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Regional Changes in Dopamine and Serotonin Activation With Various Intensity of Physical and Psychological Stress in the Rat Brain

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INOUE, T., K. TSUCHIYA AND T. KOYAMA. *Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain.* PHARMACOL BIOCHEM BEHAV **49**(4) 911-920, 1994.— The present study examined whether regional patterns of brain dopamine (DA) and serotonin (5-HT) activation after physical and psychological stress depend on the intensity of that stress. Monoamine concentrations (DA, 5-HT, and their metabolites) were measured using high-performance liquid chromatography with electrochemical detection in eight brain regions of rats exposed to two different intensities of foot shock stress for 30 min (1.5 mA or 2.5 mA) or conditioned fear stress (CFS, after single or repeated foot shock). A low level of foot shock selectively increased the DA metabolism in the medial prefrontal cortex (mPFC), whereas a high level of foot shock increased it in most of the brain regions examined in the present study. A low level of foot shock did not increase the 5-HT metabolism in any regions, but a high-intensity shock increased the 5-HT metabolism in the mPFC, nucleus accumbens, and lateral hypothalamus. Rats that received high-intensity shock displayed more freezing than those that received low-intensity shock in a conditioned fear paradigm (24 h after receiving foot shock, the animals were placed in a shock chamber without being given shock), indicating an augmentation of conditioned fear. The increased DA and 5-HT metabolism were especially marked in the mPFC after CFS following a single foot shock session (2.5 mA). Rats that were repeatedly exposed to 2.5 mA foot shock for a period of 10 days displayed a greater degree of freezing induced by CFS than those given only one foot shock session, indicating an augmentation of fear and stress intensity. CFS after repeated foot shock, like foot shock per se, increased the DA metabolism in most of the brain regions except for the striatum and increased the 5-HT metabolism in the mPFC, nucleus accumbens, and amygdala. These results suggest that regional patterns of brain DA and 5-HT activation after physical and psychological stress depend on the intensity of that stress, although there are some differences between these stress; and that the more widespread activation of DA and 5-HT after more severe stress might relate to behavioral changes that reflect the augmentation of fear.

Dopamine Serotonin Footshock stress Stress intensity Conditioned fear stress Psychological stress Freezing behavior Medial prefrontal cortex Anxiety

REGIONALLY dependent changes in rat brain dopamine (DA) activation after various types or intensity of stress have been suggested by several lines of evidence (8,9,16,35). Very mild stress, such as psychological stress (8,16,23) and mild electric shock (32), selectively activates the mesocortical DA system. In contrast, more severe stress, such as severe electric foot shock stress (9,11,16) and cold restraint (10), activates not only the mesocortical DA system but also the mesolimbic and nigrostriatal DA systems. Thus, it has been suggested that regional patterns of brain DA activation after stress are dependent upon the intensity of that stress (9), although it is not clear whether regional differences between psychological stress and physical stress are due to differences in the quality of stress or the intensity of stress. The effect of stress intensity on these variations in brain dopaminergic responses has received little systematic study until now.

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Stress also activates the brain serotonin (5-HT) neuron system. Previous studies have reported that foot shock and restraint stress increased the 5-HT metabolism in a number of brain regions (2,5,11,19,24,25). Recently, we found that conditioned fear stress (CFS, exposure to environmental stimuli paired previously with inescapable foot shock), which is regarded as mild psychological stress and an animal model of anxiety or fear, selectively increased the 5-HT metabolism in the medial prefrontal cortex (mPFC) like the DA responses to CFS, while electric foot shock stress increased the 5-HT metabolism in several other brain regions (the mPFC, nucleus accumbens, and amygdala) (19). Thus, the regional patterns of activation of 5-HT during psychological stress are different from those during physical stress. Because 5-HT has been suggested to be associated with CFS in behavioral pharmacological studies (18,30), the selective activation of 5-HT during CFS has led to the suggestion that mPFC 5-HT neurons may play an important role in the control of negative emotional states. However, it is not clear whether the intensity of physical stress and psychological stress, a quantitative variable, determines which terminal regions of serotonergic neurons are activated.

In behavioral studies, the strength of fear conditioning increases with shock intensity and repeated conditioning: conditioned fear-induced freezing, regarded as an index of fear, increases with shock intensity and repeated contextual conditioning (14,15). Therefore, the conditioned freezing response is useful for assessing the degree of behavioral influence of various intensity of foot shock and the intensity of CFS. As mentioned above, CFS is related to the brain DA, noradrenaline, and 5-HT neuron systems (3,8,16,19,36); and conditioned freezing is attenuated by 5-HT_{1A} agonists (18,30), benzodiazepines (15,18), and a corticotropin-releasing hormone antagonist (22). Although the functional significance of regional DA and 5-HT responses to various intensities of stress is unclear, the results from this ethological paradigm might provide evidence of how regional DA and 5-HT activation is related to behavioral changes following increasing intensity of foot shock and CFS.

In an attempt to clarify the relevance of the intensity of psychological stress and physical stress to regional patterns of brain DA and 5-HT activation, we have compared the effects of different intensity of CFS and electric foot shock stress on the DA and 5-HT metabolism in several brain regions in rats.

METHOD

Animals

Male Sprague–Dawley rats obtained from the Shizuoka Laboratory Animal Center (Shizuoka, Japan), weighing 250–300 g, were housed four per cage and maintained in a 12 L : 12 D, temperature-controlled environment, with free access to food and water. All experiments were performed between 0800 and 1300 h.

Electric Foot Shock Procedure

Rats were individually subjected to inescapable electric foot shock stress for 30 min [1.5 mA or 2.5 mA scrambled shock, on a variable interval schedule with a mean intershock interval of 30 s (5–55 s) and shock duration of 30 s] in a chamber with a grid floor (19 × 22 × 20 cm, Medical Agent Co., Kyoto, Japan). Electric shock was provided by a Model SGS-02D Shock Generator (Medical Agent Co., Kyoto, Japan). This provides a high-voltage, high-resistance circuit with resistance controlled by dial settings calibrated by the manufacturer in a short circuit current. A 1.5 mA foot shock was sufficient to induce flinching and some vocalization but not jumping, while 2.5 mA of foot shock additionally evoked motoric escape behavior such as jumping. Control rats were placed in the shock chamber, but no current was applied to the floor of the chamber. The animals were removed from the chamber and sacrificed by decapitation immediately after the session. These procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee.

