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Pontine Lesions Attenuate Physostigmine Suppression of Self-Stimulation in the Rat

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SAITOH, Y. AND H. KAWAMURA. *Pontine lesions attenuate physostigmine suppression of self-stimulation in the rat.* PHARMACOL BIOCHEM BEHAV 37(1) 187–191, 1990.—In male Wistar albino rats with chronically implanted electrodes, self-stimulation behavior was compared before and after making bilateral pontine lesions involving the subcoeruleus area and adjacent tegmental field. Before lesioning, slight suppression of bar pressing after subcutaneous injection of 0.05 mg/kg physostigmine, marked stable suppression after 0.1 mg/kg, and very strong suppression after 0.2 mg/kg were observed. After making pontine lesions, the suppressive effects of physostigmine were clearly attenuated. With 0.05 and 0.1 mg/kg, no effect or only occasional slight suppression was observed in most cases. With 0.2 mg/kg, total suppression was induced in association with some peripheral effects, but the duration was obviously less than in the prelesion controls. Control saline injection did not produce a suppressive effect. From these findings, it is suggested that suppression of self-stimulation by physostigmine up to 0.1 mg/kg is due to the inhibition of motor activity through the cholinoceptive dorso-lateral pontine tegmental area and not due to a direct effect on the forebrain cholinergic process, presumably involving reinforcement or motivation.

Physostigmine Intracranial self-stimulation Nucleus subcoeruleus Bar pressing Rat

IMPROVEMENT of learning and memory after administration of cholinergic agonists has been reported by many authors (1, 5, 7, 9). Also, there are several reports of trials of anticholinesterase therapy for senile dementia of the Alzheimer type (2, 6, 22, 24), in connection with findings on the initial deficit of forebrain cholinergic neurons in the disease (4,28).

However, it is also evident that operant conditioning is blocked by physostigmine in both the escape and food pecking response in pigeons as well as in squirrel monkeys (25,26). Intracranial self-stimulation (ICSS) in the rat is also blocked by physostigmine (8), which is antagonized by scopolamine (17). This is considered to be a central effect, because equimolar neostigmine, which does not penetrate the blood-brain barrier, has no such suppressive effect up to a certain dose.

The sites of action of physostigmine within the brain affecting these two behaviors may be different. Thus, in this paper, we have tested our assumption that suppression of physostigmine on intracranial self-stimulation is not due to a direct effect on the cholinergic forebrain mechanism involving reinforcement or motivation, but due to an effect on the cholinoceptive pontine dorso-lateral tegmental area which suppresses spinal motoneurons, presumably through the medullary motor inhibitory center (15).

METHOD

Data were obtained from twenty-one Wistar strain male albino rats weighing 280 to 350 g. Under deep pentobarbital anesthesia (50 mg/kg intraperitoneal injection), concentric needle electrodes (outer diameter, 0.6 mm; distance between exposed tips, 0.5 mm) were implanted stereotaxically using the atlases of König and Klippel (14) and Pellegrino *et al.* (19). The electrodes were inserted in the lateral hypothalamic area for reward stimulation and bilaterally in the dorso-lateral pontine tegmental areas for electrolytic lesions. These electrodes were connected to a 9 pin Amphenol plug which was cemented onto the skull. After recovery, the animals were placed individually in a Skinner box in which priming stimulation was given whenever the rat neared and touched the pressing bar. Final establishment of self-stimulation suitable for use in drug testing occurred when a rat, placed in the Skinner box, started bar pressing immediately and continued the pressing without obvious rate changes for at least 120 min and without intervals of longer than 30 sec. The stimulus parameters were 100 Hz, 1 msec with 25 pulses in one train with intensity from 1.5 to 3.0 V.

In each experimental session, after a stable response rate lasting more than 30 min was recorded, either a drug or control saline was given. When suppression of bar pressing was observed, the length of time until the rat again pressed the bar spontaneously was measured. No priming was done during this period. When the bar pressing was stopped for longer than 30 sec after drug injection, the length of time until bar pressing resumed was defined as the suppression time of self-stimulation. A reduction in the bar pressing rate was not included in the suppression time.

