A [14C]2-deoxy-D-glucose study of brain structures related to conditioned emotional response in the rat.

Sugioka, K

Yamadori, T

(Citation)
The Kobe journal of the medical sciences,38(4):255-270

(Issue Date)
1992

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/E0034072

Kobe J. Med. Sci. 38, 255/270, August 1992

A [^{14}C]2-DEOXY-D-GLUCOSE STUDY OF BRAIN STRUCTURES RELATED TO CONDITIONED EMOTIONAL RESPONSE IN THE RAT

KOZO SUGIOKA AND TAKASHI YAMADORI

First Division, Department of Anatomy
Kobe University School of Medicine

INDEXING WORDS

[^{14}C]2-DG method; conditioned emotional response; anterior cingulate gyrus; prefrontal cortex; rat

SYNOPSIS

We used [^{14}C]2-deoxy-D-glucose (2-DG) to determine activated brain structures related to conditioned emotional response (CER) in rats. The experimental groups were conditioned with paired conditioned-stimulus (CS; flickering light and clicking sound) and unconditioned-stimulus (US; foot-shock) for either 25 or 50 trials. The control groups were also exposed to the same stimuli but in unpaired or random sequence. Two days after conditioning, rats were intravenously injected with [^{14}C]2-DG and then exposed to the CS alone (CER test) in a shock box. Mean optical densities of 44 brain structures were measured with an autoradiogram, and their optical density ratios were compared by 2-by-2 (paired vs unpaired and 25 vs 50 trials) analysis of variance. Those brain structures were of 2 types; the first type showed similar changes of 2-DG uptake in both paired and unpaired groups (Areas 7 and 40 of the cerebral cortex, the habenula and the colliculus inferior), while the second type showed that 2-DG uptake increased in the paired groups but decreased in the unpaired groups (Areas 24, 10, 6, 4 and 3 of the cerebral cortex), as a function of number of trials. Because changes of 2-DG uptake in the first type structures and in Areas 3, 4 and 6 of the second type structures are regarded to reflect learning-nonspecific effects and task- or stimuli-related symmetrical activation, respectively, we concluded that Areas 24 (anterior cingulate cortex) and 10 (prefrontal cortex) were specifically related to conditioned emotional response.

Received for publication : August 24, 1992
Authors' name in Japanese : 杉岡幸三, 山鳥　崇

INTRODUCTION

The 2-deoxy-D-glucose (2-DG) autoradiographic technique for detecting local changes in cerebral metabolism, developed by Sokoloff and coworkers[35, 36], has provided a new approach to assess the functional activities of the brain. Many studies have been performed using this technique to determine the neural structures activated during the performance of a given brain function under various conditions[42]. However, this technique has been rarely used in studying the neural basis of the cognitive process, because of the technical difficulties in carrying out quantitative measurements of metabolic changes in freely moving animals, and the methodological difficulties in comparing intergroup 2-DG uptake to distinguish neural activity related to a specific function from that related to non-specific factors[31].

This study was performed to determine which neural structures are related to conditioned- and unconditioned-stimulus (CS-US) association learning using a conditioned emotional response (CER) paradigm with the [^{14}C]2-DG technique. Several previous studies using a similar CER paradigm have shown that increased overall regional cerebral glucose utilization (rCGU) in the brain[5], increased relative rCGU in the central band of the hippocampus[12] and increased regional cerebral blood flow (rCBF) in the medial amygdala and the hypothalamus[20] were found during CS exposure (conditioned fear) after aversive conditioning.

However, it should be noted that these studies compare the values of rCGU or rCBF in conditioned rats with those in unconditioned (unstimulated) rats, or conditioned rats presented with no CS during the CER test. Hence, the differences between the groups in rCGU or rCBF values might be attributed to learning-nonspecific factors, such as sensory, motivational, or locomotive correlates of the cognitive process, rather than to learning-specific factors directly related to the CS-US association itself. Therefore, we used unpaired CS-US presentation groups which received exactly the same stimuli as did the conditioned groups but in random sequence as control groups. Thus, these groups differed only in whether the CS had served as an informative cue preceding US onset. By comparing 2-DG uptake in the paired CS-US group with that in the unpaired CS-US group, it is possible to detect those activated structures specifically related to the CS-US association learning. Furthermore, we also examined whether the 2-DG uptake changed as a function of the duration of the association learning by giving either 25 or 50 trials for both paired and unpaired groups. Comparative data for a paired-25 group and an un-shocked control group exposed only to CS have been reported elsewhere[43].

MATERIALS AND METHODS

Materials

Twenty male albino rats of Jcl: Wistar strain of 350 $\pm$ 15 g mean body weight were used. They were randomly divided into 2 experimental groups (paired-25: n=6; paired-50: n=5) and

2 control groups (unpaired-25: n=4; unpaired-50: n=5). Animals were housed in colony and given food and water *ad libitum.*

Conditioning apparatus

For classical aversive conditioning, a conditioning box (inside dimensions: 460×210×300 mm) made of acrytic plate with a grid floor which consisted of copper rods (3 mm in diameter) spaced 10 mm aparts was used.

