A non-image-forming visual circuit mediates the innate fear of heights

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Abstract: The neural basis of fear of heights remains essentially unknown. We observed mice displaying stereotyped aversive behaviors when encountered height threats, resembling human height vertigo. Visual input plays a crucial role in mouse reactions to heights, which can also be influenced by vestibular input and prior height experiences. Unexpectedly, fear of heights in inexperienced mice does not rely on image-forming visual processing by the primary visual cortex. Instead, a subset of neurons in the ventral lateral geniculate nucleus (vLGN) connecting to the lateral/ventrolateral periaqueductal gray (l/vlPAG) drives the expression of height-related fear. Additionally, a subcortical visual pathway linking the superior colliculus to the lateral posterior thalamic nucleus inhibits the defensive response to height threats. These findings highlight an unconscious fear response to height threats through a rapid subcortical visual and defensive pathway from vLGN to l/vlPAG.

One-Sentence Summary: A non-image visual mechanism of fear of heights

Fear of heights is a common and instinctive reaction accompanied by anxiety, dizziness, muscle stiffness, and other vegetative symptoms (*1*). Physiological fear of heights, also known as height vertigo, is considered an innate behavior that helps prevent injuries from falling (*2*). However, an irrational fear of heights can impair posture balance and motor control and compromises the quality of life (*3*). Despite its documented presence in ancient literature dating back thousands of years, the fundamental neural mechanisms underlying fear of heights remain poorly understood. Current theories attribute the physiological fear of heights to posture instability at elevated locations (*4-6*). According to these theories, when the distance between an observer and visible stationary contrasts becomes critically large, visual input becomes less effective in detecting body sway. This leads to a mismatch between visual and vestibular-proprioceptive information, resulting in increased posture instability and generating fear of falling and anxiety (*4-8*). This hypothesis predicts that height vertigo is a type of "distance vertigo", as both are associated with increased posture instability. However, this hypothesis fails to explain the lack of fear and anxiety when looking at a distance. Therefore, systematic experimental investigations are necessary to determine whether fear of heights arises from direct neural responses to visual height stimuli or if it is a consequence of posture instability. Additionally, it is important to explore whether a specialized brain circuit exists specifically for processing height threats.

In this study, we took advantage of enriched methodologies for monitoring and manipulating behavior and brain circuit activities in free-behaving mice to uncover the neural mechanisms underlying the physiological response to height threat.

Mice display stereotyped aversive reactions to height threat

Adult naive mice placed on an open high platform (OHP, 20 cm x 20 cm, 30 cm off the ground) exhibit a characteristic 3-phase behavioral pattern during exploration (Fig. 1A, B, movie S1). Initially, they spend approximately 0-9 seconds exploring the center of the OHP (phase 1). Subsequently, mice cautiously approach the platform's edges. They extend their heads and forepaws towards the edge while keeping their hind paws in the central region of the platform, resulting in lowering and elongation of the body. Some mice manage to poke their heads out of the platform before withdrawal (out and half-out), while others extend their noses out or simply reach the edge without poking their heads out (nose in). After a brief edge exploration, animals avoid crossing the edge but either crawl back or turn away from the edge to initiate another round of edge exploration-retraction/turning cycle (phase 2, Fig. 1A, B). After several rounds of edge exploration lasting approximately 0.5-70 seconds, the mice enter a motionless period lasting around 1-17 seconds, during which they exhibit visible body trembling (movie S2) and occasional grooming following retraction from the edge (Fig. 1A, B). Once the trembling subsides, they resume the cycle of exploration, retraction/turning, trembling/grooming (phase 3), with variations in the duration of each behavioral condition (Fig. 1A, B). Particularly noteworthy is the lack of vertical exploration (rearing up behaviors) of mice throughout their exploration of the OHP (Fig. 1E).

The stereotyped exploration-retraction-trembling cycle (phase 3) was absent when mice explored same-sized platforms enclosed by either transparent (TWP) or non-transparent (gray, GWP) walls (Fig. 1C, D; movie S3, S4). This suggests that the detection of height threat depends on the head poking over the edge of the high platform. Compared to mice on the OHP, mice on both enclosed platforms exhibited significantly longer locomotion, more frequent self-grooming and vertical exploration, and no visible trembling (Fig. 1F-H). The lowering of the body and the lack of vertical exploration of mice on the OHP align with the human tendency to reduce anxiety by crouching at heights (*1*). In rodents, the level of self-grooming is inversely correlated to the aversiveness of tasks at a medium to high anxiety levels (*9*). Hence, less self-grooming on the OHP than on enclosed platforms also indicates

higher anxiety levels on the OHP. Additionally, male and female mice displayed similar anxiety level on the OHP (fig. S1).

Experience-dependent facilitation of fear of heights

When naïve mice were exposed to two trials of 30-minute height exposure with a 2-day interval, they exhibited more severe aversive reactions at the second trial. This was evidenced by reduced locomotion, decreased peripheral exploration, less exploration over the platform edge (nose outside), shorter latency for initiating trembling, and longer duration of trembling in the second trial compared to the first trial (Fig. 1I, J; fig. S2). These enhancements of defensive responses were observed regardless of whether the second trial utilized the same OHP in the same environment (Fig. 1I, fig. S2) or a different-colored OHP in a different environment (Fig. 1J), indicating a generalized cross-session facilitation of fear of heights. Interestingly, fear facilitation in the second trial persisted even when the interval between the two trials was as long as 30 days (fig. S2), indicating the presence of a long-lasting memory triggered by a single exposure to height threat. To minimize potential interference of prior height exposure on mouse behavior, all subsequent experiments were conducted using naïve mice.

Visual input is essential for fear of heights

By comparing the locomotion, the distance explored the peripheral zone, the distance explored over the edge (nose outside), and the trembling duration, we observed a gradual increase in overall fear level with height. The fear level reached saturation when the height exceeded 20 cm (Fig. 2A). The threshold for inducing noticeable body trembling was approximately 10 cm. When the height of the OHP was below 5 cm, most mice jumped or climbed off the OHP within 30 minutes. However, very few mice were able to descend from the OHP when the height exceeded 10 cm. When the animals explored the OHP in the dark, behaviors such as locomotion, peripheral exploration, exploration over the edge, and trembling did not increase with height (Fig. 2B), suggesting that fear of heights is visually dependent. In the dark, no mice descended from the OHP, even when it was 5 cm tall (Fig. 2B).