The drugs tested were physostigmine sulfate (Sigma) and neostigmine bromide (Tokyo Kasei). The drugs were dissolved into 0.9% saline for subcutaneous injection. After prelesion drug

testing, all 21 rats received pontine lesions. Lesions of the pontine tegmental area were made under ether anesthesia by passing a 0.5 mA DC current between the two tips of the electrodes for 10 to 90 sec repeatedly. After a recovery period of 5 days or longer, when the stable response rate of self-stimulation showed the prelesion level, the effects of the drug were again tested in the rats.

After the completion of each experiment, the rats were sacrificed by an overdose of pentobarbital, and the brains were perfused with physiological saline followed by 10% formalin solution. After fixation with formalin for 7 days or more, the frozen brains were sectioned to 50 μm thickness and stained with cresyl violet. Acetylcholinesterase staining in the dorso-lateral tegmental area of the pons was performed by the method of Koelle and the localization of well-stained cells in the locus subcoeruleus area and dorsal tegmental field was confirmed.

RESULTS

In twenty out of 21 rats, the stimulation electrode was found within the lateral hypothalamic area. In one rat, the tip of the stimulation electrode was slightly dorsal to the lateral hypothalamic area, but nevertheless was effective for reward stimulation. Before the production of pontine lesions, the suppressive effects of physostigmine on self-stimulation were observed in all 21 rats. Only a single injection was given to an animal in one day. After injection of 0.05 mg/kg physostigmine, an interval of at least 2 days was allowed before the next injection. After injection of 0.1 and 0.2 mg/kg physostigmine, a minimum 3 days to maximum 15 days interval was allowed. In 3 rats, the procedure was repeated for 3 times at each dose before and after lesioning. In one rat without lesions, the injection with 0.1 mg/kg was repeated 19 times at 3-day intervals as an additional control experiment, but no particular tendency toward an increasing or decreasing effect was observed (mean ± S.E.M.; 18.9 ± 1.4 min). In 5 animals, injection of 0.9% saline (vehicle) solution produced no obvious effects on self-stimulation before or after pontine lesioning.

In 11 out of 21 rats, attenuation of the suppression period by physostigmine injection was recognized after production of bilateral lesions of the dorso-lateral pontine tegmentum. In the remaining 10 rats, such clear attenuation was not observed. The upper three records in Fig. 1 show a sample record in which injection of physostigmine dose-dependently suppressed self-stimulation behavior. Subcutaneous injection of 0.05 mg/kg physostigmine slowed down the response rate. At this dose, the effect on the response rate was not necessarily stable. After injection of 0.1 mg/kg physostigmine, bar pressing was totally suppressed after a latency period of about 5 min, for 35 min in the illustrated case. During the suppression period, the animal crouched and rarely moved around on the floor. After injection of 0.2 mg/kg physostigmine, the behavior of the rat was similar to that after the 0.1 mg/kg injection, but the total suppression time was longer (51 min, in this case). Some of the animals experienced tooth chatter or twitches of the head muscles occasionally.

The lower 3 records in Fig. 1 show the effects of physostigmine injection in the same rat following the production of bilateral lesions of the dorso-lateral tegmental area of the pons. After lesioning, no cessation of bar pressing could be seen with either the 0.05 or 0.1 mg/kg dose. Only after injection of 0.2 mg/kg physostigmine did suppression appear, but the duration was reduced to 18 min compared to 51 min before lesioning.

In Fig. 2, the available data from 9 of the 11 rats that showed attenuation of the effect of physostigmine after dorsal pontine tegmental lesioning are summarized. The data from 2 rats are not included because of a lack of histological preparation. Mean values and standard errors of the total suppression period of

