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Sex Differences in the Effects of Inescapable Footshock on Central Catecholaminergic and Serotonergic Activity

ROB P. W. HEINSBROEK, FRANS VAN HAAREN, MATTHIJS G. P. FEENSTRA, HUGO VAN GALEN, GERARD BOER AND NANNE E. VAN DE POLL

Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands

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HEINSBROEK, R. P. W., F. VAN HAAREN, M. G. P. FEENSTRA, H. VAN GALEN, G. BOER AND N. E. VAN DE POLL. *Sex differences in the effects of inescapable footshock on central catecholaminergic and serotonergic activity.* PHARMACOL BIOCHEM BEHAV **37**(3) 539–550, 1990.—In two experiments sex differences in changes in central noradrenergic, dopaminergic and serotonergic activity were measured immediately after a 30-min session of inescapable footshocks. In Experiment 1 concentrations of noradrenaline, dopamine, serotonin and their major metabolites were determined in the frontal cortex, hypothalamus, amygdala, striatum, mesencephalon and the medulla-pons area. Inescapable shock increased the activity of all 3 transmitter systems, as evidenced by increased metabolite concentrations in specific brain areas. Shock-induced increments in metabolite levels were larger in females than in males, especially for the serotonergic system. In addition, shock presentation resulted in a decrement in the noradrenaline content in most areas studied. In the frontal cortex, noradrenaline was reduced by inescapable shock in males but not in females. In Experiment 2, sex-dependent neurochemical consequences of predictable versus unpredictable shocks were studied in the frontal cortex and the medulla-pons area. Similar to Experiment 1, both brain parts showed large shock-induced increments in the activity of the catecholaminergic systems. Differential effects of predictable and unpredictable shock were not found (frontal cortex) or were rather small (medulla-pons) and appeared sex-dependent for serotonin in this area. The sex differences in neurochemical changes found in the first experiment were largely replicated in the second experiment. The relevance of the observed sex differences in central neurotransmitter reactivity for sex differences in behavior is discussed.

Inescapable shock Serotonin Noradrenaline Dopamine Sex differences Rats

IN rats, sex differences in both behavioral and physiological responses to aversive stimulation have consistently been found. Response inhibition observed in passive avoidance and taste aversion procedures is larger and longer lasting in male rats than in female rats (18, 47–52). In active avoidance procedures female rats invariably show faster acquisition of avoidance responding (11,53), whereas extinction of avoidance behavior has been found to be slower in female rats (12). Inescapable aversive stimulation was also found to affect behavior sex-dependently, learning to escape from shock in a shuttle-box task was disrupted by prior exposure to inescapable shock (IS) and this effect was stronger in males than females (29,43).

In addition to these behavioral differences between the sexes, the magnitude of a number of physiological responses to aversive stimulation has been found to be sex-dependent. Different procedures, including restraint, footshock, ether, forced running and novelty, all invariably produced larger increases in pituitary-adrenocortical activity in female rats than in male rats (20, 27, 30, 33, 34). Furthermore, larger increases in plasma levels of oxytocin, vasopressin and prolactin were observed in female rats than in male rats after restraint (19,58). Restraint also resulted in higher plasma catecholamine concentrations in females than in males (34,35). Finally, decreases in growth hormone found after forced running, restraint, footshock or surgery were more prominent in males than in females (13,27).

Although a considerable amount of data is available with respect to sex differences in behavioral and peripheral physiological responses to aversive stimulation, information on sex differences in changes in central transmitter activity is virtually lacking. The effects of aversive stimulation on central transmitter activity have been shown to be rather severe in males (5, 8, 21, 44). Aversive stimulation produces increased utilization of central noradrenaline (NA) which initially is compensated for by increased synthesis, but prolonged stimulation eventually leads to reduced NA levels. Central serotonin (5-HT) is probably affected in a similar way, although it appears to be less sensitive to aversive stimulation than NA. Various stimuli have been found to activate mesocortical and mesolimbic dopamine (DA) projections. In particular, the frontal cortex (mesocortical DA) has been found to be very sensitive to aversive stimulation, whereas the nigro-

striatal DA system has been found to be relatively unresponsive (5, 8, 10, 21, 44).

In males, neurochemical responses have been directly related to changes in behavioral performance as a consequence of aversive stimulation. In particular, exposure to inescapable uncontrollable aversive stimulation seriously disrupts behavior. According to Weiss *et al.* (54–56), this behavioral disturbance is essentially an impairment of motor activity related to a decrease in brain NA. In addition to NA, reduced activity of DA and 5-HT as well as increased activity of central acetylcholine (ACh) have been proposed to mediate behavioral deficits induced by uncontrollable aversive stimulation (4, 6, 7, 24, 25, 41). Given the fact that behavioral differences between the sexes have been observed in response to aversive stimulation and that behavioral responses in males have been correlated with specific changes in central neurochemical parameters, it seemed important to establish whether or not males and females differ in central neurochemical responses to aversive stimulation.

Experiment 1 was thus designed to compare the effects of aversive stimulation on central NA, DA and 5-HT activity of male and female rats. Effects of IS on central neurotransmitter activity were studied in the following six brain areas: the frontal cortex, the hypothalamus, the amygdala, the striatum, the mesencephalon and the medulla-pons. These brain parts include major monoaminergic projection areas (frontal cortex, striatum, hypothalamus and amygdala), and nuclei of origin for DA and 5-HT (mesencephalon) and for NA (medulla-pons). Moreover, these areas have often been selected for studying the effects of aversive stimulation on central monoaminergic activity (8, 21, 23).

EXPERIMENT 1

METHOD

Subjects

Sixteen female and 16 male Wistar rats were obtained from Animal Supply House, TNO (Zeist, The Netherlands). The animals were 10 weeks old upon arrival in the laboratory and were maintained on a reversed light-dark cycle (lights on from 3:30 p.m. to 3:30 a.m.). When the animals were 15 weeks old they served as subjects in an open-field experiment (a single 5-min exposure). Ten weeks later the present experiment was started. Subjects were group-housed in standard cages (single sex). Food and water was always available at lib in these home cages.

Apparatus

Animals were placed in a large dark box (40 × 40 cm). The floor of the box consisted of 27 grids, spaced 1.4 cm apart (centre to centre). Scrambled footshocks could be delivered by manually operating a Grason-Stadler shock generator (model 700) attached to the grid floor.

