biochemical data for the nerve terminal from that obtained in the axon.

Dopamine-β-hydroxylase is present in the vesicles in both the adrenal medulla and the sympathetic nerves in bound and soluble forms (*5, 17*). The soluble form is that released by disruption of the vesicle by osmotic shock. The portion of DBH that is most likely to be discharged after stimulation of nerves is the soluble form. Experiments with the adrenal medulla where, depending on the species, 20 to 50 percent of the DBH activity can be liberated from chromaffin granules by osmotic shock, have shown that the soluble portion of the DBH is that which is depleted by stimulation (*17*). The results reported here agree with other reports in which a lower proportion of soluble DBH appears to be present in vesicles from sympathetic nerves than is present in the adrenal gland (*5*).

Since 85 percent of DBH is in bound form and the estimated half-life of the sympathetic nerve vesicle is 3 weeks (*8*), it is likely that the vesicle is reused. The ratio of amine to DBH released from the vas deferens is higher (although close) to that found in tissue. This might result from preferential release of newly synthesized norepinephrine (*14*). Preferential discharge of newly synthesized amine from a population of vesicles that have already liberated some or all of their releasable DBH could explain the apparent increase in release of norepinephrine relative to DBH into the bath fluid. Finally, the assumption that diffusion from the synaptic cleft of norepinephrine, a molecule with a molecular weight of 169, occurs at the same rate as that of DBH, a protein with a molecular weight of 300,000 (*3*), might be incorrect. Unequal diffusion rates would contribute further to the increase in the ratio of amine to enzyme found in the bath fluid.

The similarity of the ratios of catecholamine to DBH in tissue and in the incubation medium, despite the possible sources of error discussed above, and the proportionality of amine to DBH discharged are compatible with the coupled release of the neurotransmitter and the enzyme from sympathetic nerves by a process of exocytosis.

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18. Supported by the Pharmacology-Toxicology program of the National Institute of General Medical Sciences (R.M.W.).

15 July 1971

Analgesia from Electrical Stimulation in the Brainstem of the Rat

Abstract. *Stimulation at several mesencephalic and diencephalic sites abolished responsiveness to intense pain in rats while leaving responsiveness to other sensory modes relatively unaffected. The peripheral field of analgesia was usually restricted to one-half or to one quadrant of the body, and painful stimuli applied outside this field elicited a normal reaction. Analgesia outlasted stimulation by up to 5 minutes. Most electrode placements that produced analgesia also supported self-stimulation. One placement supported self-stimulation only in the presence of pain.*

The management of prolonged pain in man has been a stubborn medical problem because the most effective analgesic drugs carry with them such undesirable side effects as tolerance and dependence. Alternative approaches have long been sought. The approach of making discrete brain lesions has not been consistently successful in alleviating either pain states in man or experimentally evoked pain in animals. This failure to excise pain or to interrupt the pathways responsible for its appreciation and expression seems to indicate that the neural substrate of pain is so diffuse or redundant as to escape focally inflicted brain damage. On the other hand, some hope that pain can be alleviated by a neurosurgical procedure has been offered by a small number of studies reporting pain reduction that accompanies electrical stimulation of discrete brain regions in man as well as lower animals (*1, 2*). The animal work has typically involved effects of small or indeterminate magnitude [however, see (*1*)], and from these reports it has not been sufficiently clear whether the effect was directly on pain perception or was a reflection of broader deficits in sensory, motor, or motivational mechanisms. Clinical attempts to utilize this approach have been understandably few, and the number of brain areas probed quite restricted. We report that analgesia from focal brain stimulation in the rat can be of such magnitude as to render the animal totally unresponsive to pain. We show that such analgesia results from stimulation in a number of subcortical loci, including those not previously tested in man. Evidence is provided that analgesia can occur without apparent accompanying sensory, motor, or motivational deficits.

