# Effect of Substance P on Neurological Status and Behavioral Responses Altered as a Result of Prolonged Brain Ischemia in Rats with Different Types of Behavior

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Substance P administered 30 min after the onset of cerebral ischemia improved the neurological status and prevented postischemic hyperactivity in rats with a passive type of behavior; had no effect on the neurological status of rats with an intermediate type of behavior but reduced their postischemic hyperactivity; aggravated the neurological status of rats with an active type of behavior without exerting a significant effect on their behavioral responses; and averted a rise in the level of depression in rats of all three groups. The results of this study indicate that there is a relationship between the type of behavior, manifestations of cerebral ischemia, and the effects of substance P.

**Key Words**: *cerebral ischemia; behavioral responses; substance P*

We found previously that substance P fragment 1-11 (SP) influences the mortality of rats during the acute (48 h) period of cerebral ischemia in different ways depending on their type of behavior. Thus, SP reduced mortality among rats with a passive type of behavior, increased it among those with an active type, and had little or no effect on the mortality of those with an intermediate type of behavior [8].

The aim of the present study was to examine SP for its impact on the neurological status and behavioral responses of rats with different (passive, active, and intermediate) types of behavior during a prolonged (3 months) period after ligation of the common carotid arteries (animal model of ischemic insult).

## MATERIALS AND METHODS

A total of 130 random-bred male rats (body weight 250-300 g) maintained on a standard diet were used. Of these rats, neurological and behavioral consequences of cerebral ischemia were studied in 79 injected with SP and in 51 without exposure to this substance. The type of behavior was determined in 10-minute "open field" and "forced swimming" tests [13] using a previously developed procedure [6,7] permitting identification of animals that significantly differ in three major behavioral parameters - the number of squares crossed, the number of upright postures, and the time of passive floating.

Cerebral ischemia was produced by bilateral ligation of the common carotid arteries [6]. SP was injected once intraperitoneally at 250 μg/kg body weight in 0.5 ml of physiological saline 30 min after ligation of the arteries. (SP was synthesized at the Institute of Molecular Pharmacology, Berlin, and kindly provided by Professor P. Oehme). The 250 μg/kg dose was used because SP at this dose level had been shown to increase resistance to stress [10,12] and to circulatory hypoxia of the brain [8] as well as to elevate for a prolonged period the brain levels of catecholamines [10], which play an important part in determining the resistance both to stress [10] and to circulatory hypoxia of the brain [11].

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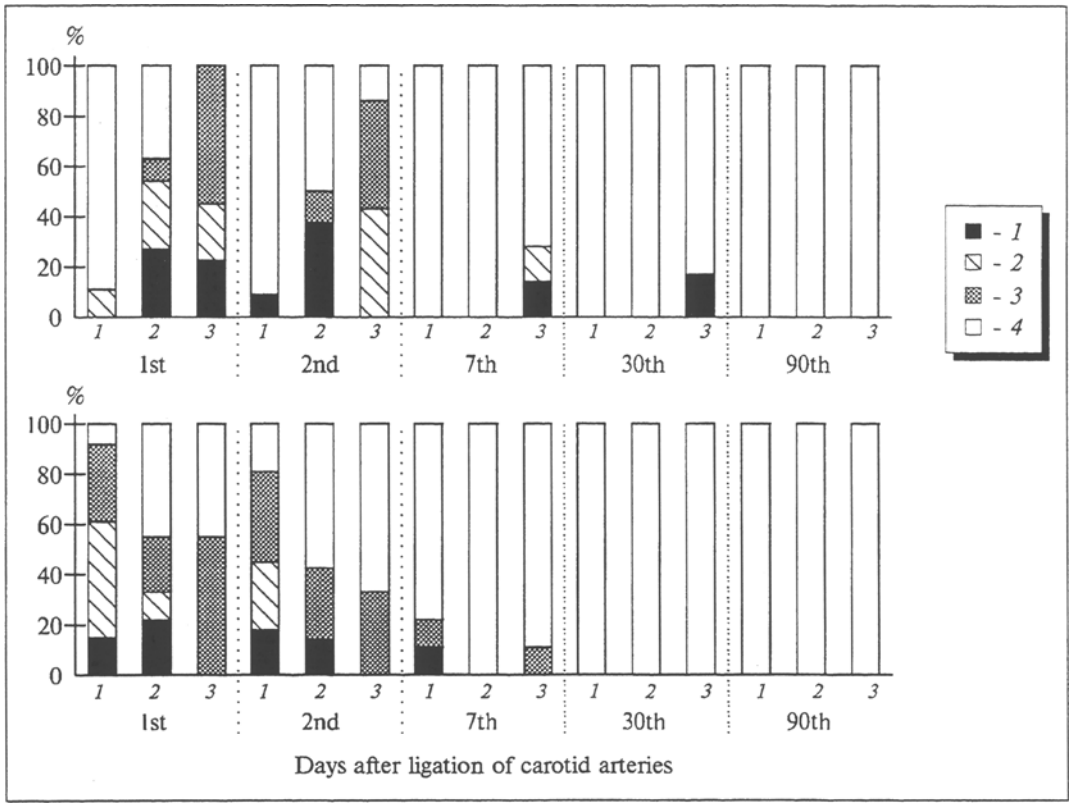


