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Review

Stressor controllability and learned helplessness: The roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor

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Abstract

The term 'learned helplessness' refers to a constellation of behavioral changes that follow exposure to stressors that are not controllable by means of behavioral responses, but that fail to occur if the stressor is controllable. This paper discusses the nature of learned helplessness, as well as the role of the dorsal raphe nucleus, serotonin, and corticotropin-releasing hormone in mediating the behavioral effects of uncontrollable stressors. Recent research indicates that (a) uncontrollable stressors sensitize serotonergic neurons in the dorsal raphe, and that a corticotropin-releasing factor-related ligand, acting at the Type II receptor, is essential to this sensitization process, and (b) the consequent exaggerated release of serotonin in response to subsequent input is at least in part responsible for the behavioral changes that occur. Finally, implications for the general role of corticotropin-releasing hormone in stress-related phenomena and for the learned helplessness paradigm as an animal model of either depression or anxiety are discussed.
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Keywords: Stress; Learned helplessness; Serotonin; Dorsal raphe nucleus; Amygdala; Bed nucleus of the stria terminalis; Corticotropin-releasing factor; Depression; Anxiety

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The main purposes of this paper are to (a) summarize recent work concerning the roles of serotonin (5-HT), the dorsal raphe nucleus (DRN), and corticotropin-releasing hormone (CRH) in mediating the behavioral phenomenon that has been called behavioral depression (Weiss, 1968) and learned helplessness (Maier and Seligman, 1976), and (b) to discuss the implications of this work for the utility of learned helplessness/behavioral depression as a model of depression or antidepressant activity. However, because different investigators have used the term 'learned helplessness' to refer to very different procedures, it will first be useful to discuss what learned helplessness is, and what it is not.

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1. Learned helplessness

In its modern context, the term 'learned helplessness' was first used with reference to experiments directed at understanding the serendipitous observation that animals that were first exposed to an aversive Pavlovian conditioning procedure, in which a light and a shock delivered to the footpads via fixed electrodes were repeatedly paired while the animal was restrained in a Pavlovian harness, later failed to learn to escape and avoid footshocks in a shuttlebox (Overmieer, 1968). The light proved to be unnecessary, and this left the shocks as the potential causative agent. By definition, the shocks delivered in a Pavlovian conditioning experiment are inescapable and unavoidable, and so escapable and yoked inescapable shocks were compared in their effectiveness in producing later failure to learn to escape. In this arrangement, each shock of the series began at the same time for the animal that could escape and its yoked partner, and terminated for *both* animals when the escape subject made an instrumental response, in this case hitting a panel located next to the restrained subject's head. Hitting the panel had no consequence for the yoked subject—shock was inescapable. The result was that only the animals that had received the yoked inescapable shocks (IS) later showed a learning deficit (Seligman and Maier, 1967). From these data it was argued that the degree of control that the organism could exert over the aversive shock was the critical ingredient, and that the organism's learning that it had no control was the key ingredient in producing later failure to learn (Maier et al., 1969). For this reason the phenomenon was called 'learned helplessness'.

At roughly the same time, Weiss was also exploring the dimension of stressor controllability. These studies followed from another serendipitous observation, namely that rats that were able to perform an avoidance–escape response were heavier in body weight than were 'yoked' animals that received similar shock, but were not given the opportunity to avoid/escape (J.M. Weiss, personal communication). Actually, the yoked animals were run as a control group for a subsequent study, but the weight differences led Weiss to compare the impact of escapable (ES) and yoked IS on food and water intake, as well as gastric lesions (Weiss, 1968). These initial studies were followed by experiments designed to determine the range of behavior impacted by stressors that were uncontrollable, relative to controllable stressors, and to explore the mechanisms by which controllability exerted these effects. It turned out that uncontrollable, relative to controllable, shock did much more than produce poor escape behavior—it reduced food and water intake, produced ulcers, reduced swimming when the animal was placed in water; reduced aggression and social dominance; produced neophobia, exaggerated fear and fear conditioning; reduced social interaction; produced opioid analgesia; reduced learning of instrumental responses for appetitive rewards; increased rewarding effects of opiates, and so on (see Maier and Watkins, 1998a for review). In addition, stressor controllability failed to modulate some sequelae of stress (Helmreich et al., 1999; Maier et al., 1986; Woodmansee et al., 1993), so it was not simply that uncontrollable stress is more potent than controllable stress.

This history is noted for several reasons. First, this history makes clear that the learned helplessness paradigm was *not* developed in order to provide an animal model of depression, or of any other clinical condition. The studies that followed initial discovery of the phenomenon were done to understand how the degree of behavioral control that an organism has over a stressor modulates behavior. Indeed, the word 'depression' did not appear in any of the original papers concerning this phenomenon, and did not do so for at least 7 years. The term behavioral depression was not used to describe stressor controllability phenomena till some 15 years later (Weiss, et al., 1981). To be sure, some of the consequences of exposure to uncontrollable stressors seem similar to the symptoms of depression (Weiss and Simson, 1986), but as a group, they are equally similar to the symptoms of extreme anxiety (Maier and Watkins, 1998a). Moreover, learned helplessness is sensitive to both antidepressants (e.g. Petty et al., 1996) and anxiolytics (e.g. Drugan et al., 1984). This should perhaps not be surprising since the development of both depression and anxiety may be influenced by stress, particularly uncontrollable stress. However, many of the investigators who have studied learned helplessness have done so not to understand depression or anxiety, but to understand the phenomenon itself, why failure to escape, exaggerated fear conditioning, and so on occur, and how the degree of behavioral control that an organism has over a stressor can so profoundly alter the behavioral, physiological, and neurobiological impact of the stressor.

