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Behavioral Correlates of a Progressive Dysfunctioning of the Deeper Layers of the Colliculus Superior: Effects of Picrotoxin

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JASPERS, R. M. A. AND A. R. COOLS. *Behavioral correlates of a progressive dysfunctioning of the deeper layers of the colliculus superior: Effects of picrotoxin.* PHARMACOL BIOCHEM BEHAV **37**(1) 1–9, 1990.—Intracaudate injections of relatively high doses of apomorphine produce a regression in motor behavior of cats collecting food pellets in a treadmill design (25). It has been hypothetized that this regression is partly due to functional disturbances in brain regions receiving (in)directly striatal output signals. In view of this hypothesis it was investigated whether experimentally induced changes in GABAergic activity within the deeper layers of the colliculus superior, which is a second order output station of the caudate nucleus, are also able to elicit a regression in motor behavior. Therefore, motor behavior of cats was tested in the treadmill paradigm before and after intracollicular injections of the GABA antagonist picrotoxin. Picrotoxin produced dose-dependently a regression in motor behavior which was comparable to that elicited by intrastriatally injected apomorphine. The noted effects were GABA-specific since muscimol attenuated the picrotoxin-induced regression. The present data are discussed in view of a model for a hierarchical organization of the brain.

Colliculus superior, deeper layers Regression Motor behavior Cats

CATS repeatedly execute sequences of distinct motor patterns in order to collect food pellets during walking on a treadmill (23). Such sequences consist of the following motor patterns: "normal walking," whereby the cat follows the speed of the belt of the treadmill (1); "gait accelerations," i.e., increases in walking speed in order to approach the front panel of the treadmill enclosure behind which a food dispenser is mounted (2); "gait transitions," i.e., changes in interlimb coordination by decreasing the steplength of the forelimbs and increasing that of the hindlimbs (3); and, finally, "food collecting attempts," whereby the cat bends its head through the opening in the opaque front panel in order to collect a food pellet (4). Recently, it has been shown that caudate nucleus injections of the dopaminergic antagonist haloperidol only reduce the number of one particular subclass of gait transitions, i.e., so-called 'nonexteroceptively directed gait transitions'; the number of the remaining motor patterns in the sequence is not reduced. Haloperidol-treated cats are still able to collect food pellets (23). Intracaudate injections of the dopaminergic agonist apomorphine have been found to prevent the haloperidol-induced reduction. Apparently, nonexteroceptively directed gait transitions, but not other motor patterns, are selectively mediated by caudate dopamine receptors. In contrast to the rather low dose of apomorphine, viz. 0.6 μg, that is able to counteract the haloperidol-induced reduction, relatively high doses of apomorphine, viz. 5–10 μg, disrupt not only nonexteroceptively directed gait transitions, but also other motor patterns (25). In fact, the motor behavior on the treadmill is broken down in a very particular way: the order in which the distinct motor patterns disappear in subsequent sequences is opposite to that in which they typically appear in intact sequences (25). Thus, apomorphine affects the distinct components of the sequence according to the rule 'last in, first out.' Since the apomorphine- (5–10 μg) induced 'regression' in motor behavior as described above is inhibited by intracaudate injections of haloperidol (25), despite the fact that at least some of the involved motor patterns are not caudate-specific (see above), it has been suggested that this regression is due to the distortion of information sent by the caudate nucleus to striatal output stations (25). Accordingly, we investigated whether functional changes at the level of an output station of the caudate nucleus also produce such a regression in the motor pattern sequence.

One of the major output stations of the caudate nucleus is the substantia nigra, pars reticulata (17,34). The substantia nigra pars reticulata gives rise to projections towards distinct thalamic nuclei,

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the mesencephalic reticular formation and the deeper layers of the colliculus superior (2, 3, 11, 16, 20, 21, 31, 38). The colliculus superior is divided into deeper and superficial layers according to the terminology of Kanaseki and Sprague (27). The deeper layers of the colliculus superior serve as an important output station funneling striatally derived signals as shown by studies on tonic EMG activity (12), stereotyped movements (10,22) and turning behavior (22, 29, 32, 33) [cf. (7,8)].

Electrophysiological (4,28), biochemical (1,37) and behavioral (12,13) studies have shown that one of the neurotransmitters of the caudato-nigral and the nigro-collicular fibers is the inhibitory amino acid gamma-aminobutyric acid [GABA; for review see (35)]. Activation of striatal dopamine receptors has been reported to decrease the release of GABA in the deeper layers of the colliculus superior (13,35).

In the present investigation, the GABAergic antagonist picrotoxin was injected into the deeper layers of the colliculus superior of cats tested in the treadmill design (25). Picrotoxin was chosen in view of the fact that this agent is known to elicit dose-dependent and GABA-specific effects in a dose range from 50 to 200 ng/0.5 μl (24). Moreover, these doses of picrotoxin produce locus-specific effects since they are neither effective when injected into the superficial collicular layers nor effective when injected into lesioned deeper layers of the colliculus superior (24). Given these considerations, the collicular injection of 25–50 ng picrotoxin would appear to be a valid tool to mimic the biochemical consequences of caudate injections of apomorphine at the level of the deeper layers of the colliculus superior (13,35). In the present study, the GABA specificity of behavioral effects induced by 50 ng picrotoxin was studied with the help of the GABAergic agonist muscimol.

