results to LC Network Connectivity*

We contrasted the change of alertness in the ANT in the placebo condition 4 hours after administration with the result 24 hours after placebo ($P = .002$) to gain a marker for alertness change compared with a non-infusion condition (higher alertness in the 4 hours after placebo condition compared with 24 hours after placebo; no difference was present contrasting the conditions 4 hours after ketamine infusion with 24 hours after ketamine). We found a significant correlation between the maker with the LC fc in the placebo infusion condition (left thalamus cluster size = 134, FDR $P < .001$; right thalamus cluster size = 60, FDR $P = .009$; Figure 4B) and with the change of the LC fc comparing ketamine and placebo infusion conditions on whole-brain level (left thalamus cluster size = 41, FDR $P = .04$): both contrasts showed a significant negative correlation to LC-thalamic fc on the whole-brain level.

## DISCUSSION

As first outcome, we could replicate and thus corroborate our former result of a ketamine-related post-infusion decrease of fc from the LC to the thalamus in 7 T fMRI during ketamine infusion (Liebe et al., 2018) and extend it to S-ketamine application. Furthermore, we extended our results by investigating ALFF and revealed a significant reduction of activity in the regions we also found disconnected from the LC due to ketamine—cerebellum and thalamus. This activity decrease in the thalamus was also related to the connectivity decrease with the LC. Furthermore, our results on alertness effects of ketamine during the attention network task show a decreased alertness shortly after ketamine was administrated and no effect of ketamine was found on orienting and conflict properties of attention. Finally, we show the relationship of the behavioral results of alertness reduction by ketamine to the LC-thalamic network connectivity decrease.

The replication of our former fMRI result of an LC-thalamic disconnection during ketamine administration further strengthens our view of an important influence of ketamine on the central sympathetic pathway. The importance of the LC-thalamic connectivity in humans was shown before in fMRI (Zhang et al., 2015; Liebe et al., 2020). The LC-thalamic connection can be interpreted in the context of findings in animal research (Devilbiss and Waterhouse, 2011; Devilbiss et al., 2012), where it was shown that the LC modulates the ascending somatosensory signaling pathway within the thalamus, and translated to the alertness function of the human brain (Périn et al., 2010; Petersen and Posner, 2012).

The activity decrease in the thalamus following ketamine, which was related to the LC-thalamic connectivity decrease, is in line with findings of other fMRI studies (Höflich et al., 2015; Abram et al., 2022). We speculate that the LC primarily influences the activity decrease of the thalamus and cerebellum by its direct NE fiber connections to the thalamus (Liebe et al., 2022), modulating the sensory signal pathway within the thalamus (Périn et al., 2010; Petersen and Posner, 2012) followed by reduced alertness (Aston-Jones and Cohen, 2005a). The relation of the LC's individual connectivity level to the degree of thalamic/cerebellar disconnection further strengthens the view of the central relevance of the LC in the overall mechanism of ketamine action.

Finally, we could reveal the proposed reduction of the alerting effect by ketamine on a behavioral basis in the ANT. We found the effect to be specific since no influence on other items of the attentional networks was present, and we could relate this behavioral effect to the central ketamine action. The ANT measures phasic alertness, which is differentiated from vigilance as it describes the response speed to behaviorally relevant objects and not long-term vigilance (Petersen and Posner, 2012). It is important for rapid change in attention following a brief event and is the basis for spatial orienting and selective attention (Petersen and Posner, 2012; Vazey et al., 2018). It is highly relevant in providing behavioral flexibility (Grella et al., 2019).

The phasic alertness score of the ANT is linked to LC phasic bursts (Corbetta et al., 2008a; Vazey et al., 2018), a specific mode of LC activity: the LC has different firing modes closely linked to attentional control (McCormick et al., 1991; Aston-Jones, 2005; Aston-Jones and Cohen, 2005b), which influence the thalamus (Devilbiss and Waterhouse, 2011) with respect to alertness (Petersen and Posner, 2012). A reduced general tonic LC activity would lead to reduced phasic bursts and reduced spillover of NE into the thalamus (Berridge and Waterhouse, 2003; Corbetta et al., 2008; Devilbiss and Waterhouse, 2011; Devilbiss et al., 2012), which we interpret to measure as LC-thalamic connectivity and thalamic activity decrease at rest during ketamine infusion in fMRI and as reduced alertness 4 hours after infusion in the ANT. Importantly, we could show the relationship of the ketamine-induced central sympathetic and the behavioral alertness changes. Thus, in a translational context, the response speed to behaviorally relevant objects could potentially be brought to a normal level by ketamine in patient groups with elevated arousal to specific information, as found in anxiety (Tully et al., 2022).

The results presented here can be linked to the antidepressant properties of subanesthetic ketamine and the involvement of the LC in depression. The well-known antidepressant effects of ketamine start around 4 hours after infusion (Murrough et al., 2013), the same timepoint for which we found the alertness reduction effect in the ANT, with a relation to the LC-thalamic connectivity decrease during ketamine infusion. Chronic stress hyperactivates the LC (Pavcovich et al., 1990), followed by an altered NE axis (Ordway et al., 1994; Klimek et al., 1997), which can induce pathological whole-brain changes as found in depression (Richter-Levin and Xu, 2018). Accordingly, in the manifestation of behavior, hyperalertness, which is tightly linked to a hyperactivated LC, was found in MDD and discussed in a model of pathological hyperstable vigilance regulation in depression (Kayumov et al., 2000; Hegerl et al., 2012; Olbrich et al., 2012; Dillon et al., 2015). Antidepressant medication was overall found to reduce LC activity and vigilance to restore appropriate functionality (West et al., 2009; Chandley and Ordway, 2012; Zacharias et al., 2020).

