Regional patterns of brain 2-DG uptake produced in mice by electrical stimulation of the septum, hypothalamus, or entorhinal cortex: Relation to functional neural pathways involved in memory mechanisms

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The regional pattern of brain 2-DG uptake was studied in different groups of mice that received electrical stimulation of the medial septum (MS), dorsomedial hypothalamus (DMH), or lateral entorhinal cortex (LEC). Site selection was based on the findings of previous experiments, in which there was an improvement in long-term retention performance following MS stimulation when applied during the first 60 sec of posttraining, following LEC stimulation (when applied 15–60 min post-training), and following DMH stimulation (when applied any time within the first 60 min of post-training). The global comparison of the patterns of stimulation-induced increases in 2-DG labeling showed that MS stimulation induced specific increases in 2-DG labeling predominantly in the hippocampal formation, whereas DMH stimulation produced increased labeling in the caudate putamen, the mediodorsal thalamus, and the entorhinal cortex. Globally, LEC stimulation produced a large cortical activation, and more particularly, it induced a specific activation of the parietal cortex. Perforant-path lesions suppressed the LEC-stimulation-induced labeling in the hippocampus but did not alter the increased labeling in the amygdala and in the cortical areas. As with previous data obtained from experiments combining training and 2-DG labeling, the functional neural networks involved in these differential labeling patterns are discussed in terms of their possible implication for early or late phases of memory processing.

Electrical stimulation of discrete areas of the brain has been used extensively as a tool to examine the involvement of certain brain structures in memory processes (Berman, 1991; Destrade, Gauthier, & Sif, 1985; Gold, Zornetzer, & McGaugh, 1974; Huston, Mueller, & Mondadori, 1977; Kesner, 1982; Kesner & Wilburn, 1974). In the consolidation paradigm, the electrical stimulation is applied during the posttraining interval and the resulting facilitation or disruption of retention is usually interpreted as an indication of the involvement of the stimulated structure in memory consolidation (Bloch, 1970; Gold & McGaugh, 1975; Huston et al., 1977; McGaugh, 1966; Routtenberg, 1975). Consolidation refers both to the perseveration of the activity of neural pathways elicited by the various aspects of a learning situation (Glickman, 1961; Hebb, 1949) and to the gradual incorporation of new stable patterns of neural activity consecutive to the perseveration phase (McGaugh, 1966). More recently, some authors have proposed that memory consolidation may involve not only the strengthening of new information but also the incorporation of other contextual information present in the learning situation (Izquierdo, 1989; Squire, 1987). This more complex view of memory consolidation implies that several processes may take place during the period that follows a learning experience. It is conceivable that the early period of memory consolidation involves the registration and temporary storage of the new elements present in a learning situation, while the late period involves comparative and integrative processes that lead to a more permanent trace.

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We have previously demonstrated in mice that electrical subconvulsive stimulation of various limbic regions, following partial training in a barpressing appetitive task, produced a noncontingent improvement in long-term retention performance (for a review, see Destrade et al., 1985). For instance, we have shown that posttraining stimulation of the medial septum (MS) improved retention performance when administered during the first 60 sec after training (Galey & Jaffard, 1992; Galey, Jeantet, Destrade, & Jaffard, 1983). In contrast, the stimulation of a cortical structure, the lateral entorhinal cortex (LEC), improved retention performance only when administered between 15 and 60 min after training (Gauthier, Destrade, & Soumireu-Mourat, 1982), and this improvement persisted after the perforant path had been lesioned (Gauthier & Destrade, 1984). On the other hand, posttraining stimulation of the dorsomedial hypothalamus (DMH) improved retention when administered at any time during the first 60 min following training (Destrade, 1982).

These results suggested to us that the distinct temporal gradients of the memory improvement observed following the electrical stimulation of these different structures reflected the differential temporal sequence of intervention of these subcortical and cortical areas during early and late memory processes (Destrade et al., 1985).

To further test this hypothesis, we examined, in nontrained animals, the effects of the electrical stimulation of the MS, the DMH, and the LEC on the patterns of relative 2-DG uptake in subcortical and cortical structures in order to identify the brain regions activated by this treatment. These experiments were completed by studying the effect of perforant-path lesions on the metabolic changes produced by the electrical stimulation of the LEC.

**METHOD**

**Subjects**

Ten-week-old male BALB/c Jlco mice (IFFA-CREDO, Lyon, France), weighing 27–30 g at the start of the experiment, were used. Throughout the experiment, the mice were maintained on a 12:12-h dark:light cycle (lights on at 7 a.m.). All animals were given ad-lib access to food and water.

