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Novel Environment and Cat Odor Change GABA and 5-HT Release and Uptake in the Rat

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FILE, S. E., H. ZANGROSSI, JR. AND N. ANDREWS. *Novel environment and cat odor change GABA and 5-HT release and uptake in the rat.* PHARMACOL BIOCHEM BEHAV 45(4) 931–934, 1993.—In hippocampal and cortical slices taken from rats moved in their home cages to a novel environment for 5 min, there were decreases in basal and K^+-evoked [^{14}C]GABA release and an increased [^{14}C]GABA uptake compared with slices taken from rats remaining undisturbed in the animal house. The changes in 5-hydroxytryptamine (5-HT) release and uptake in response to a novel environment were markedly time dependent. In rats killed immediately after the 5-min exposure, there was decreased hippocampal [^{3}H]5-HT uptake and higher hippocampal basal release, whereas in rats killed 30 min later there was increased [^{3}H]5-HT uptake and lower basal release in both hippocampal and cortical slices. Rats exposed to cat odor in the novel environment showed increased release and decreased uptake of GABA in both brain areas compared with the group exposed to a neutral odor in the same novel environment, and these differences between the two odor groups were found both immediately and 30 min after the odor exposure. In contrast, only one measure of 5-HT function differed between the neutral and cat odor groups, with the latter showing increased cortical [^{3}H]5-HT uptake 30 min after odor exposure.

5-HT GABA Release Uptake Novelty Cat odor Anxiety

AS part of a series of experiments exploring changes in presynaptic function in various anxiogenic situations, we investigated changes in the release and uptake of 5-hydroxytryptamine (5-HT) and GABA after two manipulations that cause increased anxiety in animal tests: withdrawal from chronic treatment with diazepam (3,13) and acute handling stress (11). Both of these manipulations resulted in an anxiogenic response as detected in the elevated plus maze (2,10) and in both cases the anxiogenic response was correlated with enhanced synaptic availability of 5-HT. However, the mechanism by which this was achieved was different in the two cases: There was an increased release of [^{3}H]5-HT from hippocampal slices during diazepam withdrawal, but a decreased uptake of [^{3}H]5-HT during acute handling stress. In contrast to the extensive changes indicating reduced postsynaptic sensitivity to GABA following benzodiazepine withdrawal (8) and handling stress (4), there was an increased [^{14}C]GABA release from hippocampal slices after withdrawal from diazepam (4 mg/kg/day for 21 days) (13) and after acute handling stress (11).

We also studied the changes in the release and uptake of 5-HT and GABA that occur when rats are exposed to animal tests of anxiety. Exposure to the elevated plus-maze resulted in increased [^{3}H]5-HT release and decreased uptake in hippocampal slices, but only the high light, familiar test condition of the social interaction test resulted in increased release of [^{3}H]5-HT from hippocampal slices (12). Exposure to all four social interaction test conditions resulted in increases in cortical uptake of [^{3}H]5-HT and all but the high light, unfamiliar test condition were associated with increases in cortical uptake of [^{14}C]GABA.

A recent factor analysis study (9) has shown that different animal tests of anxiety are measuring different underlying states. The different patterns of 5-HT and GABA release and uptake we found following exposure to the elevated plus-maze and social interaction tests would support this suggestion. The purpose of the present study was to explore changes in the release and uptake of [^{3}H]5-HT and [^{14}C]GABA from hippocampal and cortical slices of rats exposed to another anxiogenic situation, exposure to cat odor (5). As well as producing marked behavioral changes during odor exposure, previous exposure to cat odor also results in anxiogenic responses that can be detected 30 min later in the elevated plus-maze (16). We therefore examined the changes in release and uptake both immediately and 30 min after exposure to cat odor. Because

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this exposure necessitated placing rats in an unfamiliar room, a comparison group was included that was placed in the unfamiliar room but exposed only to a neutral odor.

METHOD

Animals

Male hooded Lister rats (Olac Ltd., Bicester, UK) weighing approximately 240 g were housed with food and water freely available in a room maintained at 22°C with lights on from 0700–1900 h. Rats were housed in groups of five until 5 days before exposure to the odors. At that point, they were singly housed and handled daily.

Procedure

Rats were randomly allocated among the following experimental groups: undisturbed home cage; novel environment; cat odor. On the test day, those allocated to the undisturbed home cage group (n = 8) were left in their home cages in the animal house until removal for sacrifice. Those allocated to the novel environment condition remained in their home cages but were moved to a novel room (with a neutral odor) for 5 min. One group (n = 6) was killed immediately after this exposure; a second group (n = 8) was returned to the animal house for 30 min before sacrifice. Rats allocated to the cat odor groups also remained in their home cages, were moved to the same small room, and in addition were exposed to cat odor (as described below) for 5 min. Two groups were tested, one killed immediately after odor exposure (n = 7) and the other returned to the animal house (n = 8) for 30 min before sacrifice.