Bipolar electrodes were implanted bilaterally in various mesencephalic and diencephalic loci in 22 male Sprague-Dawley rats. Electrodes were made of twisted, stainless steel wires (200 μm in diameter) insulated except at the cut cross sections of their

tips. In ten animals (group 1), brain stimulation consisted of 100-msec trains of 60 hz a-c delivered at two per second through a 7-megohm series resistance. Current values ranged between 15 to 50 μa root mean square. Analgesia tests were conducted by placing the animal in a small chamber with an aperture in the rear through which the tail was drawn. Noxious stimuli were shocks applied to the tail through a pair of nonpolarizing disk electrodes. Shocks consisted of a 100-msec train of 0.2-msec rectangular waves delivered at 50 per second by a Grass stimulator. For each animal, a current intensity was selected (4 to 8 ma), which was reliably noxious as evidenced by the elicitation of squeaking, lurching, and vigorous escape attempts. Brain stimulation was judged analgesic if these and all other shock-elicited reactions were totally absent.

For the remaining 12 animals (group 2), brain stimulation was provided by a constant-current stimulator (Bio-Engineering Research Laboratory, type 220A) and consisted of biphasic rectangular pulse pairs delivered at 50 per second in 100-msec trains. The pulse pair consisted of two 50-μsec pulses of opposite sign separated by 100 μsec. Trains of pulses were delivered at a rate of three per second. Current intensity was 3000 μa. Analgesia tests were conducted by applying pressure with a needle-nosed pliers to the four limbs and tail. Normally, even relatively mild pinching with this instrument elicited vigorous withdrawal and squeaking. The degree of analgesia was rated independently by two observers on a five-point scale. Inter-rater reliability was high. An attempt was made to elicit a response by pinching several times and at a number of locations on each member before assigning a rating. Brain stimulation was judged analgesic only when both observers assigned a maximum rating on a given limb or the tail. A maximum rating signified that there was no discernible response, not even a withdrawal reflex, to application of even severe, tissue-destructive pressure.

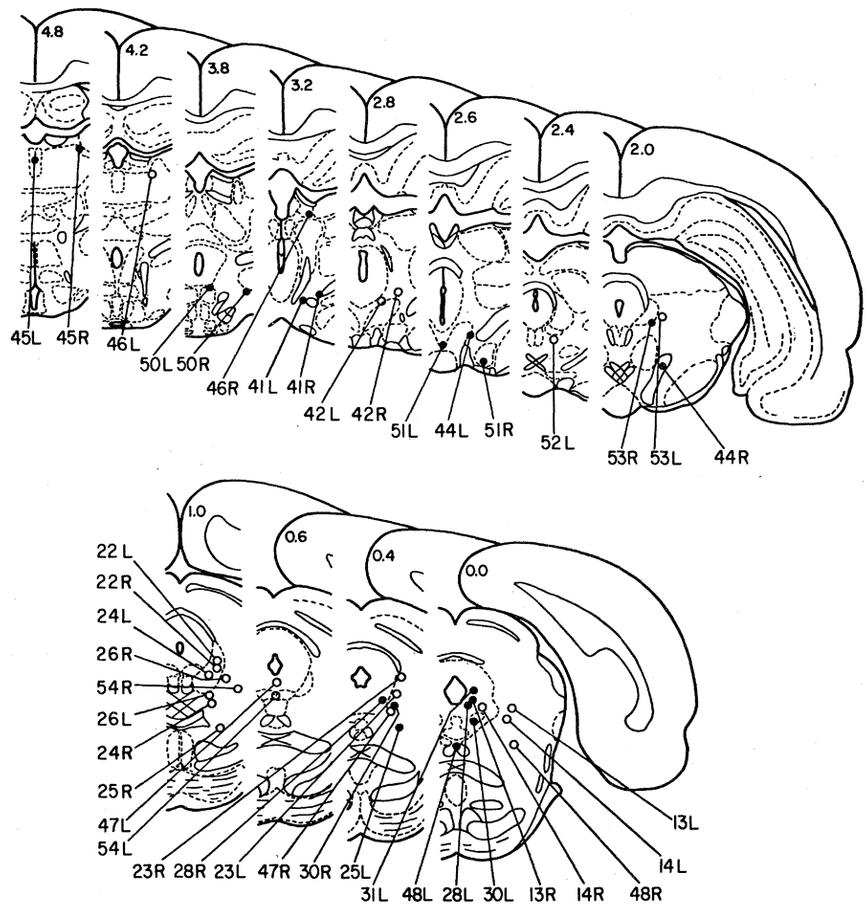

Fig. 1. Schematic reconstruction of the location of electrode tips at various frontal planes showing sites at which stimulation produced (filled circles) or failed to produce (open circles) analgesia [diagrams modified from the stereotaxic atlas of Pellegrino and Cushman (*4*)]. Designations *L* (left) and *R* (right) after the placement number refer to the side of the brain in which the electrode was located.

