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**Intracranial self-stimulation increases differentially in vivo hydroxylation of tyrosine but similarly in vivo hydroxylation of tryptophan in rat medial prefrontal cortex, nucleus accumbens and striatum**

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**Abstract**

We have examined using microdialysis the effect of intracranial self-stimulation (ICSS) on the in vivo hydroxylation rate of tyrosine and tryptophan in the medial prefrontal cortex (mPFC), nucleus accumbens (NAC) and striatum (STR). A decarboxylase inhibitor NSD-1015 was included in the perfusate, which enabled the simultaneous measurement of 3,4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP) as an index of the in vivo hydroxylation level of tyrosine and tryptophan. When rats were exposed to 1 h of ICSS at the medial forebrain bundle (MFB), their extracellular levels of DOPA significantly increased in the mPFC, NAC and STR, but with a different magnitude and time course. The same stimulation produced a delayed increase in extracellular 5-HTP, compared to DOPA, in these brain regions. The profile of 5-HTP response demonstrated no apparent difference among the regions. These findings indicate that ICSS of the MFB can increase differentially the in vivo hydroxylation of tyrosine but similarly the in vivo hydroxylation of tryptophan in the mPFC, NAC and STR.

**Keywords:** 3,4-Dihydroxyphenylalanine; 5-Hydroxytryptophan; Intracranial self-stimulation; Medial prefrontal cortex; Nucleus accumbens; Striatum

Neurochemical studies on rewarding and stressful behaviors have paid much attention to central monoaminergic neurotransmission. Microdialysis experiments have demonstrated that various types of rewarding [12,24,25] and stressful [1,7,13,25] stimuli increase the release and metabolism of dopamine (DA) in the terminal regions of the ascending dopaminergic system. It was evident from these studies, however, that both stimuli affect DA release in the different terminal regions to different degrees: in general, the reward- or stress-related increase is larger in the medial prefrontal cortex (mPFC) than in the nucleus accumbens (NAC) and smallest in the striatum (STR). Furthermore, several studies using microdialysis has also shown that the rewarding [5,14,17,26] and stressful [8,19,21] behavior activates the serotonergic system that is co-distributed with dopaminergic system in these brain regions [10,11,22]. However, our previous study found that immobilization stress activates similarly the in vivo activity of tryptophan hydroxylase, a 5-HT synthetic enzyme, whereas it activates differently the in vivo activity of tyrosine hydroxylase, a synthetic enzyme of DA and noradrenaline, in the mPFC and NAC [13]. Thus, the stressful behavior could influence 5-HT activity in the different dopaminergic terminal to similar degrees.

In the present study, therefore, in order to investigate whether there is a regional similarity for the activation of 5-HT in association with the rewarding as well as stressful behavior and also to extend the regional specificity for the DA activity to the synthesis, we have examined the effect of intracranial self-stimulation (ICSS) as a rewarding manipulation on the in vivo hydroxylation level of tryptophan and tyrosine in the mPFC, NAC and STR. A microdialysis method was used to estimate the in vivo hydroxylation rate of tryptophan and tyrosine from the accumulation rate of 5-hydroxytryptophan (5-HTP) and 3,4-dihydroxyphenylalanine (DOPA), respectively, in dialysates following inhibition of aromatic L-amino acid decarboxylase activity by 3-hydroxybenzylhydrazine (NSD-1015).

All the procedures for animal treatment and surgery were in accordance with the guidelines established by Institute for Experimental Animals of Hamamatsu University School of Medicine and were approved by the university committee for animal experiments. Male Wistar rats (230–250 g, Japan SLC Inc., Shizuoka, Japan) were individually housed in cages, which were maintained on a 12-h light/dark cycle (lights on at 07:00 h) in a temperature controlled environment (238C), with food and water available ad libitum. Under sodium pentobarbital anesthesia (50 mg/kg, i.p.) a guide

