

Augmentation of ventral tegmental area stimulation-induced feeding by both stimulation and lesion of the contralateral ventral tegmental area in the rat

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Abstract. Unilateral lesions of the ventral tegmental area (VTA) facilitate behavioral responses (feeding and exploration) induced by electrical stimulation of the VTA in the contralateral hemisphere. It was hypothesized that this facilitation may result from a lesion-induced compensatory increase in dopamine transmission in the intact hemisphere. In the present study we tested on the functional level the hypothesis that the activity of bilateral mesocorticolimbic systems is inversely related. For this purpose we compared the effect of unilateral subthreshold activation with the effect of subsequent unilateral lesion of VTA on feeding response evoked by electrical stimulation of the contralateral VTA. In male Wistar rats implanted with bilateral VTA electrodes stimulation-induced feeding was tested in a latency to feed - stimulation frequency curve-shift paradigm. One electrode was used for induction of feeding reaction and the other electrode was used for concurrent stimulation (with the subthreshold current) and subsequent electrolytic lesioning of the contralateral VTA. It was found that both contralateral stimulation and subsequent lesion performed through the same electrode facilitated a feeding response that manifested as a decrease in the reaction's threshold and a leftward shift of the latency-frequency curve. The paradoxical similarity of the effects of the stimulation and lesion is discussed in terms of functional organization of the mesocorticolimbic system and adaptive changes in dopaminergic transmission.

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INTRODUCTION

The ventral tegmental area of the midbrain (VTA), a key structure of the mesocorticolimbic system (Swanson 1982, Oades and Holliday 1987), is engaged in the control of various adaptive appetitive behaviors including food intake (Wise and Rompre 1989, Le Moal and Simon 1991). Electrical stimulation of this area or its ascending projections in the medial forebrain bundle induces feeding in satiated animals (e.g.: Delgado and Anand 1953, Valenstein 1969, Wise 1971, Trojnar and Staszewska 1994) and causes release of dopamine (DA) in the nucleus accumbens (Fiorino et al. 1993). In a previous study (Trojnar and Staszewska 1994) we found that electrolytic lesion of VTA in one hemisphere facilitated feeding response elicited by electrical stimulation of the symmetrical VTA in the other hemisphere. This effect may involve DA-ergic and GABA-ergic VTA neurons as well as their regulatory inputs (Trojnar et al. 1997, Trojnar and Klejbor 1999).

A facilitatory effect of the unilateral lesions was also found using other behavioral models. Colle and Wise (1987) described augmentation of contralateral lateral hypothalamic self-stimulation after large unilateral ablation of the prosencephalic structures. Trojnar and Klejbor (1999) found facilitation of VTA stimulation-induced locomotor response after contralateral VTA lesion and Trojnar and Wise (1992) observed augmentation of VTA stimulation-induced feeding after electrolytic lesion of the contralateral tegmental pedunculopontine nucleus.

Augmentation of function of the intact hemisphere after acute unilateral brain injury is an interesting phenomenon because of its potential for clarifying the compensatory mechanisms involved in the process of recovery. It may reflect a mechanism of immediate compensation since facilitatory effects of the lesion are already apparent on the second postlesion day (Trojnar and Staszewska 1994, Trojnar and Klejbor 1999) and may last for an indefinite time postinjury (unpublished observation).

As was found in numerous biochemical studies on the nigrostriatal system (Nieoullon et al. 1977, Glowinski et al. 1979, Leviel et al. 1979, Zetterstrom et al. 1986, Robinson and Wishaw 1988), unilateral manipulation of DA transmission in one hemisphere results in immediate changes in DA levels in the opposite direction in the contralateral hemisphere. These changes may influence the functional state of the striatal neurons (Hull et al. 1974,

Castellano and Rodriguez 1991). In their push-pull cannula studies performed on anesthetized cats Nieoullon et al. (1977) and Leviel et al. (1979) found for the first time that unilateral iontophoretic application to the substantia nigra of pharmacological agents that enhance firing rate of DA neurons caused an increase in DA release in the ipsilateral neostriatum and suppression of DA release in the contralateral neostriatum. Agents that suppressed the firing rate of the nigral DA neurons caused symmetrical changes in the opposite direction. It should be mentioned however that these findings were challenged by a more recent *in vivo* microdialysis study in rats (Santiago et al. 1993).