**Surgery**

The animals were anesthetized with sodium thialpental (90 mg/kg). A polyethylene catheter (Biotrol Pharma, Paris; 0.70 mm outer diam; 0.30 mm inner diam) was inserted and secured in the jugular vein. Patency of the cannula was maintained with heparinized brain. The value for each structure was then expressed as the ratio

**Procedure**

One week after surgery, the individual current intensity to be used in stimulation experiments was determined using a previously described procedure (Gauthier & Destrade, 1984). Experiments were carried out between 9 a.m. and 1 p.m. The animals were given a single daily session consisting of a 4-sec unilateral stimulation (sinewave = 100 Hz, 200 msec on, 200 msec off) of the implanted structure in order to determine their individual after-discharge (AD) thresholds. During the 200-msec-off interval, the EEG was continuously recorded through the bipolar stimulating electrode. AD thresholds were determined by starting with a 10-μA (peak–peak) current and increasing the current intensity in 15-μA increments each successive day until ADs were elicited. As a function of the structure, the AD threshold ranged between 50 and 100 μA. The current intensities used for each animal in the present experiment were one half of the individually determined AD values (DMH: 30 μA ± 2.7 μA [mean value ± SEM]; MS: 45 μA ± 3.2 μA; LEC: 45 μA ± 4.2 μA). During the 5 min preceding the injection of 2-DG and the 35 min following its injection, electrical stimulation was delivered continuously for 4-sec periods (100 Hz, 200 msec on, 200 msec off), which were interspersed with 4-sec-off periods (when no electrical stimulation was delivered). The EEG for each animal showed that no ADs were present. Three control animals were implanted with electrodes in either the DMH, the MS, or the LEC, but no current was passed.

The mice were injected through the jugular-vein catheter with a solution of 0.1 ml of (1-14C)2-deoxyglucose (Commissariat a l’Energie Atomique, Paris; specific activity: 46 mCi/mmol in 5% ethanol) diluted in 0.9 ml of a sterile saline solution. The injection volume (approx. 0.1 ml, adjusted according to the animal’s weight) resulted in the administration of 15 μCi per 100 g of body weight. Forty min after the injection, the animals were decapitated and the electrode withdrawn. The animals’ brains were rapidly removed and then frozen in liquid nitrogen and stored at −80°C. Twenty-μm frontal or sagittal sections were cut in a cryostat at −20°C, and were then placed on coverslips and dried rapidly on a hot plate at 50°C.

The autoradiographs were analyzed by quantitative densitometry with a Biocon 200 (Les Ulis, France) computerized image processing system with the RAG 200 software package. Twenty-seven brain regions were anatomically defined according to Lehman (1974). The mean optical density of each structure was measured bilaterally using a minimum of four consecutive sections in each brain. The value for each structure was then expressed as the ratio of the optical density of the particular structure and the mean optical density of the corpus callosum within the same brain used as a reference. It was determined that there was no significant difference between any of the groups in labeling in the corpus callosum (see Table 1). The data were analyzed using the Mann-Whitney U-test to assess statistical differences between the groups. Optical-density ratios, which are a linear function of
It is important to note that relative metabolic activity as defined above is not equivalent to the local cerebral glucose utilization measure (Sokoloff et al., 1977). Two main shortcomings of the relative metabolic activity method are the reduced accuracy in the estimate of metabolic changes and the absence of controls for the variability of some parameters, such as the kinetics of glucose transport between, and metabolism in, different physiological compartments (for a discussion, see Destrade, Messier, Bontempi, Sif, & Jaffard, 1992; Maxwell & Fink, 1988). Nonetheless, the relative metabolic activity method can be used to detect large changes that result either from electrical stimulation of brain structures (Bruce et al., 1984; Maxwell & Fink, 1988; Sif, Meunier, Messier, Calas, & Destrade, 1989; Watson, Edinger, & Siegel, 1983; Watson, Siegel, & Siegel, 1985; Watson, Troiano et al., 1983; Watson, Troiano, Poulakos, Weiner, & Siegel, 1982) or from behavioral procedures (Bontempi, Sif, Jaffard, & Destrade, 1991; Catarelli, Astic, & Kauer, 1989; Dunn, 1986; Gonzalez-Lima, 1989; Gonzalez-Lima, Finkenstaedt, & Ewert, 1989; Horster et al., 1989; Matsunami, Kawashima, & Satake, 1989; Sarter, Bodewitz, & Steckler, 1989; Sif et al., 1991). One major advantage of the relative metabolic activity method is that it can provide useful information on functional metabolic changes in freely moving unanesthetized animal preparations that are not compatible with all aspects of the cerebral local glucose utilization method (Destrade et al., 1992).

