Regional differences in desensitization of
c-Fos expression following repeated
self-stimulation of the medial forebrain
bundle in the rat.

著者	Ishida Y, Nakamura M, Kuwahara I, Todaka K, Nishimori T
journal or publication title	Neuroscience
volume	90
number	3
page range	1013-1020
year	1999-03
URL	http://hdl.handle.net/10271/2062

doi: 10.1016/S0306-4522(98)00510-7

**REGIONAL DIFFERENCES IN DESENSITIZATION OF C-FOS EXPRESSION
FOLLOWING REPEATED SELF-STIMULATION OF THE MEDIAL
FOREBRAIN BUNDLE IN THE RAT**

D. NAKAHARA,^{*} Y. ISHIDA,[‡] M. NAKAMURA,^{*} I. KUWAHARA,[‡] K. TODAKA[‡]
and T. NISHIMORI[§]

^{*}Department of Psychology, Hamamatsu University School of Medicine, 3600
Handa-cho, Hamamatsu 431-3192, Japan

[‡]Department of Psychiatry, [§]Division of Biology, Miyazaki Medical College, 5200
Kihara, Kiyotake-cho, Miyazaki-gun 889-1692, Japan

Abstract:

The acute self-stimulation of the medial forebrain bundle was reported to induce the expression of c-Fos, the protein product of *c-fos*, an immediate early gene, in the central nervous system. In the present study, we examined regional changes in c-Fos expression in several reward-related areas of rat brain in response to short-and long-term exposure to self-stimulation of the medial forebrain bundle. Short-term one-hour stimulation of the medial forebrain bundle for one day after training, which evoked steady self-stimulation behavior, significantly increased the number of c-Fos-positive neurons bilaterally in all of 15 brain structures assayed, as compared to the non-stimulation control. Among them, structures showing a larger number of the stained neurons on the stimulated side were the anterior olfactory nucleus, amygdala, medial caudate–putamen complex, lateral septum, bed nucleus of the stria terminalis, ventral pallidum, substantia innominata, lateral preoptic area, medial preoptic area, lateral hypothalamus rostral to the stimulating electrodes, and substantia nigra. Long-term stimulation of the medial forebrain bundle once daily for five successive days, which maintained consistently stable self-stimulation behavior, also increased the number of c-Fos-positive neurons in the aforementioned structures, as compared to the control. However, the long-term rewarding stimulation diminished the increased number of labeled neurons, as compared to the short-term rewarding stimulation. Seven areas, medial caudate–putamen complex, ventral pallidum, substantia innominata, lateral preoptic area, medial preoptic area, rostral lateral hypothalamus and substantia nigra, showed asymmetrical, ipsilateral predominance after the short-and long-term stimulation. However, the stained neuron count in those areas after the long-term stimulation was reduced to less than 50% of that found after the short-term stimulation with the exception of lateral preoptic area and rostral lateral hypothalamus.

The results suggest that the development of desensitization of c-Fos response may differ among the reward-relevant brain regions as a consequence of repeated self-stimulation. They also indicate that a larger portion of neurons in the lateral preoptic area and rostral lateral hypothalamus may be implicated in both short-and long-term self-stimulations of the medial forebrain bundle.

Key words:

c-Fos, immediate early genes, lateral hypothalamus, lateral preoptic area, medial forebrain bundle, self-stimulation.

Abbreviations:

AON, anterior olfactory nucleus; BST, bed nucleus of the stria terminalis; LH, lateral hypothalamus; LPO, lateral preoptic area; LS, lateral septum; m-CPU, medial caudate–putamen complex; MFB, medial forebrain bundle; MPO, medial preoptic area; NAC, nucleus accumbens; PBS, phosphate-buffered saline; SI, substantia innominata; SN, substantia nigra; VP, ventral pallidum; VTA, ventral tegmental area.

Expression of c-Fos, the protein product of the immediate early gene *c-fos*, is induced rapidly by a variety of stimuli and is used widely as a marker of neuronal activation.^{30,32} It has been reported that, in general, acute treatment with various stimuli such as stress,³⁷ electroconvulsive seizures³⁸ or drug treatments^{5,6,15,29,34} induces c-Fos protein in the brain, whereas chronic treatment with the same stimuli reduces the c-Fos responsiveness. Similarly, recent studies using this marker have demonstrated that acute self-stimulation of the medial forebrain bundle (MFB) causes unilateral activation in many forebrain regions.^{3,14} Among these, the reward-related brain regions which revealed a higher density of labeled neurons with ipsilateral predominance were: medial prefrontal cortex, orbital cortex, septum, nucleus accumbens (NAC), bed nucleus of the stria terminalis (BST), substantia innominata (SI), lateral preoptic area (LPO), medial preoptic area (MPO), amygdala, lateral habenula, medial hypothalamus, lateral hypothalamus rostral to the stimulating electrodes (rostral lateral hypothalamus), or anterior ventral tegmental area (VTA).^{3,14} However, it is at present unknown as to whether the expression of c-Fos in the brain desensitizes in response to chronic self-stimulation. In the present study, therefore, we investigated regional changes in the expression of c-Fos-like protein, in the above reward-related areas of rat brain, in response to short-and long-term exposure to self-stimulation of the MFB.

