Human sleep consolidates allergic responses conditioned to the environmental context of an allergen exposure

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Allergies are highly prevalent, and allergic responses can be triggered even in the absence of allergens due to Pavlovian conditioning to a specific cue. Here we show in humans suffering from allergic rhinitis that merely reencountering the environmental context in which an allergen was administered a week earlier is sufficient to trigger an allergic response—but only if participants had slept after allergen exposure. This context-conditioning effect was entirely absent when participants stayed awake the night after allergen exposure or were tested in a different context. Unlike in context conditioning, cue conditioning (to an odor stimulus) occurred independently of sleep, a differential pattern that is likewise observed for conditioning in the behavioral domain. Our findings provide evidence that allergic responses can be conditioned to contextual information alone, even after only a single-trial conditioning procedure, and that sleep is necessary to consolidate this rapidly acquired maladaptive response. The results unravel a mechanism that could explain part of the strong psychological impact on allergic responses.

sleep | Pavlovian conditioning | allergic rhinitis | learning | placebo

In 1886, John N. MacKenzie published a famous case report on a woman who developed an asthmatic attack after seeing an artificial rose (1). More recently, Bennett G. Braun described several patients with multiple personalities in whom an allergic disorder was present with one but not the other personality (2). "Placebo responses" in patients suffering from allergies are among the strongest observed in clinical studies (3, 4), and their great magnitude often results in insufficient statistical power for detecting verum effects (5). These observations underscore the importance of psychological factors in allergic disorders, which are widespread with an increasing prevalence worldwide, exacting a high societal burden (6).

Immune responses, including allergic reactions, are known to be subject to Pavlovian conditioning; that is, after learning an association between an immune-active agent (e.g., an allergen) and an immunologically neutral stimulus (e.g., a distinct odor), the neutral stimulus alone can trigger the immune response (7–9). Two experimentally well-controlled studies in humans have demonstrated the development of conditioned allergic reactions after pairing an allergen with a specific cue (10, 11). Another experimental study in humans added to these findings by showing that antiallergic responses also can be conditioned after pairing an antihistaminergic drug with a novel taste (12). These human experiments complement early studies in animals demonstrating conditioned mast cell responses (13, 14).

Conditioning processes can serve as mechanisms underlying the strong placebo responses in allergic diseases described above (15). Surprisingly, whereas the conditioning of diverse immune responses to distinct cues (i.e., cue conditioning) has been shown repeatedly, the specific role of context conditioning (i.e., the association of a response to its environmental context) in the Pavlovian learning of allergic responses has not yet been scrutinized experimentally (15–17), although context conditioning effects are known to substantially contribute to maladaptive responses in other domains (e.g., of fear and addiction behaviors) (18).

Sleep is generally thought of as an adaptive process, and one of its major functions is to support memory formation (19, 20). Thus, sleep also might promote learned allergic responses, despite these responses being maladaptive. The role of sleep in the conditioning of immune responses has not yet been investigated. Against this backdrop, here we assessed the effects of sleep versus wakefulness after conditioning of an allergic rhinitis response in humans. We were especially interested in comparing cue conditioning and context conditioning of allergic responses, because previous studies indicated that sleep selectively enhances context-conditioned, but not cue-conditioned, responses in the behavioral domain (21–24). Accordingly, here we expected postencoding sleep to specifically enhance context-conditioned, but not cue-conditioned, allergic responses.

Results

We subjected our otherwise healthy participants with clinically verified seasonal allergic rhinitis to a single-trial combined context/cue-conditioning procedure consisting of a Learning session and a Test session. Both sessions comprised a 45-min Context

Significance

Allergic disorders are widespread and can strongly affect quality of life. Here we show in humans that an allergic response can be triggered even in the absence of allergens by simply reencountering the environmental context in which an allergen was previously administered. This effect occurred only when participants slept during the night after allergen administration and was entirely absent when they stayed awake on that night, demonstrating that sleep is necessary for consolidating the association between environmental context and allergen. These findings have important implications for understanding the often observed "placebo" allergic responses occurring in the absence of allergens.

