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ACTIVATION OF THE VENTRAL MEDIAL PREFRONTAL CORTEX DURING AN UNCONTROLLABLE STRESSOR REPRODUCES BOTH THE IMMEDIATE AND LONG-TERM PROTECTIVE EFFECTS OF BEHAVIORAL CONTROL

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Abstract

The degree of behavioral control that an organism has over a stressor determines the behavioral and neurochemical sequelae of the stressor, with the presence of control preventing the typical outcomes that occur when the stressor is uncontrollable (e.g. failure to learn, exaggerated fear, dorsal raphe nucleus (DRN) 5-HT activation). Furthermore, an experience with a controllable stressor blocks the consequences of later uncontrollable stressors (“immunization”). These effects of control have been argued to be mediated by control-induced activation of ventral medial prefrontal cortex (mPFCv) output to the DRN. The experiments that have led to this interpretation have all involved the inactivation of the mPFCv with muscimol, showing that inactivation during the stressor eliminates the stressor-resistance produced by control, with the controllable stressor now acting as if it were uncontrollable. The present experiments in rats employed the opposite strategy, activating the mPFCv during the stressor. mPFCv microinjection of picrotoxin during the stressor eliminated the DRN 5-HT activation that normally occurs during the uncontrollable stressor, as well as the escape learning deficit and exaggerated fear that normally follows uncontrollable stress. Furthermore, mPFCv activation during an initial exposure to an uncontrollable stressor led the uncontrollable stressor to produce behavioral and neurochemical immunization when the subjects were later exposed to an uncontrollable stressor. That is, the conjoint activation of the mPFCv and exposure to an uncontrollable stressor led the uncontrollable stressor to act as if it were controllable. These results provide strong support for the argument that behavioral control produced stress-resistance by activating the mPFCv.

Keywords

stress resilience; learned helplessness; 5-HT; dorsal raphe nucleus; microdialysis; picrotoxin

Physically identical aversive events produce vastly different behavioral and neurochemical sequelae depending on whether the condition is, or is not under behavioral control (the ability to alter the onset, offset, duration, or intensity of adverse conditions by behavioral actions) (Maier and Seligman, 1976; Weiss, 1968). Potent stressors over which the organism has no control produce changes in behavior resembling both depression (Weiss and Simson, 1986) and anxiety (Maier and Watkins, 1998), while physically identical but controllable stressors do not. Interestingly, control does more than blunt the impact of the stressor that is being

controlled. The experience of control also blocks the behavioral and neurochemical consequences of uncontrollable stressors that occur *at a later time*, a phenomenon called “immunization” (Williams and Maier, 1977).

Uncontrollable and controllable stressors produce vastly different behavioral sequelae, at least in part, because uncontrollable stressors (typically inescapable shocks, IS) activate serotonergic (5-HT) neurons in the dorsal raphe nucleus (DRN) much more than do controllable stressors (escapable shocks, ES) (Grahn et al., 1999), thereby leading to sensitization of these neurons (Amat et al., 1998). However, it has recently been argued that information about the presence/absence of control is processed not by the DRN but by ventral regions of the medial prefrontal cortex (mPFCv), which then regulate DRN 5-HT activity (Amat et al., 2005, 2006). The essence of the argument is that a) intense stressors per se activate DRN 5-HT neurons, and b) the detection of behavioral control activates glutamatergic output neurons from the mPFCv to the DRN that synapse preferentially onto GABAergic interneurons within the DRN that inhibit 5-HT cells (Jankowsky and Sesack, 2004). Thus, detection of the presence of control would actively inhibit DRN 5-HT activity and behavioral effects that depend on DRN 5-HT activation.

The evidence for the above conclusions derives entirely from experiments in which mPFCv output was inhibited by microinjection of muscimol during IS and ES exposure (Amat et al., 2005, 2006; Baratta et al., 2007). Inhibition of the mPFCv during IS did not alter either the DRN activation or behavioral consequences produced by IS. However, intra-mPFCv muscimol increased the DRN 5-HT activation produced by ES to the same level produced by IS, and led ES to produce the behavioral consequences normally produced by IS. Furthermore, mPFCv inactivation during an initial ES exposure prevented the behavioral and neurochemical immunization that ES produces upon subsequent exposure to IS. As a summary, inactivation of the mPFCv during ES led ES to act as if it were IS.