To further investigate the role of visual cues in fear of heights, we compared mouse behavior on the OHP of equal height (30 cm) with or without light during the first two minutes (Fig. 2C-I, movie S5). Mice in the dark explored the edge more often than mice in the light (Fig. 2C, D). Based on how far the animal poked its head over the edge, we divided the edge exploration events into 4 different categories including head-out, half-out, nose-out, and nose-in explorations (Fig. 1B, 2E). Mice in the dark had a significantly higher frequency of “head-out” and “nose-out” explorations (Fig. 2C, E, H), but a significantly lower frequency of immediate retraction following head protrusion out (Fig 2F, H). Moreover, the body trembling following a bout of edge exploration, either immediately following the edge exploration or after the animal has turned back to the platform center, was less frequent in the dark and shorter in total duration than that in the light within the initial 2 minutes on the OHP (Fig. 2C, G, H).

During exploration, an animal's body lowering with simultaneous elongation indicates cautiousness and anxiety. We noticed that mice in the dark had more frequent body elongation in the platform center and less frequent elongation in the peripheral zone when compared to mice in the light (Fig. 2I). Overall, comparisons of animal behaviors in light and dark supported a vision-dependent fear of heights that is absent in the dark. Furthermore, mice in the dark displayed increased anxiety at the OHP edge, as indicated by elongated bodies in the center zone (Fig. 2I) and a lack of jumping or climbing off from low platforms (Fig. 2B). However, the anxiety level in the dark did not increase with height, suggesting that

it is more likely a fear of uncertainty or posture instability associated with the edge rather than a fear of height.

We also found that mice on an OHP with a transparent base had significantly longer duration of trembling than mice on an OHP with a non-transparent base (Fig. 2J). This finding further supports the positive correlation between the visual height stimulus from below and the fear level at heights. Additionally, monocular vision alone was sufficient to trigger fear of heights (Fig. 2K).

Vestibular input is dispensable to fear of heights

Fear of heights was considered a motion sickness caused by the conflict between visual and vestibular inputs (*5, 6*). To investigate the involvement of vestibular input in fear of heights, we first conducted chemical labyrinthectomy by a 2-week treatment with the ototoxic aminoglycoside gentamicin (200 mg kg^{-1}, twice a day) via intraperitoneal injection (*10*). Gentamicin-treated mice exhibited deficits in the righting reflex (fig. S3A), impaired posture balance during swimming (fig. S3B, movie S6), and a significant reduction in the frequency of moderate and heavy climbing of the pool wall (fig. S3C, movie S7), indicating impaired vestibular function. However, the fear of heights of gentamicin-treated mice was not affected in the OHP test (Fig. 2L). Gentamicin treatment had little effect on the central zone exploration in the open field test (OFT) (fig. S3D) or the exploration of the open arm in the elevated O-maze test (EOM) (fig. S3E), only reduced mouse locomotion in the open field arena (fig. S3D). We further examined the effect of more specific unilateral and bilateral deprivation of peripheral vestibular input on the fear of heights by intratympanic application of sodium arsanilate (*11*) (100 mg ml^{-1}) and validated the resulting vestibular deficits using the righting reflex test (fig. S3F) and the swimming test. We found that both unilateral and bilateral vestibular deprivation significantly increased the animal locomotion in the OFT without affecting their center exploration (fig. S3G) or their open arm exploration in the EOM (fig. S3H). In the OHP test, neither unilateral nor bilateral intratympanic treatment with sodium arsanilate significantly affected locomotion or edge exploration of the animal; however, bilateral intratympanic treatment significantly increased the animal trembling on the OHP (Fig. 2M). Moreover, treatment of mice with the vestibular depressant and anti-vertigo agent difenidol (11 mg kg^{-1}) (*12*) failed to alter the fear of heights in the OHP test (fig. S3I). These results do not support an essential role of vestibular input in triggering fear of heights. Finally, shaving whiskers did not have a significant impact on mouse behaviors on the OHP (Fig. 2N), indicating that somatosensory input from whiskers is dispensable for the sensation of height threat in mice.

The superior colliculus suppresses the fear of heights

To investigate how visual input drives the fear of heights in naïve mice, we conducted a c-Fos-based mapping of neuronal activation in response to height exposure. We focused on the visual pathway and several brain regions known to be involved in innate and conditioned fear responses. Following the mapping of neuronal activation, we manipulated the neuronal activities in these brain regions to examine their effects on animal behavior on the OHP.

The superior colliculus (SC) is a subcortical center for ocular-motor reflex and innate defensive response to visual threats (*13-15*). Glutamatergic projection from the superficial layers of the medial region of SC to the lateral posterior thalamus nucleus (LPTN) has been implicated in freezing response to overhead looming stimuli (*14, 16, 17*). We found that height exposure led to a significant increase in Fos-positive cells in the superficial layers of the medial region of the SC (Fig. 3A, C) and the rostral medial area of the LPTN (LPMR, Fig. 3B, D).

Next, we chemogenetically inhibited neuronal activity in specific brain regions of interest in mice using Clozapine-N-oxide (CNO) treatment (3.5 mg kg^{-1}, intraperitoneal

injection). The mice were previously injected with AAV virus encoding the inhibitory construct hM4Di in the targeted brain regions. The effectiveness of neuronal activity inhibition was confirmed using whole-cell patch-clamp recordings in brain slices (fig. S4A, B) and Fos-staining on virus-expressing brain sections (fig. S4C, D). Surprisingly, chemogenetic inhibition of pan-neuronal activities (AAV-*hSyn*-hM4Di) in either the SC (Fig. 3E, F) or the LPMR (Fig. 3I, J) significantly enhanced fear of heights in the OHP test, as indicated by reduced locomotion and edge exploration and increased trembling duration. Chemogenetic inhibition of glutamatergic neurons (AAV-Vglut2-hM4Di) in SC produced a similar effect (Fig. 3G, H). Moreover, specific inhibition of the SC-LPMR projecting neurons by CNO treatment of mice injected with AAV-retro-*hSyn*-Cre-EGFP in the LPMR and AAV-*hSyn*-DIO-hM4Di-mCherry in SC also significantly increased the fear of heights (Fig. 3K, L). Chemogenetic inhibition of neurons of SC also significantly reduced the open arm exploration of mice in the EOM without affecting the locomotion and central zone exploration of the same group of animals in the OFT (fig. S5 A, B). This suggests that activation of the SC-LPMR circuit by height exposure specifically suppresses fear of heights without affecting the general anxiety level.

