Alterations in Brain Norepinephrine Metabolism Induced by Environmental Stimuli Previously Paired with Inescapable Shock

Abstract. *Footshock stress produced an immediate increase in brain concentrations of 3-methoxy-4-hydroxyphenylglycol sulfate (MHPG-SO_4), a major metabolite of the neurotransmitter norepinephrine, in the rat brain. Twenty-four hours after footshock stress, when concentrations had returned to baseline, increases in MHPG-SO_4 and emotional behavior could be elicited by previously neutral environmental stimuli that had been paired with the stress.*

Stimuli in the environment can alert and prepare an organism for the onset of potentially rewarding or stressful events. Despite extensive research on the behavioral and physiological changes that take place when an organism reacts to such signals, however, we have little understanding of the neurochemical brain mechanisms underlying observed behavioral responses.

Since the early studies of Cannon (*1*), the increased release of catecholamines (dopamine, norepinephrine, and epinephrine) in the peripheral and central nervous system during stress has been extensively documented (*2, 3*). Only recently, however, has the presence of environmental stimuli that had been associated with stressful events been demonstrated to be sufficient to markedly increase peripheral measures of norepinephrine and epinephrine in rats (*4*). Moreover, the release or specific activity of intracerebrally administered, radioactively labeled norepinephrine are altered when rats are exposed to environmental stimuli previously paired with aversive (*5*) or appetitive (*6*) stimuli. In addition, the histofluorescence of catecholamine-containing cells increases in brains of isolated rats exposed to environmental stimuli previously associated with sweetened milk (*7*). However, alterations in the release and metabolism of endogenous norepinephrine in the brain under such conditions have not yet been documented by direct biochemical measurements.

Concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG) or its sulfate conjugate, a naturally occurring, major metabolite of endogenous norepinephrine in the brain, have been suggested as a measure of the functional activity of central noradrenergic neurons (*8*). We now report that stress-induced alterations in norepinephrine release and metabolism in the rat brain, as reflected by increased concentrations of the sulfate conjugate of MHPG (MHPG-SO_4), may be subject to stimulus control.

In our initial experiments, we determined the extent to which a single session, or repeated sessions, of moderately intense footshock stress altered endogenous MHPG-SO_4 concentrations in the rat brain. We used male Sprague-Dawley rats (180 to 200 g) housed individually in a room with a 12-hour light-dark cycle and with unlimited access to food and water.

For the single stress session, shocked animals received 80 trials of inescapable footshock (1.05 mA) on a variable-interval schedule of 1 minute. Shock was distributed simultaneously to all grids of the floor of an experimental chamber by a solid-state shocker/distributor (Coulbourn Instruments), and each trial of footshock was 15 seconds long (*9*). Control animals, matched for body weight, were handled identically and were placed in the experimental chamber but received no footshock. Immediately after the session, animals were decapitated; whole brains were rapidly removed, frozen by immersion into dry ice and ethanol, stored overnight at −80°C, and then assayed for MHPG-SO_4 by the fluorometric assay of Meek and Neff (*10*). Data are expressed as the means ± standard errors (S.E.) of the means of 10 to 16 animals per group.

Concentrations of MHPG-SO_4 were significantly increased [$t(20) = 3.75$, $P < .005$] in the brains of rats that had been exposed to a single session of footshock (765 ± 64 pmole per gram of brain tissue) when compared with nonshocked animals (478 ± 34 pmole/g). These results confirm previous findings of other investigators who used higher levels of footshock to study the effects of acute footshock on MHPG-SO_4 levels in the rat brain (*11*).

For repeated sessions, rats were given one session of footshock on each of 3 days (as described above), and were killed immediately after the third session. Control animals were placed in the environmental chamber on days 1 through 3 but received no footshock. Concentrations of MHPG-SO_4 were still significantly higher [$t(25) = 7.43$, $P < .001$] in animals after the third session of footshock (848 ± 33 pmole/g) than in control animals (496 ± 30 pmole/g). These findings agree with the results of other studies (*2*) showing that stress-induced increases in norepinephrine metabolism and turnover are not attenuated after repeated sessions of various stressors, including footshock (*12*). Shocked rats made few attempts to escape from (inescapable) footshock on days 2 and 3 but, rather, rigidly clung to or crouched on the grid floor of the experimental chamber.

