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Influence of three different anesthesia protocols on aged rat brain: a resting-state functional magnetic resonance imaging study

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Abstract

Background: Resting-state functional magnetic resonance imaging (rs-fMRI) is a promising method for the study of brain function. Typically, rs-fMRI is performed on anesthetized animals. Although different functional connectivity (FC) in various anesthetics on whole brain have been studied, few studies have focused on different FC in the aged brain. Here, we measured FC under three commonly used anesthesia methods and analyzed data to determine if the FC in whole brain analysis were similar among groups.
Methods: Twenty-four male aged Wistar rats were randomly divided into three groups ($n = 8$ in each group). Anesthesia was performed under either isoflurane (ISO), combined ISO + dexmedetomidine (DEX) or α-chloralose (AC) according to the groups. Data of rs-fMRI was analyzed by FC in a voxel-wise way. Differences in the FC maps between the groups were analyzed by one-way analysis of variance and *post hoc* two-sample t tests.
Results: Compared with ISO + DEX anesthesia, ISO anesthesia caused increased FC in posterior brain and decreased FC in the middle brain of the aged rat. AC anesthesia caused global suppression as no increase in FC was observed.
Conclusion: ISO could be used as a substitute for ISO + DEX in rat default mode network studies if the left temporal association cortex is not considered important.
Keywords: Anesthetics; Aged; Rat; Functional connectivity; Magnetic resonance imaging

Introduction

Advances in imaging technology have enabled the study of brain anatomy and function by non-invasive methods. Blood-oxygen-level-dependent (BOLD) signal in resting-state functional magnetic resonance imaging (rs-fMRI) can be used to monitor functional responses in human brain, and has widened the scope of brain research.[1] Functional connectivity (FC), a signal analysis method mainly focused on correlations in low (<0.1 Hz) spontaneous fluctuations in the BOLD signal in the resting-state, has been applied both in human studies[2] and pre-clinical experiments.[3-5] Further, FC changes have been observed in human neurodegenerative diseases, including Alzheimer disease (AD) and Parkinson disease (PD),[6,7] and in pre-clinical model of neuroinflammation,[8] indicating that FC can be used to investigate brain function, drug targets, and disease mechanisms in central nervous system (CNS).

Considering that most animals are not required to perform tasks during rs-fMRI data acquisition and several disease models are readily available, investigating FC in these pre-clinical models is an attractive option for studying disease pathophysiology. In most cases, animals have to be anesthetized during scanning process to minimize head motion artifacts and stress. Although various anesthetics have been successfully used to anesthetize animals during rs-fMRI scanning,[6,9-12] anesthetics have been proved to disrupt FC[13] and different anesthetics have different FC patterns.[14] Furthermore, some neural networks are preserved while others are suppressed under anesthesia,[15] making it difficult to determine the effect of medical treatment or severity of disease.

Although awake data has been used in the pre-clinical study,[13] animal stress and spontaneous movement cannot be avoided during data acquisition. In addition, the animals have to undergo a training which may last for up to 8 days[16] and is time-consuming and labor intensive. Thus, anesthetics are still necessary. Being one of the most commonly used anesthetics, isoflurane (ISO) has been widely used in neuroimaging study for its ability in rapid

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induction and recovery, as well as flexible control of anesthesia dose during maintenance.[17] However, high dose ISO anesthesia (≥1.8%) was reported to disrupt naturally occurred FC of rat brain by inducing synchronous cortico-striatal fluctuations and silencing subcortical activity.[18] α-Chloralose (AC), one of the most commonly used long-acting anesthetic agent in rs-fMRI studies, has been reported to be not the best choice for anesthesia due to its suppression on several important pharmacological binding sites.[19] So far, the combined use of ISO inhalation and intra-muscular injection of low-dose dexmedetomidine (DEX) has been shown to be effective for preserving default mode network (DMN)[20] and similar anesthesia methods have been used in several pre-clinical experiments.[8,21] Because neuroinflammation has been proved to be a major cause for AD[22] and PD[23] in aged patients, aged rats are an ideal choice for mechanism studies.[24,25] However, doses of inhalational anesthetics in aged rats differ from those used in adults.[26] Meanwhile, the levels of important neural receptors in CNS, including γ-amino butyric acid$_A$ (GABA$_A$) receptors[27] and N-methyl-D-aspartate receptors[28] also modulate with aging, indicating that the effect of anesthesia on aged brain requires further study. Moreover, despite the increasing amounts of pre-clinical rs-fMRI studies, few have focused on different FC in the aged rat brain.

