The long-term effects of single and repeated subanaesthetic ketamine administration on regional cerebral blood flow in healthy dogs measured with ^{99m}Tc-HMPAO SPECT

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ABSTRACT

Subanaesthetic ketamine has recently been established as an effective and rapid treatment for major depressive disorder showing antidepressant effects for up to 1 week on average. The use of repeated ketamine infusions has been put forward to augment and to prolong the antidepressant response and increase the remission rates. The underlying neurobiological mechanisms responsible for ketamine's antidepressant effects remain unclear. Nevertheless, it has been shown, both in dogs and humans, that ketamine can alter neuronal perfusion and therefore neuronal function in brain regions involved in psychiatric and behavioural disorders. Consequently, the aim of the current placebo controlled study was to assess the long-term effects on cerebral perfusion of single and repeated subanaesthetic ketamine infusions in dogs. Twelve healthy, laboratory dogs were scanned at six different time points following single and repeated ketamine administration, using Single Photon Emission Computed Tomography with the radiotracer ^{99m}Tc-hexamethylpropylene amine oxime. We hypothesised that repeated infusions could lead to more prolonged perfusion alterations in brain regions critical for behaviour regulation. We found that repeated subanaesthetic ketamine administration did not result in more prolonged cerebral perfusion alterations compared to a single ketamine administration.

1. Introduction

Ketamine is a dissociative anaesthetic which is routinely used in veterinary and in human medicine, especially in paediatric anaesthesia and field medicine (Morgan and Curran, 2012). Over the past decade, ketamine has gained high interest among psychiatrists as an effective treatment for major depressive disorder (Coyle and Laws, 2015; Dewilde et al., 2015; Fond et al., 2014; Lee et al., 2015). Subanaesthetic doses, given as a 40-min constant rate infusion, have been shown to produce rapid-onset antidepressant effects in patients with severe and treatment-resistant depression. Whereas traditional antidepressants typically require several weeks to show therapeutic efficacy, ketamine exerts its actions within hours. Moreover, these effects are sustained beyond ketamine's 3-h half-life, with persistent responses for up to 1 week on average (Coyle and Laws, 2015; Dewilde et al., 2015; Lee et al., 2015). Furthermore, the antidepressant response can even be prolonged when repeated ketamine infusions are given (Murrough et al., 2013; Rasmussen et al., 2013; Shiroma et al., 2014). The underlying neurobiological mechanisms responsible for these antidepressant effect remain unclear (du Jardin et al., 2016; Mathew et al., 2012), but a possible explanation could be that ketamine interacts with cerebral perfusion. Brain imaging studies using positron emission tomography (PET), single photon emission computed tomography (SPECT) and functional magnetic resonance imaging (fMRI) have reported abnormal regional cerebral blood flow (rCBF) in certain brain regions of dogs (Peremans et al., 2003; Vermeire et al., 2009) and humans (Deckersbach et al., 2006; Eren et al., 2003; Etkin and Wager, 2007; Fitzgerald et al., 2008; Järnum et al., 2011; Rogers et al., 2004) suffering from anxiety and mood disorders. Following administration of subanaesthetic ketamine doses, altered perfusion in these brain regions was reported in both humans and dogs (Holcomb et al., 2005, 2001; Pollak et al., 2015; Rowland et al., 2010; Waelbers et al., 2015). All these studies focused on rCBF evaluation during or immediately following a single ketamine infusion. However, ketamine's antidepressant effects occur
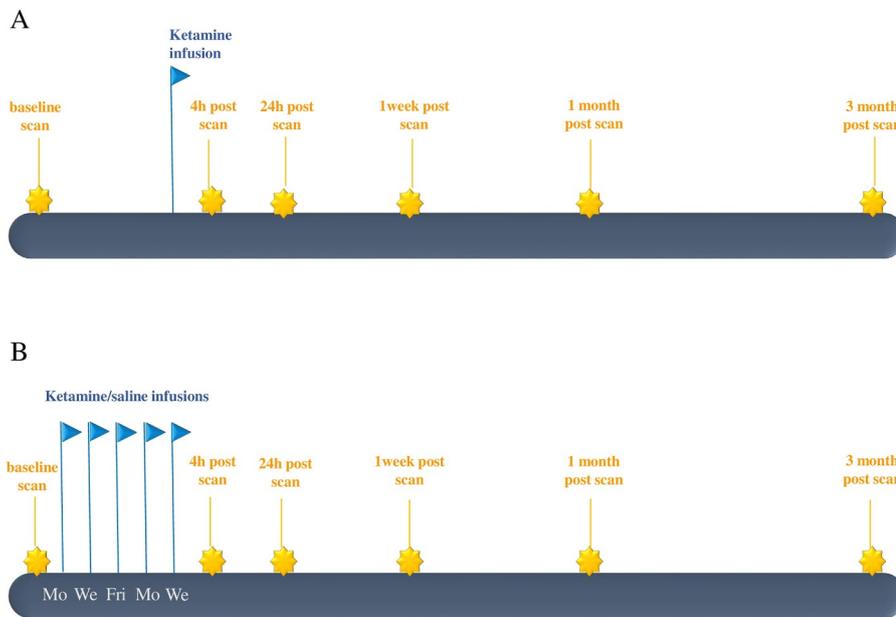
Fig. 1. Schematic overview of the study design. Condition KET1 is shown in (A), condition KET5 and condition saline are demonstrated in (B). The animals in group A first underwent the infusion/scan protocol as shown in (A), followed by the infusion/scan protocol demonstrated in (B). The animals in group P received 5 saline infusions, as shown in (B).