Procedure

Experimental subjects (8 males and 8 females) were taken from their home cage and transported to the test room one at a time. They were confined to the test box for a period of 30 min. During this period a total of 60 scrambled inescapable footshocks (1 mA, 2 sec duration) were administered at a fixed-time (FT) 30-sec schedule. Immediately after the 60th shock, subjects were decapitated and brains were rapidly dissected. Control subjects (8 males and 8 females) were also removed individually from their home cage and were decapitated and dissected in the test room. The frontal cortex was obtained by cutting the frontal pole in a dorso-caudal to ventro-rostral direction and removal of small parts of the tuberculum olfactorium from the ventral side. Subsequently, a transversal cut was made through the optic chiasm in a dorso-caudal direction (slight angle). The striatum was removed from the rostral part. From the caudal part the hypothalamus was dissected, following the hypothalamic fissures. The amygdala was sampled by making horizontal cuts through the tissue lateral to the hypothalamus, this sample also contained the overlying pyriform cortex. The cerebellum was removed thereafter and the medulla-pons area was obtained by making a transversal cut rostral to the pons. The mesencephalon was obtained from the remaining part between the two transversal cuts by removing the overlying cortex and hippocampi and remains of the striatum. Samples were frozen on dry ice and stored at −80°C until assayed. Free levels of NA, 5-HT, DA, 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxyindoleacetic acid (5-HIAA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by using a modification (Feenstra, unpublished results) of the HPLC procedure with electrochemical detection described by Westerink (57).

RESULTS

Concentrations of NA, 5-HT, DA, their metabolites and the ratio of metabolite and transmitter concentrations are shown in Figs. 1 to 3 for separate brain parts of experimental and control male and female rats. Effects of IS and sex were evaluated for each neurochemical variable separately by using analysis of variance (anova) with treatment (IS, control) and sex as main factors. Post hoc comparisons were conducted by using Newman-Keuls tests ($\alpha = 0.05$).

NA and MHPG

Concentrations of NA and MHPG and the NA/MHPG ratios are presented in Fig. 1. With the exception of the striatum the main factor treatment was significant for all brain parts ($p<0.01$), NA concentrations were decreased after IS. In the frontal cortex a significant interaction was observed between sex and treatment, $F(1,28) = 5.76$, $p<0.05$. A decrease in NA after IS was found in the frontal cortex of males but not in the frontal cortex of females.

In the present study, MHPG could not be measured reliably in the striatum. In all other brain parts, MHPG was significantly increased after IS ($p<0.01$). Similarly, in all brain parts an increment in the MHPG/NA was found after IS ($p<0.001$). For MHPG a significant interaction of treatment and sex was found in the mesencephalon, $F(1,23) = 5.16$, $p<0.05$ and in the amygdala, $F(1,27) = 4.04$, $p = 0.05$. The MHPG/NA ratio in the amygdala also revealed a significant interaction between sex and treatment, $F(1,27) = 5.69$, $p<0.05$. Although the IS-induced increase in MHPG and MHPG/NA was significant for both males and females, this change was larger in females as compared to males.

5-HT and 5-HIAA

Data for 5-HT, 5-HIAA and 5-HIAA/5-HT are depicted in Fig. 2. No main effect of treatment was found for 5-HT. However, two significant interactions between sex and treatment were observed: one in the frontal cortex, $F(1,28) = 6.50$, $p<0.05$, and one in the mesencephalon, $F(1,23) = 5.03$, $p<0.05$. Figure 2 illustrates that in both brain parts 5-HT levels after IS decreased in males and increased in females. Irrespective of treatment, 5-HT levels in the medulla-pons were significantly higher in females as compared to males, $F(1,28) = 4.56$, $p<0.05$.

With the exception of the hypothalamus, significant interactions between sex and treatment were found for 5-HIAA in all

