Effect of Subanesthetic Ketamine on Intrinsic Functional Brain Connectivity

A Placebo-controlled Functional Magnetic Resonance Imaging Study in Healthy Male Volunteers

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ABSTRACT

Background: The influence of psychoactive drugs on the central nervous system has been investigated with positron emission tomography and task-related functional magnetic resonance imaging. However, it is not known how these drugs affect the intrinsic large-scale interactions of the brain (resting-state functional magnetic resonance imaging connectivity). In this study, the effect of low-dose S(+)-ketamine on intrinsic brain connectivity was investigated.

Methods: Twelve healthy, male volunteers received a 2-h intravenous S(+)-ketamine infusion (first hour 20 mg/70 kg, second hour 40 mg/70 kg). Before, during, and after S(+)-ketamine administration, resting-state brain connectivity was measured. In addition, heat pain tests were performed between imaging sessions to determine ketamine-induced analgesia. A mixed-effects general linear model was used to determine drug and pain effects on resting-state brain connectivity.

Results: Ketamine increased the connectivity most importantly in the cerebellum and visual cortex in relation to the

What We Already Know about This Topic

- Positron emission tomography and task-related magnetic resonance imaging have been used to investigate the effect of psychoactive drugs on the central nervous system

What This Article Tells Us That Is New

- Using resting state functional magnetic resonance imaging in volunteers, the authors demonstrated changes in brain connectivity in areas involved in the effects of low-dose ketamine, including pain modulation

medial visual network. A decrease in connectivity was observed in the auditory and somatosensory network in relation to regions responsible for pain sensing and the affective processing of pain, which included the amygdala, insula, and anterior cingulate cortex. Connectivity variations related to fluctuations in pain scores were observed in the anterior cingulate cortex, insula, orbitofrontal cortex, and the brainstem, regions involved in descending inhibition of pain.

Conclusions: Changes in connectivity were observed in the areas that explain ketamine's pharmacodynamic profile with respect to analgesia and psychedelic and other side effects. In addition, pain and ketamine changed brain connectivity in areas involved in endogenous pain modulation.

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THE noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine has been used since the early 1960s as an anesthetic agent. It is the most potent NMDA receptor antagonist currently clinically available. At subanesthetic concentrations, ketamine is a potent analgesic and is used in the treatment of acute and chronic pain.[1] Ketamine use is hampered by central side effects, including serious psychedelic effects.[1] The influence of ketamine on central sites (*i.e.*, within the central nervous system) has been investigated extensively with various imaging techniques, including positron emission tomography and task-related functional magnetic resonance imaging (fMRI).[2–8] Several brain regions display ketamine-dependent activity changes. For example, during a short-term ketamine infusion, frontal and temporal brain regions are affected (these regions may be

involved in the psychedelic effects of ketamine), as are several regions involved in pain processing, such as the anterior cingulate cortex, the insular cortex, and the thalamus.[3,4]

Positron emission tomography and task-related fMRI studies have several disadvantages, including limited temporal and spatial resolution, radiation dose restrictions in positron emission tomography, and the need to postulate an *a priori* hypothesis about the site of drug action for task-related fMRI. Furthermore, large-scale network interactions of brain regions on which central nervous system functions depend cannot be adequately captured with these techniques.[9] A new approach in central nervous system drug research is resting-state fMRI (RS-fMRI), which measures these intrinsic interactions at baseline activity of the brain (*i.e.*, not task-related).[10] This technique shows that spontaneous fluctuations in RS-fMRI signal form coherent networks (resting-state networks [RSNs]) that represent connections between brain areas of similar functionality.[11,12] Studying drug effects on brain connectivity using RS-fMRI provides direct evidence of drug-induced changes in brain dynamics and consequently on brain function.

In the current study, we test the effect of low-dose ketamine *versus* placebo in healthy volunteers using a crossover and randomized design. The effect of two doses of ketamine (or placebo) on RS-fMRI and on pain scores during noxious cutaneous heat stimulation is assessed. By incorporation of pain scores in the statistical model, we will be informed on the influence of both ketamine (*i.e.*, intended effect and side effects) and pain processing on brain connectivity. We hypothesize that RS-fMRI is able to detect ketamine-induced alterations in large-scale network patterns and will identify changes in connectivity for (1) brain areas involved in ketamine's pharmacodynamic profile with respect to intended (analgesia) and side effects (most importantly psychedelic effects) and (2) areas involved in pain processing.

Materials and Methods

Subjects

Twelve healthy male volunteers (age 19–36 yr; body mass index: 21–27 kg/m²) were recruited to participate in the study after approval by the local ethics committee (Commissie Medische Ethiek, Leiden, The Netherlands). Oral and written informed consent was obtained from all participants, and before participation all subjects received a physical examination. Exclusion criteria for participation were: age younger than 18 yr or older than 45 yr; a medical disease, such as renal, liver, cardiac, vascular (including hypertension), or infectious disease; presence or history of a neurologic or psychiatric disease (*e.g.*, increased cranial pressure, epilepsy, psychosis); glaucoma; obesity (body mass index greater than 30 kg/m²); history of chronic alcohol or drug abuse; use of central-acting medication; presence of metal

†† www.trialregister.nl. Accessed April 4, 2012.

implants (*e.g.*, pacemaker, hip/knee prosthesis, cochlear implants, vessel clips); and claustrophobia.