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phase during which the participant remained in a standardized experimental room, allowing encoding of and acclimatization to the environmental context (*Materials and Methods* and Fig. 1). Then the Cue phase started with a 6-s presentation of the cue (conditioned stimulus [CS])—a distinctive odor (isobutyraldehyde)—which was immediately followed by the administration of the unconditioned stimulus (UCS) consisting of a nasal spray containing pollen allergens. A physiological measure (mucosal tryptase) and a clinical symptom measure (Lebel score) of allergic rhinitis reactions were assessed during the Context phase (preodor measurements) and after cue-conditioning (postodor measurements) (Fig. 1). Following the Learning session, the participants had either a night of regular sleep for 8 h (Sleep group) or stayed awake in bed in a semisupine position for the same 8-h period (Wake group). The Test session took place 1 wk later; this session was identical to the Learning session, except that the nasal spray presented during the Cue phase contained only a saline solution.

Polysomnography data showed that participants in the Sleep group slept on average for 465 min (Table 1 provides sleep parameters), and that sleep architecture was comparable to that of healthy individuals examined in other studies from our laboratory as well as other laboratories (25, 26). Participants in the Wake group were under constant supervision by the experimenter to ensure that they did not fall asleep during the sleep deprivation period between 23:00 and 7:00 h. Actigraphy data confirmed that none of the participants slept during the day after the sleep deprivation night, except for one participant who might have fallen asleep for approximately 90 min while watching TV in the afternoon of this day. Basic physiological parameters, including heart rate, blood pressure, and body temperature, were not significantly different between the groups in the evening before the sleep manipulation (*SI Appendix*, Table S1).

During the Learning session, all participants developed an allergic reaction to the UCS, confirming their allergy ($P < 0.001$ for increases in tryptase levels and the Lebel score after UCS presentation—i.e., postodor values—with reference to values before UCS presentation—i.e., preodor values—serving as baseline) (*SI Appendix*, Fig. S1). There were no significant differences between the Sleep and Wake groups in terms of tryptase levels and Lebel score in the Learning session (main effect "Sleep/Wake" and "Sleep/Wake" × "Preodor/Postodor" interaction; $P > 0.832$).

During the Test session 1 wk later, participants showed already in the Context phase (i.e., before the odor presentation) distinctly increased tryptase levels compared with preodor levels of the Learning session, demonstrating a conditioned response to the context (main effect "Session": $F_{(1,21)} = 6.957$, $P = 0.015$). Importantly, the context-induced increase in tryptase levels occurred only in the participants who had slept after the Learning session ($t_{(1,11)} = 2.693$, $P = 0.021$, $d = 0.74$) and was entirely absent in the Wake group ($P = 0.341$ for the post hoc pairwise t test; $F_{(1,21)} = 6.237$, $P = 0.021$ for "Sleep/Wake" × "Session" interaction) (Fig. 2*A* and *SI Appendix*, Fig. S2*A*). There was no significant difference in preodor values between the Learning and Test sessions for the Lebel score ($P > 0.336$ for main effect "Session" and "Session" × "Sleep/Wake" interaction) (Fig. 2*A* and *SI Appendix*, Fig. S2*A*).

The data from the Test session also revealed a distinct cue-conditioned response to the odor cue, as evidenced by significant