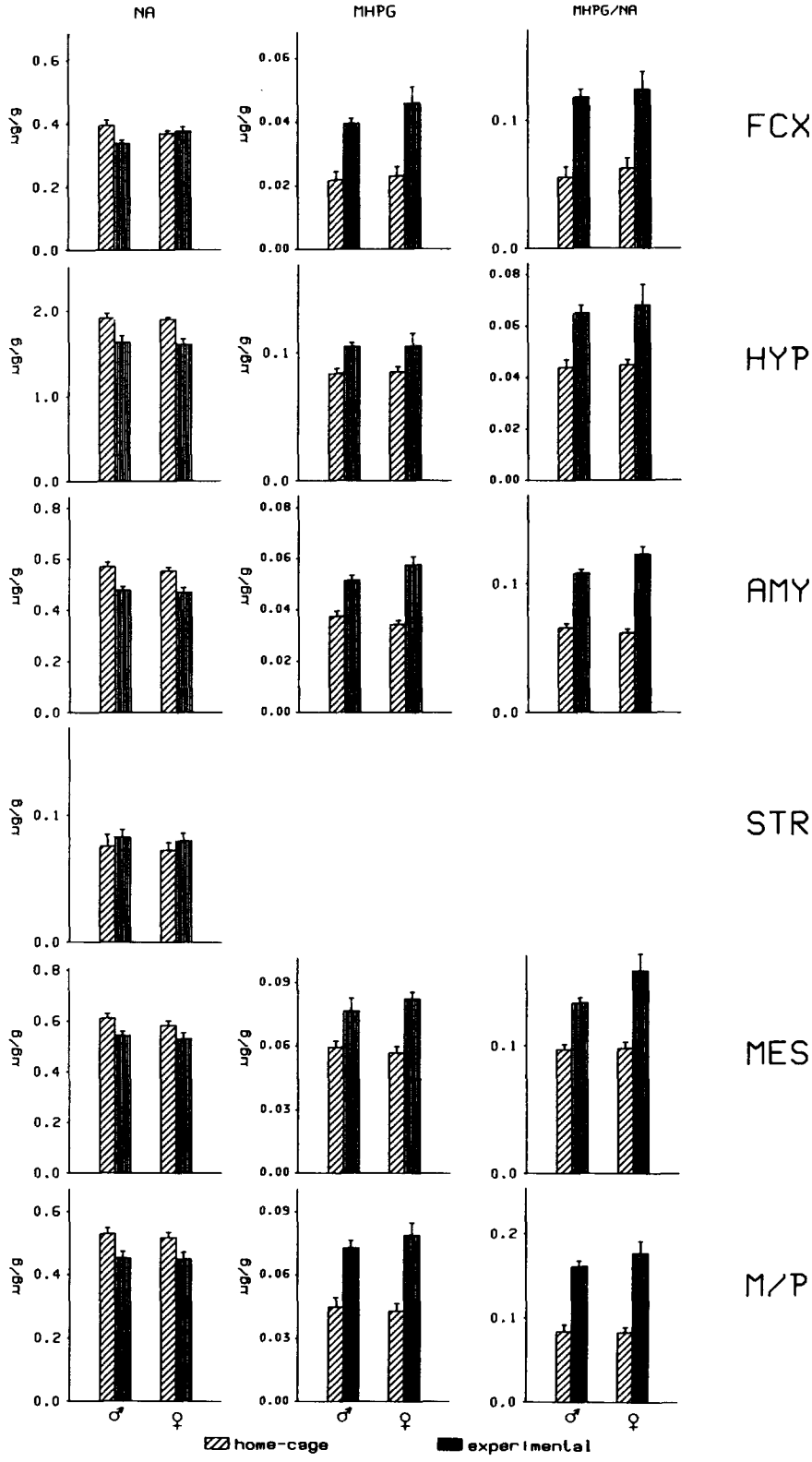

FIG. 1. Mean concentrations (±s.e.m.) of NA and MHPG, as well as the MHPG/NA ratios in the frontal cortex (FCX), hypothalamus (HYP), amygdala (AMY), striatum (STR), mesencephalon (MES) and medulla-pons (M/P) of experimental (shocked) and home cage control male and female rats.

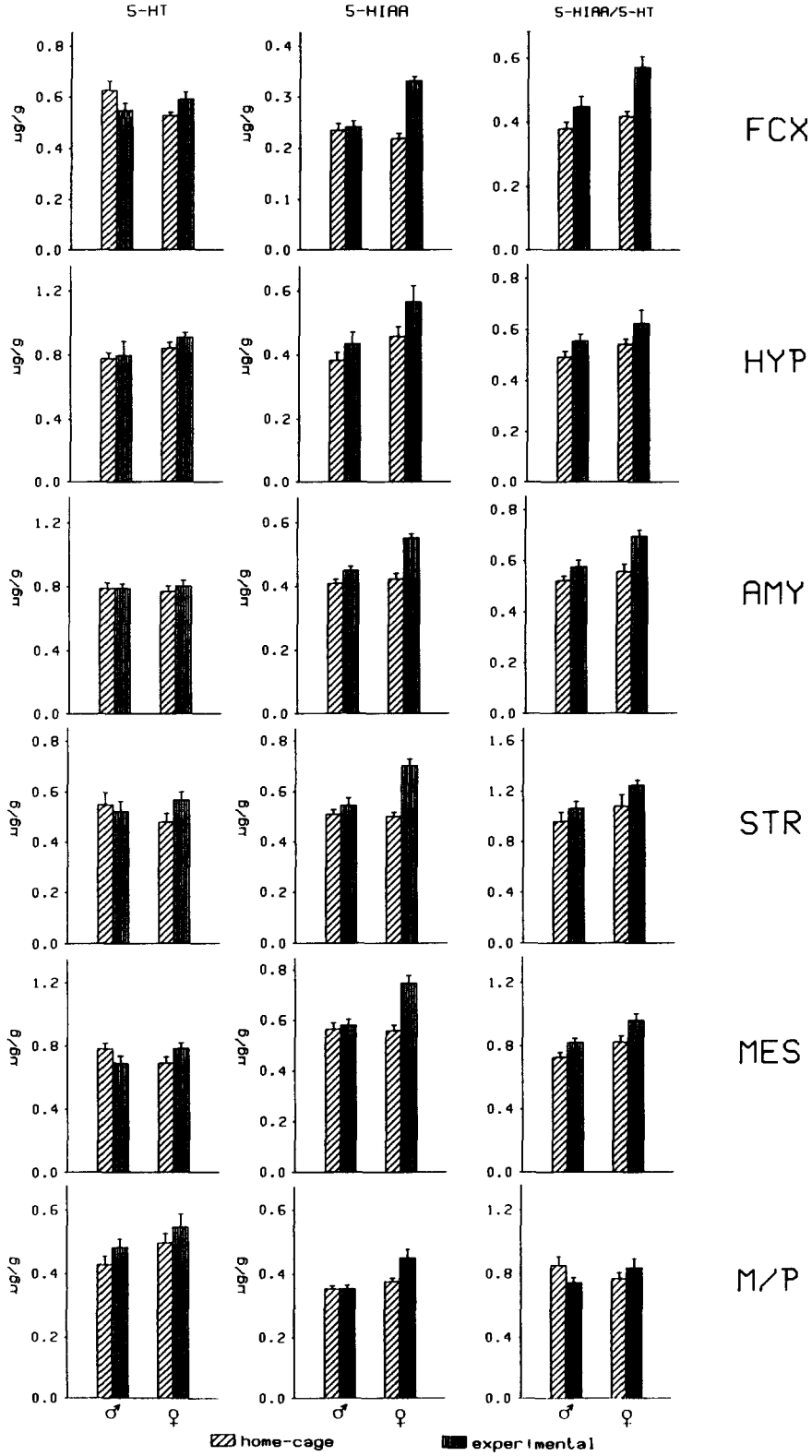

FIG. 2. Mean concentrations (±s.e.m.) of 5-HT and 5-HIAA, as well as the 5-HIAA/5-HT ratios in the frontal cortex (FCX), hypothalamus (HYP), amygdala (AMY), striatum (STR), mesencephalon (MES) and medulla-pons (M/P) of experimental (shocked) and home cage control male and female rats.

brain parts ($p<0.05$). Post hoc tests showed that in these 5 brain parts IS increased 5-HIAA levels in females but not in males. In the hypothalamus IS significantly increased 5-HIAA independent of sex, $F(1,24)=4.74$, $p<0.05$. IS also significantly increased the 5-HIAA/5-HT ratio in the frontal cortex, amygdala and mesencephalon ($p<0.01$), but significant interactions between sex and treatment were not found for the 5-HIAA/5-HT ratio. Irrespective of treatment, 5-HIAA levels in the hypothalamus were higher in females as compared to males, $F(1,24)=7.05$, $p<0.05$.

DA, DOPAC and HVA

Figure 3 presents the data for DA, DOPAC and the DOPAC/DA ratio. Results for HVA and HVA/DA were very similar to DOPAC and DOPAC/DA and are not presented graphically. DA increased as a consequence of IS in nearly every brain part ($p<0.05$), only the striatum was unaffected. Although the increment in DA after IS was usually larger in females than in males, interactions between sex and treatment were not observed for DA. A significant sex difference in DA content was found in the medulla-pons, females having higher levels as compared to males, $F(1,28)=4.41$, $p<0.05$.

