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5-HT_{1B} receptor mRNA levels in dorsal raphe nucleus: inverse association with anxiety behavior in the elevated plus maze

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Abstract

Serotonergic neurons in the dorsal raphe nucleus, the major source of forebrain serotonin projections, synthesize a terminal autoreceptor that inhibits serotonin release—the 5-HT_{1B} autoreceptor. Overexpression of this autoreceptor is hypothesized to contribute to anxiety. Antidepressants decrease (while learned helplessness increases) 5-HT_{1B} mRNA in dorsal raphe neurons, and viral-mediated overexpression of 5-HT_{1B} here increases anxiety behavior after stress. However, 5-HT_{1B} mRNA levels in dorsal raphe are substantially elevated in unstressed rats in two models of stress resistance. Thus, the role of dorsal raphe 5-HT_{1B} autoreceptors in anxiety is complex. Therefore, we tested whether different stressors differentially affect dorsal raphe 5-HT_{1B} mRNA [via in situ hybridization histochemistry] and anxiety behavior (using the elevated plus maze). Rats were assigned to a stressor (either forced swim, water restraint, dry restraint, or electric tail shock) or a control condition, then were tested and sacrificed 24 h later. Overall, controls exhibited less anxiety than stressed rats as indicated by a higher ratio of open arm to total arm entries (OTR). The stressors did not differentially affect the OTR, nor did any alter dorsal raphe 5-HT_{1B} mRNA levels. There was, however, a significant positive correlation between the OTR and 5HT_{1B} mRNA intensity in controls (r=.64; P=.006), but not in stressed rats (r=.16, P=.36), providing further evidence that elevated dorsal raphe 5-HT_{1B} levels are associated with reduced anxiety in animals that have not been exposed to stress.

Keywords: Autoreceptor; Depression; Stress; Tail shock; Restraint; Forced swim

1. Introduction

Serotonin$_{1B}$ (5-HT_{1B}) autoreceptors are expressed in the forebrain terminals of serotonergic axons that project from the midbrain raphe nuclei (Jacobs and Azmitia, 1992). 5-HT_{1B} autoreceptor activation acutely inhibits serotonin release from axon terminals and also reduces serotonin synthesis (Hoyer and Middlemiss, 1989; Hjorth et al., 1995). Accordingly, 5-HT_{1B} autoreceptors exert negative feedback control of serotonin neurotransmission by coupling local serotonin levels at terminals to subsequent release. The midbrain dorsal raphe nucleus is the origin of most of the serotoninergic terminals expressed throughout the forebrain (Molliver, 1987; Jacobs and Azmitia, 1992). Therefore, altered regulation of the 5-HT_{1B} gene in the dorsal raphe may have particularly important consequences for serotonin neurotransmission in the forebrain. Consistent with this hypothesis, rats that develop learned helplessness, an animal model of depression induced by inescapable stress (Seligman and Maier, 1967), exhibit increased 5-HT_{1B} mRNA in the dorsal raphe (Neumaier et al., 1997). Learned helplessness is also characterized by a reversible deficit in serotonin release in prefrontal cortex (Sherman and Petty, 1980; Petty et al., 1992). Additionally, 5-HT_{1B} mRNA in the dorsal raphe is selectively down-regulated by serotonin-selective reuptake inhibitors in a time-dependent and reversible manner, an effect not observed in hippocampus, striatum, or frontal cortex (Neumaier et al., 1996a,b; Anthony et al., 2000). Finally, using viral-mediated gene transfer into dorsal raphe neurons, we found that the forced swim stress induced greater anxiety behavior in rats that overexpressed the 5-HT_{1B} autoreceptor compared to animals that overexpressed a control construct (Clark et al., 2002). Collectively, these observations suggest that increased 5-HT_{1B} autoreceptor

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expression contributes to symptoms of depression and anxiety, and that down-regulation of 5-HT_{1B} autoreceptor by antidepressants may help normalize serotonergic neurotransmission and thereby contribute to the symptomatic relief of depression or anxiety (Briley and Moret, 1993).