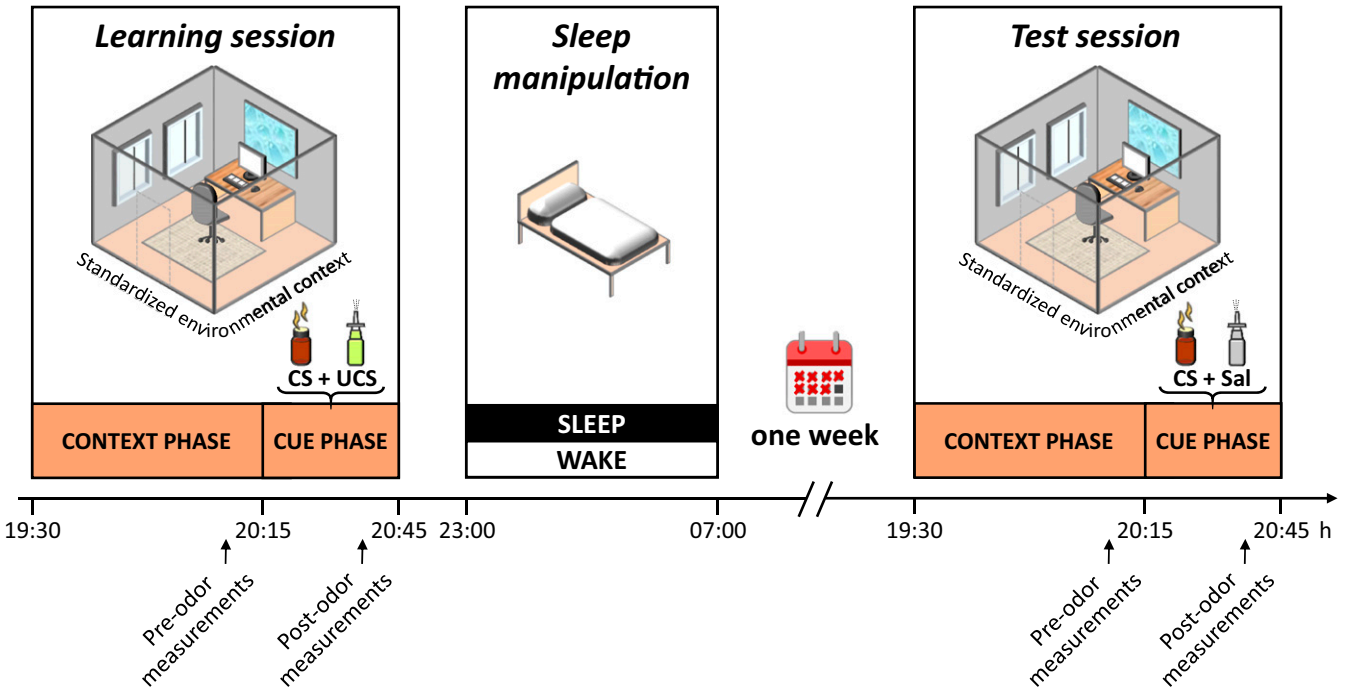

Fig. 1. Study design. The Learning session started with a 45-min Context phase, during which the participant remained in a standardized experimental room to allow for encoding of the context and acclimatization to the room. In the ensuing Cue phase, the conditioned stimulus (CS)—a distinct odor—was delivered by holding a bottle containing a solution of isobutyraldehyde for 6 s below the participant's nose. Immediately thereafter, the unconditioned stimulus (UCS)—pollen allergens delivered via a nasal spray—was presented. Allergic reactions (assessed by mucosal tryptase levels and the Lebel score) were determined at the end of the Context phase (preodor measurements) and at 5 min after presentation of the CS and UCS (postodor measurements). On the night after the Learning session, one-half of the participants slept during an 8-h period in the sleep laboratory, while the other half stayed awake (until the next evening). At 1 wk later, the Test session took place in the same standardized experimental room following identical procedures, except that the nasal spray contained only saline solution (Sal) without the UCS. Context-conditioned responses were assessed by comparing values at the end of the Context phase (preodor measures) of the Test session with those of the Learning session (baseline). Cue-conditioned responses were assessed by comparing postodor measures with preodor measures of the Test session.

Table 1. Sleep parameters

Parameter	Duration, min, mean ± SEM	Percentage of total sleep time, mean ± SEM
Total sleep time	464.8 ± 3.3	100
S1	39.9 ± 3.2	8.6 ± 0.7
S2	226.6 ± 11.2	48.6 ± 2.2
SWS	80.5 ± 4.5	17.3 ± 0.9
REM	83.1 ± 6.2	17.9 ± 1.3
WASO	28.9 ± 11.9	6.3 ± 2.7

S1, sleep stage 1; S2, sleep stage 2; SWS, slow-wave sleep, REM, rapid eye movement sleep; WASO, wake after sleep onsettblBody.