The experiments reported here examined the role of the mPFCv in stressor controllability phenomena by using the inverse strategy, that is, pharmacological *activation* of the mPFCv during IS and ES. The position being tested would expect that mPFCv activation during the stressor would lead *IS to function as if it were ES*. Thus, IS should not produce DRN 5-HT activation or later behavioral outcomes typical of IS. Furthermore, now IS should *protect* the organism against the later effects of IS, a counterintuitive prediction.

EXPERIMENTAL PROCEDURES

In all experiments, subjects were male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) weighing 275–350 g, housed four per cage on a 12-h light/dark cycle (on at 07:00 h and off at 19:00 h). Experiments were conducted between 08:00 and 1600 h. All procedures conformed to international guidelines on the ethical use of animals and were approved by the Institutional Animal Care and Use Committee of the University of Colorado at Boulder. An effort was made to reduce animal number and suffering to a minimum.

Overall organization

In a first set of experiments we studied how stimulation of the vPFCm modifies the impact of controllable and uncontrollable stressors on DRN activity and behavior (learned helplessness); and, in a second set, how the same vPFCm manipulation changes the effect of a stress experience on a subsequent uncontrollable stress (behavioral immunization, Amat et al., 2006). Common methodology will be described first followed by methods and results specific to each of the two sets of experiments.

Surgery and cannulation

Surgery was carried out under anesthesia with a mixture of 100 mg kg⁻¹ Ketamine (Fort Dodge Animal Health, Fort Dodge, IA, USA) and 6.4 mg kg⁻¹ xylazine and 1.6 mg kg⁻¹ acepromazine (Vedco Inc., St. Joseph, MO, USA). All rats were implanted with dual cannula guides for microinjections (26 gauge) 1 mm center-to-center distance (Plastics One, Roanoke, VA, USA). In most rats, the tips of the cannulae were aimed at the prelimbic/infralimbic (PL/IL) junction within the mPFCv: 2.2 mm rostral to bregma, 3.5 mm ventral from the dura mater and 0.5 mm relative to midline. Eight rats were implanted with cannula guides for microinjection 2 mm caudal to the PL/IL regions (cingulate cortex area 2 (Cg2)) as site-specificity control. A group of rats were implanted with a second cannula guide for microdialysis probes (CMA 12), with the tip terminating just above the caudal DRN: 8.3 mm caudal to bregma and 5 mm ventral from the dura matter at the midline (Amat et al., 2005). A screw cap of a 15-ml conical centrifuge tube, whose central lid portion was removed, was also affixed to the skull so that its threads were exposed and it encircled the cannulae guide. This was done so that the skull assembly could be protected during microdialysis. Rats were allowed to recover for 1–2 weeks before experimentation. All surgical procedures to any given animal were performed the same day.

Picrotoxin (PIC) microinjection

Animals were injected with 0.5 μ l of either 100 ng of PIC (Sigma, St. Louis, MO, USA), or saline vehicle (VEH) in each side of the mPFCv. Dual 33-gauge microinjectors (Plastics One) attached to PE 50 tubing were inserted through the guides, from which they protruded 1 mm. The other end of the tubing was connected to a 25 μ l Hamilton syringe that was attached to a Kopf microinjection unit (Model 5000). The volumes were injected over a period of 30 s, and the injector was left in place for 2 min to allow diffusion. Any given injection was considered successful if fluid could be readily dispensed from the injector tips after it was withdrawn from the brain.

Wheel-turn escape/yoked IS procedure

Each rat was placed in a Plexiglas box (14×11×17 cm) with a wheel mounted in the front and a Plexiglas rod extending from the back. The rat's tail was taped to the Plexiglas rod and affixed with copper electrodes. Rats received shocks in yoked pairs (ES and IS). The treatment consisted of 100 trials with an average intertrial interval of 60 s. Shocks began simultaneously for both rats in a pair and terminated for both whenever the ES rat met a response criterion. Initially, the shock was terminated by a quarter turn of the wheel. The response requirement was increased by one quarter turn when each of three consecutive trials was completed in less than 5 s. Subsequent latencies under 5 s increased the requirement by 50% up to a maximum of four full turns. If the requirement was not reached in less than 30 s, the shock was terminated and the requirement reduced to a single quarter turn. This procedure was used to insure that the ES rats learned an operant response. Shock intensity was 1.0 mA for the first 30 trials, 1.3 mA for the second 30 trials and 1.6 mA for the last 40 trials, to maintain good escape responding. Nonshocked home cage control (HCC) rats remained undisturbed in the colony, except during the microdialysis experiments, where they remained undisturbed in the dialysis room.

