Impact of the glymphatic system on the kinetic and distribution of gadodiamide in the rat brain:

Observations by dynamic MRI and effect of circadian rhythm on tissue gadolinium concentrations.

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Short title

Glymphatic for GBCA distribution in rat brain

Abstract

Objectives: The glymphatic system is a recently hypothesized waste clearance system of the brain in which perivascular space constitutes a pathway similar to the lymphatic system in other body regions. Sleep and anesthesia are reported to influence the activity of the glymphatic system. As rats are nocturnal animals, the glymphatic system is expected to be more active during the day. We attempted to elucidate the influence of the glymphatic system for intravenously injected gadodiamide in the rat brain by two experiments. One was an MRI experiment to evaluate the short-term dynamics of signal intensity changes after gadodiamide administration. The other was a quantification experiment to evaluate the concentration of retained gadolinium within the rat brain after repeated intravenous administration of gadodiamide at different times of day and levels of anesthesia.

Materials and Methods: The imaging experiment was performed on 6 rats that received an intravenous injection of gadodiamide (1 mmol/kg) and dynamic MRI for 3 hours at 2.4-minute intervals. The time course of the signal intensity changes was evaluated for different brain structures. The tissue quantification experiment was performed on 24 rats divided into four groups by injection time (morning, late afternoon) and anesthesia (none, short, long) during administration. All animals received gadodiamide (1.8 mmol/kg, 8 times over 2 weeks). Gadolinium concentration of dissected brain tissues was quantified 5 weeks after the last administration by inductively coupled plasma mass spectrometry.

Results: In the imaging experiment, muscle and the 4th ventricle showed an instantaneous signal

intensity increase immediately following gadodiamide injection. The signal curve of the cerebral cortex and deep cerebellar nuclei reached the peak signal intensity later than the 4th ventricle but earlier than that of the prepontine cistern. In the gadolinium quantification experiment, the concentration in the group with the morning injection showed a significantly lower concentration than the late afternoon injection group. The lowest tissue gadolinium concentrations were found in the groups injected during anesthesia.

Conclusion: Instantaneous transition of gadodiamide from blood to cerebrospinal fluid was indicated by dynamic MR imaging. The gadodiamide distribution to the cerebral cortex and deep cerebellar nuclei seemed to depend on both blood flow and cerebrospinal fluid. As previous studies demonstrated that the CSF is one potential pathway of GBCA entry in the brain. For the distribution and clearance of the gadodiamide from brain tissue, involvement of the glymphatic system seemed to be indicated in terms of the influence of sleep and anesthesia.

Key words

Glymphatic system; gadodiamide ; circadian rhythm; sleep; anesthesia; rat brain; cerebrospinal fluid; magnetic resonance imaging; dynamic contrast enhance; inductively coupled plasma mass spectrometry

Introduction

The first report on increased signal intensities on unenhanced T1-weighted images in the dentate nucleus and globus pallidus of patients who received repeated contrast-enhanced MRI was published in March 2014 by Kanda et al (1). They also reported that the high signal in the dentate nuclei can be seen with linear gadolinium-based contrast agents (GBCAs), but it was not seen after repeated administration of macrocyclic GBCAs, suggesting that chelate stability affects the amount of Gd present in the brain (2). The presence of Gd after administration of linear GBCA in the brain tissue was histologically proven by McDonald et al (3). They compared autopsy cases of patients with and without prior administration of linear GBCA and found a linear dose relationship between the signal intensity on T1-weighted images in the globus pallidus and the dentate nucleus, and the cumulative administered Gd dose. Signal intensity changes and the presence of the gadolinium in the brain were also observed in preclinical studies on rats. The repeated administration of linear GBCA in healthy rats is associated with progressive and persistent T1-signal hyperintensity in the deep cerebellar nuclei and increased levels of Gd in the brain tissue (4). The report also indicated that the macrocyclic GBCA showed no effect on T1-weighetd MRI and a much lower presence of Gd in the brain tissue. A study on renal impaired rats and healthy controls suggests that renal insufficiency potentiates the retention of Gd in the brain. This report also indicates that low concentrations of Gd were found in the cerebrospinal fluid (CSF) 6 days after the injection (5),

The mechanism of gadolinium presence in the brain has not yet been clarified. According to one

hypothesis, proposed by Kanda, GBCA migrates through the blood-brain barrier and deposits remain in the tissue after transmetallation with an existing metal transporter (6). Recently, several reports mentioned the possibility of the involvement of the “glymphatic system” in the entry, distribution and elimination of GBCA in and from the brain (7, 8). According to a recently introduced hypothesis the glymphatic system comprises the perivascular space, which functions as a conduit for CSF to flow into the brain parenchyma, while the perivascular space around the arteries allows CSF to enter the interstitial space of the brain tissue through water channels (9). In a recently published rat study, the possibility of involvement of CSF as a potential initial pathway of GBCA entry into the brain was explored. The infiltration of GBCAs from blood into the CSF was evaluated, and it was demonstrated that GBCAs can penetrate from blood into the CSF (10). In this study, dynamic MRI suggested a passive distribution and wash-out driven by convection and CSF flow.