EXPERIMENTAL PROCEDURES

Animals and surgical procedures

Male Wistar rats (Japan SLC Inc., Shizuoka, Japan), weighing 230–250 g at the time of surgery, were used. The animals were individually housed in cages, which were maintained on a 12 h light/dark cycle (lights on at 07.00) in a temperature controlled environment (23°C), with food and water available *ad libitum*. All the procedures for animal treatment and surgery were in accordance with the guidelines established by the Institute for Experimental Animals of Hamamatsu University School of Medicine and were approved by the local academic committee for animal experiments. The animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a Narishige stereotaxic apparatus with the skull flat between the bregma and lambda. The skull was exposed and holes were drilled, through which monopolar electrodes were bilaterally implanted in the MFB at the level of the lateral hypothalamus (LH) using the stereotaxic

coordinates of 3.8 mm posterior to bregma, 1.6 mm lateral from the midline and 8.5 mm ventral from the surface of the skull.²⁴ Electrodes consisted of a stainless steel wire (0.2 mm in diameter) coated with polyurethane, except for the tip. The reference electrode, a 1.2 mm watch screw, was attached to the frontal bone. The electrode assembly was fixed to the skull with dental cement and anchor screws.

Self-stimulation procedure

Self-stimulation training was begun following five to seven days of post-operative recovery. The rats were trained to press a lever for brain rewarding stimulation of the MFB in a 25 × 30 × 28.5 cm transparent acrylic box using conventional shaping procedures. Depression of the lever delivered a 0.3 s train of 60 Hz sine waves on a continuous reinforcement schedule. The current intensity was varied by the experimenter and finally fixed at the value between 30 and 80 μ A that sustained stable lever-pressing. A stable level was operationally defined as at least 1500 lever presses/30 min session, which was counted by a computer. Self-stimulation was estimated for both electrodes but the electrode producing the stable self-stimulation without side effects including stimulation-induced movements and/or seizures was selected for further testing. The rats were then trained to press the lever using the selected electrode for another 30 min session on the same day, and were returned to home cages. Testing started at least 48 h later to wait for c-Fos expressed following the training procedure to disappear. The animals were placed in the self-stimulation box and were allowed to press the lever for brain-stimulation reward for 1 h for one day (short-term self-stimulation group; $n = 6$) or for five consecutive days (long-term self-stimulation group; $n = 6$). Rats placed in the testing box without receiving the stimulation for 1 h for one day ($n = 4$) or for five days in a row ($n = 3$) were used as controls (control group; $n = 7$).

Immunohistochemistry

Two hours following the final self-stimulation, each rat received an overdose of sodium pentobarbital (100 mg/kg, i.p.) and was perfused transcardially first with cold saline followed by cold freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed immediately and postfixed at 4°C for 1 h in the above fixative. After fixation, the samples were immersed at 4°C for 1 h in 0.1 M phosphate

buffer with 10% sucrose and cryo-protected at 4°C overnight in the same buffer with 30% sucrose. The brains were subsequently cut on a freezing microtome into 50 µm coronal sections. The sections were collected in phosphate-buffered saline solution (PBS; pH 7.4) and processed for c-Fos immunohistochemistry according to the manufacture's instructions for use of the streptavidin–biotin system (Histofine SAB-PO(R) kit, Nichirei, Tokyo, Japan). After incubation in 10% normal goat serum for 20 min, the sections were incubated at 44C overnight with the antibody to c-Fos, a rabbit polyclonal antibody raised against a peptide corresponding to human *c-fos* amino acid residues 3–16 (Santa Cruz Biotechnology, Inc., CA) diluted 1:5000 with 0.1% Triton X-100, 0.1% sodium azide and 2% bovine serum albumin in PBS. Sections were again rinsed three times in PBS and incubated at temperature for 45 min with a secondary biotinylated goat anti-rabbit antibody, rinsed three times in PBS, and further incubated at room temperature for 15 min with a streptavidin–peroxidase complex. After three rinses in PBS, the reaction products of biotinylated goat anti-rabbit antiserum and streptavidin-conjugated horseradish peroxidase were visualized using 0.01% diaminobenzidine tetrahydrochloride (Sigma) and 0.0003% hydrogen peroxide and intensified by pretreatment with 0.25% cobalt chloride. The sections were mounted on gelatin-coated glass slides, air-dried, dehydrated, cover-slipped and analysed with light microscopy. Omission of the primary antibody from the immunohistochemical method eliminated c-Fos-like immunoreactivity. Cell counting was made from two or three sections per structure in each animal under $\times 10$ magnification using a microscopic 0.25 mm-square grid. The average number of c-Fos-positive cells in each structure for the stimulated and unstimulated sides of the brain was computed. Representative photomicrographs were taken with Technical Pan film (Kodak).

