Brain mast cells link the immune system to anxiety-like behavior

Katherine M. Nautiyal\textsuperscript{a}, Ana C. Ribeiro\textsuperscript{b}, Donald W. Pfaff\textsuperscript{b,1}, and Rae Silver\textsuperscript{c,d,2}

\textsuperscript{a}Department of Psychology, Columbia University, 1190 Amsterdam Avenue, New York, NY 10027; \textsuperscript{b}Laboratory of Neurobiology and Behavior, The Rockefeller University, 1230 York Avenue, New York, NY 10021; \textsuperscript{c}Department of Psychology, Barnard College, 3009 Broadway, New York, NY 10027; and \textsuperscript{d}Department of Pathology and Cell Biology, Columbia University, 630 West 168th Street, New York, NY 10032

Contributed by Donald W. Pfaff, September 23, 2008 (sent for review August 5, 2008)

Mast cells are resident in the brain and contain numerous mediators, including neurotransmitters, cytokines, and chemokines, that are released in response to a variety of natural and pharmacological triggers. The number of mast cells in the brain fluctuates with stress and various behavioral and endocrine states. These properties suggest that mast cells are poised to influence neural systems underlying behavior. Using genetic and pharmacological loss-of-function models we performed a behavioral screen for arousal responses including emotionality, locomotor, and sensory components. We found that mast cell deficient Kit\textsuperscript{Wsh/sh} (sash\textsuperscript{−/−}) mice had a greater anxiety-like phenotype than WT and fluorozygote littermate control animals in the open field arena and elevated plus maze. Second, we show that blockade of brain, but not peripheral, mast cell activation increased anxiety-like behavior. Taken together, the data implicate brain mast cells in the modulation of anxiety-like behavior and provide evidence for the behavioral importance of neuroimmune links.

The immune and central nervous systems are traditionally thought to meet different types of requirements for survival. Importantly, there is evidence for dynamic interactions between the two (1–3). While it is known that some immune cells, such as microglia are resident in the brain for surveillance and clearance (4–6), the roles of other immune cells are underexplored. We focus here on mast cells, which are localized not only in the periphery but are also resident in the brain of all mammalian species studied (7–9).

Mast cells are a heterogeneous population of granulocytic cells of the immune system. They contain numerous mediators, including neurotransmitters, cytokines, chemokines, and lipid-derived factors (10). Mast cells in the brain are constitutively active (11), releasing their contents by means of piecemeal or anaphylactic degranulation (12). Additionally, their activity is increased by a wide range of stimuli including immune and non-immune signals such as hormones, like corticotrophin releasing hormone, and various neuropeptides like Substance P and neurotensin (13). Of mast cells over 50 mediators, some are synthesized upon activation (e.g., substance P, somatostatin, cytokines) while others are preformed and stored in granules, allowing for very fast release (e.g., serotonin, histamine) (10, 14, 15). Due to their ability to migrate, they can serve as “single cell glands” delivering mediators “on demand” and influencing neuronal activity (16, 17). Mast cells can act via autocrine and paracrine mechanisms (18), and their secretions can reach a large spatial volume [supporting information (SI) Fig. S1]. Their granule remnants can even be acquired by neurons through endocytosis (19).

The residence of mast cells in meninges and perivascular locations on the brain side of the blood–brain barrier (7, 20), primarily in thalamic and hippocampal regions (21, 22), indicate that they are strategically situated to initiate neural and vascular responses. However, the function of mast cells in the brain is unknown. A role in normal physiology and behavior is suggested as the brain population of mast cells fluctuates with endocrine status and changes after stress and handling (23–26). Not surprisingly, there are individual differences in the number of brain mast cells within species (27), perhaps associated with behavioral and/or experiential differences.

We explored the possibility that mast cells in the brain might contribute to the modulation of behavior. The analysis entailed a behavioral screen assessing three components of generalized arousal (28), including anxiety-like behavior, locomotor activity, and sensory responsiveness using high-throughput automated assays (29). The availability of the Kit\textsuperscript{Wsh/sh} (sash\textsuperscript{−/−}) mutant provided a powerful genetic tool for the in vivo analysis of the role of mast cells (30, 31). While lacking mast cells, these mice have levels of major classes of other differentiated hematopoietic and lymphoid cells that are normal (31). Parallel pharmacological manipulation of mast cells permitted confirmation of the function(s) suggested in the mast cell deficient adult. The present work describes genetic and pharmacological loss-of-function studies that examined the relationship of brain mast cells and neural systems modulating behavior.