Study Design

The effect of ketamine on resting-state brain function was assessed using a single-blind, randomized, placebo-controlled crossover study design with two occasions (at least 1 week between sessions). Subjects received S(+)-ketamine (Ketanest-S, Pfizer BV, Capelle aan de Ijssel, The Netherlands) on one occasion and placebo (NaCl 0.9%) on the other. No details regarding treatment effects were given apart from the possibility of experiencing "drug high" during treatment.

Upon arrival, subjects were given two intravenous lines (in separate arms), one for drug infusion and one for blood sampling. A baseline blood sample; baseline measurements for heat pain, nausea, vomiting, and psychedelic effects; and a baseline anatomical (T1) and RS-fMRI scan were obtained. Next, drug infusion with either S(+)-ketamine or placebo was started at t = 0, at a low dose (20 mg/70 kg/h) for 1 h, followed by a high dose (40 mg/70 kg/h) for another hour. During infusion, heat pain rating, nausea, vomiting, and psychedelic effects were scored at 15-min intervals using visual analog scales as described below (sections Subjective Effects and Pain Assessment). RS-fMRI scans were obtained during the last 10 min of the low-dose and high-dose infusion period. After 2 h, drug administration was terminated. Heat pain, nausea, vomiting and psychedelic effects were monitored at regular intervals for another 1.5 h, and two more RS-fMRI scans were performed during the drug elimination phase. The study was registered in the Dutch Trial Register under number NTR2717.††

Blood Sampling and S(+)-ketamine and S(+)-norketamine Analysis

Venous blood was collected from a venous line inserted into the arm of the subject. Blood samples were obtained at fixed time points (t = 0, 15, 30, 60, 75, 90, 120, 130, 160, and 200 min) after the start of drug administration. Blood was centrifuged (3,500 rpm for 10 min) within 15 min after collection to separate the plasma. Plasma samples were stored at −25°C until analysis. For the construction of S(+)-ketamine and S(+)-norketamine calibration lines, solid substances were obtained from Parke-Davis (Dallas, TX) and Tocris (St. Louis, MO), respectively. S(+)-ketamine and S(+)-norketamine concentrations were determined by high-performance liquid chromatography on a Gemini C18 column (Phenomenex, Utrecht, The Netherlands) at 40°C. The detection of both analytes in the eluent was performed at 195 nm with a photodiode-array detector (PDA 100; Dionex, Amsterdam, The Netherlands). The lower limit of quantification was set at 10 ng/ml for both drugs.

Subjective Effects

Psychedelic effects were scored at fixed time points (t = 0, 13, 28, 58, 73, 88, 118, 128, 158, and 198 min) after the

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start of drug administration. Psychedelic effects were monitored using visual analog scales ranging from 0 (no effect) to 10 cm (maximum effect) of the Bowdle questionnaire.[13] Three factors of psychedelic effects can be derived from this questionnaire: drug high, internal perception, and external perception.[14] Internal perception reflects inner feelings that do not correspond with the reality and is derived from questions regarding the hearing of unrealistic voices or sounds and having unrealistic thoughts and paranoid or anxious feelings. The external perception indicates a misperception of an external stimulus or change in the awareness of the subject's surroundings and is derived from questions regarding the change of body parts, the change of surroundings, the altered passing of time, the difficulty of controlling thoughts, and the change in color and sound intensity.

Pain Assessment

Heat pain stimuli were applied between imaging sessions. Heat pain was induced using the Pathway Neurosensory Analyzer (Medoc Ltd., Ramat Yishai, Israel) at fixed time points after the start of drug infusion (t = 0, 17, 32, 62, 77, 92, 122, 132, 162, and 202 min). A fixed location on the skin of the volar side of the nondominant arm was stimulated with a 3- $\times$ 3-cm thermal probe. To quantify the pain intensity of the noxious stimulus, subjects scored the perceived pain using a computer-connected slider on an electrical potentiometer that ranged from 0 (no pain) to 100 mm (worst pain imaginable). This allows electronic monitoring of the visual analog scale during heat pain stimulation. To induce heat pain, baseline temperature was set at 32°C. Next, the temperature of the probe gradually increased (0.5°C/s) toward a preset peak temperature, after which the temperature rapidly (6°C/s) returned to baseline. A peak temperature causing a pain score between 60 and 70 mm was used during the study for evaluation of the analgesic effect of ketamine. Individual peak temperatures were determined on every experiment the day before the start of drug administration.

