malian CNS. Since many human neurons display a Thy-1 molecule on their surface (*5*), these results may have implications for the regeneration of human CNS tissue (*27*).

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6. The purity of the preparations of 2G12 and MRC OX 7 have been described [(*2*); D. W. Mason and A. F. Williams, *Biochem. J.* **187**, 1 (1980)]. Both antibodies were prepared in mice. Evidence that 2G12 recognizes Thy-1 was obtained in a series of blocking experiments. For example, rabbit antibody to Thy-1 (supplied by A. F. Williams) abolished the ability of rat cortical glycoproteins to bind 2G12 in a concentration-dependent manner (*2*). Antibody MRC OX 7 has been well characterized by Williams and colleagues and is specific for the Thy-1.1 allelic site in rats and some strains of mice, such as AKR and RF. We have found by indirect immunofluorescence that both 2G12 and MRC OX 7 recognize the Thy-1 antigen in the rat retina. Antibody 2G12, unlike MRC OX 7, does not react in immunofluorescence studies with the retina of the AKR mouse. The same second antibodies (both rhodamine- and fluorescein isothiocyanate–labeled goat antibodies to mouse immunoglobulin G) were used in each case. Furthermore, specific binding of radioactively labeled 2G12 in the rat can be competitively inhibited by MRC OX 7 as well as by unlabeled 2G12. We believe that these results indicate that 2G12 probably recognizes an antigenic determinant different from that recognized by MRC OX 7 but that the sites are close together on the Thy-1 glycoprotein of the rat, since binding of 2G12 can be blocked by MRC OX 7 (*4*). Additional immunofluorescence studies have shown that 2G12 also reacts with rat thymocytes and embryonic muscle. Since in these tissues Thy-1 differs in the carbohydrate moiety but not in the amino acid structure, it is unlikely that 2G12 recognizes the carbohydrate portion of the Thy-1 glycoprotein. There remain two small blocks of difference in the amino acid sequences of rat and mouse Thy-1 (residues 26 to 29 and 63 to 67) and ten other single amino acid substitutions (*4*). Hence monoclonal antibodies against two apparently different but neighboring antigenic determinants on the Thy-1 molecule produce the same physiological effect.
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9. Four freshly dissected retinas were incubated twice (30 and 20 minutes, respectively) at 37°C in Hanks saline solution (Gibco) containing Hepes (5 m*M*), $NaHCO_3$ (1 m*M*), glucose (16 m*M*), $CaCl_2$ (1.25 m*M*), $MgCl_2$ (0.5 m*M*), $MgSO_4$ (0.5 m*M*), bovine serum albumin (0.2 mg/ml), DL-cysteine (0.2 mg/ml), and papain (12.5 U/ml; Worthington) and adjusted to *p*H 7.3 with 0.3*N* NaOH. After being rinsed, the retinas were mechanically dispersed by triturating through a glass pipette. The total number of ganglion cells recovered after the dissociation was about 1 percent of the retinal cells, accurately reflecting their distribution in vivo. The dissociation procedure was modified from P. V. Sarthy and D. M. K. Lam [*Brain Res.* **176**, 208 (1979)] and C. R. Bader *et al.*, [*J. Physiol. (London)* **296**, 1 (1979)].
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21. Repeated freezing and thawing of 2G12 decreased its binding activity as well as its ability to enhance process growth by retinal ganglion cells.
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Metabolic Mapping of the Brain During Rewarding Self-Stimulation

Abstract. *Local rates of cerebral glucose utilization were measured in rats by the quantitative 2-deoxy-D-[^{14}C]glucose autoradiographic method during electrical stimulation of the ventral tegmental area. Rats trained in intracranial self-stimulation showed a pattern of changes in forebrain metabolic activity distinctly different from the pattern seen in rats stimulated by the experimenter. These findings provide information about the distribution of local cerebral activity specific to reinforced instrumental behavior.*