$n = 12$; for one participant, the EEG could not be completely scored due to quality problems, but the part that could be scored (>6 h) indicated regular sleep.

increases in postodor tryptase levels and the Lebel score compared with preodor values (main effect "Preodor/Postodor": $F_{(1,21)} = 4.567$, $P = 0.045$, $d = 0.64$ for tryptase levels; $F_{(1,23)} = 18.232$, $P < 0.001$, $d = 1.08$ for the Lebel score) (Fig. 2*B* and *SI Appendix*, Fig. S2*B*). Notably, unlike the context-conditioned response, this cue-conditioned allergic response was independent of whether or not the participants had slept on the post-conditioning night ("Sleep/Wake" × "Preodor/Postodor" interaction: $P > 0.526$ for both parameters).

To validate that the context-conditioning effect observed in the Sleep group was specific to the environmental context in which the learning had taken place, we added a "Context control" group, which was subjected to the same experimental procedure as the Sleep group of the main experiment (including the sleep period after conditioning), except that the Test session took place in a different environmental context than the Learning session. As predicted, participants in this group did not show an increase in preodor tryptase levels during the Test session compared with preodor levels of the Learning session. Thus, no context-conditioned response was evident when the context of the Test session differed from that of the Learning session, even though the participants slept after the Learning session ($P = 0.713$ for the pairwise t test; $P = 0.018$ for the contrast between the Sleep group and the Context control and Wake groups) (Fig. 3 and *SI Appendix*, Fig. S3). The data for the Context control group confirmed a cue-conditioned response to the odor (details in *SI Appendix*, Fig. S4).

Discussion

We show here that an allergic response can not only be conditioned to a specific cue but also can be triggered by merely reencountering the environmental context in which an allergen was previously encountered. Although the possible occurrence of context-conditioned allergic responses was suggested by previous observations (27), this is the first experimentally controlled demonstration of an allergic response specifically conditioned to the context and distinct from the response to the conditioned cue. Of central importance is our finding that only the participants who slept after the Learning session, but not participants of the Wake group, developed a context-conditioned allergic response, which underscores the critical role of sleep in the formation of a memory for such responses, regardless of whether or not the learned response is maladaptive. Thus, beyond its generally adaptive function, sleep may contribute to the aggravation of allergies.

It is worth mentioning in this context that patients with allergies often report disturbed sleep (28, 29). This disturbance may represent an epiphenomenon with some adaptive value, as it could impair the consolidation of potential context-conditioned allergic responses, although the negative health consequences of sleep disruption likely outbalance such an adaptive effect (30). In the present study, disturbed sleep was an exclusion criterion; therefore, whether allergic patients with disturbed sleep develop less pronounced context-conditioned responses than those without sleep disturbances remains an intriguing question.

The sleep effect focusing on the context-conditioned allergic response and being absent for the cue-conditioned response is a pattern that aligns well with studies of conditioning in the behavioral domain: Postconditioning sleep has been consistently shown to support context-conditioned fear responses, whereas sleep generally does not affect cue-conditioned fear responses (21–23). A major difference in these responses is that only the formation of context-conditioned behavior, but not cue-conditioned behavior, essentially depends on hippocampal function (e.g., refs. 31–33). Hippocampal engagement is critical for the reactivation of context-related memory representations during sleep (34); therefore, it is tempting to speculate that similar neuronal mechanisms involving hippocampal activity also contribute to the sleep-dependent formation of a context-conditioned allergic tryptase release.

The increase in tryptase levels in response to the context had a medium effect size but was not associated with an increase in clinical symptoms, as assessed by the Lebel score, and was considerably smaller than the response to the UCS, representing a concentrated allergen delivered directly into the nostrils of the participants. However, for the experimental purpose of a conceptual proof, here we performed only a single pairing of the allergen with the experimental context and cue, which diverges