In vivo microdialysis

The afternoon before the experiment, a CMA 12 microdialysis probe (0.5 mm in diameter, 1 mm membrane with a 20-kD molecular weight cut-off; CMA/Microdialysis Inc., North Chelmsford, MA, USA), was introduced through the cannula guide so that the membranous tip of the probe was within the DRN. A portion of a 15-ml Eppendorf tube was screwed onto the skull-mounted screw cap, through which the dialysis tubing, protected within a metal

spring, entered and attached to the probe. Each animal was placed individually in a Plexiglas bowl (Bioanalytical Systems, West Lafayette, IN, USA) and infused with isotonic Ringer's solution (Baxter, Portage, MI, USA) at a rate of $0.2 \mu\text{l min}^{-1}$ overnight. At 09:00 h the next day, the flow rate was increased to 1.5 l min^{-1} and a 90-min stabilization period was allowed. The infusion flow remained constant throughout the experiment. Samples were collected every 20 min. After stabilization, six (learned helplessness experiment) or four baseline samples (immunization experiment) were collected. Next the rats were placed in Plexiglas wheel-turn boxes that were designed to accommodate the dialysis tubing. There they received 100 ES or yoked IS tail shocks. Five samples were collected during the session. After this, the rats were transferred back to the Plexiglas bowls where three post-shock samples were collected. During collection of the last sample, brisk movements of the skull-mounted screw cap were performed to test for possible 5-HT increase due to rat head movement during the dialysis. The data from the rat were discarded if that procedure caused 5-HT increase. Two HCC control groups received bilateral mPFC injections of either VEH or PIC.

5-HT analysis

5-HT concentration was measured in dialysates by HPLC with electrochemical detection. The system consisted of an ESA 5600A Coularray detector with an ESA 5014B analytical cell and an ESA 5020 guard cell. The column was an ESA MD-150 (C-18, $3 \mu\text{m}$, $150 \times 3.2 \text{ mm}$) maintained at 37°C , and the mobile phase was the ESA buffer MD-TM. The analytical cell potentials were kept at -75 mV and $+250 \text{ mV}$ and the guard cell at $+300 \text{ mV}$. Dialysate ($25 \mu\text{l}$) was injected with an ESA 542 autosampler that kept the dialysates at 6°C . External standards (Sigma) were run each day to quantify 5-HT by means of peak height, using an ESA software, the experimenter being blind to experimental condition.

Cannula and dialysis probe verification

At the end of the experiment an overdose of pentobarbital was administered and brains were removed and frozen. A cryostat was used to take $40\text{-}\mu\text{m}$ sections, which were then stained with Cresyl Violet for cannula placement verification. The mPFC injections were considered successful if the injector tip was within the prelimbic or infralimbic regions of the mPFC, at about 2.2 mm rostral to bregma. Only rats with the dialysis probe at least 70% within the intermediate and caudal DRN (-7.8 to -8.8 from bregma) were included.

Fear conditioning and shuttle-box escape learning

Fear conditioning and escape learning occurred in shuttle boxes using procedures previously described (Maier et al., 1995). Freezing was measured for the first 5 min after placement in the shuttle boxes. Each subject's behavior was scored every 8 s as being either freezing or not freezing. Freezing was defined as the absence of all movement except that required for respiration. The observer was blind with regard to treatment condition, and interrater reliability has been calculated to be greater than 0.92. This observation period was followed by two scrambled foot shocks (0.6 mA) that could be terminated by crossing to the other side of the shuttle box (fixed ratio-1 (FR-1) trials). IS does not alter FR-1 shuttle-box escape latencies (Maier et al., 1983) and therefore IS and other rats are here exposed to shocks of equal duration. FR-1 latencies were measured in the present experiment, and, as is typical, there were no group differences. These two foot shocks were followed by a 20-min observation period during which freezing was scored. This observation period was followed by three further FR-1 escape trials and then 25 FR-2 escape trails. On FR-2 trials, the rats were required to cross to the other side of the shuttle box and back to terminate each shock. It is here that IS-induced escape deficits typically occur. Each shock terminated after 30 s if an escape response had not occurred.