Conditioned Fear Stress Procedure (After Single or Repeated Foot Shock Sessions)

Rats were subjected to inescapable electric foot shock of 2.5 mA (the same parameters as above) for 30 min for a period of either 1 or 10 days. Control rats were also placed in the shock chamber for 30 min for 1 or 10 days, but shocks were not delivered. Twenty-four hours after the last session, the rats of both groups were again placed in the shock chamber for 30 min without receiving any shock and were sacrificed by decapitation immediately after (shock-no shock or no shock-no shock). In these single and repeated foot shock experiments, two additional groups of rats were exposed to either the shock or the control condition for 1 or 10 days, and 24 h after the last session were taken from their home cages (HC) and immediately sacrificed (shock-HC or no shock-HC). Thus, four groups of eight rats each were used in both the single and repeated foot shock experiments: no shock-HC, shock-HC, no shock-no shock, or shock-no shock (conditioned fear).

Biochemical Analysis

After decapitation, the brains were immediately removed, frozen, and stored at −80°C. These were later cut at 300 μm thickness in a cryostat at −10°C. The following brain regions were punched out with small stainless steel needles according to the method of Palkovits et al. (29): the mPFC, nucleus accumbens, medial striatum, lateral striatum, paraventricular nucleus of the hypothalamus (PVH), amygdala, lateral hypothalamus, and hippocampus. The tissue obtained was homogenized by ultrasonication in 250 μl of ice-cold 0.2 M perchloric acid containing 20 μg/ml *l*-cysteine. After centrifugation at 14000 × *g* for 1 min, DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), tryptophan, 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) in supernatants were assayed by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) as previously described (19). The HPLC system consisted of an EP-10 liquid chromatograph pump (Eicom, Kyoto), an ERC-3310 degasser (ERMA, Tokyo), a reversed phase ODS column-chemcosorb 5-ODS-H 250 × 4.6 mm (Chemco, Tokyo), an ECD-100 electrochemical detector (Eicom, Kyoto), and a Chromatocorder 12 (SIC, Tokyo). The mobile phase for DA, DOPAC, HVA, 5-HT, and 5-HIAA assay was 0.1 M phosphate buffer pH 3.2 containing 11% methanol, 1.7% tetrahydrofuran, 60 mg/l Na_2EDTA, and 350 mg/l octanesulfonic acid. The mobile phase for tryptophan assay was 0.1 M sodium acetate buffer pH 4.5 containing 10% methanol and 20 mg/l sodium octyl sulfate. The buffer was filtered before use. Separations were conducted isocratically at 35°C with a flow rate of 1.0 ml/min. The electrochemical detector was set at a potential of 0.7 volt for DA, DOPAC, HVA, 5-HT, and 5-HIAA assay, and 0.85 volt for tryptophan assay. Protein levels in the pellets

were measured by the Lowry et al. method (27), using bovine serum albumin as a standard.

Behavioral Observation

The rats were subjected to inescapable electric foot shock of 1.5 mA or 2.5 mA (the same parameters as above) for 30 min. Twenty-four hours after foot shock, the rats of both groups were again placed in the shock chamber and observed for 15 min, but no current was applied to the floor of the chamber. In another experiment, the rats were subjected to 1, 5, or 10 days of foot shock (2.5 mA) at a frequency of 1/day with a duration of 30 min/session. Twenty-four hours after the last session, all the subjects were again placed in the shock chamber and observed for 30 min without shocks. During these observational periods, freezing behavior and defecation score (bolus count/30 min) were recorded. Behavior was recorded using the time-sampling procedure (14) for a period of 15 or 30 min. Every 10 s, the behavior that the animal was currently engaged in was classified as either freezing or activity. Freezing was defined as the lack of all observable movement of the body and vibrissae, except those related to respiration. All other behavior was scored as activity. The percentage scores of freezing were calculated for successive 5-min blocks during the observational period.

Data Analysis

All the data are presented as the means ± SEM of the individual values of the rats from each group. Statistical differences between two groups were made using the Student's *t*-test. Multiple group comparisons were made using the one-way analysis of variance followed by Duncan's test.

RESULTS

Effects of Two Intensities of Foot Shock on Monoamines

DA levels were significantly elevated in the PVH and hippocampus only by a 2.5 mA foot shock (Table 1). After a 1.5 mA foot shock, DOPAC levels selectively increased in the mPFC. The 2.5 mA foot shock resulted in increases in the DOPAC levels in seven of the brain regions but not the hippocampus relative to the controls and the 1.5 mA shock group (Fig. 1). The 2.5 mA foot shock elevated the HVA levels in

TABLE 1

EFFECTS OF TWO INTENSITIES OF ELECTRIC FOOT SHOCK STRESS ON DOPAMINE, HVA, TRYPTOPHAN, AND 5-HIAA LEVELS (pmol/mg PROTEIN) IN DIFFERENT BRAIN REGIONS

