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A double dissociation in the effects of 5-HT_{2A} and 5-HT_{2C} receptors on the acquisition and expression of conditioned defeat in Syrian hamsters

Marquinta L. Harvey, Cody L. Swallows, and Matthew A. Cooper

University of Tennessee, Knoxville

Abstract

Previous research indicates that serotonin enhances the development of stress-induced changes in behavior, although it is unclear which serotonin receptors mediate this effect. 5-HT₂ receptors are potential candidates because activation at these receptors is associated with increased fear and anxiety. In this study we investigated whether pharmacological treatments targeting 5-HT₂ receptors would alter the acquisition and expression of conditioned defeat. Conditioned defeat is a social defeat model in Syrian hamsters in which individuals display increased submissive and defensive behavior and a loss of territorial aggression when tested with a novel intruder 24 hours after an acute social defeat. The nonselective 5-HT₂ receptor agonist mCPP (0.0, 0.3, 1.0 or 3.0 mg/kg) was injected either prior to social defeat training or prior to conditioned defeat testing. Also, the 5-HT_{2A} receptor antagonist MDL 11,939 (0.0, 0.5 or 2.0 mg/kg) was injected either prior to social defeat training or prior to conditioned defeat testing. Injection of mCPP prior to testing increased the expression of conditioned defeat, but injection of mCPP prior to training did not alter the acquisition of conditioned defeat. Conversely, injection of MDL 11,939 prior to training reduced the acquisition of conditioned defeat, but injection of MDL 11,939 prior to testing did not alter the expression of conditioned defeat. Our data suggest that mCPP activates 5-HT_{2C} receptors during testing to enhance the display of submissive and defensive behavior, whereas MDL 11,939 blocks 5-HT_{2A} receptors during social defeat to disrupt the development of the conditioned defeat response. In sum, these results suggest that serotonin acts at separate 5-HT₂ receptors to facilitate the acquisition and expression of defeat-induced changes in social behavior.

Keywords

social defeat; stress; serotonin; anxiety; 5-HT₂ receptor

Social stress is a common type of stress experienced by humans, and when severe or prolonged it can lead to a variety of psychiatric disorders including major depression, acute stress disorder, and post-traumatic stress disorder (Taylor, Gooding, Wood, & Tarrier, 2011). However, the mechanisms by which social stress increases the risk for mood disorders remain unclear. Animal models that examine social conflict are particularly useful for determining how social stress alters brain function and subsequent behavior. Many social stress models use social defeat, which involves chronic or acute aggressive encounters between two individuals. Social defeat produces robust activation of the hypothalamic-pituitary-adrenal (HPA) axis (Huhman, Moore, Mougey, & Meyerhoff, 1992; Koolhaas, De Boer, De Rutter, Meerlo, & Sgoifo, 1997) and marked behavioral changes including decreased locomotor activity (Meerlo, Overkamp, Benning, Koolhaas, & Van den

Hoofdakker, 1996; Rygula et al., 2005), increased social avoidance (Berton et al., 2006), disruption of circadian rhythms (Harper, Tornatzky, & Miczek, 1996; Meerlo, Sgoifo, & Turek, 2002), and altered feeding and body weight (Bartolomucci et al., 2004; Foster, Solomon, Huhman, & Bartness, 2006). In Syrian hamsters social defeat results in a loss of species-typical territorial aggression and an increase in submissive and defensive behavior in future encounters with a novel intruder. This defeat-induced switch in agonistic behavior is called conditioned defeat (Huhman, 2006).