Statistical analysis

Data were analyzed by repeated measures analysis of variance (ANOVA) followed by Fisher's protected least significant difference test (PLSD) post hoc comparison (alpha set at 0.05).

Experiments 1 and 2

Effects of mPFCv PIC during IS and ES on extracellular DRN 5-HT and on learned helplessness behaviors (fear conditioning and escape learning).

Methods

Extracellular 5-HT in the caudal DRN—During dialysis, rats received ES or equal yoked IS. Half of each group received mPFCv microinjections of PIC (P-ES or P-IS) or saline VEH (V-ES or V-IS). Thus the design was a two (VEH vs. PIC) $\times$ two (stress condition) factorial (HCC groups were not run for dialysis since we had observed no change under this condition). The microinjections were administered after taking three baseline samples, then three additional post-injection samples were taken, followed by the stress session.

Fear conditioning and escape learning—A two (VEH vs. PIC) $\times$ three (stress condition: IS, ES or HCC) factorial design was used to assess the effects of PIC on IS-induced behavioral changes. Behavioral shuttle-box testing was conducted 24 h after IS, ES, and HCC, as described above.

RESULTS

Wheel-turn escape learning

The logic of the experiments requires that the rats microinjected with PIC before the ES session be able to learn the wheel-turn escape (control) response. Thus, rats received 100 escapable tail shocks while restrained in the wheel-turn boxes where they could terminate the shock. Either PIC or VEH was microinjected into the IL/PL region of the vPFCm (see Experimental Procedures) 60 min before the session started. As in our previous studies (see Amat et al., 2005) the number of wheel turns required to terminate the shock (in quarter turns of the wheel) was increased as the rats became more proficient at escape (see Experimental Procedures). Thus, the response requirement attained (that is, the number of quarter turns to terminate the shock) across trials is the best measure of the quality of escape performance. As shown in Fig. 1, rats that received PIC learned to escape quite well. Repeated-measures ANOVA did not indicate any difference between PIC- and VEH-treated groups on response requirement ($F_{1,11}=0.955$), and there was no interaction with trials ($F_{19,209}=1.38$). The locations of the injector tips within the vPFCm for this and the subsequent experiments are shown in Fig. 2.

Extracellular 5-HT in the caudal DRN—Our laboratory has previously showed that IS increases extracellular 5-HT concentrations within the DRN to a much greater degree than does equal ES (Maswood et al., 1998). We have also shown that inhibiting the vPFCm with muscimol increases the 5-HT released by ES to that produced by IS (Amat et al., 2005). Thus this experiment was conducted to determine whether injecting PIC, which activates output pyramidal cells of the vPFCm (Berretta et al., 2005), has an inhibitory effect on 5-HT release during uncontrollable and controllable stress. We measured 5-HT efflux in the caudal DRN before, during and after the stress session (see probes placements for these and the following experiments in Fig. 3).

Baseline 5-HT levels were: 1) VEH-IS group, 0.6922 ± 0.079 pg/25 μ l, 2) VEH-ES, 0.8 ± 0.187 pg/25 μ l, 3) PIC-IS, 0.505 ± 0.093 pg/25 μ l, and 4) PIC-ES, 0.648 ± 0.09 pg/25 μ l. As shown in Figs. 4 and 5, there was a small transitory increase in extracellular 5-HT following the PIC and

VEH injections, probably due to the handling involved. As in prior studies, following VEH administration IS produced a large and sustained increase in 5-HT efflux that persisted even after the stress session (Fig. 4), but ES produced only a transient effect, with 5-HT returning to baseline during the stressor exposure (Fig. 5). Remarkably, PIC injection suppressed both the persistent IS (Fig. 4) and the transient ES (Fig. 5) increases of extracellular 5-HT. The initial differential effect of VEH-ES and PIC-ES (Fig. 5), is probably due to the fact that during VEH-ES, it takes some time for the rat to learn that it has control, so, initially, stress is capable of activating DRN 5-HT neurons. However, during PIC-ES, that initial activation is suppressed due to the already activated mPFCv output neurons, because PIC was administered 1 h before ES began. ANOVA on the baseline samples taken before the stressor did not show any difference (all F values <1.0). During the stressor there were significant effects of groups ($F_{3,15}=5.93$, $P<0.007$), 20 min periods ($F_{4,60}=4.37$, $P<0.0036$), and the interaction of groups and 20 min periods ($F_{12,60}=2.772$, $P<0.0045$). Fisher's PLSD indicated that IS is different from ES in VEH-injected rats ($P<0.016$), and that the PIC-injected IS group is different from the VEH-injected IS group ($P<0.009$). The PIC-ES group was not different from the VEH-ES group across the summed stressor period (data points corresponding to the period indicated by the horizontal gray bar in Fig. 5).