Unexpectedly, however, we recently observed 63–73% higher 5-HT_{1B} mRNA values in dorsal raphe from stress-resistant male rats from two models of differential stress reactivity (congenital learned helplessness and handling/maternal separation) compared to the stress-sensitive animals (Neumaier et al., 2002). Animals in that study were not subjected to inescapable stress before sacrifice. Similarly, in rats not subjected to forced swim stress, viral-mediated overexpression of the 5-HT_{1B} autoreceptor in dorsal raphe neurons actually decreased anxiety behavior (Clark et al., 2002).

Thus, existing data suggest a paradox, i.e., the state of stress underlying learned helplessness and the trait of stress resistance are both associated with increased dorsal raphe 5-HT_{1B} synthesis. We viewed this conclusion cautiously, however, because only one study specifically examined the effect of inescapable stress/helplessness on dorsal raphe 5-HT_{1B} synthesis (Neumaier et al., 1997), and just two studies examined the latter in relation to stress reactivity (Clark et al., 2002; Neumaier et al., 2002).

Accordingly, we sought to determine whether other experimental stressors increase dorsal raphe 5-HT_{1B} synthesis and to correlate synthesis with anxiety-like behavior in typical outbred rats. Therefore, Sprague–Dawley rats were subjected to one of four well-established experimental stressors or two control conditions, evaluated in the elevated plus maze assay of anxiety-like behavior 24 h later, and sacrificed for quantification of 5-HT_{1B} mRNA in the dorsal raphe.

2. Methods

2.1. Animals

All animal procedures were approved by our institution's animal care committee and conducted in accordance with National Institutes of Health guidelines. Male Sprague–Dawley rats (280–320 g) were group housed in an AALAC-accredited specific pathogen-free (SPF) facility for these studies. Animals were maintained on a 12:12 light/dark cycle and had free access to food and water.

2.2. Forced swim procedure

This procedure was performed as previously described (Porsolt et al., 1977; Detke et al., 1995). The forced swim container was a 40-cm tall Plexiglas cylinder 20 cm in diameter mounted on a Plexiglas base. It was filled with tap water (25 °C) to 30 cm, a level deep enough to prevent the rat from resting on its extended tail. The forced swim stress session consisted of placing the rat in the chamber for 15 min, removing and towel drying it, and allowing it to recover under warm lamps before return to the home cage.

2.3. Water restraint

The water restraint stressor was modified from a previously described procedure (Pare, 1994). The restrainer was a 23-cm length of 6.4 cm diameter heavy-duty plastic hose riveted to taper so as to fit snugly along the animal's body and prevent gross movements. It had numerous holes (7 and 11 mm in diameter) to permit water to enter freely. Rats were suspended for 15 min to shoulder level in 25 °C water for 15 min using a forced swim chamber. The animals were then wiped briefly with a towel, placed under a warming lamp until dry, and returned to their home cage.

2.4. Dry restraint

Rats in this condition were restrained for 1 h in a clear plastic restrainer consisting of a semicircular roof and flat floor with an adjustable partition (San Diego Instruments, San Diego, CA). Custom-made plastic wedges limited head movement and the ability to twist about the axial plane. This device allows very limited movement.

2.5. Electric tail shock

Animals assigned to this condition were restrained as in the dry restraint condition. A total of 60 tail shocks, each at 0.6 mA for 5 s, were delivered over 1 h using a regulated animal shocker (Coulbourn Instruments, Allentown, PA). One shock occurred within each of 60 consecutive 1-min intervals. The 5-s shock interval within each minute was established a priori using a random number generator with the constraint that the intershock interval would be at least 5 s. Shock delivery was controlled by a computer and custom-written software.

2.6. Sham stress

Rats assigned to this condition were transported in their home cages from the housing facility to our laboratory ($\sim$150 m via cart and a five-story elevator ride) and placed for 1 h on a bench remote from animals being tested elsewhere.