Large increases were seen in DOPAC levels of IS subjects in most brain parts ($p<0.001$), but again the striatum was not affected by IS. Similarly, values of DOPAC/DA were increased by IS in all but the striatum sample ($p<0.001$). Increments in HVA content after IS were observed in all brain parts including the striatum ($p<0.001$). IS-induced increases in the HVA/DA ratio were significant for all brain parts with the exception of the hypothalamus ($p<0.01$). Both the DOPAC and HVA levels in the mesencephalon showed significant interactions between sex and treatment [DOPAC, $F(1,24)=8.13$, $p<0.01$; HVA, $F(1,24)=4.78$, $p<0.05$]: the response to IS was larger in females as compared to males. Significant interactions between sex and treatment were not found for the DOPAC/DA and HVA/DA ratios. Finally, independent of treatment, significantly higher values for DOPAC and for the DOPAC/DA ratio were observed in the hypothalamus of females as compared to males, $F(1,24)=5.88$, $p<0.05$ (DOPAC) and $F(1,24)=7.06$, $p<0.05$ (DOPAC/DA).

DISCUSSION

Data of this experiment show that 30 min of inescapable footshock (IS) resulted in a number of changes in neurochemical variables in both male and female rats. Large IS-induced increments in the metabolite concentrations were found for all 3 transmitter systems. These findings are in line with previous reports showing that metabolite levels provide a sensitive index of increments in central transmitter utilization (5, 8, 10, 21, 44). The present data add to this the observation that the magnitude of the neurochemical response to IS is sex-dependent. Large increases in central levels of 5-HIAA were found in females but not in males. This 5-HIAA response to IS was the most prominent sex-dependent response to the experimental manipulations. In addition, MHPG, DOPAC and HVA increments after IS were also larger in females as compared to males, although less conspicuous and consistent as observed for 5-HIAA.

Increments in metabolite concentrations are associated with an elevated utilization of the transmitter, whereas tissue levels of the transmitter itself reflect both synthesis and utilization (5, 8, 21, 44). Reductions in brain levels of NA found after aversive stimulation are most likely a consequence of an increased utilization of the transmitter which is only partly compensated by an increase in synthesis of NA (23). Although IS in the present experiment appeared to induce a larger utilization rate of NA in the female brain, a concomitant reduction in the NA tissue level was more conspicuous in the male brain, in particular in the frontal cortex. Similarly, the large increase in 5-HT utilization in the female brain was not accompanied by reduced tissue levels of 5-HT in the females. On the contrary, in females, IS tended to increase 5-HT levels, whereas in males the opposite pattern was found. In females, the 5-HIAA/5-HT ratio was less conspicuously affected by IS than 5-HIAA itself which also shows that, in females, both 5-HT and 5-HIAA were increased by IS. Finally, an IS-induced increment in DA was observed in most brain areas and was equal or slightly higher in females than in males. Taken together, exposure to IS resulted in a larger neurotransmitter utilization in females than in males which appeared to be accompanied by a higher synthesis rate in females as compared to males.

EXPERIMENT 2

Experiment 1 revealed that the central neurotransmitter activity is sex-dependently affected by IS. Research primarily conducted on male subjects has revealed that behavioral and physiological responses to aversive stimuli are modulated by the predictability of the stimulation. A reliable preference for predictable over unpredictable shock has been found (9), and various physiological responses like gastrointestinal pathology and pituitary-adrenocortical activity are differentially affected by predictable and unpredictable shock (2). Physiological responses to shock appear to be exaggerated by unpredictability if sessions are relatively short, whereas the reverse pattern may be found when the session is extended (2). In Experiment 1, shocks were presented on an FT schedule and therefore predictability of shock may have interacted with the neurochemical responses to shock per se. Evidence is available that predictability manipulated by temporal regularity of shock presentation has similar physiological consequences as compared to warning signals (1). It cannot be excluded that sex differences observed in Experiment 1 may have involved sex differences in response to predictability of shock rather than in response to shock per se. In particular, as behavioral sex differences consistently found in aversively motivated procedures suggest that male and female rats differentially respond to stimuli that predict onset of aversive stimulation. Females acquire efficient performance in signalled active avoidance more rapidly than males (11,53), and during extinction, avoidance behavior in response to presentation of the warning signal extinguished more slowly in females than in males (12).

Experiment 2 was designed to establish possible sex-dependent effects of predictability of shock. Inescapable shocks were presented on a variable time (VT) schedule and predictability and unpredictability were manipulated by presenting stimuli (light + tone) that were or were not correlated with shock. Neurochemical measurements in Experiment 2 were limited to the frontal cortex and the medulla-pons. The frontal cortex was selected on the basis of findings of Experiment 1. First of all, the sex-dependent effect of IS on NA was most conspicuous for the frontal cortex. Further, the changes in 5-HT variables induced by IS in the frontal cortex did not differ from those observed in other areas. Finally, the frontal cortex as part of the mesocortical DA system is more sensitive to aversive stimulation than the mesolimbic or nigrostriatal DA systems (8,10). The medulla-pons sample containing the locus coeruleus area was included on basis of experimental data suggesting that the locus coeruleus as the main origin of forebrain NA, plays a critical role in the response of this transmitter system to aversive stimulation (23, 31, 56).