In this study, the effects of three different but commonly used anesthesia methods on FC in the aged rat brain were examined by rs-fMRI. Because the restrosplenial cortex (RSC) has been recognized as a major region of interest (ROI) for whole brain analysis in many rs-fMRI pre-clinical experiments, it was used as a seed region. Considering that anesthesia with ISO + DEX has been widely used during rs-fMRI data acquisition, it was primarily used as a control. The rs-fMRI data were analyzed by FC in a voxel-wise way.[29-31] We hypothesize that the FC are similar among groups although different anesthesia methods were used.

Materials and methods

Ethical approval

All animal procedures were approved by the Ethical Committee of Capital Medical University (Beijing, China; approval No. AEEI-2019-052), as complied with the guide for Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health. All efforts were made to minimize the pain and suffering of the animals.

Animal preparations

A total of 24 adult male Wistar rats (19 months, 650–800 g; Dashuo Biotechnical Co, Ltd, Chengdu, Sichuan, China) were housed in an animal facility under a 12/12 h light-dark cycle at 22 ± 2°C with 50% to 60% humidity. Food and water were available *ad libitum*. The animals first underwent behavioral test after randomization and then for rs-fMRI scanning [Figure 1A].

Spatial working memory training with the Morris Water Maze (MWM)

The animals were trained using a MWM for testing of learning and spatial working memory to assess the capacity of the rats for further testing. Rats whose cognitive function was poor due to aging were eliminated from this study. The water maze was comprised of a circular tank 180 cm in diameter and 76 cm in depth with several clues around. The inner surface was painted in black. Before each session, the tank was filled with warm water (22°C) to a depth of 35 cm. A hidden platform was located 1.5 cm below the water surface in one fixed quadrant. A video camera was suspended from the ceiling above the water tank to record animal behavior.

The training process was performed as described in our previous study with some modifications.[25] Briefly, the
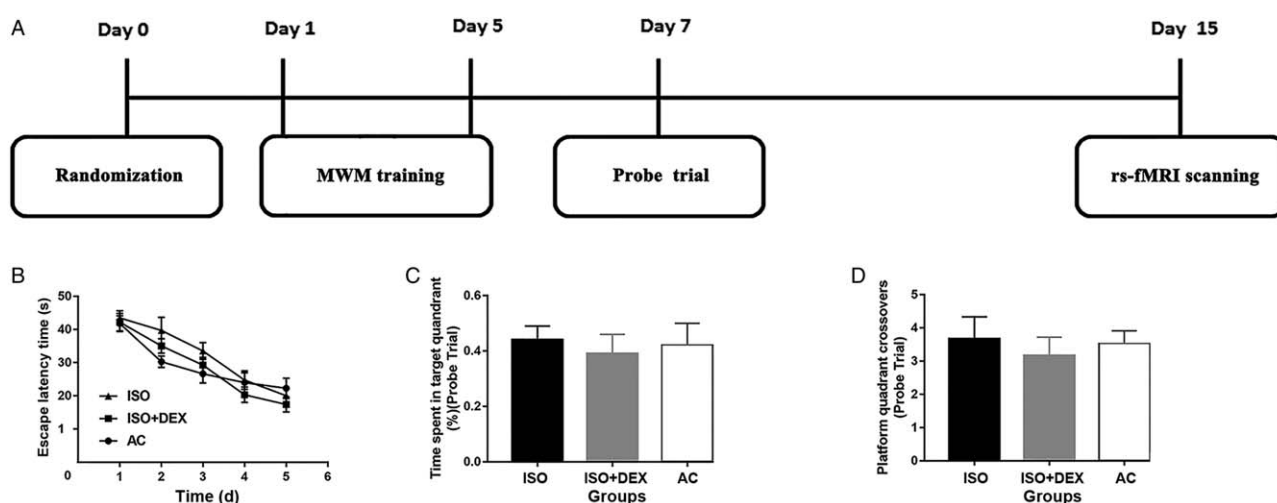

Figure 1: Protocol (A) and performances (B–D) in Morris Water Maze test. Data are presented as mean ± standard errors of mean and analyzed via ANOVA for repeated measurements followed by Dunnett multiple comparisons or one-way ANOVA followed by Dunnett *post hoc* analysis. $P < 0.05$ was considered as statistically significant. ANOVA: Analysis of variance.