METHOD

Animals and Apparatus

Adult male cats (3.0–4.5 kg) were selected from a breeding colony of the Catholic University of Nijmegen. They were housed in iron cages (1.90 × 1.20 × 1.60 m) in groups of 4 to 7 animals. Except during training and experiments (see below) food (Hope Farms) and water were present ad lib. The apparatus has been previously described (23,25). Therefore, the reader is referred to (23) for an extensive description of training procedures and apparatus. In short, cats were trained to walk on the motor-driven belt (speed 1.0–1.25 km/hr) of a roofed treadmill (120 × 20 × 65 cm). A remote-controlled food dispenser was attached at the outer side of the opaque front panel. The cat was able to collect food pellets (specially shaped, Hope Farms) by bending its head through an opening (10 × 12 cm) in the front panel. The food dispenser was constructed in such a manner that the cat, while walking, was unable to detect the delivery of a food pellet.

Surgical and Histological Procedures

After the training phase, cats were stereotaxically equipped with stainless steel cannulas under sodium pentobarbitone anaesthesia (40–45 mg/kg, IP) according to previously reported procedures (5,24). In order to avoid damage to the tectal tissue, the tip of the guide cannula (outer diameter: 0.8 mm) was directed at a point 4 mm above the injection locus: coordinates A 1.5, L 3.5, H 6.5 (36). Two weeks after the implantation, the treadmill experiments were started. After the final experiment, the cats were deeply anaesthetized with sodium pentobarbitone and intracardially perfused with a 4% formaldehyde solution. Subsequently, the brains were removed and cross sections (30 μm) were cut with help of a cryostat microtome (−20°C). The slices were mounted on slides and stained with cresyl violet to estimate the precise localization of the injection spots.

Experimental Paradigm

The animals were deprived of food for a period of 24 hr before the start of an experiment. At the beginning of each experiment (t = 0 min), the cat was placed on the static belt of the treadmill. An experiment consisted of three test periods which started at t = 15, 35 and 55 min. Each test period lasted 5 min and was recorded on video-tape. At the beginning of a test period the belt was activated; at the end of the test the belt was turned off. All solutions were bilaterally injected with help of a Hamilton syringe (diameter of the needle with sharpened tip: 0.4 mm) as has been described before (24). Each cat participated in three experiments that were spaced by at least one week. Seven cats received 25 and 50 ng picrotoxin (Serva; dissolved in 0.5 μl distilled water) at t = 25 min during the first and the second experiment, respectively; these cats received distilled water (0.5 μl; control for the picrotoxin injections) at t = 25 min during the third experiment. Seven other cats received 0.5 μl distilled water at t = 25 min (control for the picrotoxin injection) and 1.0 μl distilled water at t = 30 min (control for the muscimol injection; see below) during the first experiment; 50 ng picrotoxin at t = 25 min and 25 ng of the GABA agonist muscimol (Serva; dissolved in 1.0 μl distilled water) at t = 30 min during the second experiment; and 0.5 μl distilled water at t = 25 min and 25 ng muscimol at t = 30 min during the third experiment that served as a control for the second experiment. The first test period (PRE) served as a control (see below). Drug-induced changes in motor behavior were analyzed during the second and the third test period (POST1 and POST2, respectively). Doses and volume of picrotoxin and muscimol, the time-schedule of the collicular injections as well as the locus-specificity of the injections were based on the outcome of a previous study in which open field behavior was studied (24).

Analysis of Motor Behavior

The behavior on the treadmill was analyzed in the same way as has been reported before. Untreated cats repeatedly execute complete sequences of the following motor patterns (25).

1. Walking in the middle of the treadmill, whereby the cat follows the speed of the belt (walking normally: WLKnm).
2. Accelerating gait in order to approach the front panel (ACC).
3. Changing the interlimb coordination by decreasing the forelimb steplength and increasing the hindlimb steplength, i.e., executing gait transitions (TRN).
4. Attempting to collect a food pellet by bending the head through the opening in the front panel of the treadmill enclosure, i.e., food collecting attempts (FCA).

Although the motor patterns were executed in a particular order (see above), each pattern was executed in a relatively independent way. The independent nature of the motor patterns was demonstrated by the observation that sometimes the cats executed a particular pattern several times before they switched to the next pattern in the sequence [see also (25)]. Walking patterns as described above were labeled as 'normal' since they were also present during PRE test periods (WLKnm). During 'abnormal' walking, that hardly occurred during PRE test periods, the cat continuously touched the back panel of the treadmill enclosure with its tail and/or hindlimbs (WLKab). A motor pattern sequence was considered to be 'complete' if it ended with FCA; it was considered to be 'incomplete' if FCA and one or more other motor patterns were absent.