The effect of PIC injection in IS rats was specific to the IL/PL region since injections 2 mm caudal to that region (0.2 mm relative to bregma in Fig. 2) did not prevent the 5-HT increase normally associated with that treatment (Fig. 6). During stress there was a significant effect of groups ($F_{1,14}=4.929$, $P=0.434$) but not for 20 min periods ($F_{4,56}=0.44$, $P>0.7$) nor an interaction between groups and 20 min periods ($F_{4,56}=0.889$, $P>0.4$). Fisher's PLSD indicated that the effect of PIC injected 2 mm caudal is different from that within the IL/PL region.

Fear conditioning and escape learning—We have shown multiple times that IS facilitates subsequent post-shock freezing and interferes with escape learning, whereas ES does not (Maier, 1990). Freezing was not observed before the two foot shocks but was observed after the foot shocks (Fig. 7). As is typical IS enhanced freezing, whereas ES did not. PIC had no effect on ES rats but reduced freezing in IS rats to the level in ES rats. ANOVA showed significant main effect of group ($F_{5,42}=9.264$, $P<0.0001$) and trial block ($F_{9,378}=216$, $P<0.0001$) and a significant trial block $\times$ group interaction ($F_{45,378}=2.886$, $P<0.0001$). Post hoc Fisher's PLSD tests ($P<0.05$) indicated that the VEH-IS group differed from all the other groups, which did not differ among themselves.

Shuttle-box escape followed a similar pattern (Fig. 8). IS clearly interfered with escape learning, whereas ES had no effect compared with controls. Again PIC had no effect in ES-treated or HCC rats, but prevented the escape deficit in IS rats. ANOVA indicated significant effects of groups ($F_{5,28}=9.758$, $P<0.0001$), trial blocks ($F_{4,112}=3.012$, $P<0.02$) and the interaction between groups and trial blocks ($F_{20,112}=2.871$, $P<0.0002$). Post hoc Fisher's PLSD comparison ($P<0.05$) indicated that VEH-IS differed from all the other groups which did not differ among themselves.

Experiments 3 and 4

Effects of IS on 5-HT release and escape learning, in rats previously exposed to stress while the mPFCv is activated by PIC microinjection.

Methods

Extracellular 5-HT in the caudal DRN—Animals received two different treatments 7 days apart. During the first treatment the animals received intra-mPFCv microinjection of PIC or VEH. PIC- and VEH-injected rats were subjected to the IS/ES or HCC protocol 1 h after

injection. Thus, the design of the day 1 Treatment was a two (PIC or VEH) $\times$ three (ES, IS or HCC) factorial.

The second treatment (7 days later) consisted of a session of IS during which microdialysis was carried out for the five groups following the same procedure described above. Thus, the DRN 5-HT response to IS was tested 7 days after ES, IS or HCC treatment, with and without mPFCv stimulation by PIC. There were seven or eight rats per microdialysis group.

Escape learning—The day 1 experimental treatments were identical to the first set of treatments described above for the DRN experiments (two (PIC vs. VEH) by three (ES, IS, HCC) factorial). The second experimental treatment 7 days later consisted of an IS session carried out in Plexiglas tubes 17.5 cm long and 7.0 cm in diameter. The rat's tail extended from the rear of the tube and was taped to a Plexiglas rod. Rats received 100 IS tail shocks (5-s duration each) at an average intertrial interval of 60 s. Current intensity varied between 1 and 1.6 mA as described above. Twenty-four hours later escape learning was carried out following the procedure described in Experimental Procedures.