Statistics

To evaluate effects of stimulation on c-Fos expression in each structure, the bilateral average cell counts of c-Fospositive nuclei for the short-and long-term self-stimulation groups were compared, using the Mann–Whitney *U*-test, to the corresponding average for the control group. To evaluate unilateral effects of stimulation on each structure, the average for each stimulated side was compared, using the Wilcoxon signed rank test, to the corresponding value for the unstimulated side in the two self-stimulation groups. To examine desensitization effects of stimulation on each structure, the average for the

stimulated side in the long-term self-stimulation group was compared, using the *U*-test, to the corresponding value for the short-term self-stimulation group. The average numbers of lever presses for the short-term self-stimulation group were compared, using the unpaired Student's *t*-test, to the corresponding average for the long-term self-stimulation group. The time-course in changes of lever press scores from the long-term self-stimulation group of animals was analysed using the one-factor repeated measures analysis of variance. Statistically significant level was set at $P < 0.05$.

RESULTS

Histology

The location of electrode tips in all animals that received rewarding brain stimulation is shown in Fig. 1. All electrode placements were located in the ventral MFB at the level of LH. There was no consistent difference in electrode loci between short-and long-term self-stimulation groups.

Self-stimulation

Figure 2 represents the mean numbers of lever presses/h in the two self-stimulation groups of animals. On day 1, lever press scores were $5056 \pm 305/\text{h}$ (mean $\pm$ S.E.M.; $n = 6$) and $5771 \pm 323/\text{h}$ ($n = 6$), respectively, for the short-and long-term self-stimulation groups, and there was no group difference in the score ($t = 1.61$, $P > 0.05$). As depicted in Fig. 2, animals in the long-term self-stimulation group showed no significant differences in responding from day 1 to day 5 of testing ($F_{4,20} = 2.41$, $P > 0.05$).

Immunohistochemistry

Self-stimulation of the MFB induced the distinct increase of c-Fos-immunoreactive cells in 15 reward-related brain regions. The numbers of labeled cells in these structures counted for each self-stimulation group are shown in Table 1. c-Fos labeling was sparse (less than five labeled cells/structure) in the brain of the control group of animals that received no stimulation. In contrast, intense labeling was observed, both on the stimulated and unstimulated sides, in the brain of rats that received short-term self-stimulation of the MFB. In all of 15 structures examined, the number of c-Fos-like-immunoreactive neurons was significantly higher for the short-term

self-stimulation group than for the control group (Mann–Whitney, $P < 0.05$). Moreover, most of these structures showed higher labeling on the ipsilateral side than on the contra-lateral side of the brain (Wilcoxon, $P < 0.05$). These included the anterior olfactory nucleus (AON), medial caudate-putamen complex (m-CPU), lateral septum (LS), amygdala, BST, ventral pallidum (VP), SI, LPO, MPO, rostral LH and substantia nigra (SN). On the other hand, a relatively weak pattern of labeling was found in the brain of animals that received five days of self-stimulation. Only 11 of 15 structures assayed in the long-term self-stimulation group exhibited a higher density of stained neurons as compared with controls (Mann–Whitney, $P < 0.05$). The remaining four structures had increased numbers of immunostained cells, but the group differences did not reach statistical significance. Moreover, only seven of the above 11 structures showed asymmetrical activation with higher labeling ipsilateral to the stimulation (Wilcoxon, $P < 0.05$). These included the m-CPU, VP, SI, LPO, MPO, rostral LH and SN. Finally, in these seven brain regions, to evaluate the extent of desensitization of c-Fos responsiveness to the stimulation, levels of c-Fos induction were compared between the long-and short-term self-stimulation. As shown in Fig. 3, substantial reductions in immunoreactive cells were seen after the long-term self-stimulation in the m-CPU, VP, SI, MPO and SN (Mann-Whitney, $P < 0.05$), but not in the LPO and rostral LH ($P > 0.05$).