1.1. Stressor controllability

Second, this history should help to define what learned helplessness is, and what it is not. This is important because attention to this issue may help to resolve apparent discrepancies in the literature (see below). The term is not appropriate for simply any behavioral or physiological consequence of uncontrollable stress. Rather, it is appropriate for consequences of uncontrollable stress *that depend on the uncontrollability of the stressor*. Thus, it is necessary to demonstrate that the endpoint measure in some experimental paradigm is indeed altered *selectively* by exposure to uncontrollable, relative to exactly equal controllable, stressors. To ignore the controllability issue is to make learned helplessness and stress virtually synonymous, which they are not. Without such a demonstration, the observed effect may be an outcome of exposure to stress per se. In these cases, whatever is being observed may not be learned helplessness. This does not mean that the particular paradigm used is not relevant to depression, and it may even be more relevant than learned helplessness. Some outcomes of uncontrollable stress are indeed insensitive to

controllability, and the controllability of some stressors likely does not matter (e.g. Maier et al., 1986). In addition, this history should make clear that learned helplessness is not simply poor escape learning. There can be many reasons for poor escape responding, only one of which is learned helplessness. Furthermore, conditions that induce learned helplessness (e.g. IS) produce many behavioral outcomes other than poor escape learning. Thus, the claim that some manipulation reverses or blocks learned helplessness (e.g. a drug) requires a demonstration that the manipulation blocks or reduces some of these other outcomes. Otherwise, the manipulation could be leaving the underlying state unaltered and be influencing only the tendency of this state to alter this specific behavior.

1.2. Trans-situationality

In addition, it can be appreciated that in the original experiments subjects received behavioral testing in an environment (shuttlebox) very *different* from that in which the uncontrollable stressor was administered (Pavlovian harness). Even the types of shock (fixed electrode versus footshock) and the rooms in which the two occurred were very different. Indeed, trans-situationality was part of the original definition (Maier et al., 1969), and for many years all, or virtually all, of the experiments conducted with regard to stressor controllability and the effects of uncontrollable stressors had this feature. Thus, for example, when Weiss set out to explore behavioral effects of uncontrollable stress (tailshocks delivered to rats restrained in small wheel turn boxes) rather than their effects on eating and ulcers, he measured swimming in a tank of water (Weiss, 1968). One of the striking features of exposure to uncontrollable stress has always been that it alters behavior in circumstances quite removed from the original experience.

However, more recently, numerous studies have been reported in which behavioral testing (typically for escape learning), and initial exposure to uncontrollable stress (typically inescapable footshocks), occur in the very same or similar environments. For example, a common procedure is now to administer inescapable footshocks, rather than tailshock, while the animal is restricted to one side of the very same shuttlebox that is used for later escape testing. This is especially frequent in investigations designed to test the impact of antidepressants and other drugs. While there is nothing inherently problematic about this shift in procedure, it may well produce a phenomenon that is mediated quite differently than the phenomenon produced when the environments in which exposure to uncontrollable stressors and later behavioral testing occur are distinctly different. This is worth discussing in some detail, as it illustrates the point that experimental arrangements that have been labeled as 'learned helplessness' are sometimes procedurally quite discrepant, and so may actually reflect quite different underlying processes and involve varying neurobiological mechanisms. A number of seeming inconsistencies in the literature may be attributable to this factor, and whether uncontrollable stress and escape testing occur in similar or in different environments may be of special significance for understanding seeming anomalies in the literature with regard to the roles of 5-HT and CRH, to be discussed below.

The importance of whether the uncontrollable stressor and escape testing occur in the same or in different environments can be illustrated by considering the timecourse of learned helplessness. In the original experiments, later failure to escape in a shuttlebox (Overmier and Seligman, 1967) and reduced swimming in water (Weiss, 1968) only occurred if behavioral testing was administered within 48–72 h of the uncontrollable stress experience. This timecourse led to the idea that the failure to escape was mediated by a motivational/emotional 'state change' produced by uncontrollable stress, rather than something associatively conditioned to the environment by the uncontrollable stressor, such as conditioned fear. This conclusion followed because state changes would be expected to dissipate, while conditioned fear is long lasting. Indeed, this timecourse led Weiss to focus on norepinephrine (NE) depletion as a mediator of learned helplessness because depletion of NE by uncontrollable stress had the same timecourse as did the behavioral effects, recovering within 48–72 h (Weiss et al., 1981). Furthermore, many of the other changes produced by uncontrollable stress, such as reduced activity (Jackson et al., 1978), exaggerated fear conditioning (Maier, 1990), reduced social interaction (Short and Maier, 1993), opioid analgesia (Grau et al., 1981), and potentiation of morphine conditioned place preference (Will et al., 1988) have this same sharp timecourse, and all involve behavioral testing in environments very different from that in which the uncontrollable stressor has occurred.

However, more recently some investigators have found poor escape following uncontrollable stress to be more durable (e.g. Malberg and Duman, 2003). Interestingly, these instances occurred using experimental arrangements in which uncontrollable stress and escape testing were administered in the same apparatus. To determine whether this factor was actually critical, we compared the timecourse of escape failure following the administration of the identical ISs in either a different environment than that used for escape testing, or the same environment. Rats first received 100 5-s 1.0 mA shocks delivered via electrodes fixed to the tail. This occurred either while the rats were restrained in Plexiglas tubes in a room different from that used for later escape testing, or freely moving in one side of the shuttleboxes that would be used for testing. A swivel arrangement allowed tailshock to be used in the shuttlebox. Controls were simply placed into either the restrainer or the shuttlebox for an equal period of time. Shuttlebox escape testing occurred 24, 48, 72, or 168 h later using our usual procedures (e.g. Maier et al., 1995b). The results are presented in Fig. 1A. Escape trails terminated automatically