	Dopamine	HVA	Tryptophan	5-HIaa
Medial prefrontal cortex				
Control	15.0 ± 0.7	8.0 ± 0.9	291.7 ± 11.4	11.0 ± 1.2
1.5 mA	14.1 ± 0.8	9.3 ± 1.5	296.2 ± 4.4	14.0 ± 2.4
2.5 mA	14.1 ± 1.0	15.8 ± 1.1†§	353.9 ± 16.5†§	15.9 ± 2.3
Nucleus accumbens				
Control	778.4 ± 44.3	52.0 ± 3.0	351.6 ± 12.9	14.9 ± 1.4
1.5 mA	720.4 ± 55.2	56.4 ± 4.3	332.6 ± 7.8	15.9 ± 2.0
2.5 mA	791.4 ± 56.4	83.6 ± 3.4†§	386.9 ± 29.7	18.1 ± 3.1
Medial striatum				
Control	1068.5 ± 35.4	46.2 ± 2.1	310.7 ± 13.8	9.1 ± 0.9
1.5 mA	1030.0 ± 24.5	49.9 ± 2.4	327.7 ± 5.5	11.6 ± 1.6
2.5 mA	1128.7 ± 52.8	67.4 ± 3.1†§	400.3 ± 6.1†§	13.6 ± 1.5
Lateral striatum				
Control	1032.0 ± 48.1	74.2 ± 3.7	257.7 ± 8.5	16.2 ± 1.2
1.5 mA	966.6 ± 25.5	76.4 ± 1.9	245.9 ± 8.7	14.6 ± 1.2
2.5 mA	1059.3 ± 56.6	95.3 ± 7.2†‡	301.3 ± 6.9†§	16.1 ± 0.4
Paraventricular nucleus of the hypothalamus				
Control	30.8 ± 3.2	13.8 ± 1.3	246.2 ± 14.1	10.0 ± 1.3
1.5 mA	34.3 ± 1.5	11.6 ± 1.1	278.3 ± 17.0	11.0 ± 0.4
2.5 mA	47.5 ± 5.0†‡	13.0 ± 2.1	300.1 ± 22.1	10.7 ± 0.9
Amygdala				
Control	49.2 ± 6.4	8.0 ± 1.1	176.1 ± 7.4	7.6 ± 0.2
1.5 mA	45.6 ± 6.9	6.8 ± 1.1	173.3 ± 5.2	9.0 ± 0.5*
2.5 mA	48.3 ± 5.6	8.7 ± 0.8	211.7 ± 5.8†§	9.9 ± 0.5†
Lateral hypothalamus				
Control	10.9 ± 0.9	7.0 ± 1.0	191.5 ± 5.6	6.2 ± 0.5
1.5 mA	11.8 ± 1.0	7.1 ± 1.0	198.7 ± 7.5	6.7 ± 0.2
2.5 mA	14.1 ± 1.2	6.7 ± 0.9	208.3 ± 4.9	6.9 ± 0.2
Hippocampus				
Control	1.10 ± 0.05	1.5 ± 0.2	76.4 ± 10.8	2.5 ± 0.3
1.5 mA	1.04 ± 0.09	1.2 ± 0.2	75.7 ± 10.8	2.1 ± 0.3
2.5 mA	1.42 ± 0.06†§	1.5 ± 0.2	106.4 ± 14.2	3.0 ± 0.4

Results are means with SEM of data obtained on seven to eight rats. Significantly different from controls (*$P < 0.05$; †$P < 0.01$) or from 1.5 mA shock group (‡$P < 0.05$; §$P < 0.01$).

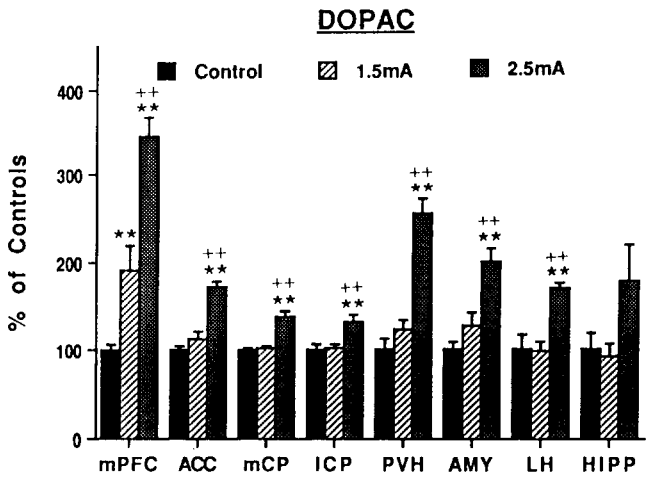

FIG. 1. Effects of two levels of electric foot shock stress (1.5 mA and 2.5 mA) for 30 min on DOPAC levels in different brain regions. Results are means with SEM of data obtained on seven to eight rats and are expressed as percentage of respective control values (pmol/mg protein, mPFC, 2.3 ± 0.2; ACC, 111.7 ± 5.1; mCP, 77.2 ± 2.5; lCP, 81.1 ± 5.1; PVH, 4.1 ± 0.6; AMY, 5.8 ± 0.5; LH, 2.9 ± 0.5; HIPP, 0.69 ± 0.13). Significantly different from controls (**$p < 0.01$) or from 1.5 mA shock group ($^{++}p < 0.01$). mPFC, medial prefrontal cortex; ACC, nucleus accumbens; mCP, medial striatum; lCP, lateral striatum; PVH, paraventricular nucleus of the hypothalamus; AMY, amygdala; LH, lateral hypothalamus; HIPP, hippocampus.

the mPFC, nucleus accumbens, medial striatum, and lateral striatum relative to the controls and the 1.5 mA shock group; but 1.5 mA foot shock did not change the HVA levels in any of the brain regions (Table 1).

Significant increases in the tryptophan levels were observed in the mPFC, medial striatum, lateral striatum, and amygdala of the rats which received 2.5 mA foot shock (Table 1). However, tryptophan levels in the 1.5 mA shock group did not differ from the controls in any of the brain regions studied. No change in the 5-HT levels was found in any of the brain regions after foot shock of either intensity (data not shown). Both 1.5 mA and 2.5 mA foot shock elevated the 5-HIAA levels only in the amygdala relative to the controls (Table 1). 5-HIAA/5-HT ratios were significantly increased by 2.5 mA foot shock in the mPFC, nucleus accumbens, and lateral hypothalamus (Fig. 2). However, 1.5 mA foot shock did not alter the 5-HIAA/5-HT ratios in any of the brain regions.

Conditioned Freezing Response

More freezing occurred in the 2.5 mA shock group than the 1.5 mA shock group in blocks 1 and 2 (Fig. 3). Defecation score (bolus count/15 min) in the 2.5 mA shock group was slightly, but not significantly, higher than that in the 1.5 mA shock group (1.5 mA group, 4.0 ± 1.1, $n = 8$; 2.5 mA group, 6.1 ± 0.6, $n = 8$).

Repeated foot shock did not change the defecation induced by conditioned fear relative to single foot shock [defecation score (bolus count/30 min): 1 day group, 6.4 ± 0.8, $n = 14$; 5 day group, 6.4 ± 0.5, $n = 14$; 10 day group, 6.4 ± 0.5, $n = 14$]. Footshock before conditioned fear was delivered to rats repeatedly: conditioned fear after 5 or 10 days of foot shock produced more freezing than that after 1 day of foot shock in block 1, indicating an augmentation of conditioned fear (Fig. 4).