later, with a significant clinical improvement 2–4 h after administration (Abdallah et al., 2015; Carlson et al., 2013; Coyle and Laws, 2015; Singh et al., 2016). In a previous study by our group, we demonstrated regional cerebral blood flow changes 24 h after single and repeated ketamine infusions in dogs. This time frame corresponds with peak antidepressant responses observed in human patients (Abdallah et al., 2015). Nevertheless, regional brain perfusion 24 h after a single ketamine infusion did not differ from rCBF measured 24 h after 5 consecutive ketamine infusions in dogs (Vlerick et al., 2018). In human literature on major depression, it is described that repeated ketamine infusions are associated with higher response and remission percentages and with a more durable antidepressant response compared to single infusions (Murrough et al., 2013; Rasmussen et al., 2013; Shiroma et al., 2014). Therefore, Vlerick et al. hypothesized that rCBF measured after a single ketamine perfusion would differ from rCBF measured after repeated infusions. Unfortunately, this hypothesis could not be confirmed, as indicated above. However, rCBF was only measured 24 h after single and repeated infusion and therefore prolonged perfusion alterations following repeated ketamine administration cannot be excluded.

Consequently, the aim of the current study was to assess the long-term effects on cerebral perfusion of single and repeated subanaesthetic ketamine infusions in healthy dogs, measured with SPECT. As opposed to rodents, dogs have a substantial frontal cortex and exhibit naturally occurring behavioural disorders, which makes them an interesting animal model for human psychiatric diseases (Cyranoski, 2010; DeFelipe, 2011; Overall, 2000; Peremans et al., 2003; Vermeire et al., 2012, 2009). Moreover, due to their shorter life expectancy, radioprotective regulations are less stringent in dogs than in humans, which allows repeated SPECT scans in a relatively short time frame. rCBF was assessed at six different time points following single and repeated ketamine infusion: at baseline, 4 h, 24 h, 1 week, 1 month and at 3 months post-infusion. We hypothesised that repeated infusions could lead to prolonged perfusion alterations in brain regions critical for behaviour regulation.