Extracellular 5-HT in the caudal DRN

Baseline 5-HT levels were: 1) group VEH-IS/IS, 1.03 ± 0.29 pg/25 μ l, 2) VEH-ES/IS, 0.92 ± 0.19 pg/25 μ l, 3) VEH-HCC/IS, 959 ± 0.11 pg/25 μ l, 4) PIC-IS/IS 1.29 ± 0.17 pg/25 μ l, and 5) PIC-HCC/IS, 1.3 ± 0.25 pg/25 μ l. As is typical, IS produced strong and persistent 5-HT efflux in the caudal DRN (Fig. 9). Also as shown previously (Amat et al., 2006) this effect of IS was prevented by a previous ES experience. The new finding is that the prevention of IS-induced 5-HT efflux by prior ES is mimicked by prior IS, if PIC is microinjected into the vPFCm 1 h before that prior IS experience. Interestingly, the effect of IS on 5-HT efflux was only partially suppressed in rats subjected to the previous PIC microinjection in the HCC condition. ANOVA on baseline samples taken before stress did not show any difference (All F values < 1.0). During stressor exposure there were significant effects of stress condition ($F_{4,25} = 11.626$, $P = 0.0001$) and the interaction between stress condition and 20 min periods ($F_{16,100} = 1.77$, $P = 0.05$). Fisher's PLSD indicated that the P-IS/IS group differed from all other groups except the V-ES/IS group, and that the P-HCC/IS group was significantly below the V-IS/IS, but significantly larger than both the V-ES/IS and the P-IS/IS groups.

Escape learning

As in prior studies (Amat et al., 2006) prior exposure to ES blocked the deficit in escape behavior produced by exposure to IS, but prior IS did not (Fig. 10). The new finding is that the mPFCv microinjection of PIC before IS led IS to duplicate the behavioral immunization produced by ES. That is, now IS was actually protective. However, the simple injection of PIC in the absence of shock had no effect whatsoever in reducing the propensity of later IS to produce shuttle-box escape failure. ANOVA showed significant effects of groups ($F_{5,40} = 6.066$, $P = 0.0003$), trials blocks ($F_{4,160} = 15.344$, $P < 0.0001$) and the interaction between groups and trial blocks ($F_{20,160} = 3.512$, $P < 0.0001$). Post hoc Fisher's PLSD comparison ($P < 0.05$) indicated that the V-ES/IS, P-IS/IS and P-ES/IS did not differ among themselves, but were different from the other three groups.

DISCUSSION

The goal of the present experiments was to activate mPFCv output neurons during the experience of an uncontrollable and controllable stressor, and determine the impact of this manipulation on DRN 5-HT activity during the stressor and on subsequent behavior. The question of interest was whether mPFCv activation during the uncontrollable stressor would provide the same protection as having behavioral control. Two methodological choices we

made require comment. First, we chose to activate the mPFCv by microinjecting PIC. This choice was made because this method has been used by a number of other investigators (e.g. Berretta et al., 2005) and has some advantages. PIC is a GABA_A receptor antagonist. It has advantages over other GABA_A antagonists such as bicuculline as it is a use-dependent non-competitive antagonist. Thus, in the presence of increased GABA it becomes more effective rather than being displaced, as would be the case for bicuculline. By reducing GABA_A currents PIC disinhibits cortical pyramidal cells, increasing their firing rates without activating fibers of passage (Chagnac-Amitai and Connors, 1989). Activation of these output neurons is, of course, our goal. These advantages have been reviewed by Berretta et al. (2005). In addition, the use of PIC to activate mPFCv output provides a symmetrical manipulation to the experiments that inhibited mPFCv output with muscimol, a GABA_A agonist (Amat et al., 2005, 2006; Baratta et al., 2007). Second, we chose to assess DRN 5-HT activation by measuring extracellular levels of 5-HT within the DRN using *in vivo* microdialysis. This measure reflects DRN 5-HT activity because 5-HT release within the DRN from axon collaterals correlates with the activity of those neurons (Tao et al., 2000). In other experiments we have assessed Fos expression in 5-HT-labeled neurons within the DRN, and this measure has correlated well with extracellular 5-HT (Amat et al., 2005, 2006).

The results of the present experiments were quite clear. Intra-mPFCv microinjection of PIC during exposure to ES and yoked IS had no effect on ES subjects. ES still failed to produce a sustained increase in DRN 5-HT or interfere with later shuttle-box escape behavior. In addition, ES still exerted its usual blunting effects on later exposure to IS—IS given 7 days after ES did not lead to sustained 5-HT efflux, nor did it produce later escape learning deficits. In contrast, intra-mPFCv completely changed the effects of IS, leading IS to precisely mimic the effects of ES. IS given while the mPFC was activated did not increase DRN 5-HT or produce later escape learning deficits. Furthermore, IS given under this condition actually produced behavioral immunization as it blocked the effects of IS given 7 days later. Here, IS did not lead to DRN 5-HT increases or subsequent escape learning deficits. Furthermore, these effects were site specific, as PIC injected 2 mm caudal was without impact.