The conditioned stimulus (CS) was a compound stimulus which consisted of flickering light (0.5 sec on and 0.5 sec off) from a 10W illumination lamp on the ceiling of the box, and the clicking sound produced by a magnetic relay synchronized with the flickering lamp. The unconditioned stimulus (US) was a mild electric shock (150 Vac, approximately 0.3 mA) delivered to the grid-floor through a shock generator, using a current-limiting resistor (250 Kohm) connected in series with grid-bars.

Surgery

A polyethylene catheter approximately 50 cm long (PE-50, Clay Adams) was inserted into the right external jugular vein under anesthesia by intraperitoneal injection of 10% chloral hydrate (3ml/kg). The other end of the catheter ran subcutaneously, exited at the back of the neck, and was coiled and enclosed in a plastic cylinder (about 50 mm long and 13 mm in diameter) sewn onto the back of the animal. The catheter was filled with 0.9% heparinized saline.

Classical aversive conditioning

Two days after the surgery, classical aversive conditioning was administered to the paired groups. After 3 min of habituation to the experimental box, rats were conditioned either 25 or 50 times (paired-25 and paired-50 experimental groups, respectively) with paired 25-sec-long CS and 1-sec-long US. The interstimulus interval (ISI) was 24 sec, and the mean intertrial interval (ITI) was 50 sec (ranging from 10 to 70 sec). The unpaired groups also received the same stimuli as the paired groups received, but in unpaired (random) sequence for 25 or 50 times (unpaired-25 and unpaired-50 control groups, respectively). Figure1 shows the patterns of CS-US presentation for the paired and unpaired groups.

Conditioned emotional response (CER) test and [^{14}C]2-DG injection

Two days after the conditioning, all groups of rats were exposed 3 times to the CS at 15-min interval in the experimental box for conditioned emotional response (CER) test. No shocks were delivered during the CER test. Immediately before the first CS presentation (3 min after placing the rat in the box), 50μCi of [^{14}C]2-DG (New England Nuclear) in 0.5 ml saline was injected through the free end of the catheter from outside the box. Two min after the

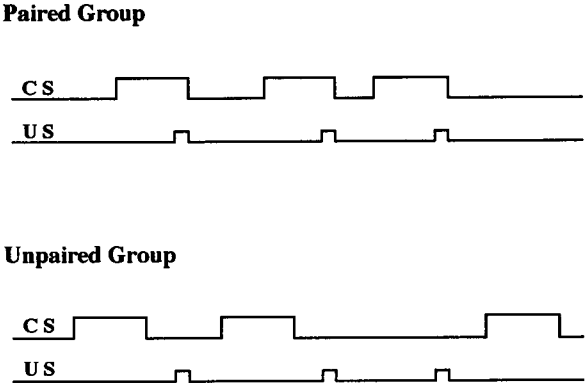

Figure 1 Schema of the CS-US presentations in the paired experimental and unpaired control groups.

presentation of the third CS, the animals was injected with overdoses of 10% chloral hydrate or saturated potassium chloride solution through the catheter to sacrifice immediately.

Behavioral observation

In general, the formation of a CER is quantitatively measured as conditioned suppression of a given operant response, such as lever-pressing in a Skinner-box. However, the establishment or maintenance of such a baseline response might affect metabolic activity of a given brain structure not involved in a CER. For this reason, to verify CER formation, we sequentially recorded the behavior of the rats before, during and after the CS-US presentations (the conditioning session) and CS exposures (the CER-test session).

Autoradiography and microphotodensitometry

Immediately after the CER test (35 min after 2-DG injection), the brain was removed, frozen, stored in cooled liquid Freon (-50℃) for a few days, and cut into serial 20μm-thick frontal sections with a cryostat microtome (-20℃). Every tenth section was prepared for autoradiography, and its adjacent sections were saved for Nissl staining. The sections for autoradiography were placed on a glass cover slip, dried on a hot plate (60℃), affixed to cardboard, and exposed to X-ray film (Fuji RX) in a cassette for 7 days at 4℃. A set of [^{14}C]methylmethacrylate standards (Amersham) was also exposed to the same film. Each film was then developed in Rendol (20℃) for 5 min and fixed in Fujifix (20℃) for 10 min.

Each autoradiogram section was cut out from the developed film and mounted on a glass-

slide. The optical density (OD) of each 200 μ m^2 unit of each section was measured using microphotodensitometer system (Sankei), scanning in the X and Y directions. OD values were stored in a personal computer (NEC 9801) and also displayed on a CRT according to an 8 color-coding system indicating OD value range. The mean ODs for 44 structures in the left hemisphere were computed using a graphic tablet. Demarcation of each structure on the autoradiogram was based on its relationship to adjacent sections stained with Cresylviolet according to Krieg atlas criteria[19] using the method reported by Yamadori et al.[43]

We calculated the optical density ratio (ODR) of each structure by dividing its mean OD by that of the corpus callosum (CC) within the same section (i.e. gray matter-to-white matter ratio). In sections having no CC, we estimated the ODR by dividing the mean OD of each structure by the mean of the CC of the whole brain.