2.7. Cage controls

These rats were left in the housing facility until just prior to behavioral testing. To minimize the stress caused by transport to the testing facility, rats remained in an adjacent room in their drape-covered home cage for 30–60 min before behavioral testing.

2.8. Elevated plus maze

The elevated plus maze apparatus was custom-built from black Plexiglas with nonreflective painted surfaces. The maze consisted of four runways (10 × 40 cm) joined by a central 10 × 10 square 50 cm above the floor. Opposing arms were either open (0.5-cm lip) or enclosed by 40 cm high walls. The maze was housed in a room illuminated by a dim overhead lamp (12 lx). Experimental methodology was based upon previously published studies of the elevated plus maze (Handley and McBlane, 1993; Hogg, 1996). Animals were introduced into the center square facing an open arm, and behavior was analyzed for 5 min using the SMART motor activity analysis program (San Diego Instruments). Entries into open and closed arms were measured, as were time and distance on open and closed arms. The center square was not considered a part of either the open or closed arms. We have previously validated the ability of our elevated plus maze to detect both anxiolytic and anxiogenic effects (Clark et al., 2002). We have optimized our elevated plus maze conditions to favor the ability to detect anxiogenic effects, seeking a ratio of open arm entries to total arm entries (OTR) in control groups of ~0.5.

2.9. In situ hybridization histochemistry (ISHH)

Rats were killed by carbon dioxide inhalation within 5 min following behavioral testing, and their brains were promptly removed and quick frozen on dry ice and later stored at −70 °C. The protocol for ISHH has been previously described (Neumaier et al., 1996a,b). Tissue sections (20 μm) were prepared in the frontal plane using a cryostat and thaw mounted on silanized glass slides. The sections were stored at −70 °C until processed for ISHH. In brief, tissue sections were thawed at room temperature and fixed in cold 4% paraformaldehyde. After rinsing in phosphate-buffered saline, sections were treated with acetic anhydride (0.25% in 0.1 M triethanolamine), dehydrated, delipidated, and air dried.

Oligonucleotide probes were designed on the basis of low sequence homology to other known receptor mRNA sequences. For the 5-HT_{1B} probes, three oligonucleotides corresponding to residues 1343–1382, 1630–1668, and 1790–1829 of the rat 5-HT_{1B} clone, MG1-$_{1B}$ (Hamblin et al., 1992), were synthesized as previously described (Neumaier et al., 1996a,b). The probes were individually labeled with [^{33}P]-dATP (Amersham, Arlington Heights, IL) using terminal deoxyribonucleotidyl transferase (Gibco, Gaithersburg, MD) and purified on NENSORB columns (Dupont NEN Research Products, Boston, MA). The specific activity of each oligonucleotide probe was 3–7 μCi/pmol.

The labeled probes were diluted (2 pmol/ml) in a hybridization buffer containing 50% formamide, 10% dextran sulfate, 0.3 M sodium chloride, 10 mM Tris (pH 8.0), 1 mM EDTA, 1 × Denhardt's (0.2% each of bovine serum albumin, Ficoll, and polyvinylpyrrolidone), 0.4 mg/ml yeast tRNA, and 10 mM dithiothreitol. Fifty microliters of the hybridization mixture was applied to each slide, and the sections were covered with silanized coverslips. The slides were incubated in moist covered trays at 37 °C overnight. Following the hybridization reaction, coverslips were removed and the slides were washed in 1 × SSC (150 mM NaCl in 15 mM sodium citrate) for 1 h at 55 °C, and again in 1 × SSC at room temperature for 1 h. The slides were rinsed in distilled water, dehydrated through a series of graded alcohol rinses containing 300 mM ammonium acetate, and air dried. Autoradiographic signal was detected using a Cyclone storage phosphor scanner (Packard Instruments, Meridian, CT) at 600 dpi resolution and were stored on CD-ROM disks; exposure times were 24–26 h.