2. Methods

2.1. Animals

Twelve healthy neutered adult laboratory dogs (10 females, 2 males; Beagles; age 3.9 ± 0.2 years; weight 12,7 ± 1.1 kg) were included in this study. None of the dogs had a history of previous neurological or behavioural abnormalities. This study was approved by the local Ethical Committee of the Faculty of Veterinary Medicine, Ghent University (EC 2016_118) and all manipulations were performed according to good animal practice. Welfare of the animals was respected at each time and great care was taken to avoid stress and anxiety. In this respect, all procedures were conducted under sedation or general anaesthesia. Sedation or premedication was always performed under minimal restraint (low stress handling) in the presence of the principal animal caretaker in a room familiar to the dogs. Following injection, the animals were allowed to relax in the shelter of a dog kennel until an appropriate level of sedation was reached. The dogs were socially-housed in small groups (2–8 dogs) on an internal surface of 15 m^2 with permanent access to an outside area of 15 m^2. The bedding material in the inner part consisted of wood shavings on top of a bottom heated concrete floor. Feeding toys, such as Kongs® were provided on a regular basis. Twice a day, the animals were allowed to run and play outside in an enclosed play area, enriched with climbing platforms, hiding places and small bushes. In addition, the dogs were regularly walked by students of the faculty of veterinary medicine.

2.2. Study design

To evaluate the effect of single and repeated subanaesthetic ketamine administration (Nimatek®, Eurovet Animal Health B.V, Bladel, Nederland) on rCBF, all dogs underwent SPECT scans at 6 different time points in a randomized design. SPECT acquisitions were performed before the start of the ketamine/saline infusions (T1), 4 h (T2), 24 h (T3), 1 week (T4), 1 month (T5) and 3 months (T6) after single or repeated infusions. The animals were randomly divided into two groups: a group receiving the active treatment (group A) and a placebo group (group P). Group A consisted of 8 dogs, while the remaining 4 dogs were included in the placebo group. In group A, each animal first received a single ketamine infusion at a dose of 2 mg/kg, followed by SPECT scans at the time points described above (condition KET1) (Fig. 1). Once the SPECT scans were completed (after T6), the animals of group A were re-enrolled in a second part of the study. In this second part, they received five consecutive subanaesthetic ketamine infusions at 2 mg/kg in a Monday–Wednesday–Friday scheme during a 10-day period, adapted from human psychiatry (condition KET5) (aan het Rot et al., 2010; Murrough et al., 2013; Shiroma et al., 2014; Szymkowicz et al., 2013; Vande Voort et al., 2016). SPECT acquisitions were again performed at the aforementioned time points (Fig. 1). The animals in group P received five saline infusions in a Monday–Wednesday–Friday schedule, followed by SPECT scans at the same time points (condition saline) (Fig. 1). In all dogs,

the total dose of ketamine or saline was administered intravenously over a 40-min time period. The dosages used are in the order of what it is described in human psychiatry and are based on the results of a previous study by our group (Coyle and Laws, 2015; Dewilde et al., 2015; Fond et al., 2014; Lee et al., 2015; Vlerick et al., 2018). In dogs, the most commonly used anaesthesia induction dose of ketamine is 5 mg/kg and 2 mg/kg is considered to be a subanaesthetic dose (Kästner, 2016).

2.3. Ketamine administration protocol

Prior to each ketamine or saline administration, all dogs were sedated intramuscular with dexmedetomidine (375 µg/m² body surface, Dexdomitor®, Orion Corporation, Espoo, Finland). Dexmedetomidine–ketamine is a frequently used combination in veterinary anaesthesia and has been shown to produce fewer sympathomimetic and haemodynamic side effects than ketamine alone (Murrell, 2016). An intravenous 22G over-the-needle catheter (Optiva®, Jelco Smiths Medical International Ltd, Rossendale, UK) was placed in one of the cephalic veins and the animals were allowed to relax in a quiet room until an appropriate level of sedation was obtained. To evaluate the sedation level, the sedation scoring system, described by Carter et al. (Carter et al., 2013) was used. The level of sedation was assessed on a numeric rating scale where 0 = no sedation; 1 = standing or sitting and head lower than before sedation; 2 = standing or sitting unbalanced, muscle weakness and refusing to lie down; 3 = lying in sternal recumbency but responsive; 4 = lying in lateral recumbence but responsive; and 5 = lying in lateral recumbency and unresponsive. Infusions were started when a sedation score of 4 or 5 was reached. A constant rate infusion (CRI) of ketamine or saline was given intravenously at a rate of 0.0125 ml/kg/min, over a 40-min time period using a syringe driver (B. Braun Melsungen AG, Perfusor® space, Melsungen, Germany). During the infusions, the principal investigator monitored the animals and cardiovascular parameters and sedation scores were evaluated every 10 min. Following termination of the infusion, the cephalic catheter was removed and the animals were allowed to recover.