| Structure | Unstimulated Controls | DMH Stimulation | MS Stimulation |
|-----------|-----------------------|-----------------|----------------|
|           | Ipsi | Contra | Ipsi | Contra | Ipsi | Contra |
| Amygdala  | 1.26 | 0.05 | 1.27 | 0.06 | 3.24* | 0.17 | 1.34 | 0.09 | 1.86 | 0.07 | 1.93 | 0.09 |
| Caudate putamen | 1.42 | 0.06 | 1.43 | 0.07 | 2.26 | 0.07 | 2.27 | 0.10 | 1.49 | 0.02 | 1.47 | 0.06 |
| Diagonal band | 1.43 | 0.06 | 1.40 | 0.09 | 3.97* | 0.18 | 1.78 | 0.10 | 4.02 | 0.30 | 3.95 | 0.27 |
| Lateral septum | 1.22 | 0.06 | 1.19 | 0.03 | 3.38* | 0.06 | 1.64 | 0.08 | 3.29 | 0.21 | 3.13 | 0.28 |
| Medial septum | 1.43 | 0.04 | 1.43 | 0.04 | 1.53 | 0.12 | 1.53 | 0.12 | 4.87 | 0.43 | 4.87 | 0.43 |
| Mammillary bodies | 2.07 | 0.11 | 2.07 | 0.11 | 4.00 | 0.10 | 4.00 | 0.10 | 3.89 | 0.31 | 3.89 | 0.31 |
| Stria terminalis | 1.27 | 0.07 | 1.25 | 0.06 | 4.24* | 0.08 | 1.28 | 0.09 | 1.95 | 0.07 | 1.92 | 0.06 |
| Subiculum | 1.32 | 0.05 | 1.24 | 0.06 | 2.08* | 0.08 | 1.50 | 0.09 | 2.53 | 0.20 | 2.45 | 0.09 |
| Hypothalamus | 1.25 | 0.09 | 1.20 | 0.04 | 4.70* | 0.10 | 1.52 | 0.10 | 1.46 | 0.08 | 1.44 | 0.10 |
| Dorsomedial | 1.29 | 0.04 | 1.25 | 0.04 | 4.73* | 0.08 | 1.34 | 0.10 | 1.77 | 0.11 | 1.78 | 0.11 |
| Lateral | 1.24 | 0.05 | 1.23 | 0.06 | 4.79* | 0.10 | 1.50 | 0.13 | 1.24 | 0.09 | 1.14 | 0.05 |
| Ventromedial | 2.01 | 0.10 | 1.98 | 0.07 | 2.52 | 0.10 | 2.49 | 0.17 | 3.18 | 0.28 | 3.09 | 0.22 |
| Thalamus | 1.70 | 0.11 | 1.69 | 0.10 | 2.27 | 0.17 | 2.32 | 0.17 | 1.97 | 0.07 | 1.98 | 0.06 |
| Anteromedial | 1.95 | 0.07 | 1.90 | 0.05 | 2.28 | 0.10 | 2.23 | 0.12 | 2.46 | 0.14 | 2.42 | 0.09 |
| Mediodorsal | 1.09 | 0.04 | 1.10 | 0.05 | 1.44 | 0.10 | 1.33 | 0.16 | 1.43 | 0.13 | 1.47 | 0.07 |
| Lateral | 1.26 | 0.06 | 1.26 | 0.04 | 1.54 | 0.06 | 1.47 | 0.08 | 1.56 | 0.09 | 1.51 | 0.06 |
| CA1 dorsal | 1.31 | 0.03 | 1.30 | 0.05 | 1.64 | 0.12 | 1.66 | 0.08 | 1.65 | 0.09 | 1.64 | 0.13 |
| CA3 dorsal | 1.10 | 0.04 | 1.13 | 0.03 | 1.58 | 0.08 | 1.48 | 0.07 | 2.48 | 0.20 | 2.53 | 0.21 |
| GD dorsal | 1.27 | 0.05 | 1.27 | 0.05 | 1.88* | 0.07 | 1.48 | 0.06 | 2.60 | 0.21 | 2.63 | 0.19 |
| GD ventral | 1.33 | 0.04 | 1.36 | 0.04 | 3.47* | 0.12 | 1.56 | 0.06 | 2.95 | 0.24 | 2.75 | 0.12 |
| Cortex | 1.46 | 0.07 | 1.43 | 0.07 | 2.23 | 0.06 | 2.29 | 0.10 | 2.13 | 0.12 | 2.16 | 0.08 |
| Anterior cingulate | 1.43 | 0.09 | 1.46 | 0.05 | 2.29 | 0.13 | 2.25 | 0.20 | 2.99 | 0.08 | 2.91 | 0.15 |
| Posterior cingulate | 1.18 | 0.04 | 1.15 | 0.05 | 1.67* | 0.12 | 1.09 | 0.07 | 1.49 | 0.17 | 1.45 | 0.13 |
| Lateral entorhinal | 1.18 | 0.07 | 1.19 | 0.04 | 1.82* | 0.07 | 1.13 | 0.05 | 1.40 | 0.18 | 1.43 | 0.15 |
| Medial entorhinal | 1.33 | 0.07 | 1.32 | 0.06 | 1.87 | 0.07 | 1.76 | 0.06 | 2.08 | 0.10 | 2.11 | 0.08 |
| Frontal | 1.31 | 0.08 | 1.59 | 0.07 | 1.49 | 0.10 | 1.50 | 0.11 | 1.76 | 0.15 | 1.79 | 0.14 |
| Parietal | 1.71 | 0.09 | 1.72 | 0.06 | 2.38 | 0.14 | 2.19 | 0.09 | 2.43 | 0.09 | 2.40 | 0.12 |

