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Original articles

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DAMAGE TO THE NUCLEUS ACCUMBENS SHELL BUT NOT CORE IMPAIRS VENTRAL TEGMENTAL AREA STIMULATION-INDUCED FEEDING

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Food intake is regulated not only by homeostatic requirements but also by emotional factors (*e.g.* palatability of food, alleviation of emotional tension *etc.*). The nucleus accumbens (Acb) is a part of the mesolimbic dopaminergic system which is responsible for a positive emotional aspect of various homeostasis-relevant stimuli. In the present work, we tested the Acb involvement in feeding behaviour using an experimental paradigm specifically designed to assess motivational *vs* motor aspect of food ingestion. In rats, feeding was evoked by electrical stimulation of the midbrain ventral tegmental area (a somatodendritic region of mesolimbic system) and assessed quantitatively with the use of the latency to feed/stimulation frequency curve-shift paradigm before and after electrolytic lesion of Acb. An impairment of stimulation-induced feeding manifesting as an elevation of the reaction threshold and a rightward, parallel shift of the stimulation frequency/reaction latency curve in the range of frequency which is sensitive to motivational aspects of food occurred after lesions localized mainly in the Acb shell. The lesions situated mainly in the Acb core were ineffective. The results obtained indicate that the Acb shell connected with the limbic system but not the motor-related Acb core affects motivational aspects of feeding behaviour.

Key words: *food intake, nucleus accumbens, ventral tegmental area, electrical stimulation, lesion*

INTRODUCTION

Food intake is regulated by a number of homeostatic factors (glucostat, lipostat, thermostat, mechano- and chemoreceptive information from the digestive tract, *etc.*) involving multiple neural (1-3) and humoral (4-8) signals. However, nutritional requirements and internal satiety signals do not entirely explain feeding behaviour, particularly food palatability-dependent overeating

and compulsive food seeking or pursuit frequently accompanying states of emotional tension, negative mood or depression (9, 10). As we found some time ago, feeding behaviour is even more directly guided by the oropharyngeal information from taste and smell receptors then by interoceptive signals of nutritional state of an organism (11).

Positive emotional (rewarding) values of exteroceptive alimentary stimuli seem to be related to activation of the so called "brain reward system" identified with the mesolimbic dopaminergic system (12,13, but see 14). The mesolimbic system begins (the somatodendritic area) in the dopaminergic A10 cells in the area of the anterior midbrain (ventral tegmental area, VTA) and the most posterior part of the lateral hypothalamus (15-17) and terminates in a number of cortical and subcortical limbic structures with the nucleus accumbens (Acb) being one of the most prominent mesolimbic nuclei (18).

The Acb, a forebrain region located ventrally to the basal ganglia (also called the ventral striatum) (19), is a part of brain circuitry integrating affective aspects of stimuli with motor programs (for review see 20-22). According to Mogenson *et al*. (23) the Acb functions as an interface between emotion and action. Correspondingly, two anatomically and functionally distinct subregions of Acb have been distinguished: the limbic related Acb shell and the basal ganglia related Acb core (24, 25).

Both the somatodendritic area (VTA) and the terminal field (Acb) of the mesolimbic system are involved in food intake. Electrical stimulation of the VTA induces feeding in satiated rats (26-29). Pharmacological inactivation of glutamate AMPA and GABA receptors in the Acb shell also increases food intake in non-deprived animals. Acb opioid receptor stimulation in rats specifically enhances ingestion of palatable solutions (21, 22) and Acb neurons were found to response to sweet taste (30). Although dopamine depletion or antagonism in Acb do not abolish natural eating it disrupts reactions necessary to obtain food which require increased effort (14).

To test for a nature of accumbal influence on feeding and the relations between the VTA- and Acb-generated food ingestion, in the present study we used an experimental procedure which enables to distinguish between motivational *vs*. motor aspects of tested reaction (31). In satiated rats feeding elicited by electric stimulation of the VTA was assessed quantitatively using the latency to eat-stimulation frequency curve shift paradigm which appeared useful in evaluation of the effects of brain damage (27, 28, 32) or stimulation (28) as well as various pharmacological agents (26, 29, 33) on food intake. The question asked was: what effect on the VTA stimulation-evoked feeding would have unilateral destruction of the Acb?

