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Central Ghrelin Resistance Permits the Overconsolidation of Fear Memory

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ABSTRACT

BACKGROUND: There are many contradictory findings about the role of the hormone ghrelin in aversive processing, with studies suggesting that ghrelin signaling can both inhibit and enhance aversion. Here, we characterize and reconcile the paradoxical role of ghrelin in the acquisition of fearful memories.

METHODS: We used enzyme-linked immunosorbent assay to measure endogenous acyl-ghrelin and corticosterone at time points surrounding auditory fear learning. We used pharmacological (systemic and intra-amygdala) manipulations of ghrelin signaling and examined several aversive and appetitive behaviors. We also used biotin-labeled ghrelin to visualize ghrelin binding sites in coronal brain sections of amygdala. All work was performed in rats.

RESULTS: In unstressed rodents, endogenous peripheral acyl-ghrelin robustly inhibits fear memory consolidation through actions in the amygdala and accounts for virtually all interindividual variability in long-term fear memory strength. Higher levels of endogenous ghrelin after fear learning were associated with weaker long-term fear memories, and pharmacological agonism of the ghrelin receptor during the memory consolidation period reduced fear memory strength. These fear-inhibitory effects cannot be explained by changes in appetitive behavior. In contrast, we show that chronic stress, which increases both circulating endogenous acyl-ghrelin and fear memory formation, promotes profound loss of ghrelin binding sites in the amygdala and behavioral insensitivity to ghrelin receptor agonism.

CONCLUSIONS: These studies provide a new link between stress, a novel type of metabolic resistance, and vulnerability to excessive fear memory formation and reveal that ghrelin can regulate negative emotionality in unstressed animals without altering appetite.

Keywords: Amygdala, Chronic stress, Corticosterone, Fear, Ghrelin, Hunger

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Ghrelin, often called the "hunger hormone," is an omnipresent circulating hormone that is synthesized and released by many organs; the stomach is the primary source of ghrelin in the bloodstream (1). In its acylated form (acyl-ghrelin), circulating ghrelin can cross the blood-brain barrier and bind to central ghrelin receptors (growth hormone secretagogue receptor 1a [GHSR1a]) (1). GHSRs are abundant in classic hypothalamic hunger regions (2). Also, ghrelin levels can be rapidly elevated within minutes during anticipatory hunger states (3), and administration of acyl-ghrelin to humans can stimulate food intake (4). However, GHSRs are widely distributed throughout the brain including brain regions not typically associated with hunger, such as the basolateral complex of the amygdala (BLA) (5), a brain region important for regulating valenced behavior, including fear. Additionally, acyl-ghrelin is tonically secreted at all times (6), not just at times when food is expected. These findings suggest that acyl-ghrelin may have a broader role than simply modulating hunger or appetitive processing.

The presence of GHSR in the BLA suggests that ghrelin signaling may modulate fear, but the role of acyl-ghrelin in BLA-dependent fear memory is controversial. While some groups report that transient elevation of ghrelin signaling promotes the excitability of BLA neurons (7), others find that ghrelin decreases BLA excitability (5). Only two studies have directly assessed the role of ghrelin in the BLA, and both reported that acute and chronic elevation of ghrelin enhance fear memory strength (8,9). In the present study, we sought to resolve the role of endogenous acyl-ghrelin in BLA-dependent fear memory.

METHODS AND MATERIALS

Blood was sampled from jugular catheters or tails at different time points around auditory Pavlovian fear conditioning in rats. We used enzyme-linked immunosorbent assay to examine plasma acyl-ghrelin and corticosterone levels in these samples. In other experiments, we administered a ghrelin receptor agonist, a ghrelin receptor antagonist, or rat acyl-ghrelin either systemically or intra-BLA. Following these manipulations, different behaviors were examined, including Pavlovian fear

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conditioning, fear recall testing, food consumption, or unconditional freezing. Some rats received chronic immobilization stress or handling; biotin ghrelin was used to assess ghrelin binding affinity in coronal brain sections containing the BLA. See Supplemental Methods and Materials for procedural details.

RESULTS

Using healthy, unstressed rats implanted with jugular vein catheters, we took blood samples at time points around auditory Pavlovian fear conditioning (Figure 1A). We found that circulating acyl-ghrelin levels were not significantly altered by the brief (<3 minutes) fear conditioning paradigm used here (Figure 1B, top), in contrast to corticosterone levels (Figure 1B, bottom). Thus, while the expectation of food can rapidly elevate acyl-ghrelin levels within minutes (3), a brief fear conditioning experience, one form of acute stress exposure, does not. We previously reported that repeated, but not acute, stressor exposure elevates acyl-ghrelin levels measured 24 hours after stressor cessation (9); this extends these findings to show that acute stressor exposure does not change acyl-ghrelin on a shorter time scale.