RS-fMRI

Neuroimaging was performed on a 3-Tesla Achieva Scanner (Philips Medical System, Best, The Netherlands) at fixed time points (t = −30, 45, 105, 140, and 170 min), where drug infusion was started at time point t = 0. The neuroimaging protocol included a high-resolution (2-mm isotropic), T1-weighted scan (repetition time = 9.7 ms, echo time = 4.6 ms, flip angle = 8 degrees, 256 $\times$ 256 $\times$ 140, isotropic resolution 1 mm, 4 min) and 5 RS-fMRI series (each 220 T2*-weighted, whole-brain volume obtained with a gradient echo planar with repetition time = 2,180 ms, echo time = 30 ms, flip angle = 80 degrees; 64 $\times$ 64 $\times$ 38, isotropic resolution 3.44 mm, 8 min). Because the subjects were taken out of the scanner between scans, and because only one anatomical T1-weighted image was acquired, each RS-fMRI was accompanied with a high-resolution, T2*-weighted echo planar (~30 s) to facilitate registering the RS-fMRI data to

the anatomical image. During scanning, heart rate was monitored with a magnetic resonance imaging-compatible pulse oximeter (INVIVO MRI 4500; Siemens Healthcare, Erlangen, Germany). Respiratory rate was recorded and registered using a flexible pressure belt (Philips Medical System, Best, The Netherlands).

Data and Statistical Analysis

To assess the effect of ketamine *versus* placebo, a repeated measures analysis of variance was performed on pain scores, drug high effect, internal perception values, and external perception with *post hoc t*-testing with Bonferroni correction for multiple comparison. *P* values <0.05 were considered significant. Data are presented as mean ± SEM and 95% confidence interval (CI) unless otherwise stated. Statistical analysis was performed in SigmaPlot version 12 for Windows (Systat Software Inc., Chicago, IL).

Each of the (12 $\times$ 5 $\times$ 2) RS-fMRI data series was preprocessed with motion correction, brain extraction, Gaussian smoothing, mean-based intensity normalization, and high-pass temporal filtering with default software parameters. Then RS-fMRI data were normalized to a standard space by first registering the middle RS-fMRI volume to the high-resolution, T2*-weighted image, which was registered to the subject's anatomical T1-weighted image, which was registered to the MNI152 standard template. These registration parameters were combined to obtain the parameters to put the RS-fMRI data in standard space. This produced standardized RS-fMRI data sets that were further analyzed to estimate functional brain connectivity. We applied a technique that we have used previously in a placebo-controlled, crossover study involving alcohol and morphine.[10] In that study, we have shown that the proposed technique reveals drug-specific and regional changes in functional brain connectivity. This method parcellates the brain into eight networks of interest (NOIs) that are consistently present in model-free analysis of the RS-fMRI data.[15] These NOIs represent 80% of the total brain volume and include the medial (NOI1) and lateral (NOI2) visual network, the auditory and somatosensory network (NOI3), the sensorimotor network (NOI4), the default mode network (NOI5), the executive salience network (NOI6), the visual-spatial network (NOI7), and the working memory network (NOI8). We then defined functional connectivity as a measure of the correspondence of the RS-fMRI fluctuations in each brain voxel in relation to characteristic fluctuations of the RS-fMRI signal in each NOI. This correspondence is expressed in terms of regional z-scores of fitting an average NOI RS-fMRI fluctuation. We refer to these statistical maps as RSN maps.

To examine the effect of ketamine over time on functional connectivity, voxel-wise statistics were performed on RSN maps. The analysis involved a mixed-effects general linear model with subject as random and time and drug as fixed within-subject variables. Because subjects received a pain stimulus between each RS-fMRI session, the corre-
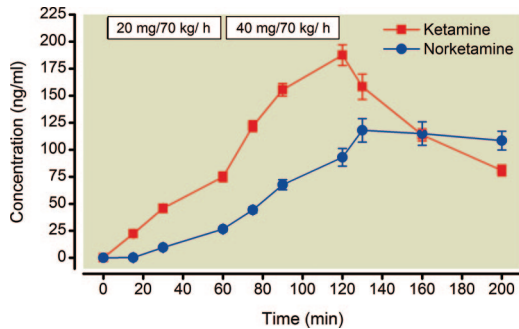
Fig. 1. The blood ketamine (*red squares*) and norketamine (*blue circles*) concentrations in nanograms per milliliter. Ketamine was administered at 20 mg/70 kg in the first hour of infusion and at 40 mg/70 kg during the second hour. A rapid increase in blood ketamine concentration (peak 187.5 ± 9.5 ng/ml) and a slow increase in blood norketamine concentration (peak 117.9 ± 10.9 ng/ml) was observed.

sponding pain relief scores (obtained 10 min before the RS-fMRI scan) were included as a regressor in the general linear model. This model accounts for possible confounding effects of pain *per se* on functional connectivity changes caused by ketamine. Voxel-wise statistical test runs the risk of false-positive results because of the problem of multiple comparisons. However, true neuronal effects are likely to happen in adjacent voxels. For this reason, correction for multiple comparisons is conducted with a method that minimizes the chances of type I errors by examining both the magnitude and size of a cluster with effects in the same magnitude range.[16] Within this criterion, we set the corrected statistical significance to $P < 0.05$ after threshold-free cluster estimation. In the *post hoc* analysis, the highest value in the significant cluster (determined as explained) was chosen to illustrate the connectivity change (z-score) over time for areas of interest. In all stages, the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL 4.1, Oxford, United Kingdom) was used.[17]‡‡

Results

Ketamine and Norketamine Concentrations

Plasma ketamine and norketamine concentrations are shown in figure 1. Peak ketamine and norketamine concentrations at the end of the first infusion hour were 74.9 ± 4.5 ng/ml and 26.7 ± 2.3 ng/ml, respectively. At the end of the second hour, peak ketamine and norketamine concentrations were, respectively, 187.5 ± 9.5 ng/ml and 93.0 ± 8.2 ng/ml. The relatively low variability in plasma concentrations indicates that the RS-fMRI data were obtained under stable ketamine and norketamine concentration conditions (between subjects).