The primary visual cortex is dispensable for fear of heights

For conscious perception of visual input, the retinal signals are transmitted to the primary visual cortex (V1) via the dorsal LGN (dLGN). We observed an increase in the number of Fos-positive cells in V1 in response to a 10-min height exposure (Fig. 4A-C). On the other hand, the dorsal lateral geniculate nucleus (dLGN), which is responsible for relaying image-forming visual information from the retina to V1, showed only a few Fos-positive cells in response to height exposure (Fig. 4D-F). Interestingly, we found height exposure significantly increased the number of Fos-positive cells in the ventral lateral geniculate nucleus (vLGN), an important nucleus that receives direct input from intrinsically photosensitive retinal ganglion cells (ipRGCs) and relays non-image-forming visual information (*18-20*).

After surgical ablation of V1 (Fig. 4G), mice exhibited impaired context discrimination in the standard fear conditioning test (Fig. 4H), indicating deficits in visual perception of the environment. However, the fear of heights of V1-ablated mice remained unaffected, as shown by a normal level of edge exploration and trembling on the OHP (Fig. 4I). Their anxiety levels in the OFT and EOM were also largely unaffected, with only a slight reduction in locomotion in the OFT (fig. S6A, B). In another experiment, mice were injected with AAV virus carrying the inhibitory construct hM4Di (AAV-*hSyn*-hM4Di) specifically in V1. Chemogenetic inhibition of neuronal activities in V1 through CNO treatment had no effect on fear of heights in the OHP test (Fig. 4K, L). In contrast, when the pan-neuronal activity in the vLGN was chemogenetically inhibited (Fig. 4M), there was a significant reduction in fear of heights, as indicated by increased edge exploration and reduced trembling on the OHP (Fig. 4N). Except for a slight decrease in locomotion, animal behaviors in the OFT were little affected by chemogenetic inhibition of the vLGN (fig. S6C). These results suggest that the physiological fear of heights in naïve mice is likely to be a non-image-forming process mediated by the vLGN, whereas the image-forming visual processing by V1 is non-essential for triggering fear of heights in naïve mice.

Glutamatergic neurons in l/vlPAG play essential roles in fear of heights

The vLGN innervates several brain regions associated with the expression of fear and anxiety, including the periaqueductal gray (PAG) (*21*). After height exposure, there was a significant increase in the number of Fos-positive cells in the lateral and ventrolateral PAG (lPAG, vlPAG) (Fig. 5A-C). However, in the lateral amygdala (LA) and the basal amygdala

(BA), another brain center for the expression of fear and anxiety in response to visual threat, there was no significant increase in Fos signal following height exposure (fig. S7A-C).

Chemogenetic inhibition of pan-neuronal activities in the l/vlPAG by treating mice with CNO after injecting AAV-*hSyn*-hM4Di in this brain region resulted in a significant disruption of the typical fear responses exhibited by mice on the OHP (Fig. 5D-H). Most CNO-treated mice did not retract at the edge of the OHP but instead continued to advance and explore underneath the platform until they either fell off the platform or hung on to the edge (Fig. 5D, E, movie S8). Some mice even jumped directly off the OHP after edge exploration. Additionally, the CNO-treated mice that remained on the OHP had a significantly reduced duration of trembling compared to the saline-treated mice (Fig. 5F). This treatment did not alter animal behavior in the OFT and EOM (fig. S8A, B). Furthermore, chemogenetic inhibition of glutamatergic activities using AAV-Vglut2- hM4Di in the l/vlPAG also caused most mice to get off the OHP (Fig. 5G, H). These findings indicate the crucial role of the l/vlPAG and its glutamatergic activity in the fear expression associated with heights.

While it has been observed that a small population of neurons in the BLA responds to height exposure (*22*), chemogenetic inhibition of BLA activities, however, slightly increased the fear of heights, as indicated by reduced exploration of the peripheral zone and outside the OHP and increased duration of trembling (fig. S7D). On the other hand, chemogenetic inhibition of the CeA had no impact on fear of heights (fig. S7E). Thus, the l/vlPAG, instead of the BLA, plays a significant role in the expression of fear of heights.

To clarify whether visual height stimulus activates the l/vlPAG through the vLGN to induce fear of heights, we conducted chemogenetic inhibition of vLGN neurons that project to the l/vlPAG. This was achieved by treating mice with CNO after injecting them with AAV-*hSyn*-DIO-hM4Di-mCherry in the vLGN and AAV-retro-*hSyn*-Cre-EGFP in the l/vlPAG (Fig. 5I). This treatment significantly reduced fear of heights, as evidenced by a significant increase in edge exploration and a decrease in trembling duration compared to mice treated with saline (Fig. 5J, K). This treatment did not affect animal behavior in the OFT and EOM (fig. S8C, D), indicating specificity of the effects to fear of heights. Immunofluorescent staining revealed that the majority of neurons projecting from the vLGN to the l/vlPAG are GABAergic (Fig. 5L), which is consistent with a previous study showing that GABAergic projecting neurons from the vLGN innervate GABAergic interneurons in the l/vlPAG (*21*).

Discussion

The aversive responses of naïve mice to height threat, such as withdrawal, trembling, low body position, absence of vertical exploration, reduced locomotion, and regulation by experience, are highly comparable to human responses to height threat. These behavioral similarities across species suggest that the fundamental mechanisms of fear of heights are phylogenetically conserved. Mice could be an ideal animal model for dissecting neural mechanisms of height vertigo and for translational medicine targeting acrophobia. Laboratory mice have no prior experience of falling trauma or scary memories associated with heights. Their fear of heights could be an innate response to height stimulus, which may utilize a hardwired brain circuit to promote adaptation and survival. Consistent with this notion, retrospective self-reports and longitudinal studies in humans have showed that the acquisition of height fear is a non-associative processes (*23*). Moreover, the cross-session facilitation of fear of heights in mice supports an experience-dependent modulation of fear of heights, which might involve plastic changes of the relevant brain circuit and may depend on the cognitive perception of the height threat upon repeated exposure. Therefore, fear of heights in a natural environment may include both an innate response and an experience-dependent component. The cross-session facilitation, instead of habituation, of fear of heights suggests

the need for a careful design of the exposure protocol in the "exposure therapy" for acrophobia to avoid reinforcing the fear by repetitive height exposure.