**Results**

We assessed the behavior of sash\textsuperscript{−/−} mice in two replicate experimental runs. In the first, we used unrelated C57BL/6 WT mice (the background strain for the sash\textsuperscript{−/−} mutant) as controls, and in the second, we tested heterozygous (sash\textsuperscript{+/−}) littermates. Anxiety-like phenotype was measured by behavioral testing in the open field arena and elevated plus maze and physiological measures of stress-induced defecation. Locomotor activity and sensory responses (to tactile, olfactory, vestibular, auditory, and pain stimuli) were also measured.

**Arousal Phenotype of Sash\textsuperscript{−/−} Mice.** Anxiety-like behavior was assessed using two tests. In the open field test, sash\textsuperscript{−/−} mice displayed more anxiety-like behavior (Fig. 1). The latency of the sash\textsuperscript{−/−} mice to enter the center square was 83 s longer than that of WT mice (sash\textsuperscript{−/−}: 141 s; WT: 58 s; P < 0.05), and nearly three times as long as their sash\textsuperscript{+/−} littermates (sash\textsuperscript{−/−}: 268 s; sash\textsuperscript{−/−}: 93 s; P < 0.01). Sash\textsuperscript{−/−} mice also entered the center square slightly, but not significantly, fewer times than WT mice (3.6 vs. 4.7 times, respectively, P > 0.05). Similarly, sash\textsuperscript{−/−} mice entered the center square fewer times than sash\textsuperscript{−/−} littermates (0.5 vs. 3.7 times, P < 0.01). The sash\textsuperscript{−/−} mice also displayed more anxiety-like behavior than


The authors declare no conflict of interest.

1To whom correspondence may be addressed. E-mail: pfaff@mail.rockefeller.edu.

2To whom correspondence may be addressed: Columbia University, Department of Psychology, 1190 Amsterdam Avenue, 406 Schermerhorn Hall, MC 5501, New York, NY 10027. E-mail: rps@columbia.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0809479105/DCSupplemental.

© 2008 by The National Academy of Sciences of the USA
WT and sash$^{-/-}$ littermate controls in the elevated plus maze (Fig. 2). Sash$^{-/-}$ mice entered open arms ~3–4 times less than WT and sash$^{-/-}$ littermate controls ($P < 0.05$). Additionally, sash$^{-/-}$ mice investigated the entrances to the open arms less than WT mice ($P < 0.01$). There was no difference between the littermate groups in the number of investigations into the open arms. In the measure of latency to enter an open arm, there was a trend, but no significant difference, between the sash$^{-/-}$ and WT animals. However, sash$^{-/-}$ mice displayed a longer latency to enter the open arm compared to their sash$^{+/+}$ littermates ($P < 0.05$).

Stress-induced defecation served as a physiological measure of anxiety-like behavior. Defecation during behavioral testing was greater in sash$^{+/+}$ mice compared to WT and sash$^{+/+}$ controls (Fig. 3). Sash$^{-/-}$ mice produced more bolus than did WT and sash$^{+/+}$ mice during the open field ($P < 0.01$ for age matched; $P < 0.05$ for littermates) and elevated plus maze tests ($P < 0.01$ for age-matched; $P < 0.05$ for littermates). Importantly, there were no differences in baseline defecation, measured by average rate or weight of defecation over a 24-h period in their home cage. There were also no differences between sash$^{-/-}$ mouse body weights and that of WT or sash$^{+/+}$ controls at time of sacrifice.

While we saw a mast cell effect on anxiety behavior, there were no differences in any of the other arousal behaviors tested (SI Methods). Sash$^{-/-}$ mice showed no differences in baseline locomotor activity compared to WT or sash$^{+/+}$ littermate controls. Additionally, there were no differences between groups in the responses to tactile, olfactory, vestibular, auditory, and pain stimuli (Figs. S2–S4).