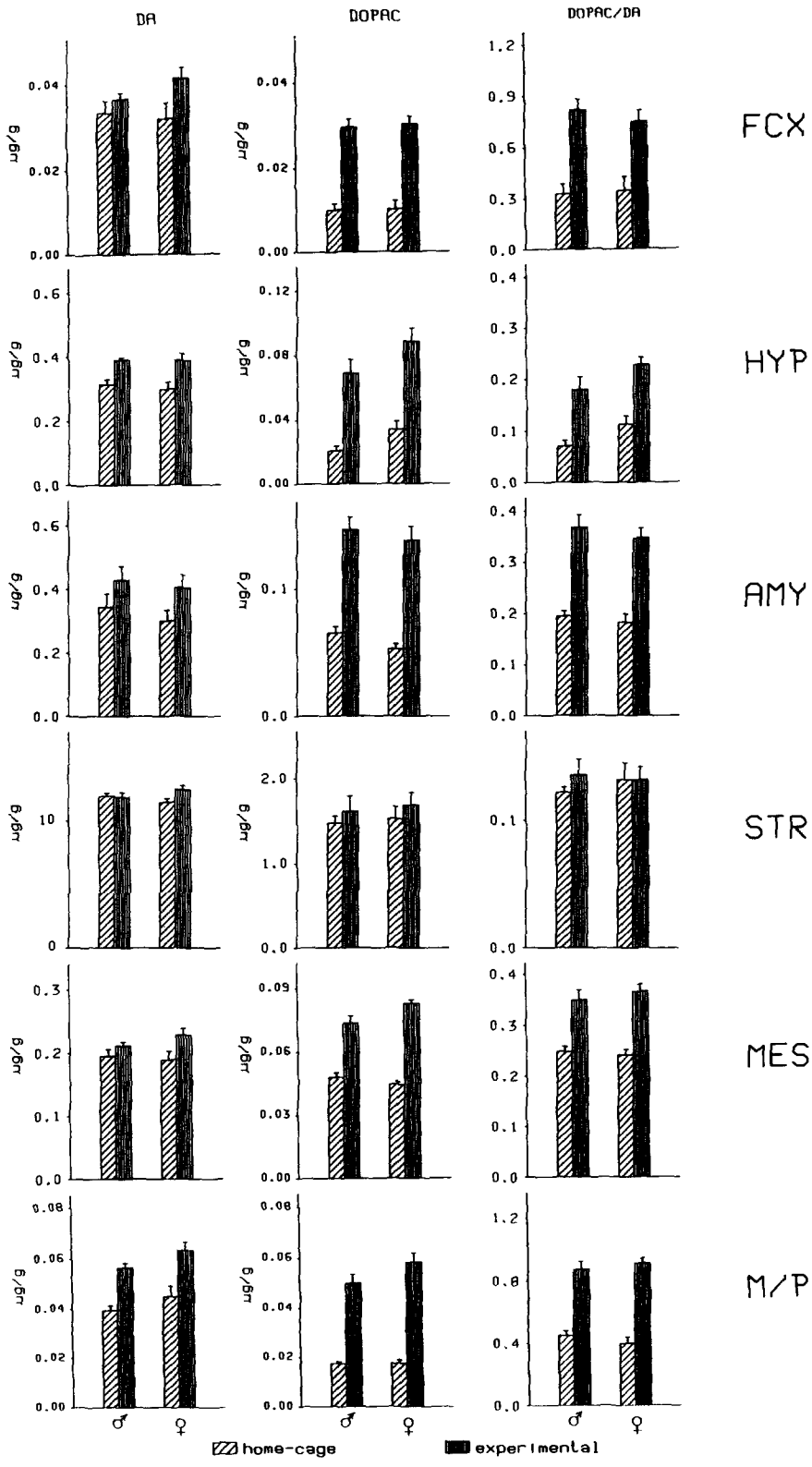

FIG. 3. Mean concentrations (±s.e.m.) of DA, and DOPAC, as well as the DOPAC/DA ratios in the frontal cortex (FCX), hypothalamus (HYP), amygdala (AMY), striatum (STR), mesencephalon (MES) and medulla-pons (M/P) of experimental (shocked) and home cage control male and female rats.

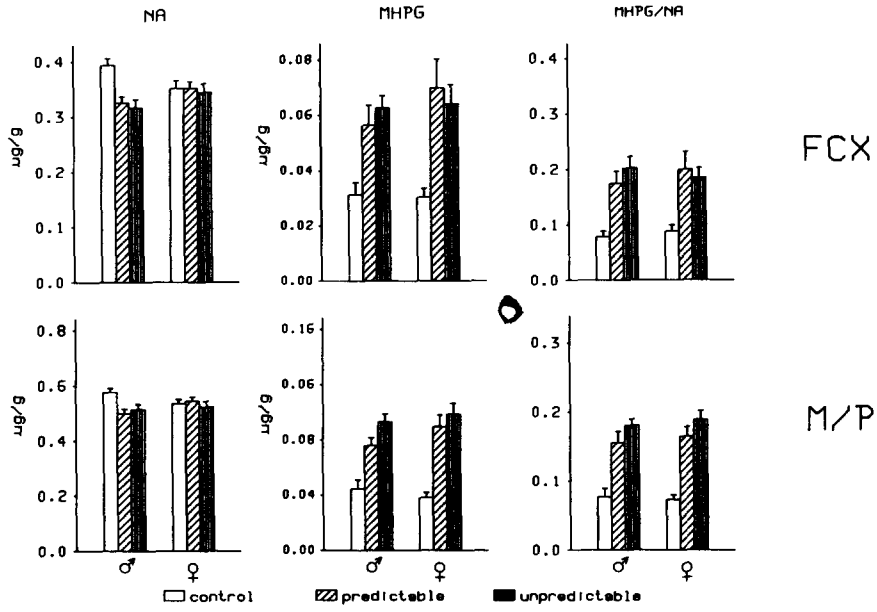

FIG. 4. Mean concentrations (±s.e.m.) of NA and MHPG, as well as the MHPG/NA ratios in the frontal cortex (FCX) and medulla-pons (M/P) of male and female rats exposed to predictable or unpredictable shock and of control rats not exposed to shock.

METHOD

Subjects

Twenty-four male and 24 female Wistar rats were obtained from the Animal House, TNO (Zeist, The Netherlands). At arrival in the laboratory animals were 9 weeks old, they were group-housed in standard cages (single-sex) and maintained under a reversed light-dark cycle (lights on from 3:30 p.m. to 3:30 a.m.). Three weeks after arrival at the laboratory experiments were started. Prior to experimentation subjects were weighed twice a week in the experimental room. In the animal quarters food and water were always available to the subjects.

Apparatus

Experiments took place in 3 Grason-Stadler (model 1111-L) rodent operant conditioning chambers. The floor (30×30 cm), consisting of 23 stainless steel grids, spaced 1.25 cm apart (centre to centre), was connected to a shock-generator (Grason-Stadler model 700). Two levers, normally located 10 cm above the floor on both sides of a pellet retrieval unit, were removed. A red and a green stimulus light were present above and slightly to the side of the original position of the levers. A white houselight was present in an upper corner of the intelligence panel. A speaker was mounted below and slightly to the side of the position of the right lever. The test chambers were enclosed in a sound attenuated compartment (Grason-Stadler model 1101) with a fan to provide fresh air. Programming of the experimental conditions was accomplished using Grason-Stadler 1200-series of programming equipment, located in the experimental room itself.