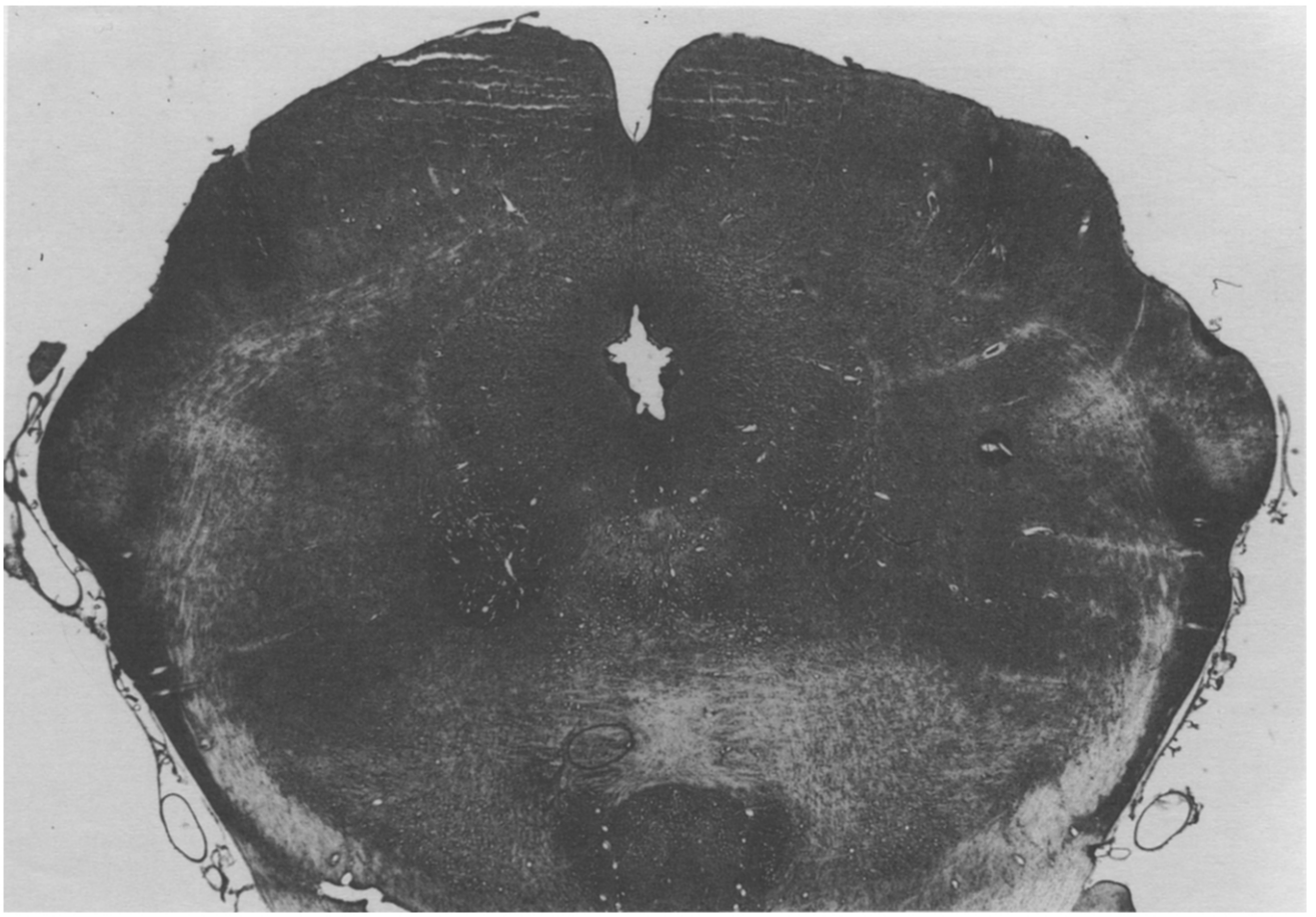

FIG. 1. A picture of a representative example of injection sites into the deeper layers of the colliculus superior.

As described previously (23,25), cats could display two types of ACC: those accompanied by the continuous visual fixation of some part of the front panel, i.e., 'exteroceptively directed gait accelerations' (ACCed), and those not accompanied by continuous fixation, i.e., 'nonexteroceptively directed gait accelerations' (ACCnd). Since the latter patterns were rarely executed during any test period, only ACCed's were analyzed. Furthermore, cats displayed two types of TRN: those accompanied by continuous tactile (with forelimbs and/or whiskers) and/or visual fixation of the belt and front panel of the treadmill, i.e., 'exteroceptively directed gait transitions' (TRNed), and those not accompanied by continuous fixation, i.e., 'nonexteroceptively directed gait transitions' (TRNnd).

As described in the Introduction section, striatal injections of 5–10 μg apomorphine have been found to produce a behavioral regression which is illustrated by the occurrence of time-dependent changes in the number of distinct motor patterns. Given the known order in which the distinct components of the sequence appear (see above), the above-mentioned parameters were used to provide direct information about drug-induced changes in the sequence under study. In addition, a frequency analysis of distinct motor patterns was used to study drug-induced changes in the ability to execute specific patterns. Therefore, the absolute number of each motor pattern was determined per test period. WLK was scored in 3-sec bins in order to allow a frequency analysis as has been described before (25). Drug effects were expressed as changes in the percentage of each motor pattern of total of patterns during the POST1 test period. The ratio of the POST-score minus the PRE-score (numerator) and the POST-score plus the PRE-score (nominator) was calculated in order to reduce the inter- and intraindividual variability (25). Ratios were compared using the Wilcoxon matched pairs signed ranks test (two-tailed) or the Mann-Whitney U-test (two-tailed).

