Indian J Physiol Pharmacol 1995; 39(4): 369-376

ELECTRICAL AND CHEMICAL STIMULATION OF THE SAME HYPOTHALAMIC LOCI IN RELATION TO AGRESSIVE BEHAVIOUR IN CATS : A COMPARISON STUDY

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(Received on February 21, 1995)

Abstract: Chemitrodes which permit electrical and chemical stimulation of the same hypothalamic loci were implanted in anterior hypothalamic and preoptic regions. These sites were stimulated electrically using biphasic square wave pulse (1 ms, 60 Hz) at a current strength ranging from 150-800 μA to evoke an aggressive response. At lower current strength of 150-200 μA, defence response, a sort of non-specific response can be elicited from these regions. Increasing the current strenght to 400 μA led to the recruitment of affective and somatic components and changed the response pattern either to affective attack or flight. The loci producing affective attack response were localized more laterally and ventrally while the loci producing flight response were located in the dorsomedial regions of the hypothalamus. In this response the animal made a goal-directed attempt to escape through an escape route. Increasing the current strength to 500 μA in the dorsomedial regions changed the flight response to violent flight, which involved vigorous running with unsheathed claws and attacking objects if obstructed. Similar increase in current strength at loci producing affective attack only led to a decrease in the latency of response and made the attack more vigorous. Microinfusion of carbachol in graded doses of 2-15 μg at all these loci produced a profound affective display. At lower doses of 2 and 5 μg, only some components of affective display like alertness, pupillary dilation and ear flatness were exhibited. Increasing the dose to 10 μg and 15 μg led to the recruitment of other affective components like piloerection, salivation, hissing and baring of teeth. Microinfusion of carbachol at all loci producing affective attack on electrical stimulation produced a prononced affective display while microinfusion of carbachol at loci producing flight response led to the development of defence posture. At six loci a typical flight response was obtained while violent flight was never exhibited at any of these sites. Microinfusion of atropine (10 μg in 1.0 μl saline) at these loci completely blocked the carbachol induced response. Both somatomotor and affective components were completely inhibited. However, the responses obtained on electrical stimulation were not totally blocked following atropine infusion and some of the somatomotor and affective components could be elicited with higher current strength. These studies indicate the involvement of cholinoceptive mechanisms in the elicitation of hypothalamically induced aggresive behaviour. Microinfustion of hexamethonium bromide, a nicotinic blocker in 50 μg doses did not affect the aggressive response.

Key words : affective attack, violent flight, preoptic area, carbachol, atropine sulphate, affective display, somatomotor components

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INTRODUCTION

Neural control of aggressive behaviour in freely moving cats is reported (1-3). Different types of aggressive responses and their modification by various environmental factors are well documented (4). Predatory attack as elicited by electrical stimulation of extreme lateral region of hypothalamus consists of minimal affective display while affective attack obtained on stimulation of the medial regions is accompanied by exaggerated affective components like piloerection, hissing, growling. The cat may run vigorously and make a goal-directed attack with unsheathed claws culminating in biting any threatening object e.g. a gloved hand, or a stick. Isolated studies by some workers (5-8) and recently by Dawra et al (9) indicating the involvement of cholinergic pathways in aggressive behaviour of affective type have also been reported. Chatterjee et al (10) have reported that the cardio-acceleratory and respiratory excitation responses produced by electrical stimulation of the preoptic area in anaesthetized cats involved the predominance of cholinergic mechanisms. Such autonomic manifestations also accompany the affective display associated with the aggressive responses. Therefore, the present study has been under-taken to study whether the aggressive responses induced by electrical stimulation of anterior and preoptic regions of hypothalamus could be blocked by microinfusion of atropine or any other blocking agent in the freely moving cats.

METHODS

A. Selection of the animals : The present study was carried out in 15 cats of either sex weighing between 2.5-4 kg. The cats were tamed and adjusted to the behaviour cage for a period of about 2 weeks to stabilize their behaviour. The tamed cats were friendly, and they were not suspicious of their surrounding.