Similar compensatory changes may occur in the VTA as the mesencephalic dopamine neurons of the substantia nigra and VTA constitute an anatomical continuum although they differ in the topography of their efferent projections (Swanson 1982, Oades and Holliday 1987). If the interhemispheric regulation of the latero-ventral (substantia nigra) and medio-ventral (VTA) mesencephalic DA cell groups is based on similar principles, one may expect that unilateral activation of VTA cells will suppress and their unilateral inactivation will enhance DA release in the contralateral efferent structures of the mesocorticolimbic system and, respectively, abolish or enhance DA-dependent behaviors (e.g. feeding) evoked from the contralateral hemisphere. This assumption was tested at the functional level in the present work. Unilateral VTA-stimulation induced feeding was measured in a latency to feed-stimulation frequency curve-shift paradigm in conditions of simultaneous subthreshold stimulation of the contralateral VTA. It was compared to the effects of the subsequent electrolytic VTA lesion performed through the same electrode.

METHODS

Twelve male Wistar rats were implanted with bilateral electrodes (monopolar stainless steel electrodes of 0.3 mm in diameter) aimed at the anterior part of VTA. Paxinos and Watson (1986) coordinates were: 4.5 mm posterior to the bregma, 1.0 mm lateral to the midline and 7.8-8.1 mm ventral to the skull surface. Five animals which ate reliably in response to VTA stimulation were used for the study.

One week after surgery each animal was screened for stimulation-induced feeding in a 250 x 350 x 440 mm box with food pellets covering the floor. Thirty minutes before testing the rats were allowed to explore the box

to allow for habituation to the experimental conditions and complete satiation. Trains of square-wave constant current of 0.1 ms duration cathodal pulses were conducted from the stimulator to the electrode by flexible wire leads. For each electrode a stimulation intensity was determined which would, at a stimulation frequency of 50 Hz, induce feeding with a mean latency of 5–8 s. The range of such intensities was 140–280 μ A. The electrode which first gave reliable response was chosen as a feeding electrode (FE) and the contralateral electrode (CE) was used for concomitant contralateral stimulation with subthreshold current.

Once reliable feeding was obtained, the rats were tested daily in a latency paradigm, where frequency of stimulation was varied from trial to trial. Latencies to feed were measured in 30-s trials. Stimulation was maintained for 30 s or until 5 s after the animal began to eat. Rest time of 20 s was given between trials. Four blocks of trials were given every day. Stimulation frequency was progressively increased (by 10% of each previous value) in the first and third blocks and decreased in the second and fourth. The range of tested frequencies was 21 to 55 Hz in control conditions and was adjusted as required under experimental conditions. The four tests were averaged to obtain a mean daily latency at each stimulation frequency. The frequency threshold for feeding defined as the stimulation frequency at which an animal began to eat with a latency of 20 s was derived from each rat's latency-frequency function by a method of linear interpolation.

Daily testing continued for each animal until the threshold stabilized. Then, in the first part of the study, both electrodes (FE and CE) were stimulated simultaneously and the rats were tested for feeding response according to the previously described procedure. Prior to each session of bilateral stimulation the current intensity threshold was determined for the CE electrode. The threshold was defined as the lowest intensity of stimulation which would induce, at a stimulation frequency of 50 Hz, the signs of behavioral activation, i.e. forward locomotion or feeding. The threshold was individually established for each animal and corresponded to a range of 25–220 μ A. Three subthreshold current intensities were applied in random order to the CE electrode during bilateral stimulation: 25%, 50% and 75% of the threshold value. Three days of unilateral stimulation (FE electrode) separated the sessions of bilateral stimulation.

In the second part of the study rats were subjected (under ketamine anesthesia; 50 mg/kg i.m.) to unilateral

electrolytic lesions of VTA performed through the CE electrode (cathodal current 1.0 mA/10 s). The rats were tested for stimulation-induced feeding for 7 consecutive days after the lesion according to the same procedure as in the baseline conditions.

Data (percentage threshold change from the baseline and latency to feed) were analyzed by a repeated measures analysis of variance (ANOVA). The factors were: CE electrode stimulation intensity and FE electrode stimulation frequency. Findings from the ANOVA were further analyzed using Tukey test at $P < 0.05$ or Student's t -test (two-tailed). Pearson correlation coefficient was calculated to assess the correlation between the effects of the contralateral stimulation and the effects of the contralateral lesion.