The cat odor was obtained by rubbing a damp cloth vigorously against the fur of a laboratory-housed domestic cat for 5 min. This procedure was carried out 1 h before the experimental session. The cat cloth was kept in a sealed plastic bag. Each cloth was used for two exposures only. Damp pieces from the same original cloth were used as a neutral odor to equate conditions for the novel environment groups.

Before the first cat odor exposure, an impregnated cloth was left in the test room for 10 min. Each rat was carried to the exposure room and placed next to an empty cage with the odor cloth wedged between the cage tops, at the opposite end from the food and water containers. All the novel environment groups with neutral odor exposure preceded the cat odor exposure to prevent any traces of cat odor influencing these groups.

Chemicals

[^{14}C]GABA (216 mCi/mmol) and [^{3}H]5-HT creatinine sulphate (14.0 Ci/mmol) were obtained from Amersham International (Arlington Heights, IL). Krebs bicarbonate buffer of the following composition was used (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.4, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, and glucose 9.5. The medium was gassed continuously with 5% CO_2 in O_2. The medium also contained aminooxyacetic acid (50 μM), pargyline (50 μM), ascorbic acid (100 μM), and EDTA (30 μM).

K^+-stimulated [^{14}C]GABA and [^{3}H]5-HT release

Immediately or 30 min after the exposure to neutral or cat odor or immediately after removal from the home cage, rats were stunned and killed by cervical dislocation. The brain was rapidly removed and the frontal cortex and hippocampus

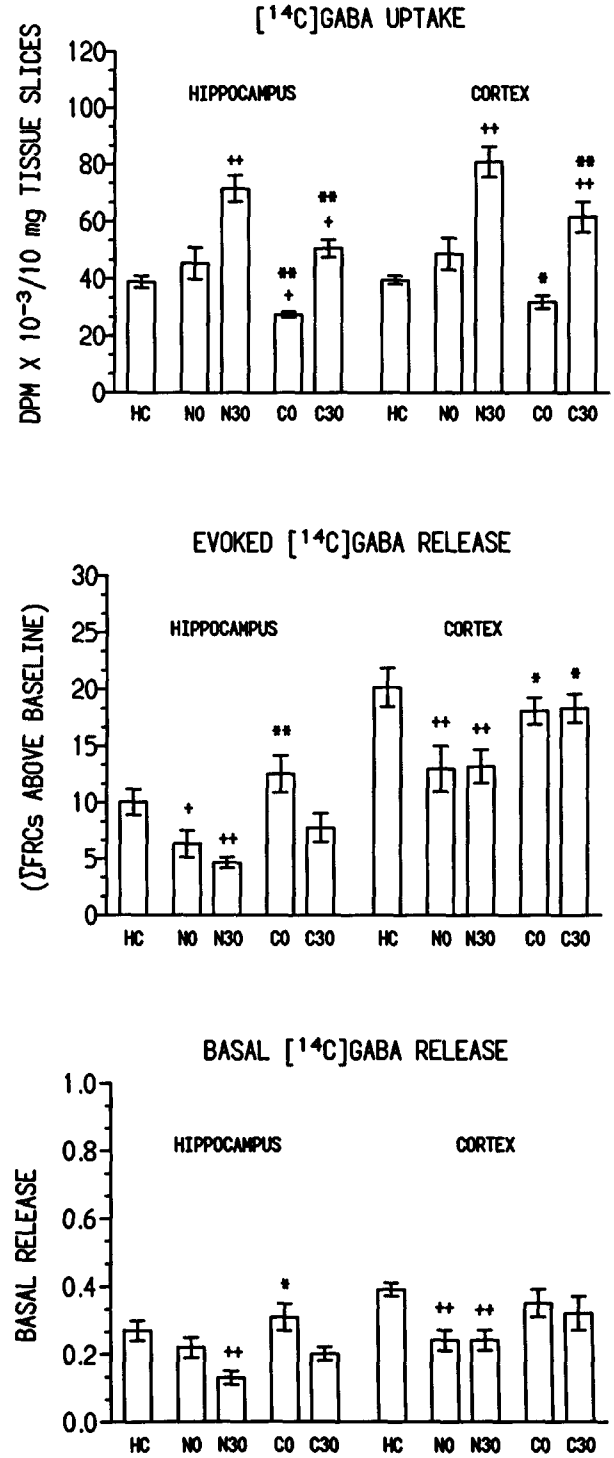

FIG. 1. Mean (±SEM) [^{14}C]GABA uptake (dpm × 10^{-3}), K^+-evoked release (sum of FRCs above baseline), and basal release (FRCs) in hippocampal and frontal cortical slices taken from rats left undisturbed in their home cages (HC), moved to a novel environment for 5 min and killed immediately (N0) or 30 min (N30) later, or exposed in the novel environment to cat odor for 5 min and killed immediately (C0) or 30 min later (C30). Each score is the mean of six to eight observations. $^{+}p < 0.05$, $^{++}p < 0.01$ compared with undisturbed home cage group; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with appropriate neutral odour group.