In the next series of experiments administering shock as before, we sought to determine whether increased concentrations of MHPG-SO_4 could be elicited by the environmental stimuli previously paired with the footshock stress. The footshock will be referred to as the unconditioned stimulus, the stress-induced increases in concentrations of MHPG-SO_4 in the brain as the unconditioned response, and the experimental chamber and all stimuli associated with it (experimenter, transfer from home cage, laboratory, and so forth) as the conditioned stimulus complex (CS).

Animals matched for body weight (200 to 220 g) were assigned to one of the following four groups (Table 1). Group 1: Animals were placed individually in the experimental chamber (CS) and were not shocked on each of days 1 through 3, but remained in the chamber for the same length of time as the shocked groups (approximately 90 minutes). On day 4, animals were not exposed to the CS but

Table 1. Increases in concentrations of MHPG-SO_4 (in picomoles per gram of brain tissue) induced by environmental stimuli previously paired with stress: multiple pairings. Abbreviations: G, group; S, shock.

G	N	Experimental condition		MHPG-SO_4 ($\bar{X}$ ± S.E.)
		Days 1 to 3	Day 4	
1	11	CS	No CS	449 ± 24
2	13	CS	CS	436 ± 15
3	13	CS + S	No CS	449 ± 25
4	13	CS + S	CS	634 ± 18*

*t-tests, $P < .001$ when compared with groups 1, 2, or 3.

Table 2. Increases in concentrations of MHPG-SO_4 (in picomoles per gram of brain tissue) induced by environmental stimuli previously paired with stress: single pairing. Abbreviations: G, groups; S, shock.

G	N	Experimental condition		MHPG-SO_4 ($\bar{X}$ ± S.E.)
		Day 1	Day 2	
1	13	CS	No CS	435 ± 21
2	10	CS	CS	440 ± 20
3	10	CS + S	No CS	429 ± 15
4	10	CS + S	CS	533 ± 18*

*t-tests, $P < .001$ when compared with groups 1, 2, or 3.

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were killed less than 1 minute after being removed from their home cages (*13*). Group 2: Animals were placed in the experimental chamber for approximately 90 minutes and were not shocked on each of days 1 through 3. On day 4, the animals were again placed in the chamber for 90 minutes and not shocked. Rats were killed immediately after this session in the experimental chamber. Group 3: Animals were placed in the experimental chamber and received 80 trials of footshock lasting about 90 minutes on each of days 1 through 3. On day 4, animals were not exposed to the CS but were killed immediately after being removed from their home cages (*13*). Group 4: Animals were placed in the experimental chamber and received 80 trials of footshock lasting about 90 minutes on each of days 1 through 3. On day 4, rats were placed in the experimental chamber for 90 minutes but not shocked. Animals were killed immediately after this session in the experimental chamber.

Concentrations of MHPG-SO_4 in the unshocked animals exposed to the CS on day 4 (group 2) were similar to those in unshocked animals who remained in their home cages and were not exposed to the CS (group 1) (Table 1). Placement in the experimental chamber, in and of itself, was thus not sufficient to alter concentrations of MHPG-SO_4 in unshocked animals. The similarity of MHPG-SO_4 concentration of group 3 to those of groups 1 and 2 indicates that 24 hours after the last session of footshock, basal MHPG-SO_4 in shocked animals was not different from that of unshocked controls. However, the animals that had been shocked on days 1 through 3 and placed in the experimental chamber with no footshock on day 4 (group 4) showed significant increases in MHPG-SO_4 (*t*-tests, $P < .001$) compared with groups 1, 2, or 3. These findings could be accounted for by either of two hypotheses: (i) that endogenous MHPG-SO_4 in the brains of previously shocked rats was increased by nonspecific environmental stimuli (sensitization) or (ii) that MHPG-SO_4 was increased in the presence of specific environmental stimuli that had been paired with footshock (conditioning) (*14*).

In another series of experiments, we explored whether MHPG-SO_4 increased in response to environmental stimuli after a single pairing with footshock stress. Animals (200 to 220 g) were assigned to one of four groups. On day 1, animals received footshock as described above (groups 3 and 4) or no footshock (groups 1 and 2). On day 2, animals were killed immediately after being removed from their home cages (groups 1 and 3) or were exposed to the CS for 90 minutes before being killed (groups 2 and 4).