Note—M, mean; SEM, standard error of mean. Data presented in bold type represent significant differences in labeling intensity in stimulated animals as compared with unstimulated controls. * Ipsilateral stimulated side was more labeled than the contralateral stimulated side and more labeled than the ipsilateral side of unstimulated controls. ** Ipsilateral stimulated side was more labeled only when compared with the ipsilateral side of unstimulated controls.
### Table 3
Relative 2-DG Uptake Following Lateral Entorhinal Cortex Stimulation

| Structure               | Intact Animals | Perforant-Path Lesioned | LEC Stimulation | |
|-------------------------|----------------|-------------------------|-----------------|----------|
|                         | Ipsi    | Contra      | Ipsi     | Contra   |
|                         | M     | SEM         | M     | SEM     | M     | SEM     | M    | SEM    | |
| Amygdala                | 3.90  | 0.23a      | 1.35  | 0.10    | 1.84bd | 0.17   | 1.01 | 0.11   |
| Caudate Putamen         | 2.20  | 0.09       | 2.19  | 0.10    | 1.99  | 0.10    | 1.92 | 0.08   |
| Diagonal band           | 3.84  | 0.14a      | 2.14  | 0.11    | 2.80bd | 0.17   | 2.02 | 0.11   |
| Lateral septum          | 4.24  | 0.08b*     | 1.58  | 0.05    | 2.73bd | 0.15   | 1.42 | 0.07   |
| Medial septum           | 3.79  | 0.22       | 3.79  | 0.22    | 1.50c  | 0.04   | 1.50 | 0.04e  |
| Mammillary bodies       | 4.37  | 0.11       | 4.37  | 0.11    | 2.86c  | 0.05   | 2.86 | 0.05e  |
| Stria terminalis        | 2.08  | 0.11a      | 1.02  | 0.06    | 1.32bd | 0.14   | 1.03 | 0.08   |
| Subiculum               | 2.34  | 0.12       | 2.29  | 0.13    | 0.99c  | 0.05   | 0.98 | 0.02c  |
| Hypothalamus            |        |            |        |         |        |        |      |        |
| Dorsomedial             | 1.42  | 0.10       | 1.46  | 0.12    | 1.77  | 0.08    | 1.60 | 0.08   |
| Lateral                 | 1.82  | 0.14       | 1.71  | 0.05    | 1.91  | 0.13    | 1.77 | 0.08   |
| Ventromedial            | 1.28  | 0.05       | 1.31  | 0.07    | 1.40  | 0.10    | 1.33 | 0.07   |
| Thalamus                |        |            |        |         |        |        |      |        |
| Anteromedial            | 3.45  | 0.10       | 3.35  | 0.12    | 2.49c  | 0.11   | 2.25 | 0.18e  |
| Mediodorsal             | 2.99  | 0.10       | 3.08  | 0.12    | 2.27a  | 0.10   | 2.28 | 0.08c  |
| Lateral                 | 3.31  | 0.13       | 3.33  | 0.19    | 2.35c  | 0.11   | 2.28 | 0.17c  |
| Hippocampus             |        |            |        |         |        |        |      |        |
| CA1 dorsal              | 1.33  | 0.07       | 1.35  | 0.10    | 1.20  | 0.06    | 1.32 | 0.03   |
| CA3 dorsal              | 1.34  | 0.06       | 1.44  | 0.10    | 1.30  | 0.06    | 1.50 | 0.04   |
| GD dorsal               | 1.41  | 0.08       | 1.40  | 0.07    | 1.24  | 0.09    | 1.35 | 0.03   |
| CA1 ventral             | 2.11  | 0.09a      | 1.56  | 0.10    | 1.61b  | 0.13   | 1.33 | 0.15   |
| CA3 ventral             | 3.65  | 0.11a      | 1.66  | 0.09    | 2.08bd | 0.27   | 1.39 | 0.13   |
| GD ventral              | 3.00  | 0.17a      | 1.46  | 0.06    | 2.01bd | 0.19   | 1.33 | 0.07   |
| Cortex                  |        |            |        |         |        |        |      |        |
| Anterior Cingulate      | 2.68  | 0.11       | 2.66  | 0.05    | 2.24  | 0.19    | 2.38 | 0.15   |
| Posterior Cingulate     | 3.36  | 0.12       | 3.17  | 0.14    | 2.99  | 0.14    | 2.80 | 0.18   |
| Lateral Entorhinal      | 4.43  | 0.14a      | 1.23  | 0.05    | 4.53d  | 0.17   | 1.88 | 0.08   |
| Medial Entorhinal       | 4.31  | 0.16a      | 1.16  | 0.04    | 4.33d  | 0.14   | 1.01 | 0.02   |
| Frontal                 | 2.06  | 0.08       | 2.11  | 0.07    | 1.92  | 0.18    | 1.68 | 0.19   |
| Parietal                | 3.07  | 0.16       | 2.96  | 0.11    | 2.76  | 0.16    | 2.60 | 0.13   |
| Pyriform                | 3.89  | 0.14       | 3.16  | 0.12    | 2.42c  | 0.10   | 2.31 | 0.12c  |

