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NEURONAL RESPONSES OF PERIAQUEDUCTAL GRAY TO PERIPHERAL NOXIOUS STIMULATION

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Abstract : Central pathways transmit pain from peripheral regions to one of the most important area of the descending pain modulatory system, the Periaqeductal gray (PAG). Independent discoveries in the past suggest that the PAG contains afferent input, output neurons and intrinsic interneurons. An attempt was made in the present study to find out the effects of more than one kind of noxious stimulus on the PAG neuronal activity. Experiments were conducted in rhesus monkeys and the effects of noxious mechanical, thermal and tooth-pulp stimulation on the activity of 14 neurons were studied. The neurons responded to more than one kind of noxious stimuli by increasing or decreasing its firing rate. No limb specificity could be identified and homogeneous distribution of the excitatory and inhibitory neurons was found.

Key words : periaqueductal gray neuronal activity tooth-pulp antinociception jaw opening reflex

INTRODUCTION

The earliest evidence for an intrinsic analgesia system was the demonstration that electrical stimulation of the midbrain Periaqeductual gray (PAG) produces a selective suppression of pain in animals (1, 2) and man (3, 4). It was subsequently shown that opiates microinjection into the PAG also produces analgesia (5). Later studies demonstrated that the PAG is rich in both endogenous opioid peptides (6, 7) and opiate receptors (8, 9, 10). Electrical stimulation of the PAG produces total body analgesia without producing concomitant motor, sensory or autonomic blockade (11,

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12, 13). This analgesia is believed to be mediated by several neurotransmitter systems, including the serotonergic, noradrenergic, glycinergic, gamma-aminobutyric acidergic. In the recent years the action of NMDA (14, 15, 16, 17), glutamate (18) calcitonin (19), CCK (20), bicuculline (21), substance P (22) and many more neurotransmitters at several overlapping sites to modulate behavious subserved by PAG has been reported.

The PAG is functionally and cytoarchitectonically heterrogenous. Afferent projections to the PAG originate in the hypothalamus, amygdala and frontal cortex and ascending spinothalamic tracts. The main descending projections of the PAG are to the nucleus raphe magnus, with limited projections to the locus coeruleus.

Studies of the physiological properties of neurons in these nociceptive modulating nuclei have also revealed significant complexity. In the PAG, cells can be divided into different physiological classes based on the discharge pattern that suggests the cell to be output inhibitory or facilitatory neuron. Recording of neuronal responses of PAG to stimulation of amygdala shows that out of 44% of cells which responded, 46% were excited and 54% were inhibited, indicating a clear segregation of cells as excitatory or inhibitory neurons (23).

The present study was undertaken to investigate the responsiveness of the PAG neurons to peripheral noxious stimuli of more than one type so as to find out if these neurons a) are affected similarly by different noxious stimuli and b) always respond by an increase or decrease in the activity.

METHODS

Animals: Adult male Rhesus monkeys (Macaca mulatta) weighing between 2.5 to 4.5 kg, bred and reared in the Central Experimental Animal Facility of the All India Institute of Medical Sciences, New Delhi, were used in this study. Food and water was provided ad libitum. Ethical guidelines were followed in accordance with the committee for research and ethical issues of IASP (24).

Anesthesia: Animals were anaesthetised with a combination of urethane (750 mg/kg, i.p.) and alpha-chlorolose (80 mg/kg, i.v.) in 3:2 proportion. Supplementary doses of urethane was given intermittently at one-fourth the original dose, to maintain a level of anesthesia sufficient to abolish the withdrawal reflex of the hindlimb induced by deep pressure to the foot pad. In addition, recording different physiological parameters, were recorded to monitor the stability of the animal, e.g. blood pressure, pulse rate, respiration and core (rectal) body temperature.

Surgical procedure: This involved two steps, first the implantation of the tooth-pulp electrodes, and thereafter, microelectode for unit recording implantation of tooth-pulp electrode were performed by fixing the anaesthetised monkey in supine position in the stereotaxic apparatus so as to gain access to the incisor tooth of the upper jaw. Atropine sulphate (7 mg/kg S.C.). was given prior to surgery to reduce tracheal secretions. The jaw-opening Reflex (JOR) was elicited by tooth-pulp (TP) stimulation and quantified by recording the electromyogram from the external pterygoid muscle (25, 26). This has been termed as dEMG in the experiment. For selective intrapulpal nerve stimulation (27, 28), two small holes were drilled into the dentine of the upper incisor and TP electrodes were implanted as reported earlier (29). The animal was then placed in prone position with the head fixed in the stereotaxy. The skin of the head was incised and reflected to expose the skull over the site to be approached. Then, a window was made on the skull on the side ipsilateral to the tooth-pulp implantation site, large enough to introduce the microelectrode at PAG according to the co-ordinates (A 3.0 to P1.5, L 0.5 to 1.5, H–1.0 to 4.0) given in the atlas for monkey brain (30). A reference electrode was attached to the skull surface with a screw.