Fear of heights was considered a type of motion sickness. It was attributed to posture instability caused by a lack of visual parallax cues and the resulting mismatch between visual information and vestibular and proprioceptive inputs (*3-6*). Consistent with this notion, vestibular input influences the distance judgment at height (*24, 25*). Vestibular exercise has been shown to improve dizziness and body sway in a group of acrophobic patients (*26*). Individuals with a complete loss of labyrinthine function seem immune to visually-induced motion sickness (*27*). Contrary to this notion, however, recent epidemiological investigations revealed that individuals with bilateral vestibulopathy did not show higher susceptibility to acrophobia than the general population (*28*), nor did they have increased anxiety levels, although they experienced more frequent falling due to impaired balance control (*29*). These findings indicate that peripheral vestibular input appears to be dispensable to fear of heights, although intact vestibular function may modulate the emotional response to height stimulus via complicated yet poorly understood mechanisms. We found that bilateral but not unilateral vestibular deprivation slightly but significantly enhanced the fear of heights instead of mitigating it in mice. In general, vestibular input does not seem to account for the fear of heights. Our findings support a direct visual mechanism for the fear of heights instead of the consequence of body imbalance at heights. Brain mechanisms of height vertigo seem to be primarily different from motion sickness.

Mice displayed increased anxiety when they explored the edge of the OHP even in the dark. However, unlike the situation in the light environment, anxiety levels in the dark did not increase with height. Therefore, edge-associated anxiety includes both a fear of heights which depends on visual input and a vision-independent component which is presumably caused by posture instability at the edge, potentially involving vestibular and proprioceptive inputs. Such vision-independent anxiety should not be considered as fear of heights but may intensify the fear and anxiety at heights.

A subcortical pathway through the SC and the LPTN (pulvinar) to the amygdala is considered to mediate the non-conscious processing of emotional visual stimuli in primates (*30, 31*). In rodents, the SC plays a central role in the defensive response to overhead visual threats including the looming stimuli (*13, 14, 16*). We found Fos activation in a subgroup of neurons in both SC and LPMR upon height exposure. To our surprise, chemogenetic inhibition of the SC-LPMR circuit enhanced, rather than blocked, the fear of heights. We note that the looming stimulus is a visual threat from above, whereas the height stimulus is a visual threat from below. It is reasonable that these visual threats from different visual fields utilize different circuits to drive protective responses. In response to height stimuli, SC neurons may activate a distinct pattern of activity, which may prevent the animal from over-reacting to height threats to benefit survival and adaptation. The difference between the height-responsive neuronal ensemble and the looming-responsive neuronal ensemble in SC remains to be clarified.

We found that neither V1 nor SC is essential to the physiological fear of heights in naïve mice, suggesting the existence of a subconscious and non-image-forming visual pathway for the onset of fear of heights. This implies that physiological fear of heights does not have to be a "fear of falling", which involves the conscious perception of the falling risk at heights. In contrast, height exposure is an imminent threat that initiates an immediate defensive response without visual awareness. However, our findings do not exclude the contribution of a conscious perception of height threat to experience-dependent fear of heights. The brain mechanisms underlying the experience-dependent regulation of fear of heights remain to be investigated.

It is generally believed that distal threats elicit activity in the prefrontal cortices, which possibly reflects the cognitive planning of avoidance strategies. As the threat becomes

proximal, midbrain structures such as the PAG dominate the defensive responses (*32*). Consistent to this notion, we discovered a circuit from vLGN to l/vlPAG that mediates the fear of heights. We found that most vLGN neurons that project to l/vlPAG are GABAergic neurons. A previous study has shown that the vLGN GABAergic neurons project to GABAergic neurons in l/vlPAG, resulting in activation of PAG glutamatergic neurons in the pain regulation by light stimulation (*21*). It is likely that vLGN activates l/vlPAG via a similar dis-inhibition mechanism upon height exposure. Among the several treatments that effectively mitigated the animal fear on the OHP, chemogenetic inhibition of l/vlPAG displayed the most striking effect. It is likely that in addition to the vLGN-l/vlPAG pathway, there may be other unknown brain circuits that converge on l/vlPAG to generate the fear of heights.

Neurons at vLGN have a large receptive field and prefer bright versus dark stimuli (*18, 19*), unlike SC neurons, which are activated by shadows (*19*). Dendritic arbors of vLGN neurons were often asymmetric, positioned to collect input from large but discrete territories (*19*). We note that height exposure generates a broad bright stimulus in the lower visual field, corresponding to the upper retina. Interestingly, a group of GABAergic ipRGCs are enriched in the dorsal temporal quadrant of the retina and their axons project to the vLGN (*20*). Whether this group of GABAergic ipRGCs contribute to the coding of visual height stimuli remains to be investigated.

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Authors declare no competing interests.

Data and materials availability:

All data used in the analysis are available to any researcher upon request to the corresponding author for reproducing or extending the analysis.

Supplementary Materials

Materials and Methods

Supplementary Text

Figs. S1 to S8

Tables S1 to S2

References (*33-34*)