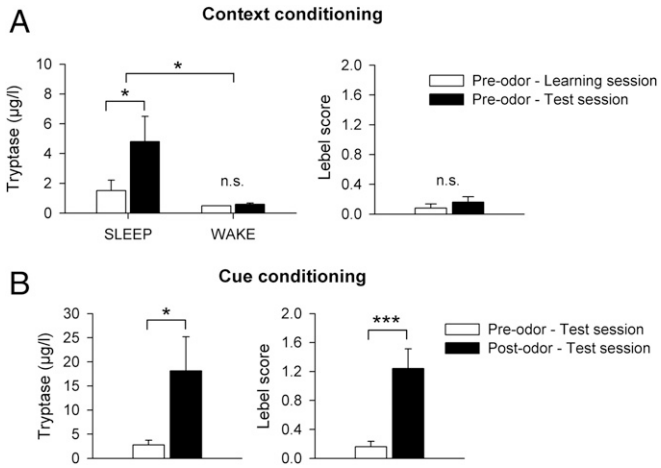

Fig. 2. Context-conditioned and cue-conditioned allergic responses measured after a single pairing of an environmental context and an odor cue (CS) with the administration of pollen allergens (UCS). (*A*) Mean ± SEM values of mucosal tryptase levels (*Left*) and the Lebel score (*Right*) for preodor measurements at the Learning session (empty bars) and at the Test session (black bars) (i.e., context-conditioning effect). $n = 12$ and $n = 13$ for tryptase levels and Lebel scores, respectively, of the Sleep group; $n = 11$ and $n = 12$ for tryptase levels and Lebel scores, respectively, of the Wake group. $^*P < 0.05$ for ANOVA "Session" (Learning vs. Test session) × "Sleep/Wake" interaction and post hoc two-sided paired t test. n.s., not significant. (*B*) Mean ± SEM values of mucosal tryptase levels (*Left*) and the Lebel score (*Right*) before (empty bars) and at 5 min after (black bars) presentation of the odor cue during the Test session (i.e., cue-conditioning effect). $n = 23$ and $n = 25$ for tryptase levels and Lebel scores, respectively. $^{***}P < 0.001$, $^*P < 0.05$ for ANOVA main effect of the factor "Preodor/Postodor" (preodor vs. postodor presentation). Because the Sleep and Wake groups did not differ in terms of the context-conditioning effect of the Lebel score ($P > 0.928$ for ANOVA "Session" × "Sleep/Wake" interaction and ANOVA main effect "Sleep/Wake") and in terms of the cue-conditioning effects of both parameters ($P > 0.526$ for ANOVA "Preodor/Postodor" × "Sleep/Wake" interactions and ANOVA main effect "Sleep/Wake") data for these effects are shown collapsed across both groups.

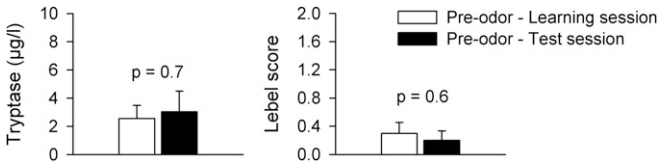

Fig. 3. Context-conditioned allergic responses after regular sleep are abolished if the Test session takes place in a context different from that of the Learning session. Mean ± SEM values of mucosal tryptase levels (*Left*) and the Lebel score (*Right*) for preodor measurements at the Learning session (empty bars) and at the Test session (black bars), which took place in an environmental context different from that during learning. Note that no sleep/wake manipulation occurred in this Context control group; all participants of this group had regular sleep after the Learning session. *P* levels refer to two-sided paired *t* tests. $n = 9$ and $n = 10$ for tryptase levels and Lebel scores, respectively.

from real-life conditions where associations of allergens with a specific context and cue are likely to occur multiple times and to be consolidated over several nights. The conditioned increase in tryptase levels to the odor cue was surprisingly large given the single-trial pairing and translated into increased expression of clinical symptoms. Repeated pairings are known to induce stronger conditioned responses that can even be comparable in size to the response to the allergen itself (11, 13, 14). Thus, context as well as cue conditioning occurring repetitively can be expected to contribute to potentially clinically relevant symptom expression in patients with allergies. On the other hand, the conditioning of allergic responses to the environmental context, as well as its dependency on sleep, might be exploited to develop and refine antiallergic treatments. Allergen immunotherapy is based on the relearning of an appropriate immune response to an allergen (35) and may profit from sleep in the same way as exposure therapy of fears does (36), especially when including multiple-context exposures (37). Ultimately, this study might provide new perspectives in the understanding of other immune-related medical conditions that are receptive to psychological influences and thus also might be affected by sleep-dependent learning processes (38–40).