The short-and long-term self-stimulations did not affect c-Fos expression in the cerebellum, a reward-irrelevant brain area (data not shown).

Photomicrographs of representative sections from animals in each self-stimulation group are shown in Fig. 4A–F.

DISCUSSION

In the present study, we examined the effect of short-and long-term self-stimulations of the MFB on brain c-Fos expression, using an anatomical technique for mapping cell bodies of activated neurons,^{9,30} which enabled us to delineate the brain in which neurons were energized by the rewarding MFB stimulation.

Effect of short-term self-stimulation on c-Fos expression

In eleven of 15 reward-related brain regions examined, the staining was asymmetrically

increased with a higher density of immunoreactive cells on the stimulated side compared to the unstimulated side. These regions included the AON, amygdala, m-CPU, LS, BST, VP, SI, LPO, MPO, rostral LH and SN, a result which is almost comparable to previous findings.^{3,14} Anatomical evidence has demonstrated that MFB axons mainly consist of unilateral ascending or descending projections.^{23,35} Behavioral experiments have shown that ablations⁸ or ibotenic acid lesions^{7,17,36} of the brain contralateral to the MFB stimulating electrodes do not affect reward efficacy. Therefore, it is conceivable, based on these findings, that the brain regions with ipsilaterally increased c-Fos staining are closely related to neural substrates mediating the rewarding effect of the MFB stimulation.

It is well known that rewarding stimulation of the MFB activates the mesolimbic dopaminergic system.^{13,18,21,22,25} However, in neurons of the ventral tegmental area (VTA) and NAC, known to be the origin and termination of this pathway, respectively, there was no asymmetric pattern of c-Fos expression. Some anatomical evidence has revealed the existence of interhemispheric ascending dopaminergic pathways which originate in the VTA and SN and send their terminals to the CPU.^{11,27,28} There is also behavioral evidence suggesting that the descending axons of the MFB synapse to the cholinergic cells of the pedunculopontine and that these cholinergic cells send bilateral projections to the VTA and SN.⁴⁰ Thus, such bilateral projections may in part account for the similarity of staining pattern on both sides observed in the VTA and NAC. Similar results were obtained from previous studies using 2-deoxyglucose autoradiography,^{12,26,39} and from a recent report using c-Fos immunohistochemistry.² Interestingly, a recent study using a double-labeling procedure identifying both c-Fos expression and tyrosine hydroxylase has demonstrated that a large number of self-stimulation-induced c-Fos-positive cells in the VTA were not co-localized with tyrosine hydroxylase.¹⁴ Since there are two types of mesocortical dopamine neurons, those that increase c-Fos expression after stress and those that do not,⁸ a possibility exists that the majority of dopamine neurons in the VTA did not increase c-Fos expression in response to self-stimulation stress, probably due to the deficiency of the biochemical phenotype necessary to synthesize c-Fos.⁴ Thus, the expression of c-Fos in this region is questionable as accurately representing the total number of neurons activated by the rewarding MFB stimulation.

Effect of long-term self-stimulation on c-Fos expression

We demonstrated for the first time that long-term self-stimulation of the MFB desensitizes the responsiveness of c-Fos expression. The number of stained neurons greatly decreased on both the ipsilateral and contralateral sides of many brain regions, when compared to the short-term self-stimulation. This c-Fos phenomenon appears to be similar to the desensitization observed after many treatments such as stress,³⁷ electroconvulsive seizures³⁸ or manipulations of monoaminergic systems.^{5,6,15,29,34} Thus, the desensitization of c-Fos expression in response to repeated exposure to the same stimuli may be a general phenomenon which occurs across a variety of stimuli. In addition, long-term treatment with the rewarding MFB stimulation caused a large decrease in induction of c-Fos, but did not alter the response rate of self-stimulation, indicating that c-Fos expression is not necessarily unique to neurons that carry the reward signal.