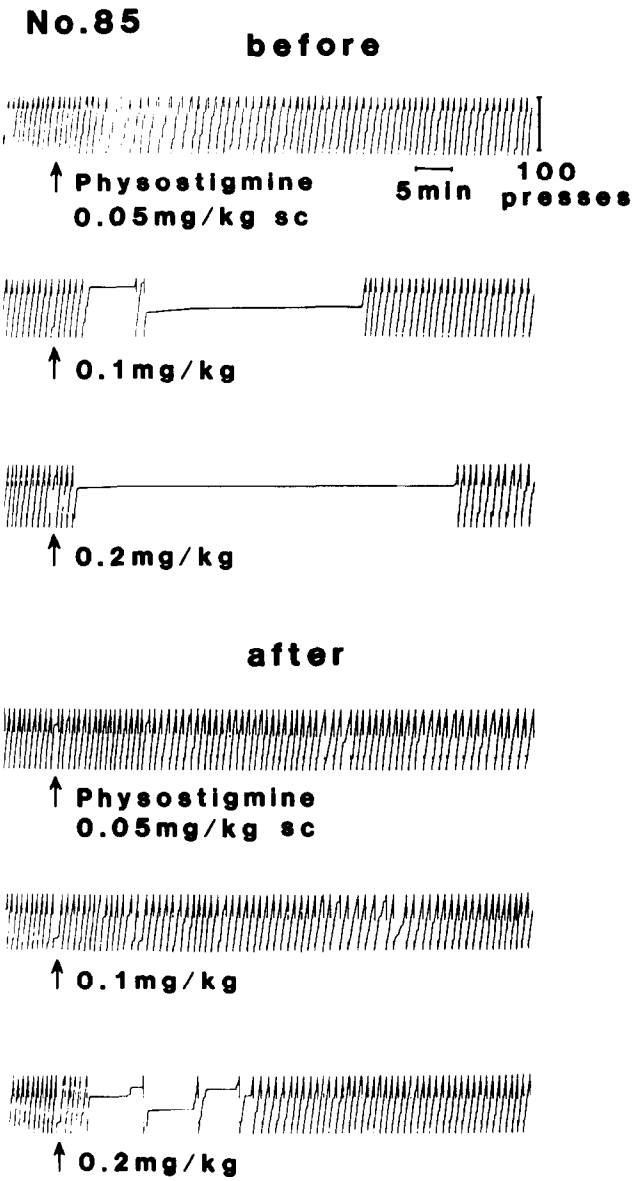

FIG. 1. Effects of physostigmine on intracranial self-stimulation before and after lesioning of the dorso-lateral pontine tegmental area. Calibration: cumulative recording of bar-pressing (one hundred pressed from the bottom to the top); time, 5 min. The upper three sample records were taken after administration of increased doses of subcutaneous physostigmine to a rat. Suppression of lateral-hypothalamic self-stimulation after injection of physostigmine (arrows) was enhanced dose-dependently. The lower three records were taken after the production of bilateral pontine lesions in the same rat. Attenuation of the suppressive effect of physostigmine is evident except after the 0.2 mg/kg injection.

self-stimulation following 0.05 mg/kg physostigmine were 12.1 ± 3.3 min and 2.6 ± 1.0 min before and after lesioning, respectively. When 0.1 mg/kg was injected, the durations of suppression were 26.2 ± 3.8 min and 6.4 ± 1.4 min, respectively. With 0.2 mg/kg, they were 45.9 ± 2.7 min and 19.5 ± 2.7 min. The numbers in parentheses in Figs. 2 and 4 are the total number of injections at each dose. Injection of 0.1 mg/kg neostigmine induced a mean suppression period of 10.9 ± 2.9 min in 4 intact rats.

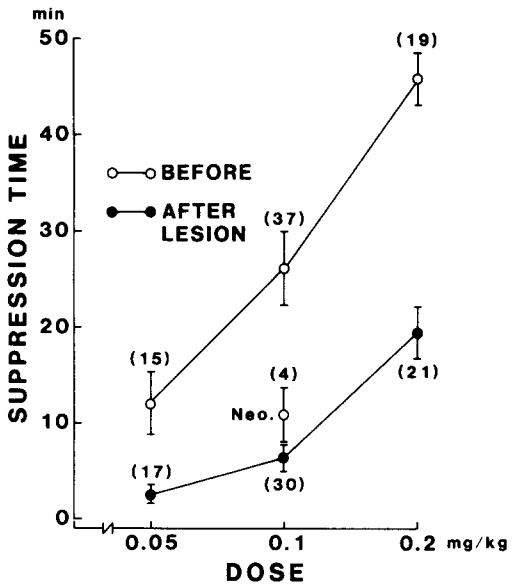

FIG. 2. Suppression of self-stimulation after physostigmine is markedly attenuated after pontine tegmental lesioning. This diagram was constructed from all available data taken from 9 rats. Abscissa: duration of suppression after physostigmine injection. Ordinate: physostigmine dose. Data indicated by open circles were taken before dorso-lateral pontine tegmental lesioning. Filled circles show values taken after lesioning. The effects of neostigmine (Neo: almost equimolar to 0.2 mg/kg physostigmine) in 4 cases are shown. Numbers in parentheses are the total number of injections in each case.