Rats will perform operant responses to deliver brief trains of electrical pulses directly to their brains. This phenomenon, known as intracranial self-stimulation (ICSS), is frequently used to study the neural mechanisms subserving goal-oriented behavior because the work animals will do to receive electrical stimulation resembles the work they will do to receive more conventional reinforcers such as food and water (*1*). The essence of such behavior is the contingent association between the response and its consequences. By definition, positive reinforcers facilitate behavior only when the rewarding event is made contingent on

the occurrence of a response. Noncontingent presentation fails to facilitate voluntary or instrumental behavior (*2*). In fact, with respect to ICSS, noncontingent brain stimulation is not positively reinforcing inasmuch as rats will learn to press a lever to escape the same stimulation for which they had previously performed a voluntary response (*3*).

The purpose of this study was to identify the neural circuitry involved in the sensory, motor, and integrative activity associated with the performance of goal-oriented self-stimulation behavior and to distinguish these circuits from those activated by electrical stimulation itself. We report that the behavioral distinctions between response-contingent ICSS and noncontingent experimenter-administered stimulation (EAS) result in distinctly different patterns of changes in local cerebral metabolic activity as measured by the quantitative 2-deoxy-D-[^{14}C]glucose autoradiographic method (*4*).

Male albino Sprague-Dawley rats (350 to 400 g) were anesthetized and unilaterally implanted with electrodes aimed at the ventral tegmental area (VTA) (*5*). After recovering from surgery, rats were screened for self-stimulation; those positive for self-stimulation behavior were trained to press a lever on a continuous reinforcement schedule (*6*). Animals were then randomly assigned to one of three groups: ICSS ($N = 8$), EAS ($N = 8$), and no stimulation (NS) ($N = 6$). During a 3- to 5-day training period, rats in the ICSS group were allowed to self-stimulate until responding stabilized; responding of rats in the EAS and NS groups was extinguished over several sessions until the animals no longer pressed the lever (*6*).

On the day of the experiment each rat was lightly anesthetized with halothane, and catheters were inserted in a femoral artery and vein. The catheters exited through the nape of the neck to permit the animals free movement during the experimental procedure. After at least 2 hours of recovery, each rat was placed in the experimental chamber and stimulation was begun, either ICSS or EAS, at the animal's preferred rate and current intensity that had been determined before extinction training. Animals in the NS group were allowed to move freely in the chamber. Ten minutes after the onset of stimulation, the experimental period was initiated by an intravenous pulse of 2-deoxy-D-[^{14}C]glucose (125 μCi/kg) and timed arterial samples were drawn for analysis of plasma deoxy-D-[^{14}C]glucose and glucose concentrations. Stimulation continued throughout the entire experimental period. After 45 minutes the rat was killed with an overdose of sodium pentobarbital, and the brain was rapidly removed, frozen, and prepared for quantitative autoradiography (*4*).

Local tissue ^{14}C concentrations were determined by quantitative densitometry of the autoradiographs (*4, 7*) in 35 structures both ipsilateral and contralateral to the side of the electrode. Rates of local

Table 1. Means and standard errors of lard cerebral glucose utilization (in micromoles per 100 g of tissue per minute) in NS ($N = 6$), EAS ($N = 8$), and ICSS ($N = 8$) animals, ipsilateral and contralateral to the placement of the electrode.