In this study, measurement of local cerebral glucose utilization (LCGU) was not possible because the drawing of blood samples would have hindered the animal's movement during the CER test. Therefore, ODR was adopted to obtain quantitative data. Although some authors (e.g. Sokoloff et al.[37]) have criticized the use of ODR, other authors (e.g. Sharp et al.[33]) have stated that this measurement is useful in the detection of relative changes in brain activity if white-matter OD is constant in all groups.

RESULTS

Behavioral observation

Generally, the observed behaviors were: head-waving, face-washing, sniffing, grooming, walking, backing, rearing, jumping, and freezing (immobility). The latter behavior included sitting-, crouching-, and rearing-freezing.

At the early stage of conditioning, the rats in both paired groups showed escape-like behaviors elicited by the shock treatment during the ISI and ITI, i.e., jumping, walking and/or rearing. However, these behaviors gradually disappeared with repetition of the stimuli, and immobility was predominantly observed during the ITI.

At the late stage of conditioning, the paired-25 group showed transient rearing and walking for a few seconds after the CS presentation, but the freezing behavior predominated during the ISI and ITI. On the other hand, the paired-50 group frequently showed rearing up, walking, or grooming during the ITI. However, these behaviors were rapidly extinguished upon CS presentation, and freezing behavior was predominantly observed during the CS period. That such a behavioral sequence was observed before and after the CS-exposure could be regarded as evidence of CER. During the CER-test session, the rats in both paired groups showed all the same behavioral patterns as observed at the late stage of conditioning.

On the other hand, the rats in both unpaired groups showed different behavioral pattern.

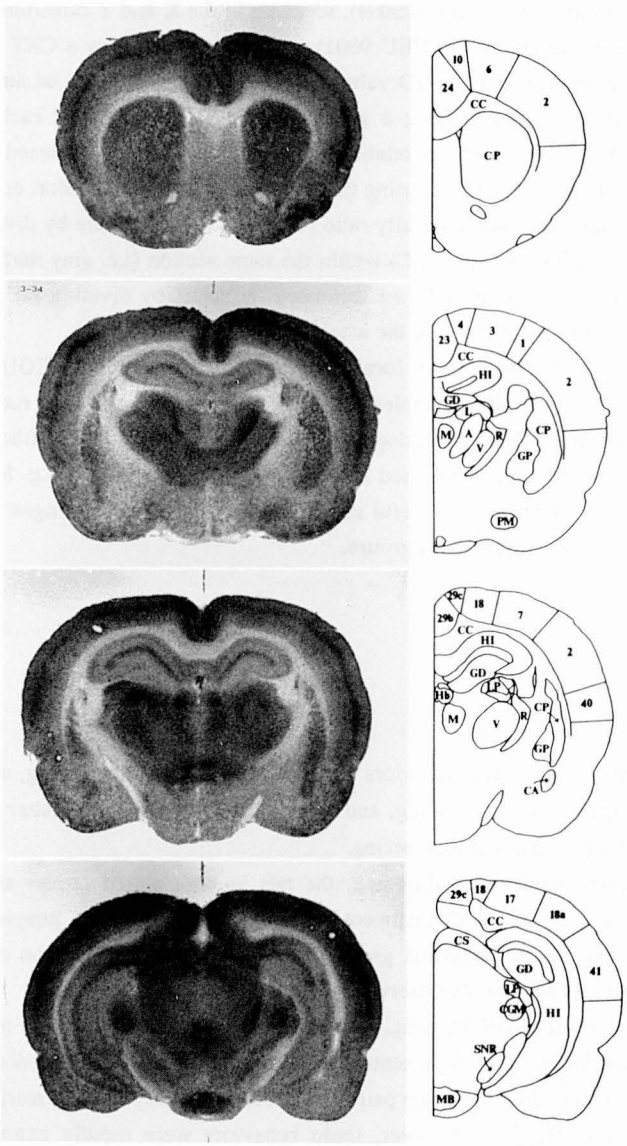

Figure 2 The autoradiograms of the brain in a rat in the paired-50 group; for abbreviations see Tables I, II and III. The darker regions of the autoradiogram indicate the regions of higher 2-DG uptake.

Although the escape-like behavior was also observed in these groups at the early stage of conditioning, no systematic behavioral change with CS or US exposure could be observed. At the late stage of conditioning as well as during the CER test, these groups predominantly showed freezing but occasionally showed walking or rearing during the CS and ITI, having no relation with the CS exposure.

Mean ODs of the CC

Mean ODs of the CC were relatively constant among the groups, being 0.296 ± 0.038, 0.269 ± 0.043, 0.266 ± 0.035, and 0.296 ± 0.034, respectively, for the paired-25, unpaired-25, paired-50 and unpaired-50 groups. A 2-by-2 (paired vs unpaired group factor and number of trials factor) analysis of variance (ANOVA) conducted on OD values showed no significant differences among the groups (all F values < 1). Thus, the ODR of a particular structure could be used to assess 2-DG uptake of that structure. Figure 2 shows the autoradiograms and demarcation of structures in a rat in the paired-50 group.

