ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1995, 55: 201-206

Bilateral lesions of the pedunculopontine tegmental nucleus affect feeding induced by electrical stimulation of the ventral tegmental area

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Abstract. The pedunculopontine tegmental nucleus (PPN) is anatomically connected with dopaminergic cells in the ventral mesencephalon, which are known to participate in the regulation of various adaptive appetitive behaviours. In the present experiment we studied a possible involvement of PPN in feeding elicited by stimulation of the ventral tegmental area (VTA). It was found that bilateral electrolytic lesioning of the PPN affected VTA-elicited feeding. However, the effects were diverse and showed dependence on the localization of the lesion within the PPN area. Lesions localized anteriorly in the PPN impaired VTA feeding whereas those involving the middle portion of the nucleus facilitated electrically elicited food ingestion. A precise alignment of the lesion and the area activated at the site of stimulation appeared crucial for the effect of the lesion. The results indicate that PPN belongs to the central feeding circuitry and it contains both activating and inhibiting elements directed to the ventral tegmental area.

Short communication

Key words: pedunculopontine tegmental nucleus, ventral tegmental area, stimulation-induced feeding, lesion

The pedunculopontine tegmental nucleus is a group of neurones mainly cholinergic but also non-cholinergic located in the ventrolateral portion of the mesencephalic tegmentum in close association with the superior cerebellar peduncle (Olszewski and Baxter 1954, Armstrong et al. 1983, Rye et al. 1987, Spann and Grofova 1992). Cholinergic PPN neurones through their projections to the thalamic intralaminar and midline nuclei (Jackson and Crossman 1983, Lavoie and Parent 1994) function as a part of a nonspecific activating system regulating sleep-waking cycle (Steriade et al. 1990). Through their projections to the dopaminergic neurones of the ventral mesencephalon (Jackson and Crossman 1983, Niijima and Yoshida 1988, Lavoie and Parent 1994) they are in a position of influencing various appetitive and reward-dependent behaviours associated with activation of the mesolimbic-mesocortical and the nigrostriatal systems (Bechara and van der Kooy 1989, 1992, Buscher et al. 1989, Yeomans et al. 1993, Olmstead and Franklin 1994).

The intimate anatomical connections of PPN with the mesolimbic and extrapyramidal structures and earlier reports on PPN engagement in brain stimulation reward and drug-rewarded reactions (Bechara and van der Kooy 1989, Buscher et al. 1989) led us to study a possible involvement of PPN in a more natural adaptive appetitive behaviour such as feeding. It is hypothesized (Wise 1988) that certain aspects of food intake, drug taking behaviour and intracranial self-stimulation may be mediated by the same neural process based on a common morphological substrate.

In our previous work (Trojniar and Wise 1992) we found that unilateral electrolytic lesions of PPN facilitated feeding elicited by electrical stimulation of the mesolimbic system at the level of the lateral hypothalamus/ventral tegmental area whereas addition of the second PPN lesion on the other side of the brain disrupted or even completely blocked electrically elicited food ingestion. This was one of the first reports on the involvement of PPN in the central mediation of feeding. The results suggested an existence in PPN of both facilitatory and inhibitory feeding-related elements and a complex interhemispheric relations within the PPN-mesolimbic circuitry. To explore further functional relations between PPN and VTA in the control of food intake, in the present experiment we studied the effect on VTA stimulation-elicited feeding of bilateral damage to the PPN area made one-stage.

A group of male Wistar rats was implanted with 2 pairs of bilateral chronic electrodes (stainless steel pins of 254 μm in diameter), one pair was aimed at the anterior VTA (Paxinos and Watson (1986) stereotaxic coordinates: 4.3 mm posterior to the bregma, 1.0 mm lateral to the midline and 7.8-8.5 mm ventral to the skull surface), the other pair was aimed at the PPN area (stereotaxic coordinates: 7.8 mm posterior to the bregma, 1.7 mm lateral to the midline and 7.2-7.3 mm ventral to the skull surface). Six animals which ate reliably in response to VTA stimulation were used for the study.

Feeding was tested in a 250 x 350 x 440 mm box with food pellets covering the floor. Thirty minutes before testing rats were allowed to explore the box to ensure habituation and complete satiation. Trains of square-wave constant current 0.1 ms duration cathodal pulses of constant intensity were conducted from the stimulator to the VTA electrode by a flexible wire leads. Pulse duration, pulse frequency and stimulation intensity were monitored by oscilloscope.