2.4. Anaesthetic protocol

SPECT acquisitions were obtained under general anaesthesia. All dogs were premedicated with intramuscular dexmedetomidine (375 µg/m² body surface) approximately 30 min prior to induction. To gain intravenous access, a 22G over-the-needle cephalic catheter was placed in one of the front legs. General anaesthesia was induced with propofol (Propovet Multidose®, Abbott Laboratories, Berkshire, United Kingdom) given intravenously to effect. After endotracheal intubation, anaesthesia was maintained with a mixture of 1.2%–1.4% isoflurane (Isoflo®, Abbott Laboratories, Berkshire, UK) vaporized in oxygen using a circle rebreathing system. During general anaesthesia, heart rate, respiratory rate, end tidal carbon dioxide concentration and arterial haemoglobin oxygen saturation were measured by means of a calibrated multigas analyser (Capnomac Ultima, Datex, Helsinki, Finland) and a pulse oximeter (N5500 Patient Monitor, Nellcor Puritan Bennett Inc., Pleasanton, CA, USA).

2.5. SPECT perfusion imaging

^{99m}Tc-HMPAO (^{99m}Tc-labelled d, 1, hexamethyl propylene amine oxime, Ceretec, GE healthcare LTD, UK) was synthesised by eluting ^{99m}TcO4 from a 99Mo generator and directly adding it to exametazime. Finally, the solution was stabilized by adding cobalt. SPECT imaging with ^{99m}Tc-HMPAO is a validated technique to study rCBF in dogs (Adriaens et al., 2013; Peremans et al., 2001). The lipophilic tracer passes the blood brain barrier and is taken up by neurons, in a manner proportionally to cerebral blood flow and neuronal function. In the neurons, it is transformed into a hydrophilic form and becomes trapped intracellular (Adriaens et al., 2013). Following premedication with dexmedetomidine, a cephalic catheter was placed and the tracer was administered intravenously (25.7 MBq/kg ± 5.2 MBq). All SPECT acquisitions were started between 10 min and

20 min after tracer injection. Prior to the acquisition, all dogs were positioned in ventral recumbence. The triple headed gamma camera (Triad, Trionix, Twinsburg, OH, USA) was equipped with low-energy ultrahigh resolution parallel hole collimators (tomographic resolution full width at half maximum = 9 mm). To facilitate comparison between dogs, camera and table positioning were standardized. Data were acquired over a circular 360-degree rotation, for 20 min in step-and-shoot mode (120 steps, 10 s per step, 3 degree per step) on a 128 × 128 matrix. Images were then processed using iterative reconstruction and a Butterworth filter (cut-off 1.6 cycle/cm, order 10). Pixel size was 1.72 mm (Vermeire et al., 2009).

2.6. Image analysis

The individual patient's perfusion images were automatically registered to a template, generated from 14 dogs (9 males, 5 females, mean age 50 months ± 20), using Brain Registration and Automated SPET Semiquantification software (BRASS, Nuclear diagnostics, Sweden) (Peremans et al., 2003). This template based automated registration method eliminates subjective operator dependent region definition and the automatic registration (allowing shifting, scaling and rotation of the data) facilitates the fitting procedure to the template, necessary to compensate for intra-individual differences in anatomical brain size and shape. On this template, a region map was generated, including 12 separate manually drawn volumes of interest (VOI) positioned over the frontal, temporal, parietal and occipital lobes of both hemispheres as well as over the cerebellum, the thalamic region, the basal ganglia and the bulbus olfactorius. To calculate the rCBF ratios (perfusion index (PI)), regional radioactivity was normalized to the radioactivity of the entire brain.

2.7. Statistical analysis

Rstudio 1.1.383 (R: A Language and Environment for Statistical Computing; R Core Team; R Foundation for Statistical Computing, Vienna, Austria, 2016, https://www.R-project.org/) with packages MASS (version 7.3-45) and Sommer (version 3.0) was used to compute all analyses.