It is well established that brain mast cells contribute to the neural pool of histamine (32–34). To confirm mast cell contribution to brain aneine levels, we measured histamine in whole brain homogenates. Sash$^{-/-}$ mice had 31.6% of WT control levels and 41.9% of sash$^{+/+}$ littermate levels of brain histamine (3.6 nM/g in sash$^{-/-}$ compared to 11.4 and 8.6 nM/g in WT and sash$^{+/+}$ littermate controls respectively; Fig. S5). The results confirm that mast cell deficiency causes a reduction in brain histamine.
Pharmacological Blockade of Mast Cells. To assess the contribution of central nervous system versus peripheral mast cells to the modulation of anxiety-like behavior in the adult mouse, we used disodium cromoglycate (cromolyn) to block mast cell degranulation (35). Cromolyn does not cross the blood brain barrier (36) and therefore we could distinguish the role of central and peripheral mast cell populations with i.p. versus i.c.v. injections. The WT mice were tested in a counterbalanced within subject experimental design to compare behavioral responses to i.p. and lateral i.c.v. cromolyn injection.

Central, but not peripheral, injection of cromolyn into WT mice increased anxiety-like behavior in the open field arena (Fig. 4). The i.c.v.-injected animals had 58% fewer entries into the center square compared to mice injected with saline i.c.v. or cromolyn i.p. [3.3 vs. 8 (saline i.c.v.) and 7.9 (cromolyn i.p.) occurrences, \( P < 0.05 \)]. There was no significant difference between mice injected i.p. with cromolyn or saline (\( P > 0.05 \)). In mice injected with i.c.v. cromolyn, there was a suggestive, but not significant, increase in the latency to enter the center of the open field arena (139 s) compared to i.c.v. saline injected (64 s) and also i.p. injected animals (\( P = 0.16 \)). Cromolyn injected i.p. did not significantly affect the latency to enter or the total number of entries into the center square compared to i.p. saline injected controls (\( P > 0.05 \)).

In the elevated plus maze, as in the open field arena, i.c.v. but not i.p. injection of cromolyn increased anxiety-like behavior (Fig. 5). When injected i.c.v., cromolyn caused a 79% decrease in the number of entries and an 86% decrease in the number of investigations into the open arms compared to i.p. cromolyn injected mice (entries: 1.0 vs. 7.5 occurrences, \( P < 0.05 \); investigations: 7.9 vs. 37.0 occurrences, \( P < 0.01 \)). Central cromolyn also increased the latency to enter the open arm compared to animals injected i.p. with cromolyn (352 vs. 33 s; \( P < 0.01 \)). The behavior in animals injected with cromolyn peripherally did not differ from that of animals injected with saline i.p. (\( P > 0.05 \)).

There were no significant effects of cromolyn on defecation. Neither i.c.v. nor i.p. injection of cromolyn caused changes in stress-induced defecation rate during the open field test or elevated plus maze.

Discussion

In the present experiments, using a genetic model, we demonstrate that a specific cellular element of the hematopoietic system, the mast cell, mediates the expression of anxiety-like behavior but has no effect on sensory arousal and locomotor responses. Using a pharmacological manipulation, we show that this is an effect of mast cells in modulating behavior of the adult rather than the result of developmental abnormalities in the mast cell deficient mouse. Additionally, the blockade of central, but not peripheral, mast cells affects anxiety-like behavior, revealing a central nervous system site of action. While the multitude of their mediators and triggers of activation prohibit the determination of which mast cell constituent(s) are responsible for the altered behavior, we show that brain histamine levels are decreased in the absence of these cells. This confirms that brain mast cells can contribute to the available CNS neurochemical pool.

Mast Cells and Emotionality. The present results are consistent with previous, highly suggestive evidence of associations between mast cells and emotionality. Food allergies, asthma and irritable bowel syndrome (all mast cell mediated pathologies) are commonly linked to trait-anxiety in humans but are potentially confounded by the stressful recurrent episodes themselves (37, 38). However, induction of an asthmatic or food allergy response in mice causes mast cell dependent increases in anxiety-like behavior and activation of the hypothalamic-pituitary adrenal axis (39, 40). Additionally, patients afflicted with systemic mastocytosis, a disease characterized by an increase in the number of mast cells (41), report low arousal states,
lethargy, and induction of coma (42, 43). These symptoms are reversed by treatment that includes histamine antagonists and cromolyn. Last, the incidence of autistic spectrum disorders is 6.75 higher in mastocytosis patients or their immediate relatives than in the general population (44).