RESULTS

Histology

Verification of the injection loci revealed that all injections were correctly placed in the deeper layers of the colliculus superior (24): coordinates A 1.0–1.5, L 3.5–4.0, H 2.0–3.0 (36). A representative example of the injection sites is shown in Fig. 1.

Motor Behavior During the PRE Test Period

The absolute number of the distinct motor patterns (median + range) during PRE test periods of all experiments is shown in Table 1. There were no significant differences between any of the experiments with respect to the number of distinct patterns during PRE test periods ($p>0.05$).

Motor Behavior During the POST1 Test Period

Injection of the solvent did not produce abnormal walking behavior in any of the cats tested. A representative example of the

TABLE 1

MEDIAN + RANGE OF ABSOLUTE NUMBER OF DISTINCT MOTOR PATTERNS IN PRE TEST PERIODS (BEFORE ADMINISTRATION OF DISTILLED WATER; SOLV), PICROTOXIN 25 AND 50 ng/0.5 μl (PT25 AND PT50, RESPECTIVELY), AND MUSCIMOL 25 ng/1.0 μl (MC25)

	PRE					
Drug (ng)	WLKab	WLKnm	ACCed	TRNed	TRNnd	FCA
SOLV*	0 (0–3)	6 (2–47)	16 (5–35)	1 (0–32)	10 (1–27)	24 (15–46)
PT25	0 (0–1)	6 (0–16)	27 (12–35)	1 (1–39)	8 (0–34)	22 (16–55)
PT50	0 (0–0)	15 (4–41)	18 (12–44)	3 (0–18)	11 (0–41)	22 (11–34)
PT50 + MC25	0 (0–2)	0 (0–5)	35 (22–48)	3 (0–13)	22 (0–56)	45 (32–67)
MC25	0 (0–0)	0 (0–5)	26 (13–59)	2 (0–15)	18 (0–54)	43 (39–72)
SOLV†	0 (0–1)	1 (0–4)	31 (16–45)	2 (0–11)	21 (0–26)	40 (22–50)

*0.5 μl injected bilaterally 5 min after the end of the PRE test period.

†0.5 μl injected bilaterally 5 min after the end of the PRE test period, and 1.0 μl injected bilaterally 5 min after the first injections.

WLKab, abnormal walking; WLKnm, normal walking; ACCed, exteroceptively directed gait accelerations; TRNed, exteroceptively directed gait transitions; TRNnd, nonexteroceptively directed gait transitions; FCA, food collecting attempts (see the Method section). Seven cats participated in three experiments: PT25, PT50 and SOLV*, respectively. Seven other cats participated in the remaining experiments: SOLV†, PT50+MC25 and MC25, respectively.

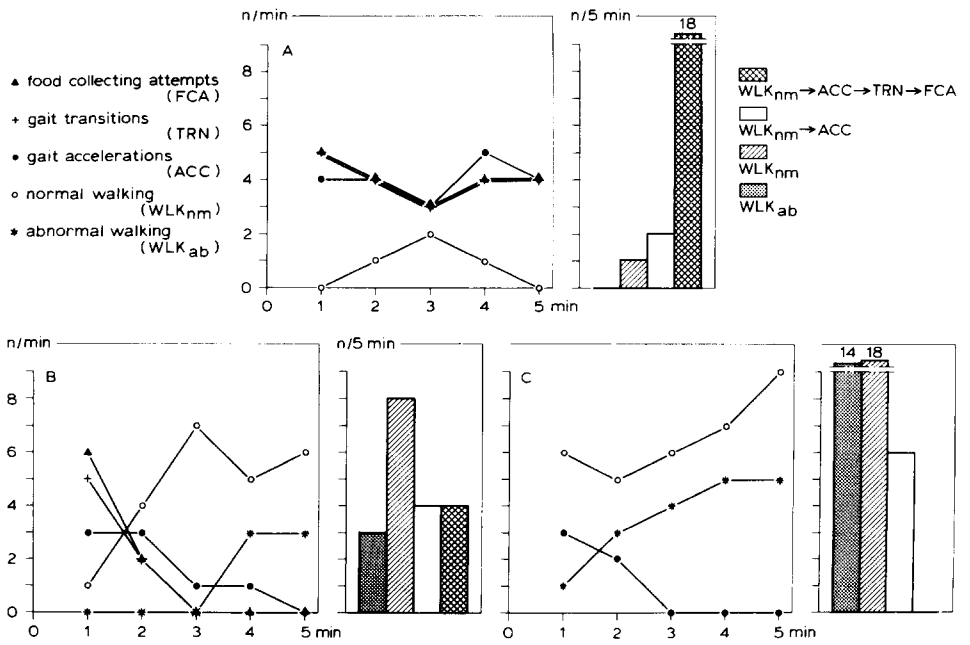

FIG. 2. Representative examples of individual scores per minute of different motor patterns during the POST1 test period, i.e., 10–15 min following the application of 0.5 μl solvent (A) or 50 ng/0.5 μl picrotoxin (B, C; left panels) as well as of absolute number of complete and incomplete sequences during POST1 (A–C; right panels).

