Supporting Information

Methods

Assessing the neuroanatomical specificity of the lesions

To further investigate the effects of damage to surrounding thalamic nuclei and neighboring brain structures, we analyzed data from GTs that were excluded from Experiment 2. GTs with missed lesions were classified into three groups: lesions that resulted in no PVT damage (PVT Miss; n=3), incomplete PVT lesions (PVT -, n=4), and lesions that encompassed the entire PVT, but also extensively damaged surrounding thalamic nuclei (PVT +, n=4).

Locomotor Response to Novelty

Following the completion of Pavlovian conditioned approach training in Experiment 2, a subset of outbred rats (GT Lesion, n=4; GT Sham, n=8; ST Lesion, n=5; ST Sham, n=8) were tested for locomotor response to a novel environment, as described previously (Stead *et al.*, 2006). Briefly, rats were taken into a novel room and placed individually into a standard acrylic cage (43 x 21.5 x 24.5 cm) with a novel floor. Locomotor activity was recorded by two rows of photocells to record both horizontal movement and rearing behavior. Photocell beam breaks resulted in activity counts that were recorded in 5 minute bins for 1 hour. Total locomotion scores were created by summing horizontal and rearing activity.

Results

Assessing the effects of PVT lesion on both sign- and goal-tracking behaviors in bLR and bHR animals

Prior to separating the bLR and bHR animals by Phenotype for analysis, linear mixed model analysis was performed for each sign- and goal-tracking measure (number of contacts, probability of contact, and latency to contact) with both Phenotype (bHR vs. bLR) and Treatment (lesion vs. sham) included as the between-subject factors, and Session as the repeated variable.

The results from these analyses are summarized in Supporting Table 1. Importantly, for all sign- and goal-tracking measures a significant Phenotype x Session and/or Phenotype x Session x Treatment interaction was found, justifying the separation of the bLR and bHR animals for additional analyses.

Assessing the effects of PVT lesions on "off target" behavior in bHR and bLR animals

Linear mixed model analyses were also performed to ensure there were no changes in "off-target" behavior, i.e. sign-tracking behavior for bLRs and goal-tracking behavior for bHRs. As shown in Supporting Figure 1A-C, there was no effect of PVT lesion on goal-tracking behavior for bHRs. Specifically, for magazine contacts during the CS period, there was no effect of Treatment ($F_{(1,19)} = 0.000$, $P = 0.986$) or Treatment x Session interaction ($F_{(11,19)} = 0.90$, $P = 0.559$). Similarly, for probability of magazine contact there was no effect of Treatment ($F_{(1,19)} = 0.04$, $P = 0.850$) and no Treatment x Session interaction ($F_{(11,54)} = 0.39$, $P = 0.955$), and the same was true for latency to magazine contact (Effect of Treatment, $F_{(1,20)} = 0.01$, $P = 0.937$; Treatment x Session interaction, $F_{(11,46)} = 0.93$, $P = 0.525$). Thus, PVT lesions did not affect goal-tracking behavior in bHR rats with a predisposition to sign-track.

For bLRs, there was no effect of PVT lesions on sign-tracking behavior (Supporting Figure 1D-F). Specifically, for lever contacts there was no effect of Treatment ($F_{(1,19)} = 19.45$, $P = 0.659$) or Treatment x Session interaction ($F_{(11,109)} = 1.15$, $P = 0.328$). Likewise, no effects were seen for probability of lever contact (Effect of Treatment, $F_{(1,9)} = 0.23$, $P = 0.639$; Treatment x Session interaction, $F_{(11,9)} = 1.43$, $P = 0.301$) and latency to lever contact (Effect of Treatment, $F_{(1,20)} = 0.05$, $P = 0.826$; Treatment x Session interaction, $F_{(11,220)} = 1.27$, $P = 0.245$). These results indicate that PVT lesion did not cause a change in sign-tracking behavior in bLRs.