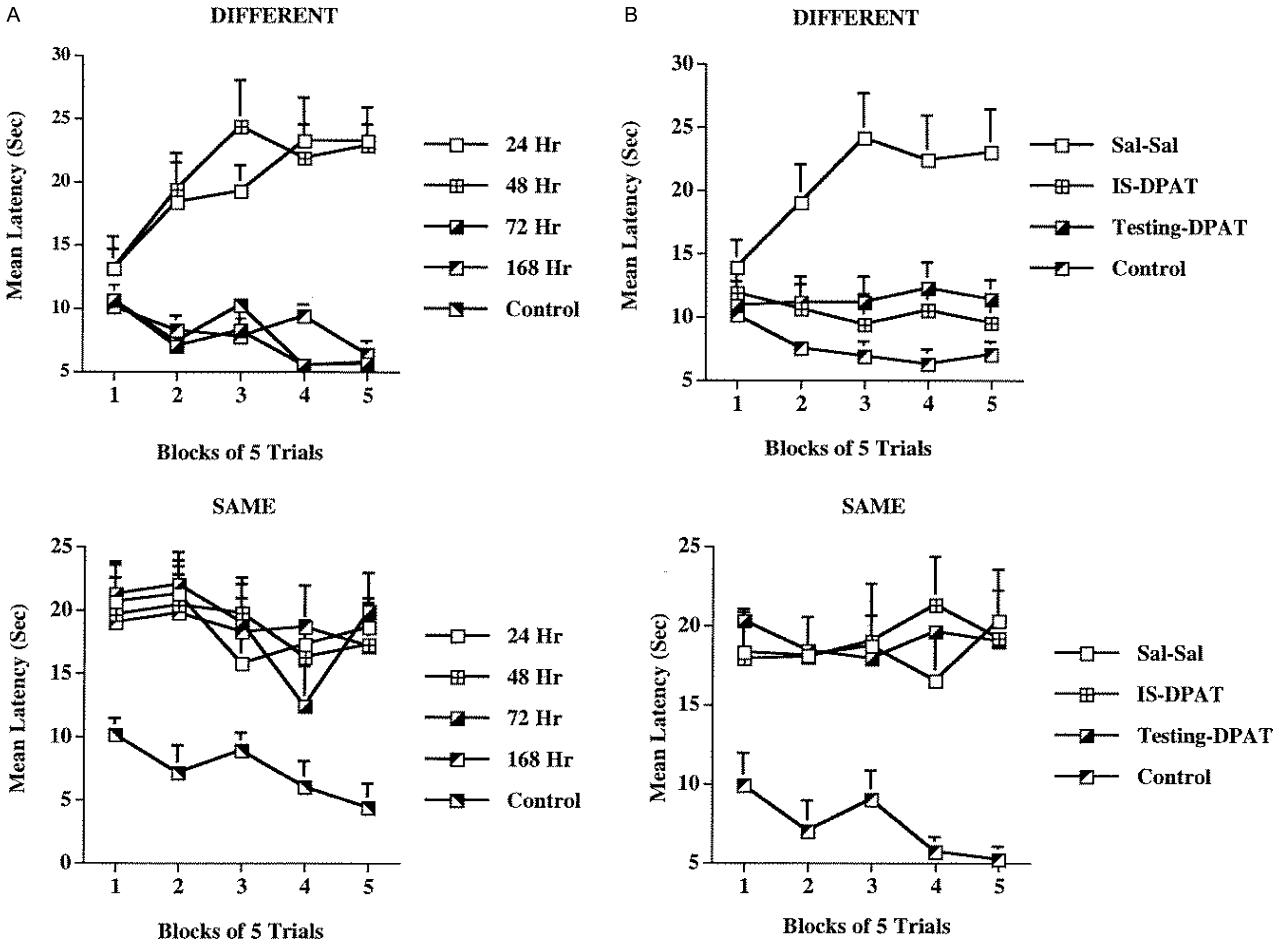

Fig. 1. A. Mean shuttlebox escape latencies for groups ($N=8$) given inescapable tailshocks and tested either 24, 48, 72, or 168 h later. The Control was restrained in the apparatus and tested 24 h later. The Top Panel shows the results for groups that received inescapable shocks in a restraining tube, while the Bottom Panel shows the results for groups that received inescapable shocks in one side of the shuttlebox. B. Mean shuttlebox escape latencies for groups (N= 8) given inescapable tailshocks and tested 24 hr later, Different groups received either saline injection (ip) before both treatments, 8-OH-DPAT before inescapable shock and saline before shuttlebox testing or saline before inescapable shock and 8-OH-DPAT before shuttlebox testing. The Control group received only saline injections and was restrained on the first day and received shuttlebox testing 24 hr later. The top Panel shows the results for groups that received inescapable shocks in a restraining tube, while the Bottom Panel shows the results for groups that received inescapable shocks in one side of the shuttlebox.

if no response had occurred after 30 s, and so group latencies close to 30 s indicate that most animals failed to escape. The data are clear in showing that when ISs were delivered in an environment different from the test shuttlebox, interference with escape only occurred if testing was within 48 h of the shocks, whereas there was no diminution of the effect over time if the shocks were first delivered in the shuttlebox.

Moreover, differences between these two experimental procedures are not restricted to the timecourse of interference with escape. Most importantly for the topic of this Special Issue, the two paradigms are *pharmacologically* distinct. For example, prior work in which testing was given in an environment distinctly different than the IS environment has shown that DRN microinjection of the 5-HT1$_A$ agonist 8-OH-DPAT, before either IS or later escape testing, blocks the effects of IS (Maier et al., 1995b). The rationale for this experiment will be described below, and here it is only necessary to understand that intra-DRN administration of 8-OH-DPAT inhibits DRN 5-HT activity since 5-HT1$_A$ receptors within the DRN function as inhibitory somatodendritic autoreceptors (see below). These somatodendritic 5-HT1$_A$ receptors are more sensitive to 8-OH-DPAT than are post-synaptic 5-HT1$_A$ receptors, and so are selectively activated by low doses of systemic 8-OH-DPAT (Kennett et al., 1987). Fig. 1B shows the results of an experiment in which a systemic dose of 8-OH-DPAT (60 (μg/kg, sc) known to selectively activate somatodendritic 5-HT1$_A$ autoreceptors (Kennett et al., 1987) was administered either before IS or escape testing 24 h later. The design included three groups that received IS and later escape testing. One received saline before both IS and testing, one received 8-OH-DPAT before IS and Saline before testing, and one received saline before IS and 8-OH-DPAT before testing. A fourth group was merely restrained on Day 1 and tested on Day 2, with saline given before both. Restrained 8-OH-

DPAT groups were not included as prior work has shown that this drug does not by itself alter escape behavior, either when given 24 h earlier or just before the escape testing (Maier et al., 1995b). The top panel shows the data for four groups in which IS occurred in Plexiglas tubes, while the bottom panel shows the data for groups in which IS occurred on one side of the shuttlebox used for escape testing. As above, IS in both cases was identical tailshock. Consistent with prior results, 8-OH-DPAT both blocked and reversed the impact of IS on escape learning when IS had been delivered in the Plexiglas tubes. However, 8-OH-DPAT had no effects at all when IS was delivered in the shuttleboxes. This was not a dose issue, as a range of doses of 8-OH-DPAT had no effects when IS occurred in the shuttleboxes. Our interpretation is that activation of 5-HT neurons are critical to the generation and expression of the state changes that mediate the effects of IS that transfer across very different situations (see below), but that conditioned processes not dependent on 5-HT neurons can also produce failure to escape. Regardless of interpretation, this sample experiment should be enough to highlight the conclusion that procedural differences within the learned helplessness paradigm can make an enormous difference in the underlying processes that actually mediate any failure to escape, or other behavioral outcome that might occur. This experiment also suggests that trans-situationality is a key variable in this regard.