Neocortex

Table I shows the mean ODRs of 19 cortical areas in the 4 groups. The same 2-by-2 ANOVA was conducted on ODR values for each cortical area. ODR value was significantly affected by number of trials in Areas 7 ($F=7.407$, $df=1/16$, $P<0.025$) and 40 ($F=13.283$, $df=1/16$, $P<0.01$), being decreased in both the paired-50 and unpaired-50 groups.

2-DG uptake was significantly affected by group-by-trial interaction in Areas 24 ($F=5.975$, $df=1/16$, $P<0.05$), 10 ($F=4.923$, $df=1/16$, $P<0.05$), 6 ($F=9.166$, $df=1/16$, $P<0.01$), 4 ($F=4.911$, $df=1/16$, $P<0.05$) and 3 ($F=4.859$, $df=1/16$, $P<0.01$). As shown in Table I, different patterns of 2-DG uptake were found in these cortical areas in the paired and in the unpaired groups; the 2-DG uptake increased in the paired groups but decreased in the unpaired groups as a function of number of CS-US presentations.

Hippocampal formation, Cerebral nuclei, Hypothalamus and Subthalamus

Table II shows the mean ODRs of 9 structures in the hippocampal formation, cerebral nuclei, hypothalamus and subthalamus in the 4 groups. The same ANOVA showed no significant differences among those groups in ODR of any structure.

Thalamus and Epithalamus

Table III shows the mean ODRs of 11 structures in the thalamus and epithalamus in the 4 groups. ANOVA showed that ODR of the habenula was significantly affected by number of trials ($F=7.500$, $df=1/16$, $P<0.025$). As shown in Table III, 2-DG uptake in the habenula in the either 50-trial group was decreased compared to that in the respective 25-trial group. There were no significant differences among the groups in ODR of any other thalamic or epithalamic

structures.

Midbrain

Table IV shows the mean ODRs of 5 structures in the midbrain in the 4 groups. ANOVA showed that ODR was significantly affected by number of trials in the colliculus inferior ($F=4.528$, $df=1/16$, $P<0.05$), suggesting that 2-DG uptake increases as a function of number of CS-US presentations. There were no differences among the groups in ODR of any other structures.

Table I. Mean ODR and standard deviation (SD) of 19 cortical areas.

	Groups			
	Paired-25	Unpaired-25	Paired-50	Unpaired-50
A24 **	1.912±0.130	2.070±0.170	2.018±0.083	1.862±0.119
A10 **	1.922±0.115	2.073±0.165	2.004±0.093	1.876±0.125
A6 **	1.880±0.081	2.043±0.131	2.056±0.090	1.896±0.122
A23	2.005±0.145	2.050±0.151	2.136±0.103	1.994±0.128
A29b	2.013±0.190	2.015±0.132	2.080±0.070	1.950±0.135
A29c	1.953±0.135	2.028±0.083	2.090±0.077	1.978±0.151
A4 **	1.875±0.082	1.983±0.136	2.036±0.103	1.894±0.130
A3 **	1.843±0.076	1.938±0.147	1.982±0.078	1.852±0.104
A1	1.825±0.105	1.860±0.134	1.962±0.103	1.830±0.103
A2	1.905±0.092	1.953±0.143	1.942±0.079	1.878±0.113
A2a	1.947±0.087	1.975±0.166	2.032±0.073	1.954±0.149
A7 *	1.903±0.112	2.003±0.126	1.834±0.090	1.764±0.094
A39	1.960±0.127	2.038±0.174	1.962±0.064	1.844±0.094
A40 *	1.910±0.110	2.035±0.116	1.786±0.104	1.714±0.131
A41	2.108±0.154	2.203±0.070	2.196±0.104	2.038±0.181
A17	1.962±0.115	2.090±0.118	2.054±0.084	1.964±0.123
A18	1.863±0.102	1.945±0.045	1.962±0.086	1.854±0.120
A18a	1.963±0.142	2.100±0.092	2.060±0.098	1.950±0.163
A36	1.833±0.196	1.940±0.133	1.820±0.156	1.880±0.107

* Significant trial effect: $P<0.025$ for A7, $P<0.01$ for A40

**Significant group-by-trial interaction: $P<0.05$ for A24, A10 and A4; $P<0.01$ for A6 and A3

Table II. Mean ODR and SD in 9 structures of the hippocampal formation, cerebral nuclei, hypothalamus and subthalamus.

	Groups			
	Paired-25	Unpaired-25	Paired-50	Unpaired-50
GD	1.472±0.091	1.490±0.027	1.444±0.031	1.380±0.086
HI	1.548±0.091	1.553±0.046	1.552±0.035	1.468±0.105
CP	1.685±0.095	1.695±0.109	1.738±0.037	1.676±0.117
GP	1.322±0.064	1.353±0.055	1.346±0.035	1.308±0.043
CA	1.615±0.137	1.618±0.071	1.588±0.147	1.498±0.122
PM	1.868±0.128	1.820±0.179	1.987±0.108	1.854±0.226
MB	2.165±0.166	2.195±0.299	2.278±0.121	2.022±0.243
SUT	1.843±0.175	1.868±0.250	1.862±0.093	1.680±0.199
ZI	1.878±0.198	1.715±0.085	1.796±0.096	1.600±0.140

GD: Gyrus dentatus, HI: hippocampus, CP: nucleus caudatus-putamen, GP: globus pallidus, CA: amygdaloid body, PM: magnocellular preoptic nucleus, MB: mammillary body, SUT: subthalamic nucleus, ZI: zona incerta

Table III. Mean ODR and SD in 11 structures of the thalamus and epithalamus.

