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Unilateral Injection of GABA Agonists in the Superior Colliculus: Asymmetry to Tactile Stimulation

G. DI SCALA, P. SCHMITT[1] AND P. KARLI

Laboratoire de Neurophysiologie, Centre de Neurochimie du CNRS
5, rue Blaise Pascal—67084 Strasbourg Cédex—France

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DI SCALA, G., P. SCHMITT AND P. KARLI. *Unilateral injection of GABA agonists in the superior colliculus: Asymmetry to tactile stimulation.* PHARMACOL BIOCHEM BEHAV **19**(2)281–285, 1983.—A unilateral microinjection of each one of three different GABA agonists (Muscimol: 1.4 nmoles; Baclofen: 0.8 nmole; THIP: 10.7 nmoles) into the superior colliculus was found to result in a reversible asymmetry in the rat's responsiveness to tactile stimulation. The rat was hyporeactive to stimulations applied contralaterally and hyperreactive to stimulations applied ipsilaterally to the infusion site. Furthermore, the rat showed ipsiversive turning in response to tactile stimulation applied either ipsi- or contralaterally to the infusion site. The results are discussed in relation with motor and sensory asymmetry produced by unilateral manipulations affecting the striato-nigro tectal system.

Rat Superior colliculus Microinjection GABA agonist Muscimol Baclofen THIP Asymmetry Tactile stimulation

SEVERAL observations suggest that the superior colliculus (SC) receives sensory inputs [6, 18, 24, 25] and plays a role in motor control as an output station for striatonigral system effects [11,12]. In particular, the suggestion was put forward that the nigrotectal pathway might mediate dopamine related behavior of striatal origin [19,26]. A unilateral lesion of various parts of the nigrostriato-nigrotectal system produces motor and sensory asymmetries [16,17]. Thus, a unilateral lesion of the SC entails a contralateral multimodal sensory neglect together with ipsiversive turning [13].

The SC receives a GABAergic inhibitory input originating from the substantia nigra [3,8]. Unilateral infusion of muscimol, a GABA agonist, into the substantia nigra was shown to produce contraversive turning together with asymmetry in responsiveness to tactile stimulation [9]. In contrast, unilateral infusion of muscimol into the SC was found to give rise to ipsiversive circling [11,12]. As the responsiveness to tactile stimulation had not yet been studied after infusion of GABA agonists into the SC, the aim of the present work was to check if such an infusion would result in an asymmetry in responsiveness to tactile stimulation, and more specifically in a contralateral decrease in responsiveness. The effects of the three following agonists were studied: muscimol [1], l-baclofen [2], and THIP [15].

METHOD

Thirteen Wistar rats weighing between 300 and 400 g were used for canula implantation. The animals were anesthetized with pentobarbital (40 mg/kg IP) and placed into a stereotaxic apparatus so that the surface of the skull was horizontal. Two guide-canulae (outer diameter: 0.4 mm) were implanted, one into the left SC, the other into the right SC. The following coordinates were used, the lambda serving as the reference: postero-anterior: 0 to 1 mm; medio-lateral: 1.7 mm, at a medio-lateral angle of 10°; dorso-ventral: 3.5 mm.

Following a recovery period of 1 week, each animal was placed into a circular enclosure (60 cm in diameter and 30 cm high). A rod coated with cotton wool was then applied to the head, to the forequarters and to the hindquarters, on either side of the rat's body. Preliminary observations had shown that the animal's response could be described in terms of the following behaviour items: lack of reaction, squeal, start, orientation of head or entire body towards the stimulus, snout contact with—or bite of—cotton wool, turning.

The experiment aimed at determining whether and how these behaviour items might be affected by a unilateral local microinjection of Muscimol (Sigma), of l-Baclofen (Ciba-Geigy), of THIP (4,5,6,7-tetrahydroisoxazolo (5-4-c) pyridin 3-ol-Sandoz) or of 9‰ NaCl. Each drug was dissolved in 9‰ NaCl, and the following doses were used on the basis of a series of preliminary observations: Muscimol: 1.4 nmoles (0.16 μg) in 0.4 μl; l-Baclofen: 0.8 nmole (0.2 μg) in 0.2 μl; THIP: 10.7 nmoles (1.5 μg) in 0.2 μl. The microinjections were performed in the freely moving animal by inserting into the guide-canula an injection canula (outer diameter: 0.28 mm) which protruded 1.0 mm beyond the outer guide. The

[1]Requests for reprints should be addressed to P. Schmitt.