RESULTS

In all rats the feeding response elicited by unilateral VTA stimulation through the FE electrode was facilitated when concomitant subthreshold stimulation was applied to the contralateral VTA through the CE elec-

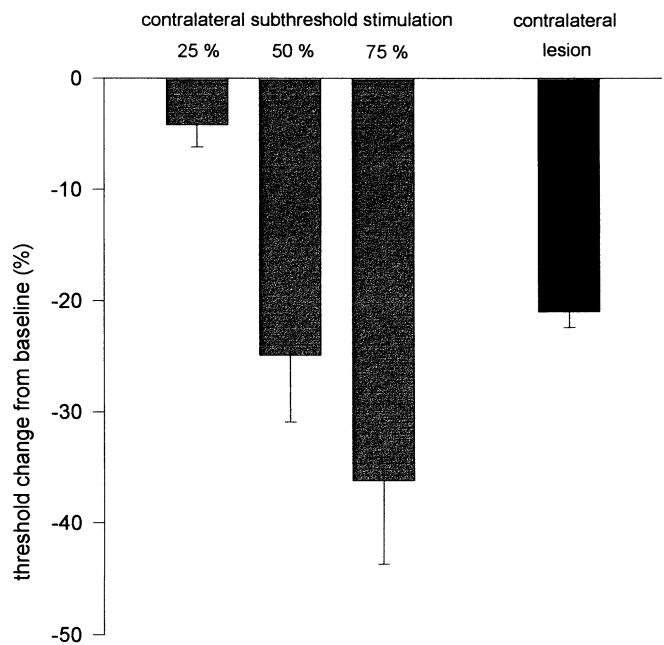


Fig. 1. Percentage change (means \pm SE) of the frequency threshold for VTA stimulation-induced feeding during simultaneous stimulation of the contralateral VTA through CE electrode and after lesion of the contralateral VTA. 25, 50 and 75% denote the percentage threshold intensity of CE stimulation. The effect of the lesion was expressed as the maximal threshold fall during the 7-day postlesion period.

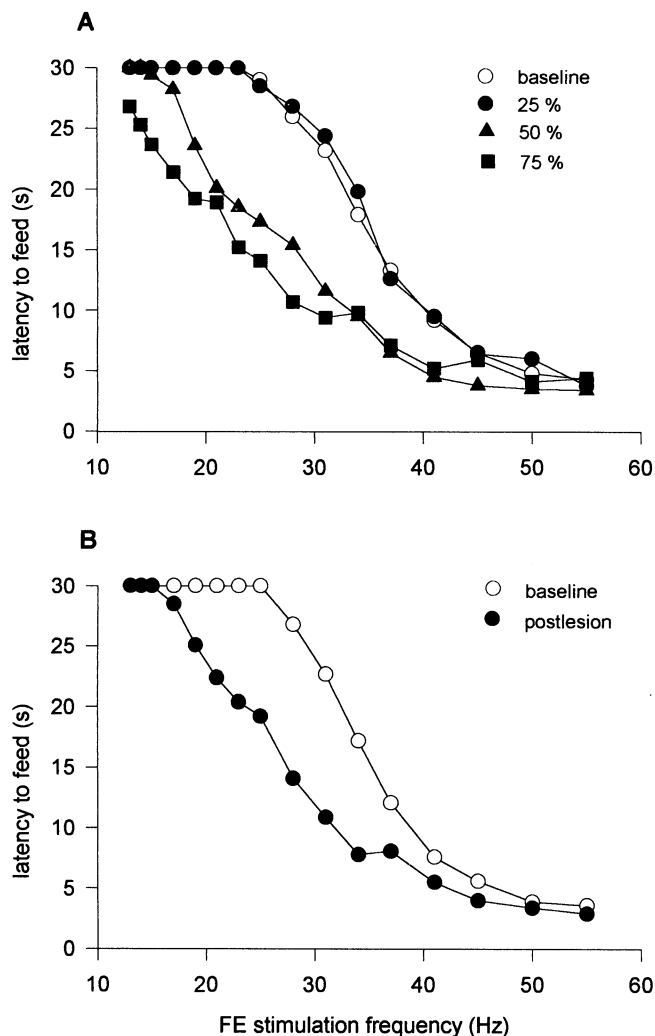


Fig. 2. Averaged latency-frequency curves for VTA stimulation induced feeding. A, during simultaneous subthreshold stimulation of the contralateral VTA (25, 50 and 75% denote the percentage threshold intensity of CE stimulation); B, after the lesion of the contralateral VTA (maximal postlesion change is presented).

trode. This facilitation manifested as a decrease in the frequency threshold for feeding (Fig. 1) and a leftward shift of the latency-frequency curve (Fig. 2A). Analysis of variance revealed a significant effect of contralateral stimulation on percentage threshold change from the baseline (averaged threshold on 3 days preceding the bilateral stimulation) ($F_{3,24} = 15.28$; $P < 0.0001$). The decrease in the threshold was intensity-dependent (Fig. 1). Tukey post hoc comparison showed that feeding threshold was significantly lower than the baseline at 50% and 75% but

not at 25% of CE current intensity. The difference between conditions of 50% and 75% of CE current intensity were insignificant.

