Research Article

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LoudNoise Exposure during Activity and Neurogenesis in the Living Rat Brain: Preliminary Study

Yoshiyuki Shimizu[,2], Shigeyuki Yamamoto[2], Dai Fukumoto[2], Hiroyuki Ohba[2], Takeharu Kakiuchi[2], Shingo Nishiyama[2], Etsuji Yoshikawa[2], Hideo Tsukada[2], Hiroyuki Okada[2] and Yasuomi Ouchi[1*]

[1]Department of Biofunctional Imaging, Medical Photonics Research Center, Hamamatsu University School of Medicine, Hamamatsu, Japan

[2]Central Research Laboratory, Hamamatsu Photonics KK, Hamamatsu, Japan,

*Corresponding author: Yasuomi Ouchi , Department of Biofunctional Imaging, Medical Photonics Research Center, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192, Japan, Tel: 053-435-2466; Fax: 053-435-2466; E-mail: ouchi@hama-med.ac.jp

Abstract

Objective: The effects of loud noise (80 dB) on neurogenesis in the sub-ventricular zone (SVZ) and the hippocampal sub-granular zone (SGZ) was investigated *in vivo* in the rat brain using positron emission tomography (PET) with 3'-deoxy-3'- [18F]fluoro-L-thymidine ([18F]FLT), a marker for thymidine kinase I (TK-1) related to cell proliferation and for neurogenesis in SVZ and SGZ.

Methods: Five young Sprague-Dawley rats were exposed to loud noise from two speakers set in front of their cages during the night for 4 nights, and 5 control rats were exposed to normal quiet conditions. PET was performed 4 days after the exposure.

Results: The accumulation of [18F] FLT in SVZ was significantly lower in the rats with noise exposure than in the control rats. The level of [18F] FLT uptake was lower in the SVZ than in the SGZ.

Conclusion: Our results indicate that loud noise exposure during the awake period may be related to the decrease in proliferative capacity of neural stem cells especially in the SVZ of the living rat brain. This finding may indicate that persistent loud noise during daily life is harmful to brain development in humans.

Keywords Neurogenesis; Noise; Rat; [18F] FLT; Positron emission tomography; Sub-ventricular zone; Hippocampal sub-granular zone

Introduction

It is widely accepted that neurogenesis occurs in the sub-granular zone (SGZ) of the hippocampal dentate gyrus and sub-ventricular zone (SVZ) adjacent to the lateral ventricles in the living rodent brain [,2]. The implications of neurogenesis are still hotly debated, but a common idea is that neural stem cells (NSCs) play critical roles in maturing the brain during the developmental process [3] and repairing the damaged brain after brain insults such as stroke, brain injury and neurodegeneration [4-6]. Accordingly, enhancement of neurogenesis is considered to be important in maturing and nurturing the rodent brain by growing in environmentally rich conditions [7] and performing motor and cognitive exercises [8]. In contrast, as expected, adverse effects on the brain such as isolation, poor environment and various types of stress can reduce neurogenesis [9,10]. Among these positive and negative changes in neurogenesis, various noise effects have been widely studied because noise is both an early and inevitably perceived stimulation during the fetal period and during the rest of life. Recent animal experiments on the effects of noise on the rodent brain showed that noise overstimulation could cause a long-lasting suppression of neurogenesis in the hippocampus of adult rats [11] along with disturbed cognitive function [12]. To date, all animal studies on neurogenesis, except for one [13], have required the sacrifice of the animals to perform immunohistochemical examinations and therefore focused on limited regions such as the dentate gyrus in the hippocampus. The advantage of positron emission tomography (PET) with 3'-deoxy-3'- [18F] fluoro-L-thymidine ([18F]FLT) is the capability of evaluating neurogenesis in the entire living brain simultaneously as described previously [13]. In addition, there has been no study on the effect of noise during activity on NSCs in SVZ and SGZ; such findings can be extrapolated to the situation of noise stress (not insomnia stress) in people who work in large urban centers [14]. Here we report the *in vivo* changes in neurogenesis in the SVZ and SGZ of rat brain in response to noise stress during the night, which is activity time period for rat, using PET with [18F] FLT.