TABLE 2

MEDIAN MOTOR PATTERN RATIO OF DIFFERENCE (NUMERATOR) AND SUM (NOMINATOR: POST1 − PRE/POST1+PRE) OF PERCENTAGES OF DISTINCT MOTOR PATTERNS DURING THE POST1 TEST PERIOD AFTER COLLICULAR INJECTIONS OF DISTILLED WATER (0.5 μl; SOLV[1]), PICROTOXIN 25 AND 50 ng/0.5 μl (PT25 AND PT50, RESPECTIVELY), PICROTOXIN 50 ng/0.5 μl 5 MIN BEFORE INJECTION OF MUSCIMOL 25 ng/1.0 μl (PT50 + MC25), 0.5 μl DISTILLED WATER 5 MIN BEFORE 1.0 μl DISTILLED WATER (SOLV[2]) AND 0.5 μl DISTILLED WATER 5 MIN BEFORE MUSCIMOL 25 ng/1.0 μl (MC25)

Drug	WLKab	WLKnm	ACCed	TRNed	TRNnd	FCA
SOLV[1]	0	−0.19	0.15	0	−0.10	−0.04
PT25	1.0*	0.16	−0.06	0	−0.19	−0.19†
PT50	1.0‡	0.50†	−0.61*	−1.0*	−0.74†	−1.0*
PT50 + MC25	0#	0¶	−0.05	0.14§	0#	0§
MC25	0	0	0.03	0	−0.11	−0.02
SOLV[2]	0	0	−0.02	0	0	0.02

*$p<0.05$, †$p<0.02$, ‡$p<0.002$: PT25, PT50, MC25 and SOLV[2] vs. SOLV[1].
§$p<0.05$, ¶$p<0.02$, #$p<0.002$: PT50 + MC25 vs. PT50.
Note that zero means no change, a positive value represents an increase and a negative value represents a decrease in the relative frequency of a pattern during POST1, compared to PRE. Each group consisted of 7 casts. The remaining abbreviations are explained in the legend of Table 1.

absolute number of each motor pattern per minute during POST1 is shown in Fig. 2A (left panel). The absolute number of complete and incomplete sequences is depicted in the same figure (2A, right panel). The display of distinct motor patterns and the execution of sequences were comparable to those seen during PRE test periods in all experiments.

In contrast to the solvent, 25 ng picrotoxin significantly affected the execution of FCA and WLKab during POST1. The ratio of FCA was significantly decreased, whereas that of WLKab was significantly increased (Table 2). Although the ratio of the remaining motor patterns, i.e., WLKnm, ACCed, TRNed and TRNnd, was not significantly changed, normal eating behavior was clearly affected. In 4 out of 7 cats, FCA's were strongly reduced, but not totally inhibited. In one other cat, this motor pattern was no longer present during POST1: this animal executed incomplete sequences that ended with TRN during the whole POST1 test period. The remaining 2 cats displayed a regression of complete sequences which was comparable to that found after 50 ng picrotoxin (see below). WLKab was increased in 4 out of 7 cats; however, only one of these animals displayed a regression in motor behavior. Figure 3 shows the relative frequency of distinct motor patterns.

A higher dose of picrotoxin, i.e., 50 ng, induced in 4 out of 7 cats a regression in the motor pattern sequence in the following way: initially, these animals executed complete sequences during POST1, but soon after the start of the test period more and more incomplete sequences appeared. The order in which motor patterns disappeared was relatively fixed; at first, FCA, TRNnd and TRNed were no longer executed while the cats still displayed incomplete sequences that ended with ACCed. During the next phase, also the latter pattern disappeared, while the frequency of WLKnm was increased. During the final part of POST1, sequences consisted of WLKnm and/or WLKab. Two other cats did not execute complete sequences at all during POST1: they started with incomplete sequences that ended with ACCed; during POST1, ACCed disappeared while the animal executed WLKnm (one cat) or WLKab (one cat) during the remaining part of POST1. The 7th animal just executed a reduced number of complete sequences throughout POST1 (5, POST1 vs. 11, PRE). In addition, this cat also displayed WLKab. Representative examples of the absolute

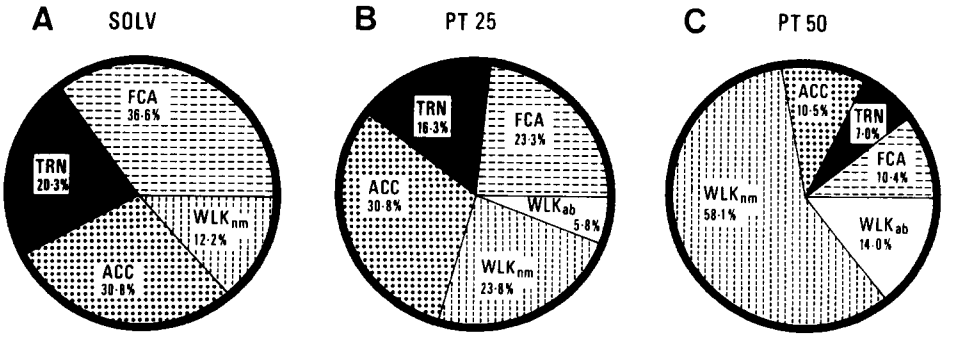

FIG. 3. Percentage of the mean frequency of the distinct motor patterns shown during POST1 test periods, i.e., 10–15 min after the injection of solvent 0.5 μl (SOLV; A), picrotoxin 25 ng/0.5 μl (PT25; B) and 50 ng/0.5 μl (PT50; C); turning clockwise, the distinct motor patterns are ordered according to their appearance in the sequence.