Effects of CFS After Single Foot Shock on Monoamines

In order to distinguish the effects of CFS on the DA and 5-HT metabolism from the long-term effects of foot shock (24 h before CFS) and the effects of transfer stress, four experimental groups were used: no shock-HC, shock-HC, no shock-no shock, or shock-no shock (conditioned fear). When the shock-no shock group significantly differed from both the shock-HC and no shock-no shock groups in neurochemical variables, those changes were considered as the effects of CFS itself. Significant differences between the no shock-HC and shock-HC groups in the DA, DOPAC, and HVA levels were not observed in any of the brain regions, except a decrease in the DA level in the PVH (Table 2, Fig. 5): long-term effects of foot shock can, therefore, be excluded. Significant differences between the no shock-HC and no shock-no shock groups in the DA and DOPAC levels were not observed in any of the brain regions (Table 2, Fig. 5). The no shock-no shock group showed small, but significant, increases in the HVA levels in the nucleus accumbens, striatum, and amygdala relative to the no shock-HC group, indicating the effects of transfer stress (Table 2). CFS after single foot shock significantly increased the DA level in the PVH relative to both the shock-HC and no shock-no shock groups (Table 2). It significantly elevated the DOPAC levels in the mPFC, PVH, and lateral hypothalamus, and the HVA levels in the mPFC and amygdala, relative to both the shock-HC and no shock-no shock groups (Fig. 5, Table 2).

Significant differences between the no shock-HC and shock-HC groups in the tryptophan, 5-HT, and 5-HIAA levels were not observed in any of the brain regions, except an increase in the 5-HT level in the PVH (data not shown). Significant differences between the no shock-HC and no shock-no shock groups in the tryptophan, 5-HT, and 5-HIAA levels were not observed in any of the brain regions, except an increase in the tryptophan level in the amygdala (data not shown). CFS after single foot shock produced no significant changes in the tryptophan, 5-HT, and 5-HIAA levels in any

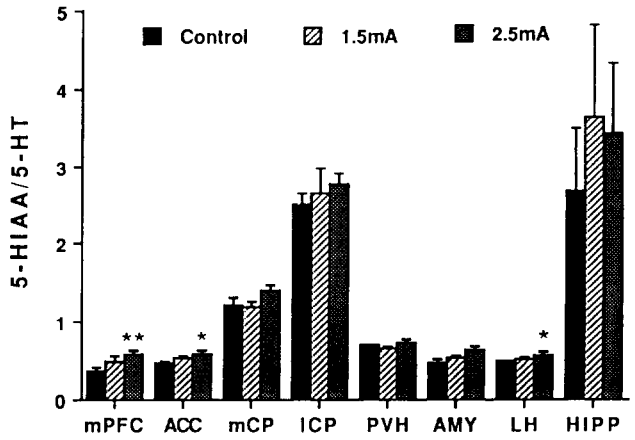

FIG. 2. Effects of two levels of electric foot shock stress (1.5 mA and 2.5 mA) for 30 min on 5-HIAA/5-HT ratios in different brain regions. Results are means with SEM of data obtained on seven to eight rats. For abbreviations see Fig. 1. Significantly different from controls (*$p < 0.05$, **$p < 0.01$).

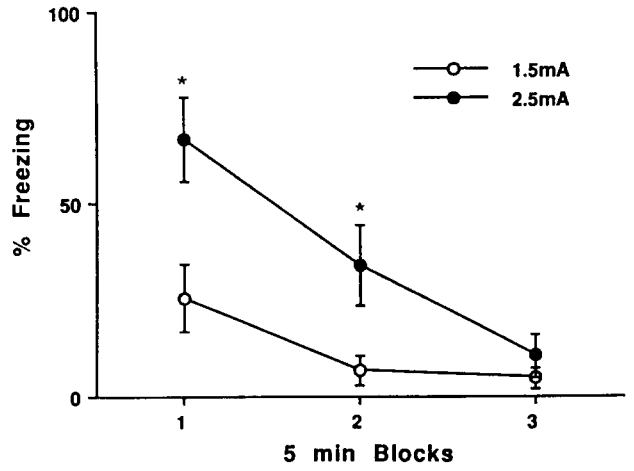

FIG. 3. Mean percent ± SEM of freezing scored for each 5-min block of testing in groups of rats (n = 8) receiving 1.5 mA or 2.5 mA shock. Behavior was sampled at 10-s intervals. Significantly different from 1.5 mA group (*$p < 0.05$).

of the brain regions (data not shown). It significantly increased the 5-HIAA/5-HT ratio only in the mPFC relative to both the shock-HC and no shock-no shock groups (Fig. 6).

Effects of CFS After Repeated Foot Shock on Monoamines

Similar to the case of CFS after single foot shock, four experimental groups were used: repeated no shock-HC (rep no shock-HC), rep shock-HC, rep no shock-no shock, or rep shock-no shock (conditioned fear). Significant differences between the rep no shock-HC and rep shock-HC groups in the DA, DOPAC, and HVA levels were not observed in any of the brain regions (Table 2, Fig. 7). Significant differences between the rep no shock-HC and rep no shock-no shock groups in the DA, DOPAC, and HVA levels were not observed in any of the brain regions (Table 2, Fig. 7). CFS after repeated foot shock significantly increased the DA levels in the PVH and hippocampus relative to both the rep shock-HC and rep no shock-no shock groups (Table 2). It also significantly increased the DOPAC levels in six of the seven brain regions but not the striatum, and increased the HVA levels in the mPFC, nucleus accumbens, and amygdala, relative to both the rep shock-HC and rep no shock-no shock groups (Fig. 7, Table 2).

Significant differences between the rep no shock-HC and rep shock-HC groups in the tryptophan and 5-HT levels were not observed in any of the brain regions, except an increase in the tryptophan level in the striatum (data not shown). Significant differences between the rep no shock-HC and rep no shock-no shock groups in the tryptophan and 5-HT levels were not observed in any of the brain regions (data not shown). CFS after repeated foot shock sessions did not change the tryptophan and 5-HT levels in any of the brain regions (data not shown). It significantly increased the 5-HIAA levels in the mPFC (Table 2), and increased the 5-HIAA/5-HT ratios in the mPFC, nucleus accumbens and amygdala, relative to both the rep shock-HC and rep no shock-no shock groups (Fig. 8).

DISCUSSION

Effects of Two Intensities of Foot Shock

The present results showed that the DA metabolism was only selectively increased in the mPFC after 1.5 mA foot shock but increased in most of the brain regions after 2.5 mA foot shock. In the mPFC, the increase in DOPAC levels after 2.5 mA foot shock was significantly larger than that after 1.5 mA foot shock, indicating an augmentation of the mPFC DA activation along with shock intensity. These results suggest that the mPFC is the most responsive to stress in agreement with many previous reports (3,11,26). Some reports indicate that DOPAC levels do not necessarily reflect the extent of synaptic DA release (4). However, because several studies have reported that various types of physical stress increase the DA efflux in the mPFC and nucleus accumbens using in vivo microdialysis studies (1,20), the increase in DA metabolism shown in the present study can be seen as reflecting an increased activity of DA neurons in the mPFC. Increases in the DOPAC levels in the nucleus accumbens and striatum were seen in the 2.5 mA shock group but not in the 1.5 mA shock group. These findings are consistent with the view that, although mild stress selectively activates the mesoprefrontal cortical DA system, more severe stress activates, not only the mesocortical DA system, but also the mesolimbic and nigrostriatal DA systems (9).

