

The results of the present study indicate a marked rhythm occurs in the bovine pineal amine serotonin which corresponds with observed changes in cattle fertility. In the cow the pineal may be involved in alterations in fertility, but only in concert with other factors, as the year long breeding capabilities of cattle are well known¹⁸.

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- 3 The authors wish to thank Roeglein's Provision Company, Beef Processing Division and especially H. Schmidt, Foreman.
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Reciprocal connections between substantia nigra and medullary reticular formation in the rat¹

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Summary. Electrophysiological analysis shows reciprocal connections between substantia nigra and medullary reticular formation (nucleus reticularis giganteo cellularis). The nigro-reticular connection appears to be monosynaptic, as shown by antidromic activation, and comprises an ipsi and a contralateral component. Its effect is mainly inhibitory. The reticulo-nigral component produces mainly excitatory effects and includes fibres from nucleus giganteo cellularis and nucleus parvocellularis in the medullary reticular formation.

It has been demonstrated that stimulation of the substantia nigra (SN) can induce changes in spinal reflexes² and produce motor responses³. As there are no known connections between the SN and the spinal cord, it has been proposed that these effects could be mediated either via nigro-tecto-spinal or reticulo-spinal pathways. There is anatomical⁴ and electrophysiological⁵ evidence of nigro-tectal connections, but evidence concerning nigro-reticular connections is more scarce. The present work was designed to obtain electrophysiological evidence of the presence of nigro-reticular connections, as the reticular formation (RF) has a well known action on spinal activity⁶, and there exists anatomical support for the presence of such connections⁷. The presence of reticulonigral fibres was also investigated.

Materials and methods. Experiments were carried out in Sprague-Dawley rats (200–300 g) anaesthetized with urethane (1 g/kg IP), and positioned in a stereotaxic frame according to the atlases of Albe-Fessard et al.⁸ and Abad-Alegria⁹. In 1 set of experiments, the SN was stimulated by means of bipolar electrodes (square wave pulses of 0.5 msec duration, repeated every 2 sec with intensities up to 300 μ A) at coordinates AP 3.2; L 2; V 3, and recordings were made using glass microelectrodes filled with Pontamine sky blue, in order to mark the recording site¹⁰. Recordings were made at coordinates AP – 3.5; L 1; V 0, taken from Abad-Alegria, in the region of the nucleus reticularis giganteo cellularis (n.r.g.c.). In another set of experiments, the situation was reversed: the stimulating electrode was positioned in the region of the n.r.g.c. or more laterally in the nucleus parvocellularis (n.p.c.), and recordings were made in the SN and adjacent ventral tegmental area (VTA). In all cases post-stimulus time histograms were made with a computer in order to assess the effects of stimulation in 100 responses. Recording and stimulating sites were histologically checked after the experiments.

Results and discussion. Stimulation of the SN produced mainly inhibitory responses on spontaneously active RF units. 22 out of 37 units showed periods of inhibition ranging in duration from 20 to 120 msec, in some cases followed by excitation. The latency of this inhibition had a

mean value of 12.5 msec, 9 units were excited and in 2 this excitation was followed by inhibition (figure 1 a, b). Some units showed reverberatory activity (cycles of excitation and inhibition) after SN stimulation. Histological analysis confirmed that all responsive units were located within the boundaries of the n.r.g.c. In some experiments recordings were made contralaterally, with similar results.

Stimulation of the n.r.g.c. evoked responses in SN and VTA units. 13 cells were excited with a mean latency of 17.5 msec., and 3 were inhibited out of a total of 40 neurons. Stimulation of the n.p.c. produced inhibition in 3 units and

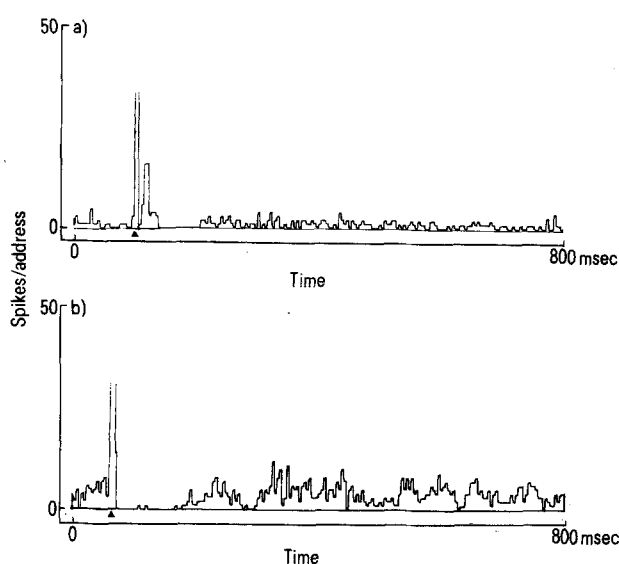


Fig. 1. Post-stimulus – time histograms of 2 neurons in the medullary reticular formation after stimulation of the SN. a) excitation followed by inhibition. b) inhibition followed by reverberatory activity. \blacktriangle , stimulus artefact. 100 responses; 4 msec/bin; 200 bins.