B. Experimental design : The general design of the experiment was to implant "Chemitrodes" (which permit both chemical and electrical manipulation of the same locus) in the anterior hypothalamic region and preoptic areas of the cats' brain. The methods of construction of chemitrodes and their permanent implantation have been decribed earlier by Dawra et al (9). After surgical implantation of chemitrodes, the animals were tested again for having achieved their normal friendliness towards the experimenter. At each locus only one chemitrode was implanted at which electrical and chemical manipulation was carried out. The construction of behavioural cage was described by Dawra et al (9). The system employed for scoring of affective display and somatomotor components were given a detailed treatment in the same paper. In this paper we have additionally observed behaviours of flight and violent flight each of which was analysed on the basis of distinctive components as included in Table I.

Electrical stimulation using biphasic squarewave pulses (1 ms, 60 Hz) was performed for 20 sec at each hypothalamic site to achieve a

TABLE I : Percentage value given to each somatic and affective components.

Pattern	*Characteristics*	*% value*	*Maximum score*
A. Defence	Retracted neck	25	
	Atching of back	25	
	Coiling of the tail	25	
	Retracting	25	100
Affective attack	Unsheathing of claws	25	
	Striking with paws	25	
	Extended neck	25	
	Biting	25	100
Flight	Searching glances and staring at the escape route.	25	
	Going to a corner	25	
	Restless movements	25	
	Leaping to foot	25	100
Violent flight	Wild running in the cage	33	
	Vigorous leaping	33	
	Unsheathing of claws	33	100
B. Affective display	Alertness	6	
	Pupillary dialation	6	
	Salivation	6	
	Flatness of ears	6	
	Respiratory acceleration	6	
	Growling	15	
	Piloerection	15	
	Hissing	15	
	Baring of teeth	15	
	Urination and Defeacation	10	100

stimulus response relationship at various graded strengths of current. Each series of stimulation was repeated three times to get the average figure for plotting the stimulus response curve and to ensure the reproducibility of the response. A minimum time gap of 5 min was allowed between two successive stimuli. In a series of experiments, cholinergic stimulation was achieved by infusion of carbachol, a cholinergic agonist in graded doses of 2, 5, 10 and 15 μg to observe if the effects were comparable to electrical stimulation. In other experiments atropine sulphate (10 μg) and hexamethonium bromide (50 μg) were infused at the same site. Responses to electrical stimulation were again elicited after infusion of each of these blockers to find out the effect on stimulus response curves. The protocol as described above was followed at each of the locus.

C. Recording of observation : The behavioural cage had a one way viewing glass so that the cat could not see the experimenter. Events of motor and affective components were marked on pre-designed protocols. Photographs of different behavioural poses were taken and in some cats video movies were also made to tally with behavioural scores. The analysis of these behavioural components was done by the quantification methods already described.

D. Histological confirmation of the site : After completion of the experiment, the animals were anaesthetized with nembutal and anodal lesion was made at the site by passing a constant current of 2 mA for 10 sec. The animals were then sacrificed, brains processed histologically and serial sections examined by enlarging them 10 time. The chemitrode tracks were reconstruc-ted for each stimulation site. The deepest point where the tip of the track had reached was confirmed by consulting the atlas of Jasper and Ajmone-Marsan (11).

RESULTS

Chemitrodes were implanted in 25 loci in the anterior regions of the hypothalamus in 15 cats. Of these, 9 were localized in the anterior hypothalamic area, 8 in the medial preoptic area and 8 in the lateral preoptic area.

Development of a response : At low strengths of electrical stimulation i.e, 150-200 uA the responses that appeared first comprised non-specific type of behavioural alerting and pupillary dilation. Increasing the current strength to 300 μA led to recruitment of more affective components like respiratory acceleration, piloerection, salivation, growling and occasionally urination. The somatomotor components which accompanied affective display included retracted neck, arching of back, coiling of tail, and retracing of steps indicating a typical defense behaviour. Further increase in current strength to 400 μA changed the response to either flight or an affective attack. On still further increase in the current strength to about 500 μA in the regions producing flight response, the dynamic somatic components of the behaviour became more vigorous, the autonomic manifestations became more exaggerated making the animal violently run to the escape route, attacking any object on the way leading to the development of a response which we have mentioned as violent flight. With such increases in current strength, the affective attack occured at a much quicker pace and more forcefully. Fig. 1 illustrates the stimulus-response relationship of flight behaviour as obtained by electrical stimulation of medial preoptic area in one set of experiment. It may be noted that the response gradually increases with increasing strength of stimulation finally leading to a fully-blown violent flight at 560 μA. Fig. 2 similarly illustrates the stimulus-response relationship of attack behaviour in another experiment.

