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Thalamic mast cell activity is associated with sign-tracking behavior in rats

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ABSTRACT

Mast cells are resident immune cells in the thalamus that can degranulate and release hundreds of signaling molecules (i.e., monoamines, growth factors, and cytokines) both basally and in response to environmental stimuli. Interestingly, mast cell numbers in the brain show immense individual variation in both rodents and humans. We used a Pavlovian conditioned approach (PCA) procedure to examine whether mast cells are associated with individual variation in the attribution of incentive-motivational value to reward-related cues. During the PCA procedure, a lever response-independently predicts the delivery of a food pellet into a magazine, and over training sessions three conditioned responses (CRs) develop: sign-tracking (lever-directed CRs), goal-tracking (magazine-directed CRs), and an intermediate response (both CRs). In Experiment 1, we measured thalamic mast cell number/activation using toluidine blue and demonstrated that sign-trackers have increased degranulated (activated) but not granulated (inactive) mast cells. In Experiment 2, we infused the mast cell inhibitor, cromolyn (200 μg/rat; i.c.v.), immediately before five daily PCA training sessions and demonstrated that mast cell inhibition selectively impairs the acquisition of sign-tracking behavior. Taken together, these results demonstrate that thalamic mast cells contribute to the attribution of incentive-motivational value to reward-related cues and suggest that mast cell inhibition may be a novel target for addiction treatment.

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1. Introduction

Mast cells are a heterogeneous population of immune cells that are generated as precursor cells in the periphery within bone marrow and can rapidly penetrate the blood vessels of the blood-brain barrier where they mature within the brain (Silverman et al., 2000). Mast cells have been identified in the brains of approximately two dozen mammalian species, including both rats and humans (Dropp, 1976, 1979). Within the brains of rats, they reside primarily within the thalamus and epithalamus (Goldschmidt et al., 1984) where they degranulate (Persinger, 1983) and release hundreds of signaling molecules (i.e., monoamines, growth factors, and cytokines) in a radius up to 50 μm (Nautiyal et al., 2008). Degranulation can amplify signaling by affecting the activity of a wide variety of surrounding cells, including neurons (Koszegi et al., 2006; Kovacs et al., 2006), astrocytes (Zeng et al., 2013), and microglia (Yuan et al., 2010; Zhang et al., 2012). Moreover, mast cells are poised to dynamically influence behavior, because their number and activity within the thalamus is highly variable under basal conditions (Florenzano and Bentivoglio, 2000, 2001), fluctuate in response to physiological states and environmental stimuli, and show behaviorally relevant patterns of regional activation (Asarian et al., 2002; Kovacs and Larson, 2006). In addition, mast cells contribute approximately 90% of thalamic histamine in rodents (Goldschmidt et al., 1985), and histamine has been implicated in appetitive behaviors and motivational processes (Torrealba et al., 2012). Although mast cells in the brain have been previously linked to depression- and anxiety-like behaviors (Chikahisa et al., 2013; Nautiyal et al., 2008), it is currently unknown whether mast cells play a role in reward-related behaviors.

Interestingly, there is substantial individual variation in the number of brain mast cells. For example, in male Sprague Dawley rats, brain mast cell numbers can vary from 1,490 to 19,103 per whole brain (Goldschmidt et al., 1984), and human mast cells can vary from 0 to 49,000 depending on the brain region (Dropp, 1979). Given this high variability in mast cell numbers, mast cells could contribute substantially to individual variation in reward-related behaviors that are relevant to vulnerability and resilience

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to addiction. The attribution of incentive-motivational value to reward-related cues is believed to underlie addiction-like behaviors (Flagel et al., 2009) and can be studied using a Pavlovian conditioned approach (PCA) procedure. During PCA training, rats are presented with a conditioned stimulus (CS; e.g., a lever) followed by the response-independent delivery of an unconditioned stimulus (US; e.g., a food pellet) into a pellet magazine. Over the course of training, three patterns of conditioned responses (CRs) develop: sign-tracking (CS-directed CRs), goal-tracking (US-directed CRs), and an intermediate response (both CRs). Whereas goal-trackers (GTs) utilize the CS as a predictor of impending food pellet delivery, sign-trackers (STs) attribute incentive-motivational value to the CS whereby it becomes a powerful motivator of behavior (Robinson and Flagel, 2009) even in the absence of the US (Ahrens et al., 2016). Previously, it has been shown that STs, compared to intermediate responders (IRs) or GTs, are more vulnerable to cue-induced reinstatement of drug-seeking behavior (Saunders and Robinson, 2010) and seek drug despite adverse consequences (Saunders et al., 2013), two hallmarks of addiction. STs compared to GTs also have increased neural activity (i.e., induction of c-Fos transcription or translation) following food- and drug-related cue presentation in the paraventricular and intermediodorsal nuclei of the thalamus as well as the lateral habenula (Flagel et al., 2011; Yager et al., 2015). Because mast cells can regulate neuronal activity in the thalamus (Koszegi et al., 2006; Kovacs et al., 2006), it is plausible that individual variation in the number and activity of thalamic mast cells between phenotypes may underlie differences in PCA behavior.