**Mast Cells Are Pluripotential.** Mast cell-dependent changes in behavior are not likely due to a single mast cell mediator, but rather to multiple interacting chemicals and neural systems. Of the many known mast cell mediators, including neurotransmitters, cytokines, and lipid derived factors (10), many have been individually implicated in the modulation of behavior. Histamine is implicated in the regulation of the sleep–wake cycle (45), as well as other arousal-related systems including sex behaviors and anxiety (46, 47). In fact, histamine has been assigned both anxiolytic (48) and anxiogenic (49) effects, with opposing roles attributed to H1 versus H2 receptors (50). Serotonin functions both as a transmitter affecting many systems including aggression, appetite, and mood (51), and also as a trophic factor influencing neurogenesis and thereby affecting emotionality and memory (52, 53). Selective serotonin reuptake inhibitors increase serotonin signaling and decrease anxiety (54, 55); therefore, a lack of mast cell derived serotonin may result in an increase in anxiety-like behaviors. Mast cell derived cytokines act as neuromodulators having effects on systems controlling behavior. Tumor necrosis factor-α (TNF-α), interleukin-1, and interleukin-6 act on the hypothalamic-pituitary-adrenal axis and stress behavior (56). TNF-α also plays a role in the regulation of body temperature and the sleep–wake cycle (57, 58). Lipid-derived factors like prostaglandin D2 also have known roles as neuromodulators, contributing to the regulation of the sleep, pain, and body temperature regulation (59–61). Given the large number of mediators, mast cells most likely have multifaceted interactions with brain systems controlling behavior.

**Mast Cells Are Active Both Constitutively and Following Stimulation.** Since a majority of thalamic mast cells are active in the basal state (11, 62), factors affecting mast cell numbers in the brain are likely to be neurophysiologically important. Many apparently unrelated manipulations, including handling, sex, and stress, increase the number of brain mast cells, and it should be noted that all of these manipulations increase CNS arousal. An early study by Persinger showed that simple gentle handling of rat pups decreased numbers of mast cells in the brain developmentally, most likely representing an increase in degranulation (25). Psychological stressors induced through social defeat and isolation stress increased the number of mast cells in the brain (26, 63). Last, gonadal hormones from testis and stress behavior (56) also play a role in the regulation of mast cells. TNF-cytokines act as neuromodulators having effects on systems controlling behavior. Tumor necrosis factor-α (TNF-α), interleukin-1, and interleukin-6 act on the hypothalamic-pituitary-adrenal axis and stress behavior (56). TNF-α also plays a role in the regulation of body temperature and the sleep–wake cycle (57, 58). Lipid-derived factors like prostaglandin D2 also have known roles as neuromodulators, contributing to the regulation of the sleep, pain, and body temperature regulation (59–61). Given the large number of mediators, mast cells most likely have multifaceted interactions with brain systems controlling behavior.

**Immune Cells in Normal CNS Physiology.** While much of the evidence for immune system effects on the brain have come from disease states, other evidence, including data presented here, points to the role of neuroimmune interactions in normal physiology. The former line of research shows that dysregulation of the immune system can negatively impact brain functioning. Cytokines released in the periphery during an immune response gain access to the brain (65) and modulate many brain systems, including effect, cognition, and pain processing (65, 66). Specifically, interferons and interleukins have been causally implicated in mediating depression (67). Interestingly, this has led to the immune system becoming a focus of novel therapeutic targets for the treatment of neurological disorders including psychiatric diseases (44, 68). However, the present study joins a growing literature investigating the role of immune cells in the healthy brain. CNS-specific T-cells contribute to hippocampal neurogenesis in adults with consequences for the formation of spatial memories (69). Also, major histocompatibility complex molecules (expressed on dendritic cells, T-cells, microglia, and mast cells in the brain) are implicated in neuronal synapse development (70) and play a role in synaptic plasticity (71).

Overall, our data provide evidence for a direct association of brain mast cells impacting anxiety-like behavior. Given that they can change the signaling milieu of the brain, we suggest that mast cells provide a functional link through which the immune system interacts with the brain. The results provide evidence of the behavioral importance of immune cells in the brain.