Movies S1 to S8

Data S1 to S2

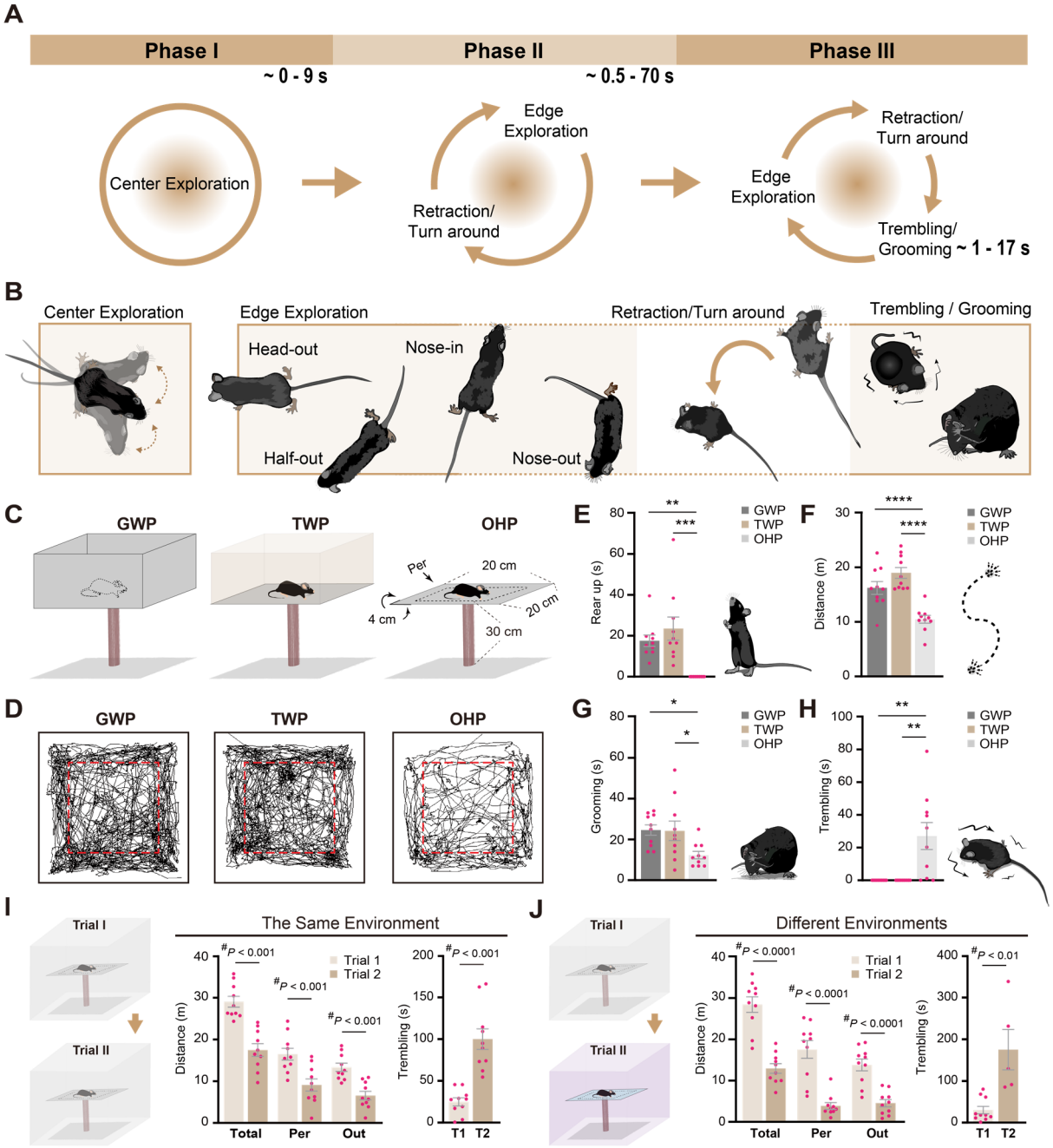

Fig. 1. Mice display stereotyped aversive responses to height exposure. (**A-B**) Different phases and typical postures of stereotyped behaviors of mouse on an open high platform. (**C**) Schematic diagrams of enclosed and open high platforms used in the behavioral tests. The two enclosed platforms had equal size (20 cm x 20 cm) and height (30 cm) with the open high platform (OHP) but were enclosed with either non-transparent (gray) walls (GWP) or transparent walls (TWP). The central 16 cm x 16 cm area of the platform was defined as the central zone, and the rest area was defined as the peripheral zone (Per). (**D**) Representative locomotion traces of mice during a 30-min session on an enclosed (GWP, TWP) or open (OHP) high platform. (**E-H**) Quantitative analysis of mice's rearing up (E), locomotion (F), grooming (G), and trembling (H) behaviors on three different types of elevated platforms. All four parameters show higher fear and anxiety level on the OHP compared to GWP and TWP. (**I, J**) Cross-session facilitation of fear of heights. Mice were tested by two trials of 30-min exposure to the OHP. The color of the OHP and the surrounding environment during the second trial were either identical (I) or different (J) from that of the first trial. Total locomotion distance, locomotion distance at the peripheral zone, the locomotion distance of

the nose tip outside the platform (Out), and the total trembling duration of animals in the two trials of OHP test are shown in the histograms. Data in E-J are mean ± S.E.M. Data points represent results from individual animals. Statistical analyses are One-Way ANOVA with post-hoc *t*-tests in E-H (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$) and a two-tailed Student's *t*-test ($^{\#}$) in I and J.