TABLE 3

MEDIAN MOTOR PATTERN RATIO OF DIFFERENCE (NUMERATOR) AND SUM (NOMINATOR: POST2 − PRE/POST2 + PRE) OF PERCENTAGES OF DISTINCT MOTOR PATTERNS DURING THE POST2 TEST PERIOD AFTER COLLICULAR INJECTIONS OF DISTILLED WATER (0.5 μl; SOLV[1]), PICROTOXIN 25 AND 50 ng/0.5 μl (PT25 AND PT50, RESPECTIVELY), PICROTOXIN 50 ng/0.5 μl 5 MIN BEFORE INJECTION OF MUSCIMOL 25 ng/1.0 μl (PT50 + MC25), 0.5 μl DISTILLED WATER 5 MIN BEFORE 1.0 μl DISTILLED WATER (SOLV[2]) AND 0.5 μl DISTILLED WATER 5 MIN BEFORE MUSCIMOL 25 ng/1.0 μl (MC25)

Drug	WLKab	WLKnm	ACCed	TRNed	TRNnd	FCA
SOLV[1]	0	0.35	0.06	0	−0.11	−0.02
PT25	0	0.18	−0.03	0	−0.16	−0.45†
PT50	1.0†	0.54	−0.08	−1.0†	−0.42*	−0.27*
PT50 + MC25	0¶	0	−0.04	0‡	0.12‡	0.03§
MC25	0	0	−0.08	0	0	0.04
SOLV[2]	0	0	−0.04	0	0	−0.03

*$p<0.05$, †$p<0.02$: PT25, PT50, MC25 and SOLV[2] vs. SOLV[1].

‡$p<0.05$, §$p<0.02$, ¶$p<0.002$: PT50 + MC25 vs. PT50.

Note that zero means no change, a positive value represents an increase and a negative value represents a decrease in the relative frequency of a pattern during POST2, compared to PRE. Each group consisted of 7 cats. The remaining abbreviations are explained in the legend of Table 1.

number per minute of distinct motor patterns during POST1 are shown in Fig. 2B and C (left panel). In the same illustration, the absolute number of complete and incomplete sequences are shown (Fig. 2B and C, right panel).

Figure 3 shows the percentages of the mean frequencies of the distinct motor patterns. This illustration reveals that picrotoxin produced a shift from a predominance of FCA, TRN, ACC and WLKnm in controls to a predominance of WLKnm in cats treated with 50 nm picrotoxin. The highest dose of picrotoxin significantly reduced the ratio of ACCed, TRNed, TRNnd and FCA, whereas it significantly increased that of WLKnm and WLKab (Table 2).

Motor Behavior During the POST2 Test Period

During POST2, picrotoxin was effective too, since some cats still showed a regression in the motor pattern sequence after 25 and 50 ng (3 and 4 animals, respectively). The relative distribution of the distinct motor patterns during POST2 was comparable to that during POST1 (data not shown). In POST2, the ratio of FCA was significantly changed after 25 ng picrotoxin, whereas that of WLKab, TRNed, TRNnd and FCA was significantly affected following 50 ng picrotoxin (Table 3).

Additional Observations

Apart from the above-mentioned regression effects, 5 out of 7 cats displayed abnormal limb movements following 50 ng picrotoxin. This effect was most pronounced during the display of ACCed and WLKnm; it was characterized by an increase in the swing-phase of one hindlimb, as the result of an increased lifting and a short interruption of the movement just before touch-down. Sometimes, this effect was also present in the forelimb ipsilateral to the affected hindlimb. During POST2, no abnormal limb movements were observed. As a final remark, neither incorrect FCA's, i.e., head movements directed towards a food pellet without collecting a pellet nor 'forced' head movements were observed in any postinjection test period.

GABA-Specificity of Picrotoxin-Induced Effects

The regression in motor pattern sequences during POST1 and POST2 did not occur in case 25 ng muscimol was injected after the application of 50 ng picrotoxin. The effect of picrotoxin on the ratio of WLKab, WLKnm, TRNed, TRNnd and FCA was significantly attenuated by muscimol (Tables 2 and 3). In contrast, the effect of picrotoxin on ACCed was not counteracted by muscimol. Finally, 25 ng muscimol itself did not induce any change in the execution of motor patterns during POST1 or POST2.

