METHODS

The order and timing of procedures is illustrated by the experimental timeline in Supplementary Fig. 1, which is reproduced from the main text.

Subjects. Thirty-two male Long-Evans rats (Charles River Laboratories) weighing between 275 and 300 g on arrival, were housed individually and placed on a 12-h light/dark schedule. All rats were given *ad libitum* access to food except during testing periods. During behavioural testing, rats were food-deprived to 85% of their baseline weight. All testing was conducted during the light period of their cycle.

Surgical procedures. Lesions were made in stereotaxic surgery using intracerebral infusions of *N*-methyl-D-aspartic acid (NMDA, Sigma) in saline vehicle. Infusions of 0.05–0.1 μl of NMDA (12.5 μg μl^{-1}) were made at 4.0 mm anterior to the bregma, and at 2.2 and 3.7 mm lateral to the midline, at a depth of 3.8 mm ventral to the skull surface and 3.0 mm anterior to the bregma, at 3.2 mm lateral to the midline (0.05 μl) and 4.2 mm lateral to the midline (0.1 μl), and 5.2 mm ventral to the skull surface. Surgical controls received saline vehicle infusions. After a 1-week recovery period, all rats were placed on food restriction. Testing began 2 weeks after surgery (Supplementary Fig. 1).

Apparatus. Testing was conducted in eight standard sized behavioural boxes ($12 \times 10 \times 12$ inches) and other equipment modules purchased from Coulbourn Instruments. A recessed food cup was located in the centre of the right wall approximately 2 cm above the floor. The food cup was connected to a feeder mounted outside of the chamber to deliver 45 mg sucrose pellets (grape or banana, Research Diets). These pellets were equally preferred but discriminable. This was tested by giving food-deprived rats ($n = 10$) access to both pellets before and after one of the two pellets was devalued by overfeeding (see Supplementary Fig. 3 for results). A house light and cue light were placed on the wall to the left or right of the food cup approximately 10 cm from the floor. Additionally, white noise and tone cues (75 dB, 4 kHz) could be delivered through speakers mounted in the centre of the wall. Data were collected by Graphic State behavioural software from Coulbourn Instruments.

Transreinforcer blocking. Transreinforcer blocking consisted of pavlovian conditioning, compound conditioning, and probe tests (Supplementary Fig. 1). Before any training, rats were reduced to 85% of their baseline weights, then they were shaped to the food cup during two training sessions in which two 45 mg sucrose pellets (grape and banana) were delivered to the food cup 16 times over the course of an hour. After these shaping sessions, all rats received 12 days of conditioning in which two visual cues (a house light and a cue light, designated A and B, counterbalanced) were paired with two distinctly flavoured yet equally preferred sucrose pellets (45 mg grape and banana flavoured sucrose pellets, designated O1 and O2, counterbalanced; Research Diets). Sessions consisted of 16 presentations of each cue, with average inter-trial intervals of 2.5 min. During the 30-s presentation of each light cue, three food pellets were delivered with one food pellet being delivered every 8–10 s.

After conditioning, all rats received one day of pre-exposure to two auditory cues (white noise and tone, designated X and Y, counterbalanced). This pre-exposure consisted of one session in which each auditory cue was delivered six times for 30 s, with an average inter-trial interval of 2.5 min. The next day, the rats began 9 days of compound conditioning (Supplementary Fig. 1). In each session, compound cues, AX and BY, were presented for 30 s. Compound cue AX was paired with O1, which was the same flavour sucrose pellet associated with A. Compound BY was also paired with O1, which was a different flavour sucrose pellet than that associated with B. Each session consisted of eight presentations of each compound. The rats also received 8 presentations of A–O1 and B–O2 as reminder training; average inter-trial intervals were 2.5 min. During compound conditioning (Supplementary Fig. 1), all rats received two probe tests, each consisting of six unrewarded 30 s presentations of A, B, X and Y; average inter-trial intervals were 2.5 min.

Conditioned reinforcement. Conditioned reinforcement testing was conducted during compound conditioning, before and again after devaluation of the predicted outcome (Supplementary Fig. 1). Each episode of conditioned reinforcement testing consisted of two 30-min sessions and was conducted in the original training chambers with response levers inserted on the right and left walls. One lever led to a 1-s presentation of a conditioned stimulus (either A or Y depending on group) on a fixed ratio two (FR2) schedule, and the second lever led to a 1-s presentation of X on an FR2 schedule. The lever-cue associations were counterbalanced for side in each group.

Devaluation. The O1 food pellet was devalued by pairing it with illness induced by 0.3 M lithium chloride (5 mg kg^{-1}, intraperitoneal injection). On days 1, 3 and 5 of this 6-day procedure, rats received access to the O1 pellet for 10 min in their home cage, followed by lithium chloride injection. On days 2, 4 and 6, rats received similar access to the O2 pellet with no injections. After all behavioural training, rats received a probe test in the home cage where they were given 10-min access to each pellet.