These data offer further support for a critical role of the mPFCv in mediating the impact of behavioral control. Prior work (Amat et al., 2005, 2006) had indicated that the activation of mPFCv is *necessary* for the presence of behavioral control to blunt the DRN 5-HT activation and the later behavioral outcomes of stressor exposure. Thus, muscimol action in the mPFCv during ES eliminated the characteristic inhibition, produced by having control, of both DRN 5-HT release and learned helplessness/behavioral depression. In the present studies activation of the mPFCv during uncontrollable stress blocked the DRN activation and later potentiation of freezing and escape learning failure produced by uncontrollable stress. Thus, mPFCv activation during a stressor is also *sufficient* to produce resistance to neurochemical and behavioral consequences of stressors. These conclusions are consistent with the known anatomy. The mPFCv sends glutamatergic projections to the DRN (Vertes, 2004) that synapse preferentially on GABAergic interneurons within the DRN that inhibit the 5-HT cells (Jankowski and Sesack, 2004). Indeed, electrical stimulation within the mPFCv inhibits DRN 5-HT cell firing (Hajos et al., 1998).

The immunization experiments require special comment. Amat et al. (2006) reported that exposure to ES 7 days before IS blocked both the DRN 5-HT increase during the IS and the behavioral sequelae (potentiated freezing after shock and failure to learn to escape) of the IS. Furthermore, intra-mPFC muscimol microinjection either before the initial controllable stressor experience or before the later uncontrollable stressor prevented immunization from occurring. The fact that intra-mPFCv muscimol microinjection at the time of IS, 7 days after the ES exposure, removed the protective effect that ES would have exerted suggests that the initial ES experience must have altered the mPFCv in such a way that the subsequent IS now

activated the mPFCv. If this were not so, then intra-mPFCv muscimol at the time of the later uncontrollable stressor should not have prevented immunization from emerging. Amat et al. (2006) also found that the intra-mPFCv microinjection of the protein synthesis inhibitor anisomycin before or immediately after the initial ES exposure prevented immunization, supporting the idea that experiencing control induces a long-term plasticity-related change. The data available at present do not allow a determination of whether mPFCv activity/output becomes associatively connected to the stressor or some aspect of the cascade of physiological changes induced by the stressor, or becomes sensitized in non-associative fashion. However, here, activation of the mPFCv during IS produced immunization. That is, IS 7 days after conjoint mPFCv activation by PIC and IS exposure now produced neither DRN 5-HT increases nor escape learning failure. Importantly, mPFCv activation by PIC in the absence of the stressor produced only a small reduction in the increase of 5-HT efflux during later IS, and no reduction at all in the behavioral effect of the IS. This lack of effect of mPFCv activation in the absence of the stressor is consistent with the view that mPFCv activation becomes associatively connected to the stressor, or some aspect of the cascade of events produced by the stressor. Regardless, these findings further suggest that the activation of the mPFCv during a stressor, either as a consequence of control over the stressor, or drug treatment, alters mPFCv function so that later stressors that would normally not activate the mPFCv now do so.

Perhaps the most important implication to be drawn is that resistance to the behavioral and neurochemical impact of stressors is not determined by the ability to exert behavioral control per se, but rather by whether mPFCv output is increased during the stressor. Behavioral control is not the only factor that reduces the consequences of stressor exposure. For example, the presentation of “safety signals” during a session of IS eliminates the shuttle-box escape learning deficits that would otherwise follow (Minor et al., 1990). Perhaps safety signals have this protective effect because they activate mPFCv output to the DRN.

It should be noted that the experiments reported here also further support the previously proposed role of DRN neurons in mediating the behavioral effects of exposure to uncontrollable stressors. This is because there was a strong correlation between whether a treatment reduced stressor-induced DRN 5-HT activation and whether it eliminated behavioral consequences. Thus, intra-mPFCv PIC during IS a) eliminated the increase in extracellular DRN 5-HT during the IS and the potentiation of post-shock freezing and poor shuttle-box escape behavior that occur 24 h later, and b) eliminated the increase in 5-HT during a subsequent exposure to IS and the shuttle-box escape failure produced by that treatment. This covariation parallels the findings of Amat et al. (2005,2006) showing that intra-mPFCv muscimol microinjection during ES leads to a) the production of the same high levels of 5-HT during the ES as is normally produced by IS, and b) the behavioral changes typical of IS. This is, of course, all correlational, but it forms a powerful pattern.