Concentrations of MHPG-SO_4 were similar in brains of all unshocked animals and shocked animals that had not been exposed to the CS on day 2 (Table 2). However, the shocked animals that had been exposed to the CS on day 2 (group 4) showed significant increases in MHPG-SO_4 (*t*-tests, $P < .001$). These findings suggest that increases in the concentration of MHPG-SO_4 were elicited by environmental stimuli after only a single pairing of the environmental stimuli and footshock.

In order to obtain preliminary behavioral and physiological data with objective but noninterventive procedures, we observed the frequency of crouching behavior in all groups ($N = 7$ per group) (*15*) and counted the number of fecal boluses excreted during the 90-minute session on day 2 (*16*). Group 4 animals excreted significantly more fecal boluses (4.0 ± 0.5) than did group 1 (1.7 ± 0.4), group 2 (0.6 ± 0.4), and group 3 (1.5 ± 0.7) [F (3,51) = 28.8, $P < .001$]. While the highest scores for crouching behavior occurred in group 4, these scores did not differ significantly among the four groups. Increases in defecation (*17*) and crouching or freezing behavior (*18*) have been reported by other investigators when rats were replaced in a chamber in which they had previously been inescapably shocked.

Although alterations in the release or metabolism of catecholamines in the peripheral nervous system in response to environmental stimuli have been demonstrated previously, we believe that the studies reported here are the first to show (i) that increases in MHPG-SO_4, a naturally occurring endogenous catecholamine metabolite in rat brain, can be elicited in stressed animals by environmental stimuli that were neutral to nonstressed animals and (ii) that these increases may be associated with certain indices of emotional behavior. Moreover, these data show that these anticipatory alterations in concentrations of MHPG-SO_4 and emotional behavior can be elicited by simple experimental procedures, and that they represent a robust, reproducible neurochemical and behavioral phenomenon. Although we cannot specify which stimuli in the animal's environmental complex led to the behavioral and neurochemical responses or whether the neurochemical response reflects sensitization or conditioning, the prior stressful experience of the animal significantly altered the animal's behavioral and neurochemical response to an environmental stimulus complex that was behaviorally and neurochemically neutral to nonstressed animals.

Finally, our findings add to a small but growing body of evidence (*5–7, 19*) that changes in neurotransmitters or neuromodulators in the brain that may mediate behavior or the brain's coding of biologically significant events can be conditioned. Such neurochemical alterations, if not extinguished, could conceivably alter the organism's sensitivity to stress-related events, its ability to cope with stressors, and its vulnerability to stress-related medical or psychiatric disorders (*20*).

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9. The experimental chamber (12 by 10 by 12 inches) contained no lever and was enclosed in a sound-attenuating cubicle equipped with a speaker that provided masking white noise and a house light (kept on during all experiments). Animals were observed through a one-way mirror in the side of the cubicle.
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14. Even though animals were not being shocked, many were braced against the grid floor or crouched on the side of the chamber in positions similar to those observed in experiment 1 on days 2 and 3 while animals were being shocked.
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20. For example, exposure to single or repeated sessions of inescapable shock increases the frequency and severity of ulcers in rats [J. Weiss, *J. Comp. Physiol. Psychol.* **65**, 251 (1968)] or tumors in mice [L. S. Sklar and H. Anisman, *Science* **205**, 513 (1979)] and induces learned helplessness in rats [S. Maier and M. Seligman, *J. Exp. Psychol.* **105**, 3 (1976)]. In humans, exposure to inescapable stress has been linked to depression [M. Seligman, *Helplessness* (Freeman, San Francisco, 1975)], and alterations in MHPG have been associated with subgroups of drug-induced or naturally occurring depressions [R. Watson, E. Hartmann, J. Schildkraut, *Am. J. Psychiatry* **129**, 263 (1972); J. Schildkraut, P. Orsulak, A. Schatzberg, J. Gudeman, J. Cole, W. Rohde, R. LaBrie, *Arch. Gen. Psychiatry* **35**, 1427 (1978)].
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Contextual Effects in Infant Speech Perception