Onto the data set a multivariate linear mixed model with heterogeneous (unstructured) variances was set up. The model was written as $E(Yt|T1,T2) = \beta_0 + \beta_1 t_1 + \beta_2 t_2 + \beta_3 t_3 + \beta_4 t_4 + \beta_5 t_5 + \beta_6 T_1 + \beta_7 T_2 + \beta_8 t_1 T_1 + \beta_9 t_2 T_1 + \beta_{10} t_3 T_1 + \beta_{11} t_4 T_1 + \beta_{12} t_5 T_1 + \beta_{13} t_1 T_2 + \beta_{14} t_2 T_2 + \beta_{15} t_3 T_2 + \beta_{16} t_4 T_2 + \beta_{17} t_5 T_2$ with Yt as response variable. The PI of the 12 VOI's (continuous) was set as response value whereas time and treatment (both categorical) were set as predictor value. The factor time (continuous) and animal (categorical) were set as random factors. The predictor time (t) denotes the different timepoints with t_1 the first of five (k − 1 = 6 − 1 = 5) dummies (= 1 if time point = "4 h post" or 0 otherwise), t_2 the second dummy (= 1 if time point = "24 h post" or 0 otherwise), t_3 (= 1 if time point = "1 week post" or 0 otherwise), t_4 (=1 if time point = "1 month post" or 0 otherwise) and t_5 (=1 if time point = "3 months post" or 0 otherwise). The treatment predictor (T) indicated the different treatment modalities with T_1 the first of two (k − 1 = 3 − 1 = 2) dummies (= 1 if treatment = "Active 1×" or 0 otherwise) and T2 (= 1 if treatment = "Active 5×" or 0 otherwise). The reference level (for each region) was set as the PI at baseline in the control group (intercept). The degrees of freedom were obtained by means of the Welch-Satterthwaite equation and the type-I error was set at 0.05 (two-tailed). The assumptions of linearity of the regression function and the normality of the error term were assessed by making diagnostics plots.

3. Results

For the single dosage (condition KET1), significant time treatment interactions were found for the second time point (4 h post-ketamine infusion) in the following brain regions: left occipital (*p*-value = 0.04), right occipital (*p*-value < 0.001), left parietal (*p*-value < 0.001) and right parietal cortex (*p*-value = 0.001) (Table 1), indicating a significant increase in rCBF in these brain regions, compared to the

Table 1

Perfusion indices (mean ± standard deviation) in the thalamic region, parietal, occipital and frontal cortex in three different conditions (condition saline, KET1 and KET5) and at six different time points (T1–T6), measured using ^{99m}Tc-HMPAO SPECT ($* \leq 0.05$; $\dagger \leq 0.005$).