2.10. Quantification of 5-HT_{1B} ISHH signal

5-HT_{1B} ISHH signal was quantified using a computer-based densitometry system (MetaView, Imaging Research, St. Catherines, ON). The intensity of hybridization signal (measured in arbitrary digital light units) was standardized using ^{14}C-plastic standard sections coexposed on each phosphor screen, which yields a linear relationship between tissue radioactivity and measured signal intensity. The rater was blinded to treatment condition of brains during sectioning, hybridization, and densitometry. Tissue sections from each brain were matched anatomically to correspond to Plate 49 (dorsal raphe, −7.8 relative to bregma) from a rat brain atlas (Paxinos and Watson, 1998). The medial subnucleus of the dorsal raphe was measured with an oval-shaped template that covered approximately 70% of the nucleus. Hybridization signal was determined from two consecutive sections. These were averaged and tissue background was subtracted for each region in each brain. After the treatment, condition of the brains was decoded and mean values were calculated for each treatment group.

2.11. Statistical analysis

Main effects of stressor type on measures of elevated plus maze performance and the 5-HT_{1B} mRNA hybridization signal were evaluated with ANOVA using the general linear model option in SPSS (Version 11.0.1, SPSS, Chicago, IL). Regression analyses were also performed using SPSS. An a priori decision was made to compare controls to the combined stress groups using an independent-groups *t* test. The OTR (defined as the number of open arm entries divided by the total number of entries into open and closed arms) was the key elevated plus maze variable assessed because factor analysis had previously shown this index to be strongly associated with fearfulness/anxiety (Ramos et al., 1997). Additional variables were also examined, however, including the total entries onto arms, the total distance traveled, the ratio of open arm distance to total distance traveled, the total time spent on arms, and the ratio of time

Table 1
Group statistics (mean ± S.E.M.) for stress conditions

	Cage control	Sham stress	Dry restraint	Water restraint	Forced swim	Tail shock	All control	All stress	*P* value[a]
n	6	13	7	9	7	15	19	38	
Total entries	15.17 ± 2.34	19.08 ± 4.03	14.43 ± 1.95	17.33 ± 2.17	14.29 ± 2.17	20.73 ± 1.42	17.84 ± 2.84	17.58 ± 1.00	.92
Open arm entries/total entries	0.56 ± 0.07	0.56 ± 0.06	0.46 ± 0.07	0.43 ± 0.05	0.46 ± 0.08	0.47 ± 0.04	0.56 ± 0.04	0.46 ± 0.03	.04
Total distance (cm)	504.00 ± 32.07	420.77 ± 40.99	439.57 ± 27.94	507.89 ± 32.10	484.57 ± 53.93	386.87 ± 40.33	447.05 ± 30.66	443.24 ± 21.80	.92
Open arm distance/total distance	0.29 ± 0.07	0.38 ± 0.07	0.34 ± 0.11	0.24 ± 0.05	0.23 ± 0.04	0.37 ± 0.06	0.35 ± 0.05	0.31 ± 0.03	.48
Total time on arms (s)	92.67 ± 18.51	118.46 ± 20.70	102.86 ± 32.07	74.64 ± 15.61	81.86 ± 21.40	77.27 ± 12.93	110.30 ± 15.28	82.20 ± 9.20	.10
Open arm time/total arm time	0.36 ± 0.07	0.50 ± 0.09	0.39 ± 0.12	0.30 ± 0.07	0.32 ± 0.08	0.31 ± 0.05	0.46 ± 0.06	0.32 ± 0.04	.05

[a] All control rats compared to all stressed rats.

on open arms to total arm time. Significance was established at α=.05, two tail.