Structure	No stimulation		Experimenter-administered stimulation		Self-stimulation	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral
Stimulation pathway						
Ventral tegmental area	59 ± 4	59 ± 4	182 ± 10*†	88 ± 4	189 ± 11*†	95 ± 7
Substantia nigra pars compacta	63 ± 3	63 ± 4	116 ± 6*†	92 ± 2	116 ± 6*†	96 ± 6
Substantia nigra pars reticulata	49 ± 3	50 ± 3	78 ± 2*	76 ± 3	79 ± 4*	74 ± 4
Medial forebrain bundle	60 ± 2	59 ± 2	103 ± 6*†	72 ± 4	116 ± 7*†	77 ± 2
Diagonal band of Broca (horizontal limb)	71 ± 4	72 ± 4	114 ± 7*†	94 ± 6	128 ± 6*†	97 ± 4
Pontine gray	60 ± 2	59 ± 3	105 ± 5*†	86 ± 5	110 ± 11*†	86 ± 7
Terminal fields of the ventral tegmental area						
Nucleus accumbens	82 ± 4	83 ± 4	88 ± 3	87 ± 3	98 ± 2*	97 ± 3
Caudate (ventrolateral)	99 ± 5	99 ± 5	101 ± 6	101 ± 5	113 ± 7	109 ± 6
Olfactory tubercle	98 ± 6	97 ± 5	105 ± 7	99 ± 7	116 ± 8	113 ± 7
Lateral septum	58 ± 3	59 ± 2	76 ± 4†	64 ± 3	74 ± 3*	71 ± 3
Bed nucleus of the stria terminalis	53 ± 1	54 ± 2	70 ± 5	63 ± 3	72 ± 4*†	65 ± 4
Amygdala (central)	42 ± 1	43 ± 1	46 ± 3	43 ± 1	51 ± 2†	46 ± 2
Amygdala (basolateral)	79 ± 4	79 ± 4	92 ± 3	89 ± 4	90 ± 4†	81 ± 4
Medial prefrontal cortex	81 ± 3	82 ± 2	74 ± 3	69 ± 3	98 ± 3†	85 ± 3
Anterior cingulate cortex	98 ± 5	97 ± 5	106 ± 3	103 ± 3	99 ± 3	103 ± 4
Entorhinal cortex	71 ± 3	69 ± 3	72 ± 3	66 ± 3	73 ± 4	74 ± 4
Lateral habenula (medial)	82 ± 5	83 ± 6	77 ± 4	78 ± 3	86 ± 5	81 ± 4
Lateral habenula (lateral)	97 ± 7	97 ± 7	111 ± 5	118 ± 4	114 ± 6	114 ± 6
Mediodorsal thalamus	100 ± 5	100 ± 6	119 ± 6†	110 ± 5	129 ± 6*	127 ± 6
Hippocampus (CA3)	70 ± 4	72 ± 4	87 ± 3†	80 ± 3	93 ± 4*	89 ± 3
Dorsal raphe	75 ± 3		151 ± 6*		143 ± 13*	
Locus ceruleus	66 ± 4	65 ± 4	87 ± 5*	82 ± 4	93 ± 4*†	76 ± 4
Medial parabrachial nucleus	51 ± 3	55 ± 4	67 ± 5	63 ± 3	73 ± 4*†	66 ± 3
Sensory-motor systems						
Motor cortex	97 ± 5	97 ± 4	119 ± 4*	125 ± 5	146 ± 9*	142 ± 9
Somatosensory cortex	97 ± 5	97 ± 5	134 ± 7*	132 ± 7	121 ± 6*	125 ± 5
Caudate (dorsomedial)	101 ± 5	102 ± 5	119 ± 3	110 ± 3	119 ± 7	117 ± 6
Caudate (dorsolateral)	99 ± 4	101 ± 4	115 ± 4	116 ± 5	122 ± 8	119 ± 7
Globus pallidus	54 ± 4	58 ± 3	79 ± 3*	74 ± 3	76 ± 5*	74 ± 3
Lateral dorsal thalamus	100 ± 5	100 ± 7	133 ± 8*	134 ± 9	135 ± 7*	131 ± 7
Ventromedial thalamus	102 ± 5	99 ± 6	127 ± 5*	134 ± 7	150 ± 10*	148 ± 10
Ventrolateral thalamus	95 ± 5	96 ± 5	134 ± 6*	140 ± 7	160 ± 7*	167 ± 9
Red nucleus	71 ± 4	71 ± 4	101 ± 2*	94 ± 3	110 ± 8*	104 ± 9
Cerebellar cortex	53 ± 1	56 ± 2	108 ± 5*	103 ± 9	95 ± 10*	116 ± 12
Internal capsule	36 ± 1	37 ± 1	40 ± 3	37 ± 2	36 ± 3	38 ± 3
Corpus callosum	30 ± 2	31 ± 3	28 ± 1	31 ± 1	37 ± 3	38 ± 3