Note—Relative 2-DG uptake for the various structures is expressed as $M \pm SEM$. Structures in italic script were significantly bilaterally more labeled in intact stimulated animals than in unstimulated controls (see Table 2). a Ipsilateral stimulated side was significantly more labeled than contralateral side; bsignificant labeling decrease in ipsilateral side of structures of lesioned animals compared with intact stimulated animals; e significant bilateral labeling decrease in lesioned animals compared with intact stimulated animals; dsignificantly greater ipsilateral labeling within lesioned animals.

### RESULTS

The quantitative data for unstimulated, MS-stimulated, and DMH-stimulated animals are presented in Table 2, and in Table 3 for LEC-stimulated animals and LEC-stimulated animals with a unilateral perforant-path lesion.

**Effects of MS Stimulation**

Figure 1 presents the major labeling increases observed after unilateral MS stimulation. Statistical analysis (see Table 2) showed that such stimulation produced bilateral increases in labeling in the amygdala, diagonal band, lateral and medial septum, mammillary bodies, stria terminalis, subiculum, lateral hypothalamus (medial forebrain bundle), anteromedial thalamus, hippocampus (ventral CA1, CA3, and GD), anterior and posterior cingulate cortices, and frontal and pyriform cortices. A small but significant increase of labeling was also observed in the contralateral lateral thalamic nucleus.

**Effects of DMH Stimulation**

Figure 2 presents the 2-DG labeling observed in DMH-stimulated animals. The statistical analysis (see Table 2) revealed that unilateral electrical stimulation of the DMH produced bilateral increases in labeling in subcortical structures (caudate nucleus, lateral septum, mammillary bodies, anteromedial and mediodorsal thalamic nuclei) and in cortical structures (cingulate, frontal, and pyriform cortices). Unilateral labeling increases on the stimulated side were observed in the hypothalamus (dorsomedial, lateral, and ventromedial nuclei), in the amygdala, diagonal band, lateral septum, stria terminalis, hippocampus (ventral CA3 and GD), and lateral and medial entorhinal cortices.

**Effects of LEC Stimulation**

Figure 3 presents the major labeling increases observed after unilateral LEC stimulation. The statistical analysis (see Table 3) showed that, compared with unstim-
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Figure 1. (14C)2-DG autoradiographs of coronal brain sections showing the distribution of 2-DG labeling following MS stimulation. Note in particular the increases in 2-DG labeling in the amygdala (A), anteromedial thalamus (AM), and hippocampal formation (CA1, CA3, and DG). MS* indicates the location of the stimulating electrode in the MS.

Figure 2. (14C)2-DG autoradiographs of coronal brain sections showing the distribution of 2-DG labeling following unilateral DMH stimulation. Note in particular the increases in 2-DG labeling in the lateral septum (LS), caudate putamen (CP), amygdala (A), mediodorsal thalamus (MD), and entorhinal cortex (EC). DMH* indicates the location of the stimulating electrode in the DMH.

Figure 3. (14C)2-DG autoradiographs of horizontal brain sections showing the distribution of 2-DG labeling following unilateral LEC stimulation. Note in particular the increases in 2-DG labeling in the anterior cingulate cortex (AC), lateral septum (LS), amygdala (A), parietal cortex (PT), lateral posterior thalamus (LP), and hippocampal regions (CA1, CA3, and DG). LEC* indicates the location of the stimulating electrode in the LEC.

Effects of Perforant-Path Lesions on LEC-Stimulation-Induced 2-DG Labeling Patterns

Statistical analysis (see Table 3) showed that, compared with unlesioned LEC-stimulated animals, perforant-path-lesioned animals showed a large decrease in the labeling intensity bilaterally in the medial septum, mammillary bodies, subiculum, lateral hypothalamus, thalamus (anteromedial, mediiodorsal, and lateral), anterior and posterior cingulate cortices, and frontal and pyriform cortices. Moreover, LEC stimulation produced relatively greater increases in ipsilateral labeling in the amygdala, diagonal band, lateral septum, stria terminalis, hippocampus (ventral CA3 and GD), and lateral and medial entorhinal cortices.