	Groups			
	Paired-25	Unpaired-25	Paired-50	Unpaired-50
A	1.950±0.188	1.998±0.240	2.004±0.135	1.884±0.151
Pt	1.853±0.166	1.853±0.155	1.842±0.070	1.796±0.188
L	1.830±0.175	1.888±0.132	1.820±0.142	1.744±0.160
V	1.878±0.121	1.835±0.092	1.832±0.100	1.800±0.119
M	1.880±0.167	1.810±0.113	1.804±0.144	1.764±0.119
LP	1.872±0.133	1.823±0.151	1.848±0.111	1.800±0.197
R	1.663±0.147	1.598±0.077	1.608±0.096	1.554±0.107
dCGL	1.845±0.172	1.773±0.127	1.810±0.081	1.764±0.146
vCGL	1.718±0.188	1.620±0.156	1.618±0.099	1.554±0.137
CGM	1.903±0.175	1.943±0.098	2.004±0.055	1.834±0.164
Hb *	1.852±0.200	1.845±0.150	1.652±0.085	1.580±0.206

* Significant trial effect: $P<0.025$

A: anterior thalamic nucleus, Pt: paratenial thalamic nucleus, L: lateral thalamic nucleus, V: ventral thalamic nucleus, M: medial thalamic nucleus, LP: lateral posterior thalamic nucleus, R: thalamic reticular nucleus, dCGL: dorsal nucleus of the lateral geniculate body, vCGL: ventral nucleus of the lateral geniculate body, CGM: medial geniculate body, Hb: habenula

Table IV. Mean ODR and SD in 5 structures of the midbrain.

	Groups			
	Paired-25	Unpaired-25	Paired-50	Unpaired-50
PT	1.935±0.163	1.865±0.181	1.990±0.107	1.774±0.204
CS	1.817±0.191	1.915±0.076	1.870±0.062	1.818±0.167
CI*	1.913±0.169	1.903±0.037	2.070±0.140	2.033±0.115
SNR	1.543±0.104	1.508±0.065	1.562±0.051	1.442±0.093
Re	1.780±0.176	1.808±0.071	1.838±0.108	1.788±0.163

* Significant trial effect: $P<0.05$

PT: pretectal area, CS: superior colliculus, CI: inferior colliculus, SNR: substantia nigra pars reticulata, Re: red nucleus

DISCUSSION

ANOVA demonstrated the presence of 2 types of brain structures, in one of which 2-DG uptake showed similar change as a function of number of CS-US presentations in both paired and unpaired groups, and in the other of which it was increased in the paired groups but decreased in the unpaired groups as a function of number of trials. However, in the investigation of sensory and motor systems involved in the learning process, learning-specific activation must be separated from learning-nonspecific or task-related symmetrical activation in CS-US association learning[17].

(1) Structures which showed decreased or increased 2-DG uptake as a function of number of CS-US presentations

The 2-DG uptake was significantly decreased in Areas 7 and 40 of the cerebral cortex and the habenula (Hb) and increased in the colliculus inferior (CI) as a function of number of trials. Changes of 2-DG uptake in these structures might reflect learning-nonspecific effects because both paired and unpaired groups showed the same pattern in 2-DG uptake.

Areas 7 and 40 of the Krieg atlas[19] are regarded as somesthetic association cortex related to integration of impulses associated with tactile, visual and auditory functions[25,29]. Area 7 of rats has been reported to receive dominant projections from cutaneous mechanoreceptors and to exhibit the characteristics of sensorimotor cortex[7,44]. Area 40 of rats is considered to be secondary somatosensory cortex with reciprocal fiber connections with a part of Area 7[39, 44]. The Hb is thought to be a controlling region for sensory and somatic systems[24]. However, the functions of the Hb apparently have not been clarified. Changes in 2-DG uptake in these

regions presumably reflect the process of habituation to the CS and US during conditioning, because the 2-DG uptake under the 50-trial condition was lower compared to that under the 25-trial condition in both the paired and unpaired groups.

On the other hand, 2-DG uptake in the CI increased as a function of number of trials. It is reasonable to assume that the CI was activated during conditioning and CER by the clicking sound component of the CS because the CS invariably preceded US for the paired group. The cause of the increased 2-DG uptake in the CI in the unpaired-50 group is not clear. Using the [^{14}C]2-DG method, Hungerbuhler et al.[15] found that pure-tone stimulation produced more rapid habituation than did noise stimulation. As the clicking sound used as the CS in the present study consisted of short multi-tone bursts, the rats might have not easily habituated to the sound.

(2) Structures which showed different patterns of 2-DG uptake in paired and unpaired groups

In Areas 10, 24, 3, 4, and 6 of the cerebral cortex, 2-DG uptake increased in the paired groups but decreased in the unpaired groups as a function of number of trials. These cortical areas may be directly or indirectly involved in CS-US association learning.