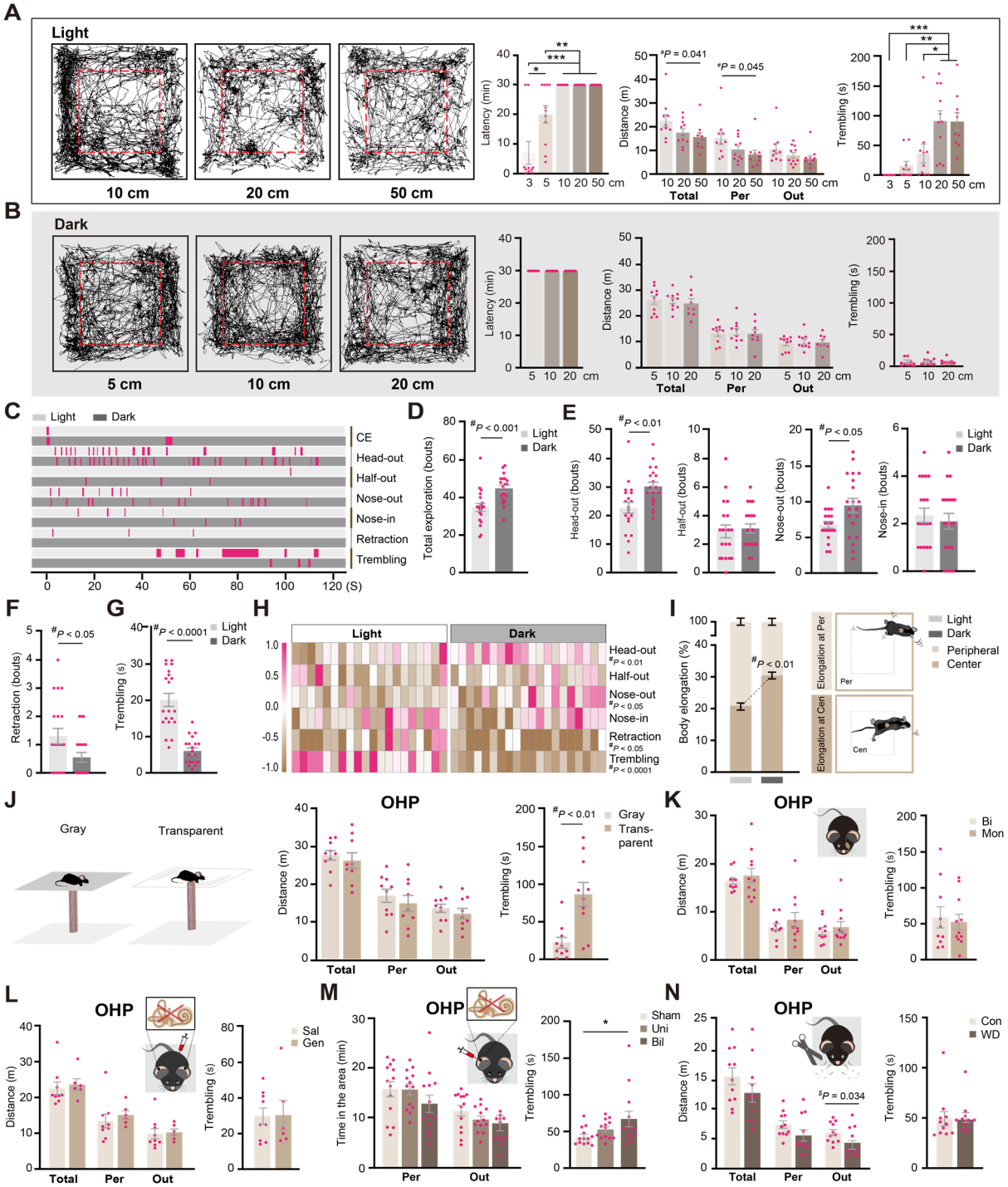

Fig. 2. Visual input plays a primary role in the fear of heights. (**A-B**) Locomotion traces (left) and quantitative analyses (right) of mouse behaviors on OHPs of different heights (3-50 cm for the light and 5-20 cm for the dark) in the light (A) or in the dark (B). Latency indicates the latency for the animal jumping or climbing off the OHP. If the animal did not get off the platform at the end of the 30-min session, the latency value was counted as 30 min. (**C**) Plots of different behavioral events (head-out, half-out, nose-out, nose-in, immediate retraction, and trembling) over time for a representative mouse in the light and a representative mouse in the dark within the initial 2 min on the OHP. Each vertical bar represents the period of an event. (**D**) Bouts of peripheral exploration of mice within the initial 2 min on the OHP in the light or dark environments. (**E-G**) Bouts or duration (in seconds) of behavioral events for mice under both light conditions (n=10) and dark

conditions (n=10) within the initial 2 minutes on the OHP. (**H**) Summary of different behavioral events for each mouse within the initial 2 minutes on the OHP under light and dark conditions. The relative level (-1.0 to 1.0, normalized across all animals) of each type of behavioral event of mice was coded by pseudo colors. Each square represents the data from one animal. (**I**) Comparing the percentage of "crawling with body elongation" events in mice at the platform center and peripheral zone in light or dark environments. (**J**) Comparison of mouse behavior on an OHP with a non-transparent (gray) and a transparent base. (**K-N**) Influence of different treatments on the fear of heights. Different treatments include monocular visual deprivation (K), systemic chemical labyrinthectomy (L), unilateral and bilateral labyrinthectomy (M), and whisker deprivation (N). Data are presented as the mean ± S.E.M. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, One-Way ANOVA with post-hoc *t*-tests. A two-tailed Student's *t*-test ($^{\#}$).