Electrodes: Tooth-pulp stimulation electrodes were made of 28G stainless steel wires cut into 1.2–1.5 cm size and insulated with insulex, keeping the tip bare (0.5 mm). For extracellular single unit recording, metal microelectrodes were used (26G diameter) approximately 8 cm long, the tip of which was etched to 2–3 um and thereafter, insulated with insulex. Electrodes with impedance of 1–3 M were selected for unit activity recording.

Application of different types of peripheral noxious stimuli: Tooth-pulp was stimulated electrically using a stimulator (SEN 3301, Nihon Kohden, Japan) through an isolation unit (SS 201J) with rectangular pluses of frequency 0.5 Hz, duration 0.3 msec and intensity 1.5–3.0 times the threshold (intensity at which the tooth-pulp stimulation evoked dEMG just appeared (25). For 10 seconds, noxious mechanical pinch was applied by uncalibrated forceps to digits of either right or left limbs for 15 seconds. Noxious thermal stimuli was applied by immersing the forepaw in the water bath for 15 seconds in graded temperatures (45°–60°C in 5°C steps).

Experimental design: After implantation of the booth-pulp electrode, the animal was placed in prone position and the head was fixed in the stereotaxy. The microelectrode was then lowered very slowly to PAG through the window made in the skull and basal unit activity was recorded for 5 minutes, after ascertaining its stability for 30 minutes. On application of peripheral noxious stimuli, recording was done for 2–3 minutes, depending on time and duration of response. An interval of 5 minutes or a return to baseline activity, whichever was earlier, was used as the criterion for studying the subsequent variable. The activity was displayed in the oscilloscope and was recorded in an audiocassette recorder (Model MR 10, TEAC, Japan) for analysis.

Analysis of data: Initially, the spikes of appropriate amplitude were selected with the help of window discriminator. This was fed on-line to a Histogram Analyser (Model QC 111J, Nihon Kohden, Japan) and histograms each having bin width 0.2 sec and total sampling time of 204.8 sec (X-axis) was recorded with frequency of firing represented in the Y-axis. The basal mean frequency of the spikes were calculated manually and compared with the experimental in terms of differences in absolute values expressed as mean ± SD.

Histology: The recording site was lesioned with 500 uA nodal current applied for 10 sec. Thereafter, the brains were perfused with 10% formalin in which 2% potassium ferricyanide was added. The fixed brains were then sectioned and processed for eosin-hematoxylin staining to visualise the site of lesion.

RESULTS

Basal discharge pattern of the PAG neurons

The total number of units recorded from PAG were 14. Out of these, 2 were from dorsal PAG, 4 were from dorsolateral PAG, 7 were from ventrolateral PAG and 1 from ventral PAG. The duration of the spikes under basal conditions varied from 0.8 msec to 1.4 msec. Spike amplitude varied from 100 uV to 140 uV. The basal mean firing rate ranged from 2.15 ± 1.69 Hz to 6.34 ± 2.11 Hz with an average of 3.97 ± 1.02 Hz in 12 out of 14 neurons. In two neurons, however, the firing rates were 17.47 ± 8.16 Hz and 23.69 ± 3.24 Hz the former being recorded from dorsal and the latter from dorsolateral PAG. There was no correlation observed between the firing rate and the recording sites in various regions of the PAG. Though the firing rates showed some variations during the recording period, no significant difference was observed in the mean firing rate of 3 to 6 successive samplings before application of noxious stimulation. The neurons which showed significant change in their firing rate during the control period were not included in the study. The duration of firing of a stable neuron on which all the experimental procedures were carried out, ranged from 2.5 hours to 4.5 hours.