Statistical analysis. Data regarding food cup responding and bar pressing were acquired using Coulbourn GS2 software. Raw data were processed in Matlab to extract rates of responding. These data were analysed using Statistica.

SUPPLEMENTAL INFORMATION

Supplemental Results

On the extent of OFC lesions: OFC lesions targeted the lateral areas on the dorsal bank of the rhinal sulcus, including the lateral and ventrolateral orbital regions and dorsal and ventral agranular regions (Figure S2). Lesions were estimated to have affected 49% of the area within these regions on average, with a range of 33-70%. The percentage did not differ between groups A and Y (A = 53%, Y = 45%; $F_{1,11} = 2.51$, $p = 0.142$).

On the preference and discriminability of grape and banana sucrose pellets: Transreinforcer blocking utilized two differently-flavored sucrose pellets (banana and grape). These pellets were equally preferred but easily discriminable. To demonstrate this, a group of food-deprived but otherwise naïve control rats were given access to equal amounts of the two pellets in their home cages, either before or after each pellet was 'devalued' by selective satiation. Before selective satiation, rats consumed identical amounts of the two pellets, showing no baseline preference between them (Figure S3). ANOVA indicated no significant difference in consumption ($F_{1,47} = 0.37$, $p < 0.54$). After selective satiation, rats consumed more of the non-devalued pellet than the devalued pellet, showing that they could distinguish between the two flavors and selectively modulate their preference based on the pellets' relative values (Figure S3). ANOVA revealed a significant effect of devaluation ($F_{1,31} = 39.5$, $p < 0.0001$), but no significant main effect nor any interaction with flavor (F's < 2.848, p's > 0.1).

Effects of training and OFC lesions on the preference and discriminability of grape and banana sucrose pellets: To test whether training experience or lesions had any effect on the preference or discriminability of the banana and grape flavored sucrose pellets, we also analyzed consumption prior to, and after, LiCl induced devaluation of the O1 pellet in the experimental groups described in the main text. Importantly the flavors assigned as O1 and O2 were counterbalanced, so that for half of the rats in each group O1 was grape and for half of the rats in each group O1 was banana. Results of devaluation analyzed in the main text and presented in Figure 1C show the effects of devaluation on O1 consumption, collapsed across flavor. However by separating these data according to which flavor was devalued, we can confirm that neither training experience nor lesions had any impact on the preference or discriminability of the banana and grape pellets. As illustrated in Figure S4, rats in each group consumed the same amount of the grape and banana pellets before devaluation and subsequently showed a selective reduction in consumption of whichever flavor was paired with illness. The effect of pairing with illness did not differ between the two flavors within a group, nor did the effect differ for either flavor between the two groups. Consistent with this interpretation, ANOVA's comparing consumption of each flavor before versus after devaluation revealed a main effect of devaluation ($F_{2,56} = 221.0$, $p<0.0001$) but no main effect nor any interactions with pellet flavor or lesion (F's < 3.280, $p > 0.09$).

Supplemental Discussion

Many important issues and potential alternative interpretations are difficult to address fully in a short format article. The following are three issues in particular that we wish to discuss.

On the evidence that affective and outcome-specific representations are dissociable entities: The first of these issues is the recognition that the significance of our results rests strongly on the ideas 1) that what are termed affective and outcome-specific representations form independently and 2) that we can effectively block affective representations using transreinforcer blocking. The evidence supporting these ideas was cited in the main text; however, it may be useful to review it more fully.

The most relevant evidence that these two types of representations may be dissociated comes from studies of Pavlovian-to-instrumental transfer (PIT) [1]. PIT refers to the observation that presentations of a previously trained Pavlovian cue will increase the rate or vigor or an instrumental response over baseline. There is "general" PIT, which occurs when the Pavlovian cue and the instrumental response have been paired with two different outcomes. This is thought to reflect the influence of the general motivational or affective properties shared across the two outcomes [1]. In addition, if the cue shares an outcome with one but not another instrumental response, it is also possible to observe "specific" PIT, which is an additional increase in instrumental responding for the shared outcome. This additional increase is thought to reflect the influence of information unique to that particular outcome that is shared between the cue and the instrumental response [1]. In other words, "specific" transfer differs from "general" transfer in that it requires the cue to activate the unique representation of the outcome predicted by the instrumental response. Importantly these two forms of transfer are dissociable on a neural level. General PIT depends on a brain circuit that includes the central nucleus of the amygdala and nucleus accumbens shell, whereas specific PIT depends on basolateral amygdala and nucleus accumbens core [2, 3]. These data suggest that affective and outcome-specific representations can be isolated behaviorally and that there are unique brain areas that mediate the mobilization of outcome-specific and general affective information to guide behavior. Notably these areas are all implicated in conditioned reinforcement.