Materials and Methods

Our study partially conformed to the RIGOR guidelines [15,16] in terms of control group and statistics. However, because of the nature of our study (small sample and open-label), it was not involved with the issues such as blinding of the study, randomization of treatment groups, and power analysis, which are all important for preclinical and clinical research.
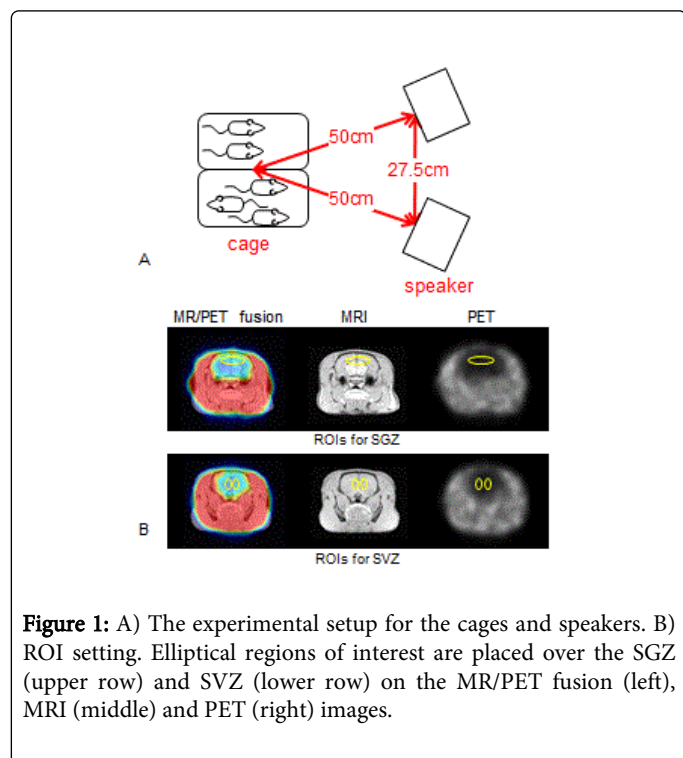
Animals

The following experiments were approved by the Ethical Committee of the Central Research Laboratory, Hamamatsu Photonics. Ten male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) weighing 230-250 g were included. These rats were arbitrarily divided into two groups: a noise group (n=5) and a control group

(n=5). The rats were housed in the laboratory with free access to food and water and were maintained on a 12-h dark/light cycle in a room with controlled temperature (23-25°C) and humidity (39-52%).

Noise exposure

Five animals in the noise group were moved from the housing cage into a sound isolation box and were housed there for 14 hours per day under dark conditions during which nocturnal animals such as rats display high locomotion activity (Figure 1A). The loud music (Symphonic paradise, Akira Miyagawa) was administered to all five rats through the speakers for 6 hours. In contrast, the other five animals in the control group spent 4 days similarly to the experimental condition without noise exposure before PET evaluation. Because the exploratory activity of rats increases markedly immediately after they are placed in a new environment [17], we observed the behavior of three rats randomly selected from both groups and determined the back-and-forth locomotion during the one hour after their transfer to a different cage on the first experimental day. The music was presented with a sample rate of 192,000 per second. Spectral analysis showed a peak at 0.38 kHz. More than 99% of the power came under less than 20 kHz. The intensity of the music was approximately 80 dB (A) on average in each cage.

Figure 1: A) The experimental setup for the cages and speakers. B) ROI setting. Elliptical regions of interest are placed over the SGZ (upper row) and SVZ (lower row) on the MR/PET fusion (left), MRI (middle) and PET (right) images.
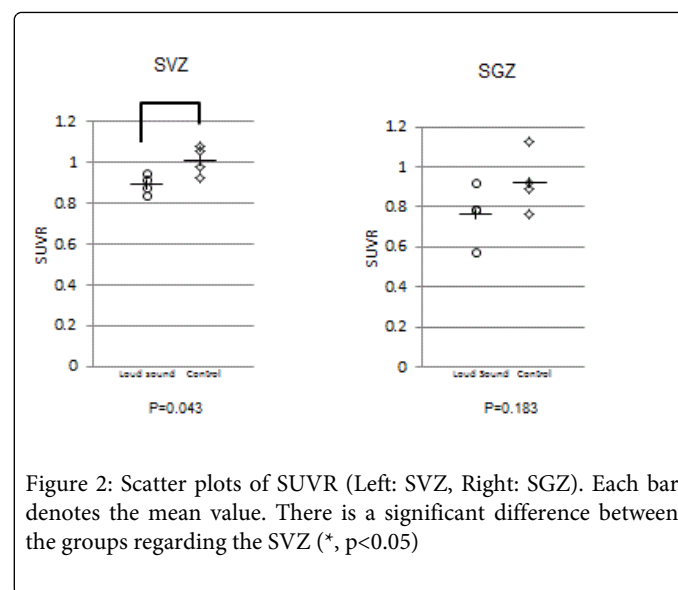
PET procedure

We used a high-resolution PET scanner for animal use (Clairvivo PET, Shimadzu Corporation, Kyoto, Japan) equipped with 107 detector rings producing 213 slice imaging. This scanner consists of depth of interaction detector modules with an axial field of view (FOV) of 151 mm and a transaxial FOV of 102 mm. The spatial resolution at the center was 1.54 mm horizontally and 1.47 mm axially at full width at half maximum. The details of this PET system were reported elsewhere [18].