3. Results

3.1. Elevated plus maze behavior

The cage-control and sham-stress control groups did not differ statistically on any of the behavioral variables assessed ($P \geq .22$) (Table 1). Therefore, these groups were combined for comparison with stressed rats. ANOVA did not reveal a significant main effect of stressors on any behavioral variable [$F(4,52) \leq 1.42$, $P \geq .24$]. However, comparison of the mean OTR score for all 38 stressed rats (0.456 ± 0.027) to that of the 19 control animals (0.559 ± 0.045) indicated a modest but significant inhibitory effect of prior acute stress on the OTR (P=.04) (Table 1). The ratio of time spent on the open arms to total arm time was also significantly different (P=.049) between stressed (0.323 ± 0.036) and control rats (0.460 ± 0.064) (Table 1). When ANOVA was restricted to only the four stress conditions, there was no indication that a particular stressor exerted a differential effect on either the OTR [$F(3,34) = 0.09$, P=.96] or the open arm time–total arm time ratio [$F(3,34) = 0.24$, P=.87].

3.2. 5-HT_{1B} gene expression

A representative autoradiograph of ISHH signal corresponding to dorsal raphe $5HT_{1B}$ mRNA is shown in Fig. 1. None of the acute stressors were associated with elevated $5HT_{1B}$ mRNA levels relative to control rats (Fig. 2), and ANOVA confirmed formally that the groupwise variation in

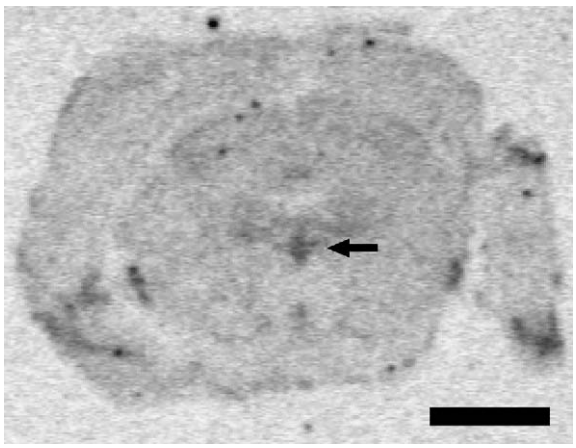

Fig. 1. Representative autoradiograph of hybridization signal for 5-HT_{1B} mRNA in dorsal raphe (arrow). Scale bar = 250 μm.

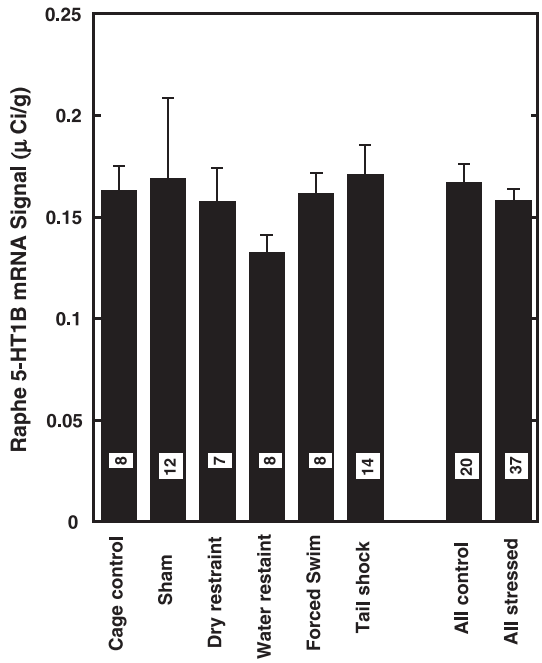

Fig. 2. 5-HT_{1B} mRNA signal in control and stressed rats. Inset values give group sizes.

5HT$_{1B}$ mRNA levels was not significant [$F(4,52) = 1.45$, $P = .22$]. The mean of the combined control group (0.167 ± 0.042 μCi/g) approximated that of the combined stress group (0.158 ± 0.037 μCi/g) ($P = .42$). ANOVA restricted to the four stressed groups did not indicate an effect of stressor type on the 5-HT$_{1B}$ mRNA signal [$F(3,33) = 1.94$, $P = .14$].