The extent of PVT damage is related to the shift from goal- to sign-tracking behavior

To assess whether the lesion effects that we saw were specifically due to the extent of PVT damage, we analyzed data from GT rats that were excluded from Experiment 2 (Supporting Figure 4). There were no significant differences revealed when a one-way ANOVA was conducted to compare post-lesion response bias score across the groups that were categorized based on the extent of the lesion. Given the small sample size and variance within the missed lesion groups, this is not surprising. Nonetheless, we believe the apparent trends evident in this dataset are meaningful. GTs in the PVT Miss group, who primarily had unilateral damage to the habenula or dorsal hippocampal damage, show a post-lesion response bias score identical to that of GT Sham controls at the conclusion of the experiment. Likewise, GTs in the PVT + group show a response bias score similar to GT Lesion rats that were included in the study. Further, GTs in the PVT - group show a response bias score between GT Sham and GT Lesion groups. Thus, it appears, at least for GTs, that the size and extent of a PVT lesion corresponds with the change in response bias. Despite the lack of statistical significance, these data suggest that it is the PVT itself, and not the surrounding nuclei, that is important for sign- and goal-tracking behaviors.

The effects of PVT lesions on Locomotor Response to Novelty

Linear mixed-effects models show no significant effect of Treatment, and no significant Treatment x Phenotype or Treatment x Phenotype x Interval interactions for locomotor response to novelty (Supporting Figure 5). These results indicate that PVT lesions did not affect novelty-induced locomotor behavior.

Supporting Table 1 – Statistical Results for Experiment 1

Measure	Session	Treatment	Phenotype	Session* Treatment	Session* Phenotype	Treatment* Phenotype	Session* Treatment* Phenotype
Lever Contacts	<0.001*	0.862	<0.001*	<0.001*	<0.001*	0.859	<0.001*
Lever Probability	<0.001*	0.353	<0.001*	0.002*	<0.001*	0.340	0.003*
Lever Latency	<0.001*	0.654	<0.001*	0.001*	<0.001*	0.652	0.001*
Magazine Contacts	0.026*	0.180	0.120	0.315	<0.001*	0.189	0.431
Magazine Probability	0.011*	0.320	0.024*	0.478	0.001*	0.187	0.847
Magazine Latency	0.005*	0.181	0.168	0.583	0.001*	0.144	0.757

Supporting Figure Legend

Supporting Figure 1. Effects of PVT lesion on the acquisition of "off target" behaviors in bHR and bLR animals. Mean + SEM for A) number of food cup contacts, B) probability of food cup contact, and C) latency to food cup contact for bHR animals (Lesion, n=9; Sham, n=12). For bLR animals (Lesion, n=12; Sham, n=10), mean + SEM for D) number of lever contacts, E) probability of lever contact, and F) latency to lever contact.

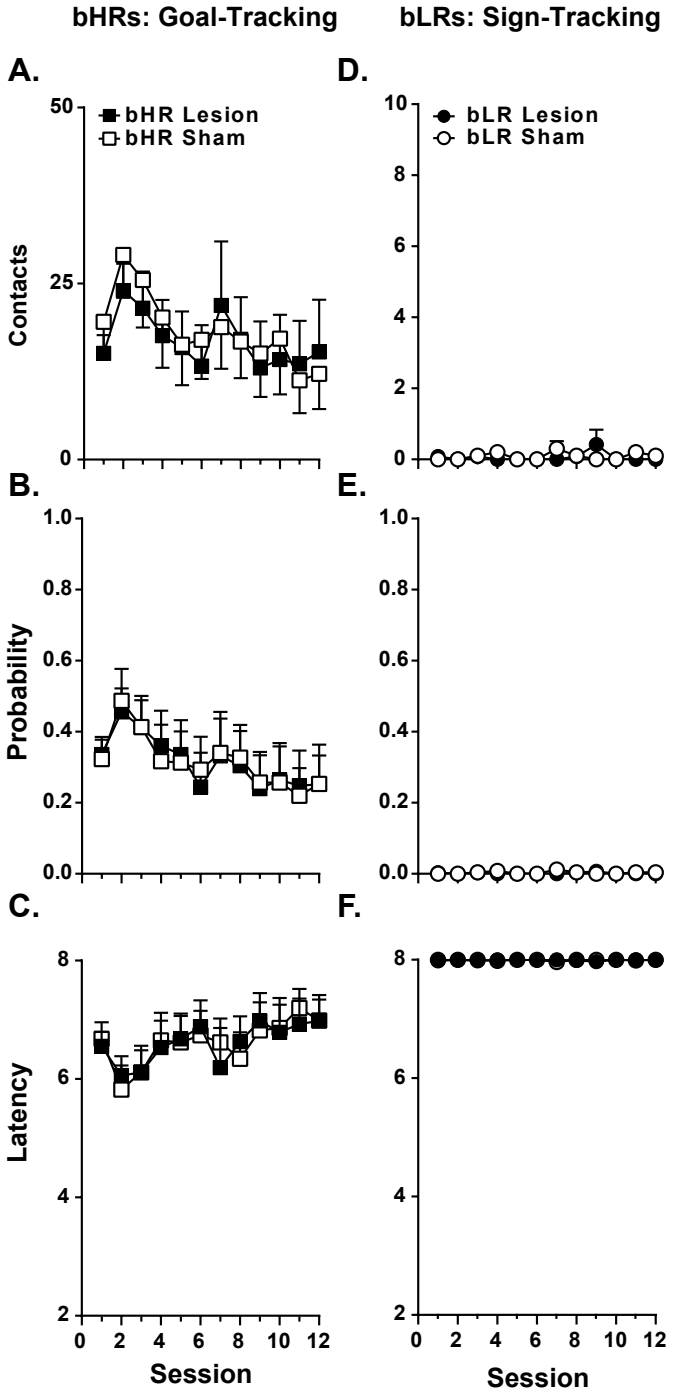
Supporting Figure 2. Activity during the inter-trial interval for bLR rats. Mean + SEM for the number of food cup contacts during the inter-trial interval. bLR Sham (n=10) and bLR Lesion (n=12) groups significantly differed on the number of food cup entries during the inter-trial interval only on session 8 ($P = 0.01$).