DISCUSSION

General

The present study shows that collicular injections of 50 ng picrotoxin produced a characteristic regression in the motor pattern sequence of cats. The order in which the distinct motor patterns subsequently disappeared was opposite to that in which they typically appeared in intact sequences. Given the known GABA- and locus-specificity of the treatment used (24), this finding shows that the picrotoxin-induced effects were due to an inhibition of GABAergic activity within the deeper layers of the colliculus superior. Apart from the decrease in ACCed, all effects were counteracted by muscimol. The finding that intracollicular injections of 25 ng muscimol alone did not alter the behavior in the present design not only excludes the possibility that the muscimol-induced attenuation of the picrotoxin-induced effects was due to a functional rather than a pharmacological antagonism, but also indicates that this dose of muscimol was too low in order to significantly stimulate the GABAergic receptors in otherwise untreated cats. Whether this implies that the baseline activity at the level of the GABAergic receptors was rather high during the performance of the treadmill behavior remains to be established.

The finding that the picrotoxin-induced reduction in the number of ACCed was not counteracted by muscimol suggests that this effect was only triggered, but not mediated, by the picrotoxin-induced attenuation of the GABAergic activity in the deeper layers of the colliculus superior. In previous experiments in which haloperidol has been injected into the caudate nucleus, a similar phenomenon has been observed: haloperidol produces both a dopamine-specific effect, viz. a decrease in TRNnd, and a dopamine-triggered change, viz. an increase in TRNed (23). In a follow-up study, it has been found that caudate injections of high

doses of apomorphine similarly affect TRNed, providing additional evidence that the drug-induced change in TRNed is at best triggered by, but not specific for, experimentally induced alterations in striatal dopaminergic activity (25). A comparable phenomenon might underlie the picrotoxin-induced decrease in the number of ACCed.

Anyhow, the overall response to picrotoxin was comparable to that elicited by caudate injections of 10 μg apomorphine: like apomorphine, picrotoxin induced a regression in motor behavior resulting in significant reductions in the number of FCA, TRNnd, TRNed and ACCed, and a significant increase in the number of WLKab (25). The picrotoxin-induced increase in the number of WLKnm, however, was not found in the apomorphine experiments (25). The latter effect might be due to the difference in the ratios of WLKnm in the corresponding control experiments [ratio, picrotoxin experiments: −0.19; ratio, apomorphine experiments (25): +0.50], suggesting that there was a so-called ceiling effect in the apomorphine experiments. In this context, it is useful to remark that the noted differences between the controls of the two series of experiments might be due to the fact that the apomorphine-treated cats were equipped with caudate cannulas in contrast to the picrotoxin-treated animals in the present study which were equipped with collicular cannulas. Anyhow, the above-mentioned data show that the response to 50 ng picrotoxin applied to the deeper layers of the colliculus superior is highly comparable to the response to 10 μg apomorphine injected into the caudate nucleus. This finding, together with the notion that intracollicular injections of picrotoxin are able to mimic biochemical consequences of striatally applied apomorphine injected into the caudate nucleus. This finding, together with the notion that intracollicular injections of picrotoxin are able to mimic biochemical consequences of striatally applied apomorphine at the level of the deeper layers of the colliculus superior (see Introduction), suggests that collicular disturbances were also involved in the regression process following intracaudate administration of apomorphine.

Specific Effects of Picrotoxin

Twenty-five ng picrotoxin, injected into the deeper layers of the colliculus superior, resulted in significant changes in the number of WLKab and FCA: the former increased, whereas the latter decreased.

Since only deprived cats are displaying FCA in the treadmill set-up (23), it is theoretically possible that picrotoxin just altered the internal state inherent in food deprivation and, as a result, attenuated the number of FCA. However, most cats did collect food pellets during the first and second postinjection test period, indicating that they were still hungry at that time. Moreover, the treated animals executed as many ACC and TRN as solvent-treated cats. Since these motor patterns enabled the cats to approach the food pellets, it appears that they were still motivated. Therefore, it is unlikely that the reduction in FCA was just due to drug-induced changes in food motivation. However, future research in which an independent measure for food motivation is used is required to verify this explanation.

Previously, collicular injections of picrotoxin (50–200 ng) have been reported to induce forced head movements in cats tested in an open field (24). In the present study, such movements were not seen in any of the tests. Apparently, these forced movements do not occur in case picrotoxin-treated cats are forced to perform particular tasks [cf. (14)]. Anyhow, the absence of these forced movements excludes the possibility that such movements could have hindered the cats to display FCA.

Recently, it has been found that the deeper layers of the colliculus superior are involved in the execution of so-called 'nonexternally guided targeting movements,' i.e., goal-directed movements that are elicited but not continuously guided by exteroceptive stimuli (7–9, 14). Since picrotoxin has been found to enhance the display of such movements (14), the picrotoxin-induced decrease in FCA found in the present study cannot be ascribed to drug-induced changes in this particular function of the deeper layers of the colliculus superior.

The fact that the behavioral regression observed in the present study was not seen in previously performed colliculus experiments at our laboratory is not difficult to understand. The experimental paradigms used in those studies were fully inappropriate to allow the cat to display any form of behavioral regression. In contrast, the paradigm used in the present study was purposely chosen to investigate this particular phenomenon. Anyhow, the available data underline the validity of our starting-point in this respect: the nature of the experimental set-up and, accordingly, the task determine whether a behavioral deficit becomes manifest or not.