3.3. Relationship between 5-HT$_{1B}$ mRNA and elevated plus maze behavior

Regression analysis restricted to nonstressed controls revealed a significant positive association between 5-HT$_{1B}$ mRNA signal strength and the OTR; indeed, 41% of the variance in this behavioral outcome was accounted for by 5-HT$_{1B}$ mRNA signal strength ($P = .006$) (Fig. 3A). By contrast, in stressed rats, this association was not significant ($r = .16$; $P = .36$) (Fig. 3B). Similarly, in controls, 5-HT$_{1B}$ mRNA signal strength was significantly associated with both the open arm distance–total arm distance ratio ($r = .73$, $P = .001$) and the open arm time–total arm time ratio ($r = .69$, $P = .002$). Again, these associations were not significant in the stressed group ($r = .13$, $P = .45$ and $r = .04$, $P = .83$, respectively).

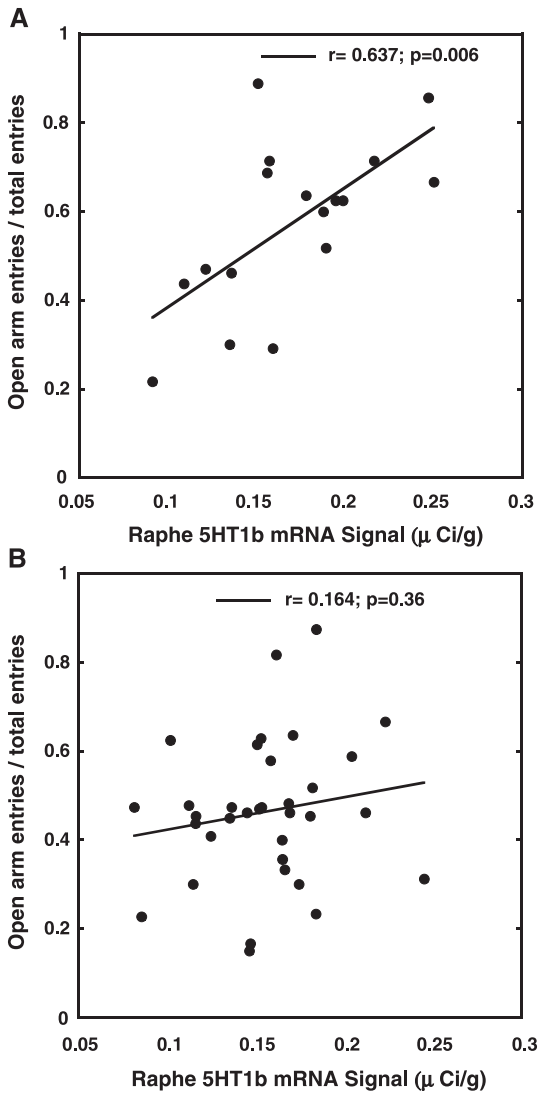

Fig. 3. Relationship between the OTR and 5-HT$_{1B}$ mRNA signal in control rats (A) and stressed rats (B).

4. Discussion

Mean values of 5-HT$_{1B}$ dorsal raphe mRNA in stressed rats did not differ from control levels in this study in contrast to previous work indicating elevated dorsal raphe 5-HT$_{1B}$ mRNA in rats exhibiting learned helplessness following inescapable stress (Neumaier et al., 1997). There are important distinctions between these studies, however. The current study involved one stressor 24 h prior to sacrifice, whereas the previous work (Neumaier et al., 1997) involved multiple stressors and more time before sacrifice. Specifically, that study involved one hundred 5-sec, 1.0-mA tail shocks, followed by individual housing (a stressor in rodents (Ruis et al., 1999; Sharp et al., 2002)), followed 24 h later by 30 trials in a shuttlebox foot shock escape paradigm, followed 24 h later by sacrifice. Hence, perhaps mean 5-HT$_{1B}$ mRNA levels did not change in the current study because our procedures were not sufficiently stressful, consistent with the absence of a marked increase of anxiety behavior in the stressed animals. It should also be noted that only those animals in the previous study that developed learned helplessness following inescapable stress exhibited a reliable increase of dorsal raphe 5-HT$_{1B}$ mRNA, while the stressed rats that did not become helpless showed a smaller nonsignificant increase in this variable (Neumaier et al., 1997).