*Combined ipsilateral and contralateral values differ from the combined values in the NS control group ($P < 0.05$) (*8*). †Difference between ipsilateral and contralateral values differs from the corresponding difference in the NS control group ($P < 0.05$) (*8*).

Table 2. Projection fields of the ventral tegmental area in which increased glucose utilization was found in ICSS and EAS animals. In bilateral fields, changes were ipsilateral and contralateral to electrode site. In ipsilateral fields, increases were confined to the side of stimulation.

Bilateral	Ipsilateral
Self-stimulation	
Nucleus accumbens	Medial prefrontal cortex
Lateral septum	Central nucleus of the amygdala
Bed nucleus of the stria terminalis	Basolateral nucleus of the amygdala
Hippocampus (CA3)	
Mediodorsal nucleus of the thalamus	
Locus ceruleus	
Medial parabrachial nucleus	
Dorsal raphe	
Experimenter-administered stimulation	
Locus coeruleus	Lateral septum
Dorsal raphe	Mediodorsal nucleus of the thalamus
	Hippocampus (CA3)

cerebral glucose utilization (LCGU) were calculated from the tissue ^{14}C concentrations and the time courses of the plasma deoxy-D-[^{14}C]glucose and glucose concentrations (*4*). For each structure, statistical comparisons of LCGU were made between stimulated (either ICSS or EAS) and NS animals and between rates of LCGU ipsilateral and contralateral to the electrode placement relative to the controls (*8*).

Histological analysis showed the electrode tips of all animals to be located within the VTA (*9*). Rats in the EAS group were stimulated at rates of 65 to 80 stimulation trains per minute, closely approximating the rates of ICSS rats (65 to 90 responses per minute). In contrast to ICSS, EAS to the VTA was not positively reinforcing. This was demonstrated in a similarly implanted and trained group of rats that pressed a lever to escape EAS to the VTA at rates and stimulus parameters that had previously supported ICSS (*10*). The distinction between these groups is, therefore, behavioral.

In the EAS animals, metabolic activity increased sharply at the site of stimulation in the VTA (Table 1 and Fig. 1), extending laterally into the substantia nigra pars compacta. Rostral to the site was evidence of discrete fiber activation through the dorsolateral aspect of the medial forebrain bundle extending into the diagonal band of Broca, whereas caudal fiber activation was more diffuse, spreading through the pontine gray. In a number of thalamic sensory-motor nuclei as well as in sensory and motor neocortex and in cerebellar cortex, LCGU was bilaterally higher in EAS than in NS animals, probably reflecting the behavioral activation of these animals relative to the unstimulated rats (Table 1). Significant alterations in glucose utilization in the terminal fields of the VTA were limited to an increase in the metabolic rate of the lateral septum ipsilateral to the side of stimulation, as well as in the ipsilateral mediodorsal nucleus of the thalamus and the ipsilateral hippocampus. Bilateral increases in the locus ceruleus and midline dorsal raphe (Table 1) were also observed. No other consistent changes in LCGU were apparent.

In the ICSS group, the metabolic activity around the site of stimulation in the VTA and the adjacent substantia nigra resembled that of the EAS animals as did the pattern of LCGU in the medial forebrain bundle, diagonal band of Broca, and pontine gray (Table 1 and Fig. 1).