cannula was implanted in either the mPFC (2.8 mm anterior to the bregma, 0.4 mm lateral to the midline, and 0.6 mm ventral from the surface of the skull), NAC (1.2 mm anterior, 1.5 mm lateral, and 5.5 mm ventral) or STR (0.5 mm posterior, 3.0 mm lateral, and 3.1 mm ventral) of the rat brain using the stereotaxic coordinates [15]. A monopolar electrode was also im¬planted in the MFB at the level of the lateral hypothalamus (3.8 mm posterior, 1.6 mm lateral, and 8.5 mm ventral). The reference electrode, a 1.2-mm watch screw, was attached to the frontal bone. The electrode/cannula assembly was fixed to the skull with dental cement and anchor screws, and then a dummy probe was placed inside the cannula. The electrode consisted of a stainless steel wire (0.2 mm in diameter) coated with polyurethane, except for the tip. The cannula guides were made from the catheter taken from a 22-G JelcoE i.v. catheter placement unit (Johnson and Johnson Medical) as described elsewhere [12]. The dialysis probe, made from the 25-G introducing needle taken from the same catheter unit, was concentric in design with a side-by-side inlet and outlet arrangement and a dialysis tubing. The effective area of the dialysis membrane (polyacrylonitrile/sodium-methalylsulfonate, 0.31 mm in outer diameter, 90 μm in wall thickness, MW cutoff: <11 000) was 3–4 mm in length. The dummy probe was made from a JelcoE i.v. stylet.

Following 5–7 days of post-operative recovery the rats were tested for self-stimulation using 0.3-s trains of 60 Hz sine waves in a 25×30×28.5 cm transparent acrylic box with a lever. The current intensity was varied by the experimenter and finally fixed at the value between 30 and 80 μA that sustained stable lever-pressing. The rats displaying the stable self-stimulation were selected for further testing. They were trained to press the lever for a 60-min session and then returned to home cages. The microdialysis collections started on the following day. At the beginning of the experiment, the dummy probe was replaced with the dialysis probe which was secured to the guide cannula using sticky wax. The animals ($n$=18) were then placed in the self-stimulation box from which the lever had been removed. The dialysis probe was perfused with a Ringer solution (138 mM NaCl, 2.4 mM KCl, 1.2 mM CaCl2, at pH 6.5–7.0) containing 20 μM NSD-1015 (Sigma), at a flow-rate of 2 ml/min. Measurement of steady-state levels of DOPA and 5-HTP was begun after a 180-min stabilization period. Three consecutive samples were collected, at 20-min intervals in small plastic vials, to determine a steady-state level (baseline). Subsequently, rats were allowed to press the lever for brain-stimulation reward for three consecutive 20-min sessions. Sample collections

continued during and for 1 h after ICSS. Dialysate samples were also collected from the mPFC, NAC or STR of unstimulated animals ($n$=18) using a comparable procedure, that were used as no-stimulation controls. The chromatographic analysis of dialysates was done by HPLC with electrochemical detection as described previously [6]. Following the experiments, all animals were deeply anesthetized with an overdose of sodium pentobarbital and perfused intracardially with 10% for-malin in saline. The brains were removed and placed in 10% formalin for more than 1 week. Brain tissues were frozen and coronally cut as 20–30-μm slices and stained with cresyl violet for the verification of the tip location of cannulas and electrodes. As shown in Fig. 1, the histological examination confirmed electrode placement within or near the MFB. All tracks of membranes were also found to be properly located across the mPFC, NAC or STR.

Neurochemical data were expressed as percentages of the mean of three steady-state samples. Time course data in lever-press rate of ICSS animals were analyzed by two-way repeated measures ANOVA. The effects of self-stimulation on extracellular levels of DOPA and 5-HTP in each brain region were analyzed using two-way repeated measures ANOVA coupled with the Fisher's PLSD test. The level of significance was set at $P$<0.05 for all comparisons.

Both DOPA and 5-HTP were clearly detected in the dialysate obtained from the rat brain in the presence of the aromatic amino acid decarboxylase inhibitor NSD-1015. Our previous experiments demonstrated that DOPA and 5-HTP increased gradually to reach steady-state levels during 120–180 min after perfusion of NSD-1015 (1–100 μM) through the probe [6] and a pilot study showed that accumbal infusion of 20 μM NSD-1015 through the probe did not affect the ICSS rate (data not shown). Based on these observations, this dosage was used as the con-centration of NSD-1015 in the perfusate and baseline sample collection was started after at least a 180-min stabilization period. The baseline values of DOPA in the mPFC ($n$=12), NAC ($n$=12) and STR ($n$=12) were 0.23±0.05 pmol/20 min, 3.07±0.29 pmol/20 min and 4.71±0.16 pmol/20 min, respectively, in the presence of 20 μM NSD-1015, while baseline 5-HTP levels were 0.27±0.02, 0.39±0.04, and 0.23±0.02 pmol/20 min, respectively.