These distinctions raise important points. First, perhaps only animals that develop a marked anxiety phenotype following stress (e.g., learned helplessness) will manifest an increase of dorsal raphe 5-HT$_{1B}$ mRNA. This hypothesis also implies that a failure to observe a significant increase in the overall mean 5-HT$_{1B}$ mRNA level following stress might mask a reliable increase in a subgroup of vulnerable animals. Alternatively, it may require multiple bouts of stress to reliably affect mean dorsal raphe 5-HT$_{1B}$ mRNA levels. Additionally, because group housing can moderate the adverse effects of stress (Ruis et al., 1999), this procedure may have opposed an increase of dorsal raphe 5-HT$_{1B}$ mRNA levels and attenuated increases of anxiety behavior. Finally, detectable poststress changes in 5-HT$_{1B}$ mRNA may occur earlier or later than the time point examined in the present study.

Our use of 5-HT$_{1B}$ mRNA as a measure of 5-HT$_{1B}$ terminal autoreceptor expression on serotonergic neurons deserves comment. It might seem preferable to use a binding assay to more directly quantify dorsal raphe 5-HT$_{1B}$ terminal autoreceptors. However, these autoreceptors reside in forebrain amid a much larger population of 5-HT$_{1B}$ hetero-

receptors expressed on nonserotonergic neurons (Offord et al., 1988; Frankfurt et al., 1994). Therefore, even a marked change of the autoreceptor population is difficult to detect with a binding assay. Hence, detecting evidence of altered expression of presynaptic 5-HT_{1B} autoreceptors currently depends on measuring mRNA in the cell bodies of origin (Clark et al., 2002). Nevertheless, studies using this approach must be evaluated with the understanding that mRNA is an imperfect proxy for actual protein expression.

The main significant outcome of our study is the strong positive association between dorsal raphe 5-HT_{1B} mRNA signal intensity and measures of elevated plus maze open arm exploration in unstressed rats. Particularly noteworthy is the correlation between 5-HT_{1B} mRNA signal intensity and the OTR (r=.64), owing to this measure's strong support and acceptance as an index of fearfulness and anxiety (Handley and McBlane, 1993; Hogg, 1996; Ramos et al., 1997). Similarly, strong associations were also observed in the unstressed group between the 5-HT_{1B} mRNA signal and each of two other open arm activity ratios, namely open arm distance−total arm distance (r=.73) and open arm time−total arm time (r=.69). None of these associations occurred in the stressed group, however. This result suggests that while stress did not alter the mean dorsal raphe 5-HT_{1B} signal, it may instead have affected 5-HT_{1B} mRNA synthesis in a manner reflecting wide individual variability. This possibility is consistent with recent work emphasizing the importance of individual differences in vulnerability to stressor-induced maladaptations in behavioral and physiological measures of anxiety in rats (Cohen et al., 2003).

Our results suggest that dorsal raphe 5-HT_{1B} mRNA levels are inversely associated with anxiety in rats that have not been subjected to stress. This concept is also supported by two previous studies from our laboratory. First, using two established rat models of differential stress reactivity, congenital learned helplessness (King et al., 1993) and handling/maternal separation (Caldji et al., 1998), dorsal raphe 5-HT_{1B} mRNA in unstressed male rats was substantially higher (63−73%) in the stress-resistant groups compared with stress-sensitive groups (Neumaier et al., 2002). Second, viral-mediated overexpression of 5-HT_{1B} receptor in dorsal raphe neurons reduced open field anxiety behavior in rats not subjected to a prior stressor (Clark et al., 2002). Accordingly, multiple studies involving very different approaches now suggest that elevated dorsal raphe 5-HT_{1B} gene expression is associated with reduced stress reactivity in rats, but that a prior stressor alters this association.