Although our final sample sizes were chosen based on a previous experimental study that demonstrated a conditioned increase in tryptase levels in patients with allergic rhinitis (10), they were relatively small and the (conditioned) allergic responses showed some variability among participants. Thus, future studies are important to extend our findings to larger populations of patients with allergies (also including females), as well as other patient groups with immune-related diseases. It also remains to be investigated why the Lebel score did not show a conditioning to the context. In contrast to tryptase levels, the Lebel score represents a conscious measure and thus likely depends more on expectations. Indeed, previous findings suggest that placebo responses for unconscious physiological measures are mediated by conditioning, whereas expectations play a significant role in placebo responses occurring in conscious measures (41). An alternative explanation for the lack of significant context conditioning of the Lebel score is that this score may simply be a less sensitive measure of allergic responses than tryptase levels. Finally, the lack of a sleep/wake manipulation in our Context control group prevents us from answering the question of whether sleep deprivation may change, in addition to the context-conditioned response, the (cue-)conditioned responses when tested in a context different from the one present at conditioning.

In summary, this study provides proof-of-principle data showing that an allergic response can be conditioned to the mere context of a conditioning procedure, and that this response depends on the occurrence of sleep after learning the association between allergen and context. These results lay the groundwork for further experiments focusing on investigating the mechanisms underlying the development of psychologically mediated allergic responses.

Materials and Methods

Participants and Experimental Design. Participants were recruited by advertisements sent via the email distribution list of members of the University of Tübingen. Before enrollment in the study, candidates were screened for a clinically relevant sensitization against birch and/or grass pollen by a skin-prick test, allergen-specific IgE titers in the serum, and a nasal provocation test. Of the 72 screened candidates, 30 were ultimately enrolled in the study, and 25 successfully completed it (mean age, 25.24 ± 4.25 y). The study was performed according to a randomized, double-blinded, mixed (within- and between-subjects) design during the pollen-free periods of 2014 to 2018. The experiment consisted of a Learning session, during which the CS (odor cue) and the UCS (pollen allergens) were paired once in a specific environmental context (a standardized experimental room; Fig. 1), and a Test session, which took place in the same standardized room and during which successful conditioning to the environmental context and to the cue was tested. On the day of the Learning session, participants arrived at the laboratory at 19:30 h. Toward the end of a 45-min Context phase, during which the participants remained in the experimental room, allowing them to encode and acclimatize to the environmental context, allergic responses (the Lebel score and tryptase levels) were assessed a first time (preodor measurements).

Following this phase, the Cue phase started with presenting a bottle containing a solution of isobutyraldehyde (diluted 1:50 with 1,2-propanediol), constituting the odor cue (CS), for 6 s. Immediately thereafter, the participants received a nasal spray (100 µL per nostril) containing either birch or grass pollen (2500 SBE/mL; Allergopharma, Hamburg, Germany). After this, the participants were asked to complete a questionnaire about the qualities of the odor, to increase the salience of the odor cue. The Lebel score was again assessed at 5 min after administration of the nasal spray, after which nasal mucus was collected for assessment of tryptase levels (postodor measurements). At 23:00 h, one-half of the participants were allowed to sleep in the sleep laboratory for an 8-h period (Sleep group), and the other half remained awake in bed in a semisupine position (Wake group) during the same period and were allowed to watch TV, listen to music, play board games, and talk to the experimenter. They were under constant supervision by the experimenter to ensure that they did not fall asleep at any time. Participants of both groups were prepared for polysomnographic recordings before bedtime, to keep the conditions comparable and keep the assignment to the respective group obscure until they went to bed. Participants wore an actigraphy device (Actiwatch 2; Philips Respironics) after leaving the sleep laboratory the next morning and completed activity diaries to confirm that they did not go to sleep before 21:00 h.