It should be emphasized that it is possible to determine a priori, for any IS and testing procedure, whether the subjects treat the two environments as similar or different. Rats and mice freeze when placed into an environment in which they have been shocked before (i.e. there is fear conditioned to the environmental cues), with the amount of freezing decreasing with increasing differences between the environments (i.e. stimulus generalization). Thus, in the above experiments there was considerable freezing measured before the occurrence of the first escape learning trial when testing and IS were in the same apparatus, but no freezing at all before the first escape trial when testing was in the shuttlebox and IS delivered in the small wheel turn boxes via tail electrodes. In experiments designed to determine the factors that mediate generalization between the IS and test environment, the presence of grid floors through which shock has been delivered has proved to be the most important. If IS is delivered via a grid floor, rats freeze as soon as they are placed in another apparatus that has a grid floor, even if it is somewhat different in appearance and ambient sound levels from the original IS.

2. 5-HT and the DRN

Given a manipulation as complex as uncontrollable versus controllable stress, and diverse behavioral consequences (failure to escape, exaggerated fear conditioning, reduced aggression, neophobia), it should be no surprise that numerous neurochemical systems are involved in the mediation of learned helplessness. However, our recent work has focused on 5-HT neurons within the DRN. Indeed, the broad array of behaviors modulated by uncontrollable stress was the starting point for a consideration of 5-HT. Although the behavioral changes produced by IS can be categorized in various ways, one way to summarize these changes is that fight/flight defensive behaviors are reduced, while fear/anxiety related behaviors are increased. It was already known that stimulation of 5-HT neurons within the DRN tends to inhibit fight/flight via projections to the dorsal periaqueductal gray, and to potentiate fear/anxiety via projections to the amygdala (see Graeff et al., 1996 for review). Thus, it seemed that the behavioral pattern produced by IS would occur if IS produced an intense activation of DRN 5-HT neurons.

Consequently, we examined the DRN 5-HT activity produced by ES and yoked IS. IS produced greater *c-fos* expression in labeled 5-HT neurons within the DRN, particularly the caudal DRN (Grahn et al., 1999b). Furthermore, IS produced greater 5-HT efflux in projection regions of the DRN such as the basolateral amygdala and medial prefrontal cortex, as measured by in vivo microdialysis (Amat et al., 1998; Bland et al., 2003). Indeed, although IS produced very large increases in extracellular 5-HT (as much as 600%), ES did not produce any elevation at all relative to controls. DRN 5-HT neurons release 5-HT within the DRN itself from axon collaterals and possibly somata and dendrites (Matos et al., 1996), as well as in projection regions. We, therefore, measured extracellular levels of 5-HT within the DRN during IS and ES, IS, but not ES, increased 5-HT within the DRN.

Although IS selectively activated DRN 5-HT neurons, this activation persisted for only several hours following exposure to IS. However, as noted above, the behavioral effects of IS persist for 48–72 h. It seemed possible that the intense activation of DRN 5-HT neurons produced by IS might sensitize these neurons for a period of time so that the later testing conditions (e.g. footshock escape training) would produce an exaggerated release of 5-HT within projection regions, thereby mediating the behavioral changes that occur. To explore this issue subjects that had received IS, ES, or control treatment were exposed to two footshocks in a different environment 24 h later. Two footshocks were insufficient to alter 5-HT efflux in the basolateral amygdala in controls. However, a 200% increase in extracellular 5-HT in response to the footshocks occurred in animals that had been exposed to IS, but not ES, 24 h earlier (Amat et al., 1998).

A variety of mechanisms could account for this sensitization of DRN 5-HT neurons by IS. For example, 5-HT neurons within the DRN are under the inhibitory control of 5-HT1_A receptors. These receptors are present on the soma and dendrites of DRN 5-HT neurons and inhibit the activity of these neurons, 5-HT synthesis within these neurons, and 5-HT release in projection regions (Casanovas and Artigas, 1996; Sprouse and Aghajanian, 1987). As

already noted, DRN neurons release 5-HT within the DRN when activated, and therefore, DRN 5-HT neurons are under autoinhibitory control. Interestingly, these 5-$HT1_A$ receptors within the DRN are especially susceptible to desensitization produced by 5-HT (Kennett et al., 1987). Thus, it is possible that the large amount of extracellular 5-HT within the DRN produced by IS desensitizes 5-$HT1_A$ inhibitory autoreceptors for a period of time, thereby sensitizing these neurons to any further inputs that might occur. Indeed, receptor binding studies indicate reduced 5-$HT1_A$ density in the DRN for a period of 48–72 h following exposure to IS (Short et al., 2000). Other mechanisms, such as increased tryptophan hydroxylase activity, are also possible.

There is nothing in the data documenting selective activation of DRN 5-HT activity by an uncontrollable stressor to indicate that it is this change that is critical to the mediation of the behavioral effects that follow. DRN 5-HT activation and sensitization could be neither necessary nor sufficient to produce the behavioral sequelae of IS. To determine whether the activation of DRN 5-HT neurons is sufficient to produce learned helplessness, experiments are needed in which it is determined whether the activation of DRN 5-HT neurons without any stressor presentation would produce the same behavioral changes as does IS. Two methods have been employed that capitalize on the facts that (a) DRN 5-HT neurons are under tonic inhibition from GABA interneurons (Tao et al., 1996), and this inhibition involves control by $GABA_A$ receptors (Celada et al., 2001), (b) these GABA interneurons express opioid receptors which inhibit their activity (Tao et al., 1996), and (c) benzodiazepines bind to the $GABA_A$ complex and facilitate GABAergic inhibition, while inverse benzodiazepines agonists interfere with GABAergic inhibition (Braestrup et al., 1980). Thus, both opiate antagonists and inverse benzodiazepines agonists should activate DRN 5-HT neurons since they both interfere with the tonic inhibition of these neurons, and both produce failure to escape and exaggerated fear 24 h after microinjection into the DRN (Grahn et al., 1999a; Maier et al., 1995a).

