Neural Systems Activated by the Aversive Stimulation of Dorsal Central Gray

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Abstract—Neural effects of aversive stimulation of dorsal central gray (DCG) were studied by \[^{14}C\] 2-deoxyglucose (2-DG) autoradiography in rats. After training the animals to escape DCG stimulation by pressing a lever, they were injected i.p. with \[^{14}C\] 2-DG and then allowed to resume the escape lever pressing for DCG stimulation. Reliable effects of the brain stimulation on the autoradiogram were found in the dorsal fasciculus of Schütz, periventricular gray and superior colliculus. Moderate effects were found in the reticular formation near the periventricular gray and in the claustrum. These data indicate that the neural signal activated by DCG stimulation is transmitted through an ascending nerve pathway over the superior colliculus via the dorsal Schütz bundle and reaches the periventricular system in the diencephalon.

It is known that electrical stimulation of the periventricular system produces aversive operant behavior and/or fear-like responses in experimental animals (1, 2). The stimulation applied to the dorsal part of the central gray (DCG) has strong aversive characteristics; and it also causes increases in arterial blood pressure, heart rate and respiration in the rat (3). Stimulation of the DCG also produces escape lever pressing (4). In a previous study (5), we suggested that the operant behavior induced by the DCG stimulation may be related to not only the central serotonergic mechanism but also related to a cholinergic mechanism. On the other hand, the currently available anatomic and neurologic data regarding the DCG indicate that the dorsolateral portion of the DCG may receive the lateral axis cylinder from the paleospinothalamic pain system (6). Conrad and Pfaff (7) also reported a mutual fiber connection between the DCG and the medial nuclei of the hypothalamus. Nauta (8) pointed out the importance of the periventricular system as a pathway of aversive stimulation. The relationship between the escape behavior in response to the DCG stimulation and the periventricular system of Nauta is of interest.

In the present study, an attempt is made to identify the site within the central nervous system that is activated by the aversive DCG stimulation, by using the \[^{14}C\] 2-deoxyglucose (\[^{14}C\] 2-DG) autoradiography method developed by Sokoloff (9). In this technique, a radioactively labeled and chemically altered form of glucose (\[^{14}C\] 2-DG) is incorporated into neural systems at a rate dependent on the functional activity of the system.

Four male rats of the Wistar strain weighing 250–300 g at the beginning of the experiment were used as subjects. They were housed two per cage in conventional plastic walled cages and were given food and water ad libitum throughout the experiment. The animals were maintained on a 12 hr light-dark cycle (lights on from 08:00 to 20:00) and at a room temperature of 22–24°C with a relative humidity of approximately 60%.
All animals were anesthetized with sodium pentobarbital at the dose of 45 mg/kg, i.p., and chronically implanted with a bipolar stainless steel electrode (250 μm in diameter, insulated except at the tip) into the DCG (A: 0.6–1.0, L: 0.4 and H: 0.6 mm) according to the stereotaxic coordinates of König and Klippel’s atlas (10). The electrodes were inserted into the target sites at a 15° angle in order to avoid piercing the sagittal venous sinus as much as possible. All animals were given 150,000 units of penicillin subcutaneously after the surgery.

The experiment was carried out in a Skinner box, with inside dimensions of 30 cm width, 25 cm depth and 27 cm height, which was constructed of transparent Plexiglass. The floor consisted of a stainless steel grid, with bars of 5 mm diameter that were spaced 1.0 cm apart to allow urine and feces to fall to the tray. The metal lever in the Skinner box was placed 4.5 cm above the grid floor and protruded 2.5 cm into the box. A lever press turned off the brain stimulation. A swivel was mounted in the ceiling of the chamber holding the electrode lead, which allowed the animal to move freely. The stimulation of DCG was derived from a square-wave stimulator (Watanabe).