		T1	T2	T3	T4	T5	T6
Saline	*Th*	1.16 ± 0.06	1.17 ± 0.03	1.17 ± 0.06	1.18 ± 0.05	1.16 ± 0.03	1.17 ± 0.04
	RP	1.12 ± 0.04	1.10 ± 0.03	1.13 ± 0.03	1.14 ± 0.04	1.14 ± 0.03	1.14 ± 0.05
	LP	1.16 ± 0.02	1.14 ± 0.04	1.15 ± 0.03	1.18 ± 0.05	1.18 ± 0.05	1.14 ± 0.05
	RO	1.15 ± 0.05	1.17 ± 0.05	1.15 ± 0.02	1.15 ± 0.04	1.20 ± 0.02	1.14 ± 0.06
	LO	1.20 ± 0.03	1.20 ± 0.01	1.15 ± 0.03	1.16 ± 0.04	1.20 ± 0.04	1.14 ± 0.04
	RF	1.10 ± 0.01	1.12 ± 0.04	1.13 ± 0.02	1.13 ± 0.02	1.10 ± 0.04	1.12 ± 0.04
	LF	1.14 ± 0.01	1.14 ± 0.01	1.15 ± 0.04	1.15 ± 0.02	1.12 ± 0.02	1.14 ± 0.04
KET1	*Th*	1.18 ± 0.05	1.26 ± 0.05	1.28 ± 0.06*	1.26 ± 0.03	1.21 ± 0.06	1.18 ± 0.05
	RP	1.13 ± 0.04	1.14 ± 0.05†	1.18 ± 0.03	1.16 ± 0.04	1.12 ± 0.05	1.13 ± 0.04
	LP	1.16 ± 0.03	1.19 ± 0.01†	1.22 ± 0.03*	1.20 ± 0.02	1.17 ± 0.05	1.16 ± 0.03
	RO	1.11 ± 0.05	1.12 ± 0.04†	1.12 ± 0.06	1.11 ± 0.05	1.10 ± 0.09	1.11 ± 0.05
	LO	1.13 ± 0.05	1.14 ± 0.05*	1.14 ± 0.05*	1.13 ± 0.04	1.12 ± 0.07	1.13 ± 0.05
	RF	1.10 ± 0.03	1.12 ± 0.03	1.14 ± 0.03	1.15 ± 0.03	1.13 ± 0.04	1.10 ± 0.03
	LF	1.15 ± 0.03	1.16 ± 0.03	1.18 ± 0.03	1.19 ± 0.02	1.16 ± 0.04	1.15 ± 0.03
KET5	*Th*	1.18 ± 0.05	1.26 ± 0.06	1.28 ± 0.06*	1.25 ± 0.07	1.23 ± 0.02	1.23 ± 0.07
	RP	1.13 ± 0.04	1.15 ± 0.04*	1.14 ± 0.05	1.16 ± 0.06	1.14 ± 0.05	1.14 ± 0.04
	LP	1.16 ± 0.03	1.18 ± 0.03*	1.18 ± 0.03*	1.20 ± 0.06	1.16 ± 0.03	1.16 ± 0.04
	RO	1.11 ± 0.05	1.12 ± 0.04	1.12 ± 0.05	1.11 ± 0.07	1.12 ± 0.02	1.12 ± 0.03
	LO	1.13 ± 0.05	1.14 ± 0.04	1.14 ± 0.04	1.13 ± 0.06	1.13 ± 0.03	1.13 ± 0.06
	RF	1.10 ± 0.03	1.13 ± 0.02	1.13 ± 0.03	1.12 ± 0.03	1.10 ± 0.02	1.12 ± 0.03
	LF	1.15 ± 0.03	1.17 ± 0.03	1.17 ± 0.01	1.14 ± 0.03	1.13 ± 0.03	1.14 ± 0.04

Th, thalamic region; RP, right parietal cortex; LP, left parietal cortex; RO, right occipital cortex; LO, left occipital cortex; RF, right frontal cortex; LF, left frontal cortex. Condition saline, five saline infusions in a Monday–Wednesday–Friday schedule during a 10-day period; condition KET1, a single ketamine infusion at a dose of 2 mg/kg; condition KET5, five ketamine infusions at 2 mg/kg in a Monday–Wednesday–Friday scheme during a 10-day period. T1, baseline SPECT scan; T2, scan 4-h post-infusion; T3, scan 24-h post-infusion; T4, scan 1-week post-infusion; T5, scan 1-month post-infusion; T6, scan 3-months post-infusion.

reference level. Only for the left occipital and left parietal cortex these changes remained significant until time point 3 (24 h post-ketamine infusion) (*p*-value respectively 0.04 and 0.05). In addition, at this time point the rCBF of the thalamic region increased significantly (*p*-value = 0.04) compared to the reference level.

When repeated infusions were given (condition KET5), changes (compared to the reference level) were only noticeable in the left and right parietal cortex at time point 2 (4 h post-ketamine infusion) (*p*-value respectively 0.01 and 0.04) (Table 1). Again, rCBF increase of the left parietal cortex was present until time point 3 (24 h post-ketamine infusion) (*p*-value = 0.04). Once again, also the thalamic region showed an increased rCBF (*p-value* = 0.04) at time point 3.