Hypothalamic localization of responses : Affective attack was obtained from ten loci localized in the lateral preoptic and ventral part of the anterior hypothalamic area. Flight response was obtained from 15 loci localized in the dorsomedial parts of anterior hypothalamic area and the preoptic area. Stimulation with increased current strength added violent components leading to violent flight at 11 of these loci. The three loci, which did not result in any violent flight response, were localized more ventrally. The anatomical reconstruction of these sites of stimulation in serial coronal sections of the cat hypothalamus as per Jasper and Ajmone-Marsen (11) is shown in Fig. 3.

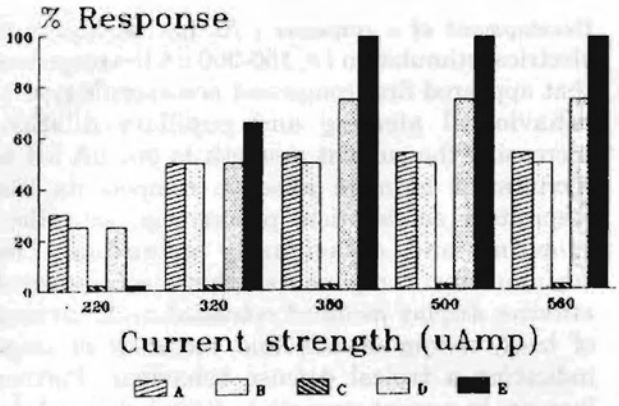


Fig. 1 : A - Affective display; B - Defence; C - Affective attack; D - Flight; E - Violent flight.

The histogram depicts that at 220 mA non-specific components of affective display, defence and flight response could be elicited. Gradual increase in the current strength led to the recruitment of affective components like hissing and baring of teeth. There was a simultaneous recruitment of flight and violent flight components and the maximum response was obtained at 500 mA.

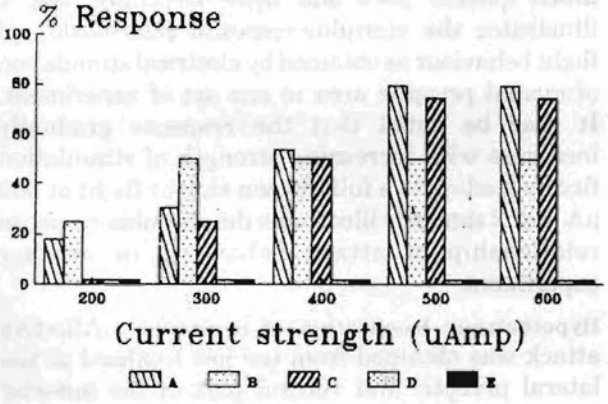


Fig. 2 : A - Affective display; B - Defence; C - Affective attack; D - Flight; E - Violent flight.

The histogram depicts that at 220 μamp only few components of affective display and defence response could be observed. Step-wise increase in the current strength led to the recruitment of more affective components like piloerection, hissing, and growling, as well as affective attack components like striking at the gloved hand with unsheathed claws and a full-fledged attack response was obtained at 500 mA.