Based on that, we tried to elucidate the involvement and potential influence of the glymphatic system on the dynamics of intravenously injected gadodiamide in the rat brain by two experiments. First, a short-term dynamic MRI experiment monitoring the signal change in the brain and the CSF after a single intravenous high-dose gadodiamide administration up to three hours post injection. And second, a gadolinium quantification experiment on the order of weeks to evaluate the concentration of gadolinium within the rat brain after repeated intravenous gadodiamide administration performed at different times of day and levels of anesthesia, in order to investigate the influence of circadian rhythm and anesthesia as an indication of the involvement of the glymphatic system activity (9, 11).

Materials and Methods

Han-Wistar rat (Crl:WI; males) were obtained from Charles River (Sulzfeld, Germany) and maintained under standard laboratory conditions. Standard food and water were provided ad libitum. The animals were handled in compliance with German animal welfare legislation, and the experiments were performed with the approval of the state animal welfare committee.

<Imaging experiment>

The MRI experiment was performed on 6 rats. Anesthesia was started with intraperitoneal administration of xylazine (2%, Rompun, Bayer Vital GmbH, Leverkusen, Germany) and ketamine (100 mg/ml, Ketavet, Pharmacia, Karlsruhe, Germany) by 1.1 ml/kg dosage (1/3 xylazine, 2/3 ketamine), and supported by intravenous anesthesia infusion (10 × dilution with saline) by 1.5 ml/h with initiation of oxygen into the magnet.

Images were obtained using a Bruker BioSpec 47/20 (4.7 T) with a transmit-receive volume coil. T1-weighted dynamic scans by RARE sequence (TR=1015ms, TE=18.2ms, Echo train length=6, Average=6, Flip angle=90°, FOV=30×30mm², matrix=135x145, slice thickness=1mm: spatial resolution 222x207x1000 μm, 25 slices) were performed for 3 hours at 2.4-minute intervals. **During the entire MRI scan the animals were in anastehsia.** All injections were performed between 8 and 12 pm. Between the 5^{th} and the 6^{th} dynamic cycle, a single dose of 1.0 mmol/kg of gadodiamide

(Omniscan, GE Healthcare Buchler GmbH & Co. KG, Braunschweig, Germany) was injected via tail vein. The injections were completed within 2 min. Signal to noise ratio, that is change from the signal normalized by noise standard deviation were measured and change of SNR (ΔSNR) from the period before gadoteridol injection were calculated. The time course of the ΔSNR was evaluated for the cerebral cortex, deep cerebellar nuclei, fourth ventricle, subarachnoid space of the prepontine cistern and temporal muscle. ROIs were manually placed as shown in Figure 1. The peak ΔSNR (ΔSNRmax) and the time to peak (TTP) were calculated numerically from each time intensity curve.

<Tissue quantification experiment>

The gadolinium-quantification experiment was performed to investigate the influence of the circadian rhythm associated with the glymphatic activity on the presence of gadolinium in the rat brain after repeated administration of gadodiamide.

The subjects of this experiment were 24 Han-Wistar rats, and they received gadodiamide 1.8 mmol/kg (equivalent to a triple standard dose in humans upon body-surface adaption (12) 8 times over 2 weeks (cumulative dose 14.4 mmol/kg). Before the first injection, the animals were randomly divided into the following four experimental groups (n=6 per group) according to the timing of the injection and the use of anesthesia:

Group1: Injection in the morning, between 6:30-7:30 am (no anesthesia)

Group2: Injection in the late afternoon, between 4:00-5:00 pm (no anesthesia)

Group3: Injection in the morning, between 6:30-7:30 am; short-term anesthesia (Isoflurane)

Group4: Injection in the morning, between 6:30-7:30 am; anesthesia ≥ 30min (Rompun/Ketavet)

The dark/light cycle was 12 hours. As shown in Figure 2, light was set on from 6 am to 6 pm, and set dark from 6 pm to 6 am. As the rat is a nocturnal animal, the administration between 6:30-7:30 am is at the beginning of rest/sleep period, while the administration between 4:00-5:00 pm is at the end of rest/sleep period. Short-term anesthesia was made under inhalation of 4% isoflurane in a box prior to administration, and the animals were anesthetized for 1-6 min. Long-term anesthesia: Rompun & Ketavet (1+2; 1mL i.p.) prior to administration, and the animals were anesthetized for 30-120 min.

Five weeks after the last injection, exsanguination in anesthesia (4% Isoflurane) was performed, and the brain dissected to sample the cerebrum, cerebellum and brain stem. The gadolinium concentrations of these structures were measured by inductively coupled plasma mass spectrometry (ICP-MS: Agilent 7900, Waldbronn, Germany).

Statistical comparisons between the different groups according to the timing of the gadodiamide injection and the use of anesthesia were performed withgroup comparison among the 4 groups (post-hoc test). The calculations were performed with “R” (ver. 3.4.3)) using a significance level of 5 %.

Results

<Imaging experiment>

Representative images at the level of the cerebrum and cerebellum shown in figure 3A, a mean time

intensity curve is shown in Figure 3B. The ΔSNR in the fourth ventricle (deep blue) increased immediately after injection, showing highest peak ΔSNR compared to other structures and decreased gradually. The shape of time intensity curve of the 4th ventricle was similar to that of the temporal muscle (green). The ΔSNR of the prepontine cistern (red) showed a gradual increase with a longer TTP than the other regions followed by a slow signal decrease. The ΔSNR in the cerebral cortex (yellow), and deep cerebellar nuclei (purple) increased only slightly after injection and signal decrease seems to take more than 3 hours.