Subjective Effects

Psychedelic effects were observed in all subjects, and scores are shown in figure 2. Mean drug high scores at 60
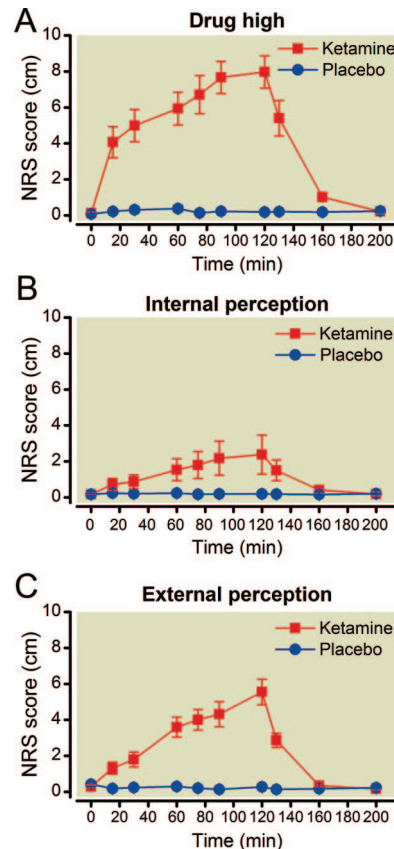
‡‡ www.fMRIb.ox.ac.uk/fsl. Accessed November 1, 2011.

Fig. 2. Psychedelic effects observed during ketamine (*red squares*) and placebo (*blue circles*) infusion presented as drug high (*A*), internal perception (*B*), which reflects inner feelings that do not correspond with reality, and external perception (*C*), which reflects a misperception of an external stimulus or change in the awareness of the surroundings. Concentration-dependent psychedelic effects were observed. NRS = numerical rating scale.