Abstract. *Infants, aged 2 to 4 months, discriminated synthetic speech patterns that varied in duration of the formant transitions; this variation provides information sufficient to signal the phonetic distinction between a stop consonant and a semivowel in adult listeners. In addition, the discriminability of a given difference in transition duration was a function of both the particular stimulus values and the total duration of the syllable. This contextual effect occurred even though the information for syllable duration came after the transition information. The obtained pattern of discontinuous discriminability was in accord with perception that is relational and categorical.*

In this study of speech perception, we found that young infants discriminated small differences in the duration of formant transitions, acoustic information that is sufficient to signal a distinction between the syllable-initial stop consonant [b] and the semivowel [w] in adult listeners. Of greater importance was the finding that for infants, as for adults, the discriminability of two transitions depended not only on the particular duration values but also on contextual information in the form of syllable duration. This is, to our knowledge, the first demonstration that contextual factors can alter the infants' categorical discriminability function for acoustic information that will soon be relevant for the detection of phonetic distinctions.

Research on the perception of speech in prearticulate infants has shown the presence of sophisticated processing abilities. For example, infants as young as a few months of age are capable of distinguishing small differences in the acoustic dimensions that underlie adult phonetic distinctions based on voicing (*1*, *2*), place of articulation (*3*), and manner of articulation (*4*). In addition, infants discriminate speech patterns much more readily when they signal different adult phonetic categories than when they signal variants of the same adult category (*1*, *5*). This discontinuity in the discriminability function has been interpreted as evidence for categorical perception in infants, a phenomenon that has often, but by no means always, been observed in adult studies of speech perception (*6*). These data suggest that infants have mechanisms for the processing of speech that divide acoustic continua into highly discriminable regions that will form the basis for adult phonetic categories.

Recent studies with adults have demonstrated that perception of phonetic elements involves more than the categorization of information along acoustic dimensions. For instance, there are "top-down" or cognitive influences on speech processing at the phonetic level (*7*). In addition, contextual factors can influence the perception of phonetic segments even when they occur several hundreds of milliseconds before or after the target information (*8*, *9*). In one study of contextual effects, Miller and Liberman (*8*) investigated the influence of an after-occurring contextual factor, syllable duration, on the locus of the phonetic boundary between the stop consonant [b] and the semivowel [w]. The stop-semivowel distinction is signaled by duration of the formant transitions, with shorter transitions identified as a stop and longer transitions as a semivowel. They found that the phonetic boundary along a continuum of transition duration increased systematically as syllable duration was increased by lengthening the vocalic portion of the syllable (*10*). With very short syllables, 80 msec in duration, speech patterns with initial transitions longer than 32 msec were perceived as the semivowel, whereas transitions as long as 47 msec were required for the same percept when the syllable duration was increased to 296 msec. Thus, there are values of transition duration that are ambiguous. Resolution of this ambiguity requires, in the absence of other information, that perception and ultimate classification of transition duration take into account the contextual factor of syllable duration, and thus be relative and not absolute.

We investigated whether the ability to make relative judgments concerning transition duration is within the speech-processing capability of young infants. Our experimental design capitalized on findings that infants are much more likely to discriminate speech patterns if sounds exemplify different phonetic categories than if they are variations of the same category (*1*, *5*). We selected our stimuli from two [ba-wa] continua that differed from each other only in total duration of the individual syllables, being 80 msec in one instance and 296 msec in the other. Within each continuum, syllables differed in duration of the formant transitions. We selected two pairs of syllables from each continuum, the stimuli

Table 1. Mean response rates (number of high-amplitude responses per minute) as function of conditions.

Group	Response measures				
	Preshift			Postshift difference scores*	
	Baseline	Minutes 1 to 7	Minutes 6 and 7	Minutes 1 and 2	Minutes 1 to 4
16-40 short ([b-w])†	25.6	41.1	47.9	+4.0	+4.6
40-64 short ([w-w])	30.4	44.2	48.4	−2.5	−2.2
16-40 long ([b-b])	28.4	40.6	50.3	−3.8	−6.5
40-64 long ([b-w])	27.9	40.1	46.4	+4.3	+3.8
Control	30.6	46.6	50.8	−4.6	−8.5

*Difference scores were obtained by subtracting the average response rate during the 6th and 7th minutes before the shift from the first 2- or entire 4-minute response rate after the shift. †The perceived consonantal quality of the stimuli according to Miller and Liberman (*8*).

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