MWM test was divided into two parts: spatial acquisition trials (days 1–5) and probe trial (day 7). The rats were rested on day 6. In spatial acquisition trials, the animals were trained to use distinctive distal visual cues surrounding the tank (one in each quadrant) to navigate a path to find the platform in the water maze. The training period lasted for 5 days with two sessions each day. Preceding each session, the rat was placed gently at the platform for 30 s. At the start of the training session (day 1), the animal was gently placed facing the wall of the MWM pool in one quadrant and was allowed to swim freely for 60 s to find the platform. If the rat was unable to find the platform, it was guided to the platform and allowed to remain there for 30 s. On day 5, rats that were still unable to find the platform were excluded from the study. A probe trial was conducted on day 7 with the platform removed. Briefly, each rat was placed in the opposite quadrant to the original location of the platform and allowed to swim for 30 s. The time to locate the platform, percentage of time spent in the target quadrant, and number of crossovers to the target quadrant were recorded and analyzed with EthoVision XT video tracking software (Noldus Information Technology BV, Wageningen, Netherlands).

Anesthesia on rats

All rats ($n = 24$) were randomly allocated to one of three treatment groups: ISO (group 1, $n = 8$; Baxter, Lessines, Belgium), ISO + DEX (group 2, $n = 8$; DEX, Hengrui Medicine Co., Ltd, Lianyungang, Jiangsu Province, China), or AC (group 3, $n = 8$; Sigma-Aldrich, Shanghai, China). The protocols for ISO, ISO + DEX, and AC were adapted from previously published studies.[8,14,19]

All rats in the ISO group and ISO + DEX group were first induced by ISO at the concentration of 3%. In the ISO group, anesthesia was then maintained with ISO at a concentration ranging from 1% to 1.3% with a respiration rate of 60 to 85/min throughout the scanning process. In the ISO + DEX group, induction with 3% ISO was followed by an intra-muscular injection of DEX (0.015 mg/kg). During the initial scanning, ISO (1%) in oxygen enriched air was delivered via a customized nose cone with a continuous intra-muscular infusion of DEX (0.03 mg·kg^{-1}·h^{-1}). After the anatomical localization scans were acquired, the ISO concentration was decreased to 0.20% to 0.25% with a respiration rate maintained at 60 to 85/min. In the AC group, the rats were all anesthetized using a single dose of AC (60 mg/kg, i.v.) and an additional bolus of 30 mg/kg every single hour. For rats in group 2, when the respiration rate increased to 90/min, the ISO concentration was adjusted to 0.5%. A small animal monitoring system (Model 1025, Small Animal Instruments Inc., New York, NY, USA), comprised of a rectal temperature probe, respiration pneumonic sensor, and fiber optic oximetry sensor or cardiogram electrodes, was used for real time monitoring. Core body temperature was maintained at 37°C via a warm water circulation system.

After scanning, the rats were sacrificed using ISO (5%) for 5 min following an intravenous injection of potassium chloride via the tail vein.

Magnetic resonance imaging (MRI)

The animal MRI measurements were performed using the 7.0T Bruker Pharmascan System (70/16 PharmaScan, Bruker Biopsin GmbH, Germany), operated via the ParaVision 5.1 software (Bruker Corporation). The same coils, including a rat brain surface coil and a quadrature resonator volume coil, were used in all the rats.

Anatomical images (T2WI) were acquired with fast-spin-echo sequence using TurboRARE with following parameters: repetition time (TR) 5000.0 ms, echo time (TE) 36.0 ms, echo spacing 12 ms, echo-train length 8, field of view 3.50×3.50 cm, matrix size 256×256, and 28 slices with a thickness of 1.0 mm. For BOLD images, EPI-SE-FOVsat sequence was used with following parameters: matrix size 64×64, flip angle = 90°, resolution = 0.55×0.55 mm, 28 slices with a thickness of 1.0 mm, slice gap = 0, TR = 2000.0 ms, TE = 18.0 ms, volume = 180.

Data processing and analysis

The data were pre-processed using spmratIHEP software based on the statistical parametric mapping (SPM12) software (Welcome Department of Imaging Science; http://www.fil.ion.ucl.ac.uk/spm) and resting-state fMRI Data Analysis Toolkit (REST) software (http://restfmri.net/Forum/Index.php? q=rest), and statistically analyzed by spmratIHEP[32,33] based on SPM12. The data were carefully examined for completeness and truncation artifacts. The FC values were analyzed and compared between the ISO group, ISO + DEX group, and AC group (using the ISO + DEX group as control). All the functional images post-processing was performed by a single experienced observer, unaware to whom the scans belonged.