min after the start of drug infusion were 5.9 ± 0.9 cm (−0.5 to 12.4 cm) for ketamine and 0.4 ± 0.2 cm (−0.8 to 1.6 cm) for placebo ($P < 0.001$). At 120 min after the start of drug infusion, mean drug high scores were 8.0 ± 0.9 cm (1.8–14.1 cm) for ketamine compared with 0.2 ± 0.08 cm (−0.4 to 0.8 cm) for placebo ($P = 0.003$). Visual analog scale scores for the outcome parameter internal perception for ketamine and placebo were, respectively, 1.6 ± 0.4 cm (−1.0 to 4.3 cm) *versus* 0.2 ± 0.1 cm (−0.5 to 0.9 cm; $P < 0.001$) at time point t = 60 min and 2.3 ± 0.6 cm (−1.1 to 6.0 cm) *versus* 0.2 ± 0.07 cm (−0.3 to 0.7 cm; $P = 0.003$) at time point t = 120 min. Mean external perception visual analog scale scores were 3.6 ± 0.8 cm (−1.3 to 8.5 cm) for ketamine and 0.3 ± 0.1 cm (−0.6 to 1.2 cm; $P < 0.001$) for placebo at time point t = 60 min and 5.6 ± 1.0 cm (−1.0 to 12.1 cm) for ketamine and 0.3 ± 0.1 cm (−0.4 to 1.0 cm) for placebo at time point t = 120 min ($P < 0.001$). Psychedelic effects rapidly returned to baseline (within 30 min) after termination of the drug infusion.
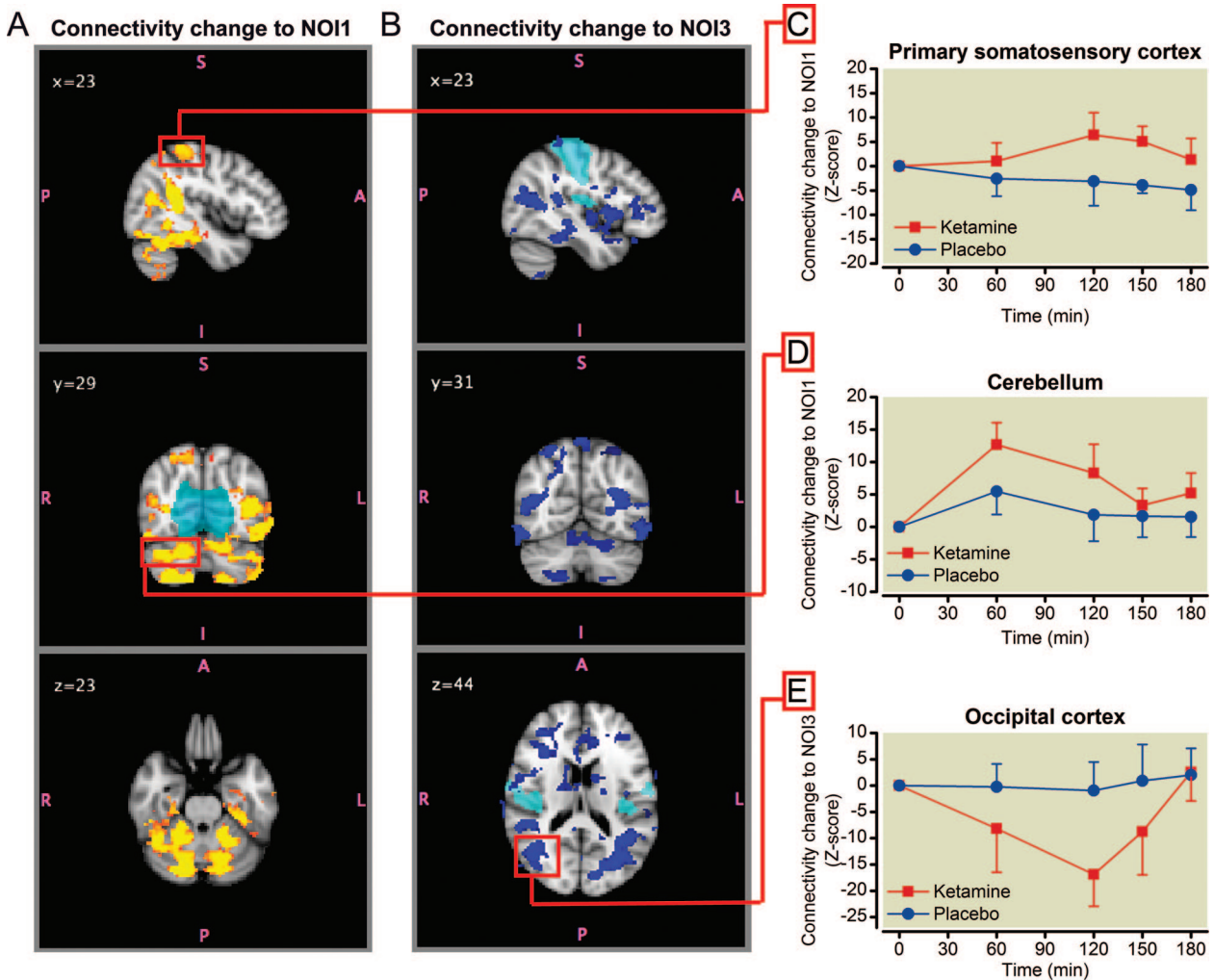
Fig. 3. Statistical maps of the ketamine-induced increase in resting state network (RSN) connectivity (*yellow*) in relation to network of interest 1 (NOI1) (*light blue*) (*A*) and decrease in RSN connectivity (*dark blue*) in relation to NOI3 (*light blue*; cluster *P* value <0.05) (*B*). The ketamine effect (*red squares*) on connectivity over time is shown for the primary somatosensory cortex (*C*), the cerebellum (*D*), and the occipital cortex (*E*). A = anterior; I = inferior; L = left; P = posterior; R = right; S = superior.

RSN Connectivity on Drug Effect

Resting-state network connectivity changes caused by ketamine administration were observed in relation to NOI1 (medial visual network) and NOI3 (auditory and somatosensory network). Figure 3A shows the statistical map (threshold-free cluster enhancement corrected *P* value <0.05 in yellow) of the areas where the connectivity in relation to NOI1 was increased. Affected areas include the frontal lobe, thalamus, primary and secondary somatosensory cortex, occipital cortex, optic radiation, cerebellum, and the supramarginal gyrus. The statistical map (threshold-free cluster enhancement corrected *P* value <0.05 in dark blue) of the areas involved in the cortical and subcortical connectivity decreases in relation to NOI3 is shown in figure 3B. The connectivity changes in the cortex were observed in many areas, with the largest effects in the occipital cortex, the anterior and posterior cingulate cortex, orbital frontal cortex, and insular cortex. The connectivity changes in subcortical areas were observed in the basal ganglia and limbic areas. Details regarding cluster

size, t-value of the cluster peak, and cluster peak location of the affected areas are provided in table 1. The ketamine effects over time on the primary somatosensory cortex and the cerebellum in relation to NOI1 are shown in figures 3C and D, respectively; the effect over time for the occipital cortex in relation to NOI3 is shown in figure 3E.