In line with this view, ketamine-induced reduction of alertness due to reduction of phasic responses (as indicated by the LC thalamic connectivity decrease and ANT results presented here) could restore physiological LC activity and provide a window that breaks through a maladaptive increased hypervigilant focus on negative thoughts (as shown as an outcome of ketamine treatment; Lehmann et al., 2016; Hasler et al., 2020) and in electroconvulsive therapy, which might similarly depend on the modulation of thalamic function (Leaver et al., 2016; Singh and Kar, 2017; Takamiya et al., 2019). The reduction of a hypervigilant focus could also open a gate for the treatment of depression (Scheepens et al., 2022). Our finding is further underpinned by a combined EEG/fMRI study revealing vigilance reduction by ketamine administration indicated by EEG (Zacharias et al., 2020).

Gender-specific differences in stress response and in the manifestation of hyperarousal were discussed previously in the context of depression, which could be linked to the LC NE system (Bangasser et al., 2018). Although in the circumscribed LC-thalamic network affected here we did not find gender differences based on our previous results (Liebe et al., 2018; supplementary Table 3; supplementary Figure 5), supported by a study about baseline LC fc (Zhang et al., 2015), future studies, especially involving patients, should assess LC fc in both genders. Such studies could investigate how gender-related differences in higher-order networks influence the LC and drive its involvement in depression.

### Limitations

There are some limitations to this study. First, the sample size was modest and consisted of male participants only. Nevertheless, we could replicate the results of our previous study of an LC-thalamic disconnection by ketamine, which involved both sexes (Liebe et al., 2018), in this new sample of healthy participants. Additionally, we re-analyzed data from our previous study (Liebe et al., 2018) with respect to gender and revealed that no gender effects influence the LC-thalamic fc decrease driven by ketamine. Even when splitting the groups for gender, the significant effect of LC-thalamic fc decrease was present in both female and male groups independently, and the extent of LC thalamic decrease did not differ between female and male participants (supplementary Table 3; supplementary Figure 5). Furthermore, the baseline LC-thalamic fc showed no gender difference (Liebe et al., 2018).

The LC is a particularly small structure and is difficult to localize in the brainstem, but we used ultra-high field MRI to gain a high signal-to-noise ratio of our MRI sequences (Neuner et al., 2022; Sbaihat et al., 2022). Furthermore, we could verify our main results by mapping the individual location of the LC and extracting the BOLD signal at the exact individual location of the nucleus (as shown in Liebe et al., 2020).

Finally, our idea of translating our results to the antidepressant effects should be underpinned by an investigation of LC-related connectivity changes by ketamine in a patient group.

## CONCLUSIONS

We could replicate our former results of racemic ketamine-induced LC-thalamic connectivity decrease also in this study conducting fMRI during S-ketamine infusion. We tested our hypothesis of an alertness reduction as a behavioral correlate of the LC-thalamic fc decrease with the ANT task and showed the proposed constrained influence of ketamine on the alerting effect. Our results shed new light on the central mechanisms of action of subanesthetic ketamine and will forward an explanation of the rapid antidepressant effects of the drug.

## Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

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## Interest Statement

M.W. is a member of the following advisory boards and has given presentations to the following companies: Bayer AG, Germany; Boehringer Ingelheim, Germany; and Biologische Heilmittel Heel GmbH, Germany. Unrelated to this investigation, M.W. has conducted studies with institutional research support from HEEL and Janssen Pharmaceutical Research for a clinical trial (IIT) on ketamine in patients with MDD. From the companies mentioned above, M.W. did not receive any financial compensation. All other authors report no biomedical financial interests or other potential conflicts of interest that are relevant to the content of this article.

## Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

## Author Contributions

Thomas Liebe (Conceptualization [Lead], Data curation [Lead], Formal analysis [Lead], Investigation [Equal], Methodology [Lead], Visualization [Lead], Writing—original draft [Lead], Writing—review and editing [Lead]), Lena Vera Danyeli (Data curation [Equal], Funding acquisition [Equal], Investigation [Equal], Project administration [Equal], Validation [Equal], Writing—original draft [Equal], Writing—review and editing [Equal]), Zümrüt Duygu Sen (Data curation [Equal], Investigation [Equal], Methodology [Equal], Project administration [Equal], Validation [Equal], Writing—original draft [Equal], Writing—review and editing [Equal]), Meng Li (Data curation [Equal], Funding acquisition [Equal], Investigation [Equal], Methodology [Equal], Project administration [Equal], Software [Equal], Validation [Equal], Writing—original draft [Equal], Writing—review and editing [Equal]), Jörn Kaufmann (Data curation [Equal], Methodology [Equal], Software [Equal], Validation [Equal], Visualization [Equal], Writing—original draft [Equal], Writing—review and editing [Equal]), and Martin Walter (Conceptualization [Equal], Formal analysis [Equal], Funding acquisition [Lead], Investigation [Equal], Methodology [Equal], Project administration [Lead], Supervision [Lead], Validation [Lead], Writing—review and editing [Equal]).

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