ODRs of almost all structures in each unpaired group were higher at the 25-trial stage but lower at the 50-trial stage compared to those of the corresponding paired group, possibly reflecting hypersensitization during the early stage due to the unpredictable shock treatment[32] followed by a process of habituation to the stimuli during the later stage of the treatment. On the other hand, ODRs of almost all brain structures in the paired group were higher at the 50-trial stage, implying that the brain became more activated with CS-US association.

In this regard it is worthwhile to consider classical aversive conditioning theory. According to the safety signal hypothesis[32], a rat can learn not only that the presence of the CS predicts the shock (danger) but also that the absence of the CS predicts the absence of the shock (safety). This hypothesis argues that the absence of the CS can serve as a safety signal at the later stage of conditioning. Once the absence of the CS has become a safety signal, it can no longer be a fear-eliciting signal. Furthermore, the CS allows the rat to make some preparatory response in anticipation of the shock[28]. In contrast, a rat for which CS and US are unpaired has no safety-signal and can make no preparatory response, and should therefore remain in a chronic fear state.

Among those areas in which the significant interaction effects were found, Areas 3, 4 and 6 showed changes of 2-DG uptake which presumably reflect task- or stimuli-related symmetrical activation. Area 3 is regarded as the primary somatosensory cortex[44], and has also been reported to constitute a zone of overlap between sensory and motor cortices[7]. Areas 4 and 6 are regarded as primary and secondary motor cortex, respectively[8].

As mentioned above, rats in the paired groups might have developed some responses

preparatory to shock during the CS. During the CER test, rats in the paired-50 group frequently showed rearing up or walking during the ITI, but, upon presentation of the CS, these behaviors soon disappeared, and freezing behavior was predominantly observed during the CS. With the termination of the CS, such behavior gradually disappeared. This freezing behavior is a species-specific defense reaction (SSDR) of the rat and their most effective defense behavior in dangerous situations[3], therefore it is regarded as a type of preparatory response. Consequently, it is reasonable to presume that such motor and sensory preparatory responses and locomotor behaviors are correlated with activation of the sensory and motor cortices. It should be noted that rats in the unpaired groups predominantly showed freezing but occasionally showed walking or rearing during the CS and ITI without systematic behavioral sequelae.

Observed changes of 2-DG uptake in Areas 24 and 10 presumably reflect learning-related activation of the cortex. Areas 24 and 10 are described as a part of the anterior cingulate cortex (ACg) and a part of the prefrontal cortex (PF), respectively. The PF of primates has been reported to receive projections mainly from dorsomedial thalamic nucleus (MD), and the PF of the rat also receives strong projections from the MD[13]. The dorsal ACg, as well as the PF, of the rat also receives strong projections from the MD[21], and these two areas in the rat are therefore thought to functionally overlap[16].

Previous studies using an aversive task have shown that lesions in the ACg produced impairment of active-avoidance learning in rats and cats[23, 27, 28]. Other studies using an appetitive task have shown that lesions in the ACg produced loss of response-inhibition to a non-rewarded stimulus in dogs[4], and impairment of performance of alternation (reward/no-reward) learning in a runway[2] and of alternating bar-press response in an operant situation in rats[1]. Lesions in the ACg in rats have also been reported to disrupt ethologically significant sequential behaviors such as maternal behaviors[34].

The PF has been thought to be a functional system in the generation of memory-based behavior and to play a role in the control of spatial orientations[11, 18]. Previous studies have shown that the PF is especially related to working memory[26] or short-term memory[9], and is involved in the temporal context of memory[22]. The PF is also reported to have an inhibitory effect on skeletal muscle activity. Stimulation of the PF in the rat has been found to suppress bar-pressing to obtain food[41] but to have no effect on general activity[40]. The rat with PF lesions, in contrast, runs more in an activity-wheel but displays no change in general activity[6].

These findings suggest that both of the PF and ACg are related to the process of learning in various tasks. Our findings are generally in agreement with these results, in that the ACg and the PF were found to be metabolically activated as a function of number of trials in the CER situation. Since previous results showed that the ACg and the PF are related to inhibition of behaviors, the increased 2-DG uptake in these areas might be related to motor function to inhibit irrelevant behaviors. Rats in the paired groups might have shown anticipatory freezing behavior contiguous with the CS exposure in an effort to reduce the pain of the shock, and thus the ACg and the PF might have functioned to inhibit or extinguish spontaneously occurring

behavioral acts during ITI. This interpretation is consistent with the hypothesis that rats with lesions in the ACg are unable to anticipate the emotional consequences, albeit reward or punishment, of their behavior[10]. It is also consistent with the findings that the PF is correlated with a kind of motor function which releases or inhibits a response and with the prospective coding of anticipatory response in animal memory processing[30].