As indicated in Fig. 2, there was no significant difference in the lever-press rate of ICSS among three brain areas ($F_{2,15}$ =0.15, n.s.). The animals received a mean±S.E.M. ($n$=18)

of 1435.8±98.0 trains of stimulations in the first 20-min ICSS session, 1607.6±184.4 in the second session, and 1545.4±176.2 in the third session.

The three brain regions showed a difference in the magnitude and time course of changes of extracellular DOPA following ICSS. ICSS caused a maximal 195.7±51.4% increase of baseline in DOPA levels in the mPFC and much smaller increases in the NAC (138.2±12.0%) and the STR (102.0±3.1%). As for the time course, the accumbal DOPA increased rapidly, peaked during ICSS in the second session, and returned to levels notsignificantly different from baseline for 40–60 min following the third 20-min ICSS session, while the frontal DOPA increased more slowly, peaked in the third session, and gradually decreased to steady state levels. The striatal increase in DOPA was slowest. On the other hand, the three brain regions showed a similarity in the pattern of ICSS-induced increases of extracellular 5-HTP. In the mPFC, 5-HTP increased to a maximum of 193.3±10.9% of baseline for 40–60 min following the third ICSS session and remained significantly elevated for 60 min afterdiscontinuing brain stimulation. The almost similar magnitude of 5-HTP response was observed in the NAC andSTR, although its onset was slightly faster in the STR than in the NAC and mPFC.

In the present study, we demonstrated that ICSS of the MFB differentially augments the in vivo hydroxylation rate of tyrosine, whereas the same stimulation similarly enhances the in vivo hydroxylation level of tryptophan, in the three brain regions examined. The medial region of the rat prefrontal cortex contains thick innervations from both DA and noradrenaline neurons in the brainstem [2,4,9,23]. A previous microdialysis study has shown that eating behavior increases extracellular levels of both DA and noradrenaline in this area but not in the NAC [3]. Thus noradrenaline as well as DA may contribute to our reward-induced increase of the in vivo tyrosine hydroxylation in the mPFC. The increased in vivo hydroxylation level of tyrosine was higher in the mPFC than in the NAC and lowest in the STR. This evidence parallels with a previous result reporting that enhancing effects of ICSS at the MFB on extracellular levels of DA and its metabolites were more prominent in the mPFC and NAC than in the STR [12]. Such elevations in the in vivo hydroxylation level of tyrosine in the NAC and STR also agree well with evidence from tissue samples [16]. Thus, the region-specific change in DA activity associated with ICSS is thought to include the synthesis as well as the release and metabolism. Furthermore, several previous studies have confirmed that stressful stimuli also enhance extracellular DA levels greatly in the mPFC and NAC compared