Serotonin is a neurotransmitter that has been implicated in stress-related changes in behavior, including the development of mood disorders. The majority of serotonin neurons that innervate stress-sensitive regions of the forebrain originate in the dorsal raphe nucleus (DRN). Exposure to stressful stimuli, or corticotropin-releasing factor itself, has been shown to increase serotonin concentration in several DRN projection regions including the basolateral amygdala (BLA) (Amat, Matus-Amat, Watkins, & Maier, 1998a; Christianson et al., 2010), dorsal striatum (Strong et al., 2011), medial prefrontal cortex (Forster et al., 2008), nucleus accumbens (Lukkes, Forster, Renner, & Summers, 2008), and ventral hippocampus (Amat, Matus-Amat, Watkins, & Maier, 1998b). Social defeat has been shown to increase neural activation in the DRN, as indicated by increased c-Fos expression in serotonin neurons (Cooper, Grober, Nicholas, & Huhman, 2009; Gardner, Thirivikraman, Lightman, Plotsky, & Lowry, 2005; Paul et al., 2011). Also, blocking serotonin signaling by activating 5-HT_{1A} autoreceptors in the DRN disrupts the acquisition and expression of conditioned defeat, whereas enhancing serotonin signaling by blocking 5-HT_{1A} autoreceptors in the DRN facilitates the acquisition and expression of conditioned defeat (Cooper, McIntyre, & Huhman, 2008). Altogether, these data suggest that social defeat activates serotonin neurons and that serotonin activity, at least in the DRN, enhances conditioned defeat. Recently, we found that pharmacological activation of 5-HT_{1A} receptors in the BLA reduces the acquisition and expression of conditioned defeat (Morrison & Cooper, 2012). While these data suggest that 5-HT_{1A} receptor activation in the BLA can disrupt conditioned defeat, it is unclear which serotonin receptors might facilitate conditioned defeat.

The 5-HT₂ receptors are potential candidates for mediating the effects of serotonin on the acquisition and expression of conditioned defeat. Activation of 5-HT_{2C} receptors is associated with the expression of stress-related behavior in humans (Charney, Woods, Goodman, & Heninger, 1987; Van Veen, Van der Wee, Fiselier, Van Vliet, & Westenberg, 2007) and rodents (Bagdy, Graf, Anheuer, Modos, & Kantor, 2001; Campbell & Merchant, 2003; Heisler, Zhou, Bajwa, Hsu, & Tecott, 2007). For example, systemic administration of a 5-HT_{2C} receptor agonist was shown to interfere with shuttle-box escape behavior and increase shock-induced freezing, suggesting that activation of 5-HT_{2C} receptors promotes learned helplessness (Strong, Greenwood, & Fleshner, 2009). Furthermore, 5-HT_{2C} receptors in the BLA are critical for the social avoidance produced by uncontrollable stress (Christianson, et al., 2010), whereas 5-HT_{2C} receptors in the dorsal striatum are critical for the deficits in shuttle-box escape behavior (Strong, et al., 2011). 5-HT_{2A} receptors are likely candidates for modulating the acquisition of conditioned defeat because their activation is critical for associative learning (Harvey, 2003). Previous research indicates that activation of 5-HT_{2A} receptors enhances eye blink conditioning in rabbits (Harvey, Gormezano, & Cool, 1982; Romano et al., 2010), whereas blockade of 5-HT_{2A} receptors disrupts it (Welsh, Romano, & Harvey, 1998). Also, accumulating evidence suggests that 5-HT_{2A} receptor blockade helps to ameliorate some of the cognitive deficits in schizophrenia (Houthoofd, Morrens, & Sabbe, 2008).

The purpose of this study was to examine the role of 5-HT_{2C} and 5-HT_{2A} receptors in conditioned defeat. We hypothesized that activation of 5-HT_{2C} receptors prior to social

defeat training would enhance the acquisition of conditioned defeat and that activation of 5-HT_{2C} receptors prior to testing would increase the expression of conditioned defeat. Similarly, we hypothesized that blockade of 5-HT_{2A} receptors prior to social defeat training would impair the acquisition of conditioned defeat and that blockade of 5-HT_{2A} receptors prior to testing would reduce the expression of conditioned defeat.