METHOD

Animals and surgery

Male Wistar rats (n=12) weighing 250-320 g at the time of surgery, were used. They were housed in individual cages with free access to food and water under 12 h light/12 h dark illumination cycle.

For 7 days the animals were handled and gradually adapted to the presence of the experimenter. Then, all animals were implanted, under pentobarbital anesthesia (50 mg/kg, i.p.) with unilateral stimulating electrode aimed at VTA and contralateral lesion electrode aimed at Acb (monopolar stainless steel electrodes, 0.3 mm diameter, insulated on the entire length except for the square-cut tip). Paxinos and Watson (34) coordinates for implantation were: VTA: 4.52-5.20 mm posterior to the bregma, 1.0 mm lateral to the midline and 8.0-8.1 mm ventral to the skull surface; Acb: 1.0-1.7 mm anterior to the bregma, 0.9-1.2 mm lateral to the midline and 7.0-7.1 mm ventral to the skull surface (skull levelled). The electrodes were anchored to four stainless steel screws with a dental acrylic; stainless steel wire soldered to one screw served as the anode for electrical stimulation.

The principles for the care and use of laboratory animals in research, as outlined by the Local Ethical Committee, were strictly followed and all the protocols were reviewed and approved by the Committee. All efforts were made to minimize both animals' discomfort and the number of animals used.

Experimental procedure

After 1-week recovery from implantation, rats were screened for VTA stimulation-induced behaviour. The testing was carried out in a 220 x 350 x 440 mm box (food covering the floor) placed in a sound-attenuating chamber. The rats were taken from their home cages, where they had free access to food and water, and were allowed to explore the test box for 30 min before testing to allow for habituation to the experimental conditions and complete satiaton. Trains of square-wave, constant current, 0.1 ms duration were conducted from the stimulator to the electrode by flexible wire leads. Pulse duration, pulse frequency and stimulation intensity were monitored by oscilloscope. Screening was carried out using a fixed stimulation frequency of 50 Hz; current intensity was raised incrementally in 30-s trials (20 s rest between trials) until feeding reaction was observed. For each rat such stimulation intensity was determined, which would, at a stimulation frequency of 50 Hz, induce behavioural response with a mean latency of 5-8 s; the range of such intensity was 95-250 μA. Once determined this stimulation intensity was used for all the subsequent tests.

Once reliable eating response was obtained, the rats were tested in a latency paradigm, where frequency of stimulation was varied from trial to trial. Latencies to eat were measured for 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 in the first and third blocks and decreased in the second and fourth. The between trial increments in stimulation frequency were 10% of each previous value. The range of tested frequencies was from 10 to 81 Hz in control condition and was adjusted as required under postlesion conditions. A total of 8-12 stimulation frequencies were tested per block, each block of trials took about 10 min to complete. The four tests were averaged to obtain a mean daily latency at each stimulation frequency. The frequency threshold for feeding response was determined for each daily testing. It was defined as the stimulation frequency at which an animal began to eat with a latency of 20 s. Threshold was calculated from each rat's latency-frequency function by a method of linear interpolation.

Daily testing continued until the threshold stabilized. Then, the animals were subjected (under short ketamine anesthesia, 85 mg/kg, i.p.) to electrolytic lesions of the contralateral Acb (in relation to stimulating electrode). Cathodal current of 2.0 mA was applied through 15 s. Seven animals were lesioned in the right hemisphere and five in the left hemisphere.

After the lesion for 14 days the rats were tested for stimulation-induced feeding according to the same procedure as in the prelesion period.

Histology

After completion of behavioural testing, the animals were sacrificed and localization of the stimulating and lesion electrodes was determined. Rats were overdosed with pentobarbital and were transcardially perfused with 200 ml of 0.9% saline, followed by 350 ml of phosphate buffered 4% formaldehyde. The brains were removed from the skull and stored in 4% formalin solution. After fixation brains were frozen and sectioned at 30 μm.

