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Effect of unilateral injection of MK-801 into the area of A10 cells on feeding evoked by stimulation of homologous area in the contralateral hemisphere

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Abstract. It was found previously that unilateral electrolytic and 6-OHDA lesions of the ventral tegmental area (VTA) and unilateral intra-VTA injection of bicuculline resulted in facilitation of behavioral responses evoked by electrical stimulation of the symmetrical VTA area in the contralateral hemisphere. We postulated that „the contralateral facilitation effect", which may reflect the yet unexplored mechanism of immediate compensation after acute unilateral brain injury, is attributable to the A10 DA neurons and their regulatory inputs. The present study was aimed at examining the possible involvement of NMDA-mediated glutamatergic transmission in VTA in the „contralateral facilitation effect". The behavioral model of the VTA stimulation-induced feeding in rats was used. Latency to eat was measured as a function of stimulation frequency before and after unilateral intra-VTA injection of non-competitive NMDA receptors antagonist, MK-801, (doses 0.0, 1.25 and 2.5 μg). MK-801 caused a dose-dependent augmentation of feeding evoked by stimulation of the contralateral VTA, which manifested as a decrease in the reaction frequency threshold and a leftward shift of the latency/frequency curve. Dose 2.5 μg replicated the facilitatory effect of electrolytic and 6-OHDA lesions. The results are interpreted in terms of MK-801-evoked depression of excitatory glutamatergic tone over A10 DA cells and compensatory increase in DA release in the contralateral hemisphere.

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Numerous neurochemical studies have shown that the activity of the dopaminergic (DA) systems of both hemispheres, at least those comprising the nigrostriatal system, is reciprocally interrelated. Unilateral depression of A9 (substantia nigra) DA neurons activity due to the electrolytic and neurotoxic lesions or pharmacological manipulations was shown to induce a compensatory increase in DA release in the neostriatum of the contralateral hemisphere (e.g.: Nieoullon et al. 1977, Leviel et al. 1979, Robinson and Whishaw 1988) and to influence the functional state of the contralateral striatal cells (Garcia-Rill et al. 1980, Castellano and Rodriguez 1991). In our previous studies we found functional evidence for the interhemispheric interrelations within the mesocorticolimbic DA system at the level of the ventral tegmental area (VTA) (A10 cells group). Unilateral electrolytic lesion of VTA resulted in immediate facilitation of feeding and locomotor behavior evoked by electrical stimulation of the contralateral VTA (Trojniar and Staszewska 1994, Trojniar and Klejbor 1999, Maliszewska-Ścisło and Trojniar 1999). Similar facilitatory effect was obtained after selective unilateral destruction of VTA DA cells with 6-OHDA (Trojniar et al. 1997) and blockade of GABA-ergic tonic inhibition of these cells by unilateral injection of bicuculline (Trojniar and Klejbor 1999), indicating that the "contralateral facilitation effect" is attributable to A10 DA neurons and their regulatory inputs. Augmentation of the lateral hypothalamic self-stimulation behavior was also described after massive contralateral ablation of the prosencephalic structures, including those containing DA elements (Cole and Wise 1987).

Among neurotransmitters regulating the activity of the midbrain DA cells glutamate focuses the greatest attention. Midbrain (A10 and A9) DA neurons receive glutamatergic afferents from the medial prefrontal cortex, pedunculopontine tegmental nucleus and subthalamic nucleus (for review see: Kalivas 1993 and Meltzer et al. 1997). Ionotropic NMDA and non-NMDA, and metabotropic glutamate receptors are present on DA neurones (Meltzer et al. 1997). Microinjections of glutamate receptors (NMDA and AMPA) antagonists into VTA were shown to decrease DA release in the VTA target structures, i.e. the nucleus accumbens (Karreman et al. 1996, Westerink et al. 1996) and the prefrontal cortex (Enrico et al. 1998, Takahata and Moghaddam 1998, Westerink et al. 1998), suggesting a tonic excitatory glutamatergic influence over DA-ergic neurons. The excitatory effect of glutamate on A10 DA neurons appears to be preferentially mediated by NMDA *versus* non-NMDA receptors (Wang and French 1993). Following these facts the present study was aimed at examining the possible involvement of NMDA-mediated glutamatergic transmission in VTA in the "contralateral facilitation effect".