As shown in Figures 3c and 3d ΔSNRmax for the cerebral cortex was 1.7±0.5 and TTP for the cerebral cortex was 27±14 min. ΔSNRmax for the deep cerebellar nuculei was 1.4±0.4 and TTP was 36±24 min. For the 4th ventricle, ΔSNRmax was 20±5 and TTP was 15±4 min. For the prepontine cistern, ΔSNRmax was 12±2 and TTP was 31±9 min. For the temporal muscle, ΔSNRmax was 11±1 and TTP was 8±1 min.

<Tissue quantification experiment>

The gadolinium concentration in the cerebrum, cerebellum and brain stem 5 weeks after the last administration of gadodiamide is shown in the Table and Figure 4. In cerebrum, the mean gadolinium concentration was 2.9 5.0, 2.4 and 1.9 (nmol/g tissue) in Groups 1, 2, 3 and 4, respectively. Likewise, the mean gadolinium concentration in the tissue was 3.6, 5.8, 2.9 and 2.5 (nmol/g) in the cerebellum, and 1.6, 1.8, 1.6 and 1.2 (nmol/g) in the brain stem. Thus, the highest concentration was shown in the group with gadodiamide

administration at the end of the rest phase, and the lowest gadolinium concentration was shown in the group with gadodiamide administration in the morning during long anesthesia. On table 1, relative changes of gadolinium concentration in the tissue (Δ Gd %) were shown compared to the conditions with maximum gadolinium concentration (late afternoon injection).

Discussion

The transportation of substances in the brain is important for the maintenance of brain homeostasis. Blood flow plays the largest role as a substance transportation route in the brain. Oxygen and glucose, which are required for the metabolism, are carried to the brain by blood and are transported into the tissue through the blood-brain barrier formed by the end feet of glial cells. However, crossing the blood-brain barrier is not the only transport route. In recent years, mass transport in the brain by CSF and interstitial fluid (ISF) has been introduced. Outside the brain the lymphatic system is involved in protein waste removal in the body. On the other hand, it was conventionally thought that there is no lymphatic system in the brain. Nedergaard and Iliff et al. recently hypothesized that the perivascular space constitutes a system corresponding to the lymphatic system in the brain and named “glymphatic system”, which is a coined word that combines “g” for glia cell and “lymphatic” system (9, 13). The outline of their hypothesis is as follows: The perivascular space functions as a conduit for the flow of CSF into brain parenchyma. The CSF is filtered from blood in the choroid plexus and then led to the perivascular space around the artery where it enters the interstitial space of the brain tissue through

water channels controlled by aquaporin 4 (AQP4). CSF entering the interstitial space removes waste proteins such as amyloid β from the tissue. The CSF that flows between the neuronal cells reaches the perivascular space around the veins and is discharged outside of the brain (13). This hypothesis of a glymphatic system has been confirmed in many studies. In addition to original microscopic study using fluorescent tracers, a MRI study using GBCA as a tracer has been reported, as well as permeability study by MRI, diffusion study by MRI and near infrared observation of the glymphatic system (14-20).

A characteristic feature of the glymphatic system is that the function of activity is modified by sleep. This was confirmed by an experiment on the drainage of amyloid β. A study on the excretion of amyloid β in healthy and AQP4 knockout mice showed that natural sleep or anesthesia are associated with a 60% increase in the interstitial space, resulting in a striking increase in the exchange of CSF with ISF (11). Another study indicates that the volume fraction of the interstitial space of the mouse brain tissue is 13 to 15% when awake, while 22 to 24% when sleeping (21, 22). A recently published animal (mice) study also indicated that sleep deprivation reduced influx efficiency along the perivascular space, disturbed AQP4 polarization and induced anxiety-like behaviors (23). Another study reported the effect of sleep for brain lactate concentration and shows that the suppression of glymphatic function via acetazolamide treatment, cisterna magna puncture, aquaporin 4 deletion, or changes in body position reduced the decline in brain lactate normally observed when mice transition into sleep or anesthesia (24). Based on these findings and our results, we hypothesize that modification

by sleep or anesthesia influences the entry, distribution and clearance of GBCAs to the brain tissue via the glymphatic system. Consequently, the amount of Gd that is present 5 weeks after the last administration of gadodiamide seems to depend significantly on the activity of the glymphatic system during or immediately after the administration.