Seven days later, participants arrived again at the laboratory for the Test session. The procedure was identical to that for the Learning session, except that this time the nasal spray contained only a saline solution without allergens. The participants and the experimenter were blinded to the content of the nasal spray and had been told that for each visit, there was a 50% chance of receiving an allergen or a saline solution without allergens. Another group of participants ($n = 7$) followed a "sham conditioning" protocol in which they received only a saline solution without allergens during both the Learning and the Test sessions, to exclude the possibility that any nonspecific aspect of the experimental procedure per se induced increases in allergic reactions ($P > 0.356$ for all context- and cue-conditioning measures) (*SI Appendix*, Fig. S5).

To verify that the conditioned increase in tryptase levels found in the Sleep group was specific to the environmental context in which the Learning session had taken place, a control experiment was performed in which the Test session took place in a different environmental context than the Learning session—the participant's home environment, reflecting a deviating physical and social context. Apart from this, the procedure was identical to the Sleep group of the main experiment. Forty-nine candidates were screened for this Context control group, of which 10 (mean ± SD age, 24.10 ± 5.00 y) successfully completed it.

Subject Enrollment. All participants had a regular sleep/wake rhythm and did not present with any sleep disturbances, as anamnestically assessed and defined by the symptoms: difficulty falling asleep, experiencing early awakenings, frequent nighttime awakenings, and/or excessive daytime

sleepiness. They were not taking any medication at the time of the experiments and were nonsmokers. Acute and chronic illnesses were excluded by medical history and physical examination. Further exclusion criteria included the use of antihistaminergic drugs or corticosteroids in the past 2 wk, strong sensitization responses, drug incompatibilities (especially to adrenaline), allergy against house dust mites, and a current allergen immunotherapy treatment. The presence of fur allergies was considered an exclusion criterion only if the participant had contact with the respective animal. Only men were recruited for the present study, to homogenize the sample and because of known sex differences in allergic disorders (42, 43), conditioning responses (44, 45), and sleep (46, 47). The participants were synchronized by daily activities and nocturnal rest. All participants spent one adaptation night in the sleep laboratory before the experiment proper to habituate to sleeping with electrodes attached and to exclude any sleep problems. The study was approved by the Ethics Committee of the University of Tübingen, and all participants provided written informed consent in accordance with the Declaration of Helsinki.

Seventy-two volunteers were assessed for eligibility for the main experiment. Forty-two volunteers were excluded either because they did not meet the inclusion criteria (29 with a previously unknown pronounced sensitization to house dust mites as assessed by skin prick test, 9 with a too-weak response to the nasal provocation test [Lebel score ≤3], 1 with other medical issues, and 1 who was a regular smoker) or because they declined to participate ($n = 2$). Thirty volunteers were enrolled in the main study. Three participants were excluded as they reported having a cold at the second session; this session could not be postponed as it had to occur 1 wk after the first session. One participant was excluded because he had unusually high tryptase levels (>20 μg/L) already at baseline (i.e., during the Context phase of the Learning session) and another was excluded because of technical problems during allergen administration. The final sample size was $n = 25$ ($n = 13$ for the Sleep group and $n = 12$ for the Wake group). The tryptase values of two participants were missing due to technical problems, resulting in sample sizes of $n = 12$ for the Sleep group and $n = 11$ for the Wake group for this parameter.

Forty-nine volunteers were assessed for eligibility for the Context control experiment. Thirty-five of these volunteers were excluded because they did not meet the inclusion criteria (20 had a previously unknown pronounced sensitization to house dust mites, 6 had a too weak response to the nasal provocation test, 6 had other medical issues, 1 showed no response to birch or grass pollen in the skin prick test, 1 showed allergy symptoms already during the recruitment phase, and 1 had already participated in a study using the same odor). Fourteen volunteers were enrolled in this experiment. One participant was excluded during the Learning session because of allergy symptoms at baseline, and another participant declined to participate after the adaptation night for personal reasons. Two participants were excluded because they had unusually high tryptase levels (>20 μg/L) already at baseline. Thus, the final sample size was $n = 10$. The tryptase values of 1 participant were missing due to technical problems during the analysis, leading to a sample size of $n = 9$ for tryptase levels.