RSN Connectivity on Pain Processing

Mean baseline pain scores are shown in figure 4A and were 63.9 ± 4.9 mm (30.6–97.2 mm) for the ketamine study day *versus* 62.3 ± 4.9 mm (28.8–95.7 mm) for the placebo study day (*P* = 0.621). Corresponding testing temperatures were 48.7° ± 0.6°C (44.9°–52.5°C) and 48.4° ± 0.7°C (43.7°–53.0°C) for ketamine and placebo, respectively (*P* = 0.296). At the end of the first infusion hour (t = 60 min), 20.1% ketamine-induced pain relief was observed with mean pain scores of 51.0 ± 7.8 mm (9.6–92.4 mm) for ketamine compared with 69.2 ± 5.6 mm (31.2–107.2 mm; *P* = 0.027) for placebo. Mean pain scores for ketamine and placebo at t =

Table 1. Ketamine Effect on Resting-state Network Connectivity (Cluster *P* Value <0.05)

	Location	Cluster Size (Voxels)	*t* Value	x	y	z
NOI1: medial visual network	L thalamus	11	3.30	45	53	37
	R frontal lobe	40	3.61	20	89	29
	R cerebellum *Cluster also includes	21,098*	4.17	36	22	14
	R primary somatosensory cortex	—	4.42	23	47	64
Calcarine, inferior precuneus, and primary visual cortex. Relays visual input through thalamus to primary visual area	L secondary somatosensory cortex	—	4.34	75	57	45
	L occipital cortex	—	3.82	64	29	41
	L optic radiation	—	4.40	62	41	46
	R supramarginal gyrus	—	5.28	19	42	42
NOI3: auditory and somatosensory network	R hippocampus	5	3.18	30	54	25
	L precuneus cortex	12	2.85	48	40	67
	R primary motor cortex	16	4.02	41	50	71
	L orbitofrontal cortex	17	3.52	56	77	23
	L premotor cortex	126	4.27	55	53	72
	R middle temporal gyrus *Cluster also includes	28,971*	5.64	16	51	33
	R thalamus	—	2.76	43	51	40
	L cerebellum	—	3.75	48	27	28
	L primary auditory cortex	—	2.68	70	52	38
Superior temporal cortex, insula, operculum, dorsocaudal anterior cingulate cortex, somatosensory cortices, and bilateral thalamus	L caudate nucleus	—	3.69	51	64	45
	R caudate nucleus	—	4.45	42	69	39
	L anterior cingulate cortex	—	3.76	46	61	52
	L lateral occipital cortex	—	4.67	72	31	27
	L posterior cingulate cortex	—	4.28	50	52	53
	L amygdala	—	3.19	60	63	26
	L superior longitudinal fasciculus	—	4.48	62	48	53
	R insular cortex	—	4.36	24	67	35
	R occipital cortex	—	3.78	17	30	26

Voxel dimension is 2 mm × 2 mm × 2 mm (voxel volume 0.008 ml).

L = left; NOI1 = network of interest 1; NOI2 = network of interest 2; R = right.

120 min (end of second infusion hour) were 33.9 ± 6.6 mm (−7.2 to 75.0 mm) and 64.7 ± 6.4 mm (21.5–107.8 mm; $P = 0.001$), respectively, with a pain relief of 46.9% by ketamine. During the drug elimination phase, ketamine pain scores rapidly returned to baseline (in approximately 20 min).

Because we included pain scores as a regressor in the general linear model, connectivity maps were obtained that assist in separating brain areas affected by ketamine from regions involved in the processing of pain. Figure 4B illustrates the statistical map of regions where the amount of connectivity variation is explained by the drug effect (threshold-free cluster enhancement corrected *P* value <0.05 in yellow) and by the fluctuations in pain scores (threshold-free cluster enhancement corrected *P* value <0.05 in green). Using this model, an increase in RSN connectivity explained by pain processing was observed only in relation to NOI1 for the anterior cingulate cortex, insula, orbitofrontal cortex, and the brainstem. No decrease in RSN connectivity was observed for any of the NOIs.

Discussion

In the last decade, neuroimaging studies increasingly focused on the spontaneous RS-fMRI signal to study large-scale brain

interactions within RSNs.[11,12,18] RS-fMRI can be used to evaluate the effect of psychoactive drugs on RSN connectivity.[10,19] In this study, we assessed the effect of low-dose S(+)-ketamine on brain connectivity. The use of low-dose (*i.e.*, subanesthetic) ketamine has increased significantly since 1990, mainly for treatment of chronic neuropathic pain syndromes (and for a few years for the treatment of therapy-resistant depression).[1] Thus, knowledge on the effect of low-dose ketamine on brain areas involved in pain processing is of importance, providing additional insight into the mechanisms of action of this increasingly popular analgesic.