Figure 3 demonstrates the location of lesions in the pontine tegmentum in a sample of 3 rats, the data for which are included in the diagram shown in Fig. 2. All showed a marked reduction in the suppressive effect of physostigmine upon self-stimulation behavior. Lesions common to all 9 rats, including the 6 rats which do not appear on the diagram, involved the dorso-lateral tegmental area ventral to the locus coeruleus. This area contains the subcoeruleus area and a part of the pontine giganto-cellular tegmental field where many intensely AChE positive cells were found.

Figure 4 summarizes the data taken from the remaining 10 rats which demonstrated no significant changes in the suppression period after lesioning of the pontine tegmental area. With 0.05, 0.1 and 0.2 mg/kg physostigmine, no obvious attenuation of the suppressive effect after lesioning of the pontine area was observed. The mean values of the suppression period of self-stimulation were 10.4 ± 3.0 min and 6.6 ± 2.3 min before and after lesioning, respectively, after the administration of 0.05 mg/kg physostigmine. With 0.1 mg/kg physostigmine, the mean values and standard errors of the suppression period before and after lesioning were 21.9 ± 2.8 and 17.7 ± 3.6 min. With 0.2 mg/kg physostigmine, they were 40.7 ± 3.4 and 42.8 ± 5.7 min, respectively.

A one-way analysis of variance (ANOVA) was performed to compare 4 data groups (A: data before lesioning in Fig. 2; B: data before lesioning in Fig. 4; C: data after lesioning in Fig. 2; D: data after unsuccessful lesioning in Fig. 4) using all the available raw suppression time scores at each of the doses shown in Figs. 2 and 4. The ANOVA revealed statistically significant differences between the groups at each dose, $F(3,51) = 3.3970$, $p<0.05$ for 0.05 mg/kg; $F(3,103) = 8.1495$, $p<0.01$ for 0.1 mg/kg; $F(3,73) = 10.5850$, $p<0.01$ for 0.2 mg/kg.

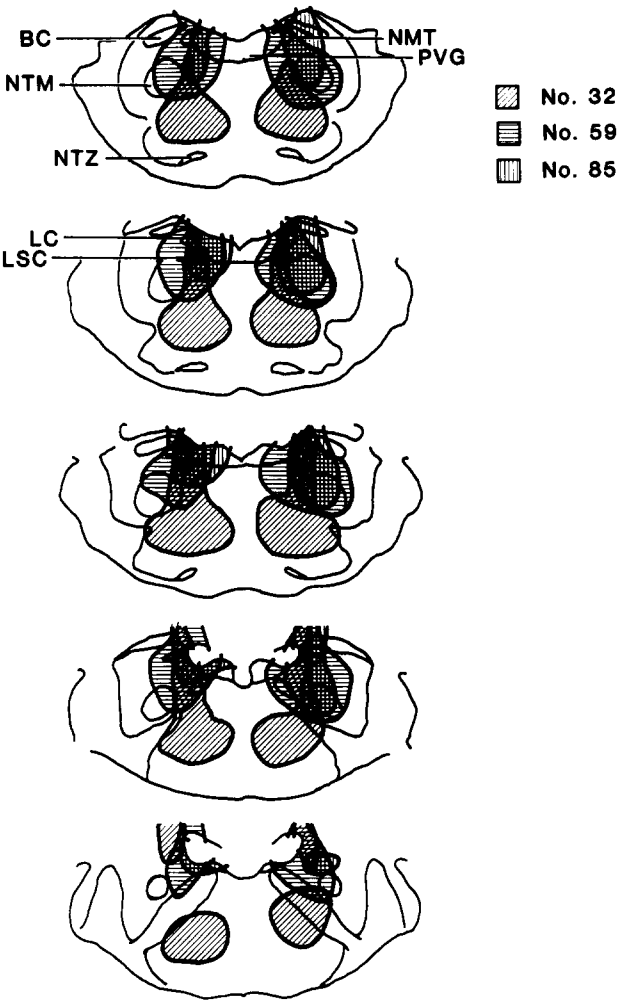

FIG. 3. The locations of pontine lesions in 3 rats showing marked attenuation of physostigmine effects on intracranial self-stimulation are illustrated. The subcoeruleus (filled regions) area and a portion of pontine tegmentum with AChE positive cells were lesioned in all 3 rats. Abbreviations: BC, brachium conjunctivum; LC, locus coeruleus; LSC, subcoeruleus area; NMT, nucleus tractus mesencephalici nervi trigemini; NTM, nucleus motorius nervi trigemini; NTZ, nucleus corporis trapezoidei; PVG, periventricular gray substance.