dissected. Slices (0.2 mm thick) of both areas were prepared with a McIlwain tissue chopper. After a preliminary incubation for 10 min at 37°C in a Krebs bicarbonate medium, [^{14}C]GABA and [^{3}H]5-HT were added to the medium to give final concentrations of 0.23 and 0.07 μM, respectively. After 30-min incubation, five slices (approximately 10 mg tissue wet wt) were inserted between nylon grids in a small chamber (volume 1 ml), where they were superfused with Krebs at 1 ml/min for 15 min before fractions (2 ml each) were collected. An evoked release was achieved by exposing the slices for 2 min, during fraction 7 (in a total of 20 fractions), to Krebs containing 30 mM KCl—this being replaced with the normal Krebs for the remainder of the collection period.

Statistics

The data were analysed with analysis of variance, followed by Duncan's tests for the significance of differences between individual groups. It is these values that are presented in Figs. 1 and 2.

RESULTS

Response to Novel Environment

[^{14}C]GABA. In hippocampal and cortical slices taken from rats moved to a novel environment, there was lower basal K^+-evoked [^{14}C]GABA release and increased uptake compared with those remaining in their home cages (see Fig. 1). The changes in release were significant immediately after a 5-min exposure to the novel room and persisted for at least 30 min, whereas the changes in uptake reached significance only after 30 min.

[^{3}H]5-HT. The changes in 5-HT release and uptake in response to exposure to a novel environment were markedly time dependent. Immediately following the 5-min exposure, there was an increase in hippocampal, but not cortical, synaptic 5-HT availability, shown by decreased uptake and a higher basal release (see Fig. 2). Thirty minutes later this was reversed and there was decreased synaptic 5-HT availability in both brain regions, shown by increased uptake and decreased basal release.

Response to Cat Odor

Rats exposed to cat odor experienced a change in environment plus the additional exposure to the odor of a predator. The changes in this group were therefore compared with those in the neutral odor group, as well as with the home cage control group.

[^{14}C]GABA. Compared with the neutral odor groups, the groups exposed to cat odor showed significantly enhanced synaptic GABA availability in both brain regions, shown by increased release and decreased uptake (see Fig. 1). These differences between the neutral and cat odor groups were found at both time intervals.

When compared with the home cage groups, those exposed to cat odor showed decreased hippocampal GABA uptake when killed immediately after the exposure whereas those killed 30 min later showed increased GABA uptake in both areas (see top panel Fig. 1).

[^{3}H]5-HT. In contrast to the effects of cat odor on the GABA system, only one measure of presynaptic 5-HT function (uptake in cortical slices at 30 min) differed significantly between the neutral and cat odor groups (see Fig. 2). Thus, the differences were all with respect to the undisturbed home