The experimental procedure adopted in the present work was as follows: latency of feeding response to the unilateral electrical stimulation of VTA area was measured as a function of stimulation frequency (Trojniar and Staszewska 1994, Maliszewska-Ścisło and Trojniar 1999) before and after contralateral microinjections of MK-801, a selective and highly potent non-competitive antagonist at the glutamate receptors of the NMDA subtype (Wong et al. 1986). The working hypothesis was that unilateral suppression of glutamatergic transmission in VTA should decrease DA level in the ipsilateral hemisphere (Karreman et al. 1996, Westerink et al. 1996, 1998, Enrico et al. 1998, Takahata and Moghaddam 1998) which would induce a compensatory increase in DA-ergic transmission in the contralateral hemisphere (e.g.: Nieoullon et al. 1977, Leviel et al. 1979, Robinson and Whishaw 1988) resulting in an enhancement of stimulation-induced feeding, a highly DA-dependent behavior (Wise and Rompre 1989).

Male Wistar rats ($n = 6$) weighing about 300 g at the time of surgery were used. They were housed in individual cages with free access to food and water under a 12 h light/12 h dark illumination cycle. All animals were implanted with a unilateral stimulating electrode (monopolar stainless steel electrodes of 0.3 mm in diameter insulated with varnish except at the square-cut tip) and a contralateral guide cannula (stainless steel cannulas 15.0 mm long, 0.6 mm diameter) aimed at VTA. Stereotaxic coordinates for implantation were: 4.5 mm posterior to the bregma, 1.0 mm lateral to the midline and 7.7-8.0 ventral to the skull surface (skull leveled). Stainless-steel jeweler screws screwed to the skull served as the earth and reference electrodes.

After one-week recovery from surgery, the rats were screened for VTA 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, 0.1 ms duration cathodal pulses were conducted from the stimulator to the electrode by flexible wire leads. Screening was carried out using a fixed stimulation frequency of 50 Hz; current intensity was raised incrementally in 30-s trials until forward

search, sniffing and eventually eating were observed. For each rat a stimulation intensity was determined which would induce feeding with a mean latency of 5-8 s; the range of such intensities was 110-230 μA. Once determined this stimulation intensity was used for all subsequent tests.

Once reliable feeding response was obtained the rats were tested daily in a latency paradigm, where stimulation frequency was varied from trial to trial. Latencies to feed were measured in 30-s trials; stimulation was maintained for a maximum of 30 s when the animal did not start eating or was discontinued 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 (i.e. blocks 1 and 2 started at the lowest and blocks 2 and 4 at the highest tested frequency). The range of tested frequencies was from 17 to 55 Hz. All frequencies were tested in each block. 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 calculated from each rat's latency-frequency function by a method of linear interpolation. Daily testing continued for each animal until the threshold stabilized.

The experimental procedure consisted in unilateral intra-VTA injection of MK-801 and in subsequent testing of the effect of this injection on the feeding reaction induced by electrical stimulation of the contralateral VTA. All injections were performed using a 10 μl Hamilton syringe through the chronically implanted guide cannulas. The needle of the syringe (0.3 mm diameter) extended about 0.2 mm past the tip of the guide cannula. Injection volume was 0.5 μl and injection time was 1 min. Another minute was allowed before the syringe was disconnected. Stainless still blockers were kept in the guide cannulas between injections. MK-801 (from RBI) was dissolved in distilled water (Aqua pro injectione, Polfa). The doses were 0.0, 1.25 and 2.5 μg, they were administered in a random order. Dose 0.0 was the solvent. Each rat received 4 injections, the last injection took place on the 26th-29th day after implantation surgery. The rats were tested for stimulation-induced feeding for about 30 min starting immediately after each injection. A three-day interval was allowed between the injections during which the rats were tested daily in a drug-free state to confirm the baseline. After completion of behavioral testing the animals were sacrificed and localization of the stimulating electrodes and injection cannulas was determined.