Stimulation at 7 of the 19 placements tested in group 1 animals resulted in complete analgesia to the tail shocks used in this study. In two animals more extensive tests were run. Even tail shocks which caused tissue damage (10-msec pulses, 200 ma, 50 per second) elicited no observable response when administered during or shortly after brain stimulation. Stimulation at 13 of the 23 placements tested in group 2 animals resulted in complete analgesia of at least one limb or the tail to the intense pinch employed in this study. In all 13 placements, analgesia outlasted brain stimulation by 30 seconds to 5 minutes. Responsiveness to other forms of noxious stimulation was tested in four animals shown to be unresponsive to pinch. It was found that a heat stimulus, left in contact with the skin long enough to produce blistering, evoked no response from these animals. Similarly, standing in ice water gave no apparent discomfort. One animal, for example, ate a food pellet for over 5 minutes while standing on its hind legs in a shallow trough of ice water. Approximately 30 seconds after brain stimulation was turned off, the animal suddenly dropped the pellet and jumped out of the trough.

A number of observations routinely made on animals showing the analgesic effect have convinced us that analgesia is not due to a general deficit in sensory, emotional, or attentional mechanisms or to a global motor incapacity or the elicitation of overt responses incompatible with those normally evoked by pain. (i) Most importantly, in all but two animals the peripheral field of analgesia was restricted, usually to one-half or one quadrant of the body. Most animals, therefore, could make normal defensive reactions during brain stimulation and did so if the noxious stimulus was applied outside the peripheral zone of analgesia. The topographic pattern of analgesia elicited by stimulation at a given electrode site remained constant from day to day. (ii) The majority of animals appeared responsive to visual, auditory, and tactile stimuli during brain stimulation. Some were in fact hyperreactive to light touch while apparently oblivious to pain. Such animals, for example, startled when the pliers touched their tails but failed to respond to strong,

sustained pinch. (iii) A number of animals squeaked and struggled when picked up or gently handled during brain stimulation. Several made organized attacks on objects placed in their visual fields. Brain stimulation typically interrupted eating, but in two animals eating was not interrupted by brain stimulation applied alone or concurrent with intense tail shock. (iv) Brain stimulation sometimes elicited forced circling or head, eye, or paw movements. However, the righting reflex was always intact; corneal, grasp, and placing reflexes generally appeared normal. Seizures with motor involvement were never observed (*3*). Locomotor activity appeared augmented in some animals during stimulation but reduced or unchanged in others. Many animals showed stimulus-elicited sniffing. In general, no relationship between analgesia and the elicitation of motor effects was evident. In fact, at several placements yielding maximum analgesia, it was not possible to tell from the general appearance of the animal when brain stimulation was being delivered. Even in cases where motor effects were prominent, behavior rapidly returned to normal within a few seconds after termination of the brain stimulation; yet, as described above, analgesia typically outlasted brain stimulation by 30 seconds or more.

Brain areas (Fig. 1) (*4*) in which stimulation produced analgesia were: dorsal tegmentum, especially ventral posterior central gray ($n = 9$); ventral tegmentum ($n = 6$); dorsal, medial thalamus ($n = 3$); and the junctural region between ventral tegmentum and posterior hypothalamus ($n = 2$). The majority of ineffective points (Fig. 1) was found in the reticular formation, lateral and ventrolateral to central gray.

Several of the analgesic areas are well-established components of the reward system, and we have shown (*5*) that high rates of self-stimulation can be achieved with electrodes in ventral central gray. A relationship between analgesia and reward, therefore, seemed possible and was investigated in all animals by systematic self-stimulation tests. Group 1 animals were again tested with 60 hz a-c, and group 2 animals with biphasic pulses. Stimulus parameters were identical to those used in the analgesia tests. Each bar-press response delivered a 100-msec train of stimulation. Animals were given every opportunity to demonstrate that brain stimulation was rewarding by response shaping and repeated testing.