Methods

Animals

Subjects were adult male Syrian hamsters (*Mesocricetus auratus*) that weighed 130–170 g (3–4 months of age) at the start of the study and were individually housed for 10–14 days prior to testing. Older hamsters that weighed 180–200 g (>6 months) were individually housed and used as resident aggressors for social defeat training. Immature hamsters that weighed 90–120 g (2 months) were group-housed (4 per cage) and used as non-aggressive intruders for conditioned defeat testing. All animals were housed in polycarbonate cages (12 cm × 27 cm × 16 cm) with corn cob bedding, cotton nesting materials, and wire mesh tops. Animal cages were not changed for at least 1 week prior to testing to allow individuals to scent mark their territory. Animals were housed in a temperature-controlled colony room (20 ± 2 °C) and maintained on a 14:10 h light-dark cycle with food and water available ad libitum. All procedures were approved by the University of Tennessee Animal Care and Use Committee and are in accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Conditioned defeat protocol

Social defeat training consisted of a single aggressive encounter in the home-cage of a larger resident aggressor during which subjects were reliably attacked and defeated. Two animals were excluded from the study because they were not defeated. When drug treatments were expected to increase conditioned defeat, subjects received a 10 min social defeat to avoid a ceiling effect on later submissive and defensive behavior at testing. When drug treatments were expected to reduce conditioned defeat, subjects received a 15 min social defeat to avoid a floor effect on later submissive and defensive behavior at testing. To standardize the amount of aggression received and the duration of social defeat, timing of aggressive encounters began at the first attack by the resident aggressor, which usually occurred within the first 60 s of the encounter. When drugs were administered prior to social defeat, defeat sessions were digitally recorded and the number of attacks and total duration of aggressive behavior were quantified using Noldus Observer (Noldus Information Technology, Wageningen, Netherlands). When drug treatment altered the conditioned defeat response, non-defeated controls were included to evaluate whether drug treatment also affected agonistic behavior in the absence of social defeat experience. Non-defeated animals were exposed to a resident aggressor's empty cage, which controlled for olfactory cues and exposure to a novel environment.

Behavioral testing occurred 24 h after social defeat training and consisted of one, 5-min encounter with a novel, non-aggressive intruder in the subject's home cage. Non-aggressive intruders are younger, group-housed animals that typically display social and nonsocial behavior during conditioned defeat testing. However, intruders attacked nine of our subjects during testing and these subjects were excluded because their agonistic behavior at testing was contaminated by the aggression they received. Testing sessions were digitally recorded and later scored by researchers blind to the experimental conditions using Noldus Observer. We recorded the total duration of four classes of behavior during the 5-min tests: (a) non-agonistic social (approach, investigate, sniff, and nose touch); (b) nonsocial (locomotion, self-groom, nest build, and feed); (c) submissive and defensive (flee, avoid, tail up, upright

and side defense, stretch-attend, head flag); and (d) aggressive (upright and side offensive posture, chase, and attack including bite) (Albers, Huhman, & Meisel, 2002). A second researcher scored a subset of testing sessions and inter-rater reliability was 91% for the duration of submissive and defensive behavior. All social defeat training and subsequent testing was performed under dim light (< 40 lux) during the first 3 h of the dark phase of the light-dark cycle.

Drug Treatment

1-(3-Chlorophenyl)piperazine (mCPP; Sigma-Aldrich) was dissolved in sterile saline as per previous research (Fox, Hammack, & Falls, 2008). mCPP is a non-selective 5-HT₂ receptor agonist which shows a preferential affinity at 5-HT_{2C} receptors (Kimura et al., 2009). α -phenyl-1-(2-phenylethyl)-4-piperidine methanol (MDL 11,939; Tocris) was dissolved in sterile saline with 1% acetic acid and the pH was adjusted to 5.5 as per previous research (Welsh, et al., 1998). MDL 11,939 is a highly selective 5-HT_{2A} receptor antagonist (Welsh, et al., 1998). All drugs were administered with a 0.3 ml intraperitoneal injection (i.p.).