Decrease in the feeding threshold due to the contralateral stimulation was accompanied by a parallel leftward shift of the function relating latency to feed to stimulation frequency (Fig. 2A). There was a significant effect on latency of stimulation frequency ($F_{15,384} = 77.44$; $P < 0.0001$) and the contralateral (CE) stimulation ($F_{3,384} = 64.46$; $P < 0.001$) as well as a significant frequency \times contralateral stimulation interaction ($F_{45,384} = 2.16$; $P < 0.001$). Latency to feed decreased with an increase in stimulation frequency, and with an increase in the contralateral VTA stimulation current intensity. Student's *t*-test comparisons showed a significant difference in the latency between the baseline and the conditions of 50 and 75% of CE current intensity at a frequency range of 31–41 Hz (31, 34, 41 Hz $P \leq 0.05$; 37 Hz $P \leq 0.01$) and 23–41 Hz (23, 25, 37, 41 Hz $P \leq 0.05$; 28, 34 Hz $P \leq 0.01$; 31 Hz $P \leq 0.001$) respectively. No significant differences were found at 15–28 Hz and 45–55 Hz (50%), and at 13–21 Hz and 45–55 Hz (75%). Latency to feed at 25% of CE current intensity was not significantly influenced. On the day after bilateral stimulation both the feeding threshold and the latency-frequency relationship returned to the baseline values and remained at this level during a 3-day period of unilateral stimulation.

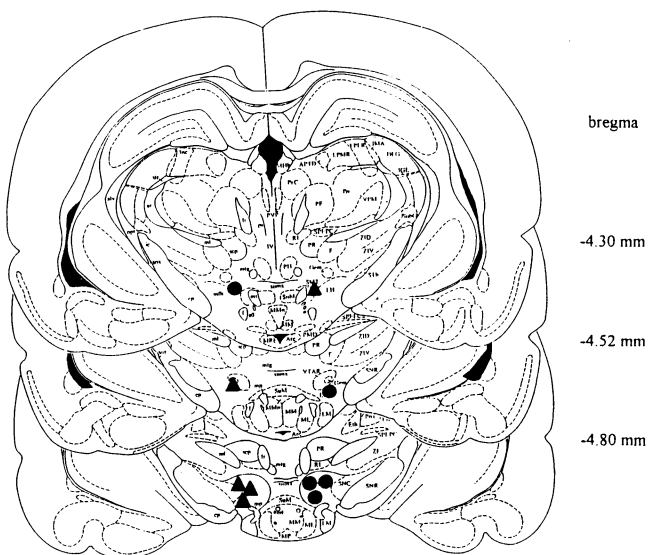


Fig. 3. Localization of the electrodes' tips superimposed on plates taken from the atlas by Paxinos and Watson (1986). Dots, FE electrodes; triangles, CE electrodes.

Subsequent electrolytic lesion of the VTA performed through the CE electrode also caused facilitation of feeding response elicited from the FE electrode which agrees with the previous findings (Trojnar and Staszewska 1994). In Figure 1 the effect of the lesion was expressed as a maximal daily decrease in the frequency threshold for feeding averaged across rats. This value ($-21.0 \pm 3.0\%$) was comparable to the effect of the moderate CE stimulation ($-24.9 \pm 6.0\%$ at 50% of CE current intensity). Figure 2B shows averaged latency/frequency curves illustrating maximal postlesion leftward shift from the baseline.

The percentage decrease in the feeding threshold due to the simultaneous CE stimulation was positively correlated with the maximal decrease in the threshold after the lesion ($r = 0.98$; $P < 0.01$ for 50% of CE current intensity, and $r = 0.84$; $P < 0.05$ for 75% of CE current intensity).

Histological verifications (Fig. 3) showed that both FE and CE electrodes were localized in the anterior part of VTA corresponding to the rostral extension of the parabrachial pigmented nucleus bordering the most posterior part of the lateral hypothalamus. Lesions encircled the CE electrodes' tips. The smallest facilitatory effect of both contralateral stimulation and the lesion was observed in a rat with the most rostral localization of the FE and CE electrodes.