In previous metabolic studies of structures activated during CER, learning-specific activation was not separated from symmetrical activation involved in the learning process. In the present study we were able to determine that the ACg and PF are specifically related to CS-US association learning by comparing the 2-DG uptake in the paired group with that in the unpaired group. However, it could be asserted that the changes of 2-DG uptake in the ACg and the PF observed in our study reflects different motivational consequences of being exposed to a dangerous situation. It is widely accepted that the ACg is a major component of the emotional circuit. A previous study disclosed enhanced fear response in animals with lesions of the cingulate cortex[23]. Furthermore, a previous study has shown that unpaired shocks are more stressful than paired ones[32]. However, a recent [^{14}C]2-DG experiment revealed that increased metabolic activation related to emotional arousal was found not in the ACg but in the posterior cingulate cortex[31]. On the other hand, a recent study has disclosed that rats with mesial prefrontal cortical lesions showed enhanced timidity which was situation-specific, and affective hyperreactivity to aversive stimuli, with no indication of any learning deficits[14]. Those findings indicate the possibility that the increased 2-DG uptake of the PF might be correlated with emotional reactivity. Further research is needed to determine whether our findings extend to situations or tasks which do not involve aversive stimuli.

ACKNOWLEDGMENTS

This article is dissertation submitted by Kozo Sugioka to Kobe University School of Medicine for the requirement of Doctor of Medical Sciences.

The authors wish to extend their sincere appreciation to Mrs. Tokiko Moriya for her valuable technical assistance and Dr. Hanie A. Rahman and Mr. Tomiyoshi Setsu for their helpful suggestions in preparing the manuscript.

REFERENCES

1. Barker, D. J.: J. Comp. Physiol. Psychol. 1967. 64. 453/460. Alternations in sequential behavior of rats following ablation of midline limbic cortex.
2. Barker, D. J. and Thomas, G. J.: Phisiol. Behav. 1966. 1. 313/317. Effects of regional ablation of midline cortex in alternation learning by rats.
3. Bolles, R. C.: Psychol. Rev. 1970. 77. 32/48. Species-specific defence reactions and

avoidance learning.

4. Brutkowki, S. and Mempel, E.: Science. 1961. 134. 2040/2041. Disinhibition of inhibitory conditioned respose following selective brain lesions in dogs.
5. Bryan Jr., R. M. and Lehman, R. A. W.: Brain Research. 1988. 444. 17/24. Cerebral glucose utilization after aversive conditioning and during conditioned fear in the rat.
6. Campbell, B. A. and Lynch, G. S.: J. Comp. Physiol. Psychol. 1969. 67. 15/22. Cortical modulation of spontaneous activity during hunger and thirst.
7. Donoghue, J. P., Kerman, K. L. and Ebner, F. F.: J. Comp. Neurol. 1979. 183. 647/664. Evidence for two organizational plans within the somatic sensory-motor cortex of the rat.
8. Donogue, J. P. and Parham, C.: J. Comp. Neurol. 1983. 217. 390/404. Afferent connections of the lateral agranular field of the rat motor cortex.
9. Fuster, J. M. and Alexander, G. E.: Science. 1971. 173. 652/654. Neuron activity related to short-term memory.
10. Glass, D. H., Isòn, J. R. and Thomas, G. J.: J. Comp. Physiol. Psychol. 1969. 69. 17/24. Anterior limbic cortex and partial reinforcement effects on acquisition and extinction of a runway response in rats.
11. Goldman-Rakic, P. S., Isseroff, A., Schwartz, M. L. and Bugbee, N. M.: In: Mussen, P., ed., Handbook of Child Psychology: Biology and Infancy Development (Wiley, New York). 1983. 281/344. The neurobiology of cognitive development.
12. Gonzalez-Lima, F. and Scheich, H.: Brain Research. 1986. 363. 239/256. Classical conditioning of tone-signaled bradycardia modifies 2-deoxyglucose uptake patterns in cortex, thalamus, habenula, caudate-putamen, and hippocampal formation.
13. Groenewegen, H. J.: Neuroscience. 1988. 24. 379/431. Organization of the afferent connections of the mediodorsal thalamic nucleus in the rat, related to the mediodorsal-prefrontal topography.
14. Holson, R. R.: Physiol. Behav. 1986. 37. 221/230. Mesial prefrontal cortical lesions and timidity in rats. I. Reactivity to aversive stimuli.
15. Hungerbuhler, J. P., Saunders, J. C., Greenberg, J. and Reivich, M.: Exp. Neurol. 1981. 71. 104/121. Functional neuroanatomy of the auditory cortex studied with [2-^{14}C] deoxyglucose.
16. Issacson, R. L.: Plenum Press, New York. 1982. The Limbic System.
17. John, E. R., Tang, Y., Brill, A. B., Young, R. and Ono, K.: Science. 1986. 233. 1167/1175. Double-labeled metabolic maps of memory.
18. Kolb, B., Sutherland, R. J. and Whishaw, I. Q.: Behav. Neurosci. 1983. 97. 13/27. A comparison of the contributions of the frontal and parietal association cortex to spatial localization in rats.
19. Krieg, W. J. S.: J. Comp. Neurol. 1946. 84. 221/275. Connections of the cerebral cortex. I. The albino rat. A topography of the cortical areas.
20. Ledoux, J. E., Thompson, M. E., Iadecola, C., Tucker, L. W. and Reis, D. J.: Science.

1983. 221. 576/578. Local cerebral blood flow during auditory and emotional processing in conscious rats.