with the STR [1,7]. Taken together, it is conceivable that both rewarding and stressful stimuli may preferentially activate the mesolimbocortical DA pathway rather than the nigrostriatal DA pathway, and that the reward- and stress-related changes in DA activity may not be related to the specific aspects of the experimental manipulation such as ICSS or immobilization but rather may be due to emotional / cognitive arousal [24,25]. There are no previous reports, to our knowledge, about the increase in the in vivo hydroxylation rate of tryptophan in the mPFC, NAC and STR of self-stimulating rats, which is the first in vivo evidence. Our previous study has reported that ICSS of the MFB induces a rise in extracellular levels of 5-hydroxyindoleacetic acid, the 5-HT metabolite, in the mPFC and NAC [12]. In view of these findings, although there still remains unanswered whether ICSS affects 5-HT release in these brain areas, we suggest that ICSS at the MFB can enhance the synthesis and metabolism of 5-HT in the mPFC and NAC. The pattern of 5-HTP responses showed no apparent difference between the mPFC, NAC and STR, suggesting that the synthesis rate of 5-HT in these brain regions may be activated to similar degrees by the rewarding MFB stimulation. Our recent microdialysis study has found that immobilization stress augments the in vivo hydroxylation rate of tryptophan in the mPFC and NAC and their magnitudes are analogous [13]. Thus, the profile of 5-HTP responses in the mPFC and NAC seen during the rewarding manipulation was found to be comparable to that observed during the stressful situation. The 5-HT neurons extensively innervate widespread areas in the brain [22]. These findings, therefore, indicate that rewarding and stressful stimuli may produce an ubiquitous activation of 5-HT synthesis in the brain. Our data strongly support the contention that central serotonergic activation occurs independently of specific behavioral changes and of specific brain areas [18,20]. In addition, since stress causes an increase in 5-HT release at nerve terminals without enhancing the firing activity of cell bodies of 5-HT neurons, it is suggested that the stress-evoked release of 5-HT may occur through presynaptic mechanisms [18,19]. Thus, it is also possible that during ICSS the synthesis of 5-HT may be locally activated by such presynaptic mechanisms.

In summary, the present finding provides in vivo evidence that in the mPFC, NAC and STR the catecholamine synthesis is differentially activated, while the 5-HT synthesis is comparably activated, in association with the rewarding MFB stimulation.

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**References**

[1] E.D. Abercrombie, K.A. Keefe, D.S. Di Frischa, M.J. Zigmond, Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex, J. Neurochem. 52 (1989) 1655–1658.

[2] M.A. Audet, G. Doucet, S. Oleskevich, L. Descarries, Quantified regional and laminar distribution of the noradrenaline innervation in the anterior half of the adult rat cerebral cortex, J. Comp. Neurol. 274 (1988) 307–318.

[3] M.A. Cenci, P. Kalen, R.J. Mandel, A. Bjorklund, Regional differences in the regulation of dopamine and noradrenaline release in medial frontal cortex, nucleus accumbens and caudate putamen: a microdialysis study in the rat, Brain Res. 581 (1992) 217–228.

[4] L. Descarries, B. Lemay, G. Doucet, B. Berger, Regional and laminar density of the dopamine innervation in adult rat cerebral cortex, Neuroscience 21 (1987) 807–821.

[5] D.F. Fiorino, A. Coury, H.C. Fibiger, A.G. Phillips, Electrical stimulation of reward sites in the ventral tegmental area increases dopamine transmission in the nucleus accumbens of the rat, Behav. Brain. Res. 55 (1993) 131–141.

[6] H. Hashiguti, D. Nakahara, W. Maruyama, M. Naoi, T. Ikeda, Simultaneous determination of in vivo hydroxylation of tyrosine and tryptophan in rat striatum by microdialysis–HPLC: relationship between dopamine and serotonin biosynthesis, J. Neural Transm. [GenSet] 93 (1993) 213–223.

[7] A. Imperato, S. Piglisi-Allegra, P. Casolini, L. Angelucci, Changes in brain dopamine and acetylcholine release during and following stress are independent of the pituitary–adrenocortical axis, Brain Res. 538 (1991) 111–117.

[8] L.G. Kirby, A.R. Allen, I. Luchki, Regional difference in the effects of forced swimming on extracellular levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid, Brain Res. 682 (1995) 189–196.

[9] O. Lindvall, A. Bjorklund, General organization of cortical mono¬amine systems, in: L. Descarries, T.R. Reader, H.H. Jasper (Eds.), Monoamine Innervation of Cerebral Cortex, Alan R. Liss, New York, 1984, pp. 9–40.

[10] L.A. Mamounas, C.A. Mullen, E. O'Hearn, M.E. Molliver, Dual serotoninergic projections to forebrain in the rat: morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives, J. Comp. Neurol. 314 (1991) 558–586.

[11] E.J. Mylecharane, Ventral tegmental area 5-HT receptors: mesolim¬bic dopamine release and behavioural studies, Behav. Brain Res. 73 (1996) 1–5.

[12] D. Nakahara, K. Fuchikami, N. Ozaki, T. Iwasaki, T. Nagatsu, 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 (1992) 164–170.