Supporting Figure 3. The acquisition of sign- and goal-tracking conditioned responses across 7 PCA training sessions. Mean + SEM for A) number of food cup contacts, B) probability of food cup contact, C) latency to food cup contact, D) number of lever contacts, E) probability of lever contact, and F) latency to lever contact. (ST Lesion n = 11, ST Sham n =13, GT Lesion n =9, GT Sham n =12)

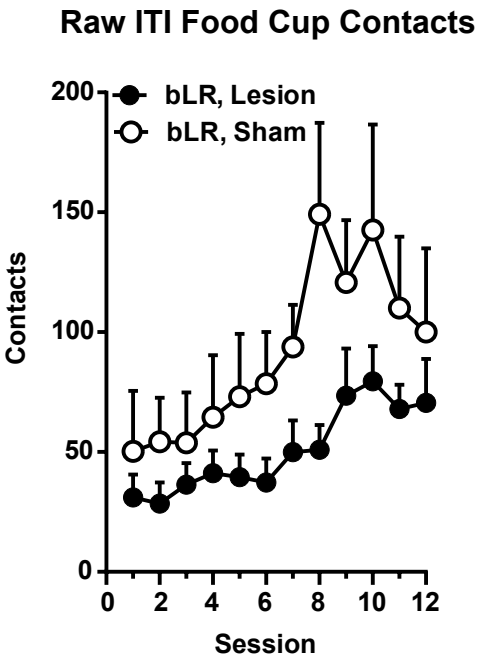
Supporting Figure 4. The extent of PVT lesion underlies change in response bias score. Mean + SEM of post-lesion response bias score (average of sessions 19-21) for GTs from Experiment 2 that were included in the study (Sham, n=12; PVT Lesion, n=9) or did not meet inclusion criteria (PVT Miss, n=3; PVT -, n=4; PVT +, n=4).

Supporting Figure 5. PVT lesions do not affect locomotor response to a novel environment. The line graph represents the mean + SEM for total locomotor score (lateral + rearing activity) across 5 minute time bins (GT Lesion, n=4; GT Sham, n=8; ST Lesion, n=5; ST Sham, n=8).