Response of PAG neurons to peripheral noxious stimuli

Depending upon the different types and grades of peripheral noxious stimuli applied, most of the neurons responded with an increase or decrease in firing rate in the at least two types of peripheral noxious stimuli. In accordance with the experimental design, two types of noxious stimuli (mechanical and thermal) were applied to all the four limbs. However, their receptive fields in the respective areas were found to be non-specific. Also, there was no limb-specific variation in the responses recorded from PAG. Instead, the responses varied with the grades of noxious thermal or tooth-pulp stimuli. Observations on few neurons, in which no effect of a noxious stimulus was obtained, are not included in the results. The predominant effect observed was an increased rate of firing with mechanical and thermal noxious stimuli and a decrease in firing rate was observed on stimulation of tooth-pulp. (Fig 1). This was more obvious on observing the number of responses than the units. This is because many units showed both increase and decrease in firing rate when response to the same stimuli was studied more then once. The response of one such neuron is depicted diagramatically in Fig 2.

Response to mechanical stimuli

Noxious mechanical stimuli in the form of pinch was applied to the palmar surface of all the four limbs i.e. left arm (LA), left leg (LL), right arm (RA) and right leg (RL)

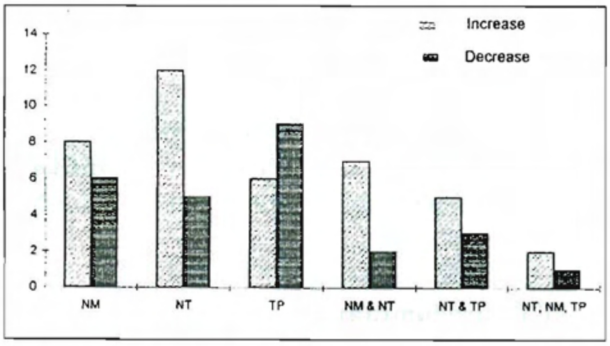

Fig. 1 : The number of neurons showing an increase or decrease in the activity (x-axis) on application of noxious mechanical (NM) noxious thermal (NT) and Tooth-pulp (TP) stimulation.

with serrated forceps for 10 sec. Effect of noxious pinch was studied on a total of 10 neurons. An increase in firing rate was observed in 8 neurons. A maximum increase of 3.14 ± 2.74 Hz was obtained from RL pinching and a minimum of 1.80 ± 1.20 Hz from pinching LL (Table I).

On the other hand, a decrease was observed in 6 neurons, which was a maximum of 10.73 Hz recorded from one neuron after RA pinch. The decrease in