Experiments 1 and 2: 5-HT_{2C} receptor agonist

Experiment 1 was designed to test whether injection of a 5-HT_{2C} receptor agonist would enhance the acquisition of conditioned defeat. mCPP (0.3 mg/kg, N = 11; 1.0 mg/kg, N = 11; or 3.0 mg/kg, N = 11) or vehicle (N = 11) was injected 15 min prior to a 10-min social defeat. Subjects were tested for conditioned defeat behavior 24 h later.

Experiment 2 was designed to test whether injection of a 5-HT_{2C} receptor agonist would enhance the expression of conditioned defeat. Hamsters received a 10-min social defeat, and 24 hours later mCPP (0.3 mg/kg, N = 10; 1.0 mg/kg, N = 11; or 3.0 mg/kg, N = 10) or vehicle (N = 11) was injected 15-minutes prior to conditioned defeat testing. No defeat controls received exposure to a resident aggressor's empty cage during training, and 24 h later mCPP (1.0 mg/kg, N = 8) or vehicle (0.0 mg/kg, N = 8) was injected 15-minutes prior to conditioned defeat testing.

Experiments 3 and 4: 5-HT_{2A} receptor antagonist

Experiment 3 was designed to test whether injection of a selective 5-HT_{2A} receptor antagonist would reduce the acquisition of conditioned defeat. MDL 11,939 (0.5 mg/kg, N = 11; or 2.0 mg/kg, N = 11) or vehicle (N = 11) was injected 30 min prior to a 15-min social defeat. For no defeat controls, MDL 11,939 (2.0 mg/kg, N = 8) or vehicle (0.0 mg/kg, N = 9) was injected 30 min prior to exposure to a resident aggressor's empty cage. Animals were tested for conditioned defeat behavior 24 h later as described above.

Experiment 4 was designed to test whether injection of a selective 5-HT_{2A} receptor antagonist would reduce the expression of conditioned defeat. Hamsters received a 15-min social defeat, and 24 h later MDL 11,939 (0.5 mg/kg, N = 11; or 2.0 mg/kg, N = 10) or vehicle (N = 10) was injected 30 min prior conditioned defeat testing.

Data analysis

For conditioned defeat testing, the total durations of submissive and defensive, non-agonistic social, nonsocial, and aggressive behavior were analyzed separately. Conditioned defeat data were analyzed using either one-way or two-way ANOVAs with Defeat Experience and/or Drug Treatment as between-subject factors. Significant main effects and interactions were followed with Fisher's protected least significant difference (PLSD) post-hoc tests. All statistical tests were two-tailed and the alpha level was $P < 0.05$.

Results

Experiment 1: mCPP and acquisition of conditioned defeat

Injection of mCPP prior to social defeat did not significantly alter the acquisition of conditioned defeat (Fig. 1). Specifically, mCPP treatment prior to social defeat training did not significantly alter the duration of submissive and defensive behavior at testing ($F(3,43) = 0.28$, $P = 0.840$). Also, injection of mCPP prior to social defeat did not alter the duration of non-agonistic social behavior ($F(3,43) = 0.39$, $P = 0.760$), nonsocial behavior ($F(3,43) = 0.35$, $P = 0.792$), or aggressive behavior ($F(3,43) = 1.31$, $P = 0.286$).

Injection of mCPP prior to social defeat training did not alter the amount of aggression resident aggressors directed toward subjects. Vehicle controls received 200.5 s (± 33.3) of aggression during social defeat training and individuals injected with 0.3, 1.0, or 3.0 mg/kg of mCPP received 250.5 s (± 28.9), 291.5 (± 24.6) and 296.0 s (± 28.4) of aggression, respectively ($F(3,43) = 0.55$, $P = 0.567$). Vehicle controls received 12.3 (± 1.1) attacks during social defeat training and individuals injected with 0.3, 1.0 and 3.0 mg/kg of mCPP received 10.8 (± 1.6), 13.7 (± 2.1) and 12.7 (± 1.9), attacks, respectively ($F(3,43) = 1.41$, $P = 0.796$).