To quantify the *in vivo* proliferation of NSCs in both niches, the SVZ and the SGZ, PET with [18F] FLT [13] was performed under anesthetic conditions. Rats, anesthetized and maintained with 2% isoflurane in a mixture of O2, were placed in a prone position on a fixation plate and then placed in the gantry hole of the PET scanner. X-CT images were obtained using a Clairvivo CT (Shimadzu Corporation, Kyoto, Japan) just before the PET measurements. Some of the rats were also scanned using 3T MRI (Signa HDxt, GE Healthcare, Milwaukee, WI, USA) with a rat-purpose coil for anatomical information. After setting a rat on the PET table, we injected 8.0 MBq of [18F] FLT as a bolus via the tail vein. The data were acquired in list-mode format from 60 to 75 min after injection of [18F] FLT. The list mode was reconstructed with a dynamic row-action maximum-likelihood algorithm (DRAMA) with efficiency, scattering, count losses, and decay correction. During PET examination, body temperature was kept constant using a hot mat (34°C).

Data analysis and statistics

For image co-registration of X-CT and PET data, the image analysis software PMOD (PMOD Technologies LTD, Zurich, Switzerland) and Image-J (National Institutes of Health, Bethesda, MD, USA) were used. As described elsewhere [19], the elliptical regions of interest (ROIs), ranging from 32-96 mm2 wide, were symmetrically placed bilaterally on the regions covering the SVZ (2.2 to -0.6 mm in relation to bregma) and the SGZ (-4.1 to -5.5 mm) and on the cerebellum (-10.4 to -11.8 mm) based on the CT data [20] (Figure1B). A standard uptake value (SUV) was calculated for each ROI, dividing the average ROI activity by the decay-corrected injected radioactive dose per body weight as an index of [18F] FLT uptake in the brain. To investigate the level of specific accumulation in the target region in each animal, the ratio index (SUVR) was estimated by dividing the target SUVs (SVZ and SVG) by the cerebellar SUV. The levels of the averaged SUVs and SUVRs were compared between the two groups using t-test. Statistical significance was assumed at p values less than 0.05.

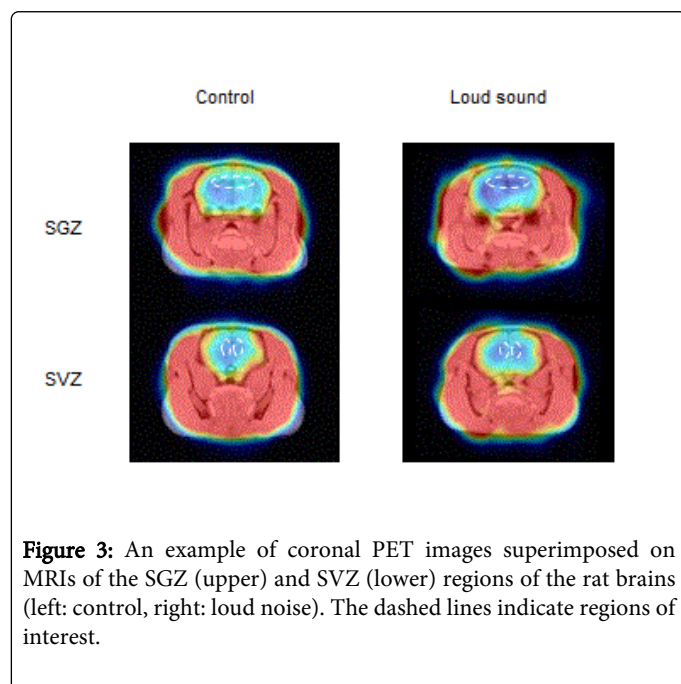Figure 2: Scatter plots of SUVR (Left: SVZ, Right: SGZ). Each bar denotes the mean value. There is a significant difference between the groups regarding the SVZ (*, p<0.05)

		[18F]FLTSUVR	
Group	region	mean	sd

Loud	SVZ	0.8935*	0.0461
Noise	SGZ	0.7674	0.1444
Control	SVZ	1.0081	0.0721
	SGZ	0.9237	0.1495

Table 1: SUVR: standard uptake value ratio; *: p<0.05: noise group vs. control group

Results

Assuming the cerebellum as the reference region for neurogenesis, we estimated the uptake of [18F]FLT in the target region as the SUVR. The T-test showed significant reduction (p<0.05) in the SUVR of the SVZ in the loud noise group compared with the SUVR of the SVZ in the control group (Table , Figure 2). However, the SUVR in the SGZ of the loud noise group was not significantly lower than that in the control group.