Procedure

One group of subjects (n=8) was exposed to predictable shock. They received 60 scrambled footshocks (1 mA, 2 sec duration) that were presented at a VT 30-sec schedule. Time between 2 successive shock presentations was at least 10 sec. Shock was preceded and partly coincided with a 7.5-sec stimulus (CS) which consisted of illumination of the houselight accompanied by a tone of 80 dB (1 kHz) against a background of 70 dB provided by the fan. CS presentations were alternated by periods during which only the red and green stimulus lights were illuminated and tone and houselight were off. Another group of subjects (n=8) received unpredictable shock. For this group of subjects the same tone and light stimuli were presented in the experimental chamber and 60 scrambled footshocks (1 mA, 2 sec duration) were presented on a VT 30-sec schedule. Shocks were, however, programmed to occur independently of the light and tone presentations. Control subjects (n=8) were exposed to tone and light parameters but shocks were never presented. Presentation of tone and light stimuli was synchronized for all 3 chambers. Session duration averaged 30 min. Immediately after session ending subjects were sacrificed by rapid decapitation and the brains were quickly removed from the skull. The frontal cortex and medulla-pons were sampled and stored as described for Experiment 1. Similar to Experiment 1, free DA, NA, 5-HT, MHPG, DOPAC, HVA and 5-HIAA was measured by using high-performance liquid chromatography with electrochemical detection.

RESULTS

The concentrations of NA, 5-HT, DA, their metabolites and the metabolite and transmitter ratios are presented in Figs. 4 to 6. Every variable was analysed separately using analysis of variance (ANOVA) with the main factors sex and treatment (control, predictable and unpredictable shock). Newman-Keuls multiple comparisons ($\alpha = 0.05$) were used for post hoc tests.

NA and MHPG

Figure 4 depicts the results for NA, MHPG and MHPG/NA. The main factor treatment was significant for NA in the frontal

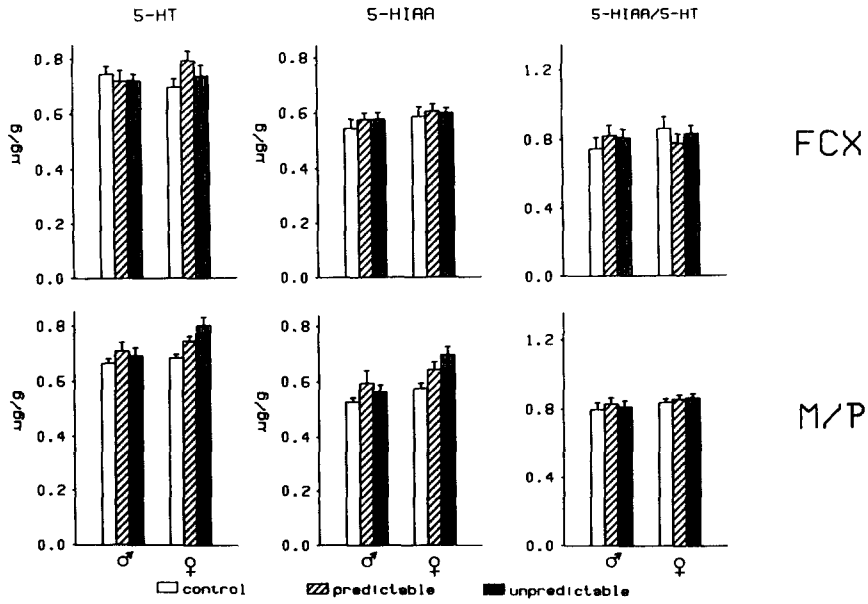

FIG. 5. Mean concentrations (±s.e.m.) of 5-HT and 5-HIAA, as well as the 5-HT/5-HIAA ratios in the frontal cortex (FCX) and medulla-pons (M/P) of male and female rats exposed to predictable or unpredictable shock and of control rats not exposed to shock.

cortex, $F(2,42)=5.70$, $p<0.01$, and marginally significant for NA in the medulla-pons, $F(2,42)=2.87$, $p=0.07$. In addition, a significant interaction between treatment and sex was found for NA in the frontal cortex, $F(2,42)=4.52$, $p<0.05$, and this interaction was marginally significant for NA in the medulla-pons, $F(2,42)=3.11$, $p=0.06$. Both predictable and unpredictable shock resulted in a reduction in the NA content of the frontal cortex and medulla-pons in males, but not in females. Differences between predictable and unpredictable shock were not observed for NA.

For both the frontal cortex and the medulla-pons a strong effect of treatment was found as a consequence of a large increment in MHPG levels in response to both predictable and unpredictable shock [frontal cortex: $F(2,42)=16.21$, $p<0.001$; medulla-pons: $F(2,42)=38.28$, $p<0.001$]. Differences between predictable and unpredictable shock were not significant and the MHPG response was not sex-dependent. The MHPG/NA ratio also revealed a significant effect of treatment in the frontal cortex, $F(2,42)=18.47$, $p<0.001$, and the medulla-pons, $F(2,42)=44.60$, $p<0.001$. Sex differences for the MHPG/NA ratio were not found, but post hoc comparisons did show that MHPG/NA in the medulla-pons was significantly higher after unpredictable shock as compared to predictable shock.

5-HT and 5-HIAA

Figure 5 presents the data for 5-HT, 5-HIAA and 5-HIAA/5-HT. No effects of treatment or sex were observed for 5-HT in the frontal cortex. 5-HT in the medulla-pons area showed a significant effect of treatment, $F(2,42)=4.84$, $p<0.05$. Unpredictable shock resulted in significantly elevated 5-HT levels in the medulla-pons, whereas predictable shock did not. The effect of unpredictable shock was particularly evident in females. However, only the main factor sex was significant, $F(1,42)=7.81$, $p<0.01$ and sex and treatment did not interact significantly. Analysis the effects of predictable and unpredictable shock separately did reveal a sex-dependent (marginally significant) effect of unpredictable shock, $F(1,28)=3.67$, $p=0.07$ (sex × shock), but not of predictable shock.

For the frontal cortex no effects of treatment or sex were found for 5-HIAA and the 5-HIAA/5-HT ratio. For the medulla-pons, treatment was significant for 5-HIAA, $F(2,42)=4.26$, $p<0.05$, but not for the 5-HIAA/5-HT ratio. Post hoc tests showed that both predictable and unpredictable shock resulted in significant elevations of 5-HIAA in the medulla-pons. A significant sex difference was also found for 5-HIAA in the medulla-pons, $F(1,42)=10.68$, $p<0.01$. 5-HIAA was higher in females than males and this was particularly evident after unpredictable shock. However, a significant interaction between sex and treatment was not observed. Separate analysis of the consequences of predictable and unpredictable shock showed that unpredictable shock resulted in a sex-dependent (marginally significant) elevation of 5-HIAA, $F(1,28)=3.40$, $p=0.08$ (sex × shock), whereas predictable shock did not.