DISCUSSION

Table 4 summarizes the major results of the present study. In the first part of this discussion section, the global patterns of metabolic activation observed following the stimulation of the MS, the DMH, and the LEC will be compared and discussed. Following that, we will discuss the implications of our data in the context of putative neural networks involved in the spatiotemporal evolution of memory processes in the brain.

Medial Septum

Electrical stimulation of the MS produced significant increases in 2-DG labeling in many subcortical structures and in the whole ventral hippocampal formation (CA1, CA3, and DG). It is particularly noticeable that we observed only slight labeling in the hypothalamus (except the medial forebrain bundle) and thalamus (except the anteromedial nucleus), while no labeling increases at all were observed in the entorhinal or parietal cortices. Globally, these data are consistent with those obtained in rats by Watson et al. (1985), using the same relative 2-DG method (except for the fact that Watson et al. did not observe any significant labeling in the hippocampus—a finding which is at variance with the well-known anatomical connections between these structures and the hippocampus).
Amygdala
Caudate putamen
Diagonal band
Lateral septum
Medial septum
Diagonal band
Stria terminalis
Mammillary bodies
Subiculum
Hypothalamus
Cortex

Table 4
Main Effects of Electrical Stimulation of the MS, DMH, and LEC on Relative 2-DG Uptake

| Structure                      | Area of Stimulation | MS | DMH | Nonlesioned | Perforant-Path Lesioned |
|-------------------------------|---------------------|----|-----|-------------|-------------------------|
| Amygdala                      |                     | +  | +   | +           | +(i)                    |
| Caudate putamen               |                     | +  | +   | +           | +(i)                    |
| Diagonal band                 |                     | +  | +   | +           | +(i)                    |
| Lateral septum                |                     | +  | +   | +           | +(i)                    |
| Medial septum                 |                     | +  |  +  | +           | +(i)                    |
| Mammillary bodies             |                     | +  | +   | +           | +(i)                    |
| Stria terminalis              |                     | +  | +   | +           | +(i)                    |
| Subiculum                     |                     | +  | +   | +           | +(i)                    |
| Hypothalamus                  |                     | +  |  +  | +           | +(i)                    |
| Dorsomedial                   |                     | +  |  +  | +           | +(i)                    |
| Lateral                       |                     | +  |  +  | +           | +(i)                    |
| Ventromedial                  |                     | +  |  +  | +           | +(i)                    |
| Thalamus                      |                     | +  | +   | +           | +(i)                    |
| Anteromedial                  |                     | +  | +   | +           | +(i)                    |
| Mediodorsal                   |                     | +  | +   | +           | +(i)                    |
| Lateral                       |                     | +  |  +  | +           | +(i)                    |
| Hippocampus                   |                     | +  | +   | +           | +(i)                    |
| CA1 dorsal                    |                     | +  |  +  | +           | +(i)                    |
| CA3 dorsal                    |                     | +  |  +  | +           | +(i)                    |
| GD dorsal                     |                     | +  |  +  | +           | +(i)                    |
| CA1 ventral                   |                     | +  | +   | +           | +(i)                    |
| CA3 ventral                   |                     | +  | +   | +           | +(i)                    |
| GD ventral                    |                     | +  | +   | +           | +(i)                    |
| Cortex                        |                     | +  | +   | +           | +(i)                    |
| Anterior cingulate            |                     | +  | +   | +           | +(i)                    |
| Posterior cingulate           |                     | +  | +   | +           | +(i)                    |
| Lateral entorhinal            |                     | +  | +   | +           | +(i)                    |
| Medial entorhinal             |                     | +  | +   | +           | +(i)                    |
| Frontal                       |                     | +  | +   | +           | +(i)                    |
| Parietal                      |                     | +  | +   | +           | +(i)                    |
| Pyriform                      |                     | +  | +   | +           | +(i)                    |

Note—+ indicates significant labeling increases as a consequence of MS, DMH, or LEC stimulation. In perforant-path-lesioned, LEC-stimulated brains, (i) indicates a significantly greater ipsilateral labeling on the stimulated side. Stimulated structures are indicated in italics.

very large increase in labeling was observed in the amygdala (see Figure 2 for an example), an increase that has also been more recently described by Maxwell and Fink (1988) after stimulation of the anterior hypothalamic area in female rats (it is very likely that in fact, these 2-DG-labeling increases in the amygdala, as well as those observed in the lateral septum and the ventral hippocampus, are due to the activation of the ventromedial hypothalamic and medial preoptic areas, which are highly interconnected with the DMH [Luiten & Room, 1980]). From these results, it can be concluded that the 2-DG patterns induced by DMH stimulation involved a great number of subcortical and cortical structures which are connected by mono- or polysynaptic pathways and stimulated ortho- or antidromically. However, the lack of 2-DG labeling in the MS and the restricted labeling in the ventral hippocampus indicate that the influence of DMH stimulation is probably not mediated by the septo-hippocampal pathway.