The general view that the mPFCv exerts inhibitory control over stress-responsive structures in other parts of the brain is consistent with recent human neuroimaging experiments, although the DRN has not been studied in this regard. Instead, there has been a focus on the amygdala, and an inverse relationship between mPFCv and amygdala activity has been noted (Kim et al., 2003). In an especially intriguing study, Urry et al. (2006) instructed subjects to either increase or decrease the emotional impact of negative images. Subjects that were successful in decreasing the negative emotional impact of the pictures revealed decreased amygdalar activity as would be expected, but also increased mPFCv activity. It was suggested that the mPFCv was exerting top-down inhibitory control of the amygdala. In rodents, the infralimbic region of the mPFCv projections to the intercalated cell mass within the amygdala (Sesack et al., 1989; Vertes, 2004), which in turn inhibits amygdala central nucleus activity (Royer et al., 1999). Indeed, stimulation within the IL inhibits fear responses (Quirk et al., 2003). Corresponding to the present studies, experiencing behavioral control over a stressor can

interfere with subsequent fear responses, and this effect is mediated by the mPFCv (Baratta et al., 2007). Thus, mPFC activation during a stressor may be a general mechanism of resistance to the impact of stressors.

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Abbreviations

ANOVA	analysis of variance
DRN	dorsal raphe nucleus
ES	escapable stress
FR-1	fixed ratio-1
HCC	home cage controls
IS	inescapable shock
PIC	picrotoxin
PL/IL	prelimbic/infralimbic
PLSD	protected least significant difference
VEH	vehicle
vPFCm	ventral medial prefrontal cortex

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Fig. 1. Efficiency of wheel-turn escape behavior during exposure to controllable tail shock measured as number of quarter turns (mean $\pm$ S.E.M.) of the wheel attained as the escape requirement on each trial. The rats had received mPFCv microinjections of PIC (closed circles) or VEH (open circles) 60 min before the shock session.

Fig. 2.
Placements of microinjection cannulae in the mPFCv. Numerals indicate distance from bregma in mm.

Fig. 3.
Placements of microdialysis probes in the DRN. Numerals indicate distance from bregma in mm.

Fig. 4. 5-HT as a mean ($\pm$ S.E.M.) percentage of baseline in the DRN in groups that received IS. IS occurred during the gray bar. Open squares received VEH and closed squares PIC in the vPFCm at Time 0.

Fig. 5. 5-HT as a mean percentage ($\pm$ S.E.M.) of baseline in the DRN in groups that received ES. ES occurred during the gray bar. Open circles received VEH and closed circles PIC in the vPFCm at Time 0.

Fig. 6. 5-HT as a mean percentage ($\pm$ S.E.M.) of baseline in the DRN in groups that received IS. IS occurred during the gray. Closed squares received PIC in the vPFCm and open squares received PIC 2 mm caudal at Time 0.

Fig. 7.

The mean ($\pm$ S.E.M.) number of 8-s intervals spent freezing, in 2-min blocks, after two foot shocks 24 h following stressor exposure. Squares represent IS, circles ES and triangles HCC. Open symbols VEH, closed symbols PIC given before day 1 treatment.

Fig. 8.

The mean ($\pm$ S.E.M.) shuttle-box escape latencies across blocks of five shuttle-box FR-2 escape trials 24 h after stressor exposure. Squares represent IS, circles ES and triangles HCC. Open symbols VEH, closed symbols PIC given before day 1 treatment.

Fig. 9.

5-HT as a mean ($\pm$ S.E.M.) percentage of baseline in the DRN in groups that received IS. IS occurred during the gray bar. These rats had received PIC and IS (closed squares), VEH and IS (open squares), or VEH and ES (open circles) 7 days earlier.

Fig. 10.

The mean ($\pm$ S.E.M.) shuttle-box escape latencies across blocks of five shuttle-box FR-2 escape trials 24 h after IS exposure. The rats had received VEH (open symbols) or PIC (closed symbols) in the vPFCm before either IS (squares), ES (circles) or HCC (triangles), 7 days before IS.