After a period of preliminary screening during which optimal stimulation intensity was determined for each VTA electrode in each subject (range 75-170 μA), the rats were tested in a latency to feed--stimulation frequency paradigm where frequency of stimulation was varied from trial to trial. Latencies to eat were measured in 30-s trial; 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 each 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 17-53 Hz in control conditions and was adjusted as required under post-lesion conditions. A total of 13 stimulation frequencies was tested per block. The four blocks were

averaged to obtain a daily mean 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.

After stabilization of feeding reaction, under short-lasting inhalant halothane anaesthesia, bilateral electrolytic lesions of PPN were performed (cathodal current 1.5 mA/15 s). For 7-14 days after the lesions, the rats were tested daily for VTA stimulation-induced feeding according to the same procedure as in the prelesion period.

The results are presented on Fig 1. Percentage change of the frequency threshold for feeding in comparison to the prelesion baseline is shown in particular subjects for each VTA electrode. Threshold change was averaged across weeks of testing.

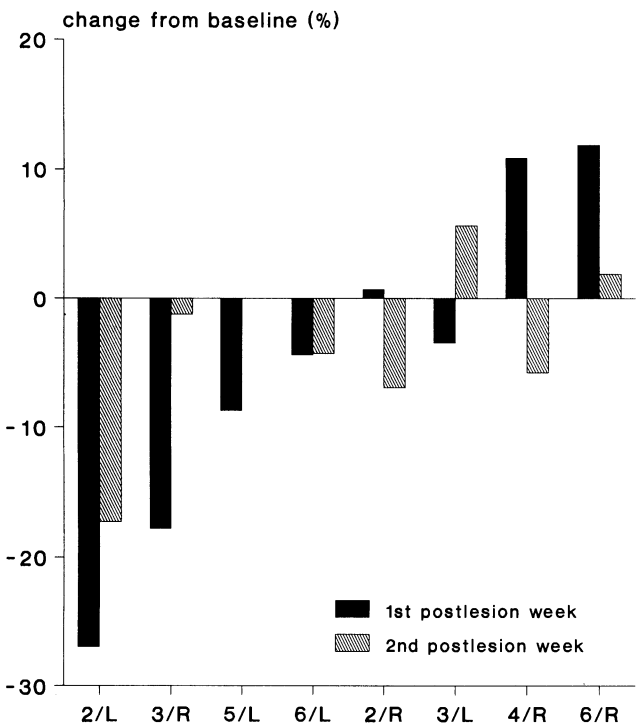

Fig. 1. Percentage change of the frequency threshold for VTA stimulation-induced feeding in particular rats subjected to the bilateral lesions of the PPN area. In 4 out of 6 tested animals feeding was elicited after stimulation by each of the VTA electrodes, and in 2 other from one electrode (left or right). The results of rat No. 1 and rat No. 4 - left electrode are not shown due to a loss of feeding reactions after the lesion. Abbreviations on the abscissa: 2-6 are rat's identification numbers; "L" and "R" mark respectively left and right VTA electrodes.

Observed effects were diverse. The predominant tendency was facilitation of feeding as a result of bilateral injury to the PPN area which manifested as a decrease of threshold and also by a leftward shift of latency-frequency function (not shown). However, animals differed in the magnitude and stability of this effect. Most pronounced facilitation occurred in rat No. 2 (left electrode) where consistent decrease of feeding threshold (up to 34.4% of the baseline on particular postlesion days) persisted up to the end of the experiment. In other cases facilitation was either shorter or smaller, or the reaction became unstable and threshold oscillated around the baseline. In some cases feeding reaction became destabilized with a tendency to an increase of the threshold. In two rats (No. 1, and No. 4 - left electrode) (not shown on Fig. 1) feeding behaviour in response to VTA stimulation became abortive. Stimulation evoked unusual agitation during which an animal was running around the cage with a food pellet in its mouth never actually eating it, and occasionally even trying to jump out of the testing box still carrying the pellet.

It is worth noticing that in the same animal the same PPN lesion might have produced different effects on feeding elicited from the right and the left VTA electrode. An extreme example is the rat No. 2 in which there was a profound facilitation of feeding elicited from the left electrode and only slight destabilization of the threshold for feeding from the right VTA electrode. The other example is the rat No. 6 in which slight facilitation of the left VTA stimulation-induced feeding coexisted with an impairment of behaviour elicited from the right VTA.

Histological examination of the lesions and localization of VTA stimulating electrodes suggests dependence of the effect on localization of the damage within the PPN area and on the precise topographical relations between the site of stimulation and the site of PPN damage. Figures 2 and 3 show histological verification of brains of rats displaying respectively a complete disorganization of feeding

behaviour (No. 1 described above) and a long-lasting facilitation of feeding from one VTA electrode (No. 2 - left) and almost lack of the effect on feeding elicited from the other electrode (No. 2 - right).