In the MRI experiment of the current study, signal changes were visualized with T1weighted imaging at high temporal resolution. The ΔSNR of the muscle showed an immediate increase after the injection which may reflect the combined signal from vasculature and interstitium, as extravasation starts immediately after GBCA arrival. The ROI placed in the 4th ventricle contains both choroid plexus and CSF, thus the signal can be considered to come from both. The ΔSNR of the 4th ventricle showed an early increase after injection, which may indicate that the gadodiamide transmits instantaneously from the blood to CSF most likely via the choroid plexus. The subarachnoid space of the prepontine cistern, which does not contain choroid plexus, showed a different time course compared to that of the fourth ventricle. This difference may be due to the time for transition of the CSF which contains gadodiamide from the 4th ventricle to the subarachnoid space of the prepontine cistern via diffusion and CSF pulsation. The cerebral cortex and deep cerebellar nuclei showed smaller signal changes compared to that of muscle or CSF space due to the blood-brain barrier. The mean TTP was longer than for muscle or for the 4th ventricle but shorter than for the prepontine cistern. This finding could be interpreted as gadodiamide transfer to the cerebral cortex or deep cerebellar nuclei

both via the blood flow and via the CSF. If the transfer of the gadodiamide is only via the blood flow, it would show a shorter TTP interval, comparable to that of muscle. The delayed TTP within the cerebral cortex and deep cerebellar nuclei indicates at least a partial transition of gadodiamide from CSF. On the other hand, T2* effects may also reduce the T1-weighted signal intensity at early time points, where the vascular gadolinium concentrations are high. This can potentially also have contributed to the delayed TTP in the cerebral cortex and deep cerebellar nuclei. The observations of our study are consistent with findings of signal enhancement in the cerebellum following intracisternal administration of GBCA and subsequent transport by the glymphatic system (14, 25).

In the gadolinium quantification experiment, gadolinium concentrations in the cerebrum and cerebellum were highest in the group given the late afternoon injection, followed by morning injection, morning injection with short anesthesia and morning injection with longer anesthesia during and after gadodiamide administration. As rats are nocturnal animals, the glymphatic system is expected to be more active during the day (11). The results of this study might be explained by a higher glymphatic clearance following the morning injection, while a reduced glymphatic activity is present after the injection in the late afternoon. Anesthesia seemed to facilitate the glymphatic clearance of gadodiamide especially for longer durations. Combining results from the current two experiments, gadodiamide seems to transfer into the brain tissue rather promptly which would be both from blood flow and CSF and as far as looking into time intensity curve of the imaging experiment, the wash out is not completed within 3 hours. As the gadolinium quantification experiment suggested that sleep and

anesthesia accelerated gadodiamide removal, the glymphatic system may play an important role in the elimination process from the brain tissue. In the current experiment, slopes of the signal increase were larger than signal decrease both in CSF and brain tissue, which can be interpreted that elimination process takes a much longer time compared to the distribution. The glymphatic system activity is reported to be larger during sleep and anesthesia (11). In the current study the degree of activity has an impact on the amount of gadolinium that can be found in the brain 5 weeks after the last administration in the current study. Thus, we consider that the glymphatic system is involved in the processes which lead to the presence and distribution of gadodiamide in the brain tissue. However, it has to be noted that the molecular forms of the gadolinium observed in the imaging experiment and in the quantification experiment in the current study may not be identical. A recently published study on the residual Gd in the rat brain after repeated administration of linear GBCAs showed that Gd in the tissue can be found in at least 3 distinctive forms – soluble small molecules, including the intact GBCA, soluble macromolecules, and insoluble forms. A large portion of the Gd from the linear GBCA was found in the insoluble fractions and to a lesser degree bound to soluble macromolecules, which is most likely responsible for the observed prolonged signal enhancement in the brain MRI. The decrease of Gd in the soluble brain tissue fractions was observed between days 3 and 24 after the last injection. (26). While the soluble forms of Gd may be transported by CSF or ISF under mechanisms such as glymphatic system, the differences between groups of the tissue quantification experiment in the current study is speculated to take place mainly in the fraction of soluble form.

Although we hypothesized that the observations of the current study suggests the involvement of the glymphatic system for the kinetics of Gd within the brain, it is not finally proven.

In order to make the dynamics of the Gd within the brain more clearly, further experiments are needed. This may include a close monitoring of MRI signal enhancement for several weeks, the gadolinium tissue quantification at various time-points after administrations and sequential quantification of the CSF Gd concentration. Laser-ablation ICP-MS can provide spatially-resolved quantification of the gadolinium in tissue (27). A sequential monitoring with this technique may allow to get a deeper insight into the kinetic of GBCA distribution within the brain.

There are several limitations in the current examination. The duration of the imaging experiment was 3 hours and the total process for elimination from the CSF could not be observed. On the other side it is very difficult to maintain good anesthesia for more than three hours. Manual ROI placement could be another limitation for the current study. However, we tried to place ROIs as uniform as possible for the different animals.

In conclusion, rather prompt transition of gadodiamide from blood to CSF via the choroid plexus in the rat brain was observed by dynamic MR imaging. However, the question whether the gadodiamide distribution within the cerebral cortex or deep cerebellar nuclei originated from the blood or from CSF cannot be reliably answered. It seems that both ways may contribute. The distribution and clearance of gadodiamide from brain tissue through the glymphatic system was suggested by the influence of circadian rhythm and anesthesia.

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Table : Gadolinium concentration in the tissue and Δ Gd %

		Cerebrum		Cerebellum		Brain stem	
		Gd conc. (nmol/g)	Δ Gd %	Gd conc. (nmol/g)	Δ Gd %	Gd conc. (nmol/g)	Δ Gd %
Group 1	Morning/ Awake	2.9±0.7	43.3	3.6±0.5	37.9	1.6±0.3	7.5
Group 2	Late afternoon/ Awake	5.0±1.3	-	5.8±1.9	-	1.8±0.2	-
Group 3	Morning/ Short anesthesia	2.4±0.5	53.1	3.0±0.4	48.9	1.6±0.3	8.3
Group 4	Morning/ Long anesthesia	1.9±0.4	61.9	2.5±0.2	56.4	1.2±0.3	30.3

Figure legends

Figure 1

The ROIs for the dynamic contrast enhanced MRI experiment. The cerebral cortex, deep cerebellar nuclei, fourth ventricle, prepontine subarachnoid space and temporal muscle are shown. The images were acquired at 10 am.