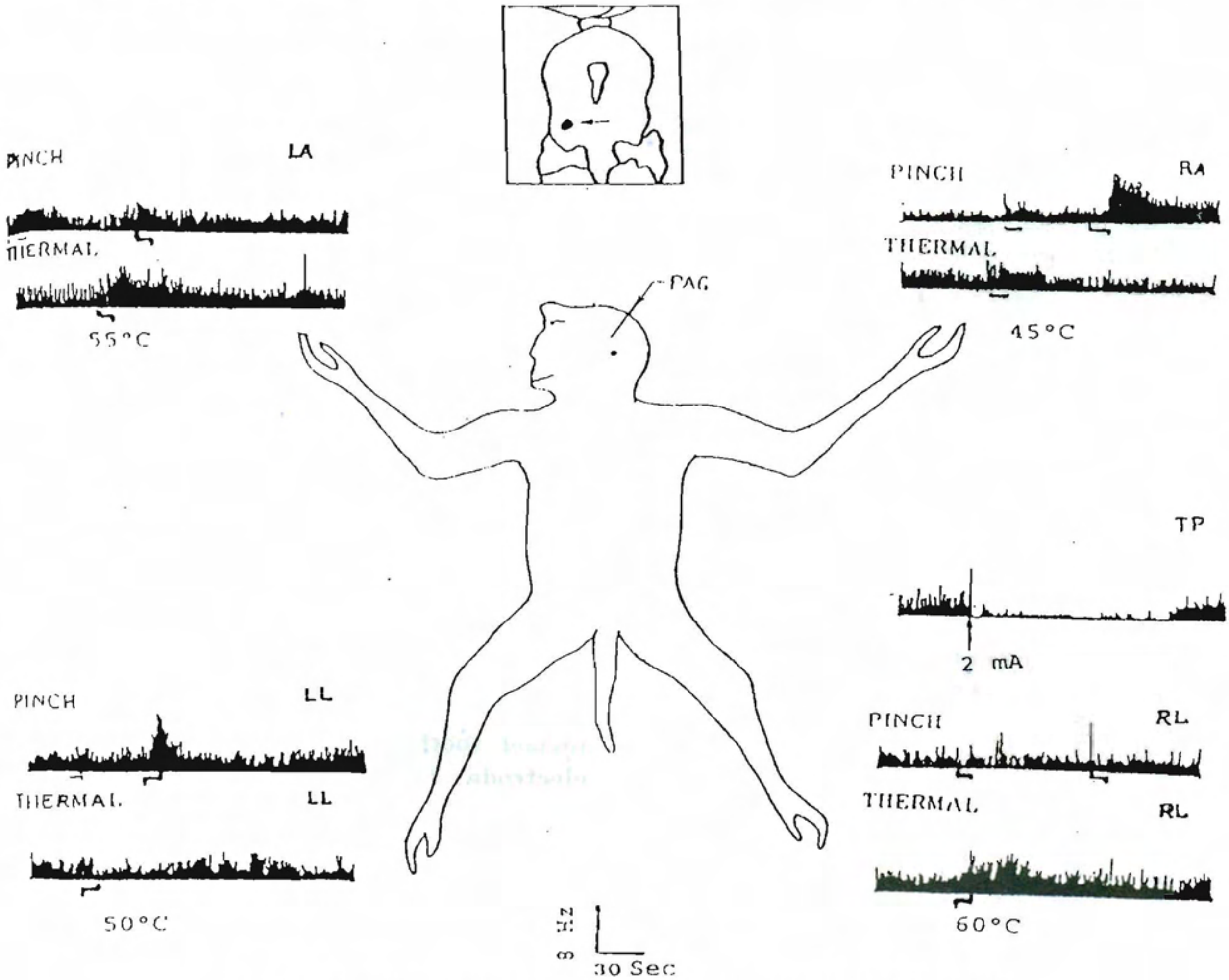

Fig. 2 : Diagramatic representation of the responses of a PAG neuron (shown in the inset) to various peripheral noxious stimuli.

other limbs varied from 1.23 ± 1.27 Hz (LA) to 1.98 ± 1.68 Hz. (Table 1). However, there was there was no limb specificity observed when the change in firing rates of neurons on stimulation of the individual limbs was considered.

Response to thermal stimuli

Noxious thermal stimuli were applied by immersion of the limb (below the wrist or ankle joints) in hot water at graded (5°C) temperatures from 45°C to 60°C for 10 sec. The data obtained from stimulation of all the four limbs with respective grades of temperature did not show any significant response specificity as compared to the other limbs.

TABLE I : The number of neurons (in parentheses) showing increase or decrease (Hz) in firing rate (mean ± SD) on application of noxious mechanical (on different limbs), noxious thermal (at various temperatures) and tooth pulp (at various intensities) stimulation.

Noxious Mechanical

Limb	*Increase*	*Decrease*
LL	2.62±1.98 (4)	1.23±1.27 (3)
LA	1.80±1.20 (6)	1.94±1.98 (4)
RL	2.10±2.57 (5)	10.73 (1)
RL	3.14±2.74 (5)	1.98±1.68 (2)

Noxious Thermal (T)

Temp	*Increase*	*Decrease*
45	0.99±0.63 (2)	0.87±0.30 (2)
50	1.34±0.37 (5)	0.75±0.37 (4)
55	1.30±0.41 (4)	0.68±0.41 (2)
60	3.18±2.52 (9)	0.62±0.46 (2)

Tooth Pulp (mT)

Intensity	*Increase*	*Decrease*
1.	3.75±2.83 (2)	0.99 (1)
2.	1.35 (1)	1.00±0.51 (3)
3.	0.55 (1)	0.51±0.44 (4)
4.	1.08±0.77 (3)	1.76±0.24 (2)
5.	0.40±0.1 (2)	1.24±0.85 (5)

The predominat effect observed was an increase in firing rate of the neurous. This rate increased with increasing temperature applied to the limb i.e. an increase of 0.99 ± 0.65 Hz observed at 45°C became 3.18 ± 2.52 Hz at 60°C (Table I). Also, the proportion of neurons showing increased firing was more with rising grades of temperature. That is, at 45°C only 50.00% of the neurons showed increase while at 50, 55 and 60°C, 55.55%, 66.67% and 88.81% of the neurons showed increase in firing rate.

In the other group with application of temperature from 45°C–60°C the neurons responded with decrease in the firing rate which was directly related to the intensity of stimulation. Nevertheless, the predominant response of the neurons recorded from PAG to increments in thermal stimuli was an increase in firing.