[13] D. Nakahara, M. Nakamura, Differential effect of immobilization stress on in vivo synthesis rate of monoamines in medial prefrontal cortex and nucleus accumbens of conscious rats, Synapse 32 (1999) 328–342.

[14] D. Nakahara, N. Ozaki, Y. Miura, H. Miura, T. Nagatsu, 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 (1989) 178–181.

[15] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic Press, New York, 1982.

[16] A.G. Phillips, A. Jakubovic, H.C. Fibiger, Increased in vivo tyrosine hydroxylase activity in rat telencephalon produced by self-stimulation of the ventral tegmental area, Brain Res. 402 (1987) 109–116.

[17] E.T. Pleim, J.A. Matochik, R.J. Barfield, S.B. Auerbach, Correlation of dopamine release in the nucleus accumbens with masculine sexual behaviour in rats, Brain Res. 524 (1990) 160–163.

[18] L.E. Rueter, C.A. Fornal, B.L. Jacobs, A critical review of 5-HT brain microdialysis and behavior, Rev. Neurosci. 8 (1997) 117–137.

[19] L.E. Rueter, B.L. Jacobs, A microdialysis examination of serotonin release in the rat forebrain induced by behavioral/environmental manipulations, Brain Res. 739 (1996) 57–69.

[20] M.R. Spoont, Modulatory role of serotonin in neural information processing: implications for human psychopathology, Psychol. Bull. 112 (1992) 330–350.

[21] H. Takahashi, Y. Takada, N. Nagai, T. Urano, A. Takada, Extracellular serotonin in the striatum increased after immobilization stress only in the nighttime, Behav. Brain Res. 91 (1998) 185–191.

[22] I. Tork, Anatomy of the serotonergic system, in: P.M. Whitaker-Azmita, S.J. Peroutka (Eds.), The Neuropharmacology of Serotonin, Annals of New York Academy of Science,Vol. 600, 1990, pp. 9–34.

[23] C.G. Van Eden, E.M.D. Hoorneman, R.M. Buijs, M.A.H. Matthijssen, M. Geffard, H.B.M. Uylings, Immunocytochemical localization of dopamine in the prefrontal cortex of the rat at the light and electron microscopical level, Neuroscience 22 (1987) 849–861.

[24] B.H.C. Westerink, Brain microdialysis and its application for the study of animal behaviour, Behav. Brain Res. 70 (1995) 103–124.

[25] A.M.J. Young, Intracerebral microdialysis in the study of physiology and behavior, Rev. Neurosci. 4 (1993) 373–395.

[26] A.M.J. Young, M.H. Joseph, J.A. Gray, Increased dopamine release in vivo in nucleus accumbens and caudate nucleus of the rat during drinking: a microdialysis study, Neuroscience 48 (1992) 871–876.

**Figure captions**

**Fig. 1.** Coronal sections showing the site of electrode tips and microdialysis tubes. Because of technical errors, the electrode tip of one rat (rat no. 138) was not confirmed. The numbers above each section indicate the distance (mm) anterior (1) or posterior (2) to bregma. Drawings are adopted from Paxinos and Watson [15].

**Fig. 2.** Effects of ICSS at the MFB on the dialysate levels of DOPA and 5-HTP in the mPFC, NAC and STR during continuous infusion of 20 μM NSD-1015. The same number of animals ($n$=6) was used for both ICSS and control (no-ICSS) groups in each brain region. The time when animals were engaged in ICSS was indicated by the period between vertical dotted lines. Values are the means±S.E.Ms. Neurochemical data were analyzed using two-way repeated measures ANOVA followed by the Fisher's PLSD test. The $F$-values for the group×time interaction were as follows. DOPA: mPFC, $F_{8,80}$=3.33, $P$<0.01; NAC, $F_{8,80}$=2.72, $P$<0.01; STR, $F_{8,80}$=2.37, $P$<0.05. 5-HTP: mPFC, $F_{8,80}$=28.56, $P$<0.001; NAC, $F_{8,80}$=30.61, $P$<0.001; STR, $F_{8,80}$=14.07, $P$<0.001. *$P$<0.05, **$P$<0.01, ***$P$<0.001 compared with respective control (no-stimulation) values.