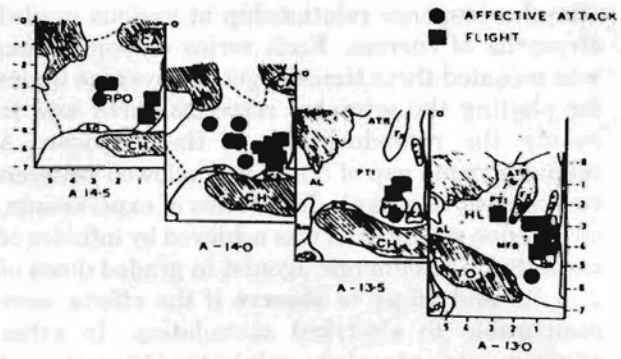


Fig. 3 : Shows the anatomical reconstruction in serial coronal section of the cat hypothalamus as per Jasper and Ajmone-Marsan (11). Abbrevations: Aco.. Cortical nucleus of amygdala, AL.. Lateral nucleus of amygdala, AM.. Medial nucles of amygdala, ATR.. Anterior thalamic radiations, CA.. Anterior Commissure, CH.. Optic chiasma, Fil.. Filiform hypothalamic nucleus, Fx.. Fornix, HL.. Lateral hypothalamic area, Hdm.. Dorsomedial nucleus of hypothalamus, MFB.. Medial forebrain bundle, RPO.. Preoptic area, Sch.. Suprachiasmatic nucleus, PTI.. Inferior thahlamic peduncle, PVH.. Praventricular hypothalamic nucleus, SO.. Supraoptic nucleus, VMH.. Ventromedial hypothalamic nucleus.

Effects of carbachol infusion : Following infusion of carbachol in graded doses of 2 to 15 μg, various affective and somatic components were exhibited. The affective display was the first to delvelop. At lower doses of 2 and 5 μg alertness, pupillary dilation, ear flatness and growling were observed. At 10 μg dosage components of piloerection, salivation and respiratory excitation were added. Raising the dose to 15 μg led to the recruitment of hissing and baring of teeth on provocation. After carbachol infusion, the animals seemed to be in an exalted state of excitation. Although they did not exhibit any spontaneous somatic response *per se,* any little provocation i.e. visual image of a stick or a gloved hand would make them respond in some of the patterns described. At all the loci producing affective attack, carbachol infusion produced mainly affective display. Only at one locus, some components of the affective attack were produced. Carbachol infusion at the loci which produced flight response led to affective display and defensive posture. Infusion at 6 of these loci produced flight or attack response on provocation, but never voilent

flight. Increased doses of carbachol not only made the response more vigorous but also decreased the latencies of various components of aggressive response, e.g. hissing, growling and salivation etc. Table II depicts the latency of some of the affective components like growling, salivation and hissing with increasing doses of carbachol.

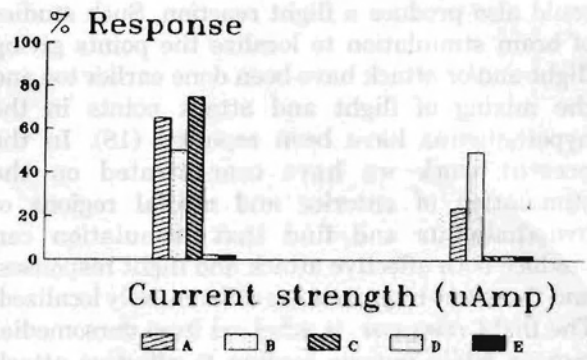
TABLE II : Depicts the mean latency of various affective components with increasing doses of carbachol.

Affective component		*2 µg*	*5 µg*	*10 µg*	*15 µg*
Hissing	Mean	9 min	6 min	5 min	4 min
(N=10)	SE	±1.8 min	±1.3 min	±1.0 min	±0.8 min
Growling	Mean	7 min	5 min	3 min	2 min
(N=15)	SE	±1.0 min	±1.2 min	±0.8 min	±0.6 min
Salivation	Mean	5 min	4 min	2 min	1 min
(N=15)	SE	±1.2 min	±1.5 min	±0.8 min	±0.8 min

*N=Number of loci
SE= Standard Error

Effects of cholinergic blocking agents :

A. Effects of atropine on electrically-induced responses : Microinfusion of atropine sulphate (10 µg) was performed at all the 25 hypothalamic loci. Electrical stimulation using graded current was carried out at each loci after half an hr of microinfusion as the blocking effect is maximal by this time. It was observed that somatic components of affective attack i.e unsheathing of claws, striking with paws were no longer exhibited while somatic components of flight namely restless movements and leaping to foot as well as violent flight such as wild running in the cage with unsheathed claws and vigorous leaping required much higher current strength. Affective display components like hissing with baring to teeth were also significantly inhibited. However, non-specific components of affective display like alterting, pupillary dilation and flatness of ear were not affected. Fig. 4 illustrates the stimulus response curve as obtained after atropine infusion at one of the loci in lateral preoptic area which clearly depicts the blockage of somatomotor components as unsheathing of claws and biting. Similar blocking of affective components as hissing and baring of teeth and piloerection was also observed after atropine infusion.