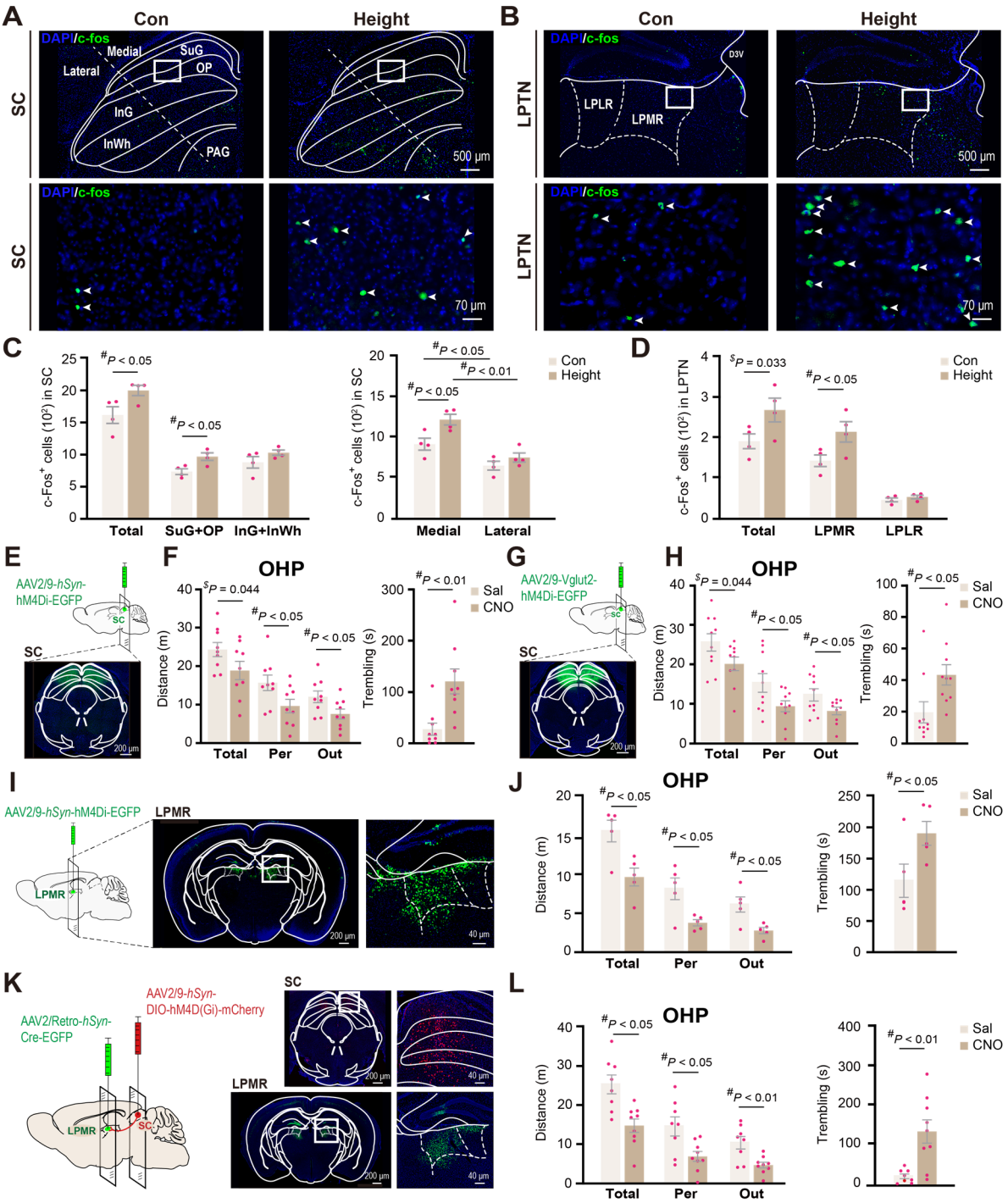

Fig. 3. The SC-LPMR circuit suppresses the fear of heights. (**A-D**) Representative images (A, B) and quantitative analysis (C, D) of c-Fos signal in response to 10-min height exposure in the superior colliculus (SC; A, C) and the lateral posterior thalamic nucleus (LPTN; B, D). LPLR and LPMR are the lateral rostral and medial rostral subregions of LPTN, respectively. (**E, F**) Effect of chemogenetic inhibition of pan-neuronal activities at the SC on fear of heights. Note the elevation of fear level of mice on the OHP after CNO treatment, as indicated by reduced peripheral exploration and increased trembling duration (F). (**G, H**) Effects of chemogenetic inhibition of glutamatergic neurons (AAV-Vglut2-hM4Di) in the SC on fear of heights. **(I, J)** Effect of chemogenetic inhibition of pan-neuronal activities at LPMR on the fear of heights. (**K, L**) Effect of chemogenetic inhibition of SC neurons that project to LPMR on the fear of heights. Data were analyzed using a two-tailed ($^{\#}$) or one-tailed ($^{\$}$) Student's *t*-test and are presented as the mean ± S.E.M.