Figure 2

The time table for the tissue quantification experiment. Green arrows indicate the injections in the morning and orange arrows indicate the injections in the late afternoon.

a: The lighting of laboratory was set light from 6 am to 6 pm, and set dark from 6pm to 6am. Thus, the gadodiamide administration between 6:30-7:30 am is at the beginning of the rest/sleep period, and the gadodiamide administration between 4:00-5:00 pm is at the end of the rest/sleep period. B: b: The subjects received gadodiamide (1.8 mmol/kg) for 8 times over 2 weeks for both the morning injection group and late afternoon injection group. Five weeks after the last injection, exsanguination under anesthesia was made, and the brain was removed and dissected.

Figure 3

a. Dynamic MR scan by T1-weighted dynamic scans by RARE sequence at the level of cerebrum (upper row) and cerebellum (lower row). Images of pre administration and 2.4, 7.2, 17.0, 36.5, 75.3

and 150.7 minutes after administration of gadoteridol are shown. Note the prompt enhancement of the CSF spaces.

b. Time intensity curves ΔSNR are presented with standard deviations. (yellow: cerebral cortex, purple: deep cerebellar nuclei, blue: 4th ventricle, red: pre-pontine subarachnoid space, green: temporal muscle) While muscle showed a prompt increase and wash out, intracranial structures indicated a delayed washout. Wash out seemed to take more than 3 hours.

c: Peak ΔSNR with standard deviation. Peak ΔSNR of the 4th ventricle was the highest compared to other structures. The ΔSNR increase of the cerebral cortex and deep cerebellar nuclei were smaller than that of other structures.

d: Time for the peak ΔSNR with standard deviation. Muscle shows most prompt signal increase and the 4th ventricle also showed prompt increase. The ΔSNR of the cerebral cortex and deep cerebellar nuclei reached to the peak later than the 4th ventricle but earlier than that of prepontine cistern.

Figure 4

Tissue gadolinium concentrations 5 weeks after repeated high-dose gadodiamide administrations with standard deviation.

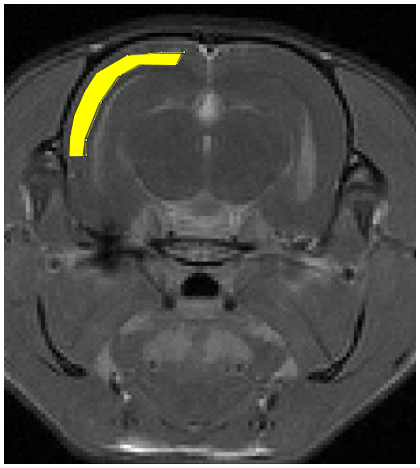
a. Cerebrum. The gadolinium concentration showed a statistically significant difference ($p<0.01$) between the group receiving the late afternoon injection and the morning injection without anesthesia. A larger difference were found between the late afternoon injection and morning injection with

anesthesia ($p < 0.001$) between the groups with the late afternoon injection and morning injection with anesthesia. As rats are nocturnal animals, these findings seem to indicate that there was a higher or effective glymphatic clearance of gadodiamide following the morning injection. Anesthesia seemed to facilitate the glymphatic clearance.

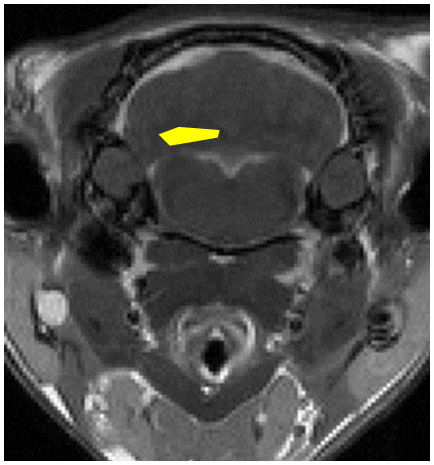
b: Cerebellum. The gadolinium concentration in the cerebellum showed a similar tendency with cerebrum.

c. Brain stem. The gadolinium concentration in the brain stem was smaller compared to that of the cerebrum and cerebellum.