I. Control

II. After atropine

Fig. 4 : The diagram depicts the effect of atropine infusion on electrically induced affective attack response. Both unsheathing of claws and striking were completely abolished and there was significant reduction in affective display score due to blocking of hissing, baring of teeth as well as piloerection.

B. Effect of atropine on carbachol-induced responses : Prior infusion of atropine (10 µg in 1.0 µl saline) at all the loci where carbachol was infused, completely blocked the responses obtained by carbachol stimulation.

C. Effects of hexamethonium bromide infusion : Microinfusion of hexamethonium bromide in 50 µg dose was performed at all these loci. Electrical stmulation was carried out after one hr of microinfusion as the blocking effect is complete by this time. These responses were neither blocked nor augumented following the infusion of this blocker.

DISCUSSION

Our results indicate that electrical stimulation of a large number of sites in the anterior hypothalamus and preoptic area produced various types of aggressive responses. Many authors have reported that predatory attack can be elicited by electrical stimulation of far lateral regions of middle and posterior hypothalamus (12, 13, 14, 15). There are other workers who have excited the anterio-medical regions of hypothalamus successfully producing affective attack with lot of affective display (4, 9, 17, 18). Flynn et al (4) have also described that from similar situations the cats

could also produce a flight reaction. Such studies of brain stimulation to localize the points giving flight and/or attack have been done earlier too and the mixing of flight and attack points in the hypothalamus have been reported (18). In the present work we have concentrated on the stimulation of anterior and medial regions of hypothalamus and find that stimulation can produce both affective attack and flight responses, and these two responses are differentially localized. The flight response is acheived from dorsomedial regions while regions leading to affective attack are located more ventral and lateral. These results are in consonance with the observations made by Sheikh et al (19). We additionally report that both flight and attack have a common base of defence reactions which is achieved at the lowest current strength from all loci. We are able to report this observation because of our scoring system to quantify the response. Grading the strength of stimulation in many steps was built in the design so that we could exactly mark the component which appear at the lowest level of stimulation and these were the components of defence response. We expect that this basic response will have a larger morphological substratum and would be based on some level of arousal constituting the minimal common denominator for all response concerned with survival.

Violent flight : A normal cat in rage exhibiting affective display indicates some noxious threat. The animal tries tries to escape it when an intelligent guess so warrants it. But if it is cornered and does not find an escape route, it attacks and runs vigorously. Such extrmely agitated types of behaviour could be produced in our study from all loci giving flight response by only a small increase in the current strength. In a normal quiet flight response the animal starts with growling and other components of affective display, goes to a corner, shifts glances, looks around searchingly, stares at the escape route (in our set-up, a small hole at the ceiling) and then makes a well-directed jump in an effort to pass through the hole. But raise the strength of the stimulation only by 30% and one finds that the animal unsheaths its claws, starts running wildly without any sense, leaps vigorously and frequently without any judgement, but just put a stick or a gloved hand in front, then it would immediately attack ferociously. This also is a normal behaviour that occurs in extremes of agitation and excitement. For the purpose of classification we have termed it violent flight in view of the rather exaggerated sense of violence that such a response depicts. We could not obtain such a response from the lateral preoptic region where it was a straight affective attack. Raising the stimulation strength only elicited it quicker and with more vigour. Again such a variant of flight behaviour has not been described or investigated earlier although flight behaviour *per se* produced by hypothalamic stimulation is a well known entity (15, 18).

Effects of cholinergic stimulation of hypothalamus: Cholinergic stimulation with graded doses of carbachol was carried out at all the 25 loci. Our studies indicate that the total aggessive response obtained on cholinergic stimulation can be broken down into simpler components with varying doses of carbachol. Exaggerated affective display was obtained from the ventral regions of anterior hypothalamic and dorsomedial regions of the preoptic area. The extent of affective display was proportional to the dose of carbachol infused. With higher doses of carbachol the total extent of affective display was increased as depicted by the histogram (Fig. 5). Flight response was obtained from medial regions of the anterior hypothalamus. To the best of our knowledge, this is the first study in which such a full-fledged flight behaviour has been obtained from the medial regions of the preoptic area. Earlier Baxter (20) obtained a defensive behaviour which he termed as retreat from the medial and posterior regions of hypothalamus. In addtion he never obtained a typical flight response associated with leaping to foot and only going to corner on provocation was exhibited by the animal. Increasing the dose at these points brought about the recruitment of additional somatomotor components of affective attack. These animals displayed mainly flight tendencies but made a goal-directed affective attack as well. In the literature there is hardly any report regarding the elicitation of affective attack from medial preoptic area. Only Romaniuk et al (21) have reported an attack response from one point in the