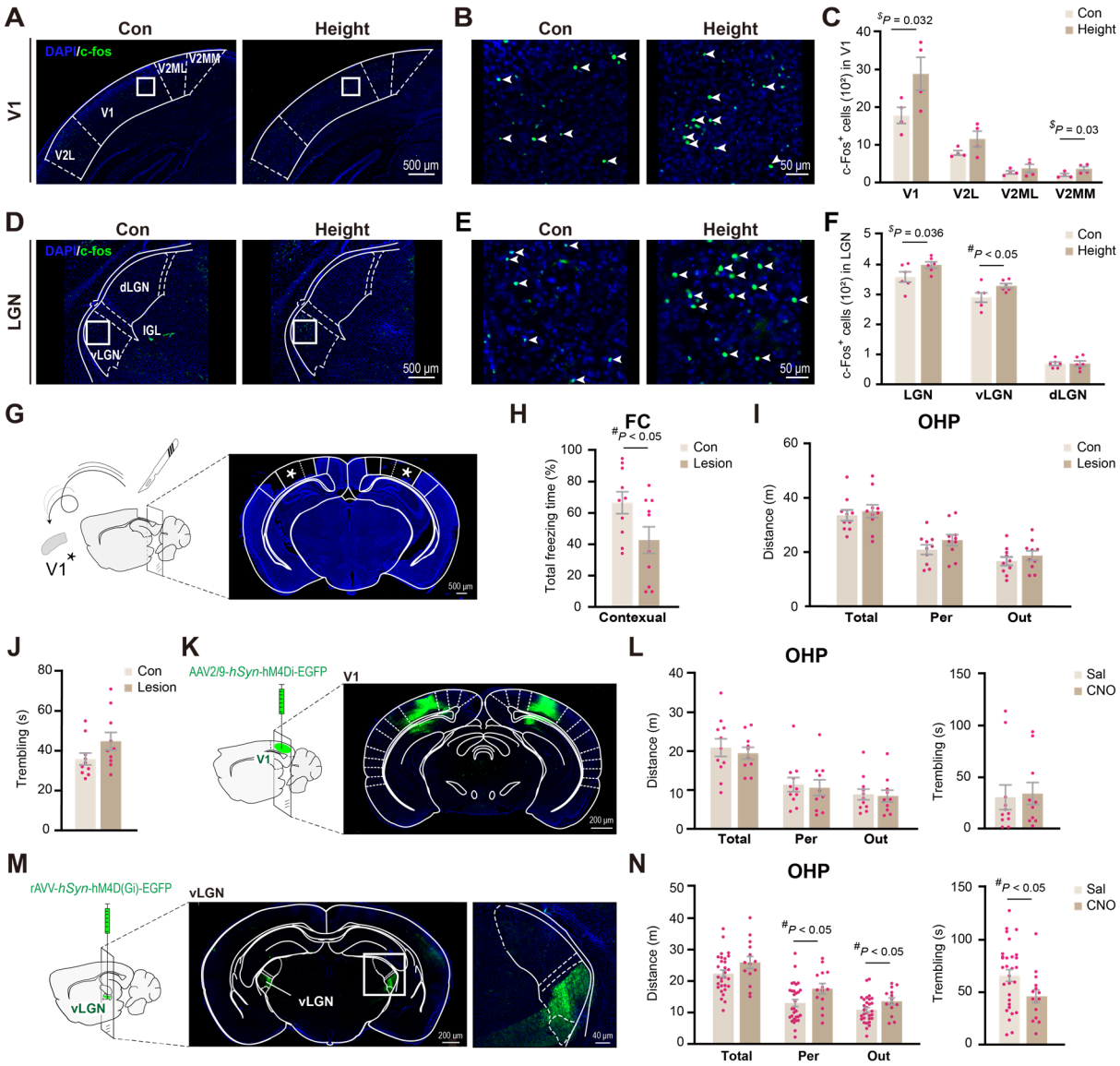

Fig. 4. The primary visual cortex is dispensable for fear of heights. (**A, B**) Representative visual cortex section (A) and high magnification images of the selected regions (squares) of the cortex (B). (**C**) The quantitative analysis of c-Fos-positive cells in response to 10-min height exposure in different subregions of the visual cortex (V1, V2L, V2ML, V2MM). (**D, E**) Representative LGN section (E) and high magnification images of the selected regions (squares) of the LGN. (**F**) The quantitative analysis of c-Fos signal in response to 10-min height exposure in different regions of LGN (total LGN, vLGN, and dLGN). (**G-J**) Effect of surgical ablation of V1 on fear of heights. Results of fear conditioning (FC, H), OHP (I, J) tests are shown. (**K-N**) Effects of chemogenetic inhibition of pan-neuronal activities of V1 (K, L) and vLGN (M, N) on the fear of heights. Data are presented as the mean ± S.E.M. with a two-tailed ($^{\#}$) or one-tailed ($^{\$}$) Student's *t*-test.

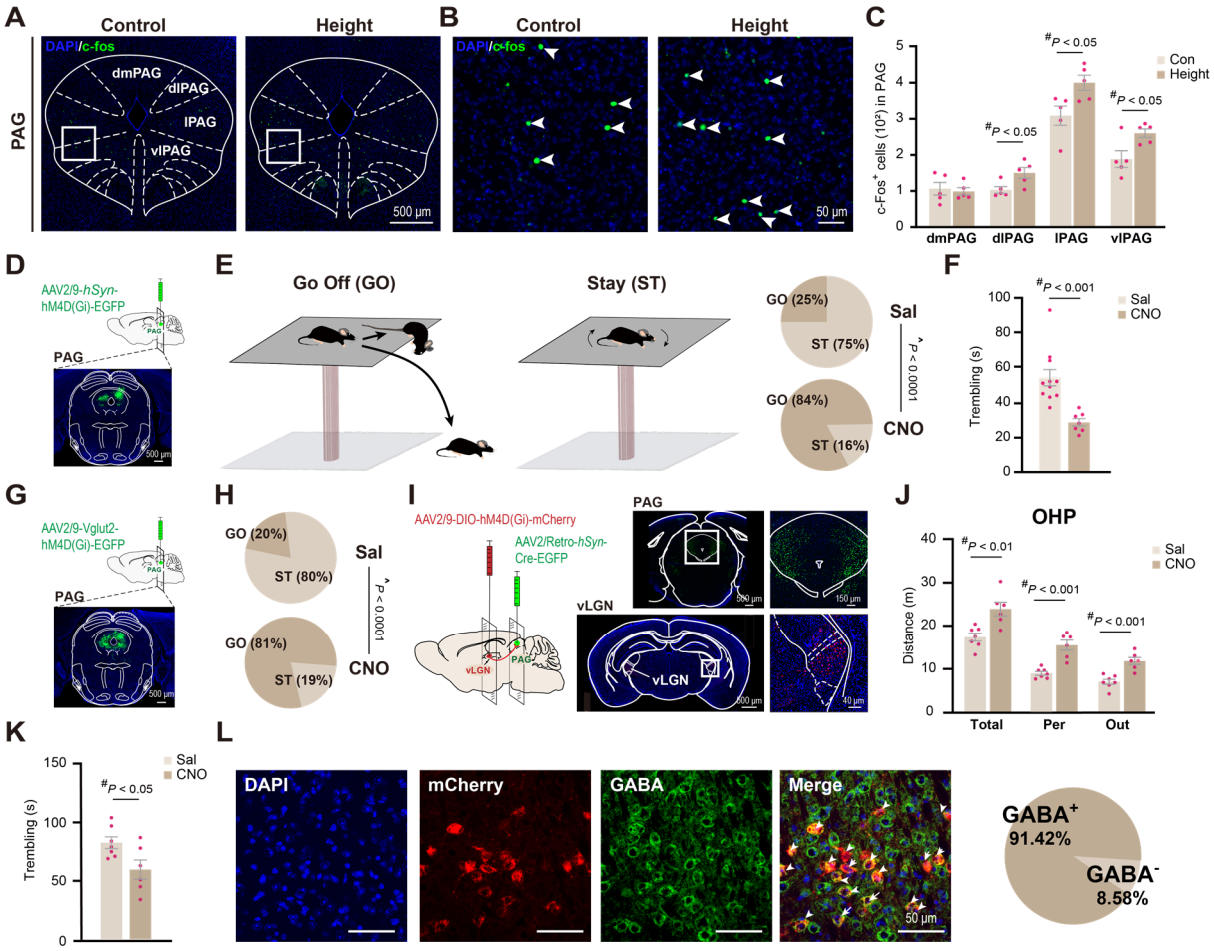

Fig. 5. The vLGN-PAG circuit mediates the fear of heights. (**A, B**) Representative images (A) and quantitative analysis (B) of c-Fos signal in response to height exposure in different subregions of the PAG (dmPAG, dlPAG, lPAG, vlPAG). (**D-H**) Effects of chemogenetic inhibition of pan-neuronal activities (D-F) or glutamatergic neuronal activities (G, H) of the l/vlPAG on fear of heights. "Go" and "ST" in (E) and (H) indicate mice that had descended or stayed on the OHP, respectively, at the end of the 30-minute test. (**I-K**) Effects of chemogenetic inhibition of neurons that project from the vLGN to the l/vPAG on the fear of heights. (**L**) Immunofluorescence fate-mapping of neurons that project from the vLGN to the l/vlPAG. The vLGN-l/vlPAG projecting neurons were labeled by retrograde tracing by injecting AAV-retro-*hSyn*-mCherry (in red) into the l/vlPAG. The vLGN brain slices were stained with the antibody against GABA (in green). The pie chart shows the percentage of $GABA^+$/$mCherry^+$ cells in all $mCherry^+$ cells. Data in histograms are presented as the mean ± S.E.M. with a two-tailed ($^{\#}$) or one-tailed ($^{\$}$) Student's *t*-test. The chi-square test (^) is used in (E) and (H).