The pattern of alterations in local metabolic rates in ICSS and EAS animals were divergent, however, in the terminal fields of the VTA. In the ICSS animals significant bilateral increases in LCGU were found in the nucleus accumbens, lateral septum, bed nucleus of the stria terminalis, mediodorsal nucleus of the thalamus, hippocampus, locus ceruleus, and the medial parabrachial nucleus. In addition, side-to-side differences were seen in the central and basolateral nuclei of the amygdala, bed nucleus of the stria terminalis, medial prefrontal cortex, locus ceruleus, and medial parabrachial nucleus (Table 1). These changes differ from those found in EAS animals in two ways (Table 2). First, in one group of structures, for example, the nucleus accumbens and medial prefrontal cortex, significant changes in metabolic rate were present in ICSS animals but not in EAS animals. A second category of differences was apparent in the lateral septum, mediodorsal thalamic nucleus, and hippocampus, in which increases in LCGU were found bilaterally in ICSS animals, but only ipsilateral to the electrode site in EAS rats. These findings demonstrate that the complex pattern of metabolic changes present in rats lever-pressing for electrical stimulation of the VTA, which we have described (*9*), is specific to the goal-oriented behavior of these animals and not merely the result of electrical stimulation of the VTA.

A number of major differences between the behaviors resulting from contingent and noncontingent electrical stimulation may account for the differences in the patterns of glucose utilization. (i) In the response-contingent condition (ICSS), the motor behavior of the rats was highly organized and directed, whereas during noncontingent stimulation (EAS) behavioral activation was nondirected. This difference is reflected in the higher rates of glucose utilization in the motor cortex of the ICSS animals (Table 1). It is unlikely, however, that motor behavior can account for all the differences in the patterns of glucose utilization, because many of the areas in which differences were observed, particularly the terminal fields of the VTA, have not been found to be activated during various motor behaviors as studied with the 2-deoxy-D-[^{14}C]glucose method (*11*). (ii) The behavior of the rats in the contingent condition was reinforced in that the electrical stimulation was contingent on pressing a lever. (iii) ICSS behavior is characterized by the performance of a learned response, in this case pressing a lever. Many of the areas in which changes were found, such as the amygdala (*12*) and hippocampus

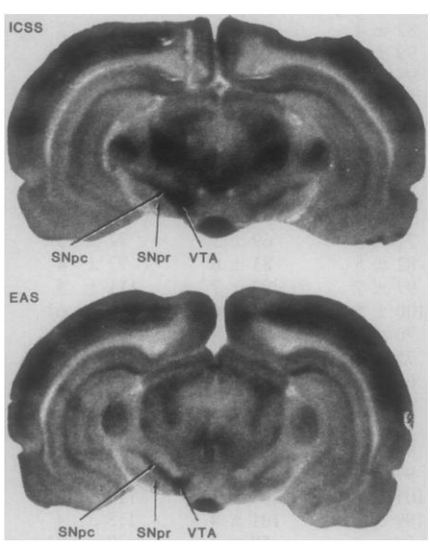

Fig. 1. Autoradiographs of coronal brain sections at the level of the midbrain from ICSS and EAS rats. Note the similarity in the area of high optical density in the lower part of the left side of both sections, corresponding to the site of electrical stimulation. Abbreviations: *VTA*, ventral tegmental area; *SNpc*, substantia nigra pars compacta; *SNpr*, substantia nigra pars reticulata.

(*13*), play roles in the acquisition and performance of learned goal-oriented behaviors.

The 2-deoxy-D-[^{14}C]glucose method (compared with techniques that can sample only one pathway at a time) has allowed the simultaneous visualization of widespread yet highly selective neural circuits specific to a goal-oriented behavior. These findings provide a basis for isolating the critical patterns of neural activity uniquely associated with the rewarding dimension of the process of reinforcement.