Data Analysis

Percentage threshold change from the prelesion baseline and the latency to feed as a function of lesion and stimulation frequency were subjected to the analysis. To test for significance of individual threshold changes the t-test based on comparison with the constant value was used. On its basis animals were assigned to the lesion-affected and lesion-unaffected groups and further analysis was performed separately for the distinguished groups. One-way analysis of variance (ANOVA) was used to test for an effect on feeding threshold of the lesion. Percentage threshold changes from the baseline (3 prelesion days) during the first and the second postlesion weeks were analyzed. Latency to feed was analyzed by two-way ANOVA; the factors were: time (weeks) postlesion and stimulation frequency. Findings from the ANOVA were further analyzed using Tukey's test at $p \leq 0.05$ or Student's t-test (two-tailed).

RESULTS

The effect of unilateral Acb electrocoagulation on VTA stimulation-induced feeding depended on localization of the lesion within the Acb. In 7 rats in which damage involved mainly the Acb shell (*Fig. 1B*) there was an impairment of feeding response which manifested as an elevation of stimulation frequency threshold (*Fig. 1A* inset) and a rightward shift of the stimulation frequency/reaction latency curve (*Fig. 1A*). In 5 rats in which lesion involved mainly the Acb core (*Fig. 2B*) no effect on VTA stimulation-induced feeding was observed (*Fig. 1A*). The rats were assigned to the "impaired" or "non-affected" groups on the basis of the t-test comparisons of the pre- and postlesion feeding thresholds. Averaged data from 3 days preceding and 14 days following Acb coagulation were compared for each subject. It occurred that in animals with damage to the Acb shell (n=7) there was a significant ($p \leq 0.0015$ to $p \leq 0.000013$) elevation of the frequency threshold whereas in rats with damage to the Acb core (n=5) statistical analysis did not yield significant differences ($p \leq 0.88$ to $p \leq 0.096$ in particular subject).

In the Acb shell-damaged group analysis of variance on the percentage threshold change from the prelesion baseline revealed a significant effect of the week of testing ($F_{2,105}=23.12$, $p \leq 0.0001$). In the first postlesion week, the threshold change (mean ± SE) from the prelesion baseline (week 0) was 21.3 ± 2.7%, and in the second week, 30.4 ± 2.9%. Tukey's test post hoc comparisons showed significantly higher feeding threshold on the second postlesion week (*Fig. 1A* inset) then in the first.