To determine whether these 5-HT changes are necessary, experiments are needed in which the DRN 5-HT changes produced by IS are prevented, and subsequent behavior examined. The argument above suggests that DRN 5-HT activity is necessary at the time of IS and at the time of behavioral testing for escape, fear conditioning, and the other behavioral sequelae of IS. It is necessary at the time of IS, because it is here that large amounts of 5-HT are released within the DRN that putatively desensitize 5-$HT1_A$ receptors, and at the time of testing because it is the exaggerated release of 5-HT in projection regions produced by these sensitized DRN 5-HT neurons during testing that is the presumed proximate cause of at least some of the behavioral effects. A number of manipulations have been employed to inhibit DRN 5-HT neurons. As already discussed, DRN 5-HT neurons are under the inhibitory control of somatodendritic 5-$HT1_A$ and $GABA_A$ receptors. Thus, if DRN 5-HT activity is necessary for the production of learned helplessness, then 5-$HT1_A$ and $GABA_A$ agonists microinjected within the DRN should block the behavioral consequences of IS, and should do so if injected either during IS or during later behavioral testing. Both the 5-$HT1_A$ agonist 8-OH-DPAT and benzodiazepines microinjected within the DRN blocked both the escape deficit and the potentiated fear conditioning produced by IS, and both did so when injected before either IS or before behavioral testing (Maier et al., 1994, 1995b). Inhibiting the DRN also blocks other effects of IS that have not been discussed here (Will et al., in press). Lesion of the DRN should, of course, also block learned helplessness, and it does (Maier et al., 1993).

3. CRH

The DRN is a small midbrain structure, containing perhaps 30,000 neurons in the rat. It is, thus, not likely to be capable of performing the complex information processing necessary to determine whether a stressor is controllable or uncontrollable, and is doubtlessly part of a more extended circuit. In addition, a number of other structures and transmitters have been shown to be important in the production of learned helplessness. It may be that the DRN is on the efferent side of the 'learned helplessness circuit', receiving inputs from some of the other regions that are of importance. For example, IS, relative to ES, strongly stimulates NE neurons within the locus coeruleus (Weiss et al., 1981), and this stimulation is important for producing some of the behavioral effects of IS (Weiss and Simson, 1986). Interestingly, the LC provides NE input to the DRN, with alpha-1 NE receptors on 5-HT neurons regulating their activity (Hopwood and Stamford, 2001). The microinjection of the alpha 1 antagonist benoxathian into the DRN before IS blocks the effects of IS on behavior (Grahn et al., 2002), supporting a role for NE input to the DRN.

As is well known, CRH plays a crucial role in integrating endocrine, autonomic, and behavioral responses to stressors (Koob et al., 1993; Tao and Auerbach; 2004., Vale et al., 1981). Thus, it is noteworthy that the DRN receives extensive CRH projections (Sakanaka et al., 1987) and expresses dense CRH immunoreactivity (Swanson et al., 1983). There are two known CRH receptors (CRHR1 and CRHR2). Both are G-protein coupled receptors that initiate similar signal transduction cascades, but differ in relative affinities for CRH-related ligands (Grigoriadis et al., 1996) and in their regional distribution (Chalmers et al., 1995). CRHR1 is widely distributed and has a relatively high affinity for CRH and low affinities for the recently discovered ligands urocortin II (UCN II, Reyes et al., 2001), and urocortin III (UCN III, Lewis et al., 2001). Conversely, CRHR2 has a much more restricted distribution (Chalmers et al., 1995), and has relatively low affinity for CRH and high affinity for UCN II and III (Reyes et al.,

2001). CRHR1 is present within the DRN, but the DRN is one of the few regions that has a dense expression of CRHR2 (Chalmers et al., 1995). Indeed, the DRN is relatively unique in that CRHR2 outnumbers CRHR1 within this structure.

The foregoing suggests that CRH, or some other CRH-related ligand, might provide important input to the DRN in the mediation of learned helplessness. To begin to explore this possibility, the CRH antagonist D-Phe CRH (12-41) was microinjected into the (caudal, see below) DRN before either IS or later behavioral testing (Hammack et al., 2002). D-Phe CRH (12-41) dose-dependently blocked the behavioral effects of IS when administered before IS, but had no effect when given before testing. This suggests that CRH input to the DRN is important in generating learned helplessness, and consistent with this idea, the intra-DRN microinjection of CRH by itself produced exaggerated fear conditioning and failure to escape footshock 24 h later (Hammack et al., 2002). CRH only induced these behavioral changes when injected into the caudal DRN, with microinjection into the rostral DRN having little or no effect (Hammack et al., 2002).

3.1. CRHR1 and CRHR2

A role for CRH within the DRN in initiating the 5-HT alterations responsible for learned helplessness would appear to be inconsistent with studies that have examined the impact of CRH on DRN 5-HT activity. This is because these studies have found CRH to generally *inhibit* DRN 5-HT neurons. Using in vivo recording techniques, Kirby et al. (2000) found both intra-DRN and ICV CRH administration to decrease DRN 5-HT unit activity. Interestingly, this inhibitory effect *decreased* as CRH dose increased, and tended to become excitatory at even higher doses. Similarly, extracellular 5-HT in the lateral septum, a projection region of the DRN, decreased in response to ICV (Price et al., 1998) and intra-DRN (Price and Lucki, 2001) CRH. Again, the inhibition decreased with increasing CRH dose, and even larger doses tended to produce increases in 5-HT efflux rather than decreases. Importantly, the inhibitory effects of CRH were reversed by a CRHR1-selective antagonist (Kirby et al., 2000). In contrast, Lowry et al. (2000) reported consistently excitatory effects of CRH in midbrain slices in the caudal DRN. Whether this difference was caused by the use of in vitro versus in vivo techniques, or by the more caudal location within the DRN of the excitatory effects reported by Lowry et al. (2000) is unknown. In this regard, we have already noted that the behavioral effects of CRH were restricted to a caudal location within the DRN.