Lateral Entorhinal Cortex
The patterns of cerebral metabolic changes induced by LEC stimulation and their modifications after perforant-path lesions are original observations. Table 4 shows that, except for the dorsal hippocampus and the hypothalamic regions (where only a slight increase was observed in the medial-forebrain bundle), LEC stimulation increased the relative 2-DG uptake in all subcortical regions, thalamic regions (where the lateral nucleus, too, presented a 2-DG labeling), and cortical regions. These results are consistent with the literature describing the principal direct and indirect connections between the entorhinal cortex and the hippocampus, as well as between the entorhinal cortex and the other subcortical and cortical areas. (For an exhaustive review of these connections, see Lopes Da Silva, Witter, Boeijinga, & Lohman, 1990.) However, it should be observed that no 2-DG-labeling increases were induced in the dorsal hippocampus, even though direct or indirect (via the lateral septum) connections between the entorhinal cortex and the dorsal hippocampal regions have been described in the rat.

Effects of Perforant-Path Lesions
Perforant-path lesion results in the disappearance of entorhinal-stimulation-induced labeling in the CA1 region of the ventral hippocampus and in a large reduction in labeling in the CA3 and dentate regions. On the other hand, labeling increases persist ipsilaterally to the stimulated side in the amygdala, the diagonal band, the lateral septum, the thalamic nuclei, and, finally, the cortical areas (parietal, cingulate, and frontal). These results suggest that the following three functionally distinct anatomical systems are involved in the present observations. First, the very large reduction of hippocampal activation after lesion of the perforant pathway confirms the view, based on anatomical data (Amaral & Witter, 1989), that the entorhinal cortex is a major source of input to the hippocampal formation. Second, and more inter-

Dorsomedial Hypothalamus
The pattern of 2-DG labeling produced by stimulation of the DMH differed from that induced by MS stimulation in that (1) it produced significant increases in 2-DG labeling in the striatum (caudate-putamen), the whole hypothalamic area (dorsomedial, lateral, and ventromedial regions), and the mediodorsal thalamus, and also in the entorhinal cortex, which is consistent with a reported direct connection between this region and the DMH (Wyss, 1981); (2) no labeling increase was observed in the MS, a result which is consistent with the data of Watson et al. (1982); (3) the 2-DG labeling in the ventral hippocampus was restricted to the CA3 and the dentate gyrus; and (4) a
estingly, the persistence, following perforant-path lesions, of the labeling observed in the amygdala, thalamus, diagonal band, lateral septum, and frontal cortex indicates that entorhinal stimulation could activate a hippocampal-independent amygdalofugal pathway, linking the amygdala to the thalamus and the frontal cortex—an interpretation supported by anatomical and physiological data (Brothers, 1985). The residual weak labeling in the ventral hippocampus following perforant-path transection could then be due to an antidromic activation exerted by this polysynaptic circuit back onto the hippocampal formation via a ventral pathway that forms a relay in the amygdala (Polettì, Kliot, & Boytim, 1984). Third, the persistence of neocortical labeling in the cingulate, parietal, and frontal areas suggests that entorhinal stimulation also triggers activation of direct cortico-cortical pathways (Kosel, Van Hoesen, & Rosene, 1982; Sorensen, 1985) which would exist independently of the hippocampus.

Relation of the Present Data to the Neural Pathways Involved in Memory Processes

The present study is to be viewed as a complement to a larger group of studies we have previously conducted that revealed, on the one hand, memory-enhancing effects of electrical stimulation of limbic structures, and on the other, 2-DG metabolic changes following behavioral training. We are, of course, aware that the structures we selected in the present study are not involved solely in memory processing; they are certainly also involved in other brain functions. It is also clear that 2-DG changes induced by electrical stimulation of these structures cannot be conceived of as mimicking memory functioning, and that brain-mapping techniques do not solely identify regions specifically activated during a memory task. Nevertheless, the present results provide further insights into the putative neuronal substrates involved in early and late memory processes. Briefly, in a first set of studies (see the Introduction), we showed that, following a partial-acquisition session involving a barpressing appetitive task, electrical stimulation of various structures produced time-dependent memory-enhancing effects. The time gradient of efficacy for the stimulation differed as a function of the structures. In a second series of studies, we investigated the modifications of 2-DG uptake that were induced by different learning tasks at different times following the end of training in normal unstimulated mice (Bontempi et al., 1991; Destrade et al., 1992; Sif et al., 1991). This second series demonstrated that early stages of memory processes and consolidation are specifically associated with immediate postraining increases in the relative metabolic activity in the septum and diagonal band, as well as in the hippocampus, anterior thalamus, and mamillary bodies. In contrast, late memory stages are associated with delayed increases (by up to 3 h) in relative metabolic activity in such cortical regions as the frontal, cingulate, parietal, and lateral entorhinal areas. Metabolic activation in the hypothalamus was not found to be specifically linked to learning; that is, it was also observed in active control animals. These data supported the hypothesis that there are early and late memory processes which specifically involve different brain circuits in a sequentially organized manner.