The voxel size of the functional datasets of all individuals was first multiplied by a factor of 5 to better approximate human dimensions, and then pre-processed using the following main steps: (1) Slice timing: the differences of slice acquisition times of each individual were corrected using slice timing. (2) Re-align: the temporal processed volumes of each subject were re-aligned to the first volume to remove any head motion, and a mean image was created over the 180 re-aligned volumes. All participants had less than 1 mm of translation in the x, y, or z axis and a 1° of rotation in each axis. (3) Spatial normalization: the re-aligned volumes were spatially standardized into the Paxinos & Watson space[34] by normalizing with the EPI template via their corresponding mean image. Subsequently, all the normalized images were re-sliced by $1.0 \times 1.5 \times 1.0$ mm^3 voxels (after zooming). (4) Smooth: the normalized functional series were smoothed with a Gaussian kernel of 2 mm^3 full width at half-maximum. (5) Removal of the linear trend: the smoothed images had any systematic drift or trend removed using a linear model. (6) Filtering: the band-pass was filtered at 0.01 to 0.08 Hz as the physiological spontaneous BOLD fluctuation mainly focus on this band and also to remove the very low frequency drift and high frequency noise. The pre-processed images were analyzed within spmratIHEP in SPM12 based on the framework of the general linear model.

The FC analysis was performed by REST software. Pearson correlation was computed between each voxel of the RSC and the other intercranial voxels to obtain an FC map for each rat. To identify differences in the FC maps between groups 1, 2, and 3, a one-way analysis of variance (ANOVA) and post-hoc two-sample t tests were performed. Regions with significant FC changes between each two groups were yielded based on a voxel-level height threshold of $P < 0.001$ (uncorrected) and a cluster-extent threshold of 20 voxels.

Statistical analysis

Data were analyzed by SPSS for windows (Version 18.0, IBM Corp., Armonk, NY, USA). Physiological factors during rs-fMRI and rats' behavioral data were all tested for homogeneity of variances and analyzed for normal distribution. The physiological factors were analyzed by one-way ANOVA followed by Dunnett *post hoc* analysis (for data in homogeneity) or Kruskal-Wallis test followed with Mann-Whitney U test as *post hoc* analysis (for data in heterogeneity). For rats' behaviors over the 5-day training period, data were analyzed by ANOVA for repeated measurements followed by Dunnett multiple comparisons. For the probe trial on day 7, the data were analyzed by one-way ANOVA followed by Dunnett *post hoc* analysis. P value <0.05 was considered statistically significant. All group level values are presented as mean $\pm$ standard errors of mean.

Results

The physiology of anesthetized rats was carefully controlled. The use of ISO + DEX caused significant bradycardia (H = 15.825, $P < 0.01$, Kruskal-Wallis test followed by Mann-Whitney U test) [Table 1] and low respiration rate ($F = 4.700$, $P = 0.021$, one-way ANOVA followed by Dunnett *post hoc* analysis) [Table 1]. These effects could be considered as normal effects of DEX.

No significant differences in behaviors were found before rs-fMRI

Considering that the aging process may impair cognitive function and influence brain activities, the MWM test was used to assess the capacity of the rats for further testing. All rats completed the MWM study within the 5-day training period; none was excluded from the study. No significant differences were observed in rats' performances during the training period ($F = 2.664$, $P = 0.075$, ANOVA for repeated measurements) [Figure 1B], indicating that all rats effectively completed the training. For the probe trial on day 7, no significant differences were found in percentage of time spent in target quadrant ($F = 1.453$, $P = 0.256$, one-way ANOVA) [Figure 1C] and number of crossovers to the target quadrant ($F = 1.792$, $P = 0.191$, one-way ANOVA) [Figure 1D]. The results suggested that baseline learning and spatial working memory in all rats included in this study were consistent.

Differences in FC induced by ISO vs. ISO + DEX in whole brain analysis

Compared with ISO + DEX, the ISO caused differences in FC of the aged rat brain mainly resulted in four clusters (one-way ANOVA, $F = 15.27$, $P < 0.001$). All of the clusters located in the middle and posterior part of the aged rat brain (Bregma: −1.7979, −2.2779, −4.1979, and −7.0779, $P < 0.001$, cluster size 20, Figure 2, respectively). Using ISO + DEX as control, areas with increased FC mainly located at the posterior part while decreased FC located at the middle part in whole brain analysis [Table 2]. In the sub-regions, only left temporal association cortex (L-TeA, cluster 4) is a component of the rat DMN.