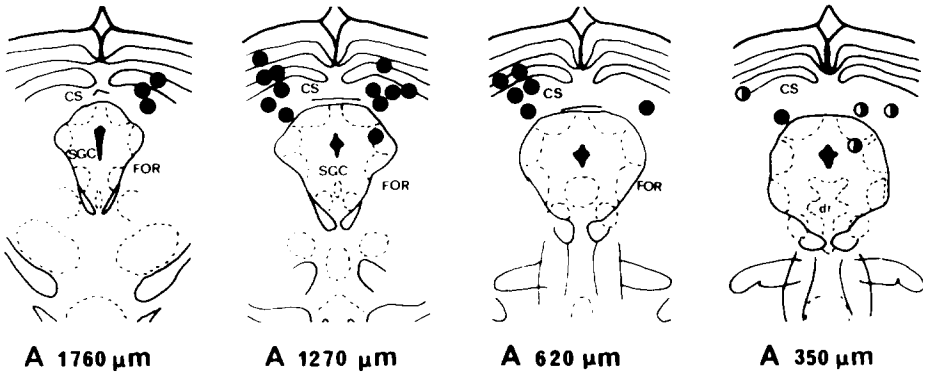

FIG. 1. Localization of the microinjection sites on corresponding frontal planes of the König and Klippel atlas [14]. The sites where partial neglect followed from the injection of GABA agonists are indicated by half-filled circles.

injection canula was linked to a Hamilton syringe by means of polyethylene tubing. The injections were performed at a speed of 0.2 µl/30 sec. At the end of the injection, the injection canula was left in its place for 30 sec. A delay of at least 4 days elapsed between two successive injections.

Procedure

The rat was first allowed to explore the enclosure for 30 minutes. Two tests were then carried out, 30 and 15 minutes before the injection. Each test consisted of 5 applications of the cotton wool to each of the three explored body regions, on either side. The three body regions were stimulated in succession without any predetermined order, but the stimulation of any given region was always followed by the stimulation of its contralateral counterpart. Following the injection the same tests were again carried out, the first two 15 and 30 minutes after the injection, and then every 30 minutes.

In addition, a sound stimulus (hand clap) was delivered between the test-periods and the induction of turning or lack of any reaction was recorded. Following the test carried out 60 minutes after the injection, the animal was placed on a grid (30×30 cm) for an anti-gravity test in order to detect a possibly existing motor incapacity. The grid was given a 80° tilt from the horizontal in such a way that the animal's head was oriented downwards and that the animal's body axis would form a 30° lateral angle with the tilted grid axis. Ten trials were carried out for either side and the way in which the animal righted itself was noted down.

Non-parametric tests were used for the statistical analysis of the results obtained [22].

On completion of the experiment, the animals were killed by means of an overdose of pentobarbital and then intracardially perfused with 9‰ NaCl followed by 10% formalin. Serial brain sections were stained with cresyl violet in order to localize the actual injection sites which were then drawn on the corresponding frontal planes of the König and Klippel atlas [14].

RESULTS

Figure 1 shows the injection sites to be located in the superior colliculus (n=24) or in the dorsal part of the periaqueductal gray (n=2).