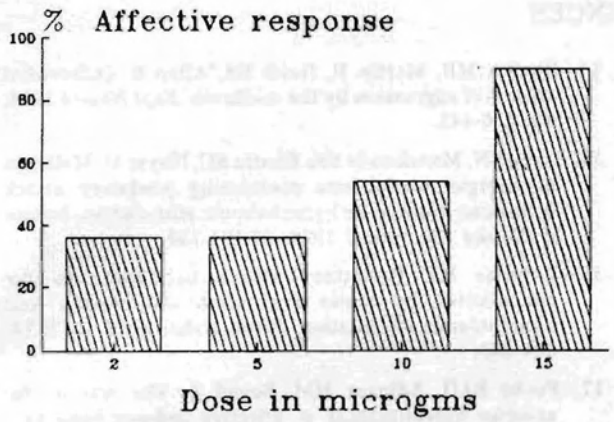


Fig. 5 : Depicts the effects of successively increasing doses of carbachol on affective display. With the lowest doses of 2 and 5 mg affective display components namely alertness, pupillary dialation, and growling were observed. At 10 mg dose, piloerection, salivation and respiratory acceleration were also displayed. Increasing the dose to 15 mg brought about the recruitment of hissing and baring of teeth, thus raising the score to its maximum.

posterior-medial region of hypothalamus. However, no details of the components elicited have been given by them. Recently Bandler (22) has reported that cholinergic stimulation of the hypothalamus and PAG facilitates the expression of aggression. This is probably the first study in which affective attack has been obtained by cholinergic stimulation of the medial preoptic regions. Cholinergic stimulation of the dorsal regions of anterior hypothalamus and medial reions of the preoptic area produced a defense response at lower doses which was intermixed with either flight or affective attack component when the dose was increased. It has been postulated that both serotinergic and adrenergic mechanisms somehow trigger the cholinergic pathways in the final expression of aggressive behaviour (23).

Effect of blockers on electrically induced response: Our results show that muscarinic cholinoceptive sites in the hypothalamus are involved in the mediation of aggressive behaviour. Microinfusion of atropine does attenuate the aggressive responses obtained on electrical stimulation of the anterior hypothalamus and the preoptic areas. This is indicated by the shifting of the curves depicting the respective relationships between affective display, affective attack, flight and violent flight on the one hand and current strength on the other hand, to the rigth. While atropine was microinfused at the loci giving affective attack, the somatic components were completely blocked, and the affective components were markedly reduced. A critical analysis of the histogram depicting the blocking effects of atropine indicates that components which were specific to a particular behaviour e.g. unsheathing of claws, striking with paws in case of affective attack or leaping to foot and starting at the escape route with respect to flight behaviour or for that matter growling or hissing or piloerection were the earliest to be affected. It is however, important that this blocking effect could be deciphered only at stimulation levels which were submaximal for the total behaviour pattern. Whenever the electrical stimulation was supramaximal and elicited a full-fledged aggressive response, atropine infusion did not have any blocking effect. On raising the stimulation strength even at those loci which were seemingly blocked by atropine, these responses could still be elicited. It is not surprising, therefore, that Romaniuk et al (6) could not demonstrate the abolishing of the flight responses on application of atropine. We feel that they were stimulating only with very high strengths of stimulation and never constructed these type of curves, thus missing the importance of the cholinergic system in the elaboration of these responses.

Recently Katz (7) has suggested there is muscarinic cholinergic mediation of the patterned reflexed in the cat in aggression and that the cholinergic synapses play a final common path for many forms of aggression. Our findings confirm the report of Belestin and Ksenija (24) who have indicated that the cholinoceptors when stimulated by intraventricular carbachol infusions trigger the whole pattern of aggressive behaviour.

CONCLUSION

The present findings indicate the involvement of muscarinic cholinergic system in the expression of aggressive behaviour from anterior and preoptic regions of hypothalamus.

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