Figure 1

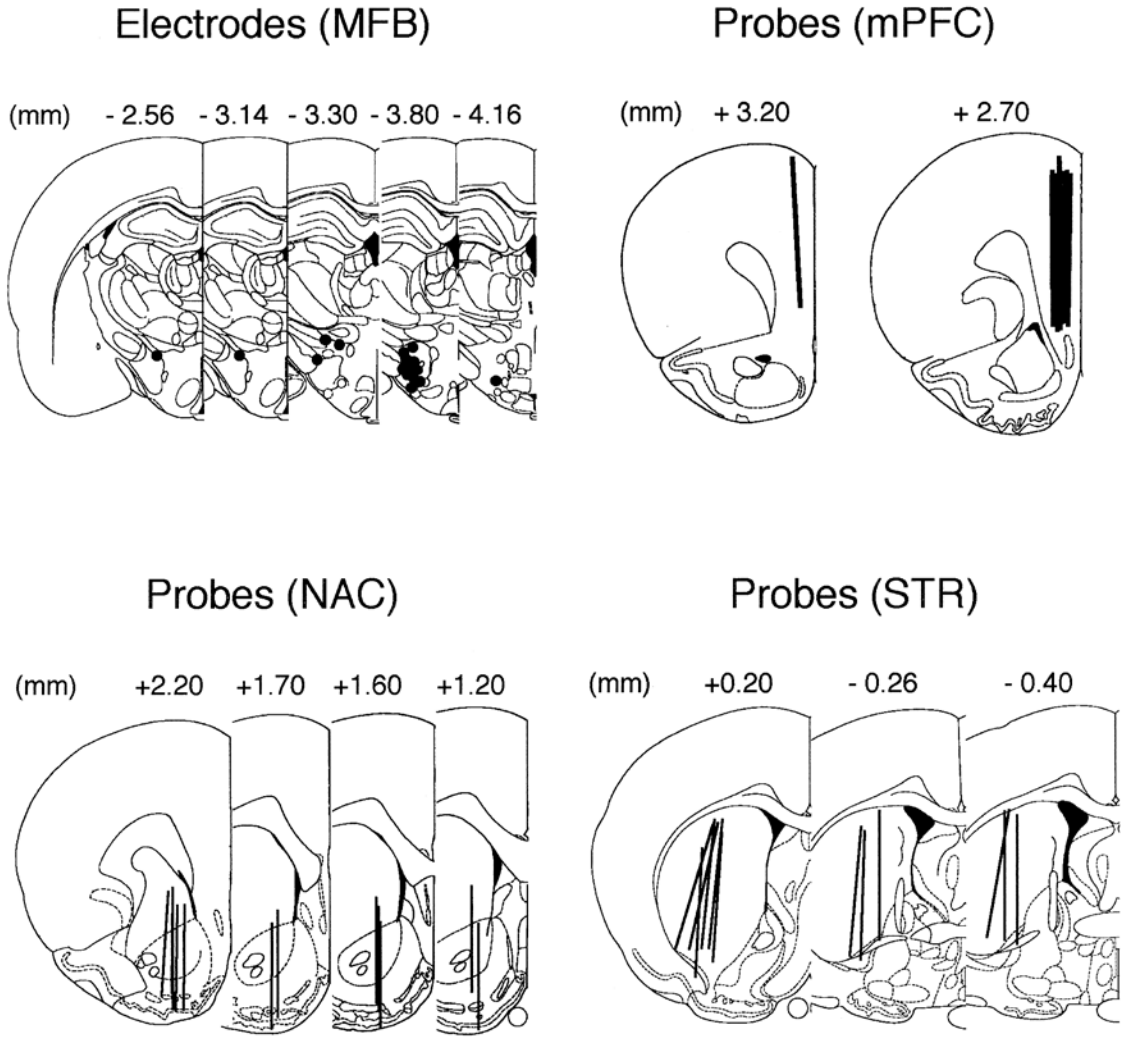
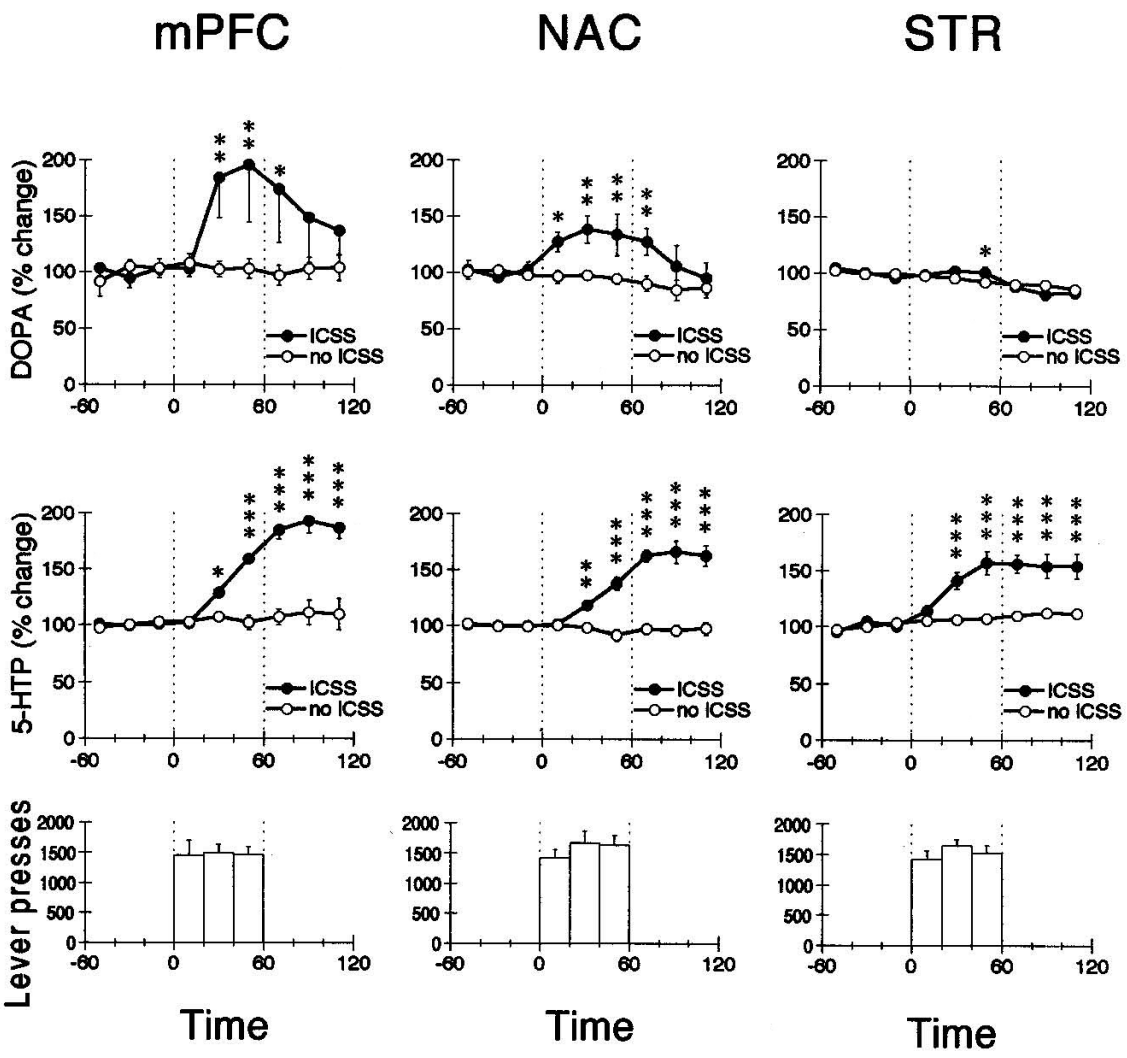

Electrodes (MFB)
(mm) - 2.56 - 3.14 - 3.30 - 3.80 - 4.16
Probes (mPFC)
(mm) + 3.20
+ 2.70
Probes (NAC)
(mm) +2.20 +1.70 +1.60 +1.20
Probes (STR)
(mm) +0.20 - 0.26 - 0.40

Figure 2


mPFC
NAC
STR
DOPA (% change)
5-HTP (% change)
Lever presses
ICSS
no ICSS
Time