Figure 2 shows how the rats' responses to tactile stimula-

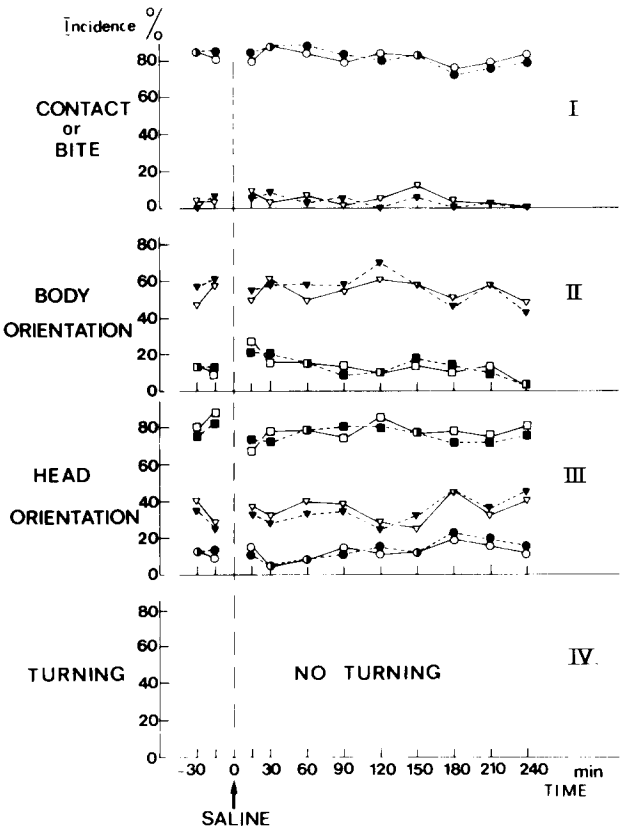

FIG. 2. Incidence of the behavioral items observed on application of a tactile stimulus, before and after a microinjection of saline into the superior colliculus. The site of stimulus application is indicated by the following symbols: head (○, ●); forequarters (▽, ▼); hindquarters (□, ■). Open symbols refer to responses provoked by stimulations applied ipsilaterally to—whereas filled symbols refer to responses provoked by stimulations applied contralaterally to—the injection site.

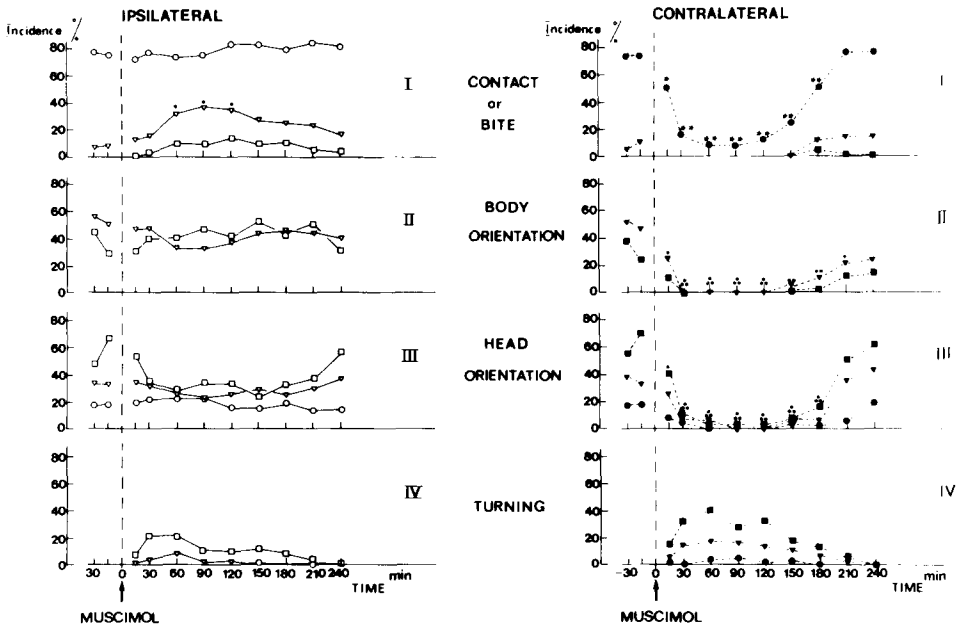

FIG. 3. Incidence of the behavioral items observed on application of a tactile stimulus, before and after a microinjection of muscimol into the superior colliculus. Responses to ipsilateral stimulation are shown on the left; responses to contralateral stimulation are shown on the right. As in Fig. 2, the site of stimulus application is indicated by the following symbols: head (○, ●); forequarters (▽, ▼); hindquarters (□, ■). Some of the statistical data are shown. The Wilcoxon test was used to compare ipsi- versus contralateral responses (★$p<0.05$; ★★$p<0.01$; ★★★$p<0.01$). The Mann Whitney test was used to compare the incidence of responses after muscimol and saline microinjections, respectively (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

tion evolved over time, before and after a microinjection of 0.4 μl of 9‰ NaCl into the SC (n=12). It should first be stressed that before any microinjection, the rats' responsiveness to tactile stimulation proved characterized by a clearly increasing postero-anterior gradient. Thus, a stimulation applied to the hindquarters elicited most often an orientation of the head (80%), an orientation of the entire body towards the stimulus being observed in only 15% of the cases. In contrast, a stimulation applied to the forequarters elicited an orientation of the entire body in 50 to 60%—and bites in 7%—of the cases. When the stimulation was applied to the head, the animal oriented only its head toward the stimulus and the percentage of bites went up to 80%.

Following the microinjection of 0.4 μl of NaCl, the animals' responsiveness changed little over time. For any given body region, the responses elicited by tactile stimulation did not reliably differ depending on whether the stimulus was applied ipsi- or contralaterally with regard to the injection site (Wilcoxon, $p>0.05$). Whether tactile or acoustic, the stimulation never elicited a turning of the animal, and the rats placed on the tilted grid righted themselves equally well to the left or to the right.