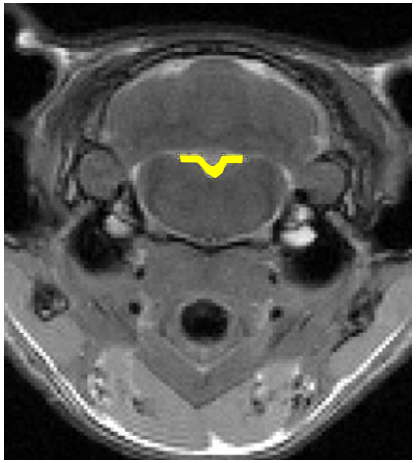
Figure 1



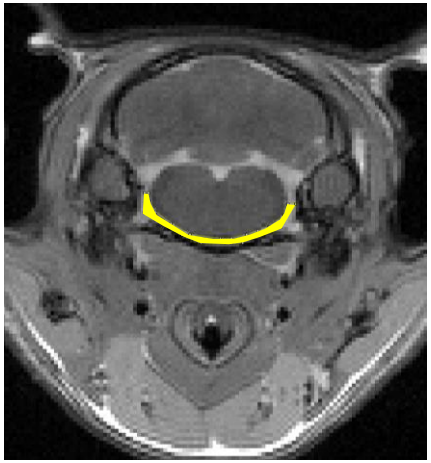
Cerebral cortex



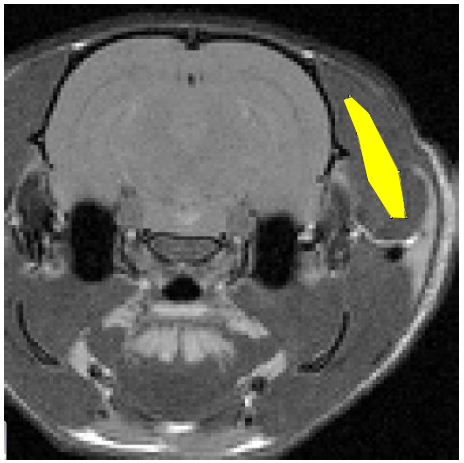
Deep cerebellar nuclei



4th ventricle



Pre-pontine subarachnoid



Muscle

Figure 2

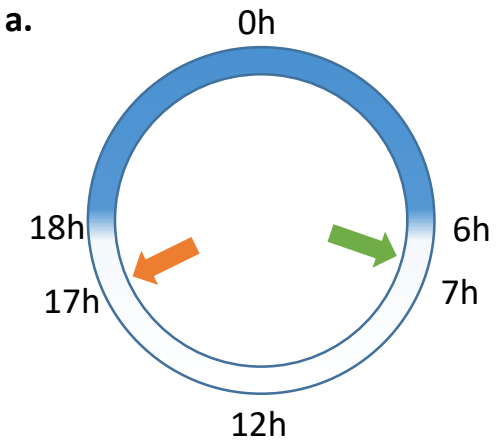
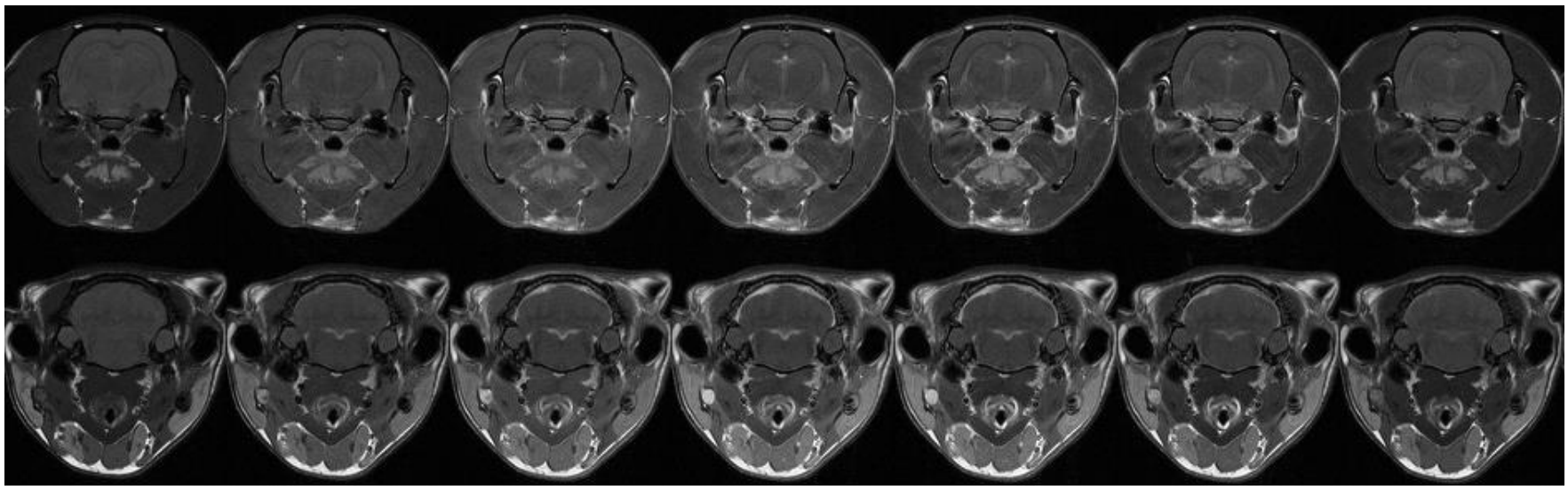