**Fig. 1.** Neurological status at different times after the onset of cerebral ischemia in rats with different types of behavior not injected (*a*) and injected (*b*) with substance P. *I*) Died (score 25 points); *II*) poor status (7–24 points); *III*) intermediate status (3–6 points); *IV*) nearly normal status (<3 points). *1*) rats with active type of behavior (9 [*a*] and 11 [*b*] animals; *2*) rats of intermediate group (11 and 9 animals, respectively); *3*) rats with passive type of behavior (10 rats in *a* and 10 in *b*). *Ordinate*: number of rats with a particular neurological status.

The condition of animals at different periods of cerebral ischemia was evaluated on a 25-point scoring scale of neurological deficit (Table 1), which is our modification of a 100-point scale previously proposed for assessing neurological deficit in rats that have suffered clinical death [4]. Behavioral changes after the onset of ischemia were determined in the open-field test (on days 2, 7, 30, and 90) and the forced-swimming test (on days 30 and 90).

The data were processed using a standard STATGRAPHICS software package. The significance of differences was estimated by Student's *t* test, the $c^2$ test, and Fisher's exact test.

## RESULTS

Whereas most of the SP-unexposed rats with severe neurological disorders (scores 7-24) after ischemia induction belonged to the group with the passive or intermediate type of behavior (Fig. 1, *a*), most of the SP-treated rats with such disorders were from the group with the active or intermediate type (Fig. 1, *b*). Among the SP-treated rats with the passive type of behavior, the proportion of symptom-positive animals after ischemia induction was lower than among the SP-untreated animals (33% *vs.* 86%; $\chi^2_1=2.52$, $p=0.05$) while the proportion of symptom-negative ones was higher (45% *vs.* 0%; $\chi^2_1=3.27$, $p=0.03$). Among the SP-treated rats with active behavior, on the contrary, the proportion of symptom-negative animals was lower (19% *vs.* 89%; $\chi^2_1=7.27$, $p=0.002$). Among the SP-treated rats with intermediate behavior, the proportion of symptom-positive animals did not differ significantly from that among the SP-unexposed animals (33% *vs.* 36%; $\chi^2_1=0.0$, $p=1.0$), nor did the proportion of symptom-negative ones (45% *vs.* 37%; $\chi^2_1=0.1$, $p=0.74$). Whereas not a single instance of convulsive seizures was recorded after ischemia induction among the SP-unexposed rats with active behavior (seizures occurred in 75% of rats with passive behavior and in 25% of those with intermediate behavior), 75% of the SP-treated rats with active behavior and 25% of those with intermediate behavior developed convulsive seizures after the onset of ischemia, but none of the SP-treated animals with passive behavior.

Following ischemia induction, SP-untreated rats showed biphasic changes in behavior (Fig. 2,