The behavioral observation of animals during the one hour after their transfer to a different cage on the first experimental day showed a reduction in the frequency of crossing the centerline of the cage with back and forth locomotion in the loud noise group (10.7 times on average) compared with the control rats (15.3 times), although the difference was not significant (Figure 3).

Figure 3: An example of coronal PET images superimposed on MRIs of the SGZ (upper) and SVZ (lower) regions of the rat brains (left: control, right: loud noise). The dashed lines indicate regions of interest.

Discussion

The present study is the first to show a significant reduction in [18F]FLT uptake in the SVZ in living rat brains of the loud noise group, indicating that loud noise exposure during an activity period could suppress neurogenesis in the SVZ. Compatible with the previous finding of an *ex vivo* study [21], our findings provide *in vivo* evidence of a loud noise-induced negative effect on neurogenesis in the living brain on a molecular basis. Consistent with the open field test finding that stress reduces the locomotive activity of rats [22], animals with

reduced neurogenesis were more likely to be less active than rats exposed to the quiet conditions in the present study. Thus, loud noise exposure during the awake state not only alters behavior but also affects NSC proliferation in the brain.

This negative effect of noise on neurogenesis has already been suggested in a noise stress study showing that noise-induced stress reduced the proliferation of neuroblasts (Type-1 and Type-2 cells) and increased neuroblast apoptosis (Type-1) [21] and in a silent vs. noisy circumstance study that showed that silence yielded a better neurogenesis outcome in the rat SGZ [23]. In the latter study, while all types of noise stimuli with different amplitudes plus silence except for white noise increased precursor cell proliferation initially, only silence remained associated with increased numbers of BrdU-labeled cells after 7 days of the experiment. In the present study, we performed PET measurement 5 days after the noise stimulation, when it is considered that the number of temporally proliferated NSCs would have been decreasing [23]. A possible mechanism of the reduced neurogenesis in the hippocampal SGZ under noise stress is that an elevation of blood corticosterone level by stress-induced activation ofhippocampal-pituitary-adrenal (HPA) axis could disrupt the NSC proliferation in the SGZ [21]. As mentioned in the introduction, a great advantage of PET is the capability of simultaneous detection of neurogenesis in any brain region in a single scan. In the present study, we focused on two different niches, the SVZ and the SGZ, where the pattern of [18F]FLT uptake in the SVZ was lower than in the SGZ. Because the NSCs in the SVZ migrate to the olfactory and frontal lobe regions [24], it is likely that the SVZ NSCs are implicated in the neuronal control of emotion, attention and higher brain functions. In this regard, the present loud noise-induced suppression of neurogenesis in the SVZ as well as the SGZ may be a more devastating event during brain development.

In the present study, we used music at an average of 80 dB (A) in volume. There was a report on the relationship between stress and the volume of noise, which showed that noise greater than 85 dB (A) increased the ACTH content and that 80 dB (A) noise induced an elevation of c-fos mRNA in some parts of the rat brain [25]. The duration of noise exposure in this study was only 30 minutes, far shorter than our 6 hours of stimulation per day. It was shown that the longer stimulation generates more stress to affect neurogenesis [21]. Because noise as low as 70 dB SPL (not A-weighting) altered hippocampal neurogenesis [23], the volume of the present noise was considered sufficient to induce stressful noise for the animals. It has also been reported that the range of good hearing of rats can reach as low as 8 kHz and as high as 42 kHz [26]. Therefore, our music at a frequency more than 20 kHz might have produced more stressful stimulation to the rats.

The present results can be extrapolated to the neurogenesis in the human brain, which may be a matter of significance beyond noise-induced auditory dysfunction. In the present study, the neurogenesis in the SVZ was more affected by the loud noise compared to the neurogenesis in the SGZ, suggesting that the NSCs in the SVZ might be more vulnerable to the noise stress. While SGZ neurogenesis is implicated in memory function, SVZ neurogenesis might be related to frontal functions such as attention and behavior. In the 6-OHDA induced Parkisonian rat model, paramipexole, which is a possible enhancer of neurogenesis, improved the reduction of NSC proliferation in the SVZ and improved behavior [27]. It was shown that in a human MRI study, the signal pattern of diffusion-weighted images in the SVZ region was different between patients with dementia and normal elderly adults [28]. Thus, again, persistent

exposure to loud noise could exert an unfavorable influence on NSC growth in the SVZ, resulting in executive dysfunction.

In conclusion, the present *in vivo* PET study showed that loud noise exposure during activity caused a negative effect on neurogenesis in the SVZ of rat brain. This suppressed neurogenesis might exert more or less adverse effects on behavioral activity in rodents.

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