Differences in FC induced by AC vs. ISO + DEX in whole brain analysis

The AC caused differences in FC of the aged rat brain mainly resulted in six clusters compared with ISO + DEX (one-way ANOVA, $F = 15.27$, $P < 0.001$). Five clusters were located in the middle and posterior part of the aged rat brain (Bregma: 0.1221, −4.1979, −4.6779, −5.6379, and 5.6379, $P < 0.001$, cluster size 20, Figure 3, respectively) and one was located in the cerebellum (Bregma: −12.8379). Using ISO + DEX as control, areas with decreased FC were all located in the brain while increased FC was located at cerebellum in whole brain analysis [Table 3]. In the sub-regions, hippocampus (Hip, clusters 2 and 3) and RSC (cluster 5) are components of the rat DMN.

Table 1: Physiological factors during the BOLD signal acquisition.

Protocols	Weight (g)	HR (beats/min)	RR (/min)	SpO$_2$ (%)
ISO	739.76 ± 29.29	313 ± 8	72 ± 2	99.5 ± 0.3
AC	773.05 ± 29.19	341 ± 14	77 ± 2	99.3 ± 0.3
ISO + DEX	732.85 ± 33.16	235 ± 2	65 ± 1	98.9 ± 0.4
Statistics	0.493[*]	15.825[†]	4.700[*]	0.905[*]
P values	0.618	<0.01	0.021	0.42

Data are presented as mean ± standard errors of mean. HR, RR, and SpO$_2$ were defined as the mean value during the BOLD signal acquisition. All the data were tested for normal distribution. Using ISO + DEX as control, HR was analyzed by Kruskal-Wallis test followed by Mann-Whitney U test as *post hoc* analysis while the others were analyzed by one-way ANOVA followed by Dunnett *pot hoc* analysis. $P < 0.05$ was considered as statistically significant. [*]F value; [†]H value. BOLD: Blood-oxygen-level-dependent; HR: Heart rate; RR: Respiratory rate; SpO$_2$: Percutaneous oxygen saturation; ISO: Isoflurane; AC: α-Chloralose; DEX: Dexmedetomidine.
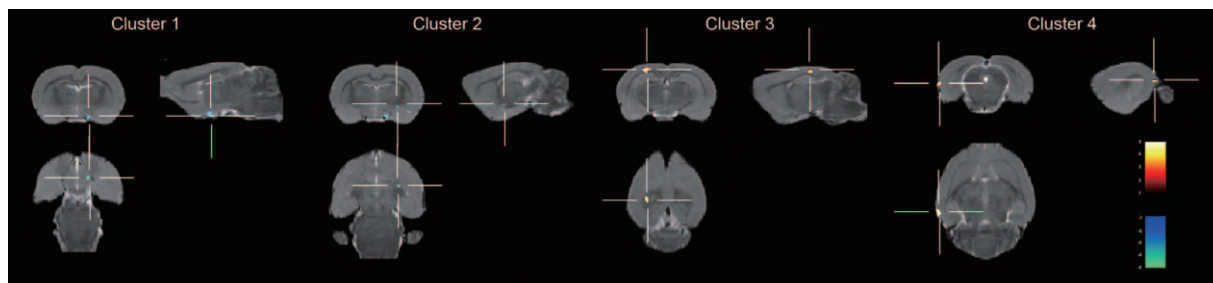
Figure 2: Differences in FC between ISO and ISO + DEX groups. The red color indicated main FC increased while blue color indicated main FC decreased in regions mentioned in Table 2, as compared with ISO + DEX group. DEX: Dexmedetomidine; FC: Functional connectivity; ISO: Isoflurane.

Table 2: Difference of FC in the whole brain analysis between ISO and ISO + DEX groups.