21. Leonard, C. M.: Brain Research. 1969. 12. 321/343. The prefrontal cortex of the rat: I. Cortical projection of the mediodorsal nucleus. II. Efferent connections.
22. Lewis, R. S.: Psychobiology. 1989. 17. 102/107. Remembering and the prefrontal cortex.
23. Lubar, J. F. and Perachio, A. A.: J. Comp. Physiol. Psychol. 1965. 60. 46/52. One-way and two-way learning and transfer of an active avoidance response in normal and cingulectomized cats.
24. Mok, A. C. S. and Mogenson, G. J.: Brain Research. 1972. 43. 343/360. An evoked potential study of the projections to the lateral habenular nucleus from the septum and the lateral preoptic area in the rat.
25. Mountcastle, V. B., Lynch, J. C., Georgopoulos, A., Sakata, H. and Acuna, C.: J. Neurophysiol. 1975. 38. 871/908. The posterior parietal association cortex of the monkey: Command functions for operations within extrapersonal space.
26. Passingham, R. E.: Behav. Neurosci. 1985. 99. 3/21. Memory of monkeys (Macaca mulatta) with lesions in prefrontal cortex.
27. Peretz, E.: J. Comp. Physiol. Phychol. 1960. 53. 540/548. The effects of lesions of the anterior cingulate cortex on the behavior of the rat.
28. Perkins, C. C., Seymann, R., Levis, D. J. and Spencer, R.: J. Exp. Psychol. 1966. 72. 190/196. Factors affecting preference for signal shock over shock-signal.
29. Sakata, H. and Iwamura, Y.: In: Gordon, G., ed., Active Touch: The Mechanism of Recognition of Objects by Manupulation (Pergamon Press, New York). 1978. 55/72. Cortical processing of tactile information in the first somatosensory and parietal association areas in the monkey.
30. Sakurai, Y. and Sugimoto, S.: Brain Research. 1986. 20. 295/301. Multiple unit activity of prefrontal cortex and dorsomedial thalamus during delayed go/no-go alternation in the rat.
31. Sater, M., Bodewitz, G. and Steckler, T.: Behav. Neurosci. 1989. 103. 1217/1225. 2-[^{3}H] deoxyglucose uptake patterns in rats exploring a six-arm radial tunnel maze: Differences between experienced and nonexperienced rats.
32. Seligman, M. E. P., Maier, S. F. and Solomon, R. L.: In: Brush, F.R., ed., Aversive Conditioning and Learning (Academic Press, New York). 1971. 347/395. Unpredictable and uncontrollable aversive events.
33. Sharp, F. R., Kilduff, T. S., Bzorgchami, S., Heller, H. C. and Ryan, A. F.: Brain Research. 1983. 263. 97/103. The ralationship of local cerebral glucose utilization to optical density ratios.
34. Slotnick, B. M.: Behaviour. 1967. 29. 204/236. Disturbance of maternal behavior in the rat following lesions in the cingulate cortex.
35. Sokoloff, L.: Brain. 1979. 102. 653/668. Mapping of local cerebral functional activity by

measurement of local cerebral glucose utilization with [^{14}C]-deoxyglucose.

36. Sokoloff, L., Reivich, M., Kennedy, C., De Rosiers, M. H., Patlak, C. S., Pettigrew, K. D., Sakurada, O. and Shinohara, M.: J. Neurochem. 1977. 28. 897/916. The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat.
37. Sokoloff, L., Kennedy, C. and Smith, C. B.: In: Bjorklund, A. and Hokfelt, T., eds., Handbook of Chemical Neuroanatomy: Vol. 1. Methods in Chemical Neuroanatomy (Elsevier, Amsterdam). 1983. 416/441. Metabolic mapping of functional activity in the central nervous system by measuring of local glucose utilization with radioactive deoxyglucose.
38. Trafton, C. L., Fibley, R. A. and Johnson, R. W.: Psychon. Sci. 1969. 14. 100/102. Avoidance behavior in rats as a function of the size and location of anterior cingulate cortex lesions.
39. Welker, C.: J. Comp. Neurol. 1976. 166. 173/190. Receptive fields of barrels in the somatosensory neocortex of the rat.
40. Wilcott, R. C.: Physiol. Behav. 1979. 23. 1041/1048. Cortical stimulation and suppression of activity in the rat.
41. Wilcott, R. C.: Brain Research Bull. 1984. 12. 63/69. Prefrontal cortex and bulbar reticular formation and behavioral inhibition in the rat.
42. Wree, A. and Schleicher, A.: Histochemistry. 1988. 90. 109/121. The determination of the local cerebral glucose utilization with the 2-deoxyglucose method.
43. Yamadori, T., Sugioka, K. and Ishimoto, Y.: Kobe J. Medical Sciences. 1991. 37. 111/127. A study on the brain structures related to conditioned emotional respose by means of [^{14}C]2-deoxy-D-glucose method.
44. Zille, K. and Wree, A.: In: Paxions, G., ed., The Rat Nervous System, Vol. 1 (Academic Press, New York). 1985. 375/415. Cortex: Areal and laminar structure.