Examining Carryover Effects of CTA on Pavlovian Conditioned Approach:

Results:

Although not directly related to our initial hypotheses, we conducted additional analyses of the behavioral responses to CSs corresponding to the *non-devalued outcomes* in order to determine whether devaluation of the SS-CSs carried over to the other non-devalued SS-CS, or non-devalued Gen-CS. First, we determined whether the pattern of conditioned approach responses to these CSs (ND Gen-CS, ND SS-CS, and Dev SS-CS) differed between Vehicle and CNO conditions, and found no effect of drug in either group (Supp. Fig. 2. Three-way RM ANOVA, no effect of drug, p=0.34; no drug x group interaction, p=0.55). We then determined whether conditioned approach differed across the CSs during devaluation testing. We found that the magnitude of conditioned approach differed across each CS regardless of viral transduction or CNO treatment (Supp Fig. 2. Three-way RM ANOVA, main effect of CS, $F_{(1, 8)}$=11.78, p<0.01; no CS by group interaction, p=0.53; no CS x drug interaction, p=0.76). Therefore, we collapsed the data across drug and viral transduction conditions (Supp Fig. 3) in order to examine conditioned approach to CS previously paired with devalued or non-devalued outcomes (ND Gen-CS, ND SS-CS, and Dev SS-CS). This revealed a strong carryover devaluation effect on conditioned approach elicited by the ND Gen-CS and the Dev SS-CS (Supp Fig. 3, One-way RM ANOVA, main effect of CS $F_{(1.44,\ 12.94)}$=5.46, p=0.02; Holm-Sidak's multiple comparisons test: $t_{(9)=}$3.00, p=0.03). In contrast a weaker devaluation effect was observed between the ND SS-CS and the Dev SS-CS (Supp Fig. 3, Holm-Sidak's multiple comparisons test: $t_{(9)=}$2.10, p=0.07). These data demonstrate carryover devaluation effects between the SS-CSs suggesting that the shared associative properties of these outcomes, renders them similar enough to support these carryover effects.

Discussion:

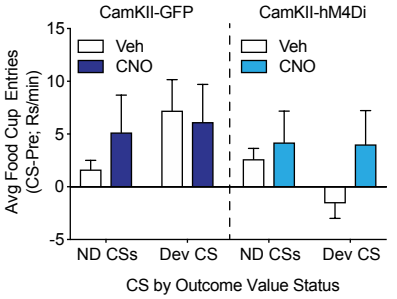
One interesting and unexpected behavioral finding in this study that is separate from the question of the role of BLA in PIT, was that rats showed “carryover” effects between outcomes trained under conditions promoting sensory-specific encoding versus general affective encoding. Specifically, we found that when CTA was performed using one of the outcomes from the CS-Os used to promote SS-PIT (e.g., O1 or O2), Pavlovian devaluation effects were seen to both the devalued SS-CS, and to the non-devalued SS-CS, but not to the non-devalued Gen-CS (Supp Fig. 3). This carryover effect while mild, was notable. Furthermore, a conceptually similar but stronger carryover effect was observed during the taste aversion choice testing (Fig. 4H). During choice testing, rats vastly preferred the non-devalued Gen-O versus the non-devalued SS-O. Critically, the flavors had been carefully counterbalanced and therefore similarity in sensory perception of outcomes alone cannot account for this effect.

These data suggest that CTA can produce aversions not just to stimuli directly associated with the devalued outcomes, but also to other stimuli (outcomes and CSs) that share the same associative modalities (i.e., SS versus Gen). In this PIT paradigm, the outcomes that support SS-PIT are each independently associated with a Pavlovian CS (CS-O) and an instrumental response R-O association. In this way the outcome is embedded in a wider S-O-R network. In contrast, the outcome that supports General-PIT is only experiences within a Pavlovian CS-O association, but not an instrumental R-O association. In this way the General-O exists in a smaller S-O network. The SS-Os share a common associative structure, which is distinct from that of the General-O, which render the outcomes trained under conditions promoting SS encoding vulnerable to effects observed. If outcomes trained within the same associative history are more vulnerable to carryover effects of CTA training, then we would expect a similar effect between a devalued General-O and a non-devalued General-O, where a non-devalued SS in this paradigm should be

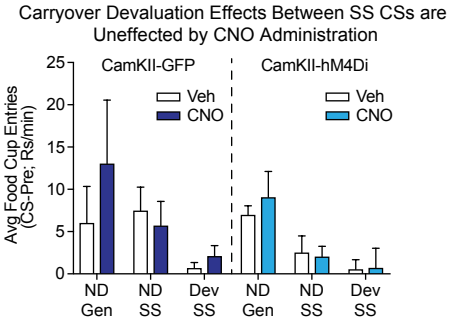
immune to carryover. This hypothesis has yet to be tested. Nevertheless, the finding here of carryover between a devalued SS-O and a non-devalued SS-O, but not a General-O, provides further support for the idea that independent encoding processes exist for sensory specific associative structures and general affective associative structures (as canonically proposed by Konorski, 1967), and presumably dissociable neural mechanisms underlying these processes.

Supplemental Figure 1

CTA Training of a General Outcome Fails to Support Outcome Devaluation Effects on Conditioned Approach



Supplemental Figure 2. Test for Pavlovian devaluation effects in the SS-Dev groups (individual group data).



Supplemental Figure 3.

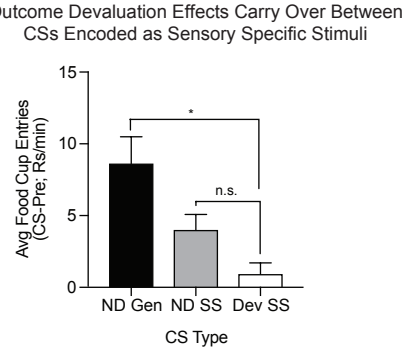


Figure Captions:

Supplemental Fig 1: Pavlovian Devaluation testing in rats where the devalued outcome was the previous General outcome (i.e, O3; Table 1). Neither CamKII-GFP nor the CamKII-hM4Di groups showed evidence of Pavlovian devaluation effects, and CNO administration did not alter this.

Supplemental Fig 2: Pavlovian devaluation testing in rats where the devalued outcome was one of the previous SS outcomes (i.e, O1 or O2; Table 1). Both CamKII-GFP and the CamKII-hM4Di groups exhibited Pavlovian devaluation effects, approaching the food cup less during presentation of the devalued CS (Dev SS) vs the non-devalued CSs (NDev Gen and NDev SS). CNO administration did not alter this behavior.

Supplemental Fig 3: Collapsed CamKII-GFP and CamKII-hM4Di group data from Pavlovian devaluation testing in rats where the devalued outcome was one of the SS outcome (i.e, O1 or O2; Table 1). Conditioned approach to the non-devalued SS-CS is lower than to the non-devalued General-CS (NDev Gen), illustrating carryover devaluation between the devalued SS-CS (Dev SS) and the non-devalued SS-CS (NDev SS).