Cluster (Total)	Sub-regions	Cluster size	t value	Paxino atlas		
				x	y	z
Cluster 1	–	80	−5.0539	1.3541	8.3350	−1.7979
	R-hypothalamus	80	−5.0539	1.3541	8.3350	−1.7979
Cluster 2	–	20	−4.2600	4.8380	7.8529	−2.2779
	R-amygdaloid body	18	−4.2600	4.8380	7.8529	−2.2779
Cluster 3	–	216	4.5586	−3.5607	0.6687	−4.1979
	L-parietal association cortex	20	4.2386	−3.5673	0.6261	−3.7179
	L-parietal cortex posterior area	9	4.0850	−3.9586	0.6457	−4.1979
	L-visual cortex	186	4.5586	−3.5607	0.6687	−4.1979
Cluster 4	–	137	4.7969	−7.2579	4.3545	−7.0779
	L-auditory cortex	39	4.4197	−7.2645	4.1703	−6.5979
	L-temporal association cortex	76	4.7969	−7.2579	4.3545	−7.0779

The regions of interest were drawn according to the rat anatomic atlas (Paxino and Watson, 4th edition). The t values were the maximum values of the two-sample t tests for the ROIs with statistical significance (showing greatest statistical significance within a cluster). A positive t value means increased FC while a negative t value means the opposite. Comparison was performed for ISO and ISO + DEX groups, with the ISO + DEX group as a control. $P < 0.001$ and a cluster size of 20 was considered as statistically significant. FC: Functional connectivity; ISO: Isoflurane; DEX: Dexmedetomidine; R: Right; L: Left.

Discussion

This study demonstrated that both anesthesia methods (ISO and AC) had significant influences on aged rat brain as compared with ISO + DEX while AC seemed to have a more significant influence, since AC anesthesia affected FC in a larger number of brain areas than ISO anesthesia.

The MWM test was performed in aged rats before rs-fMRI scanning because aging is correlated with cognitive dysfunction[35] and FC change.[36] Meanwhile, cognitive function is also associated with FC, as previously mentioned. Consistent cognition baseline is essential for whole brain analysis and further statistical analysis. In this study, no statistically significant differences were observed in MWM behavioral performances in acquisition and probe trials, indicating consistent baseline cognition in the rats of all three groups. High quality data are also essential for further analysis. A spin-echo BOLD sequence was used because the signal suffers less from motion artifacts and physiological noise,[37] and it has a better capillary level specificity in high magnetic fields.[38] Because the BOLD signal is also dependent on various physiological factors, mainly the concentration of deoxyhemoglobin in the blood,[1] heart rate, respiratory rate, and SpO_2 were monitored. No significant differences were observed in these parameters between the three groups, indicating that the influence of physiological factors on the BOLD signal was minimized.