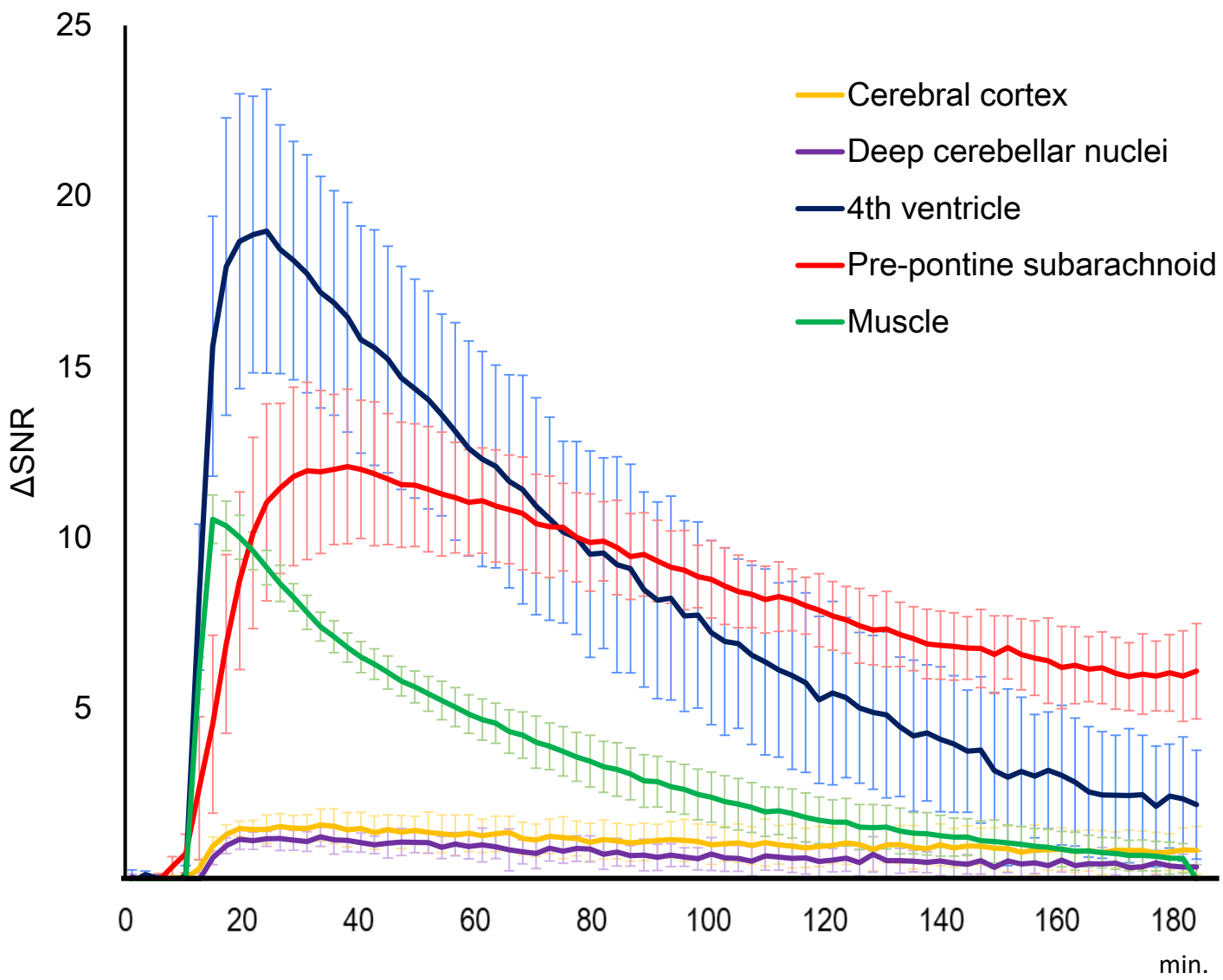
Figure 3

a.

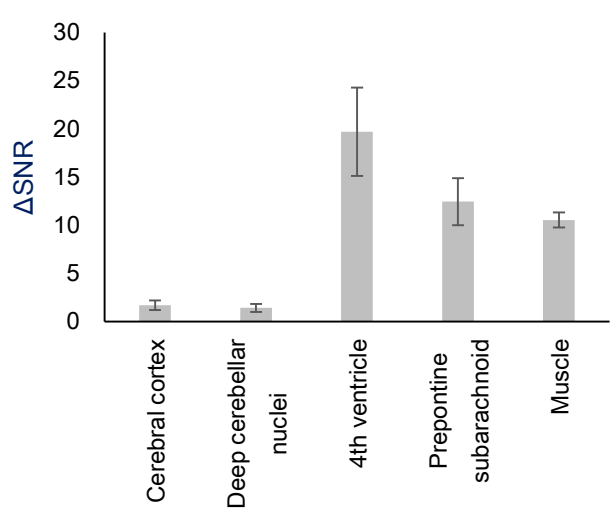


Pre 2.4 7.2 17.0 36.5 75.3 150.7 min.

b.



c.



d.