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6. The ICSS screening and training were conducted in a 20 by 20 by 40 cm Plexiglas box containing a single lever in one wall. Stimulation was delivered by a constant current stimulator (Nuclear-Chicago model 7150) and consisted of biphasic symmetrical rectangular waves with alternating 0.2-msec positive and negative pulses with a 0.2-msec delay between pulses, delivered at 100 Hz in 400-msec trains at current levels of 250 to 300 μA. Current was monitored by an oscilloscope. Training continued for 3 to 5 days until responding stabilized. After establishing preferred rates of ICSS at currents of 250 to 300 μA, the EAS and NS animals underwent extinction training by being placed in the experimental chamber with the electrode lead attached but the lever disconnected from the stimulator. During the experimental procedure, brain stimulation delivered to the EAS rats was randomly presented at rates at which these animals had previously stimulated themselves.
7. Optical density measurements were made with a manual densitometer (Sargent-Welch) or by means of a computerized image-processing system [C. Goochee, W. Rasband, L. Sokoloff, *Ann. Neurol.* **7**, 359 (1980)].
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Multiple Microtektite Horizons in Upper Eocene Marine Sediments?

Keller *et al.* recently suggested (*1*) that there are several middle Eocene to middle Oligocene microtektite horizons and implied that these horizons indicate separate tektite events. Although there is no a priori reason why there could not be multiple tektite events during this period, Keller *et al.* do not provide any descriptive, petrographic, or compositional data to support their identification of microtektites from previously unreported stratigraphic layers. Furthermore, the lack of data on abundance versus depth and of compositional data does not allow the reader to decide if the microtektite occurrences are due to several events, as Keller *et al.* claim, or merely to one event with scattered younger occurrences attributable to reworking.

For example, Keller *et al.* show (*1*) three microtektite layers at site 292 (cores 29, 36, and 38). I have examined cores 36 and 38. The microtektites in core 36 are small (< 125 μm in diameter), rare, and scattered over most of the core. In core 38 the microtektites are generally larger (up to 1 mm in diameter), are more abundant, and occur in a well-defined layer. The microtektites from core 36 are petrographically and compositionally similar to those in core 38; thus the microtektites in core 36 are probably from the same event as those found in core 38, but they have been reworked into younger sediment. I have found similar results for site 94, cores 14 and 15, where Keller *et al.* also claim to have found two different events. Keller *et al.* further claim (*1*) to have found four layers of microtektites at site 242 (cores 10, 15, 18, and 19). I have searched for microtektites in 10-cm^3 samples taken at 20-cm intervals through cores 18 and 19 and did not find a single microtektite. If microtektites are present in these two cores, they must be rare and may therefore be reworked.

Keller *et al.* also conclude (*1*) that no faunal extinctions can be correlated with any of the late Eocene to middle Oligocene microtektite layers. However, it has been shown that the last abundant appearance of several species of Radiolaria (for example, *Thyrsocyrtis bromia*, *T. tetracantha*, *T. finalis*, and *Calocyclas turris*) is closely associated with a late Eocene microtektite layer at ten sites ranging from the Caribbean Sea to the Indian Ocean (*2*). Keller *et al.* suggest that the coincidence between the extinctions and the microtektite layer is due to a hiatus. Indeed, they suggest that most of the late Eocene to middle Oligocene microtektite occurrences are associated with a hiatus or dissolution zone. However, they discuss the evidence for a hiatus at only two of the sites and in both cases the evidence is at best ambiguous; the exact relation between the "hiatus" and the microtektite layer is not defined.

Another puzzling aspect of the report of Keller *et al.* (*1*) is their discussion of the age of the North American tektite-strewn field, which they say is 37.5 to 38.0 million years. They fail to mention the potassium-argon and ^{40}Ar-^{39}Ar data for the North American tektites, which confirm an age of 34 to 35 million years.

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References

1. G. Keller, S. D'Hondt, T. L. Vallier, *Science* **221**, 150 (1983).
2. B. P. Glass and J. R. Crosbie, *Bull. Am. Assoc. Pet. Geol.* **66**, 471 (1982).

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Glass comments that the two late Eocene and middle Oligocene microtektite layers represent a single meteorite impact (this conclusion is based on petrographic and chemical analyses) and that the multiple layers are due to reworking