Our behavioral data were also somewhat surprising because, in a prior series of studies, we had found a peripherally administered nonpeptide CRH antagonist (antalarmin) to be completely ineffective in blunting the behavioral impact of IS (Deak et al., 1999). In addition, very large amounts of intra-DRN CRH (between 0.5 and 1.0 μg r/hCRH) had to be administered to produce later failure to escape and potentiated fear conditioning. Although there are many possible reasons why systemic antalarmin was ineffective and intra-DRN D-Phe CRH (12–41) completely blocked learned helplessness, it can be noted that antalarmin is relatively selective for CRHR1, while D-Phe CRH (12-41) is non-selective between CRHR1 and CRHR2 (Chen et al., 1996). In addition, CRH is relatively selective for CRHR1, with high dosages being required to activate CRHR2 (Reyes et al., 2001). Thus, the behavioral data are consistent with the idea that the effects of CRH within the DRN in producing learned helplessness are mediated by CRHR2. Moreover, the shape of the dose–response curves found in the electrophysiological and neurochemical studies above is consistent with the idea that CRHR1 activation inhibits DRN 5-HT activity, while CRHR2 activation excites 5-HT neurons. This is because at low doses of CRH only CRHR1 should be activated, and as dose increases, CRHR2 should then be brought into play. Since CRHR1 and CRHR2 activate similar signal transduction cascades, such an inhibition/excitation arrangement could only occur if the two receptors were expressed on different cell populations within the DRN. For example, this pattern would occur if CRHR1 were differentially expressed on GABAergic interneurons, while CRHR2 were expressed on the 5-HT neurons themselves.

For these reasons we have begun to explore the roles of CRHR1 and CRHR2 within the DRN. Along with Jose Amat, Sondra Bland, and Julie Tamblyn we have conducted a series of studies in which the selective CRHR2 agonist UCN II was microinjected into the caudal DRN. Fig. 2 shows the levels of extracellular 5-HT within the basolateral amygdala, a projection region of the DRN. Clearly, this is an excitatory pattern, and an examination of *c-fos* expression in 5-HT labeled cells revealed a similar pattern. At a behavioral level, Hammack et al. (2003) found that the intra-DRN administration of the selective CRHR2 agonist UCN II dose-dependently produced deficits in escape behavior and potentiated fear conditioning 24 h later, and that it did so at a molar concentration 100 to 1000-fold lower than that required by r/hCRH. Furthermore, intra-DRN injection of the relatively selective CRHR2 antagonist antisauvagine-30 (ASV-30, Higelin et al., 2001) before IS dose-dependently blocked the later behavioral changes produced by IS, while the CRHR1 selective antagonist 2-methyl-4-(*N*-propyl-*N*-cycloproanemethylamino)-5-chloro-6-(2,4,6-trichloranilino)pyrimidine (NBI27914, 9) was without effect.

Interestingly, the idea that CRHR1 inhibits DRN 5-HT neurons and that CRHR2 is excitatory suggests more than that CRHR2 is involved in the mediation of learned helplessness. Counterintuitively, it suggests that selective CRHR1 stimulation, such as would occur with low doses of CRH, should actually *block* the development of learned helplessness. To test this idea Hammack et al. (2003) microinjected either 0.5, 5.0 or 50.0 ng oCRH into the

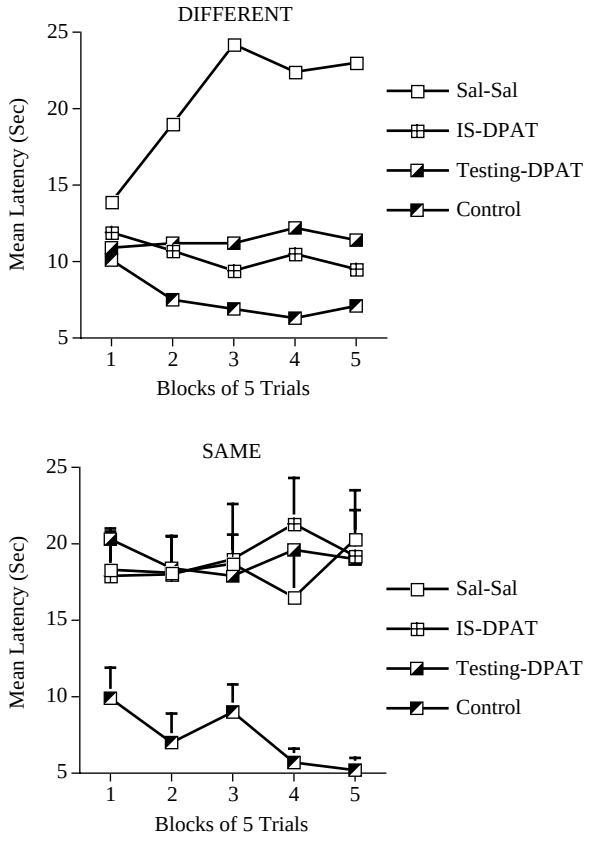

Fig. 2. Mean levels of extracellular 5-HT in the basolateral amygdala as a percentage of baseline, for groups that received saline or 1, 10, 50, or 100 ng intra-DRN CRH. Injection occurred at time 0.

caudal DRN before either IS or intra-DRN UCN II administration. The lowest dose had no effect on the interference with escape and potentiated fear conditioning that occurs 24 h after IS or UCN II. However, the 5.0 ng dose of oCRH blocked the behavioral effects of IS and of UCN II. Importantly, as the dose increased to 50.0 ng, the effects of oCRH disappeared. It can be noted that the 5.0 ng dose is roughly equivalent to the dosage found to be inhibitory by Kirby et al. (2000), Price et al. (1998), and Price and Lucki (2001).