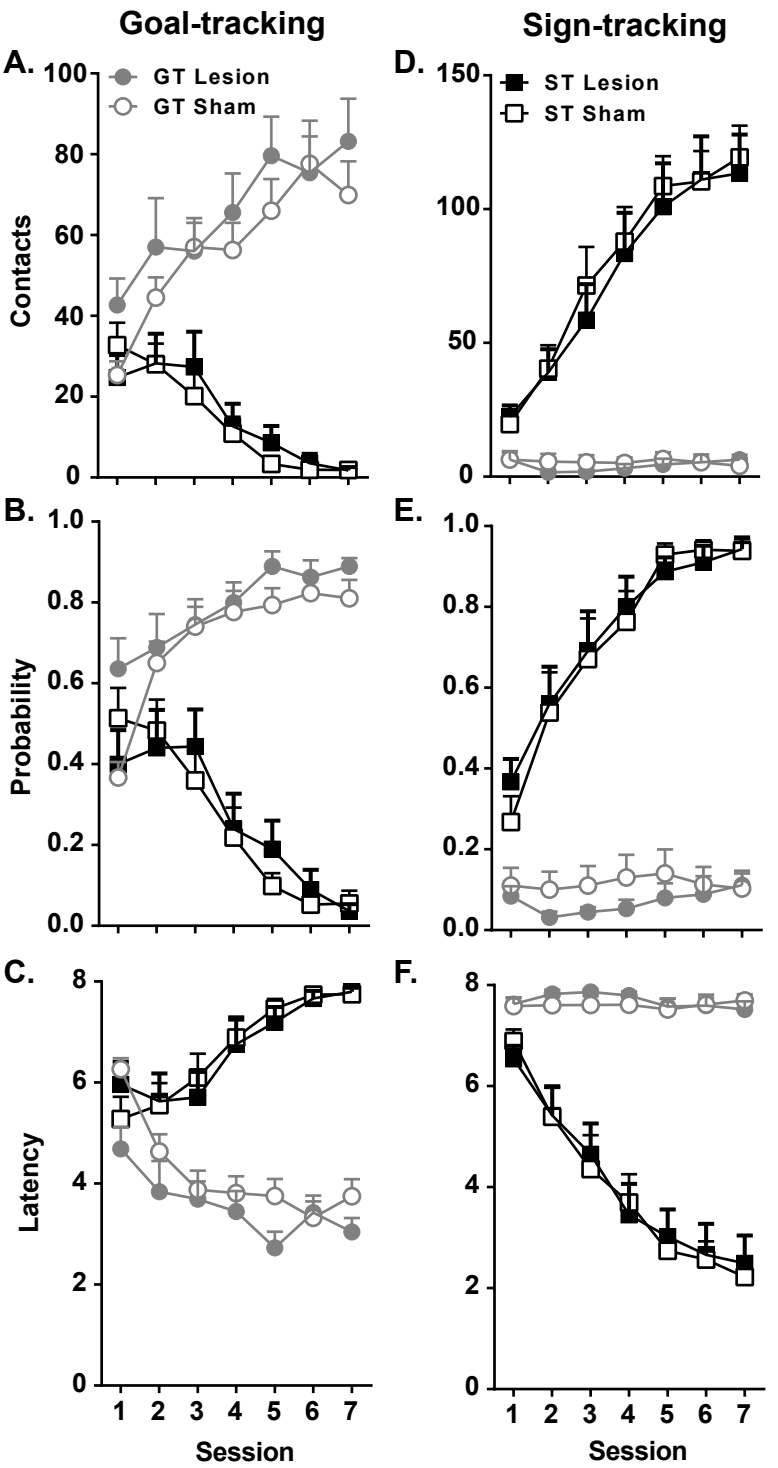
Supporting Figure 1



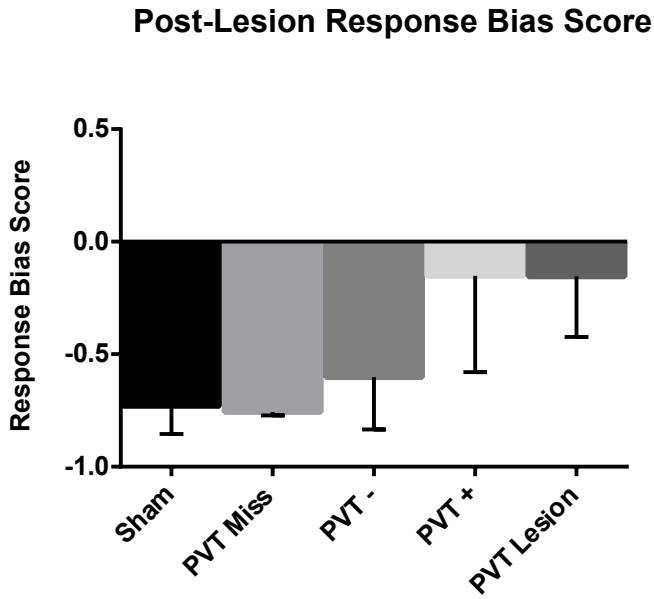
Supporting Figure 2



Supporting Figure 3



Supporting Figure 4



Supporting Figure 5