Ketamine acts *via* antagonism of the excitatory glutamatergic NMDA receptor. The NMDA receptor has a high expression in the temporal cortex, hippocampus, basal ganglia, cerebellum, and brainstem, all regions significantly affected by ketamine in our study.[20,21] Ketamine most significantly affected the cerebellum (relative to NOI 1 and 3). The cerebellum has an important role in motor learning and coordination,[22] and we showed previously in rats that ketamine produces motor dysfunction.[23] Because of its connections with nonmotor cortical and subcortical areas, including the limbic system and prefrontal cortex, the cerebellum is
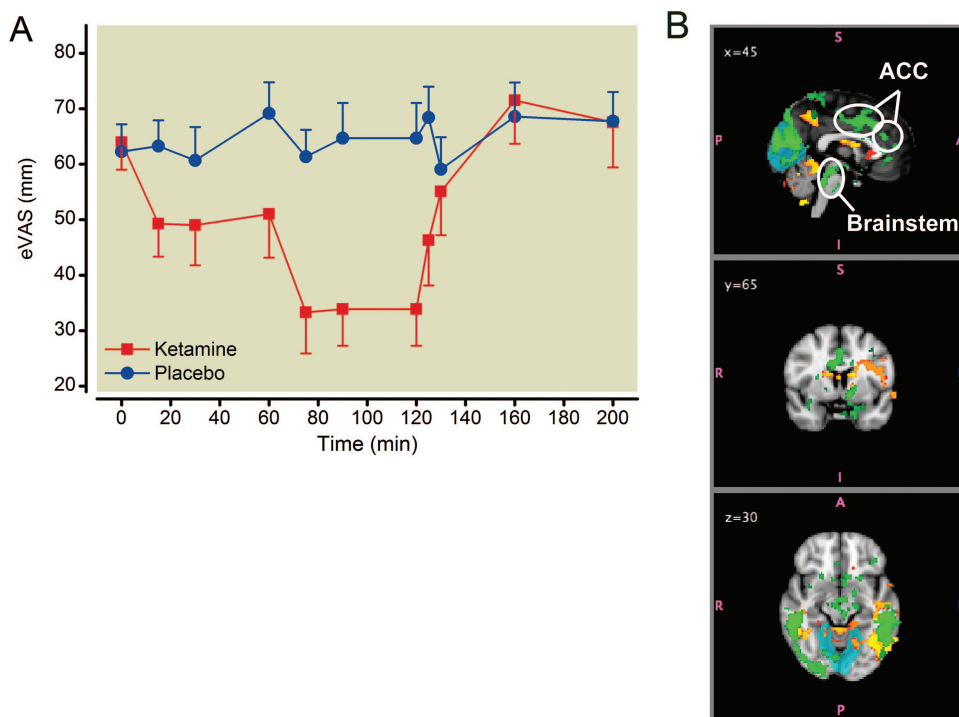
Fig. 4. Pain scores to the fixed heat stimulus during and after ketamine (*red squares*) and placebo (*blue circles*) infusion. Concentration-dependent analgesia (46.9% at t = 120 min) was observed during ketamine infusion, which rapidly disappeared after termination of the infusion. No analgesia was observed during the placebo infusion (*A*). Statistical maps of the variations in connectivity explained by the drug effect (*yellow*) and by pain relief (*green*). The network of interest (NOI) is represented in *light blue* (*B*). A = anterior; ACC = anterior cingulate cortex; eVAS = electronic visual analog scale; I = inferior; L = left; P = posterior; R = right; S = superior.

thought to play a role in emotional processing (mainly anxiety) and thought coordination.[22] Therefore, alterations in connectivity of the cerebellum (increases to NOI1 and decreases to NOI3) may be related to some of the psychedelic side effects observed during ketamine infusion. Large connectivity changes were also observed in the visual cortex and the optic radiation to NOI1, which may explain the visual hallucinations observed during ketamine infusion and perhaps the symptoms of blurry and double vision.[5,24,25] Additional connectivity changes to NOI3 were observed in the (pre)frontal cortex, orbitofrontal cortex, temporal cortex and gyrus, anterior and posterior cingulate cortex, thalamus, and precuneus cortex. In agreement with our observations, task-related fMRIs, evaluating ketamine-induced psychedelic effects as model for schizophrenia, showed similar activity changes.[3,5,24–30] Indeed, ketamine has long been recognized as a model for schizophrenia because many of the ketamine-induced psychedelic side effects show similarities with the positive (psychotic symptoms such as hallucinations) and negative (emotional blunting, lack of initiation) symptoms of schizophrenia.[26]

Ketamine decreased resting-state connectivity in most of the known pain-processing–related structures to NOI3, including the thalamus, amygdala, insula, anterior and posterior cingulate cortex, orbitofrontal cortex, and primary and secondary sensory cortices. The observed effect on the amygdala (which is part of the limbic system) may explain ketamine's effect on the loss of the affective component of pain. Our findings are in close agreement with positron emission topography and task-related fMRI studies, which show the involvement of the brainstem, thalamus, amygdala, insula, anterior and posterior cingulate cortex, orbitofrontal cortex, and the sensorimotor cortices in pain processing.[31–33] Overall, these data indicate that RS-fMRI is a reliable and relatively simple (more efficient) method for identifying how drugs affect the brain. A single RS-fMRI study can recognize all pharmacologically affected regions in the brain simultaneously in contrast to task-related fMRI studies.