Subsequently, Duncan's multiple range test was performed in order to test the significance of differences between two arbitrarily selected groups from the 4 data groups at each dose. This test showed significant differences in all combinations between C (data taken from complete lesions) and the other three groups (A, B and D) at all doses except 0.05 mg/kg. The difference between C and D at 0.05 mg/kg was not significant [C and A ($p<0.05$), C and B ($p<0.05$) at 0.05 mg/kg; C and A ($p<0.01$), C and B ($p<0.01$), C and D ($p<0.05$) at 0.1 mg/kg; C and A ($p<0.01$), C and B ($p<0.01$), C and D ($p<0.01$) at 0.2 mg/kg]. There were no significant differences in the other combinations. In other words, the suppression time of ICSS after physostigmine injection decreased significantly only in the group shown in Fig. 2 with large pontine lesions involving the subcoeruleus area and dorsal tegmental field observed bilaterally.

In Fig. 5, the sizes of the lesions in 2 rats whose data appear in Fig. 4 are demonstrated as examples. In the diagram, it is clear

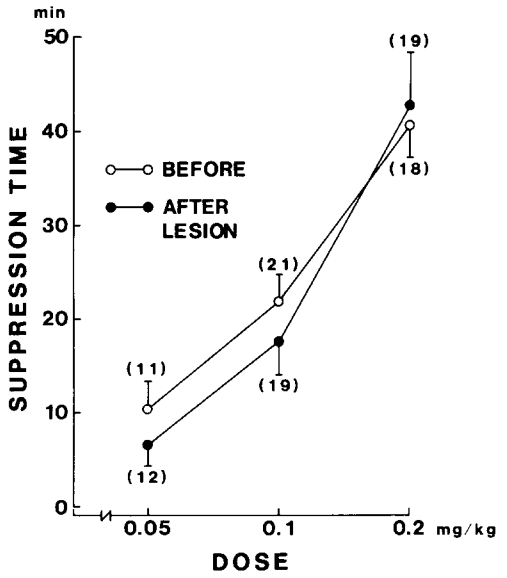

FIG. 4. Summarized data taken from 10 rats not showing attenuation of physostigmine suppression after pontine lesioning.