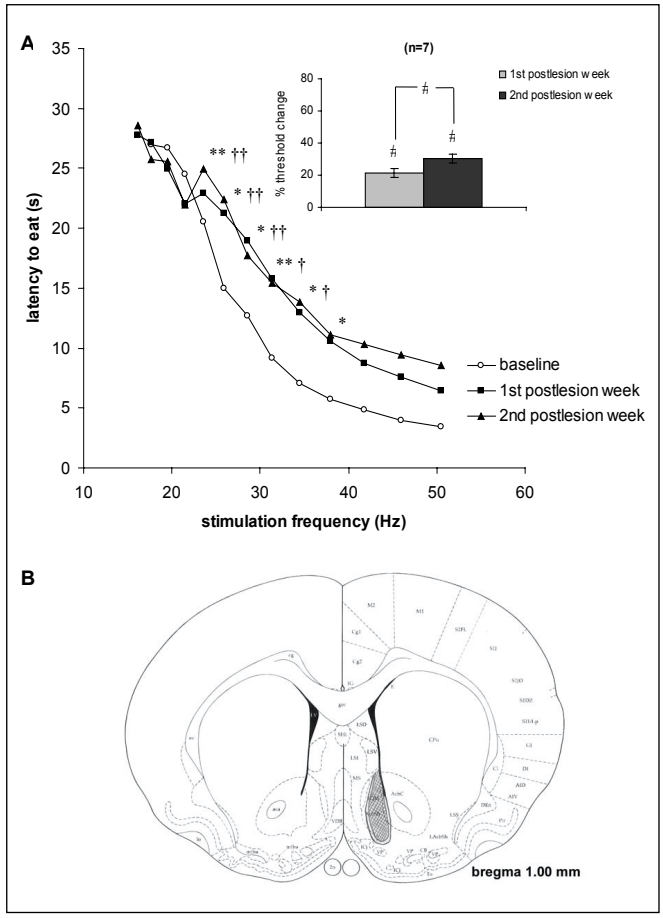

Fig. 1. Impairment of the VTA stimulation-evoked feeding after Acb shell lesion. (A) Function relating latency to feed to stimulation frequency before the lesion (control baseline) and on the first and second postlesion weeks, Student's t-test: * p≤0.05, ** p≤0.01 first postlesion week, † p≤0.05, †† p≤0.01 second postlesion week, significance of difference from the baseline. Inset: Mean (± SE) percentage change from the prelesion baseline of the feeding frequency threshold during the first and the second week postlesion. p≤0.05 (Tukey's test): # above the bars indicate significance of difference from the baseline, # in brackets, difference between the postlesion weeks. (B) Acb shell destruction (hatched area) in representative rat, superimposed on plate from the atlas by Paxinos and Watson (34).

Elevation of the frequency threshold for stimulation-induced feeding response was accompanied by a parallel rightward shift of the function relating latency to feed to stimulation frequency. There was a significant effect on latency of stimulation frequency ($F_{5,72}$=13.58; p≤0.0001) as well as the lesion ($F_{1,72}$=18.43; p≤0.0001). No frequency x lesion interaction was found ($F_{5,72}$=1.1; p≤0.37). Latency to initiate feeding response (*Fig. 1A*) decreased with the increase in stimulation frequency. It was significantly longer after the lesion. Significant (Student's *t*-test) lengthening (in comparison to the baseline) of the latency in the first postlesion week concerned stimulation frequency range from 23.6 to 38 Hz (23.6 Hz, p≤0.006; 26 Hz, p≤0.03; 28.5 Hz, p≤0.02; 31.4 Hz, p≤0.007; 34.5 Hz, p≤0.02; 38 Hz, p≤0.05) but not asymptotic part of the curve (41.8-50.5 Hz). In the second postlesion week the lengthening of response latency concerned the frequency range from 23.6 Hz to 34.5 Hz (23.6 Hz; p≤0.009; 26 Hz; p≤0.003, 28.5 Hz, p≤0.005; 31.4 Hz, p≤0.02; 34.5 Hz, p≤0.03) but not asymptotic values 38-50.5 Hz.

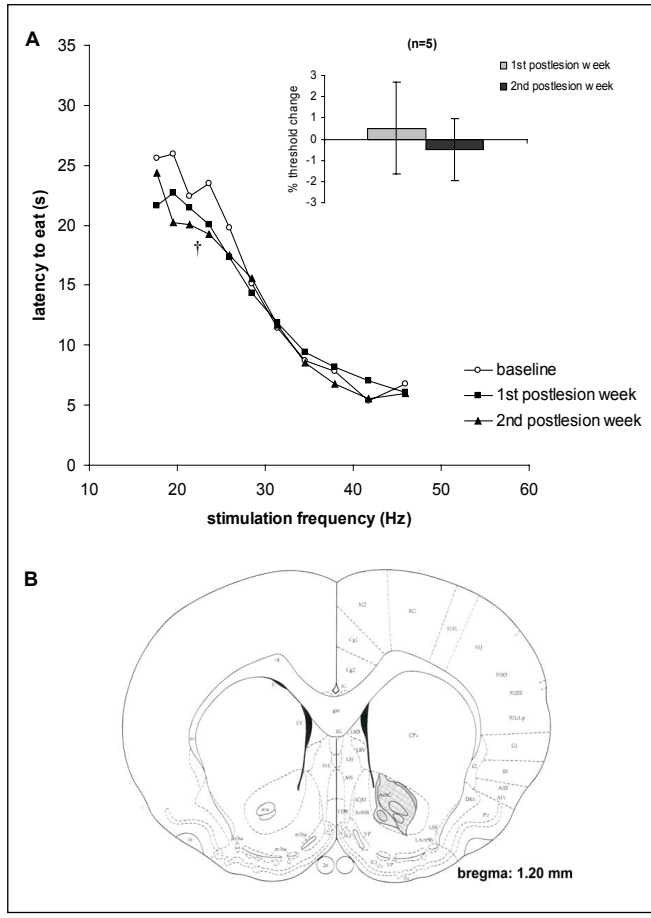

Fig. 2. Lack of effect of the unilateral Acb core lesion on VTA stimulation-induced feeding. (A) For explanation, see *Fig. 1.* (B) Acb core destruction (hatched area) in representative rat, superimposed on plate from the atlas by Paxinos and Watson (34).