Experiment 2: mCPP and expression of conditioned defeat

Injection of mCPP prior to behavioral testing dose dependently increased the expression of conditioned defeat (Fig. 2). A nearly significant drug by defeat interaction was found for the duration of submissive and defensive behavior ($F(1,37) = 4.12$, $P = 0.051$). This result indicates that 1.0 mg/kg of mCPP increased submissive and defensive behavior in defeated subjects more than it did in no-defeat controls. A separate analysis of all doses of mCPP revealed a significant effect of drug treatment in defeated subjects ($F(3,41) = 4.20$, $P = 0.012$). Injection of 1.0 or 3.0 mg/kg of mCPP significantly increased submissive behavior compared to vehicle controls ($P = 0.002$, $P = 0.046$, respectively), whereas injection of 0.3 mg/kg did not ($P = 0.492$). To measure the selectivity of mCPP on submissive and defensive behavior, we quantified the duration of non-agonistic social, nonsocial, and aggressive behavior. Injection of mCPP prior to behavioral testing did not alter the duration of non-agonistic social ($F(1,37) = 0.08$, $P = 0.778$), nonsocial ($F(1,37) = 1.27$, $P = 0.268$), or aggressive behavior ($F(1,37) = 0.52$, $P = 0.478$).

No-defeat controls exhibited less submissive and defensive behavior than did defeated subjects ($F(1,37) = 17.382$, $P < 0.001$), which indicates that a 10-min social defeat produced conditioned defeat at testing (Fig. 2). Importantly, injection of mCPP in no-defeat control animals did not alter the duration of submissive and defensive ($P = 0.248$), aggressive ($P = 0.351$), non-agonistic social ($P = 0.649$), or nonsocial behavior ($P = 0.541$). These results indicate that mCPP treatment did not alter agonistic behavior in non-defeated animals.

Experiment 3: MDL 11,939 and acquisition of conditioned defeat

Injection of MDL 11,939 prior to social defeat dose dependently decreased the acquisition of conditioned defeat (Fig. 3). A significant drug by defeat interaction for the duration of submissive and defensive behavior ($F(1,38) = 6.31$, $P = 0.017$), indicated that 2.0 mg/kg of MDL 11,939 reduced submissive and defensive behavior in defeated subjects but did not in no-defeat controls. A separate analysis of all MDL 11,939 doses in defeated subjects showed a significant effect of drug treatment ($F(2,32) = 3.53$, $P = 0.042$). Injection of 2.0 mg/kg MDL 11,939 significantly reduced submissive and defensive behavior compared to vehicle controls ($P = 0.021$), but injection of 0.5 mg/kg did not ($P = 0.759$). Also, injection of MDL 11,939 prior to social defeat did not alter the duration of other classes of behavior including

non-agonistic social ($F(1,38) = 0.20$, $P = 0.661$), nonsocial ($F(1,38) = 0.01$, $P = 0.912$), or aggression ($F(1,38) = 0.19$, $P = 0.661$).

No-defeat controls showed more aggression ($F(1,38) = 9.41$, $P = 0.004$) and less submissive and defensive behavior at testing ($F(1,38) = 17.95$, $P < 0.001$) compared to defeated subjects (Fig. 3). Also, injection of MDL 11,939 in no-defeat controls did not alter the duration of submissive and defensive ($P = 0.279$), aggressive ($P = 0.689$), non-agonistic social ($P = 0.729$), or nonsocial behavior ($P = 0.845$). These results indicate that no-defeat controls did not show a conditioned defeat response and MDL 11,939 did not alter their agonistic behavior.