The findings summarized above might seem inconsistent with prior studies that have examined the role of CRH in 'learned helplessness'. Both Mansbach et al. (1997), and Takemori et al. (2001) have reported that the systemic administration of a CRHR1 selective nonpeptide antagonist before IS blocks the escape deficits that follow. Two important factors can be noted. First, both investigations employed systemic injection, and thus CRH receptors in regions other than the DRN would have been reached. As already noted, the DRN is likely part of a much more extended circuit, and CRHR1 is widely distributed throughout the brain. There is nothing in the findings that indicate an important role for CRHR2 in the DRN to suggest that CRHR1 might not be involved in some of these other brain structures that are part of the 'learned helplessness circuit' (see below). In addition, both the Mansbach et al. (1997), and Takemori et al. (2001) studies administered IS in the same apparatus as was used for later escape testing, a procedure that produces a different phenomenon from the trans-situational procedures used in the CRH studies described here. Consistent with the importance of this procedural factor, Deak et al. (1999) found this same CRHR1 selective nonpeptide antagonist to be without effect at any dose when IS and testing were conducted in different environments. It can be added that not even serotonergic lesions reduce escape deficits if IS and testing are conducted in the same environment (Siegel and Broun, 1988; Soubrie et al., 1986).

4. Implications

4.1. CRH and 5-HT

It is clear that CRH and other CRH-like ligands are intimately involved in mediating stress-related and emotional/mood phenomena, with CRHR1 generally being regarded as the critical mediator. CRHR2 has generally not been accorded a major role in such processes. Indeed, deletion of the CRHR2(α) gene has been reported to either produce a phenotype characterized by increased anxiety-like behavior (Bale et al., 2000; Kishimoto et al., 2000), or to have no effect on anxiety (Coste et al., 2000). However, these results could have been produced by developmental compensations, and indeed, Bale et al. (2000) found elevated levels of CRH and UCN in the genetically altered subjects. The results of studies employing antisense oligonucleotides that target CRHR2 have also been conflicting (e.g. Heinrichs et al., 1997 vs. Ho et al., 2001). The results of ICV administration of ASV-30 have also been inconsistent, with both increases (Radulovic et al., 1999) and decreases (Pelleymounter et al., 2002; Swanson et al., 1983) in anxiety-like behavior having been reported.

These inconsistencies in the outcomes of CRHR2 manipulations may result from CRHR2 playing very different roles in different brain regions, a possibility suggested by Takahashi (2001). If this is so, then the impact of any manipulation that alters CRHR2 function across many brain regions, would be the result of the summed effects across these regions, as would occur with genetic deletion or ICV administration of oligonucleotides or antagonists. Different behavioral tasks (e.g. shock-induced freezing, the elevated plus maze, escape learning) and psychological processes (e.g. anxiety, depression) will depend more on the activity of some brain regions than others, and so different results would be expected. Consistent with this line of reasoning, blockade of CRHR2 within the lateral septum appears to consistently reduce anxiety-related behavior (Bakshi et al., 2002), and the data reviewed here indicates that blockade of these

receptors within the caudal DRN blocks learned helplessness.

This concept of regional specificity of function of CRHR2 also suggests that the interplay between CRHR1 and CRHR2 will vary by region. The internal circuitry of the DRN may be such that CRHR1 and CRHR2 stimulation has opposed actions, but this is not necessarily so in other brain regions. Indeed, this arrangement may not even hold throughout the DRN, as the DRN is a complex structure composed of anatomically distinct subregions that differ with regard to CRH innervation and internal organization (Commons et al., 2003; Lowry, 2002; Valentino et al., 2001).

The data regarding the role of 5-HT in stress-related phenomena is equally inconsistent, with reports of stressor-induced changes in 5-HT ranging from little or no effect, to large increases, to decreases. As noted by Kirby et al. (1997), the effects of stress on 5-HT are likely to be stressor and region specific, and the work reviewed here underscores this conclusion. Caudal DRN 5-HT neurons would appear to be especially responsive to intense uncontrollable stressors such as IS, likely because this region receives a unique set of inputs (Peyron et al., 1998). These neurons are critical in the mediation of the behavioral sequelae of IS, likely because of the unique projections of these neurons (Vertes, 1991). However, other 5-HT containing regions may be more responsive than the caudal DRN to different stressors than those used here (e.g. Dilts and Boadle-Biber, 1995), and may be more important in mediating other phenomena. For example, both 5-HT (Le et al., 2002) and CRH (Le et al., 2000) are involved in the reinstatement of drug taking produced by footshock, but here the MRN is the key 5-HT-containing nucleus (Funk et al., 2003; Le et al., 1999). Thus, CRH and 5-HT interact to also produce the reinstatement phenomenon, but here the median raphe nucleus (MRN) is the site of interaction, and the CRHR1 receptor is likely the key receptor involved (Shaham et al., 1998). This line of reasoning suggests that 'global' statements concerning the roles of 5-HT, CRH, and their interaction will be problematic.

4.2. Depression and anxiety

Do the data reviewed here have any implications for the use of learned helplessness as a model of depression? This is a difficult question, and the answer depends, at least in part, on the purpose(s) toward which the paradigm is utilized. If the paradigm is utilized to attempt to understand the etiology and underlying neurobiology of depression, then a number of issues become key. Some are: (1) Is the experience of specifically uncontrollable stress important in the etiology of depression, because it is the essence of learned helplessness? (2) Are the symptoms of learned helplessness and depression similar? (3) Is the underlying neurobiology of learned helplessness and depression similar? On the other hand, if the paradigm is utilized as a drug screen, then different questions become important, with the core involving whether the paradigm is selectively sensitive to antidepressant drugs.

We will only comment briefly on the issue of learned helplessness as a drug screen, because this is not the source of our interest in the phenomenon. Certainly, under some conditions learned helplessness is sensitive to antidepressants (e.g. Gambarana et al., 2001). However, effective antidepressant drugs may not themselves be selective to action in depression. For example, SSRIs are now known to be effective in a number of anxiety disorders (e.g. Van Der Linden et al., 2000), and are currently often the drug of choice. In this context, learned helplessness, at least under some conditions, is extremely sensitive to blockade (Drugan et al., 1984), and perhaps reversal (Maier et al., 1994; Short and Maier, 1993), by anxiolytics such as benzodiazepines. If there ultimately proves to be a substantial overlap in the neural mediation of different disorders such as depression and anxiety, then non-selectivity between drugs that alter these two conditions may prove to be a virtue.