The Caudato-Nigro-Collicular Feedforward Loop

Previously, it has been found that striatal injections of haloperidol significantly reduce the number of a particular motor pattern without affecting the ability to execute complete sequences during the treadmill task (23). In a subsequent study it has been found that intracaudate injections of 5.0 μg apomorphine produce a regression in motor behavior without changing the number of certain motor patterns such as normal walking during the treadmill task (25). The same holds true for the effects elicited by intracollicular injections of 25 ng picrotoxin (present study). All these data together indicate that the picrotoxin-induced regression in motor behavior was not simply the consequence of the order in which distinct motor patterns had to be executed during the treadmill task.

Some of the mentioned picrotoxin-induced effects, namely the increase in the number of WLKab and the decrease in the number of FCA, have previously been observed in treadmill experiments, in which the substantia nigra pars reticulata was manipulated (19). So, it has been found that decreasing the nigral GABAergic activity results in (a) abnormal body positions and postures in cats being free to move and (b) abnormal limb movements in cats being challenged to walk (6, 15, 19, 39). Moreover, a decrease in nigral GABAergic activity has been found to suppress FCA (19). As mentioned, collicular injections of picrotoxin too elicited such effects. Still, there is evidence that these nigral effects are not mediated by the colliculus superior (15). Since the picrotoxin treatment used in the present study was selective and specific for the deeper layers of the colliculus superior (24), it can also be excluded that the picrotoxin-induced changes were due to diffusion of picrotoxin to the substantia nigra. Thus, the effects elicited by collicular injections of 25 ng picrotoxin were neither due to distortion of the information sent by the substantia nigra to the colliculus superior nor due to leakage of collicularly injected picrotoxin to the substantia nigra. Nevertheless, the picrotoxin treatment of the colliculus superior produced effects which were similar to those elicited by a picrotoxin treatment of the substantia nigra. In fact, these data show that intracollicularly administered picrotoxin anyhow produced a functional 'shut-off' of the substantia nigra pars reticulata, viz. its first order input station. Whether or not this phenomenon was mediated by direct or indirect colliculo-nigral connections remains open for future research [cf. (18) and (30)].

The functional shut-off of the substantia nigra pars reticulata produced by 50 ng picrotoxin was even greater than that elicited by 25 ng: for 50 ng picrotoxin resulted not only in effects seen after 25 ng, viz. a decrease in the number of FCA and an increase in the

number of WLKab, but also in clearcut abnormal limb movements, viz. behavioral consequences of a stronger dysfunctioning substantia nigra pars reticulata (15,19). As mentioned in the Results section, 50 ng picrotoxin also reduced the number of TRNnd in a GABA-specific manner. Given the known function of the caudate nucleus in directing the display of the latter motor pattern (23), this finding shows that 50 ng picrotoxin even produced a functional shut-off of the caudate nucleus. Comparable phenomena have been observed in experiments in which the behavioral regression following intracaudate injections of kainic acid were studied (26). This treatment resulted acutely in the display of caudate-specific movements which disappear as soon as nigra-specific movements appear, whereas the latter, in turn, disappear as soon as colliculus-specific movements appear. The latter data together with the present data may reveal a basic principle of cerebral organization: particular changes in a hierarchically lower brain structure can produce a functional shut-off of hierarchically higher order brain structures.

Apart from the above-mentioned effects, 50 ng picrotoxin produced changes in TRNed, ACCed and WLKnm, viz. effects which were not yet elicited by 25 ng picrotoxin. The finding that picrotoxin inhibited the display of ACCed is in agreement with previously reported data showing that this drug reduces the display of externally guided targeting movements (14). Anyhow, as long as the brain structures mediating these effects are unknown it is not relevant to discuss these effects in more detail.

The present study shows that picrotoxin injected into the deeper layers of the colliculus superior produced a regression in motor behavior which was comparable to that elicited by striatal injections of 10 μg apomorphine. Since collicular injections of picrotoxin or caudate injections of apomorphine both produce similar GABAergic changes at the level of the deeper layers of the colliculus superior (13,35), it is likely that a common mechanism at the level of the colliculus is involved. Further studies examining the ability of collicular injections of muscimol to block the regression in motor behavior elicited by intracaudate injections of apomorphine would clarify this point. Anyhow, the present data are in line with the hypothesis that experimentally induced changes at the level of the caudate nucleus can produce behavioral deficits inherent in dysfunctioning output stations. It is evident that this notion has far-reaching consequences for the interpretation of the consequences of the progressive pathology of psychomotor diseases such as Parkinson's disease. For instance, it would imply that classical parkinsonian symptoms such as hypokinesia, rigidity, etc., become only manifest when the caudate pathology has resulted in a malfunctioning of one or more striatal output stations.

Furthermore, the present data suggest that collicular injections of a low dose of picrotoxin (25 ng) produced a functional shut-off of the substantia nigra pars reticulata, and that the injections of a higher dose of picrotoxin (50 ng) produced, in addition, a functional shut-off of the caudate nucleus. Overall, the latter data might reveal a basic principle of the cerebral organization: changes in a hierarchically lower order brain structure are able to produce a functional shut-off of hierarchically higher order brain structures. The mechanisms underlying this principle remain to be investigated.

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