Effect of Destruction of Gyrus Cinguli in Rat Brain on the Development of Tolerance to the Analgesic Effect of Morphine and Physical Dependence on Morphine

S. K. Sudakov*,, I. V. Rusakova*,**, M. N. Trigub*, V. Yu. Shakhmatov***, A. I. Kozel'***, and G. E. Smith******

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We studied the effect of bilateral laser destruction of rat anterior cingulate gyrus on the analgesic effect of morphine and development of tolerance and physical dependence on morphine. Bilateral laser destruction of the anterior cingulate gyrus did not modulate pain sensitivity, analgesic effect of morphine, and development of morphine tolerance. Destruction of the cingulate gyrus alleviated symptoms of the abstinence syndrome in morphine-dependent animals. We showed that morphine-induced analgesia and morphine tolerance are not associated with activity of the anterior cingulate gyrus. However, this structure plays a key role in the development of physical dependence on morphine and abstinence syndrome.

Key Words: *morphine; physical dependence; withdrawal syndrome; cingulate gyrus; rats*

Chronic opiate intake leads to a variety of pathological changes, including tolerance and physical dependence, related to deep reconstruction of intraneuronal processes produced by narcotics [6]. The effect of opiates progressively decreases, while withdrawal produces abstinence syndrome, which is determined by the degree of physical dependence (direct dependence). Much attention was paid to neurochemical processes in brain neurons at various stages of chronic opiate intake. Functional changes in opiate receptors [12] and variations in gene expression were studied in previous experiments [2].

However, little is known about neurophysiological processes in the central nervous system during long-term opiate intake. Changes in two structural and functional systems were previously reported. The first system includes the paragigantocellular nucleus, solitary tract nucleus, and locus coeruleus [4]. The second mesocorticolimbic dopamine system consists of ventral tegmental neurons and various brain regions contain terminals of these neurons (*e.g.*, nucleus accumbens and cingulate gyrus) [3,10]. The mechanism of consecutive involvement of brain structures in the development of tolerance and physical dependence remains unclear.

The cingulate gyrus is a limbic structure that plays a role in emotional behavior. Cingulectomy suppresses narcotic consumption, which is related to reduction of pathological addiction [5,8,11]. Destruction of the cingulate gyrus is followed by an increase in blood β-endorphin concentration [9].

Here we studied the effect of bilateral laser destruction of rat anterior cingulate gyrus on the analgesic effect of morphine and development of tolerance and physical dependence on morphine.

MATERIALS AND METHODS

Experiments were carried out on 40 male Wistar rats weighing 180-200 g. The animals were housed in ca-

*National Research Center of Narcology, Russian Ministry of Health and Social Development; **Institute of Morphology, Russian Academy of Medical Sciences, Moscow; ***Chelyabinsk State Institute of Laser Surgery; ***Department of Physiology and Pharmacology, Medical Faculty, Wake Forest University, Winston-Salem, USA. ***Address for correspondence:*** s-sudakov@mtu-net.ru. S. K. Sudakov

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ges (8-10 rats per cage) under 12:12-h light/dark regimen and had free access to standard food and water.

The rats were scalped and the skull was drilled. A rigid optical fiberglass light guide was bilaterally introduced through the hole into the anterior cingulate gyrus according to stereotaxic coordinates (Bregma 0; L, 0.5; H, 2.5) [7]. The light guide was connected to an ALTO diode laser.

The anterior cingulate gyrus in experimental rats (n=20) was destructed with laser (0.2 W) delivered through the light guide for 10 sec. Sham-operated animals were not exposed to laser.

Physical dependence on morphine was induced in 10 animals of each group 3 days after surgery. The rats received morphine hydrochloride in increasing doses of 10-60 mg/kg for 8 days (2 times a day, 12-h interval). Other controls and experimental animals received an equivalent volume of isotonic NaCl twice a day. The latency of tail flick from hot water (56°C) was estimated 30 min before the 1st and last injection of morphine or isotonic NaCl. The abstinence syndrome was modeled 6 h after the last injection of opiate receptor antagonist naloxone in a dose of 1 mg/kg. Ten minutes after treatment, the rats were placed in an automatic open-field device for 3 min to record specific abstinence reactions (shaking, dyspnea, ptosis, writhing, bruxism, and diarrhea). The general index of abstinence (total score) and frequency of signs were determined for each animal.

The results were analyzed by unpaired t test. The differences were significant at p<0.05. Morphological verification of destruction was performed on serial sections (100 μ).

RESULTS

Morphological study was performed 1 month after surgery. Laser irradiation was followed by the appearance of a round connective-tissue scar (1 mm) in the anterior cingulate gyrus.