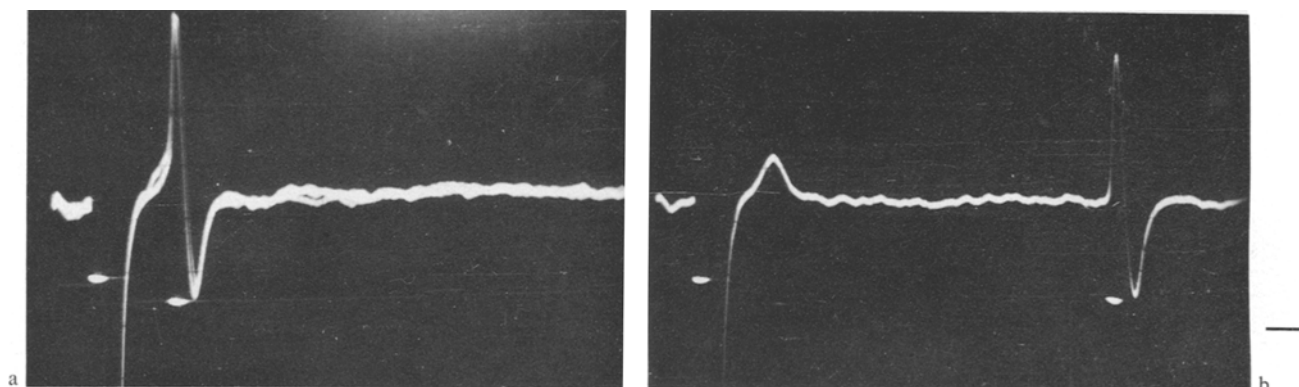


Fig. 2. Antidromic activation and cancellation by a spontaneous spike in a neuron in the SN, after stimulation of the n.r.g.c. a) Antidromic spike showing constant latency (3 superimposed sweeps). b) Cancellation by a spontaneous spike which triggered both the stimulator and the scope sweep. Only the SD component is present as the stimulus failed to evoke a full spike. Dots indicate occurrence of stimulus artefact and of spikes. Calibration: 5 msec; 100 μ V.

excited 2 units with similar latencies. On 3 occasions while stimulating from the n.r.g.c., neurons showing antidromic activation (constant latency and ability to follow frequencies up to 500 Hz) were detected, and cancellation by a spontaneous spike was obtained (figure 2).

The present results provide electrophysiological evidence of a reciprocal link between the SN and the medullary RF, the connection between the SN and the RF being ipsi and contralateral. Although fibres en passage could have been activated, the finding of antidromically activated neurons, demonstrates a direct link of a monosynaptic nature. At present it is not known whether there are separate projections to both sides of the brain or whether there is a branching of the same axons which can cross the midline to innervate the contralateral side. This pathway could be the

output from the SN to the spinal cord, through reticulo-spinal neurons, as has already been suggested^{2,11}. It is interesting to note that both ipsi and contralateral effects have been reported after SN stimulation^{2,12}, albeit of a different nature. More recently, alterations in EEG activation from the RF have been described after making lesions in the SN¹³. It is still premature to assign a function to the reticulo-nigral connection found in this study, but SN units show responses to peripheral stimulation not mediated by diencephalic structures¹⁴, and these effects could be relayed via the RF. The n.r.g.c. is known to receive afferents from peripheral receptors¹⁵, and it may be that feedback information from several receptors including muscle afferents is being conveyed to the SN as part of its regulatory role in motor control.

- 1 This work was aided by a grant from CONICIT (projecto 31.26.S1-0412).
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Methionine enkephalin inhibits the bursting activity of the Br-type neuron in *Helix pomatia* L.

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Summary. The present study demonstrates that methionine enkephalin can inhibit the normal bursting activity pattern of the RPal or Br-type neuron and this inhibition can be blocked by prior treatment with naloxone, the selective opiate antagonist. The study demonstrates indirectly the presence of opiate-like receptors in *Helix pomatia*.

Recent reports on the pharmacological effects of methionine enkephalin and morphine on bivalves^{3,4} have strongly suggested the presence of opiate receptors in invertebrates. Specific binding analysis using ¹²⁵I-labeled FK 33824 on *Mytilus edulis* pedal ganglia demonstrated the existence of stereo-specific opiate receptors⁵. A more recent study has localized enkephalin and B-endorphin in the earthworm⁶. As a result of the above, the present study examined the effect of methionine enkephalin on the bursting pattern of

the Br-type neuron, since this neuron responds to dopamine and this effect is antagonized by haloperidol and naloxone⁷.

Materials and methods. Specimens of *Helix pomatia* employed for this study were collected locally in the woods surrounding the Biological Research Institute at Tihany. The preparation of the tissue, cellular identification and recording technique have been described previously^{7,8}.