Data (percentage threshold change from the baseline and latency to feed) were analyzed by a repeated measures analysis of variance (ANOVA). The factors were: treatment (dose) and stimulation frequency. Findings from ANOVA were further analyzed using Tukey test at $P<0.05$ or Student's t-test (two tailed).

Unilateral injection of MK-801 to A10 cells area resulted in facilitation of feeding response to stimulation of the symmetrical contralateral area which manifested as a decrease in the reaction's frequency threshold (Fig. 1) and a parallel leftward shift of the function relating latency to feed to stimulation frequency (Fig. 2). No such effect was found after a control injection of distilled water (dose 0.0 μg). No effect of repetition of drug injection was observed either. One way ANOVA with factor dose revealed significant effect of the treatment on percentage threshold change from the baseline (averaged threshold on 3 days immediately preceding the injection, i.e. each experimental injection was compared to its own baseline) ($F_{3,32} = 20.57$, $P<0.001$). Mean ± SE percentage threshold change was -16.5 ± 3.9 % after 1.25 μg of MK-801, -20.5 ± 3.4 % after 2.5 μg and -0.2 ± 1.8 % af-

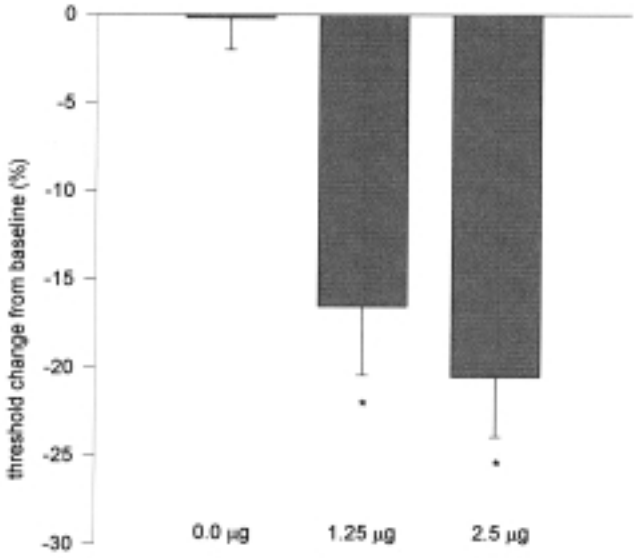

Fig. 1. Percentage change (mean ± SE) of the frequency threshold for VTA stimulation-induced feeding after MK-801 injections. Asterisks: Tukey test differences ($P<0.05$) from the preinjection baselines.

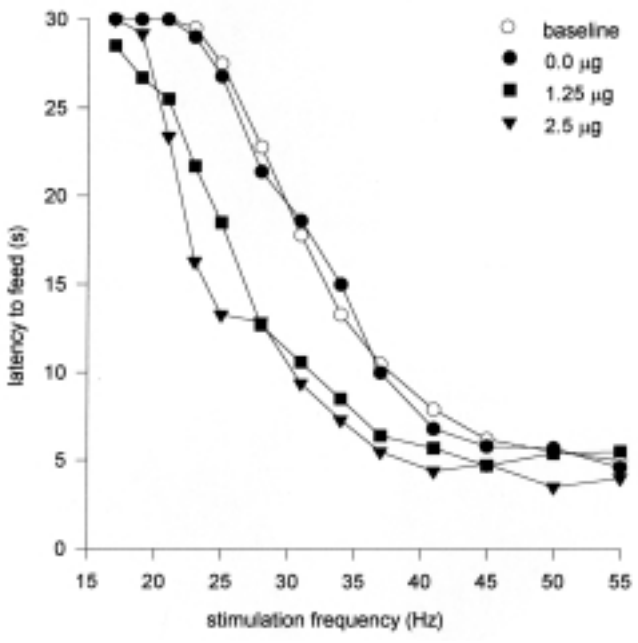

Fig. 2. Dependence of the mean feeding latency (ordinate) on the frequency of the VTA stimulation (abscissa) before and after injections of different concentrations of MK-801. The baseline is an average of 3 baselines immediately preceding each experimental injection.

ter 0.0 mg. Tukey test post hoc comparisons showed a significant difference between doses 1.25 and 2.5 µg and the baseline, and a lack of difference between both MK-801 doses. Individual differences in the reaction to MK-801 were observed. In two rats the dose 1.25 µg had stronger facilitatory effect than the dose 2.5 µg.