In case No. 1 lesion destroyed the anterior part of PPN bordering the caudal edge of the substantia nigra. Damage extended to the middle part of the PPN and involved substantial portions of the retrorubral field and surrounding tegmental tissue. Stimulating electrode was localized in the most anterior part of VTA bordering the lateral hypothalamus. In case No. 2 PPN lesions were symmetrically placed in the middle portion of PPN laterally to the superior cerebellar peduncle. The VTA electrodes differed slightly in the dorso-ventral dimension, the more affected being localized more ventrally. It is worth noticing that the end of the lesion in rat No. 1 overlaps the beginning of the lesion in rat No. 2. The lesions and electrodes of the remaining animals fell approximately within the limits between the most anterior sections shown on Fig. 2 and the most posterior sections shown on Fig. 3.

The results obtained in the present experiment are to some extent consistent with those of our earlier study (Trojniar and Wise 1992). Both facilitatory and inhibitory effects on VTA stimulation-elicited feeding were observed after damage to the PPN

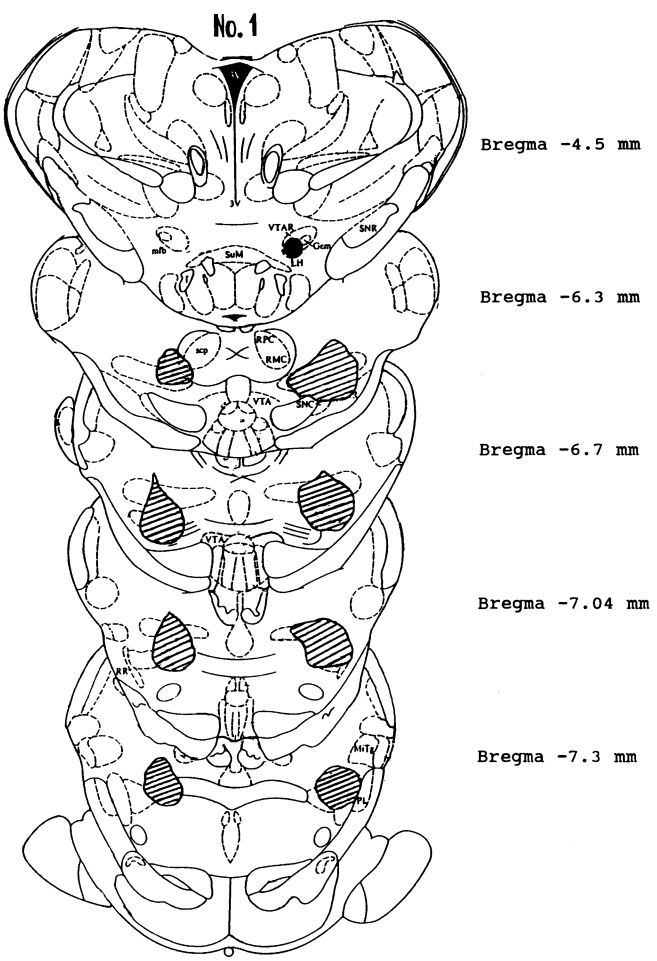

Fig. 2. Histological verification of the lesion (shaded areas) and localization of stimulating electrode (filled dot) in the rat (No. 1) in which VTA stimulation-elicited feeding was lost after the lesion. Plates were taken from atlas by Paxinos and Watson (1986).