HVA levels were found to have increased in the mPFC, nucleus accumbens, and striatum after 2.5 mA foot shock, but not at all after 1.5 mA foot shock. In the PVH, amygdala, and lateral hypothalamus, the absence of significant changes in the HVA levels at 2.5 mA shock level was somewhat puzzling when compared with the increased DOPAC levels in these regions following foot shock. Although few data exist that directly compare DOPAC and HVA levels in a variety of regions in postmortem brains of animals exposed to stress, one report did also show that the HVA results were more variable than the DOPAC, and that they did not always parallel increases in the DOPAC levels (11,12). These discrepancies between regional changes in DOPAC and HVA levels after stress might be ascribed to any of the following three facts: first, HVA is derived from DOPAC and 3-methoxytyramine

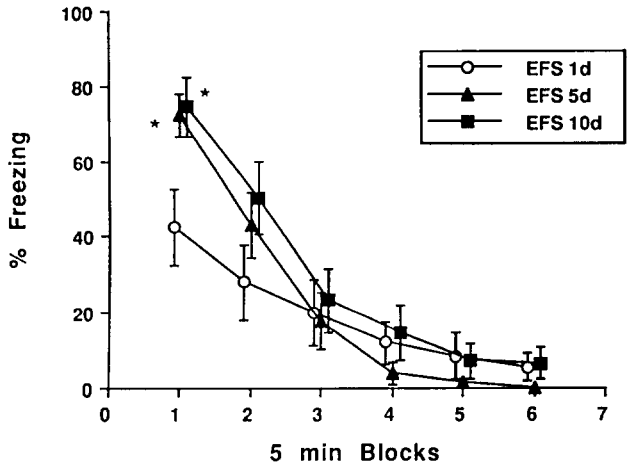

FIG. 4. Effect of repeated foot shock stress of different durations on conditioned fear stress-induced freezing. Rats were subjected to electric foot shock stress (EFS) for 30 min every day for 1, 5, or 10 days. They were placed for 30 min in the shock box 24 h after the last shock session but current was not applied to the floor of the box. Values are mean percent ± SEM of freezing scored for each 5-min block of testing obtained on 14 rats. Behavior was sampled at 10-s intervals. *Significant difference from 1 day group (EFS 1d), $p < 0.01$.

TABLE 2

EFFECTS OF CONDITIONED FEAR STRESS AFTER SINGLE OR REPEATED FOOT SHOCK ON DOPAMINE, HVA, AND 5-HIAA LEVELS (pmol/mg PROTEIN) IN DIFFERENT BRAIN REGIONS

	After Single Footshock		After Repeated Footshock		
	Dopamine	HVA	Dopamine	HVA	5-HIAA
Medial prefrontal cortex					
No shock-HC	12.1 ± 0.9	2.49 ± 0.09	12.8 ± 0.6	3.01 ± 0.22	7.3 ± 0.7
Shock-HC	15.8 ± 1.0	3.27 ± 0.19	13.6 ± 0.6	3.21 ± 0.18	6.9 ± 0.7
No shock-no shock	16.3 ± 0.9	3.62 ± 0.35	12.1 ± 0.7	3.28 ± 0.27	7.2 ± 0.8
Shock-no shock	14.8 ± 1.8	6.97 ± 0.70†§	13.7 ± 0.9	5.17 ± 0.24†§	10.1 ± 0.9*‡
Nucleus accumbens					
No shock-HC	853.4 ± 35.6	41.4 ± 2.1	866.8 ± 38.3	40.4 ± 1.5	15.7 ± 1.7
Shock-HC	832.6 ± 42.1	41.1 ± 2.4	907.2 ± 19.3	39.8 ± 2.3	17.7 ± 1.4
No shock-no shock	830.4 ± 42.6	50.4 ± 2.8¶	870.3 ± 31.1	41.2 ± 2.5	17.6 ± 1.7
Shock-no shock	720.0 ± 45.5	52.4 ± 2.7†	856.5 ± 39.5	62.2 ± 2.3†§	21.4 ± 1.8
Striatum					
No shock-HC	1153.0 ± 29.2	70.5 ± 1.9	937.7 ± 44.4	51.7 ± 2.4	15.5 ± 2.3
Shock-HC	1181.6 ± 37.0	68.0 ± 3.7	991.0 ± 32.2	57.3 ± 3.4	16.6 ± 2.1
No shock-no shock	1222.4 ± 39.8	83.1 ± 2.3#	920.2 ± 20.7	60.5 ± 3.4	16.4 ± 2.1
Shock-no shock	1159.0 ± 45.2	81.3 ± 3.6†	927.1 ± 22.5	69.3 ± 3.8*	17.4 ± 2.1
Paraventricular nucleus of the hypothalamus					
No shock-HC	35.5 ± 2.1	2.79 ± 0.28	34.6 ± 2.8	3.73 ± 0.25	10.8 ± 1.5
Shock-HC	28.3 ± 2.0#	2.41 ± 0.29	35.9 ± 2.7	4.13 ± 0.39	12.4 ± 0.9
No shock-no shock	37.3 ± 1.8	3.25 ± 0.17	32.7 ± 2.2	3.23 ± 0.39	13.4 ± 0.7
Shock-no shock	48.3 ± 2.0†§	3.85 ± 0.24†	49.3 ± 5.1*§	3.92 ± 0.29	15.5 ± 0.4*
Amygdala					
No shock-HC	53.6 ± 5.3	2.55 ± 0.13	45.2 ± 5.0	2.24 ± 0.18	10.8 ± 0.7
Shock-HC	49.4 ± 3.8	3.02 ± 0.18	46.1 ± 5.8	2.46 ± 0.22	11.8 ± 0.6
No shock-no shock	51.9 ± 7.5	3.25 ± 0.15#	53.2 ± 7.5	2.54 ± 0.26	11.2 ± 0.8
Shock-no shock	55.1 ± 3.6	4.05 ± 0.33†‡	53.9 ± 3.8	3.71 ± 0.22†§	12.8 ± 0.5
Lateral hypothalamus					
No shock-HC	19.0 ± 1.3	1.49 ± 0.14	10.1 ± 0.8	1.89 ± 0.20	7.5 ± 0.5
Shock-HC	17.2 ± 1.1	1.37 ± 0.14	10.8 ± 0.9	2.02 ± 0.14	7.3 ± 0.4
No shock-no shock	20.2 ± 0.9	1.59 ± 0.08	11.1 ± 1.2	2.34 ± 0.16	7.4 ± 0.4
Shock-no shock	19.0 ± 0.8	1.78 ± 0.19	13.5 ± 1.0	2.41 ± 0.12	7.5 ± 0.4
Hippocampus					
No shock-HC	1.25 ± 0.38	0.63 ± 0.06	0.40 ± 0.03	0.46 ± 0.02	5.9 ± 0.3
Shock-HC	0.86 ± 0.13	0.59 ± 0.11	0.46 ± 0.05	0.56 ± 0.05	6.5 ± 0.5
No shock-no shock	0.90 ± 0.11	0.73 ± 0.05	0.42 ± 0.03	0.53 ± 0.03	6.3 ± 0.4
Shock-no shock	0.95 ± 0.11	0.89 ± 0.10	0.89 ± 0.09†§	0.65 ± 0.03‡	5.9 ± 0.3