Two way ANOVA on the latency data showed a significant effect of stimulation frequency ($F_{12,260} = 89.19$, $P<0.001$) and MK-801 injection ($F_{3,260} = 23.78$, $P<0.001$). No significant frequency x injection interaction was found ($F_{36,260} = 1.32$, $P<0.1$). Latency decreased with an increase in stimulation frequency and after MK-801 injection. After 1.25µg of MK-801 it was significantly shorter in comparison to its baseline (Student's *t*-test) in a frequency range of 25-45 Hz, except for 41 Hz (25, 31, 34, 45 Hz $P< 0.05$; 28, 37 Hz $P< 0.01$) but not at 17-21 and 50-55 Hz (asymptotic values). After 2.5 µg of MK-801 latency was significantly shorter at a range of 23-50 Hz, except for 45 Hz (23-28, 37, 41, 50 Hz $P< 0.05$; 31 and 34 $P< 0.01$) but not at 17-21 and 55 Hz. No significant influence of water injection on the reaction's latency was found.

Histological verification showed that all electrodes and cannulas were located in the anterior VTA bordering the most posterior part of the lateral hypothalamus, i.e. in the area of A10 cells (German and Manaye 1993).

In the present experiment we have shown that unilateral blockade of VTA NMDA receptors resulted in augmentation of feeding reaction evoked by electrical stimulation of the contralateral VTA. 2.5 µg of MK-801 replicated the effect of the electrolytic and 6-OHDA lesions that usually caused approximately 20% decrease in the reaction's threshold (Trojniar and Staszewska 1994, Trojniar et al. 1997, Maliszewska-Ścisło and Trojniar 1999, Trojniar and Klejbor 1999). It can be supposed that facilitatory effect found in this study is attributable to the interference of MK-801 with the activity of A10 DA cells.

As was mentioned glutamate plays an essential role in regulating the activity of the mesocorticolimbic DA system, exerting a tonic excitatory influence on VTA DA neurons (Meltzer et al. 1997) preferentially through the NMDA receptors (Wang and French 1993) which are present in VTA on both DA and non-DA cells (Seutin et al. 1990, Wang and French 1995). MK-801, when administered directly into unilateral VTA was shown to lower DA level in the ipsilateral nucleus accumbens (Karreman et al. 1996, Westerink et al. 1996) and prefrontal cortex (Enrico et al. 1998, Takahata and Morghaddam 1998, Westerink et al. 1998). Recently, Kretschmer (1999) showed that intrategmental infusion of MK-801 increased a somatodendritic DA release in VTA which through the inhibitory feedback mediated by autoreceptors might result in a decrease in the synaptic release of DA in the VTA target structures. Also phencyclidine, another non-competitive NMDA receptor blocker, was shown to decrease the basal firing rate of the VTA DA neurons (French 1986). It may be supposed that according to the "inverse relationship model" of interhemispheric control of DA activity (Nieoullon et al. 1977, Leviel et al. 1979) augmentation of feeding reaction was due to compensatory increase in DA transmission in the contralateral VTA target structures. As there are no direct data on DA level in the contralateral mesolimbic structures after unilateral administration of MK-801 to VTA, our assumption is only speculative. For example Zhang et al. (1992) found no effect on basal DA-ergic activity of iontophoretic application of MK-801 into VTA of anesthetized rats. Rao et al. (1990) observed an increase in DA metabolites concentration in the ipsilateral pyriform cortex after unilateral microinjections of MK-801 into VTA with no changes on the contralateral side. Whatever the mechanism in-

volved, the present experiment provides further functional evidence on the interhemispheric interdependence in the mesocorticolimbic system, in which a glutamatergic control of DA activity may play a significant part. We believe that changes which are not yet well understood in the intact hemisphere after unilateral alterations of the activity of A10 DA cells may be of importance for immediate compensations after acute unilateral brain injury.

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