After allowing the rats at least one week to recover from the implantation surgery, each animal was placed in the Skinner box, and the stimulation cable was connected to the electrode plug mounted in the animal’s head. The DCG was stimulated with a negative square-wave. The stimulation conditions were 5 trains/sec, 100 msec train duration, 60 pulse frequency and 0.5 msec pulse duration, with current in the range of 100–600 μA. The stimulation current was gradually increased until the subject began to show aversive behaviors such as rapid running around the box and jumping, defecation and urination. These rats were trained to escape DCG stimulation by pressing a lever in the box and learned the escape behavior in one or two days. DCG stimulation resumed immediately to 5 sec after the prior lever pressing response, and the period of the stimulation was for 45 min. Only animals that showed a stable latency in the escape lever pressing were used in the [14C] 2-DG experiment.

After a 5 min period of escape lever pressing in a Skinner box, i.e., after confirming the stable lever pressing, the rat was injected i.p. with 30 μCi/rat of [14C] 2-DG (Amer sham, specific activity of 53.7–60 mCi/mmol) and replaced in the box to resume the escape lever pressing for DCG stimulation. At the end of the 45 min allowed for isotope incorporation, the rats were anesthetized by the i.p. injection of sodium pentobarbital (45 mg/kg) and perfused intracardially for 30 sec with 10% formalin. After the electrode was extracted, the brain was removed, rapidly frozen in liquid freon at −55°C, allowed to equilibrate in a cryostat (Bright) at −18°C and sectioned at 20 um. Each section was picked up on a chilled coverslip (24×24 mm) and dried on a warming tray at 40°C. The coverslips were mounted on cardboard and exposed to Kodak SB-5 X ray film for 10 days (11). The sections were subsequently stained with thionine, and these sections were used to identify brain portions on the autoradiogram. The autoradiographic images were analyzed by Computerized Image Processing (Magiscan-2).

First the effects of [14C] 2-DG on the latency of escape behavior in response to DCG stimulation were determined. The rats pressed at the mean latency of 2.2–3.8 sec for 5 min before [14C] 2-DG administration and at the mean latency of 2.7–5.5 sec for 45 min after the administration, indicating that the escape lever pressing for DCG stimulation was not influenced by [14C] 2-DG administration.

The effect of unilateral DCG stimulation on the [14C] 2-DG autoradiogram was observed on the ipsilateral site to the stimulation. Figure 1 shows the effects of DCG stimulation on a representative [14C] 2-DG autoradiogram (Rat No. 952) by means of color windows of Computerized Image Processing. The effects of the stimulation are determined by comparing a structure on the stimulated side to the homonymous structure on the unstimulated side. Moderate effects of DCG stimulation were in the pvr (abbreviations are illustrated in Table 1) at level B (A–3400 μm). The most reliable effects were in the CS, SGCD, SGCL and FLDT at level C...
Fig. 1. Color displays of $^{14}$C 2-DG autoradiogram from level A–D (A-3700 μm–A-1000 μm) for the rat (rat No. 952) that received aversive stimulation of the dorsal central gray (DCG). The DCG was stimulated by the right side electrode. The degree of $^{14}$C radioactivity is displayed as rainbow color, i.e., the portions displayed by the blue black color show the site of high glucose utilization and the portions displayed by the green or yellow show the site of low glucose utilization. Black arrows in the picture indicate the site where the DCG stimulation effect was marked. E (near) indicates the site near the stimulation electrode. pvr: nucleus periventricularis rotundocellularis; CS: colliculorum superiorum; SGCD: substantia grisea centralis, pars dorsalis; SGCL: substantia grisea centralis, pars lateralis; FLDT: fasciculus longitudinalis dorsalis (Schütz), pars tectalis; FOR: formatio reticularis; SAP: stratum album profundum colliculi superioris; and E (near): near the electrode.