We sought to determine whether acyl-ghrelin or corticosterone levels during fear learning determined subsequent long-term fear memory strength in rats. Long-term auditory fear memory strength was assessed 2 days after fear conditioning, a time point beyond the time in which short-term memory undergoes synaptic consolidation to form long-term memory (10). We computed 12 linear regressions with freezing as the dependent variable and acyl-ghrelin level at each time point (0–180 minutes) to determine whether acyl-ghrelin levels at individual time points around fear conditioning were related to long-term fear recall measured more than 24 hours later (see Supplemental Methods and Materials).

Acyl-ghrelin levels measured at 120 and 180 minutes after fear conditioning each individually accounted for a considerable amount of the variance in freezing behavior (R^2 = .94 and .93) (Supplemental Figure S1A, B). Because the number of subjects for this experiment was low, we also computed the predicted residual sum of squares statistic for each regression. The predicted residual sum of squares statistic is a leave-one-out cross-validation method that provides an estimate of how well an equation will generalize to new data. The low predicted residual sum of squares values for the regressions against acyl-ghrelin at 120 and 180 minutes after conditioning (Supplemental Table S1) confirmed that the values at these time points were the most strongly correlated with freezing during the long-term memory recall test. We used Lasso regression (see Supplemental Methods and Materials and Supplemental Figure S2A) to determine whether measurements of acyl-ghrelin and/or corticosterone levels at multiple time points could better explain freezing data than any single measurement. Two Lasso regressions were performed, with acyl-ghrelin or corticosterone levels from all time points as the independent variables. The best-fitting linear model included acyl-ghrelin plasma levels at both 120 and 180 minutes after fear conditioning (Figure 1C). Higher levels of endogenous circulating ghrelin across these time points robustly constrained auditory fear memory strength. Despite a substantial literature implicating glucocorticoids in memory formation [see (11) for review], none of the selected models retained corticosterone levels as a measurement to explain freezing behavior (Supplemental Figure S2B and Supplemental Table S1). Endogenous acyl-ghrelin levels did not correlate with shock reactivity during fear conditioning (Supplemental Figure S1E) or with the hypothalamic-pituitary-adrenal response induced by fear conditioning (Supplemental Figure S1F). Because postconditioning acyl-ghrelin plasma levels were correlated with long-term auditory fear memory recall but not fear acquisition, this suggests that endogenous acyl-ghrelin might negatively regulate fear memory consolidation.

It could be argued that hunger and fear are incompatible states and thus compete with each other within neural circuits. This hunger and aversive behavior tradeoff has been noted in rodents in seminaturalistic foraging environments (12) and has been described for a specialized subset of hypothalamic neurons (13). By this logic, high levels of acyl-ghrelin, which under some circumstances can promote hunger (14), may also facilitate exploration to increase the opportunity to find food. For example, increasing levels of acyl-ghrelin might facilitate motor hyperactivity, thereby decreasing freezing behavior. However, we did not observe correlations between gross motor activity and acyl-ghrelin levels across the subjects from Figure 1 (Supplemental Figure S1G).

To further determine whether acyl-ghrelin was broadly related to fear memory strength, rather than simply to freezing behavior, we examined risk assessment behavior, in which the body remained immobile, but the head was moved to scan the environment (see Supplemental Methods and Materials), during the long-term auditory fear recall test (Figure 1D). Risk assessment behaviors are observed when threat levels are determined to be low or moderate (15) and are thought to be important for reducing defensive behaviors such as freezing when danger is no longer present (16). As perceived threat transitions from high to low levels, fear also shifts from high to low levels; increased risk assessment behaviors accompany this shift. No risk assessment behavior was observed in any rat before the tone onset of each fear recall test (data not shown), confirming that this behavior is specifically elicited during a fear state. We computed linear regressions with risk assessment behavior as the dependent variable and acyl-ghrelin level at each time point (0–180 minutes) to determine whether acyl-ghrelin levels at individual time points around fear conditioning were related to this second measure of fear memory strength. We found that acyl-ghrelin levels measured at 120 and 180 minutes after fear conditioning were also associated with earlier onset of vigilance behaviors (Figure 1D; Supplemental Table S2), suggesting that rats with higher endogenous acyl-ghrelin levels more rapidly transition to a low fear state during the fear recall test. Thus, endogenous acyl-ghrelin was correlated with two distinct measures of fear memory strength. Collectively, these data suggest that plasma acyl-ghrelin levels are related to the strength of fear memories, rather than simple motor hyperactivity.

Because ghrelin can be elevated by hunger (17), it might be hypothesized that interindividual variability in acyl-ghrelin arises when fear conditioning suppresses postconditioning food consumption to different degrees in individuals in the hours after conditioning. However, acyl-ghrelin levels were similar before and after fear conditioning (Figure 1B), and fear conditioning did not suppress food consumption (Supplemental Figure S1H).