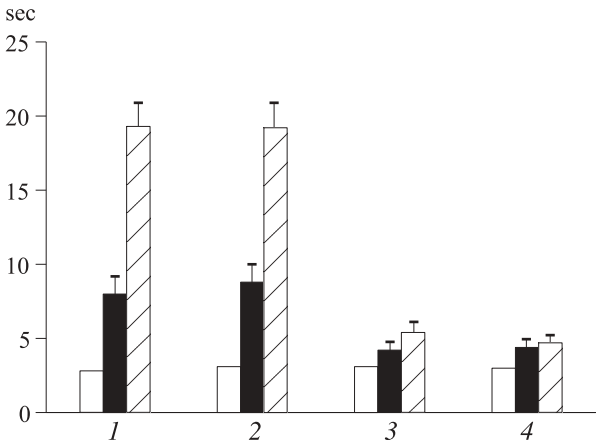

Fig. 1. Latency of tail flick from hot water under basal conditions (light bars) and 30 min after the 1st (dark bars) and last injection of morphine or isotonic NaCl (shaded bars). Here and in Fig. 2: sham-operated rats receiving morphine (*1*), operated rats receiving morphine (*2*), sham-operated rats receiving isotonic NaCl (*3*), and operated rats receiving isotonic NaCl (*4*).

Bilateral laser destruction of the anterior cingulate gyrus did not modulate the sensitivity of animals to pain and analgesic effect of morphine (Fig. 1).

The sensitivity of rats to the analgesic effect of morphine decreased after chronic morphine consumption. Tail-flick latency in these animals was estimated after administration of 60 mg/kg morphine. Destruction of the anterior cingulate gyrus did not modulate the development of tolerance to morphine-produced analgesia (Fig. 1).

Chronic consumption of morphine in increasing doses for 8 days induced physical dependence. The abstinence syndrome in rats manifested in a sharp decrease in horizontal locomotor activity in the open field (Fig. 2, *a*) and appearance of various pathological symptoms ("wet dog shaking", shaking of limbs and

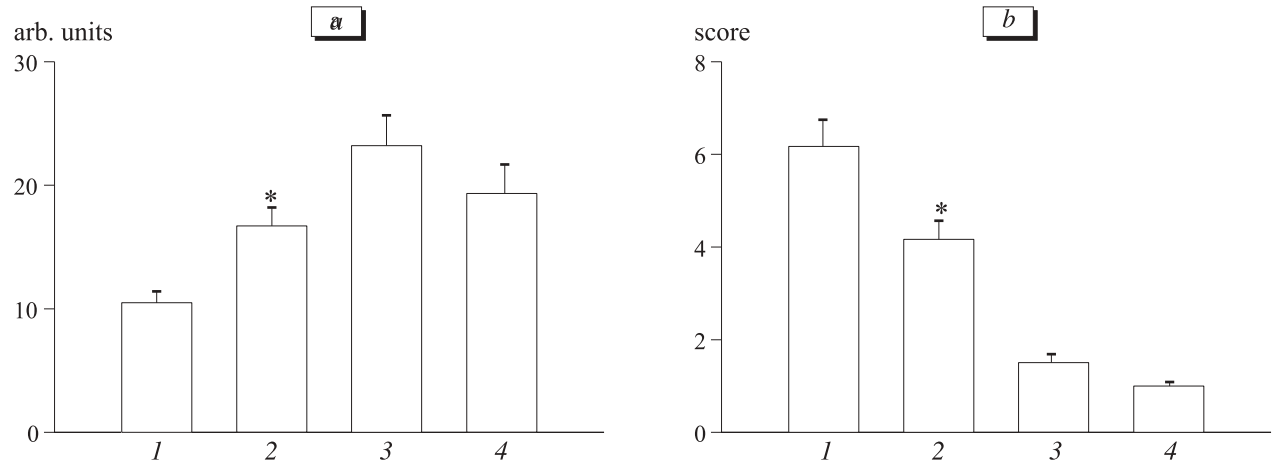

Fig. 2. Horizontal locomotor activity of animals (*a*) and total score of abstinence (*b*). *p<0.05 compared to sham-operated animals.