Injection of MDL 11,939 did not alter the amount of aggression subjects received during social defeat training. Vehicle controls received 307 s (± 57.9) of aggression during social defeat training and individuals injected with 0.5 or 2.0 mg/kg of MDL 11,939 received 303 s (± 47.5) and 277.3 s (± 53.3), respectively ($F(2,32) = 0.10$, $P = 0.909$). Vehicle controls received 21.7 (± 1.2) attacks during social defeat training and individuals injected with 0.5 or 2.0 mg/kg of MDL 11,939 received 19.5 (± 2.5) and 16.4 (± 3.3) attacks, respectively ($F(2,32) = 0.86$, $P = 0.436$).

Experiment 4: MDL 11,939 and expression of conditioned defeat

Injection of MDL 11,939 prior to behavioral testing did not significantly alter the expression of conditioned defeat (Fig. 4). Specifically, MDL 11,939 treatment prior to testing did not significantly alter the duration of submissive and defensive behavior ($F(2,30) = 0.25$, $P = 0.782$). Also, injection of MDL 11,939 prior to conditioned defeat testing did not alter the duration of non-agonistic social behavior ($F(2,30) = 1.23$, $P = 0.276$), nonsocial behavior ($F(2,30) = 2.62$, $P = 0.090$), or aggressive behavior ($F(2,30) = 0.31$, $P = 0.579$).

Discussion

We found that injection of mCPP increased the expression, but not acquisition, of conditioned defeat. In contrast, we found that injection of MDL 11,939 reduced the acquisition, but not expression, of conditioned defeat. Our results suggest that mCPP activates 5-HT_{2C} receptors during testing to enhance the display of submissive and defensive behavior. Also, mCPP treatment did not alter agonistic behavior in no-defeat controls, indicating that 5-HT_{2C} receptor activation enhanced only defeat-induced changes in agonistic behavior. Our results also suggest that MDL 11,939 blocks 5-HT_{2A} receptors during social defeat to disrupt the development of the conditioned defeat response. MDL 11,939 treatment did not alter the amount of aggression received during social defeat training and did not alter agonistic behavior in no-defeat controls. In sum, these data suggest that serotonin acts at 5-HT_{2A} receptors during social defeat to facilitate the development of conditioned defeat and at 5-HT_{2C} receptors during testing to facilitate the display of submissive and defensive behavior.

In humans with panic disorder administration of mCPP increases anxiety, including panic attacks (Kahn, Wetzler, van Praag, Asnis, & Strauman, 1988). Also, mCPP increases the expression of anxiety-like behavior in several animal models including the social interaction test (Bagdy, et al., 2001), light/dark transition box test (Bilkei-Gorzo, Gyertyan, & Levay, 1998), and open field test (Campbell & Merchant, 2003). Although mCPP is often used as a 5-HT_{2C} receptor agonist, its pharmacology is complex. mCPP activates several other receptors and binds with equal affinity to 5-HT_{2C} and 5-HT_{2B} receptors. mCPP has a ten-fold greater affinity for 5-HT_{2C} receptors compared to 5-HT_{2A} receptors and a two-fold greater affinity for 5-HT_{2C} receptors compared to 5-HT_{1A} receptors (Campbell & Merchant, 2003; Roth et al., 1995). The non-selective binding of mCPP at serotonin

receptors can explain its non-linear dose response curve. In our study, 1.0 mg/kg of mCPP increased conditioned defeat whereas 3.0 mg/kg was less effective. Our data are consistent with other research showing inverted U-shaped dose response curves for mCPP effects (Campbell & Merchant, 2003). One possibility is that mCPP fails to increase conditioned defeat at high doses because it binds to other receptors, such as the 5-HT_{1A} receptor which inhibits conditioned defeat (Morrison & Cooper, 2012). In a learned helplessness model, 5-HT_{2C} receptor agonists have been shown to impair escape behavior in the absence of prior stress (Strong, et al., 2009). In contrast, activation of 5-HT_{2C} receptors in our study did not increase the display of submissive and defensive behavior in non-defeat subjects. Thus, activation of 5-HT_{2C} receptors at testing appears to interact with social defeat training to enhance conditioned defeat; however it does not produce a conditioned defeat response itself.