This concept, however, runs counter to the hypothesis that increased dorsal raphe 5-HT_{1B} autoreceptor tone would predispose animals to increased anxiety by reducing serotonin availability in forebrain terminal fields (Edwards et al., 1991; Neumaier et al., 1997; Clark et al., 2002). Furthermore, this hypothesis is not easily dismissed because in addition to the support provided by the learned helplessness model (Neumaier et al., 1997) and the viral-mediated gene transfer approach (Clark et al., 2002) discussed above, 5-HT_{1B} terminal autoreceptors are implicated in the adaptation of dorsal raphe neurons to serotonin-selective reuptake inhibitors, agents with efficacy in many anxiety disorders (Bergqvist et al., 1999; Sayer et al., 1999) that also prevent learned helplessness (Martin et al., 1990; Takamori et al., 2001; Tordera et al., 2002). Specifically, 5-HT_{1B} mRNA is selectively down-regulated in a time-dependent and reversible manner in dorsal raphe, but not in hippocampus, striatum, or frontal cortex, by fluoxetine or paroxetine (Neumaier et al., 1996a,b; Anthony et al., 2000). Accordingly, multiple findings do support the concept that elevated gene expression for dorsal raphe 5-HT_{1B} autoreceptors is associated with established states of anxiety. Reconciling this concept with the finding that increased dorsal raphe 5-HT_{1B} mRNA is also associated with resistance to learned helplessness and with reduced anxiety behavior in unstressed rats poses an interesting challenge worthy of further research. Meanwhile, a hypothesis advanced by Maier and colleagues is relevant to part of the challenge (Maier et al., 1994).

The hypothesis is that learned helplessness actually derives from exaggerated dorsal raphe serotonin release and is supported by several lines of research. Compared to escapable stress, inescapable stress more strongly increases c-Fos immunoreactivity in dorsal raphe serotonin neurons (Grahn et al., 1999) and results in greater serotonin efflux in the ventral hippocampus (Amat et al., 1998a,b), basolateral amygdala (Amat et al., 1998a,b), and dorsal raphe (Maswood et al., 1998; Amat et al., 2001). Freewheel running both opposes the development of helplessness and reduces dorsal raphe c-Fos expression following inescapable stress in rats (Greenwood et al., 2003). Lesions or pharmacological interventions that reduce dorsal raphe serotonergic activity during inescapable stress block development of behavioral escape deficits (Maier et al., 1993, 1994, 1995; Amat et al., 2001). A key postulate of Maier's model is that exaggerated serotonin release by the dorsal raphe during inescapable stress sensitizes this nucleus to an exaggerated response during subsequent escape testing, which is the critical factor in impairing escape behavior (Maier et al., 1995; Amat et al., 1998a,b).

Maier's model predicts that state- or trait-related receptor changes that prevent exaggerated serotonin release during inescapable stress or during escape testing would impede the development and expression of learned helplessness, respectively. Consistent with this model, Greenwood et al. (2003) reported that reduced vulnerability to learned helplessness accompanied a state-related (exercise-induced) up-regulation of mRNA corresponding to the 5-HT_{1A} somatodendritic inhibitory autoreceptor on dorsal raphe serotonergic neurons. Maier's model similarly predicts that a trait-related increase of dorsal raphe 5-HT_{1B} gene expression would reduce vulnerability to learned helplessness, thus providing one way to interpret our previous finding that stress-resistant rats have elevated dorsal raphe 5-HT_{1B} mRNA (Neumaier et al., 2002). Finally, the inverse association between anxiety behavior

and 5-HT_{1B} mRNA observed in the in the present study suggests that Maier's model may even apply to anxiety behavior elicited by the mild stress of testing, a concept with potentially important implications for understanding the relationship between everyday life stress and anxiety.

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