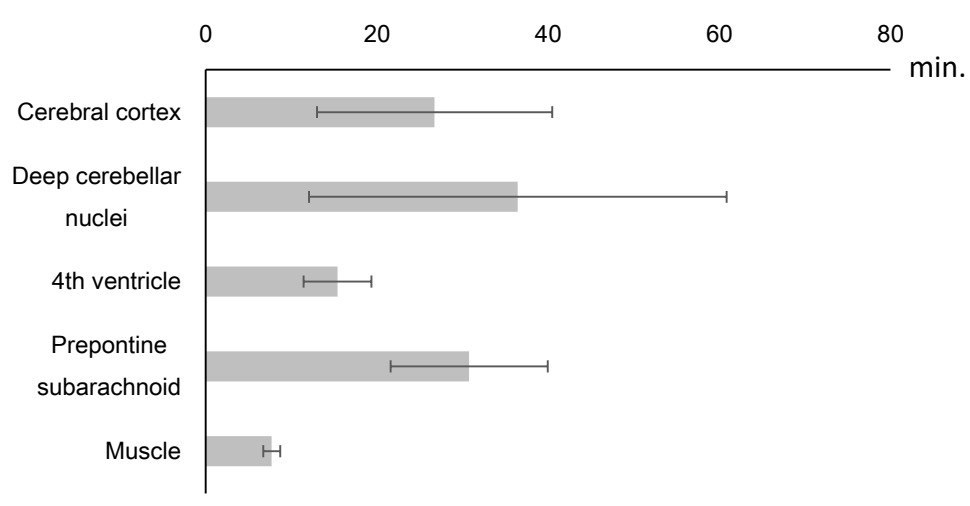
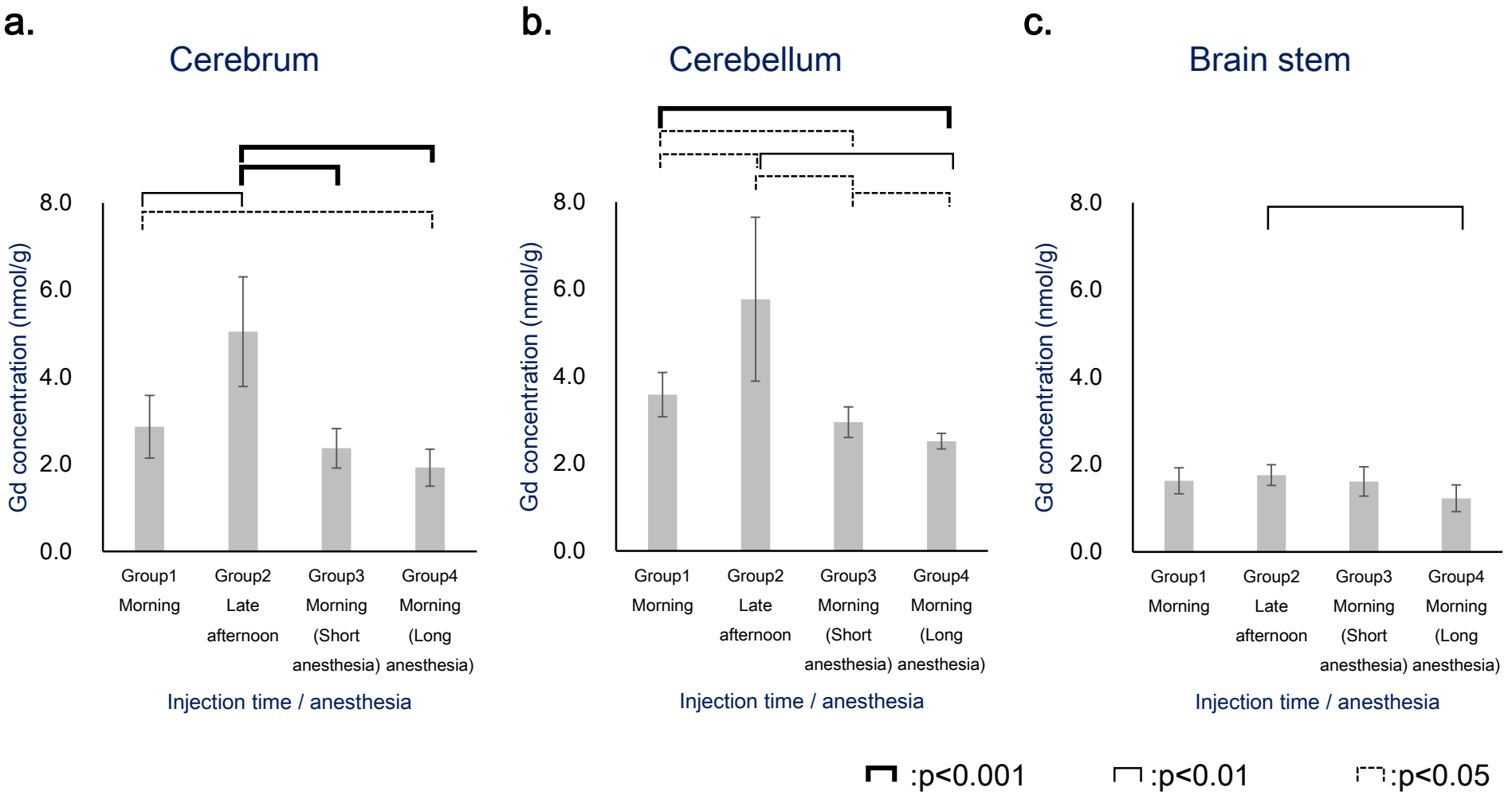


Figure 4


a.
Cerebrum
b.
Cerebellum
c.
Brain stem
Gd concentration (nmol/g)
8.0
6.0
4.0
2.0
0.0
Group1 Morning
Group2 Late afternoon
Group3 Morning (Short anesthesia)
Group4 Morning (Long anesthesia)
Injection time / anesthesia
:p<0.001
:p<0.01
:p<0.05