Regional differences in the desensitization rate of c-Fos expression

About half of the brain regions examined exhibited asymmetrical predominance of stained neurons after both short-and long-term MFB stimulations. However, in the group that received the long-term rewarding stimulation, the cell count was reduced to about 22-70% of that shown by the group that received the short-term rewarding stimulation. Thus, our data again revealed desensitization of the c-Fos response in the ipsilateral m-CPU, VP, SI, MPO and SN, but lack of desensitization of the response in the ipsilateral LPO and rostral LH. It is postulated that self-stimulation of the MFB activates, in the first place, the descending myelinated axons, synapsing to cholinergic neurons of the pedunculopontine which project on dopaminergic neurons in the VTA and SN.⁴⁰ Thus, ascending dopaminergic fibers appear to be postsynaptically activated to release dopamine in their forebrain terminals.^{13,18,21,25} Current physiological¹⁹ and behavioral^{2,20} data strongly suggest that some of the first-stage axons subserving self-stimulation of the MFB arises from cell bodies near the junction of the anterior LH and the LPO.³³ Therefore, a majority of neurons in the LPO and rostral LH, which are activated directly and antidromically,² could result in persistent c-Fos expression after the repeated MFB self-stimulation. On the other hand, immunohistochemical staining revealed that the magnocellular preoptic area and surrounding regions receive dopaminergic inputs from the VTA and SN.³¹ It was also found that the VTA sends

dopaminergic axons to the VP.¹⁶ Moreover, an anterograde axonal marker, *Phaseolus vulgaris* leucoagglutinin, was used to show that the pontine parabrachial area sends ascending projections to the basal forebrain regions, the BST and neighboring areas.¹ Beside these direct projections, axons of the MFB may include some other ascending fibers terminating in the forebrain regions. Therefore, there is a possibility that stimulation of the MFB activates the reward-related descending axons and concurrently these ascending pathways within the same stimulation field. Thus, the expression of c-Fos in many neurons of the m-CPU, VP, SI, MPO and SN, which are postsynaptically activated through the above ascending projections, could be selectively reduced after the repeated MFB self-stimulation. However, it was demonstrated that continuous stimulation can result in persistent trans-synaptically-induced c-Fos expression¹⁰ and there is no direct evidence supporting that c-Fos expression desensitizes preferentially in neurons activated postsynaptically, but not directly, after repeated stimulation. Therefore, the mechanism underlying the desensitization of c-Fos expression in response to the repeated self-stimulation is not clear and should be elucidated by future investigation.

CONCLUSION

The present study demonstrated that short-term, unilateral rewarding stimulation of the MFB induces an asymmetry of c-Fos expression with ipsilateral predominance in the AON, amygdala, m-CPU, LS, BST, VP, SI, LPO, MPO, rostral LH and SN, which are closely related to the reward-related brain regions. However, only seven of these regions revealed a similar asymmetrical pattern of c-Fos staining in response to long-term rewarding MFB stimulation: the m-CPU, VP, SI, LPO, MPO, rostral LH and SN. Furthermore, in comparing the number of stained neurons activated by short-and long-term stimulations for each structure, a substantial reduction of c-Fos induction was observed after the long-term stimulation in the m-CPU, VP, SI, MPO and SN, but not in the LPO and rostral LH. It is suggested, therefore, that the desensitization of c-Fos expression may develop differentially among the reward-related brain areas as a consequence of repeated self-stimulation, and that a greater portion of neurons in the LPO and rostral LH may contribute to the mediation of the rewarding effects of both acute and chronic MFB stimulations.

Acknowledgements:

The authors thank Drs Natalie Alexander and David B. Kelley for helpful comments on the manuscript and Kazuhisa Kadohata for the construction of the operant box. Part of this work was presented at the 1997 Meeting of the Society for Neuroscience. This study was supported by Grants-in-Aid for Scientific Research, the Ministry of Education, Science and Culture of Japan, Nos. 07610072, 08610078, 09410024.

REFERENCES

- 1 Alden M., Besson J. M. and Bernard J. F. (1994) Organization of the efferent projections from the pontine parabrachial area to the bed nucleus of the stria terminalis and neighboring regions: a PHA-L study in the rat. *J. comp. Neurol.* **341**, 289–314.
- 2 Arvanitogiannis A., Waraczynski M. and Shizgal P. (1996) Effects of excitotoxic lesions of the basal forebrain on MFB self-stimulation. *Physiol. Behav.* **59**, 795–806.
- 3 Arvanitogiannis A., Flores C., Pfaus J. G. and Shizgal P. (1996) Increased ipsilateral expression of Fos following lateral hypothalamic self-stimulation. *Brain Res.* **720**, 148–154.
- 4 Asmus S. E. and Newman S. W. (1994) Colocalization of tyrosine hydroxylase and Fos in the male Syrian hamster brain following different states of arousal. *J. Neurobiol.* **25**, 158–168.
- 5 Beck C. H. M. and Fibiger H. (1994) Chronic desipramine alters stress-induced behaviors and regional expression of the immediate early gene, *c-fos*. *Pharmac. Biochem. Behav.* **51**, 331–338.
- 6 Chang S. L., Kengs V., Moldow R. and Zadina J. E. (1995) Chronic treatment with morphine and ethanol, but not cocaine, attenuates IL-1b activation of Fos expression in the rat hypothalamic paraventricular nucleus. In *The Brain Immune Axis and Substance Abuse* (eds Sharp B. *et al.*), pp. 201–208. Plenum, New York.
- 7 Colle L. M. and Wise R. A. (1987) Opposite effects of unilateral forebrain ablations on ipsilateral and contralateral hypothalamic self-stimulation. *Brain Res.* **407**, 285–293.
- 8 Deutch A. Y., Lee M. C., MGillham M. H., Cameron D. A., Goldstein M. and Iadarola M. J. (1991) Stress selectively increases Fos protein in dopamine neurons