Table 1. Individual placement, self-stimulation, and analgesia test results for group 1. Self-stimulation score is expressed as the mean rate (responses per minute) during the hour of maximum responding for trains of 0.1-second duration. CG, central gray matter, TEG, tegmentum adjacent to central gray matter.

Placement	Self-stimulation	Analgesia
31L (CG)	112	yes
28L (CG)	87	yes
13R (CG)	76	yes
30L (CG)	69	yes
25L (TEG)	52	yes
28R (CG)	51	yes
23L (TEG)	23	no
14R (CG)	20	no
22R (TEG)	14	no
30R (TEG)	13	yes
23R (TEG)	6	no
25R (TEG)	6	no
26R (TEG)	5	no
26L (TEG)	4	no
14L (TEG)	2	no
22L (TEG)	2	no
24L (TEG)	1	no
24R (TEG)	1	no
13L (TEG)	0	no

Table 1 (group 1) and Table 2 (group 2) provide self-stimulation rates for individual placements and show whether or not analgesia resulted from stimulation at these sites. Placements yielding analgesia also tended to yield the highest rates of self-stimulation. This relationship was statistically reliable (Table 1, $r = .83$, point-biserial correlation, $P < .01$ and Table 2, $r = .42$, point-biserial correlation, $P < .05$). On the other hand, several placements characterized by high self-stimulation rates gave no evidence of analgesia, and stimulation at one site, which was apparently not rewarding, gave rise to excellent analgesia. Thus, areas from which analgesia can be evoked appear to only partially overlap those of the reward system. Evidently, the strength of the relationship between analgesia and reward (or whether or not such a relationship exists) will vary from structure to structure (*6*). In any case, analgesia cannot be ascribed simply to pleasant sensations that mask or divert attention from unpleasant ones.

Table 2. Individual placement, self-stimulation, and analgesia test results for group 2. Self-stimulation score is expressed as the mean rate (responses per minute) during the 30-minute session of maximum responding. DT, dorsal tegmentum; DMT, dorsal, medial thalamus; M-D, mesencephalic-diencephalic juncture; VT, ventral tegmentum.

Placement	Self-stimulation	Analgesia
41R (VT)	202	yes
41L (VT)	196	yes
52L (DT)	185	no
44L (VT)	178	yes
48L (DT)	168	yes
50R (M-D)	160	yes
50L (M-D)	131	yes
54L (DT)	96	no
51L (VT)	66	yes
53L (DT)	60	no
51R (VT)	50	yes
44R (VT)	30	yes
47L (DT)	27	no
45R (DMT)	26	yes
53R (DT)	24	yes
46R (DMT)	16	yes
42L (DT)	0	no
42R (DT)	0	no
45L (DMT)	0	yes
46L (DMT)	0	no
47R (DT)	0	no
48R (DT)	0	no
54R (DT)	0	no

Analgesia was tested during self-stimulation in a number of animals by making tail shocks contingent on the bar-press response, which also delivered brain stimulation, or by applying tail shocks or pinches randomly during the self-stimulation session. In both cases, the placements previously determined to yield analgesia, yielded comparable analgesia when brain stimulation was self-administered, and the self-stimulation rate was unaffected by even intense shock or pinch. By contrast, in those animals which had high rates of self-stimulation but were not analgesic, tail shocks elicited vigorous nociceptive responses and the self-stimulation rate was greatly reduced.

The one placement which supported analgesia but never self-stimulation afforded an opportunity to assess whether the apparent pain-reducing ability of brain stimulation could alone reinforce an operant response. This animal was given access to two bars; one, when operated, delivered a 1-second train of brain stimulation, and the other (control) did nothing. Normally the animal pressed the control bar more often than the active bar, indicating that brain stimulation, if anything, was slightly aversive. However, during each of ten 1-minute periods when electric shocks (1 msec, 12 ma) were applied to the tail at a rate of one per 2 seconds, many more responses were made on the active than on the control bar ($P < .001$, one-tailed t-test). The same result was obtained when the positions of active and control bars were reversed. This suggests that nonrewarding brain stimulation can reinforce an operant response in the presence of pain. The reinforcement presumably derives from pain reduction. We conclude that brain stimulation can reduce or abolish not only the observable

motor responses elicited by a noxious stimulus but also the perceived aversiveness of that stimulus.