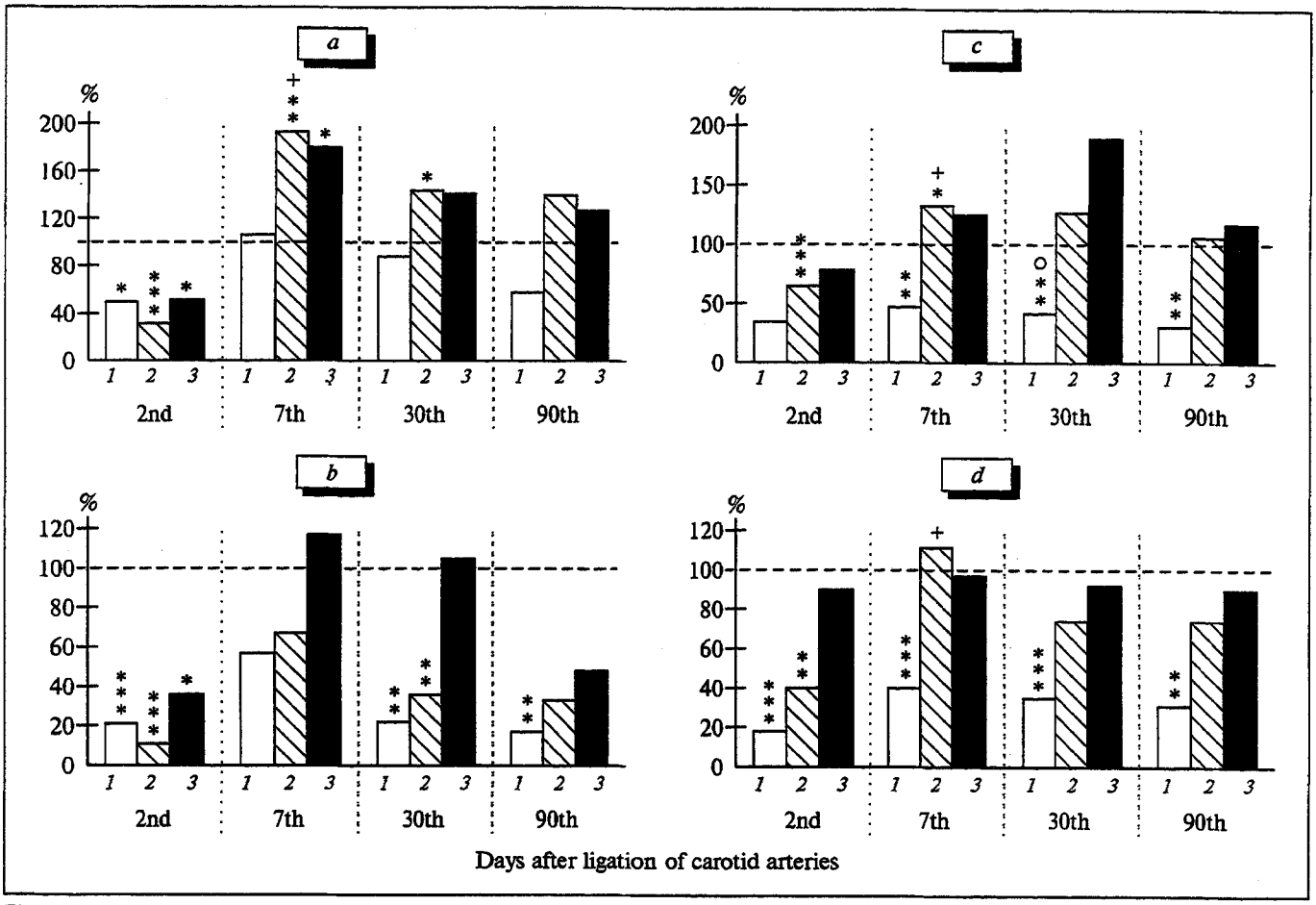


**Fig. 2.** Behavioral parameters as tested in an open field at different times after the onset of cerebral ischemia in rats with different types of behavior not injected (*a* and *b*) or injected (*c* and *d*) with substance P. *a* and *c*) number of squares crossed; *b* and *d*) number of upright postures. Figures are percentages of the initial numbers taken as 100% (denoted by dashed lines). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ in comparison with initial values; $^{+}p<0.05$ between groups 2 and 3; $^{\circ}p<0.05$ between groups 1 and 3. Other designations as in Fig. 1.