- innervating the prefrontal cortex. *Cerebral Cortex* **1**, 273–292.
9. Dragunow M. and Faull R. (1989) The use of *c-fos* as a metabolic marker in neuronal pathway tracing. *J. Neurosci. Meth.* **29**, 261–265.
 10. Edelstein K. and Amir S. (1996) Constant light induces persistent Fos expression in rat intergeniculate leaflet. *Brain Res.* **731**, 221–225.
 11. Fass B. and Butcher L. L. (1981) Evidence for a crossed nigrostriatal pathway in rats. *Neurosci. Lett.* **22**, 109–113.
 12. Gallistel C. R., Gomita Y., Yadin E. and Cambell K. A. (1985) Forebrain origins and terminations of the medial forebrain bundle metabolically activated by rewarding stimulation or by reward-blocking doses of pimozide. *J. Neurosci.* **5**, 1246–1261.
 13. Gratton A., Hoffer B. J. and Gerhardt G. A. (1988) Effects of electrical stimulation of brain reward sites on release dopamine in rat: an *in vivo* electrochemical study. *Brain Res. Bull.* **21**, 319–324.
 14. Hunt G. E. and McGregor I. S. (1998) Rewarding brain stimulation induces only sparse Fos-like immunoreactivity in dopaminergic neurons. *Neuroscience* **83**, 501–515.
 15. Karen E., Bednarz L., Nikkel A. and Perner R. (1995) Rotation and striatal *c-fos* expression after repeated, daily treatment with selective dopamine receptor agonists and levodopa. *J. Pharmac. exp. Ther.* **273**, 1483–1490.
 16. Klitenick M. A., Deutch A. Y., Churchill L. and Kalivas P. W. (1993) Topography and functional role of dopaminergic projections from the ventral mesencephalic tegmentum to the ventral pallidum. *Neuroscience* **50**, 371–386.
 17. Lestang I., Cardo B., Roy M. T. and Velley L. (1985) Electrical self-stimulation deficits in the anterior and posterior parts of the medial forebrain bundle after ibotenic acid lesion of the middle lateral hypothalamus. *Neuroscience* **15**, 379–388.
 18. Miliaressis E., Emond C. and Merali Z. (1991) Re-evaluation of the role of dopamine in intracranial self-stimulation using *in vivo* microdialysis. *Behav. Brain Res.* **46**, 43–48.
 19. Murray B. and Shizgal P. (1996) Physiological measures of conduction velocity and refractory period for putative reward-relevant MFB axons arising in the rostral MFB. *Physiol. Behav.* **59**, 427–437.
 20. Murray B. and Shizgal P. (1996) Attenuation of medial forebrain bundle reward by