Consideration of how brain stimulation exerts its analgesic action is impelled by its obvious importance to our understanding of neural mechanisms of pain perception. For instance, electrical stimulation of these particular brain regions may abolish pain by producing a functional lesion at the stimulation site, which disrupts the normal processing of the afferent pain message. However, this seems unlikely since destruction of an area from which analgesia is produced (the central gray matter, for example) does not clearly lead to a reduction in pain sensitivity (*7*) even though this area has been implicated on electrophysiological grounds in the coding of pain (*8*).

Rather, we propose that brain stimulation attenuates pain by activating a neural substrate that functions normally in the blockage of pain. That such a substrate exists and is capable of being selectively activated is supported by a number of studies concerning the site and mechanism of the analgesic action of morphine. The integrity of certain neurotransmitter systems appears necessary for morphine to exert its analgesic effect (*9*). This suggests that morphine acts, at least in part, by activating a neural pathway in which these transmitters are released. The neurons or chains of neurons in this pathway, then, comprise the substrate of analgesia. The locus of this substrate is suggested by studies that employ intracerebral microinjections of morphine or its antagonist nalorphine. From these studies it appears that morphine acts at certain specific sites in the brain, including the hypothalamus (*10*), the midbrain central gray matter, and the more caudal periventricular regions (*11*). These are areas showing at least partial overlap with those where we find analgesia to result from electrical stimulation. Also, a high transection of the spinal cord abolishes the inhibitory effect of morphine on sensory transmission through the spinal cord (*12*), which suggests the existence of a descending inhibitory influence from those brain regions activated by morphine. Our observation that the spinally mediated flexion or withdrawal reflex was totally suppressed in analgesic animals supports this suggestion.

It seems plausible to us that the analgesia we have observed results from activation of a neural system in the brain which has an ultimate inhibitory action on sensory transmission in the spinal cord. The existence of such a system, a central control mechanism influencing a spinal "gate" for pain perception, has already been proposed (*13*) to account for the powerful modulating effects psychological factors are known to have on nociception. Our results suggest that this system can be effectively activated by focal brain stimulation. These results reinforce continuing attempts to apply this technique to the problem of pain management in man.

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5 August 1971

Stage 4 Sleep: Influence of Time Course Variables

Abstract. *Age, length of prior wakefulness, length of time asleep, and a circadian influence all affect stage 4 sleep. The amount of stage 4 sleep decreases as subject's age increases and as time asleep increases. Longer periods of wakefulness before sleep result in greater amounts of stage 4 sleep in the first 3 hours of sleep. Sleep periods that begin at times other than the regular onset time tend to produce less stage 4 sleep; this decrease suggests a circadian effect.*

For many years we have been stalking a segment of sleep that is known as stage 4. We have studied the effects of stage 4 sleep (*1*), and others have studied such variables as drugs (*2*), psychopathology (*3*), exercise (*4*), and growth hormones (*5*). This report is a review and analysis of data from our laboratory on the responsiveness of stage 4 sleep to changes in four commonly variable features of human sleep: age, length of prior wakefulness, length of the sleep period, and time of sleep onset (a circadian effect). The first three variables are a regular part of daily living. With increase in work shifts spread throughout the 24-hour day and in jet travel, the last variable becomes increasingly important for human sleep. We have asked, to what extent can we predict the amount of stage 4 sleep in humans relative to variations in the normal sleep-wakefulness distributions?

Stage 4 is one of five stages of sleep which can be reliably detected by an electroencephalogram (EEG). Stages 1 to 4 are related to depth of sleep, and a fifth stage, 1-REM, is associated with rapid eye movements, an EEG characteristic of stage 1, and visual dreaming in humans. These stages form a complex and changing pattern throughout sleep. For example, there is an average of 32 changes in stage when young adults sleep at night in the laboratory.