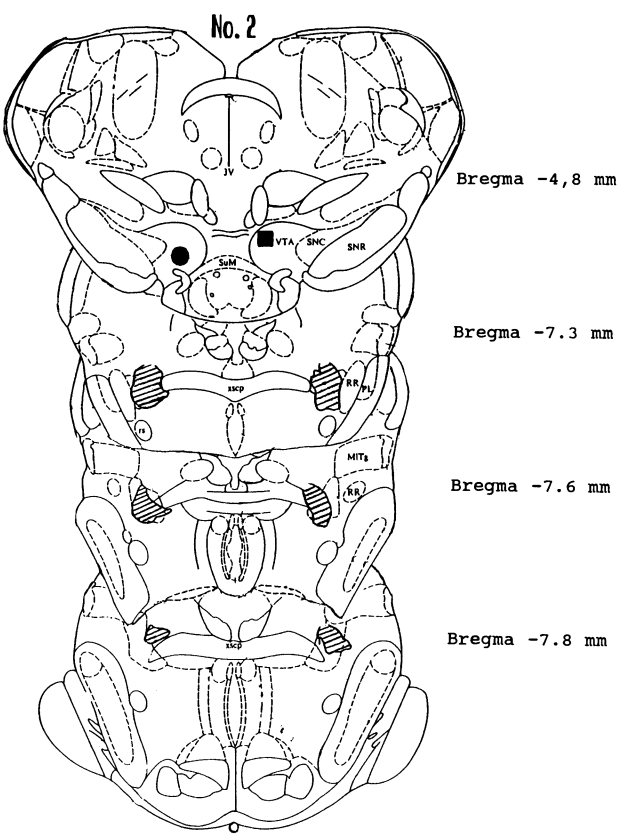

Fig. 3. Histological verification of the lesions (shaded areas), and localization of stimulating electrodes in the rat (No. 2) in which after the lesion VTA stimulation-induced feeding was facilitated from the left electrode (filled dot) and almost unchanged from the right electrode (filled square). Plates were taken from atlas by Paxinos and Watson (1986).

area. The analysis of lesion placements suggests that impairment of feeding is associated with lesions placed anteriorly, whereas facilitatory effect follows lesions located in the middle part of PPN. Lesions involving both divisions of the PPN area give either mixed effects or lead only to destabilization of reaction to stimulation. Our data also suggest rather narrow localization of feeding relevant neuronal elements connecting VTA and PPN. Slight variation in dorso-ventral localization of VTA electrodes in two hemispheres might have been responsible for different effect of the lesion on feeding elicited from the left and the right electrode in rat No. 2 and No. 6. Thus, precise alignment of the lesion and fibres activated at the stimulation site seems to be crucial for the effects observed in this experiment. Similar suggestion had been made by Murray and Shizgal (1991) on the basis of their study on the effect of electrolytic lesions on lateral hypothalamic self-stimulation. In the future studies the proper alignment should be found out between the VTA stimulation sites and effective PPN areas. However, these anatomical relations may appear even more complicated if we regard bilateral connections between PPN and mesencephalic dopaminergic area (Jackson and Crossman 1983, Lavoie and Parent 1994). Future studies should also resolve the question of the involvement of the retrorubral vs. anterior PPN area in inhibition of stimulation-induced feeding.

The mechanism through which PPN influences feeding behaviour is not certain. There are only two other studies exploring the involvement of PPN in food intake control. Dunbar et al. (1992) found no effect of the excitotoxic PPN lesions on spontaneous feeding. Our rats did not display any change in daily food intake either as may be judged from the normal body weight gain during the experimental period. On the other hand Bechara and van der Kooy (1992) found deficit in place conditioning with food reinforcement in satiated but not in food deprived PPN-lesioned rats. This suggests that PPN may be involved in rewarding rather than homeostatic aspect of food. In our model we studied feeding in response to stimulation of VTA which together with its efferent structures is supposed to determine rewarding value of various classess of stimuli. Thus, our data may be treated as compatible with that of Bechara and van der Kooy (1992).

We are not aware of any other report on facilitatory effect of PPN lesions on feeding. The only data which may correspond with our finding were reported by Bachus and Gale (1986) who described enhancement of amphetamine and cocaine-induced locomotor activity by stimulation of $GABA_A$ transmission in the PPN area by means of bilateral injections of muscimol. If we assume that VTA stimulation-elicited feeding and stimulant drugs--elicited forward locomotion derive from activation of a common anatomical (mesolimbic-related) substrate (Wise 1988), thus the effect of bilateral PPN lesion in the present study was comparable to that produced by an enhancement of GABA-ergic transmission in PPN. It may suggest that both experimental manipulations lead to suppression of a process or neural pathway which normally inhibits or competes with mesolimbic-derived activities. Bachus and Gale (1986) pointed to the anatomical specificity of their effect. Localization of their effective muscimol injections corresponds to that of our facilitatory PPN lesion shown on Fig. 2.

In summary, the present results support our previous finding (Trojniar and Wise 1992) that PPN belongs to the circuitry through which realizes the feeding behaviour initiated by activation of the mesolimbic system. They indicate the functional diversity of the PPN area and may constitute the basis for further, more detailed investigation of VTA-PPN relations.

Authors wish to thank Mrs Małgorzata Maliszewska for her valuable help in preparing the manuscript. This work was supported by Grant No. 4 0546 91 01 from the Scientific Research Committee of Poland.

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Received 21 November 1994, accepted 21 April 1995