Figure 3 shows how the rats' responsiveness was affected by a unilateral microinjection of 1.4 nmoles of muscimol into the SC (n=24). Such an injection markedly suppressed or even abolished the normally elicited responses when—and only when—the stimulation was applied to the side opposite to the injection site. Thus, a stimulation applied contralaterally to the head did no longer elicit any bite, whereas the ipsilateral stimulation remained efficient (Wilcoxon, $p<0.01$). This contralateral suppressant effect was apparent as soon as 15 min after the injection and it lasted 180 min. For 3 out of the 24 injection sites, only the responsiveness to stimulations applied to the fore- or hindquarters proved to be suppressed.

A comparison of the effects of muscimol with those of NaCl confirmed the marked contralateral suppressant effect on the rats' responsiveness that resulted from a microinjection of muscimol (Mann Whitney U test, $p<0.05$). It further showed an enhanced responsiveness to stimulations applied to the ipsilateral side of the animals' body. Following a microinjection of muscimol, the incidence of bites elicited by a stimulation of the forequarters was indeed reliably higher (Mann Whitney, $p<0.05$) than that observed following a microinjection of NaCl (row I in Figs. 2 and 3). This increased incidence of bites entailed in return a reduced incidence of other behaviour items noted down such as orientation of head or entire body. On stimulation of the hindquarters, 6 rats responded with bites, which never occurred before the injection of muscimol. At the head level, this increased responsiveness expressed itself through an increased vigor of the bites, but the measurements carried out did not allow to clearly quantify this observation.

Figure 3 shows further (row IV) that whether ipsi- or contralaterally applied tactile stimulation of either fore- or hindquarters elicited turning that went always ipsilateral with regard to the injection site. Moreover, in 83% of the cases, the sound stimulus elicited turning of low amplitude and speed. It is worth adding that such turning did also occur spontaneously during the first minutes following the injection. On the tilted grid most rats (17/24) proved able to right

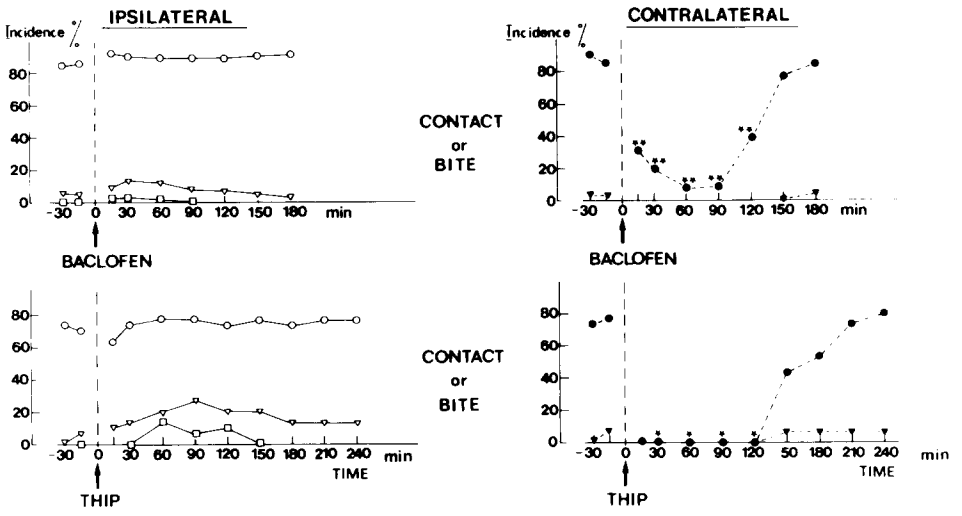

FIG. 4. Incidence of contact and bite before and after microinjection of l-baclofen (upper rows) or THIP (lower rows) into the superior colliculus. Responses to ipsilateral and contralateral stimulations are shown on the left and on the right, respectively. The site of stimulus application is indicated by the following symbols: head (○, ●); forequarters (▽, ▼); hindquarters (□, ■).

themselves to either side, even though they all showed a marked preference for righting themselves ipsilaterally with regard to the injection site. The 7 remaining animals did never right themselves contralaterally.

In order to exemplify the effects of microinjections of l-baclofen (0.8 nmole; n=18) or of THIP (10.7 nmoles; n=6), Fig. 4 shows the incidence of bites elicited by tactile stimulations applied to the head. The effects of these two drugs were qualitatively similar to those obtained with muscimol, as they provoked a markedly suppressed responsiveness to contralateral stimulation—with regard to the injection site—together with an increased responsiveness to ipsilateral stimulation. At 2 out of the 18 injection sites (of which one had not been tested with muscimol), l-baclofen provoked a suppression of responsiveness only when the fore- or hindquarters were stimulated, but not when the stimulation was applied to the head.