In terms of the use of the learned helplessness paradigm to understand the etiology and underlying neurobiology of depression, there is again an overlap with anxiety. As already noted, learned helplessness is a model in which to study the importance of stressor controllability and the mechanisms that are involved, and the experience of uncontrollable stressors has been argued to be important in the etiology of both depression and certain anxiety disorders (e.g. Foa et al., 1992). At the level of behavior and symptoms, IS induces behaviors that resemble symptoms of depression (Weiss and Simson, 1986) and anxiety (Maier and Watkins, 1998a,b). At the level of underlying neurobiology, the similarity between learned helplessness, depression, and anxiety is difficult to assess for a number of reasons. Of most relevance here, there are neurobiologically distinct phenomena that have been called learned helplessness in the literature. Thus, conclusions derived from one do not necessarily apply to another. This may also be true of depression and anxiety themselves.

The studies described above clearly indicate that an uncontrollable stressor, IS, activates 5-HT neurons within the DRN, relative to controllable stress. Furthermore, it is also the case that many of the behavioral sequelae that follow IS that occur in situations distinct from the IS environment are mediated by exaggerated DRN 5-HT activity during the behavioral testing. This is true not only for potentiated fear conditioning and escape failure, but also for decreased social interaction (Short and Maier, 1993), opioid analgesia (Sutton et al., 1997), potentiated morphine conditioned place preference (Will et al., 2002), and increased anxiety on a circular elevated maze (Short et al., 2000). Although the role of 5-HT in depression is not completely clear, it is far easier to relate enhanced 5-HT activity to anxiety, than to depression. Although the literature relating anxiety to 5-HT is quite complex (see

Millan, 2003 for a recent review), there are numerous instances in which inhibition of DRN 5-HT activity reduces anxiety in animal models, and increasing DRN 5-HT activity increases anxiety-related behavior (see Millan, 2003 for review). It might seem that the effectiveness of SSRIs in a number of anxiety disorders argues against an involvement of increased, rather than decreased, 5-HT in anxiety. However, the acute effect of SSRI treatment is often to increase anxiety (Sramek et al., 2002), and the chronic administration of SSRIs has been shown to desensitize/downregulate a number of post-synaptic 5-HT receptors whose activation by agonists often increases anxiety-related behaviors (Van Oekelen, 2003).

The data reviewed in this paper may be relevant to a number of issues related to 'anxiety'. One concerns the very definition of anxiety and its distinction from fear or conditioned fear. Fear has generally been regarded as a motivational system elicited by either learned (e.g. a tone that signals a footshock) or unlearned (e.g. the sight and smell of a cat to a rat) signals for danger, whose outputs in the rat are freezing, increases in respiration, etc., and which functions for species defense. It may be that anxiety is not different, but merely that the signals are less identifiable and more diffuse. However, animals that have received IS show a pattern of *exaggerated fear* in diverse situations. It has already been noted that they show more rapid and stronger explicit fear conditioning and show exaggerated anxiety on measures such as social interaction. Interestingly, IS also leads animals to avoid novel objects. For example, if a rat is presented with a familiar and a novel object, it will approach and explore the novel object. However, an animal that has had IS will avoid the novel object (unpublished data). Also as discussed above, this potentiation of fear responses occurs in situations far removed from the original IS experience and has a timecourse, dissipating in 2–3 days. This suggests that these behavioral alterations are mediated by an IS-induced change in a *state* of the organism. Perhaps this is what is meant by anxiety, a state of the organism that leads to the exaggeration of fear if there is a potential source of threat or ambiguity, either learned or unlearned. This is different from fear. Fear is driven by and tied to a discrete stimulus, either learned or unlearned. It is induced by the stimulus, and disappears rapidly when the stimulus is removed.

There is much known concerning the neural circuitry that underlies fear. There is a substantial literature implicating the amygdala in this process. One conception would be that IS sensitized DRN 5-HT neurons modulate the fear circuit, potentiating some aspects of its activity. Here, the DRN would not be involved in mediating 'normal' fear, but only in potentiating fear should the fear circuitry be activated. Indeed, lesion of the DRN does not alter fear conditioning in controls, but does prevent the exaggeration of fear conditioning produced by IS (Maier et al., 1993).

In a fashion similar to the argument made here, M. Davis and colleagues have also recently distinguished between fear and anxiety. For example, Walker et al. (2003) have distinguished between long duration responses that build and dissipate slowly, versus responses that are tightly driven by discrete stimuli. Interestingly, they argue that the phasic fear response involves mediation by the central nucleus of the amygdala, while the more diffuse anxiety response involves mediation by the bed nucleus of the stria terminalis. It has been known for some time that lesion of the central nucleus of the amygdala do not prevent IS-induced escape deficits (Maier et al., 1993). Whether such lesions block IS-induced potentiation of fear conditioning could not be assessed, as the lesions blocked fear conditioning in controls as well. Intriguingly, Hammack et al. (2004) have recently found that in contrast to amygdala lesions, bed nucleus lesions do block the escape deficit produced by IS. These lesions did not reduce basal fear conditioning in controls, but did block IS-induced potentiation of fear conditioning. Thus, the present data converges with the work of Davis and colleagues in distinguishing between fear and anxiety. Clearly, the bed nucleus of the stria terminalis is a key structure, and it can be noted that this structure is rich in CRH cell bodies that may project to the DRN (Gray and Magnuson, 1992). Conversely, the DRN sends 5-HT projections to the bed nucleus. It is possible that the bed nucleus activates the DRN under conditions of anxiety, that the DRN regulates the bed nucleus, or both.

Finally, the experiments reviewed here support the proposal recently made by Lowry (2002) that 5-HT neurons projecting from the caudal DRN to limbic and cortical structures form a mesocorticolimbic 5-HT system that is involved in the mediation of anxiety, in a manner analogous to the role of the mesocorticolimbic dopamine system in reward. Our data suggest that uncontrollable stressors selectively activate this system, thereby inducing a state of anxiety that persists for a number of days, and that CRH, or a CRH-related ligand acting at CRHR2, is at the core of this process.

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