The present study investigated whether thalamic mast cell number and activity is different between PCA phenotypes and influences PCA behavior. In Experiment 1, mast cell number and activity was quantified seven days after five daily PCA training sessions using toluidine blue staining. In Experiment 2, rats were implanted with intracerebroventricular (i.c.v.) cannulas and infused with cromolyn (200 μg/rat), a mast cell inhibitor, immediately before five daily PCA training sessions in order to determine whether mast cell inhibition alters the acquisition of sign- or goal-tracking behavior.

2. Material and methods

2.1. Animals

Adult male Sprague Dawley rats (275–300 g) were purchased from Charles River Laboratories. Rats were maintained on a 12:12-h light/dark cycle, and food and water were available *ad libitum* for the duration of experimentation. All procedures were approved by the University Committee on the Use and Care of Animals (University of Michigan; Ann Arbor, MI).

2.2. Drugs

Toluidine blue (#T3260), cromolyn (#C0399), and pontamine sky blue (#C8679) were used (Sigma-Aldrich, Inc.; St. Louis, MO).

2.3. Pavlovian conditioned approach: apparatus

Sixteen modular conditioning chambers (24.1 cm width × 20.5 cm depth × 29.2 cm height; MED Associates, Inc.; St. Albans, VT) were used for Pavlovian conditioning. Each chamber was located in a sound-attenuating cubicle equipped with a ventilation fan to provide ambient background noise. During PCA training sessions, each chamber was equipped with a pellet magazine, a retractable lever (counterbalanced on the left or right side of the magazine), and a red house light on the wall opposite to the magazine. The magazine contained an infrared sensor to detect magazine entries, and the lever was calibrated to detect lever deflections in response to 10 g of applied weight. Whenever the lever was extended into the chamber, an LED mounted inside the lever mechanism illuminated the slot through which the lever protruded.

2.4. Pavlovian conditioned approach: procedure

For two days prior to the start of training rats were familiarized with banana-flavored pellets (45 mg; Bio-Serv; Frenchtown, NJ) in their home cages. Rats were then placed into the chambers for one pretraining session during which the red house-light remained on but the lever was retracted. Fifty food pellets were delivered on a variable time (VT) 30-s schedule (i.e., one pellet was delivered on average every 30 s, but varied 0–60 s). Rats were not food-deprived at any point during experimentation. Each trial during a PCA training session consisted of presentation of the illuminated lever (the CS) into the chamber for 8 s on a VT 90-s schedule (i.e., time randomly varied 30–150 s between CS presentations). Retraction of the lever was immediately followed by the response-independent delivery of one food pellet (the US) into the magazine. The beginning of the next inter-trial interval commenced immediately after pellet delivery. Each test session consisted of 25 trials of a CS-US pairing. If rats did not consume all the pellets that were delivered, they were excluded from further behavioral testing.

2.5. Experiment 1: toluidine blue staining procedure

One week following the last session of PCA training, rats were anesthetized with a solution of ketamine (90 mg/kg) and xylazine (10 mg/kg), then transcardially perfused with a 4% paraformaldehyde solution in 0.1 M phosphate buffered saline (pH 7.32–7.36). Next, brains were post-fixed in a 4% paraformaldehyde solution for 24 h then placed in a 20% sucrose solution containing 0.01% sodium azide. After sucrose saturation, brains were flash frozen in isopentane over dry ice. Then, brains were sectioned on a cryostat (40 μM; Leica CM1850; Leica Microsystems, Inc.; Buffalo Grove, IL) through the thalamus (anterior-posterior [AP]: −1.8 to −4.56 mm measured from bregma; Paxinos and Watson, 2007) and finally processed using toluidine blue staining. Every third section was processed for mast cells, resulting in 24 sections per rat.

When toluidine blue is acidified, it metachromatically stains highly anionic, sulfated proteoglycans within mast cell secretory granules (Ronnberg et al., 2012b). As a result, granulated mast cells are stained dark purple, and degranulated mast cells are stained lighter shades of purple depending on the degree of degranulation. Toluidine blue staining was adapted from Florenzano and Bentivoglio (2000). First, a 2% stock solution of toluidine blue was made. Brain sections were placed in a 0.01% toluidine blue solution containing acidified ddH_2O (pH = 2.5) for 30 min. Second, brain sections were dipped briefly in ddH_2O, then progressively dehydrated through a series of washes: 50% ethanol in ddH_2O (15 s), 70% ethanol (45 s), 95% ethanol (60 s), 100% ethanol (60 s), and 100% ethanol (60 s). Brain sections were kept in the final ethanol solution, individually dipped in xylene, then coverslipped using Permount® (#SP15; Thermo Fisher Scientific, Inc.; Pittsburgh, PA). Following mounting, mast cells were quantified using a light microscope.

2.6. Experiment 2: cannulation surgery

Rats were anesthetized with a solution of ketamine (90 mg/kg) and xylazine (10 mg/kg) and placed into a stereotaxic frame (David Kopf Instruments; Tujunga, CA). Rats were implanted with a stainless-steel guide cannula (26-gauge, cut 3 mm below the

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