Including pain scores as a regressor in the statistical model for testing the ketamine *versus* placebo fingerprint on the brain revealed brain areas whose connectivity was explained by pain processing aside from drug effect. Pain relief scores were associated with increased connectivity in relation to NOI1 in the anterior cingulate cortex, orbitofrontal cortex, insula, and brainstem, all of which are regions involved in pain sensing and processing.[31–33] Various studies have demonstrated the involvement of these same brain areas in descending inhibition of pain.[34–39] Descending inhibition of pain is a modulatory system originating at spinal and supraspinal sites, modifying afferent pain signal propagation. Examples of this system are conditioned pain modulation (which is the central inhibition of a focal pain stimulus by

administering a second noxious stimulus at a remote area), placebo analgesia, and stress-induced analgesia.[34,35] These top-down pain modulatory pathways dampen pain signal propagation at the level of the spinal cord dorsal horn and are thought to be defective in various chronic pain states.[40,41] The current study suggests a modulatory role for ketamine on descending pain inhibition. We previously tested the effect of ketamine treatment on endogenous pain modulation in patients with chronic pain and observed an increase in descending inhibition as tested by conditioned pain modulation (unpublished observation by Marieke Niesters, M.D., M.Sc., Leiden, The Netherlands; April 2011). Our current study corroborates the hypothesis that ketamine is able to influence endogenous pain modulation.

An important potential of the current technique lies in the development of new NMDA receptor antagonists for treatment of chronic pain. By applying the current RS-fMRI paradigm, new agents can be evaluated on their effects on the brain areas currently identified as involved in analgesia *versus* those involved in the side effects of ketamine, the most important, potent, and prototypical NMDA receptor antagonist currently available. One such agent could be traxoprodil (Pfizer, New York, NY), a selective NR2B NMDA receptor antagonist.[21] In rats, we previously showed that this drug produces analgesic effects similar to that of ketamine but without significant side effects (such as absence of motor dysfunction and agitation). It would be of interest to assess whether this drug is effective in humans and whether the lack of side effects coincides with the absence of alterations in activity (connectivity) in the cerebellum, frontal cortex, and visual cortex. The current study provides support for the use of the RS-fMRI technique to evaluate even newer NMDA receptor antagonists, which may allow prediction of the toxicity-efficacy balance before application in patients.

Only a limited number of drugs have been tested using RS-fMRI.[10,19,42–44] Previously we assessed the effect of morphine and alcohol in healthy volunteers using the same paradigm as applied in the current study.[10] Morphine influenced resting-state connectivity in all NOIs, with the most extensive effects between NOI4 and NOI6 and the thalamus, brainstem, insula, putamen, and cerebellum, some of which are areas involved in descending inhibition of pain. Alcohol effects were limited, with the most important changes relative to NOI1, NOI3, and NOI4 (areas: posterior parietal cortex, cerebellum, brainstem, and visual cortex). Although some overlap in connectivity changes was present between morphine and alcohol *versus* ketamine, the overall connectivity change pattern observed after ketamine was different from that of morphine and alcohol. Further RS-fMRI studies include a study with psilocybin, a psychedelic found in mushrooms, which at a dose causing changes in consciousness (sedation) produced a decrease in connectivity between medial prefrontal cortex and posterior cingulate cortex. This may be related to its psychedelic effects.[19] Anesthetic doses of propofol caused changes in corticocortical and thalamocortical connectivity relative to frontoparietal networks. These propofol changes correlated linearly with the level of consciousness.[42–44] Although we do not know the biologic meaning of the observed connectivity changes, the finding of drug and state-of-consciousness specific changes in connectivity are plausibly related to drug-specific neuronal modulation (*i.e.*, a drug-specific fingerprint on the brain).

In the current study we applied two low (subanesthetic) doses of ketamine. In previous studies we showed that in volunteers and patients these doses had no effect on the level of consciousness.[45–47] We cannot exclude that some (minor) sedative effects did occur in our subjects that may have affected our results. However, we did not find any changes in brain connectivity relative to the default- and executive-control networks. Connectivity changes relative to these frontoparietal networks play an important role in the generation of sedation and unconsciousness.[45,46]

Both respiratory stimulation and depression have been reported after ketamine administration.[48–50] Changes in carbon dioxide concentration may affect the cerebral blood flow and possibly RS-fMRI connectivity globally in the brain. In our study, we observed no changes in cerebral blood flow in the areas of the brain where we report connectivity changes, as measured by arterial spin labeling (data not shown). Furthermore, we measured respiratory frequency during imaging and observed no changes during ketamine infusion relative to placebo. Finally, we incorporated breathing frequency as a regressor in our statistical model. This did not affect the ketamine-induced changes in RS-fMRI connectivities.

In conclusion, RS-fMRI is a useful and efficient method for assessing drug effect on the brain. In the current study, this was exemplified by assessing the effect of the NMDA receptor antagonist ketamine on resting-state brain connectivity in healthy volunteers. Low-dose ketamine induced connectivity changes in brain areas involved in motor function, psychedelic effects, and pain processing. With respect to pain processing, ketamine's analgesic effect may arise from multiple pathways. We observed a decreased connectivity in regions of the pain matrix responsible for the perception of pain (pain sensing) and the affective processing of pain. In addition, ketamine affected connectivity in brain areas involved in endogenous pain inhibition.

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