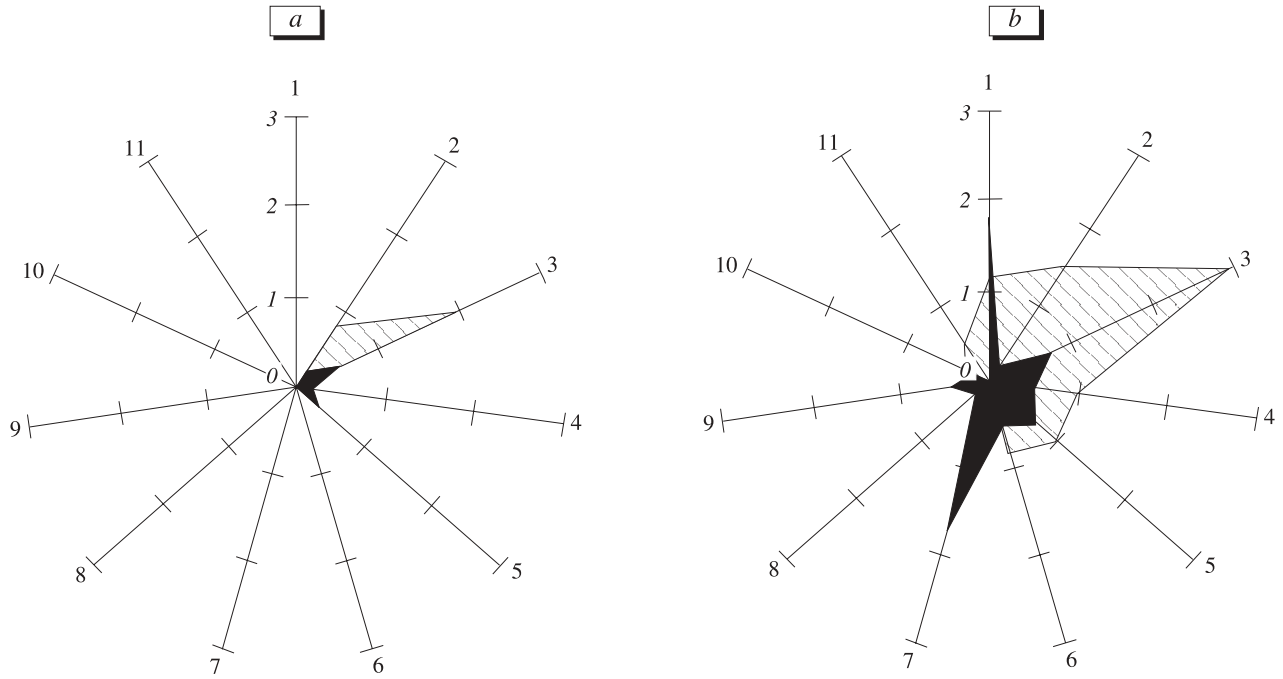

Fig. 3. Abstinence syndrome in animals receiving isotonic NaCl (*a*) and morphine (*b*). Dark area: sham-operated animals. Shaded area: operated animals. Rays of diagram (italic type): average number of signs for the withdrawal syndrome. "Wet dog shaking" (*1*), shaking of limbs (*2*) and head (*3*), dyspnea (*4*), ptosis (*5*), bruxism (*6*), diarrhea (*7*), piloerection (*8*), disturbances in posture (*9*), writhing (*10*), and rhinorhea (*11*).

head, dyspnea, ptosis, bruxism, piloerection, disturbances in posture, writhing, rhinorhea, and diarrhea). Naloxone injection produced shaking of limbs and head, dyspnea, and ptosis in some control animals receiving isotonic NaCl (Fig. 3).

Bilateral laser destruction of the anterior cingulate gyrus relived symptoms of the abstinence syndrome in morphine-treated rats. Horizontal activity increased, while the incidence of limb shaking and dyspnea decreased after surgery. The severity of other symptoms in these rats was lower than in sham-operated animals (except for disturbances in posture and diarrhea, Fig. 3). The total score of abstinence syndrome in operated rats was lower than in sham-operated animals (Fig. 2, *b*).

Our results indicate that the anterior cingulate gyrus does not play a key role in the regulation of pain sensitivity, which is contradictory to published data [1]. Probably, other forms of pain (except for terminal pain) mediated by various neurophysiological mechanisms are closely related to activity of the anterior cingulate gyrus. Morphine-produced analgesia and morphine tolerance are not associated with functions of the anterior cingulate gyrus. However, the anterior cingulate gyrus plays a role in the development of physical dependence on morphine and abstinence syndrome.

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REFERENCES

1. P. G. Bernad and H. T. Ballantine, *Comp. Radiol*., **11**, 117-123 (1987).
2. X. L. Fan, J. S. Zhang, X. Q. Zhang, and L. Ma, *Neuroscience*, **122**, 997-1002 (2003).
3. F. Georges and G. Aston-Jones, *Neuropsychopharmacology*, **28**, 1140-1149 (2003).
4. A. D. Johnson, J. Peoples, R. L. Stornetta, and E. J. Van Bockstaele, *Brain Res*., **15**, 72-84 (2002).
5. T. S. Kanaka and V. Balasubramanaim, *Appl. Neurophysiol*., **41**, 86-92 (1978).
6. E. J. Nestler, *Am. J. Addict*., **10**, 201-217 (2001).
7. G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates*, San Diego (1986).
8. T. Sharma, *Texas Med*., **70**, 49-52 (1974).
9. G. C. Smith, G. L. Willis, D. L. Copolov, *et al*., *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **12**, 683-688 (1988).
10. S. Spiga, G. P. Serra, M. C. Puddy, *et al*., *Eur. J. Neurosci*., **17**, 605-612 (2003).
11. C. L. Trafton and P. R. Marques, *J. Comp. Physiol. Psych*., **75**, 277-285 (1971).
12. M. Waldhoer, S. E. Bartlett, and J. L. Whistler, *Annu. Rev. Biochem*., **73**, 953-990 (2004).