Results are means with SEM of data obtained on seven to eight rats.

No shock-HC: rats exposed to the apparatus without shock for 1 or 10 days and left quietly in their home cages 24 h after the last session; shock-HC: rats shocked for 1 or 10 days and left quietly in their home cages 24 h after the last shock session; no shock-no shock: rats exposed to the apparatus without shock for 2 or 11 days; shock-no shock (conditioned fear): rats shocked for 1 or 10 days, but merely exposed to the apparatus without shock 24 h after the last shock session.

Significantly different from the shock-HC (home cage) group (*$P < 0.05$; †$P < 0.01$), from the no shock-no shock group (‡$P < 0.05$; §$P < 0.01$) or from the no shock-HC group (#$P < 0.05$; ¶$P < 0.01$).

(3-MT), although about 80% of HVA is formed from DOPAC (37). Second, about 40% of DOPAC is not O-methylated to HVA but is removed from the brain by energy dependent active transport mechanisms (37). Third, some HVA is generated postmortem from 3-MT, which increases dramatically after death (17).

The present data demonstrated that foot shock (only at the 2.5 mA level) and CFS increased the DA concentrations in the PVH and hippocampus but not any other regions. These findings suggest an enhanced synthesis of DA, and are consistent with previous reports that stress enhanced DA concentrations in the hypothalamus (7,31). However, inconsistent with our data, other reports have shown that stress produced no changes in the DA levels in the hypothalamus (11,13) or hippocampus (11). De Souza et al. demonstrated that a 2-min restraint increased the DA concentrations in the hypothalamus at 30 min after the onset of the stress but not at 5 or 15 min, and that the same restraint increased the synthesis rate of DA in the hypothalamus at 30 min after the onset of the stress (7). Thus, increases in the DA concentrations in the PVH during stress may be attributable to increased DA synthesis.

Taking the 5-HIAA/5-HT ratio as an index of 5-HT turnover, our results show that the 5-HT turnover was increased in the mPFC, nucleus accumbens, and lateral hypothalamus

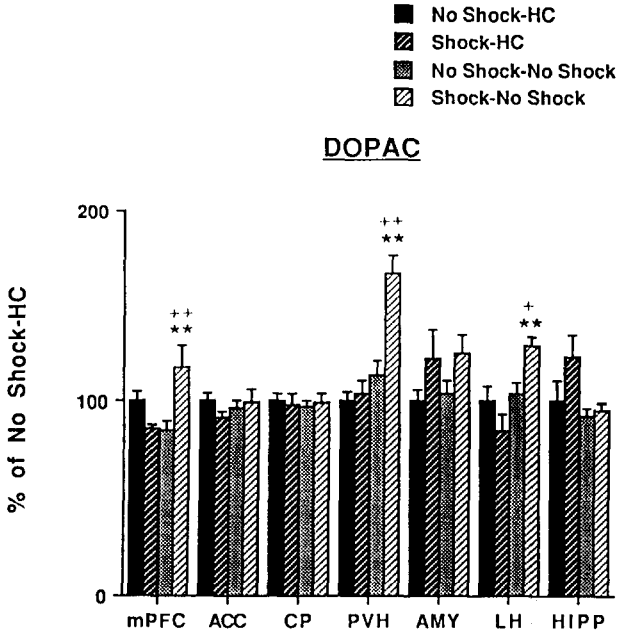

FIG. 5. Effects of conditioned fear stress after single foot shock on DOPAC levels in different brain regions. Results are means with SEM of data obtained on seven to eight rats and are expressed as percentage of respective no shock-HC values (pmol/mg protein, mPFC, 3.91 ± 0.20; ACC, 130.7 ± 4.5; CP, 107.4 ± 4.5; PVH, 3.37 ± 0.23; AMY, 5.87 ± 0.36; LH, 3.46 ± 0.27; HIPP, 0.54 ± 0.06). Abbreviations are as shown in Fig. 1. No shock-HC: rats exposed to the apparatus without shock on day 1 and left quietly in their home cages on day 2; shock-HC: rats shocked on day 1 and left quietly in their home cages on day 2; no shock-no shock: rats exposed to the apparatus without shock on both days 1 and 2; shock-no shock (conditioned fear): rats shocked on day 1, but merely exposed to the apparatus without shock on day 2. **Significant difference from the shock-HC group, $p < 0.01$; $^{+}$Significant difference from the no shock-no shock group, $p < 0.05$; $^{++}$Significant difference from the no shock-no shock group, $p < 0.01$.

following 2.5 mA foot shock, consistent with our previous data (19). However, 1.5 mA foot shock did not increase the 5-HIAA/5-HT ratio in any of the brain regions, while it selectively increased the DA metabolism in the mPFC. These suggest that 5-HT responses are less responsive to mild physical stress than DA responses are. In contrast to the DA metabolism, which increased in most of the brain regions after 2.5 mA foot shock, the 5-HT metabolism increased in only a few. Thus, there are some differences between DA and 5-HT responses to foot shock stress.