- anterior lateral hypothalamic lesions. *Behav. Brain Res.* **75**, 33–47.
21. Nakahara D., Ozaki N., Miura Y., Miura H. and Nagatsu T. (1989) Increased dopamine and serotonin metabolism in rat nucleus accumbens produced by intracranial self-stimulation of medial forebrain bundle as measured by *in vivo* microdialysis. *Brain Res.* **495**, 178–181.
 22. Nakahara D., Fuchikami K., Ozaki N., Iwasaki T. and Nagatsu T. (1992) Differential effect of self-stimulation on dopamine release and metabolism in the rat medial frontal cortex, nucleus accumbens and striatum studied by *in vivo* microdialysis. *Brain Res.* **574**, 164–170.
 23. Nieuwenhuys R., Geeraedts L. M. G. and Veening J. G. (1982) The medial forebrain bundle of the rat. I. General introduction. *J. comp. Neurol.* **206**, 49–81.
 24. Paxinos G. and Watson C. (1986) *The Rat Brain in Stereotaxic Coordinates*, 2nd edn. Academic, Sydney.
 25. Phillips A. G., Blaha C. D. and Fibiger H. C. (1989) Neurochemical correlates of brain-stimulation reward measured by *ex vivo* and *in vivo* analyses. *Neurosci. Biobehav. Rev.* **13**, 99–104.
 26. Porrino L., Esposito R., Seeger T., Crane A., Pert A. and Sokoloff L. (1984) Metabolic mapping of the brain during rewarding self-stimulation. *Science* **224**, 306–309.
 27. Pritzel M., Hutson J. P. and Sarter M. (1983) Behavioral and neuronal reorganization after unilateral substantia nigra lesions: evidence for increased interhemispheric nigrostriatal projections. *Neuroscience* **9**, 879–888.
 28. Pritzel M., Sarter M., Morgen S. and Hutson J. P. (1983) Interhemispheric nigrostriatal projections in the rat: bifurcating nigral projections and loci of crossing in the diencephalon. *Brain Res. Bull.* **10**, 385–390.
 29. Rosen J. B., Chuang E. and Iadarola M. J. (1994) Differential induction of Fos protein and a Fos-related antigen following acute and repeated cocaine administration. *Molec. Brain Res.* **25**, 168–172.
 30. Sagar S. M., Sharp F. R. and Curran T. (1988) Expression of *c-fos* protein in brain: metabolic mapping at the cellular level. *Science* **240**, 1328–1331.
 31. Semba K., Reiner P. B., McGeer E. F. and Fibiger H. C. (1988) Brainstem afferents to the magnocellular basal forebrain studied by axonal transport, immunohistochemistry, and electrophysiology in the rat. *J. comp. Neurol.* **267**,

433–453.

32. Sharp F. R., Sagar S. M. and Swanson R. A. (1993) Metabolic mapping with cellular resolution: *c-fos* vs 2-deoxyglucose. *Crit. Rev. Neurobiol.* **7**, 205–228.
33. Shizgal P. (1989) Toward a cellular analysis of intracranial self-stimulation: contributions of collision studies. *Neurosci. Biobehav. Rev.* **13**, 81–90.
34. Simpson J. N., Wang J. Q. and McGinty J. F. (1995) Repeated amphetamine administration induces a prolonged augmentation of phosphorylated cyclase response element-binding protein and Fos-related antigen immunoreactivity in rat striatum. *Neuroscience* **69**, 441–457.
35. Veening J. G., Swanson L. W., Cowan W. N., Nieuwenhuys R. and Geeraedts L. M. G. (1982) The medial forebrain bundle of the rat. II. An autoradiographic study of topography of the major descending and ascending components. *J. comp. Neurol.* **206**, 82–108.
36. Wagner U., Segura-Torres P., Weiler T. and Hutson J. P. (1993) The tuberomammillary nucleus region as a reinforcement inhibiting substrate: facilitation of ipsihypothalamic self-stimulation by unilateral ibotenic acid lesions. *Brain Res.* **613**, 269–274.
37. Watanabe Y., Stone E. and McEwen B. S. (1994) Induction and habituation of *c-fos* and *zif/268* by acute and repeated stressors. *NeuroReport* **5**, 1321–1324.
38. Winson S. M., Hayward M. D., Nestler E. J. and Duman R. S. (1990) Chronic electroconvulsive seizures down-regulate expression of the immediate-early genes *c-fos* and *c-jun* in rat cerebral cortex. *J. Neurochem.* **54**, 1920–1925.
39. Yadin E., Guarini V. and Gallistel C. R. (1983) Unilaterally activated systems in rat self-stimulating at sites in the medial forebrain bundle, medial prefrontal cortex, or locus coeruleus. *Brain Res.* **266**, 39–50.
40. Yeomans J. S., Mathur A. and Tampakeras M. (1993) Rewarding brain stimulation: role of tegmental cholinergic neurons that activate dopamine neurons. *Behav. Neurosci.* **107**, 1077–1087.

Table 1. Means and S.E.M.s of cell counts/structure in unstimulation control ($n = 7$), short-term self-stimulation ($n = 6$) and longterm self-stimulation ($n = 6$) groups of animals, ipsilateral and contralateral to the placement of the electrode