Response to tooth-pulp stimulation

In this case, the plup of the upper left incisor tooth was stimulated with bipolar electrode at graded intensities of 1 mA to 5 mA (increment of 1mA) at a frequency of 0.5 Hz and pulse duration 0.3 msec, for 10 sec. The single neuronal activity was also recorded from ipsilateral (left) PAG. Out of a total of 10 neurons recorded from PAG, 6 showed an increase in the firing rate of 1.66 ± 2.03 hz while 7 neurons showed a decrease

(Table I). An increase in firing rate was observed when tooth-pulp was stimulated at 1 and 4 mA. This increase was of 3.75 ± 2.83 Hz and 1.08 ± 0.77 Hz. However, a decrease was observed on stimulation at 2, 3 and 5 mA. This decrease was in the magnitude, which varied from 0.51 ± 0.4 Hz to 1.24 ± 0.85 Hz respectively.

Thus, with tooth-pulp stimulation, majority of the neurons showed a decrease in firing rate. Also, no definite correlation was observed when the magnitude of response was compared with the grades of stimulation. However, maximum decrease in firing rate was observed at the highest intensity of stimulation (5 mA) (Table I).

DISCUSSION

In our study we used three different types of peripheral noxious stimuli to identify the neurons responsive to noxious stimuli. It was observed that, mechanical and thermal stimuli produced a predominant increase in the firing rate of the neurons. However, tooth-pulp stimulation produced a decrease in firing in most of the neurons recorded from PAG. It is known that PAG contains the on and the off cells which project to the spinal cord. The off cells which transmit information away from the PAG, are tonically inhibited by GABAergic neurons. It has been suggested that activation of mu receptors inhibit the GABA neurons thereby allowing the off cells of the PAG to interact with NRM. The NRM serotonergic system, in turn, activates the spinal analgesic system through a similar mechanism. This opioid disinhibition of the PAG neurons to the RVM as the predominant mechanism underlying opiod-induced analgesia in the PAG has been demonstrated (31, 32). Using patch clamp technique Vaughhan and Christie (33) demonstrated that opioids inhibit both GABAergic and glutamatergic synoptic transmission in all PAG neurons. These observations are consistent with the pioid disinhibition model of PAG induced analgesia. The on cells when stimulated, produce depression of PAG induced analgesia. These cells may be the GABA cells referred to above (34, 35, 36).

Since our results showed a predominat increase in the firing rate of PAG neurons on application of noxious mechanical and thermal stimuli, it is possible that spinothalmic pathway carrying the peripheral noxious input give a collateral on its way to thalamus, which converge on the 'on' cells at PAG which gets activated showing an increase in frequency of firing. The 'on' cells in turn might inhibit the descending serotonergic neurons from PAG to NRM and from NRM to spinal cord, thereby suppressing the modulatory response and facilitating the noxious input. Also, these collaterals to PAG might act on the enkephalin cells, which impinge on the GABA cells. Thus, activation of the enkephalin cells will inhibit the activity of the GABA cells, which results in removal of its tonic inhibition on the descending serotonergic cells resulting in analgesia. Therefore, the cells recorded here might be the on cells or the enkephalinergic cells or off cells which respond with an increase in firing.

The neurons, which respond to noxious mechanical or thermal stimuli were not limb specific, as stated earlier are therefore, can be said to have large receptive fields. At the same time, the magnitude of response to thermal stimuli, increased with increasing intensity of stimulation exhibiting a clear capacity to encode thermal stimuli in noxious range.

Stimulation of the tooth-pulp produced a predominant decrease in the firing rate of PAG neurons, which did not show a linear relation with stimulus intensity. The collaterals from the trigeminothalamic tract in this case, might impinge on the GABA neurous which inhibit the off cells. Therefore, recording from an off cell shows a decrease in firing rate, which might be the same neuron, getting activated with noxious mechanical and thermal stimuli, impinging on the enkephalin cells. Contrary to the study by da Casta (23), the neurons showing an inhibitory response did not have a higher baseline firing frequency, as in their study and are therefore not likely to be the tonically active inhibitory interneurons.

Further the neurons excited or inhibited by noxious stimuli were homogeneously distributed throughout the PAG.

Finally analysis of the direction of response obtained in various neurons reveals that when tested with more than one kind of noxious stimulus the neurons showed both excitatory and inhibitory responses and were not stimulus modality specific.

The present study in its limited way suggests that the PAG neurons may be getting activated by more than one physiological mechanism by the afferent pathways and then affects the descending pathways by increasing or decreasing the activity of output neurons.

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