A high-intensity foot shock significantly elevated the brain tryptophan levels in the mPFC, medial striatum, lateral striatum, and amygdala, but a low-intensity foot shock did not. These findings are consistent with previous reports (6,11, 12,19,24,25). Kennet et al. showed that administration of valine, which interferes with brain tryptophan uptake and can prevent the increase in brain tryptophan that follows restraint, also prevented the increase in 5-HIAA (24). They suggested that the increase in 5-HIAA in the brain was at least partly dependent on the increase in brain tryptophan (24). Thus, in the mPFC, the increased 5-HT metabolism during high-intensity foot shock might be explained in part by elevated levels of brain tryptophan. However, because CFS after both single and repeated foot shock increased the brain 5-HIAA levels without any changes in the brain tryptophan levels as we have demonstrated in the previous and the present studies (19), not all increases in the 5-HT metabolism following physical stress can be explained by the increase in brain tryptophan. The increased levels of brain tryptophan during foot shock might be produced by physical stimuli such as pain and not attributable to emotional reaction.

As discussed in our previous paper (19), an increased metabolism of 5-HT is not always indicative of increased neuronal activity (4). Recently, while electrophysiological studies have not confirmed it (21), in vivo microdialysis studies have suggested that the increased metabolism of 5-HT after stress reflects an increase in the release of synaptic 5-HT (our unpublished data, 33). Thus, the increases in the 5-HT metabolism shown in the present study, at least, relate to an increase in the activity of serotonergic neurons.

The present data provide evidence to support the hypothesis that the regional patterns of brain DA and 5-HT activation after stress depend on the intensity of the stressor, a quantitative characteristic (9,35). Furthermore, the present experiment demonstrated that rats that received high-intensity shock displayed more freezing than those that received low-intensity shock in the conditioned fear paradigm. These findings are consistent with other reports (14,34), and suggest a functional significance for regional DA and 5-HT responses to various intensity of foot shock: DA and 5-HT activation in the more extended brain regions, including the mPFC, may be a result of the strength of fear conditioning that reflects the degree of emotional reaction caused by foot shock.

Effects of CFS After Single and Repeated Foot Shock

In contrast to physical stress, which increased the DA metabolism in most of the brain regions when the intensity of that stress was severe enough [(9,10,11,16), the present study), some studies have consistently reported that psychological

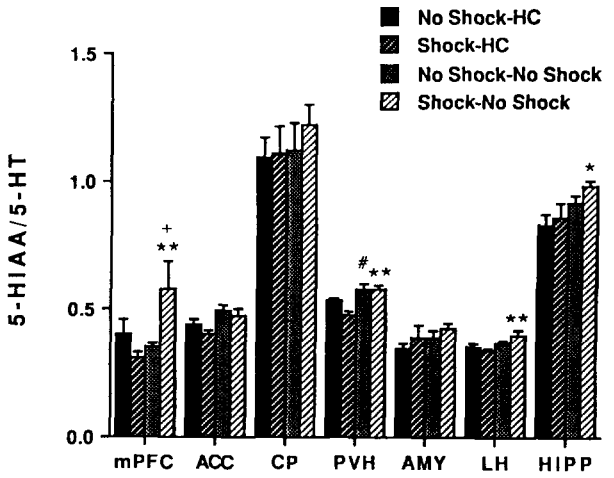

FIG. 6. Effects of conditioned fear stress after single foot shock on 5-HIAA/5-HT ratios in different brain regions. Results are means with SEM of data obtained on seven to eight rats. Abbreviations are as shown in Figs. 1 and 5. *Significant difference from the shock-HC group, $p < 0.05$; **Significantdifference from the shock-HC group, $p < 0.01$; $^{+}$Significant difference from the no shock-no shock group, $p < 0.05$; $^{\#}$Significant difference from the no shock-HC group, $p < 0.05$.

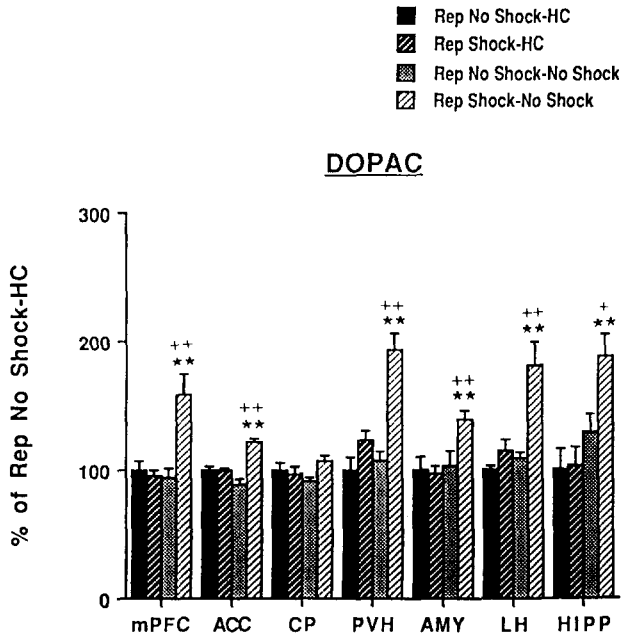

FIG. 7. Effects of conditioned fear stress after repeated foot shock on DOPAC levels in different brain regions. Results are means with SEM of data obtained on seven to eight rats and are expressed as percentage of respective rep no shock-HC values (pmol/mg protein, mPFC, 2.23 ± 0.15; ACC, 157.1 ± 5.5; CP, 93.0 ± 5.4; PVH, 3.87 ± 0.39; AMY, 4.83 ± 0.47; LH, 2.00 ± 0.07; HIPP, 0.54 ± 0.06). Abbreviations are as shown in Fig. 1. Rep no shock-HC: rats repeatedly exposed to the apparatus without shock for 10 days and left quietly in their home cages on day 11; rep shock-HC: rats repeatedly shocked for 10 days and left quietly in their home cages on day 11; rep no shock-no shock: rats repeatedly exposed to the apparatus without shock for 11 days; rep shock-no shock (conditioned fear): rats repeatedly shocked for 10 days, but merely exposed to the apparatus without shock on day 11. **Significant difference from the rep shock-HC group, $p < 0.01$; $^{+}$Significant difference from the rep no shock-no shock group, $p < 0.05$; $^{++}$Significant difference from the rep no shock-no shock group, $p < 0.01$.

stress, such as CFS, only selectively activates the mesoprefrontal cortical DA system (8,16,23). Herman et al. (16) and Deutch et al. (8) reported that CFS (after single foot shock) selectively increased the DOPAC levels in the mPFC of rats but not in any other brain regions. Kaneyuki et al. (23) also found that another type of psychological stress (exposure to the emotional responses of foot shocked rats) selectively increased the DOPAC levels in the mPFC of rats. Thus, three reports indicate the regional specificity of psychological stress-induced activation of the mesoprefrontal cortical DA system. In the present study, we found that CFS after single foot shock increased the DOPAC levels in the mPFC, PVH and lateral hypothalamus, and increased the HVA levels in the mPFC and amygdala. Although the present study fails to demonstrate selective CFS-induced DA activation in the mPFC, our results are still consistent with the previous studies (8,16,23) because they did not examine the effects of CFS on DOPAC levels in the PVH and lateral hypothalamus. With respect to changes in HVA levels during CFS, there have been few reports until now. Our present data indicate that the DA activation induced by CFS after single foot shock is especially marked, but not selective, in the mPFC of rats.