In the Acb core damaged group the percentage feeding threshold change was insignificant ($F_{2,82}$=0.09; p≤0.9). In the first postlesion week the mean (±SE) threshold change was 0.51 ± 2.2%, and in the second week -0.49 ± 1.5% (*Fig. 2A* inset). Two-way ANOVA revealed a significant effect on feeding latency of stimulation frequency ($F_{6,56}$=11.53; p≤0.0001) but not the lesion ($F_{1,56}$=0.02; p≤0.89).

In the "impaired" animals the shared regions of the Acb damage involved mainly its shell subregion at the level of 1.6-0.48 mm anterior to the bregma, whereas ineffective lesions (non-affected group) involved mainly the Acb core localized in the middle part of the nucleus at the level of 1.6-0.7 mm anterior to the bregma. In all animals the stimulatory electrodes were localized in the area of the A10 dopaminergic cells at the level of the posterior hypothalamus/anterior VTA (17) (bregma -4.42 to -5.20 according to the atlas by Paxinos and Watson; 34).

DISCUSSION

The results obtained in the present study can be summarized as follows: (i) feeding response to electric stimulation of the VTA was impaired after contralateral destruction of the Acb, mainly in its shell subregion. The inhibiting influence of Acb shell lesion had a progressive character and was anatomically specific because damage to the Acb core had no effect. (ii) In the Acb shell-lesioned animals there was an increase in the reaction threshold (by about 20-30%) and a rightward shift of the latency to eat/stimulation frequency function indicating that rats were more reluctant to initiate feeding and needed higher stimulation frequency to respond with the same latency as before the lesion. The curve shift was parallel and significant at the range of current frequencies sensitive to motivational aspect of tested reaction (31). Although there was some elevation of the asymptotic part of the curve (highest frequencies and lowest latencies) sensitive to motor impairment, but it was insignificant. Neither of this was observed in the Acb core damaged animals.

These results correspond with those reports which indicate the role of Acb in motivational rather than homeostatic aspect of food intake. Our rats were neither food deprived nor oversatiated before the stimulation session and their basal food intake was not affected by the Acb lesion as may be inferred from the unchanged body weight gain. Also other authors did not find reduction of primary feeding or instrumental feeding behaviour which did not require much effort in the Acb dopamine-depleted animals (14, 35). However, when access to food required to overcome work-related response costs as in the case at certain schedules of reinforcement, the performance of the Acb dopamine-depleted animals was impaired (14). This points to the role of dopaminergic innervation of the Acb. Our data indicate that the Acb and VTA are interconnected functionally in respect to control of motivational aspect of feeding and this relationship concerns specifically the Acb shell. The Acb receives direct dopaminergic innervation from the VTA but it is also a part of limbic circuitry involving Acb afferents from the other VTA innervated structures such as the hypothalamus, amygdala, hippocampus, prefrontal cortex, *etc.* (20-22). In turn, the Acb sends efferents directly or through the ventral pallidum to the VTA, regulating its activity (36). Our results indicate that this Acb-VTA interrelation involves both cerebral hemispheres as VTA stimulation-induced feeding was impaired by the Acb lesion in the contralateral hemisphere. Which of many possible (direct or indirect) connections between the VTA and Acb shell are essential for the control of feeding is not certain at present. Some hypothesis have been recently put forward by Kelley *et al.* (21, 22). Regarding motivational nature of the Acb shell damage-evoked feeding impairment, hypo- or overactivity of this putative neuronal circuitry may be involved in pathologies of feeding behaviour (anorexia, hyperphagia leading to extreme obesity *etc.*) not related to metabolic and other homeostatic disturbances.

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