Measurement of Allergic Responses. Allergic responses were assessed using the following measures. Levels of tryptase, a physiological indicator of allergic rhinitis response (48), were measured in nasal mucus collected with a surgical cotton tamponade that was placed into one nostril for 5 min. The mucus was then centrifuged at 1,300 × g for 5 min at 4 °C and stored at −20 °C until the assay (ImmunoCAP; Thermo Fisher Scientific; sensitivity, 1 μg/L, intra-assay and interassay coefficients of variation <10%). Fifty-nine of 128 measurements were below the limit of detection; however, 47 of these were baseline/preodor measurements, in which such low tryptase levels are expected. For statistical analyses, values below the limit of detection were set to 0.5 μg/L (i.e., one-half the value of the assay sensitivity). Because of the small volume of the nasal secretion samples, we concentrated on the measurement of one physiological parameter and selected tryptase because it is a most valid biomarker of the early allergic response and is more sensitive and closely related to mast cell degranulation than other markers, such as histamine (48–50).

The Lebel score, which represents a clinical assessment of allergic rhinitis symptoms (51), comprises the ratings of several allergy symptoms to which points are allocated depending on the severity and/or presence of the respective symptom: zero to two sneezes, 0 points; three to four sneezes, 1 point; five or more sneezes, 3 points; anterior rhinorrhea, 1 point; posterior rhinorrhea, 1 point; strong anterior and posterior rhinorrhea, 3 points; difficult nasal breathing, 1 point; one nostril blocked, 2 points; two nostrils blocked, 3 points; pruritus of the nose, 1 point; pruritus of palate or ear, 1 point; conjunctivitis, 1 point. The total score ranged from 0 to 12 points. Symptoms were scored by a trained experimenter and were assessed at 5 min after delivery of the nasal spray. All sneezes occurring during the entire 5-min period were counted.

Sleep Recordings. To objectively measure sleep, standard polysomnographic recordings were obtained including electroencephalography (EEG) recordings from electrodes attached at C3 and C4 (according to the International 10–20 System) as recommended (52). Eye movements were recorded electrooculographically with electrodes placed diagonally ∼1 cm above and below and slightly lateral to the outer canthus of each eye (combined measurement of vertical and horizontal eye movements). Electromyography recordings were obtained from electrodes attached to the chin. Signals were amplified (Brain Amp; Brain Products) and digitized, with the EEG sampled at a rate of 500 Hz and filtered between 0.16 and 30 Hz. Sleep stages were determined off-line for subsequent 30-s recording epochs following standard criteria (52).

Statistical Analysis. Statistical analysis was performed using mixed ANOVA followed by two-sided paired t tests if ANOVA revealed significant interaction effects. To determine the response to the UCS in the Learning session, differences between the measurements before (preodor values serving as baseline) and after (postodor values) UCS presentation were analyzed. The respective ANOVA included the repeated measures factor "Preodor/Postodor" (preodor vs. postodor values of the Learning session) and the group factor "Sleep/Wake" (Sleep group vs. Wake group).

To assess whether the experimental procedure induced conditioning of allergic responses to the experimental context (i.e., context conditioning), differences between the preodor values of the Learning session and those of the Test session (i.e., the values obtained during the Context phase of both sessions) were analyzed. The respective ANOVA included the repeated-measures factor "Session" (preodor values of the Learning session vs. Test session) and the group factor "Sleep/Wake."

To assess whether the experimental procedure induced conditioning of allergic responses to the odor cue (i.e., cue conditioning), differences between preodor and postodor values of the Test session were analyzed. The respective ANOVA included the repeated-measures factor "Preodor/Postodor" (preodor vs. postodor measures of the Test session) and the group factor "Sleep/Wake."

Differences in the context-conditioned increase in tryptase levels between the Sleep group, the Wake group and the Context control group were analyzed using planned contrasts. For the within-subject comparisons of the control groups, two-sided paired t tests were calculated. Effect sizes are indicated as Cohen's d ($d = 0.2$ for small, $d = 0.5$ for medium, and $d = 0.8$ for large effect sizes).

Data Availability. All data relevant to the conclusions of this paper are included in the text and *SI Appendix*. Any additional data are available on request.

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