The BLA and bed nucleus of the stria terminalis (BNST) are critical for the expression of conditioned defeat (Jasnow, Cooper, & Huhman, 2004; Markham, Norvelle, & Huhman, 2009), and we expect that mCPP activates 5-HT_{2C} receptors in these brain regions at testing to increase submissive and defensive behavior during conditioned defeat testing. Systemic mCPP administration has been shown to increase the expression of c-Fos in the anterolateral BNST (Singewald, Salchner, & Sharp, 2003). Also, the anxiogenic effects of mCPP have been linked to 5-HT_{2A/2C} receptors expressed by BNST projection neurons (Hammack et al., 2009). Activation of 5-HT_{2C} receptors in the BLA increases anxiety-like behavior in both an open-field test and ultrasonic vocalization test (Campbell & Merchant, 2003). Similarly, blockade of 5-HT_{2C} receptors in the BLA prevents the reduction in social exploration observed after uncontrollable stress (Christianson, et al., 2010). Based on our inverted U-shaped dose response curve, it is likely that 1 mg/kg of mCPP acts at 5-HT_{2C} receptors to enhance the expression of conditioned defeat. In sum, our results suggest that activation of 5-HT_{2C} receptors is sufficient to enhance the expression of conditioned defeat, although future research will be required to determine whether endogenous serotonin activity at these receptors is necessary for this effect.

Several lines of evidence suggest that MDL 11,939 reduced the acquisition of conditioned defeat in our study by blocking 5-HT_{2A} receptors. MDL 11,939 has at least a 160-fold higher affinity for 5-HT_{2A} receptors than for 5-HT_{2C} receptors in rabbits, rats, and humans (Aloyo & Harvey, 2000; Pehek, Nocjar, Roth, Byrd, & Mabrouk, 2006; Welsh, et al., 1998), and has very low affinity for other serotonin receptors, including 5-HT_{2B} (Pehek, et al., 2006). The doses of MDL 11,939 used in our study are consistent with the doses reported in the literature (1.5 - 3.0 mg/kg: Harvey, Quinn, Liu, Aloyo, & Romano, 2004; Jiang et al., 2011; Welsh, et al., 1998). A wide range of data also implicates 5-HT_{2A} receptors in the development of memories for aversive events. For example, pharmacological activation of 5-HT_{2A} receptors has been shown to enhance eyeblink conditioning in rabbits (Harvey, et al., 1982), whereas blockade of 5-HT_{2A} receptors impairs it (Welsh, et al., 1998). Activation of 5-HT_{2A} receptors has been shown to enhance the formation of active avoidance in rats (Alhaidar, Ageel, & Ginawi, 1993). Blockade of 5-HT_{2A} receptors after predator stress has been shown to prevent stress-induced increases in acoustic startle and anxiety-like behavior on an elevated plus maze (Adamec, Creamer, Bartoszyk, & Burton, 2004). Also, daily injection of MDL 11,939 prior to three consecutive days of uncontrollable shock has been shown to prevent the stress-induced enhancement of acoustic startle (Jiang, et al., 2011).

There are several brain regions in which MDL 11,939 might act to impair the acquisition of conditioned defeat, including the hippocampus, prefrontal cortex, and BLA. The BLA is a particularly interesting candidate brain region because numerous studies indicate that neural plasticity in the BLA is critical for the acquisition of conditioned defeat (Day, Cooper,