4. Discussion

To unravel the underlying neurobiological mechanisms responsible for ketamine's antidepressant effects, this healthy dog study investigated changes in rCBF after single (condition KET1) and repeated (condition KET5) ketamine infusions at six different time points. The main finding of this study was that, in contrast to our initial hypothesis, repeated sub-anaesthetic ketamine infusions, administered through a Monday–Wednesday–Friday scheme, did not provoke more prolonged cerebral perfusion alterations compared to a single ketamine infusion. However, several studies in human literature have described a progressive increase in response and remission rate with increasing amount of ketamine infusions (Murrough et al., 2013; Shiroma et al., 2014; Singh et al., 2016). Moreover, repeated ketamine infusions prolonged the durability of antidepressant response compared with a single infusion, even after the infusions were discontinued (Murrough et al., 2013; Shiroma et al., 2014; Vande Voort et al., 2016). In one study evaluating antidepressant response to 6 thrice-weekly ketamine infusions, the antidepressant response was still maintained at the end of the 4-week follow-up period in 45% of responders after the last infusion (Shiroma et al., 2014). In another study using the same treatment paradigm, the median time to relapse among responders was 18 days after the final ketamine infusion (Murrough et al., 2013). The durability of antidepressant response after a single ketamine infusion is markedly shorter, with relapse rates ranging between several days to 1 week (Ibrahim et al., 2012; Zarate et al., 2006). However, in our study, perfusion alterations after five consecutive ketamine infusions were no longer significant 1 week after the last infusion. These results indicate that ketamine's sustained antidepressant effects after repeated infusions are not associated with sustained perfusion changes. Nevertheless, to our knowledge there are no functional imaging studies in human medicine evaluating the long-term effects of repeated ketamine infusions on rCBF. It could be that the increase in rCBF is only required as the initial triggering factor for ketamine's long-term clinical effects. Further downstream neuronal signalling and effects on neurotransmitters may be involved in the long-term behavioural effects of ketamine. On the other hand, this study was conducted in healthy dogs with normal cerebral perfusion. It could be possible that ketamine influences rCBF in a different way in dogs with abnormal behaviour if they have altered cerebral perfusion compared with behaviourally healthy dogs. To assess this hypothesis, examination of the long-term effects of single and repeated ketamine infusions on rCBF in a comparable population of dogs with anxiety disorders would be needed. The secondary advantage of a comparison study is that true clinical effects could be assessed, something neither relevant nor possible here.

In healthy dogs, a single ketamine infusion caused increases in rCBF in the parietal, occipital and thalamic region. In the thalamic region, the increase in rCBF was evident 24 h after the infusion. This time point corresponds with peak antidepressant effects of ketamine in human patients (Abdallah et al., 2015). Several human studies also demonstrated increased rCBF in the thalamic region during or immediately following ketamine infusion (Holcomb et al., 2005, 2001; Rowland et al., 2010). The changes in rCBF in the parietal and occipital cortices were already detectable 4 h after ketamine administration. This time frame corresponds with the rapid onset of antidepressant effects after intravenous ketamine administration (Abdallah et al., 2015; Carlson et al., 2013; Coyle and Laws, 2015; Singh et al., 2016). Furthermore, various studies demonstrated increased rCBF or increased regional glucose metabolic rate in parietal and occipital cortices during or immediately following subanaesthetic ketamine infusion in human patients (Carlson et al., 2013; De Simoni et al., 2013; Langsjo et al., 2004; Vollenweider et al., 1997). In addition, in one healthy dog study, increased rCBF was found in right parietal cortices during subanaesthetic ketamine infusion, using a slightly higher dose than in the current study (Waelbers et al., 2015). Furthermore, various studies in human medicine have reported decreased parietal and occipital perfusion in patients

suffering from major depressive disorder (Biver et al., 1994; Bonne et al., 1996a; Bonte et al., 2001; Drevets, 1998; Ishizaki et al., 2008; Nagafusa et al., 2012). Moreover, response to antidepressant therapy is generally associated with normalization of rCBF abnormalities in these brain regions (Bonne et al., 1996b; Ishizaki et al., 2008; Kohn et al., 2007; Mervaala et al., 2001).

Twenty-four hours after a single ketamine infusion, perfusion was still increased in the left parietal and in the left occipital cortex, but not in the same regions of the right hemisphere. Also 24 h after repeated ketamine infusions, perfusion was increased in the left parietal but not in the right parietal cortex. These differences between both hemispheres may be explained by a relatively small sample size, resulting in limited power. Nevertheless, there is some scientific evidence that reductions in brain perfusion in MDD patients are predominantly situated in the left hemisphere (Nagafusa et al., 2012; Navarro et al., 2002; Rogers et al., 2004).