ISO (1.0%) is a commonly used anesthetic in rs-fMRI data acquisition. In the adult rat brain, anesthetic does of ISO (1.5%–2.5%) induce synchronous cortico-striatal fluctuations and suppresses subcortical activity, although these side effects can be minimized by reducing the inhaled concentration.[18] In our study, compared with ISO + DEX, the use of low dose ISO inhalation (3% for induction and 1%–1.3% for maintenance) resulted in FC differences in the brain, while the cerebellum was not affected. Published data have suggested that ISO inhalation results in increased FC in the fronto-cortical regions and heavily suppressed thalamo-cortical, subcortical and intra-subcortical connections compared with the awake data.[14,19,39,40] Considering the sub-regions in each cluster, our study showed decreased FC for subcortical ROIs (clusters 1 and 2) and increased FC for cortical ROIs (clusters 3 and 4), which is consistent with previous findings.[14,19] However, for other cortical and subcortical ROIs, whole brain analysis showed no significant differences. In an ROI-wised analysis, ISO induced higher FC in both cortical and subcortical regions compared with combined ISO + DEX, although they used a higher
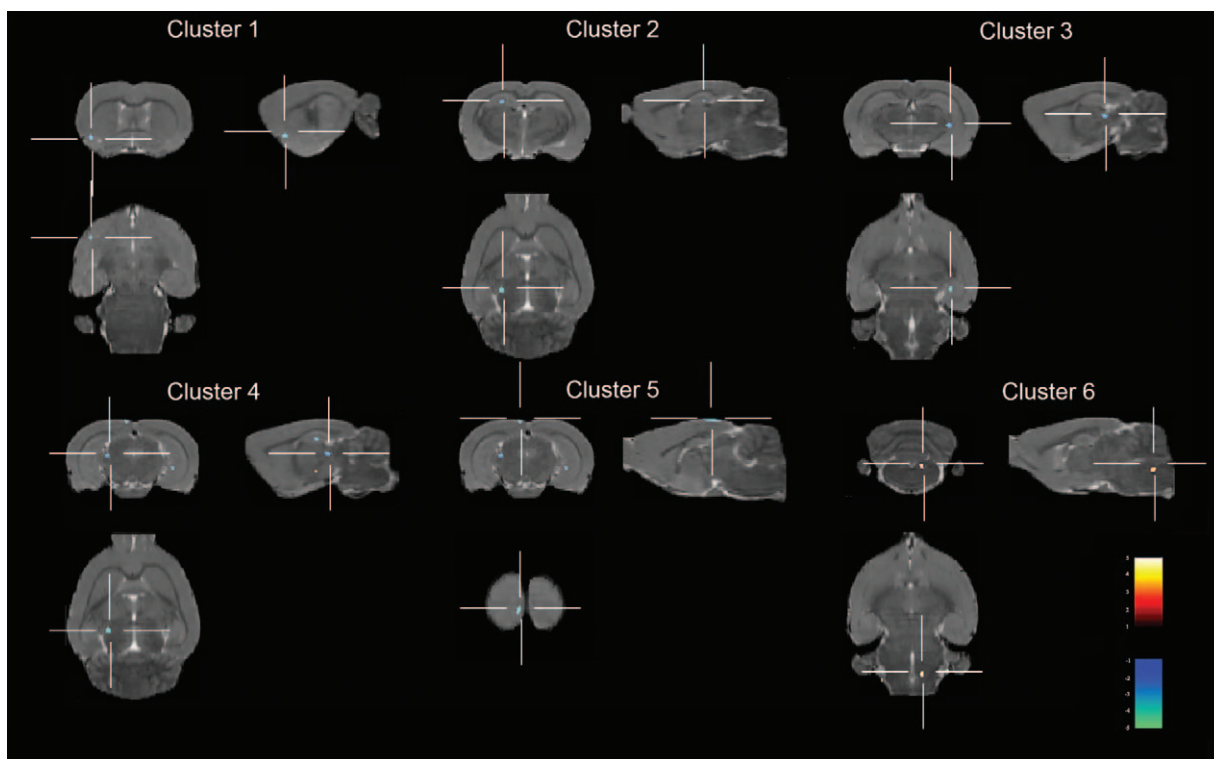
Figure 3: Differences in FC between AC and ISO + DEX groups. The red color indicated main FC increased while blue color indicated main FC decreased in regions mentioned in Table 3, as compared with ISO + DEX group. AC: α-Chloralose; DEX: Dexmedetomidine; FC: Functional connectivity; ISO: Isoflurane.

Table 3: Difference of FC in the whole brain analysis between AC and ISO + DEX groups.

Cluster (Total)	Sub-regions	Cluster size	t value	Paxino atlas x	y	z
Cluster 1	–	30	−4.7713	−5.2290	6.4758	0.1221
	L-claustral layer	18	−4.7713	−5.2290	6.4758	0.1221
	L-insular cortex	4	−4.5037	−5.2356	6.4332	0.6021
	L-piriform cortex	8	−4.4802	−5.0972	6.5828	0.6021
Cluster 2	–	30	−4.1626	−2.7649	1.8829	−4.1979
	L-hippocampus	30	−4.1626	−2.7649	1.8829	−4.1979
Cluster 3	–	84	−4.3381	4.1963	5.6905	−4.6779
	R-thalamus lateral nucleus group	32	−4.3381	4.1963	5.6905	−4.6779
	R-hippocampus	52	−4.2948	4.3413	5.7411	−5.1579
	R-supraoptic region	5	−4.3356	4.3347	5.6985	−4.6779
Cluster 4	–	39	−4.7468	−3.1430	4.0409	−5.6379
	L-thalamus lateral nucleus group	39	−4.2085	−3.2814	4.1745	−5.6379
Cluster 5	–	66	−5.4829	−0.7383	0.2097	−5.6379
	L-retrosplenial cortex	46	−5.2766	−0.8767	0.0761	−5.6379
Cluster 6	–	26	4.3959	1.1080	6.9554	−12.8379
	R-posterior lobe of cerebellum	9	4.3959	1.1080	6.9554	−12.8379
	R-medulla oblongata	3	4.1285	1.1080	7.2563	−12.8379
	R-tegmentum of pons	14	4.3608	1.2291	7.1040	−12.8379