Markham, & Huhman, 2011; Markham, Taylor, & Huhman, 2010). 5-HT_{2A} receptors are located on GABAergic interneurons in the BLA that express the calcium binding protein, parvalbumin (McDonald & Mascagni, 2007). Activation of 5-HT_{2A} receptors provides inhibitory drive onto BLA projection neurons, which is the dominant effect of serotonin in the BLA (Jiang et al., 2009; Rainnie, 1999). Also, uncontrollable shock down-regulates both 5-HT_{2A} receptor mRNA and protein in the BLA (Jiang, et al., 2009; Wu et al., 1999), and MDL 11,939 is capable of preventing stress-induced 5-HT_{2A} receptor down-regulation (Dave, Harvey, & Aloyo, 2007; Harvey, et al., 2004). Thus, in our study MDL 11,939 might have prevented the down-regulation of 5-HT_{2A} receptors in the BLA during social defeat which in turn would have preserved 5-HT_{2A}-mediated inhibition of BLA projection neurons during testing. Interestingly, we found that mCPP did not alter the acquisition of conditioned defeat and others have shown that mCPP does not activate parvalbumin-expressing GABAergic interneurons in the BLA (Hale et al., 2010).

The current study extends our research on the mechanisms by which serotonin signaling modulates conditioned defeat. Social defeat increases c-Fos expression in serotonin cells within the DRN (Cooper, et al., 2009). Reducing serotonin activity by pharmacological activation of 5-HT_{1A} autoreceptors in the DRN impairs both the acquisition and expression of conditioned defeat (Cooper, et al., 2008). The current study suggests that serotonin facilitates the acquisition and expression of conditioned defeat by acting at 5-HT_{2A} and 5-HT_{2C} receptors, respectively. Further characterization of the mechanisms by which serotonin modulates stress-induced changes in behavior may contribute to the development of novel treatments for stress-related psychiatric disorders.

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Figure 1.

5-HT_{2C} receptors and the acquisition of conditioned defeat. Durations (mean $\pm$ SE) of submissive and defensive, aggressive, non-agonistic social, and nonsocial behavior are shown during conditioned defeat testing. Subjects received an injection of mCPP (0.3 mg/kg, N = 11; 1.0 mg/kg, N = 11; or 3.0 mg/kg, N = 11) or vehicle (N = 11) 15 min before social defeat training.

Figure 2.

5-HT_{2C} receptors and the expression of conditioned defeat. Durations (mean $\pm$ SE) of (a) submissive and defensive, (b) aggressive, (c) non-agonistic social, and (d) nonsocial behavior are shown during conditioned defeat testing. Defeated animals received an injection of mCPP (0.3 mg/kg, N = 10; 1.0 mg/kg, N = 11; or 3.0 mg/kg, N = 10) or vehicle (N = 11) 15 minutes before conditioned defeat testing. Likewise controls received an injection of mCPP (1.0 mg/kg, N = 8) or vehicle (N = 8) 15 min before conditioned defeat testing. * indicates significantly different than defeated, vehicle controls. ** indicates a significant main effect of social defeat.

Figure 3.

5-HT_{2A} receptors and the acquisition of conditioned defeat. Durations (mean $\pm$ SE) of submissive and defensive, aggressive, non-agonistic social, and nonsocial behavior are shown during conditioned defeat testing. Defeated animals received an injection of MDL 11,939 (0.5 mg/kg, $N = 11$ or 2.0 mg/kg, $N = 11$) or vehicle ($N = 11$) 30 min before social defeat training. Likewise, controls received an injection of MDL 11,939 (2.0 mg/kg, $N = 8$) or vehicle ($N = 9$) 30 min before exposure to a resident aggressor's empty cage. * indicates significantly different than defeated, vehicle controls. ** indicates a significant main effect of social defeat.

Figure 4. 5-HT_{2A} receptors and the expression of conditioned defeat. Durations (mean $\pm$ SE) of (a) submissive and defensive, (b) aggressive, (c) non-agonistic social, and (d) nonsocial behavior are shown during conditioned defeat testing. Defeated animals received an injection of MDL 11,939 (0.5 mg/kg, N = 11 or 2.0 mg/kg, N = 10) or vehicle (N = 10) 30 min before conditioned defeat testing.