In the present study, the augmentation of conditioned fear produced by repeated conditioning, like foot shock per se, resulted in DA activation in most of the brain regions studied except for the striatum in contrast to CFS after single foot shock, which increased the DA metabolism markedly in the mPFC. Both the lack of direct physical stimuli (e.g., pain) in CFS and the psychological interpretation that CFS is a simple animal model of fear or anxiety suggest that this brain DA activation during CFS directly reflects emotional or cognitive processes, and is not due to any nonspecific physical influence. All these things make it clear that regional patterns of brain DA activation during psychological stress are dependent on the intensity of that stress; and that, although mild psychological stress markedly activates the mesoprefrontal cortical DA system, more intense psychological stress activates DA systems in more extended brain regions.

The DA metabolism in the striatum was increased by electric foot shock, but not altered by CFS after repeated foot shock. There are three possible explanations for this difference between foot shock and CFS in the striatal DA response: first, because electric shocks induce intense motor activity whereas conditioned fear causes a reduction in motor activity, i.e., freezing, it could be argued that this difference relates to the level of activity of the animals. This explanation is supported by previous data showing that the DOPAC and HVA levels in the striatum are increased when rats are induced to walk on a rotarod, which is compatible with the view that nigrostriatal DA neurons are strongly associated with motor function (35). Furthermore, Nabeshima et al. reported a decrease, rather than no change, in the DA turnover in the striatum of rats that exhibited a conditioned suppression of motility, a similar paradigm to CFS (28). They suggested that this reduction in the nigrostriatal DAergic function is related to the reduction of locomotion being expressed as a conditioned suppression of motility. Second, this difference may be related to the difference between foot shock and CFS in that the latter has no physical stimuli, foot shock producing pain but CFS producing none. However, this seems unlikely because previ-

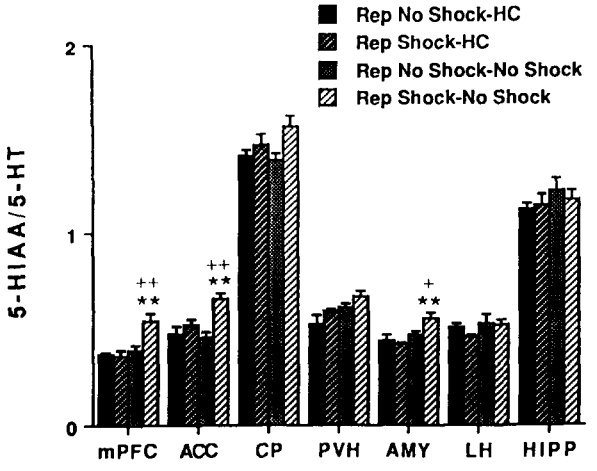

FIG. 8. Effects of conditioned fear stress after repeated foot shock on 5-HIAA/5-HT ratios in different brain regions. Results are means with SEM of data obtained on seven to eight rats. Abbreviations are as shown in Figs. 1 and 7. **Significant difference from the rep shock-HC group, $p < 0.01$; $^{+}$Significant difference from the rep no shock-no shock group, $p < 0.05$; $^{++}$Significant difference from the rep no shock-no shock group, $p < 0.01$.

ous studies indicated that mild foot shock, which does not evoke changes in locomotor behavior, does not affect nigrostriatal DA neurons (35); and because we show that mild foot shock did not change the DA metabolism in the striatum. Third, this difference might be explained by assuming that CFS after repeated foot shock is insufficient to induce the same degree of intense emotional reaction as foot shock. To clarify this question, additional experiments are needed to further examine the effects of more intense psychological stress on the striatal DA response than that used in our present study.

As we previously reported (19), CFS after single foot shock selectively increased the 5-HT metabolism in the mPFC, taking the 5-HIAA/5-HT ratio as an index of 5-HT turnover. However, the augmentation of CFS, like 2.5 mA foot shock, increased the 5-HT metabolism in more brain regions, including the mPFC, nucleus accumbens, and amygdala. Accordingly, the regional differences between foot shock and CFS in brain 5-HT activation, which we indicated previously (19), might be, in part, due to the intensity of stress. Thus, regional patterns of brain 5-HT activation during CFS are also dependent on the intensity of that CFS, similar to the case of physical stress and brain DA activation by CFS. This 5-HT activation in more extended brain regions following CFS after repeated foot shock may be related to the augmentation of conditioned fear as indicated by the enhancement of the freezing response in behavioral analysis.

In addition, it must be noted that CFS after single foot shock increased both the DA and 5-HT metabolism in the mPFC, while mild foot shock increased the DA but not 5-HT metabolism in the mPFC. Furthermore, the increase in the DA metabolism in the mPFC during mild foot shock (192% in the controls) was larger than that during CFS after single foot shock (137% in the shock-HC group). This comparison between mild foot shock and CFS after single foot shock suggests that the mPFC 5-HT activation is more responsive to psychological stress than to physical stress. Interestingly, recent studies reported that freezing behavior induced by CFS is attenuated by a 5-HT_{1A} agonist and suggested that conditioned fear is associated with brain serotonergic neuron systems (18,30). Because CFS after single foot shock produced marked behavioral changes such as freezing behavior, 5-HT activation in the mPFC might be closely related to the feeling of anxiety or fear.

In conclusion, the present study suggests that regional patterns of brain DA and 5-HT activation after physical stress and psychological stress depend on the intensity of that stress, although there are some differences between these stress (i.e., 5-HT activation in the mPFC after psychological stress and striatal DA activation after physical stress); and that the more widespread activation of DA and 5-HT after more severe stress might relate to behavioral changes which reflect the augmentation of fear.

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