In three other animals, the effects of unilateral DCG stimulation were also observed on the ipsilateral site to the stimulation as shown in Table 1. In rat No. 810, most reliable effects of DCG stimulation were in the SGCD, SGCL and SAP at level D (A-1000 μm). In data not shown in the figure, a moderate effect was observed in the CL at the level of P-400 μm. In rat No. 830, moderate effects were in the FLD at the level of A-3000 μm, and weak effects were in the SGCL, SGCV and FLDG at the level of A-2220 μm and in the SGCD, SGCL and FLD at the level of A-600 μm. The strongest effects were in the SGCL, SGCV and a part of the CL near the DCG electrode at the level of A-450 μm; and weak effects were observed in the SGCV and FLDG at the level of P-300 μm. In rat No. 928, the most reliable effects of brain stimulation were found in the FLD and FPVT at level of A-2800 μm, SGCD and CS at level of A-1000 μm, and SGCD and a part of the CL near the electrode.
The effect of unilateral DCG stimulation on the $[^{14}C]$ 2-DG autoradiogram was observed throughout the periventricular system on the ipsilateral site to the stimulation in all rats. This effect was not observed in the whole central gray matter near the site of stimulation, but was localized to the dorsal area and its neighboring regions. No stimulus effect was noted on the ventrodorsal side of the central gray matter, the site at which an analgesic effect appears (12). Another unaffected area was the dorsal raphe nucleus, the site at which intracranial self-stimulating (reward) behavior appears (13). These suggest that the region related to aversive stimulation of the DCG is localized to a narrow area near the dorsolateral central gray matter.

Near the stimulation site, darkening of the autoradiogram was pronounced, and this became weaker as the distance from the site of stimulation was increased. An intense and reproducible effect from stimulation was noted in the lateral bundle of Schütz (FLD, FLDT and FLDG). A mild effect was seen in the midbrain FOR adjacent to the peri-aqueductal gray matter (No. 952), and a slight effects was noted in the medial hypothalamus.

Nauta (8) has suggested that the periventricular system is involved as the neuronal route of the aversive pathway. This system connects the cerebral cortex, the forebrain limbic system and the dorsomedial midbrain. The ascending fibers originate from the midbrain central gray matter and were reported to ascend through the dorsal bundle of Schütz into the medial hypothalamus. The descending fibers have been shown to pass through the basal forebrain reaching the thalamus, hypothalamus and the midbrain. Grofova, et al. (14), using a horseradish peroxidase technique, reported the presence of centrifugal fibers originating from the DCG and radiating to the superior colliculus. The results of the present study suggest that following DCG stimulation, the activation signal is transmitted through an ascending nerve pathway over the CS via the dorsal bundle of Schütz and finally reaches the periventricular medial hypothalamus in the diencephalon. This is in agreement with Nauta’s periventricular ascending system (8).

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| Table 1. Sites of DCG stimulation effect on $[^{14}C]$ 2-deoxyglucose autoradiogram in four rats |
|---|---|---|---|
| Effect \ Rat | No. 952 | No. 810 | No. 830 | No. 928 |
| Most reliable | SGCD, SGCL | SGCD, SGCL | FLD, FPVT | SGCD, CS, CL |
| Strongest | FOR, SAP | SGCL, SGCV | CL |
| Moderate | pvr, CL | FLD | |
| Weak | FLDG, SGCV | |

Detailed descriptions on the section level are given in the text. Abbreviations: SGCD: substantia grisea centralis, pars dorsalis; SGCL: substantia grisea centralis, pars lateralis; SGCV: substantia grisea centralis, pars ventralis; FLD: fasciculus longitudinalis dorsalis (Schütz); FLDT: fasciculus longitudinalis dorsalis (Schütz), pars tectalis; FLDG: fasciculus longitudinalis dorsalis (Schütz), pars tegmentalis; CS: colliculorum superiorum; pvr: nucleus periventricularis rotundocellularis; FPVT: fibrae periventriculares thalami; CL: claustrum; SAP: stratum album profundum colliculi superioris; and FOR: formatio reticularis.

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