*a* and *b*). Thus, on day 2 after the onset of ischemia, motor (Fig. 2, *a*) and exploratory (Fig. 2, *b*) activities were greatly inhibited in all such rats, regardless of the type of behavior they displayed before ischemia was produced (intergroup differences were insignificant), and these behavioral changes went along with neurological disturbances of varying severity in the different groups (Fig. 1, *a* ); on day 7 after ischemia induction, however, behavioral responses were observed to correlate with the type of behavior: motor activity, as assessed by the number of squares crossed, was sharply increased in rats with the passive type (from 45.1±8.5 before ischemia to 81.5±15.7; $p<0.05$) and even more so in those with the intermediate type (from 67.2±5.1 to 129±10.1; $p<0.01$), and it rose to the baseline level in animals with the active type of behavior (from 88.4±7.3 before ischemia to 93.9±21.4 on day 7 of ischemia; $p>0.1$) (Fig. 2, *a*). These changes in motor activity were accompanied by statistically insignificant changes in exploratory activity (number of upright postures) (Fig. 2, *b*) and occurred when external manifestations of the neurological deficit were absent or slight (Fig. 1, *a*). On day 7 of ischemia, some rats with the passive type of behavior exhibited signs of spontaneous aggressivity and hyperactivity in response to external provoking stimuli. A characteristic feature of hyperactive rats was rapid, disordered motor activity in the open field: the animals moved on straightened legs without pressing the body against the floor ("ischemic gait") [14]. One month after the onset of ischemia, motor activity was restored in rats with the passive type of behavior (difference from the baseline level was insignificant) while remaining significantly above baseline in those with the intermediate type (Fig. 2, *a*). The enhanced motor activity in the latter rats combined with significantly reduced exploratory activity (Fig. 2, *b*). External manifestations of the neurological deficit were not observed (Fig. 1, *a*). Three months after the onset of

ischemia, motor activity was restored in rats with the intermediate type of behavior (Fig. 2, *a*) and significantly reduced in those with the active type (Fig. 2, *b*).

SP produced differential impacts on the behavior of rats altered by circulatory hypoxia of the brain, depending on their type of behavior. In rats with the passive type, SP significantly reduced the magnitude of both the initial hypoxia-induced reduction in exploratory activity (9.2±1.3 in SP-treated rats *vs.* 3.9±1.8 in SP-untreated ones on day 2 after ischemia induction; $p<0.05$) (Fig. 2, *d*) and the subsequent (posthypoxic) enhancement of motor activity (45.0±6.9 in SP-treated rats *vs.* 81.5±15.7 in SP-untreated ones on day 7 after ischemia induction; $p<0.05$) (Fig. 2, *c*). As a consequence, the motor and exploratory activities of SP-treated rats with the passive type of behavior, unlike these activities of SP-untreated rats, did not significantly deviate from the baseline levels at various times after ischemia induction. SP also significantly reduced the degree to which motor activity was enhanced on day 7 after ischemia induction in rats with the intermediate type of behavior (72.3±11.6 in SP-treated animals *vs.* 129.7±10.1 in SP-untreated ones; $p<0.01$) (Fig. 2, *c*), but failed to reduce significantly the degree of posthypoxic reduction in exploratory activity by that time in rats with the active type (10.8±2.6 in SP-treated animals *vs.* 22.2±6.3 in SP-untreated ones; $p=0.2$) (Fig. 2, *d*).

One and three months after the onset of cerebral ischemia, the level of depression was found to be increased to varying degrees in all groups of SP-unexposed rats (Fig. 3, *a*) and SP prevented this increase (Fig. 3, *b*).

The results of this study show that motor activity was restored more rapidly in rats with the active type of behavior (by day 7 after the carotid arteries were ligated) than in those with the passive type (by day 30 postligation) or in those with the intermediate type (by day 90 postligation). In other words, restorative processes proceeded at faster rates in animals with the active type of behavior than in those of the other two groups. The enhanced motor activity (increased number of squares crossed) in the rats with the passive and intermediate types of behavior was not paralleled by enhanced exploratory activity (increased number of upright postures), which can be interpreted as evidence of their general psychomotor excitation (a feature characteristic of the state of "agitated anxiety") rather than of enhanced exploratory motivation. The observed aggressivity and hyperactivity in response to external provoking stimuli appear to be signs of enhanced general excitability of the central nervous system. Changes in conditioned response activity suggesting that inhibitory processes in the central nervous system were suppressed (against a background of intensified excitatory processes) have been detected in rats with long-lasting hypoxic hypoxia [3] or cerebral ischemia caused by circulatory arrest [5]. The lack of concordance between changes in behavioral variables characterizing motor and exploratory activities, which (as we showed earlier [7]) are closely linked up, attests to disintegration of the interrelated components of an integrated behavioral response, and this, together with central nervous system activation, is one of the symptom-forming factors in neurotic states [1]. The hyperactivity observed in rats with the intermediate type of behavior one month after the onset of cerebral ischemia went