In the present study, we investigated the 2-DG changes produced by electrical stimulation of three structures. It cannot be determined as yet whether stimulation in mice tested in learning/memory tasks could possibly result in a 2-DG uptake pattern sensibly different from the present effects found in behaviorally naive mice. Preliminary data obtained in the course of previous experiments (Sif, Bontempi, Messier, & Destrade, 1990) indicated that in the MS, postraining stimulation in trained mice yielded patterns of increased 2-DG labeling that were qualitatively similar to those observed in the present study in nontrained mice. Thus, the interpretations of the different brain circuits engaged in the various time-dependent phases of memory processing proposed in the following paragraphs must be considered somewhat speculative until the systematic verification, currently under way, of the patterns of increased 2-DG labeling between stimulated nontrained and stimulated trained groups of mice has been completed.

Nevertheless, comparing structures showing increased 2-DG labeling after MS stimulation (effective on memory for less than 10 min) with those showing increased 2-DG labeling after DMH stimulation (effective on memory for 1 h after training), we observe that the former, but not the latter, produces increased 2-DG labeling in the ventral CA1 hippocampal region and that, accordingly, the labeling in the other hippocampal fields is generally greater after MS stimulation than it is after DMH stimulation. Globally, DMH stimulation produces a more widespread brain metabolic activation than MS stimulation; for example, it induces 2-DG labeling increases in the striatum (caudate and putamen), medio-dorsal thalamus, and several cortical areas, including the lateral and medial entorhinal cortices, none of which are observed following stimulation of the MS.

The specific labelings produced by MS stimulation in the CA1 region and in the whole extent of the ventral hippocampus strengthen the hypothesis, already proposed by several authors (see Dunnett, 1985; Eichenbaum, Otto, & Cohen, 1992; Jaffard & Meunier, 1993; Rawlins, 1985), that the MS intimately belongs to a hippocampal-centered system critically involved in the early stage of memory formation. The lack of 2-DG changes induced by DMH stimulation in the septum and hippocampus, or the weakness of those that do occur, implies that the memory-enhancing effect of DMH stimulation is very unlikely to be mediated by the septo-hippocampal system. Because DMH stimulation yields 2-DG activation in a large array of other subcortical and cortical structures, one can speculate that the memory-enhancing effect of DMH stimulation at both early and late memory stages could reflect an undifferentiated activation of several nonhippocampal subcortical and cortical pathways. Such a view could account for the fact that, in unstimulated trained animals (Bontempi et al., 1991; Sif et al.,
hippocampal system that is assumed to be critically implicated in early memory processes, stimulation of the entorhinal cortex does not enhance learning performance when applied at short posttraining intervals. While these data may seem contradictory, they are, however, consistent with recent behavioral studies which demonstrated that although it provides a major source of sensory input to the hippocampus (and, to a lesser extent, to the amygdala), the entorhinal cortex plays an important role by itself in some memory functions, and that this role is different from that of the hippocampus proper (e.g., Meunier, Bachevalier, Mishkin, & Murray, 1993; Otto & Eichenbaum, 1992; Zola-Morgan, Squire, Amaral, & Suzuki, 1989). Recent investigations of the effects of selective (neurotoxic) entorhinal lesions indeed indicate that, in contrast to the hippocampus, the entorhinal cortex would contribute predominantly to late memory processes (Cho, Bércacocha, & Jaffard, 1993; Levisohn & Isaacson, 1991; Myhrer, 1989). Unlike MS stimulation, entorhinal-cortex stimulation produces a large amount of cortical activation, and, in particular, specific activation of the parietal cortex, a structure which also makes a major contribution to memory processing (Kesner, 1991, 1993; Kesner & DiMattia, 1984). Interestingly, this cortical labeling is spared following transection of the perforant path. Taken together, these results thus suggest that the delayed (by between 15 and 60 min) memory-enhancing effect of posttraining electrical stimulation of the entorhinal cortex could be mediated mainly by cortical pathways, independently of the hippocampus, and that it is involved in the long-term storing of information.

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