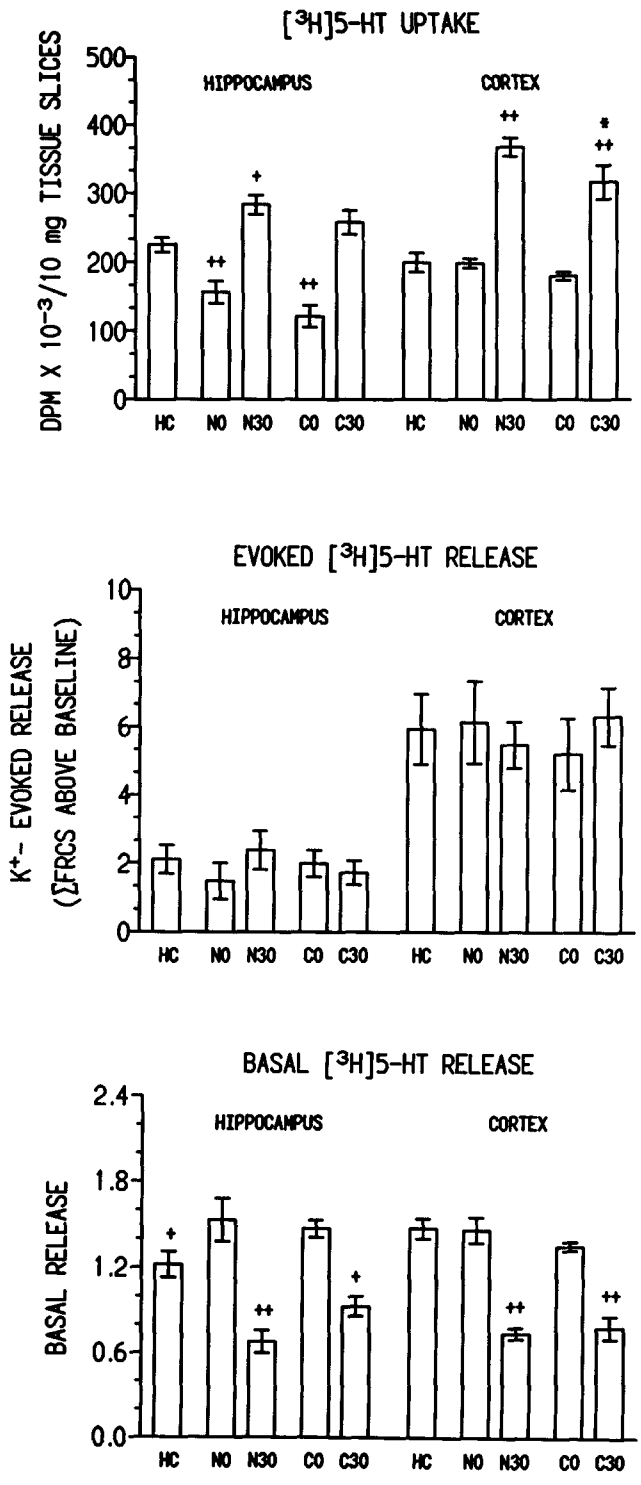

FIG. 2. Mean (±SEM) [^{3}H]5-hydroxytryptamine (5-HT) uptake (dpm × 10^{-3}), K^+-evoked release (sum of FRCs above baseline), and basal release (FRCs) in hippocampal and frontal cortical slices taken from rats left undisturbed in their home cages (HC), moved to a novel environment for 5 min and killed immediately (N0) or 30 min (N30) later, or exposed in the novel environment to cat odor for 5 min and killed immediately (C0) or 30 min later (C30). Each score is the mean of six to eight observations. $^{+}p < 0.05$, $^{++}p < 0.01$ compared with undisturbed home cage group; $^{*}p < 0.05$, compared with appropriate neutral odour group.

cage group. The only significant change immediately after exposure was a decrease in hippocampal uptake. After 30 min, there was increased uptake in the cortex and decreased basal release in both regions. Thus, the changes in 5-HT release and uptake were time dependent, as in the neutral odor group.

DISCUSSION

When the cat odor group was compared with the neutral odor group, there were increases in [^{14}C]GABA release in both the cortex and hippocampus and accompanying decreases in uptake. This pattern of results is similar to that found in rats exposed to an acute handling stress when compared with those habituated to handling (11). However, it is not a pattern that is general to all anxiogenic situations and has not been reported after withdrawal from chronic benzodiazepine treatment or after exposure to the elevated plus-maze or social interaction tests. The changes in release and uptake of GABA after exposure to cat odor were opposite in direction to those found simply after exposure to the novel environment. Compared with the home cage control group, rats exposed to the neutral odor in the novel environment had decreased [^{14}C]GABA release and increased uptake in both brain regions studied. The changes induced by novelty and exposure to cat odor were more or less equal in extent but opposite in direction, resulting in little difference between the home cage controls and the cat odor groups. In light of the differing postsynaptic changes in the GABA system that have been found after exposure to different stressful situations (6,7,14), it is probably not surprising that we found different presynaptic changes after exposure to different anxiogenic situations. To fully understand any test situation, it will be necessary to identify the contributions of the various components of the test as well as the contribution of novelty and any handling stress. We proposed that the increased release of hippocampal 5-HT plays a major role in the increased anxiety that occurs upon benzodiazepine withdrawal (1) and increases in hippocampal 5-HT availability might also contribute to the anxiogenic response to acute handling stress. However, it has been argued that increased hippocampal 5-HT availability is not always associated with an increased anxiogenic response in the elevated plus-maze (15) and it does not correlate with anxiogenic responses in the social interaction test (12). No changes in the release or uptake of hippocampal 5-HT were found as a result of exposure to cat odor. It must therefore be concluded that anxiogenic responses are not always associated with changes in hippocampal 5-HT release. However, there may be specific instances, such as benzodiazepine withdrawal, where they are of major importance.

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