**Materials and Methods**

**Mast Cell Deficient Mice.** Mast cell deficient KitW−/− W- (sash-/-) mice were obtained from Jackson Laboratory (B6.Cg-KitW−/−H2bNatr3JaeMs6J; strain 5051). Sash-/- mice carry a mutation upstream from the white spotting (W) locus causing disruption of the S' regulatory sequences and therefore reduced signaling through c-kit tyrosine kinase (30). This reduced c-kit expression in the bone marrow causes an inability of hematopoietic stem cells to differentiate into mast cell precursors resulting in a complete lack of mast cells (51, 72). No other known disruptions in the development of hematopoietic stem cell derivatives or any other irregularities in c-kit receptor mRNA or protein expression, with the exception of melanocytic (causing irregular fur pigmentation), have been found (30).

The studies were first completed with male sash-/- (n = 8) and unrelated age-matched C57BL6 WT control mice (n = 8). A second cohort, run with sash-/- (n = 7) serving as controls for sash-/- mice (n = 6) allowed us to control for other genetic variability and epigenetic factors that might affect behavior. All animals were male and housed in a 12:12 light–dark cycle and food and water were available ad libitum. All experimental procedures were in accordance with the protocols of the Institutional Animal Care and Use Committee at Rockefeller University.

**Cromolyn Blockade of Mast Cell Degranulation.** For examination of the response to disodium cromoglycate (cromolyn, Sigma Aldrich), C57BL6 WT animals (n = 14, Jackson Laboratory) were used. All mice were male. Mice were anesthetized with a Ketamine (100 mg/kg):Xylazine (13 mg/kg) mixture and implanted stereotaxically (David Kopf Intruments) with chronic indwelling cannulas (Plastics One). Tips of the cannula were directed 0.2-mm dorsal to the right lateral ventricle (bregma, 0.5 mm; mid, 1.2 mm; skull, 2.5 mm) for use with an injector computer. Animals were available for 2 × 2 study design receiving either saline or cromolyn via i.p. or i.c.v. injections. Cromolyn doses were 10 mg/kg (i.p.) and 50 µg/µl (i.c.v.). Cromolyn or saline was injected for 3 days, and behavioral testing was completed on the third day after injection.

**Behavioral Testing**

**Open field arena.** For assessment of open field behavior, mice were placed individually into a 40 × 40 cm brightly lit, Plexiglas open field arena. The bottom was demarcated into a 5 × 5 grid making 25 equal-sized (8 × 8 cm) squares. Mice were allowed to explore the arena undisturbed for 5 min. Video tapes were scored by two experimenters blind to the study for latency to enter and number of entries into the center square.

**Elevated plus maze.** The elevated plus maze was elevated 30 cm from the ground with a 5 cm × 5 cm center platform and 4 radial arms (5 cm wide × 30 cm long) with two opposing “closed” arms encased by 15-cm high opaque black walls (Rockefeller University Instrument Shop, New York, NY). Mice were placed in the center of the maze oriented toward an open radial arm and allowed to explore undisturbed and videotaped for 10 min. Video tapes were scored by experimenters blind to the study for latency to enter an open arm, number of entries into open arms, and instances of investigations into open arms.

**Defecation.** The number of bolus was counted for each animal after behavioral testing. For measures of baseline defecation, animals were placed individually in bins with a grid bottomed floor without bedding for 24 h. Dry weight and number of bolus were recorded. Defecation data are represented as average rates (number of bolus/minute).

**Statistical Analysis.** Comparisons between sash-/- mice and WT controls or heterozygous littersmates were made using one-way ANOVA. Results from the pharmacological loss-of-function experiment were compared using two-way ANOVA (treatment × injection route). Pairwise multiple comparisons were performed with Tukey’s HSD test when appropriate.

**Acknowledgments.** We thank Akhila Iyer and Isabelle Carron-LeSauter for help with behavioral scoring and Drs. Frances Champagne, Joseph LeSauter and Matthew Butler for their helpful comments on a previous version of this manuscript. Support provided by Grants NICHD 05751–33 (to D.P.) and NIMH 67782 and NSF IOS-05–54514 (to R.S.).