**TABLE 1.** Neurological Status of Rats (as Assessed in Scores) after Bilateral Ligation of the Common Carotid Arteries

| Parameters | Score |
|---|---|
| 1. Respiration: | |
| normal | 0 |
| increased/decreased rate | 2 |
| with participation of accessory muscles, irregular | 4 |
| 2. Signs of ptosis: | |
| none | 0 |
| unilateral | 1 |
| bilateral | 2 |
| 3. Tone of limb muscles: | |
| normal | 0 |
| slightly increased | 1 |
| increased | 2 |
| absent | 4 |
| 4. Convulsions: | |
| none | 0 |
| clonic | 2 |
| tonic | 4 |
| 5. Response to external stimuli: | |
| normal | 0 |
| depressed | 2 |
| exaggerated and inappropriate | 2 |
| absent | 4 |
| 6. Behavioral reactions: | |
| normal | 0 |
| hyperactivity, aggressivity | 2 |
| hypoactivity | 2 |
| could not be determined due to gravity of condition | 6 |
| 7. External appearance: | |
| groomed | 0 |
| ungroomed | 1 |

**Note.** Score 25: death; scores 7–24: gravely ill; scores 3–6: moderately ill; scores 0–3: near–normal condition.

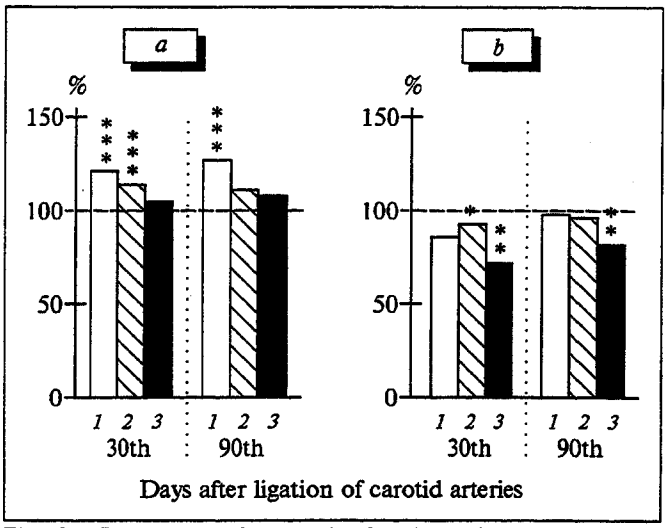


**Fig. 3.** Percentage changes in the time of passive floating (level of depression) in Porsolt's test after ischemia induction in SP—untreated (*a*) and SP—treated (*b*) rats with different types of behavior. Same designations as in Figs. 1 and 2.

along with an elevated level of depression in Porsolt's test, indicating that an anxiety-depressive syndrome was developing in the rats.

Our experimental findings are in line with the clinical observations that a neurasthenic syndrome accompanied by anxiety/depressive disorders is one of the more frequent long-term sequelae of circulatory hypoxia affecting the brain [2].

In rats with the passive type of behavior, a single SP injection improved the neurological status during the acute period of cerebral ischemia and prevented the occurrence of posthypoxic hyperactivity subsequently. SP did not influence the neurological status of rats with the intermediate type of behavior (although it reduced their posthypoxic hyperactivity) and worsened the neurological status of rats with the active type of behavior without exerting a significant effect on their behavioral responses altered by cerebral ischemia. In rats of all three groups, SP prevented a rise in the level of depression in Porsolt's test.

The observation that the same compound has a beneficial antihypoxic effect in animals with one type of behavior and a different or even opposite effect in those with another type is not unexpected. Indeed, adaptation to hypoxia enhances, as do antihypoxic agents, the resistance to oxygen deficiency only in animals with an initially low or medium level of tolerance to hypoxia, whereas the resistance of animals with an initially high level of tolerance remains unchanged or decreases [9].

To sum up, a single dose of SP exerts a beneficial therapeutic effect on the long-term sequelae of circulatory brain hypoxia in rats with the passive type of behavior, produces some benefit to rats with the intermediate type, and does not significantly influence these sequelae in rats with the active type of behavior.

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