The regions of interest were drawn according to the rat anatomic atlas (Paxino and Watson, 4th edition). The t values were the maximum values of the two-sample t tests for the ROIs with statistical significance (showing greatest statistical significance within a cluster). A positive t value means increased FC while a negative t value means the opposite. Comparison was performed for AC and ISO + DEX groups, with the ISO + DEX group as a control. $P < 0.001$ and a cluster size of 20 was considered as statistically significant. FC: Functional connectivity; AC: α-Chloralose ISO: Isoflurane; DEX: Dexmedetomidine; R: Right; L: Left.

concentration (1.3% *vs.* 1%).[14] This difference may attribute to the aging process. As the minimum alveolar concentration of inhalation anesthetics can decrease with aging,[41] a lower dose of ISO may be sufficient during scanning. Moreover, ISO suppresses more activities in the cortical and subcortical regions compared with similar

concentration in adult rat. The TeA (cluster 4) is an important component of DMN in rats,[20] indicating this type of anesthesia may also disrupt DMN connectivity, although only one region was affected. Considering that ISO does not affect long-term neurocognitive outcomes in aged rats,[42] it could be used as a substitution for combined ISO + DEX anesthesia when the TeA is not considered as an important outcome.

AC (60 mg/kg) is another commonly used anesthetics during rs-fMRI scanning. Although the specific binding sites remain undefined, intravenous AC infusion is thought to enhance GABA$_A$ receptor activity.[27] With ISO + DEX as control, our study demonstrated that AC anesthesia can cause global suppression in the aged rat brain, which is basically consistent with findings of previous studies.[14,43] However, using an ROI-wised analysis, the above mentioned studies demonstrated higher FC in whole brain analysis when compared with combined ISO + DEX anesthesia, which is not consistent with our findings. Published data have shown that the level of GABA receptors decreases during aging,[44,45] the aged rat brain should show a theoretically higher FC with AC anesthesia when compared with combined ISO + DEX. Few studies have focused on the levels and responsiveness of alpha-2 receptor in aging. Considering our results, we hypothesize that alpha-2 receptor levels also decreased with aging. Thus, the efficacy of DEX was reduced, resulting in an abnormal higher FC compared with AC anesthesia. Notably, in the whole brain analysis, the cerebellum had a high FC (cluster 6). Human studies have found that the ability to modulate GABA$_A$-nergic inhibition appears to related to motor ability in older adults.[46,47] Considering that the cerebellum is an efferent system that processes information from the brain and participates in motor function, we hypothesize that an increase in FC maybe a compensatory effect of the global suppression caused by AC.

In whole brain analysis, both the Hip (clusters 2 and 3) and RSC (cluster 5) are significant regions in rat DMN. Meanwhile, when this type of anesthesia was used, decreased FC was also observed in other cortical (cluster 1) and subcortical (cluster 4) regions. Thus, the result indicates this type of anesthesia may globally suppress FC network structures as well as a more obvious DMN disruption in aged rat brain.

The current study has several limitations. First, awake data was not provided for comparison. Although several studies have mentioned the use of awake data,[13,14] however, this method may be time consuming (training for up to 8 days) and labor intensive. Moreover, awake data requires greater scan volume to minimize head motion artifacts and other physiological noise compared with animals anesthetized.[14] Related to this, the rs-fMRI scanning parameters might differ among groups, making comparison challenging. Since data from combined ISO + DEX anesthesia has been shown to better resemble awake data compared with several other commonly used anesthetics and preserves DMN connectivity,[14,20] this method was used as control. Second, no adult rats were used for further analysis. The physiological parameters of aged rats (eg,

body shape and weight) differ from adult rats. The simultaneous use of the same scanning parameter is not suitable for both aged and adult rat, making the result comparison challenging. Furthermore, the effects of anesthetics are highly associated with aging,[48] thus the FC is difficult to compare under the same dose of anesthetics among aged and adult rats. Third, although the physiological parameters were strictly controlled and behavior performance was measured before rs-fMRI scanning, there might be other factors affecting baseline cognition in aged rats.

Conclusions

Compared with ISO + DEX, ISO anesthesia caused increased FC in posterior brain area and decreased FC in middle brain area of aged rats while AC anesthesia caused global suppression. However, the effect of AC on FC was more significant compared with that of ISO. The L-TeA was the only component of rat DMN that exhibited significant FC change. ISO could be used as a substitute for ISO + DEX in DMN related studies if the L-TeA is not considered an important outcome.

Conflicts of interest

None.

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