Perfusion changes in the parietal, occipital and thalamic region can also be linked to the dissociative state caused by ketamine. Dissociative states in patients with depersonalization disorder and post-traumatic stress disorder (PTSD) have been associated with increased activity of the parietal and occipital cortex (Irle et al., 2007; Lanius et al., 2002; Simeon et al., 2000). In addition, the thalamus plays an important role in dissociation, and changes in activity of this brain region have been reported in patients with the dissociative subtype of PTSD and dissociative identity disorder (Felmingham et al., 2008; Krause-Utz et al., 2017; Schlumpf et al., 2014). However, dissociative symptoms caused by subanaesthetic ketamine are usually considered mild and typically resolve within 2–4 h following administration of ketamine (aan het Rot et al., 2010; Shiroma et al., 2014; Singh et al., 2016; Vande Voort et al., 2016). The fact that the perfusion of the parietal, occipital and thalamic region was still altered 24 h after ketamine administration, questions the possibility of a link between these changes and dissociation caused by ketamine.

In line with rCBF abnormalities found in humans suffering from depression and anxiety disorders, also in dogs with pathological anxiety alterations in rCBF have been reported (Vermeire et al., 2009). This is an interesting finding, as it means that ketamine could be a valuable alternative treatment for dogs with an incomplete response to standard behavioural therapy and pharmacotherapy or for dogs with the need of a faster response. Indeed, the management of behavioural disorders is challenging and treatment outcome is often unsatisfactory (Beata et al., 2007; Landsberg et al., 2008; Sherman and Mills, 2008; Takeuchi et al., 2000). This results in rehoming, relinquishment to an animal shelter or even euthanasia of healthy dogs (Scarlett et al., 2002; Sherman and Mills, 2008). Consequently, faster and more effective treatments are required.

Since sedation or anaesthesia is required during image acquisition in veterinary medicine, dogs were under general anaesthesia during brain imaging. Due to the rapid intra-neuronal trapping mechanism of the tracer used in this study, the influence of sedation and anaesthesia is generally considered to be minimal (Adriaens et al., 2014; Waelbers et al., 2012). Additionally, a placebo group was included to further eliminate possible confounding effects of sedatives and anaesthetics.

In conclusion, ketamine caused increases in rCBF in the parietal, occipital and thalamic regions, but not in the frontal cortex, in this healthy dog study. Additionally, repeated subanaesthetic ketamine infusions, administered through a Monday–Wednesday–Friday scheme, did not provoke more prolonged cerebral perfusion alterations compared to a single ketamine infusion. These results indicate that sustained antidepressant responses following repeated ketamine administration are not associated with sustained perfusion changes in brain regions critical for behaviour regulation. The study also emphasizes the advantages of the dog as animal model for human psychiatric conditions. As radioprotective regulations are less stringent in dogs, longitudinal studies with repeated SPECT scans in a relatively short time frame are possible. Since such a study protocol is less feasible in human patients, studies in dogs can be of great value. Additionally, also dogs with anxiety disorders could benefit from this study as they show similar changes in brain activity and perfusion compared to humans with anxiety disorders. Indeed, treatment of canine anxiety disorders also includes similar first line

psychopharmacological support as in human patients with a similar lag time before effect. Due to its rapid onset of action, ketamine in subanaesthetic doses may therefore also be useful as induction therapy in the canine species. However, as the study was conducted in healthy dogs, clinical effects of repeated subanaesthetic ketamine administration could not be assessed. Therefore, it would be interesting to examine the long-term effects of single and repeated ketamine infusions on behaviour and on regional cerebral perfusion in a population of dogs with anxiety disorders with abnormal cerebral perfusion.

Contributors

Authors LV, KP, RD and IP conceived the study and planned the design. The SPECT scans, the ketamine/saline infusions and the statistical analysis were performed by LV and RD. Image analysis was performed by KP. LV managed the literature search and wrote the first draft of the manuscript. IP and KP provided supervision and critical review of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest.

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