DA, DOPAC and HVA

In Fig. 6 the results for DA, DOPAC and DOPAC/DA are presented. No figure is presented for HVA and HVA/DA, the results for these parameters will be described. DA in the frontal cortex was not affected by the experimental manipulations. On the other hand, DA in the medulla-pons showed a large increment in response to predictable and unpredictable shock. This response was larger in females than in males, but the interaction between sex and condition was only marginally significant, $F(2,42)=2.54$, $p=0.09$.

DOPAC and HVA in the frontal cortex showed large increments in response to predictable and unpredictable shock ($p<0.001$). Similarly, DOPAC/DA and HVA/DA were increased in the groups that were subjected to shock ($p<0.001$). However, the response of the DA metabolites in the frontal cortex did not depend on sex of the animal or the predictability of shock. DA

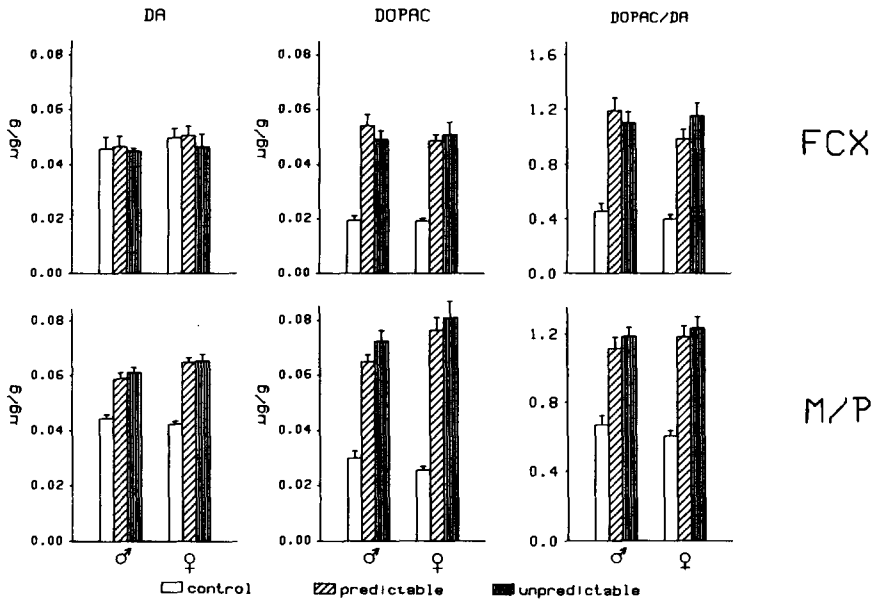

FIG. 6. Mean concentrations (±s.e.m.) of DA and DOPAC as well as the DOPAC/DA ratios in the frontal cortex (FCX) and medulla-pons (M/P) of male and female rats exposed to predictable or unpredictable shock and of control rats not exposed to shock.

metabolites in the medulla-pons were significantly increased after shock presentation. ANOVA revealed significant effects of treatment for DOPAC, HVA, DOPAC/DA and HVA/DA ($p<0.001$). The response of the DA metabolites in the medulla-pons was not affected by the predictability of shock. The DOPAC response to shock was larger in females than in males but similar to DA in the medulla-pons, the sex by treatment interaction was only marginally significant, $F(2,42)=2.40$, $p=0.10$.

DISCUSSION

Similar to Experiment 1, Experiment 2 showed large increments in NA and DA metabolite concentrations induced by exposure to IS and reflecting increased utilization of these transmitters in response to IS. Metabolite responses were somewhat larger in females than in males, although differences between the sexes were not significant. Concomitant IS-induced reductions in NA concentrations were again seen only in males and not in females. Increments in DA, induced by IS presentation, were found in the medulla-pons and were somewhat larger in females than in males. These findings show that results obtained for both catecholamine systems were very similar for Experiment 1 and Experiment 2. For 5-HT in the medulla-pons a larger IS-induced elevation of both 5-HT and 5-HIAA in females than in males was again noted. This was, however, only observed after unpredictable shock and not after predictable shock. In the frontal cortex, 5-HIAA appeared to be unaffected by IS presentation. However, the 5-HIAA concentration in the frontal cortex of control subjects was relatively high, suggesting that mere placement in the test chamber resulted in an activation of 5-HT in the frontal cortex. This interpretation is supported by the high 5-HIAA/5-HT ratio found in the frontal cortex of control subjects of Experiment 2 as compared to the home-cage control subjects of Experiment 1.

Experiment 2 was specifically designed to study effects of predictability on the neurochemical response of males and females to IS. Differential effects of predictability were, however, small or absent. The MHPG/NA ration in the medulla-pons showed a larger elevation in response to unpredictable than to predictable shock in both males and females. Furthermore, a larger activation of 5-HT in the medulla-pons of females than in the medulla-pons of males was noted after unpredictable, but not after predictable shock. This latter finding suggests that the large sex difference in 5-HT reactivity observed in Experiment 1 may have involved a sex-dependent response to predictability. Sex-dependent effects of predictability were not found for the catecholamines. Moreover, findings of Experiment 2 were generally in line with findings of Experiment 1, although a VT schedule of shock presentation was applied in Experiment 2 and a FT schedule in Experiment 1. These results suggest that sex differences in IS-induced changes in catecholamine variables are independent of possible sex-dependent effects of predictability.

Previous findings indicate that it may be of interest to extend these observations over a wider range of conditions. In a recent report (3), studying the effects of predictability of shock in male rats, it was shown that unpredictable shock was more effective in reducing central NA levels than predictable shock when material was sampled at a 30-min interval after shock presentation. After a 2-hour interval, levels of NA were elevated above control values in the unpredictable group but not in the predictable group. In addition to NA this study also revealed a differential response of the 5-HT system to predictable and unpredictable shock. The activity of the 5-HT system was more substantially increased by unpredictable shocks and this was particularly evident after the longer 2-hour interval. These data indicate that it may be profitable to study the effects of predictability at longer intervals after aversive stimulation.