DISCUSSION

Contrary to what has been assumed, opposite experimental manipulations of the activity of VTA did not result in an opposite influence on contralateral VTA stimulation-induced behavior. Paradoxically, both stimulation and lesion of one VTA facilitated feeding evoked from the contralateral VTA. In searching for a possible mechanism for this paradoxical effect, the following facts should be taken into consideration: (1) unilateral electrical stimulation of VTA causes DA release in the ipsilateral nucleus accumbens (Fiorino et al. 1993); (2) an increase in DA level in the nucleus accumbens is associated with induction of feeding behavior (Hoebel 1992); (3) unilateral suppression of DA activity in one hemisphere results in an increase in DA level in the terminal DA-ergic structures of the other hemisphere as was found in the nigrostriatal system (Nieoullon et al. 1977, Glowinski et al. 1979, Leviel et al. 1979, Zetterstrom et al. 1986, Robinson and Wishaw 1988). These facts may explain the facilitatory effects of our VTA lesions as was previously suggested (Trojnar and Staszewska

1994). If one accepts the view of the inverse interdependence of the bilateral dopamine systems (Nieoullon et al. 1977, Glowinski et al. 1979, Leviel et al. 1979), then in the case of bilateral stimulation two opposite processes might be assumed to operate simultaneously. Each hemisphere is both activated by the ipsilateral and inhibited by the contralateral VTA stimulation. Unilateral activation of DA-ergic pericarya, either electrically (Fiorino et al. 1993) or pharmacologically (Leviel et al. 1979) was found to release DA from axon terminals in the ipsilateral hemisphere and at the same time to lower DA level in the symmetrical DA-ergic terminal structures in the other hemisphere (Leviel et al. 1979). As may be inferred from the present study, the activating ipsilateral effect overrides the inhibitory one deriving from the contralateral hemisphere and bilateral activating influences summate resulting in facilitation of the behavioral output. If the effects of two stimulations are additive it is supposed that they summate on the same synapse. Where those synapses are located in the mesocorticolimbic circuitry is not certain. The other possibility is that subthreshold CE stimulation, due to the somatodendritic DA release and activation of the autoreceptor mechanism, may rather cause a diminution of DA-ergic transmission in the VTA target structures of the same hemisphere evoking a compensatory increase in DA release in the homologous structures of the other hemisphere (Nieoullon et al. 1977, Glowinski et al. 1979, Leviel et al. 1979) which summate with the activatory effects of FE suprathreshold stimulation. This results in augmentation of behavior. Therefore, the effect of subthreshold CE stimulation would act in the same direction as the lesion effects.

However, the mode of functional interactions between bilateral mesocorticolimbic systems may be more complex than that deriving from the "inverse interdependence model". In our preliminary c-Fos studies (unpublished) unilateral VTA stimulation-induced activation spreads to the symmetrical structures of both hemispheres (mainly cortical, limbic and thalamic). Although activation is higher in the stimulated hemisphere it is also substantial in the contralateral hemisphere. C-Fos staining does not discriminate between activation of the excitatory and inhibitory neurons but in the self-stimulation studies bilateral increase in DA level in the medial frontal cortex and nucleus accumbens was found during unilateral electrical stimulation of the medial forebrain bundle (Nakahara et al. 1992). Bilateral spread of activation during medial forebrain bundle self-stimulation was also postulated on the basis of electro-

physiological experiments (Malette and Miliareissis 1995). Nevertheless, significant augmentation of the lateral hypothalamic self-stimulation was described after contraletal ablation of the prosencephalic strutures (Colle and Wise 1987).

It may also be supposed that in our experiment facilitatory effects of the lesion and stimulation reflect different mechanisms of interhemispheric integration. The exact way by which the mesolimbic system of both hemispheres communicate in the control of feeding and other appetitive behaviors is not clear. VTA efferent projections are mainly ipsilateral but about 10% of the fibers are crossed at different levels of the neuraxis (Swanson 1982, Oades and Holliday 1987). Communication may thus occur at the level of VTA target structures. Indeed, interhemispheric projections were found at the level of the medial prefrontal cortex, and between the medial prefrontal cortex and nucleus accumbens or other subcortical structures (Ferino et al. 1987, Brog et al. 1993).

No definite neurochemical explanation of the present data can be offered before direct measurements of the extracellular level of DA in conditions of bilateral stimulation and unilateral lesion of VTA are made. Irrespective of a possible mechanism involved, the present results provide functional evidence for bilateral interdependence within the mesocorticolimbic system, a problem that has not been addressed until now in either biochemical or functional studies. Augmentation of function both after inactivation and activation of the contralateral hemisphere indicates that interhemispheric functional relations are complex and may involve both reciprocal and non-reciprocal influences.

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