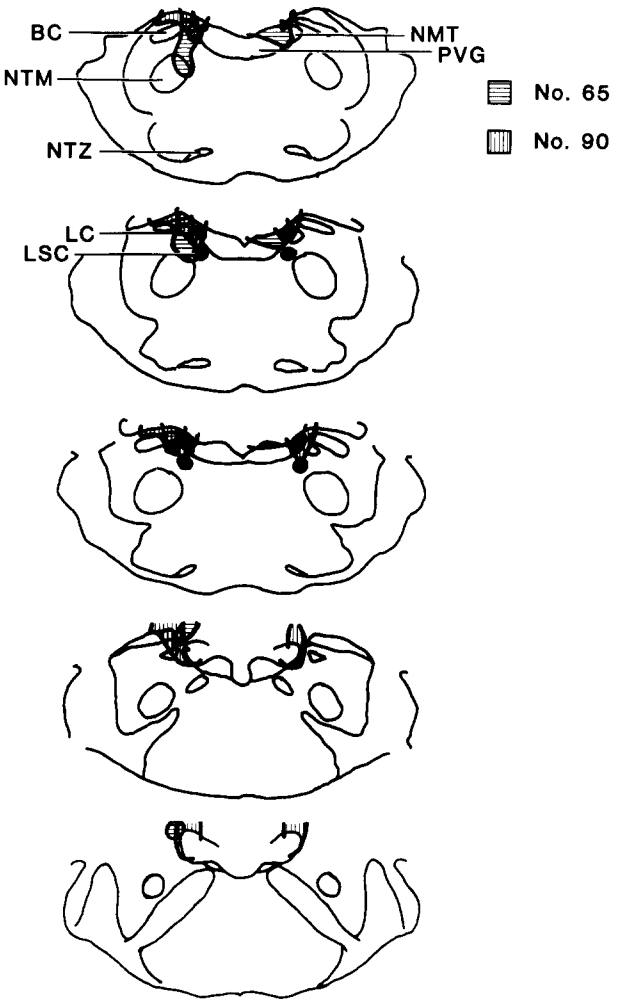

FIG. 5. Pontine lesions in 2 rats which did not show attenuation of the physostigmine effect. Note that the AChE positive subcoeruleus area and adjacent pontine tegmental field are almost intact. Abbreviations: same as in Fig. 3.

that although the locus coeruleus may be damaged after electrolytic lesioning, the bilateral subcoeruleus areas and dorsal tegmental field ventral to them are totally free of lesions. All the 8 other rats showed a more or less similar pattern of lesioning excluding the subcoeruleus area and adjacent tegmental field.

DISCUSSION

It has been long known that physostigmine administration induces behavioral quietness in spite of marked ECoG desynchronization suggesting alertness (3). The inhibition of neuronal activity in the locus coeruleus, subcoeruleus area with the simultaneous increase in neuronal activity in the giganto-cellular tegmental field in the pons during muscle atonia is induced by physostigmine (11,20). It is also known that microinjection of cholinergic agonists into the brain stem including the pontine dorsal tegmental area produces a suppression of motor activity with muscle atonia (10, 12, 23, 27). According to Kimura *et al.* (13), there are many cholinergic and presumably cholinoceptive neurons in the locus coeruleus and subcoeruleus area of the cat. There are also scattered but significant cholinergic and cholinoceptive neurons in the giganto-cellular tegmental field in the pons of the cat. Acetylcholinesterase-containing neurons are also found in these areas of the rat (18). Sakai *et al.* (21) suggested that muscle atonia during paradoxical sleep is due to the activity of the peri-LC (locus coeruleus)-alpha area. The activation of this area affects medullary inhibitory reticular formation which induces inhibition of spinal motoneurons leading to muscle atonia. Apparently, the area including the subcoeruleus and adjacent dorsal pontine tegmental area is sensitive to cholinergic agonists.

Our findings reveal a dose-related enhancement of the suppressive effect of physostigmine on intracranial self-stimulation and the attenuation of this effect after the formation of dorsal pontine tegmental lesions. The results clearly suggest that such a suppressive effect is due to the cholinergic activation of the subcoeruleus area and the pontine dorsal tegmental area, which in turn elicits motor suppression, presumably through the medullary inhibitory center. Induction of neck muscle atonia by physostigmine injection in the cat after midbrain transection (16) also supports our notion. This phenomenon is similar to that observed during paradoxical sleep. In the group of rats that did not show any attenuation of suppression after pontine lesioning, there are many presumably cholinoceptive cells remaining in the dorsal tegmentum, so physostigmine is able to activate these cells and cause motor suppression.

The dose of neostigmine equimolar to 0.2 mg/kg physostigmine is less than 0.1 mg/kg (0.093 mg/kg). As shown in Fig. 2, 0.1 mg/kg neostigmine injected into intact animals induces a weak suppressive effect. This may be due to some peripheral effects, because neostigmine does not penetrate the blood-brain barrier. Also, muscle twitches were sometimes observed with 0.1 mg/kg neostigmine. Likewise, a major part of the suppressive effect of 0.2 mg/kg physostigmine (19.5 ± 2.7 min) after pontine lesioning may arise from similar peripheral effects. Presumably the difference between the intact animals (45.9 ± 2.7 min) and the pontine-lesioned rats (19.5 ± 2.7 min) with 0.2 mg/kg physostigmine injection represents a real central effect.

The present findings suggest that cholinergic suppression of intracranial self-stimulation by physostigmine at up to 0.1 mg/kg is not due to the direct interference of physostigmine on forebrain processes involving motivation or reinforcement. Rather, it is due

to a direct effect on the motor system through the cholinoceptive pontine subcoeruleus and tegmental field region that results in inhibition of the self-stimulation behavior primarily due to inhibition of spinal motoneurons.

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