GENERAL DISCUSSION

Both Experiment 1 and 2 revealed sex differences in the changes in central transmitter activity induced by exposure to IS. Increments in metabolite concentrations after IS were generally

higher in females as compared to males. In the first experiment this was observed for MHPG, DOPAC and HVA and was particularly evident for 5-HIAA. Although less conspicuous, the data from the second experiment revealed a similar trend. Tissue levels of the transmitters were also affected sex-dependently by IS. DA showed increases in response to IS which were somewhat larger in females than males, and 5-HT was increased by IS only in females. NA was decreased after IS and this response was more conspicuous in males than in females.

Although aversive stimulation is claimed to increase both synthesis and utilization of NA, tissue concentrations of NA are often reduced after exposure to aversive stimulation. This reduction is explained by assuming that synthesis cannot compensate for the increase in utilization (23). The present findings indicated that in response to IS, utilization of NA was somewhat higher in females than in males. In males, however, tissue levels of NA were reduced more clearly by IS than in females, suggesting that the female rat brain is better protected against IS-induced depletion of central NA. Synthesis and reuptake of NA are both increased by aversive stimulation (54) and the present results possibly relate to sex-dependent effects of IS on these mechanisms.

The findings of the present experiments are of particular interest with respect to sex differences in the behavioral consequences of IS. It has been shown that exposure IS resulted in a more severe disruption of subsequent shuttle-box escape behavior in males than in females (29,43). Depletion of central NA has been claimed to be causally related to IS-induced behavioral disturbance. Reduced central levels of NA diminish the capacity to initiate and maintain high levels of motor activity necessary for effective escape performance (55,56). In the present experiment, sex-dependent reductions in NA were noted in the frontal cortex (Experiment 1 and 2) and the medulla-pons (only Experiment 2). Selective depletion of NA was also seen in the frontal cortex and locus coeruleus area (LC) of the medulla-pons in males exposed to inescapable but not to escapable shock (56). However, NA depletion in the medulla-pons and, more specifically in the LC, correlated better with suppression of activity than the NA reduction found in the frontal cortex. Recently, evidence has been accumulated suggesting a role of forebrain NA in mediating another aspect of behavioral effects of IS. Depletion of forebrain NA (cerebral cortex and hippocampus) was proposed to subserve a deficit in selective attention (36,37). These findings, combined with the results of the present experiments, make it thus seem very likely that sex differences in behavioral disruption after IS are related to a sex-dependent reduction in central NA levels.

In Experiment 1 a larger response of 5-HIAA in females was found in all brain areas studied. A similar result was obtained after unpredictable shock for the medulla-pons area in Experiment 2, but not after predictable shock. Sex differences in the 5-HIAA response to aversive stimulation were also noted by Kennett *et al.* (28) who subjected male and female rats to restraint. Two hours of restraint induced a higher 5-HIAA response in the frontal cortex of males as compared to females. These findings, as well as the present ones, indicate that aversive stimulation sex-dependently affects 5-HT metabolism, but that the magnitude and the direction of differences between the sexes largely depends on the procedure and the duration of aversive stimulation.

Increments in central levels of 5-HIAA induced by aversive stimulation are generally not accompanied by decrements in 5-HT levels. The synthesis of 5-HT is increased by aversive stimulation and apparently compensates for the elevated utilization (5, 8, 21). The significant increment in 5-HT utilization suggested by the response of 5-HIAA in IS-females was not accompanied by a decrease in 5-HT concentration. In fact, concomitant small increments in 5-HT were seen in females in several brain areas. As a consequence, changes in the 5-HIAA/5-HT ratio were not as obvious as compared to the metabolite itself. These results suggest that IS evoked an increment in both the utilization and the synthesis of 5-HT in the female brain. This finding may relate to the fact that in females higher central levels of 5-HT and 5-HIAA are generally found (17). A higher 5-HT synthesis rate in females than in males has been proposed to underlie sex differences in basal 5-HT and 5-HIAA levels and may partly explain for the presently observed sex-dependent effects of IS on 5-HT variables. Sex-dependent activity of central 5-HT neurons may in turn be relevant for sex differences in behavioral reactions to aversive stimuli. Reduced activity of central 5-HT was suggested to contribute to the behavioral interference found after exposure to IS (38). As already noted, the IS-induced interference effect was found to be more pronounced in males than in females, and it can be suggested that a more active 5-HT system in females is involved in this sex difference. Other recent findings strongly suggest that 5-HT is involved in sex differences in behavioral changes induced by aversive stimuli. The sex differences in passive avoidance behavior were found to be abolished after pharmacological suppression of the central 5-HT activity (26).

Metabolites of DA showed substantial increments indicative of an IS-induced increase in the utilization of DA. DA concentrations were unaffected or increased by IS in the present experiment. In literature, increases in the DA metabolites induced by aversive stimulation have also been reported to be accompanied by unchanged or elevated levels of DA (22, 40, 42, 46). Synthetic activity appears to be increased in DA neurons as a consequence of aversive stimulation (40). Nevertheless, reduced levels of DA have been found after aversive stimulation as well (14, 32, 45). Lavielle *et al.* (32) showed that DA concentrations in the frontal cortex shows a rapid initial decline in response to shock, but that a further reduction is prevented probably by an increase in DA synthesis. Findings of Experiment 1 and 2 suggest that the experimental procedure increased both utilization and synthesis of DA in various brain areas, and that the increment in utilization and synthesis was generally larger in females than in males. Primarily pharmacological manipulations have led to the suggestion that altered DA activity in response to IS contributes to the behavioral effects of IS. The deleterious behavioral effects of IS were prevented by increasing DA activity pharmacologically. In addition, drugs that inhibit DA function were found to mimic the behavioral effects of IS (6, 7, 39). These results therefore suggest that decreased DA functioning subserves the behavioral effects of IS. Sex differences in the effects of aversive stimulation on central DA are also indicated by findings that the behavioral response to d-amphetamine is sex-dependently affected by a single (16) and repeated (15) exposure to inescapable aversive stimulation. The significance of sex differences in DA responsiveness for sex-dependent effects of IS remains to be established.

The results of previous experiments have consistently shown that male and female rats react differentially to aversive stimulation. The present findings showed that neurotransmitter activity is altered in a sex-dependent way after exposure to IS. These combined findings suggest that behavioral differences between the sexes in aversively motivated procedures are related to sex differences in central monoaminergic transmitter systems in response to aversive stimulation. Whether or not sex differences in brain and behavioral changes after aversive stimulation are causally related remains to be investigated in future experiments.

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