Structure	Unstimulation		Short-term self-stimulation		Long-term self-stimulation	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral	Ipsilateral	Contralateral
Anterior olfactory nucleus	11.8 ± 0.6	1.2 ± 0.4	55.7 ± 12.8**†	20.5 ± 5.5	13.9 ± 2.1**	8.4 ± 2.7
Cingulum	0.9 ± 0.3	1.1 ± 0.6	20.4 ± 2.7**	14.1 ± 4.3	12.6 ± 5.2	6.2 ± 3.6
Amygdala	1.3 ± 0.8	0.8 ± 0.4	26.0 ± 4.3**†	6.6 ± 1.3	7.9 ± 2.5	2.3 ± 0.8
Medial caudate–putamen complex	3.0 ± 2.3	2.4 ± 1.6	59.7 ± 11.4**†	38.2 ± 7.6	25.0 ± 7.6*†	13.8 ± 4.4
Lateral caudate–putamen complex	1.0 ± 0.4	0.6 ± 0.5	6.2 ± 1.8**	3.4 ± 1.1	4.8 ± 1.8	2.8 ± 1.1
Nucleus accumbens	0.1 ± 0.1	0.1 ± 0.1	19.2 ± 9.5**	10.5 ± 4.9	2.8 ± 1.1**	1.6 ± 0.7
Lateral septum	1.9 ± 1.0	2.5 ± 1.3	65.8 ± 16.8**†	31.5 ± 7.4	12.4 ± 4.5*	9.5 ± 3.8
Bed nucleus of the stria terminalis	1.8 ± 0.8	1.9 ± 0.8	26.8 ± 4.5**†	11.5 ± 3.2	8.3 ± 3.5	5.1 ± 2.1
Ventral pallidum	0.6 ± 0.3	0.4 ± 0.2	24.3 ± 4.9**†	13.1 ± 4.3	11.8 ± 2.8**†	6.6 ± 1.8
Substantia innominata	1.1 ± 0.5	0.8 ± 0.3	33.2 ± 7.2**†	15.1 ± 3.5	7.3 ± 2.5*	2.7 ± 0.9
Lateral preoptic area	2.1 ± 0.8	2.1 ± 0.8	36.5 ± 5.7**†	19.6 ± 5.1	25.4 ± 8.8*†	10.9 ± 4.9
Medial preoptic area	2.6 ± 0.9	2.1 ± 0.9	50.3 ± 7.7**†	21.5 ± 3.5	14.2 ± 4.5*†	7.1 ± 2.5
Rostral lateral hypothalamus	1.5 ± 0.9	1.3 ± 0.8	94.8 ± 22.2**†	21.4 ± 4.2	53.5 ± 21.7**†	13.1 ± 4.5
Ventral tegmental area	0.6 ± 0.2	0.3 ± 0.2	18.5 ± 11.6**	11.2 ± 5.9	4.9 ± 1.6*	1.7 ± 0.6
Substantia nigra	0.4 ± 0.2	0.2 ± 0.1	25.2 ± 6.1**†	17.7 ± 5.1	9.3 ± 2.2**†	4.7 ± 1.4

* $P < 0.05$, ** $P < 0.01$; combined ipsilateral and contralateral counts differ from the combined values in the unstimulated control group.

† $P < 0.05$; differences between ipsilateral and contralateral counts in the short-term self-stimulation and long-term self-stimulation groups.

Figure captions

Fig. 1. Stimulation sites of the electrode tips for all rats exposed to short-(left side) and long-term (right side) self-stimulations. The alpha numeric listing on the side of each section identifies the subject. The numbers inside each section indicate the distance (mm) posterior (-) to bregma. Drawings are adapted from the atlas of Paxinos and Watson.²⁴

Fig. 2. Lever press scores (mean $\pm$ S.E.M.) of rats exposed to short-(open circle) and long-term (filled circle) self-stimulations.

Fig. 3. Ipsilateral cell counts of c-Fos-immunoreactive nuclei expressed as the ratio of long-to short-term stimulation counts. m-CPU, medial caudate–putamen complex; VP, ventral pallidum; SI, substantia innominata; LPO, lateral preoptic area; MPO, medial preoptic area; r-LH, lateral hypothalamus rostral to the stimulating electrodes; SN, substantia nigra.

Fig. 4. Photomicrographs of c-Fos-immunoreactive cells in the lateral preoptic area (LPO; A, B, C) and nucleus accumbens (NAC; D, E, F) ipsilateral to the stimulation. (A, D) Sections from unstimulated control rats. Few c-Fos-positive cell are visible. (B, E) Sections from rats exposed to short-term self-stimulation. One-hour self-stimulation for one day induced the expression of c-Fos in both the LPO and NAC. (C, F) Sections from rats exposed to long-term self-stimulation. One-hour self-stimulation once daily for five successive days diminished c-Fos expression to a larger extent in the NAC, but to a lesser extent in the LPO. The box in the drawing indicates the location of areas where photomicrographs were taken. Scale bar = 100 μ m. c-Fos after short-and long-term MFB stimulations

Figure 1

Figure 2

Figure 3

Figure 4