DISCUSSION

Previous studies have shown unilateral infusions of GABA agonists into the superior colliculus (SC) to result in postural asymmetry and weak ipsilateral circling [11,12]. The present study further shows that a unilateral application of any of these different GABA agonists (muscimol, l-baclofen, THIP) within the SC produces a reversible asymmetry in the rat's responsiveness to tactile stimulation. This asymmetry results from both a contralateral decrease and an ipsilateral increase in responsiveness to tactile stimulations applied to different parts of the body. Furthermore, turning ipsiversive to the infusion site can be induced by tactile stimulation applied either ipsi- or contralaterally. The present study does not allow to conclude that the described effect of GABA agonist injection specifically relates to superior colliculus sites. As a matter of fact, efficient sites were also found within the central gray. Despite the relatively large dose used as compared to those used by others [11,12], a partial neglect could be observed after infusion at 4 sites located in the caudal part of the SC. Such a result could hardly be obtained had the diffusion area been large.

The superior colliculus, where cells were found to be responsive to various sensory stimuli [6, 24, 25] among which tactile stimuli [18], seems to be involved in the orientation toward various stimuli and it is considered to be an output station for motor control [11]. However, one can hardly conclude definitely from the present results whether infusion of GABA agonists produces an imbalance of only sensory thresholds or an imbalance of only motor reactions or both. The fact that the treated rat bites the cotton wool applied to the forequarters ipsilateral to the infusion site whereas in the saline injected rats, such a stimulation elicits mainly "orientation of the body," suggests a lowering of sensory threshold. Moreover, the fact that in 4 rats the infusion affected the responsiveness to stimulations applied to fore- and hindquarters, but not to stimulations applied to the head could suggest that there exists some kind of somatotopy similar to that shown to exist for somatosensory fields in the SC of the golden Hamster [7]. However, some of our data are also suggestive of a motor bias. Indeed, in the antigravitational test, 7 rats righted themselves exclusively to the ipsilateral side and the others, while using both sides for righting themselves showed nevertheless a preference for the ipsilateral side. Furthermore, even though the rat proved able to react to a stimulus applied contralaterally to the infusion site, its actual reaction was unexpectedly a turning ipsiversive to the infusion site. This could be due to a blockade of motor reactions contralateral to the infusion site, but also to an inappropriate localization of the stimuli as suggested by Kirvel [13].

Interestingly, GABA agonists applied within the SC seem to mimic the effects of a unilateral lesion of the SC. Thus, such a lesion was found to produce a contralateral multimodal sensory neglect [13,23] as well as a hyperresponsiveness to stimulations applied to the side ipsilateral to the

lesion, at least in the cat [23]. Taken as a whole, these results suggest that the main effect produced by a unilateral lesion may result from the exclusion of a neuronal system which is normally inhibited by GABA perhaps released by the GABAergic nigro-tectal input [3]. It is worth adding that preliminary data indicate that infusion of GABA antagonists results, in some cases, in an ipsilateral neglect. However, this neglect is not as easy to demonstrate as it is with GABA agonists, since GABA antagonist infusion produces also vigorous escape ([4, 11], and personal observations).

Various manipulations affecting the nigro-striato-nigro-tectal system result in asymmetry in responsiveness to sensory stimuli. Thus, unilateral 6-hydroxydopamine lesions of the dopaminergic nigro-striatal pathway result in a contralateral sensory neglect [16,17]. Furthermore, unilateral injection of a GABA agonist into the substantia nigra was found to result in a contralateral hyperreactivity and an ipsilateral hyporeactivity [9], a result just opposite to that obtained in the present study following a microinjection of a GABA agonist into the SC. Therefore, our results agree well with the proposition that the striatum may be able to control responsiveness to sensory stimuli via the striato-nigral and nigro-tectal pathways [21]. They are also in good agreement with the view that the striatal dopaminergic input has—via a GABAergic striato-nigral pathway—an inhibitory effect on substantia nigra pars reticulata neurons, the latter neurons exerting—via the nigro-tectal GABAergic pathway—an inhibitory effect upon those tectal neurons that are involved in the processing of sensory information [5,26]. The failure to reverse by a microinjection of picrotoxin into the SC the sensory neglect produced by 6-hydroxydopamine lesions of the substantia nigra [20] however questioned this hypothesis, and further studies are needed to clarify this problem.

ACKNOWLEDGEMENTS

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