In addition there is evidence that the procedure we used, transreinforcer blocking, also dissociates two types of information, creating a cue (Y) that preferentially evokes outcome-specific information. The evidence for this is that this Y cue will support specific PIT and does not seem to increase instrumental responding more generally [4]. Similar evidence comes from an analogous procedure using an aversive US [5]. This evidence suggests that transreinforcer blocking results in a cue, Y, which acts through a representation of the specific outcome with which it has been paired and does not trigger general affective or emotional representations. Of course the idea that Y differs from a normally conditioned cue, in its propensity to act through a representation of the outcome, is also supported by the observation presented in the main text here that responding to Y is highly sensitive to devaluation, whereas responding to A is not.

On alternative interpretations for blocking: The second issue that we would like to address here are a number of alternative explanations for blocking. In the main text, we adopt the relatively popular explanation for blocking in which one of the two simultaneously presented cues evokes previously acquired representations that block learning for the new cue. By this account [6], each cue is treated as an independent element. However an alternative account has been advanced in which the two cues are

treated as a compound [7]. By this account, learning for the new cue occurs but only as part of the compound. It still is unable to evoke any representations when presented alone. Although our study was not directed at distinguishing between these explanations, it is important to consider whether any of them might impact the validity of our interpretation.

Whether the mechanism of normal blocking relies on one elemental cue blocking the other (as we have written) or a configural explanation is orthogonal to our question, since in either case one must still resort to discussing outcome-specific processing to account for the increased responding observed to Y versus X (in both conditioned reinforcement as well as in the extinction probe test). This is confirmed by the sensitivity of Y responding to devaluation of the specific outcome that it predicts. In addition, if the rats were required to create a compound cue and then learn about that cues associations with the new outcome, one might expect an initial drop in conditioned responding in the compound training phase. We saw no such change in responding.

It has also been suggested that transreinforcer blocking might involve occasion setting. Occasion setting refers to the observation that an animal can learn to respond to a cue differently when another cue is present. Thus, one cue serves to "set the occasion" for responding [8]. It has been suggested to us that transreinforcer blocking may be a situation in which Y sets the occasion for whether B is to be followed by O1.

Although this is an interesting argument, we believe there are a number of pieces of evidence that argue strongly against it. First, occasion setting is an unlikely explanation because it is difficult to obtain even when proper procedures are used. Our procedures differ in critical ways from the ones normally used [8]. Perhaps most importantly, the occasion setter must be presented before the target cue to have a reasonable chance of working properly (see Schmajuk et al [8], "When Does a CS Behave as an Occasion Setter?" for a direct discussion and modeling of this question). In our case, the two cues were always presented simultaneously. And even then, it appears to require quite a bit more training than we provide.

Second, one would expect little or no responding to the occasion-setter, Y, when it is later presented alone, since it is only thought to gate (lower or raise the threshold) for activation of the correct unconditioned stimulus (US) representations. It is not thought to actually activate these representations directly, and the target cues are never presented with Y in the critical test sessions. However, we do see significant responding to Y presented alone.

Third, even if one were to assume that this responding is generalization, then we would expect to see similar responding to X since the same explanation should hold there. Yet we see neither Pavlovian nor instrumental responses supported by X in our study. Other studies using this sort of blocking procedure report a similar dissociation in the properties of X and Y [4, 5].

Finally, even if we were to assume that Y were operating as an occasion-setter – modulating the threshold for activating the US representation, and that it would do this in the absence of the target cue, and that it somehow obtained this property when X does not, we would still expect responding to Y to show the same properties as responding evoked by a normally conditioned cue. This is because both are operating on the same US representation presumably. And yet this is not the case. In our experiment, responding to Y is completely abolished by devaluation (or OFC lesions), whereas

responding to the normally conditioned cue remains unaffected (similar to Parkinson et al., 2005). Obviously these effects might be explained by noting that there is a new outcome present; however, in that case one is again resorting to discussing outcome-specific processing to account for the unique responding to Y.

In sum, while both compound conditioning and occasion setting are interesting and thought provoking explanations, neither seems to provide as clear and straightforward an explanation for what we observe as the one we have advanced. In particular, in order to explain differential responding to Y, both explanations must still resort to discussing outcome-specific representations. Since it is not our intent to explain the mechanisms underlying transreinforcer blocking but only to use it as a tool to test for the influence of outcome-specific processing on conditioned reinforcement (and the role of OFC in this process), it seems to me that these explanations do not negate the significance of our results.

On the possible role for orbitofrontal cortex in attentional processing: One alternative explanation for the effect of orbitofrontal lesions that we report in the main text is that orbitofrontal cortex might serve an attentional function. According to Pearce and Hall [7], omitting the O2 outcome on BY trials results in increased attention to B and Y, thereby facilitating learning. If orbitofrontal cortex were critical to this attentional function, then one might expect there to be effects of orbitofrontal damage on learning for the Y cue. Thus orbitofrontal cortex would play a role in regulating the associability of cues via an attentional function, similar to that served by the central nucleus of the amygdala [9], rather than being directly involved in signaling the associative representations. Although we cannot fully rule out this intriguing possibility, there are several issues, including our own data, that make this interpretation less attractive than the one we have advanced.

First, this account would contradict results from numerous prior experiments on orbitofrontal function [10]. In many of these experiments, the effects of orbitofrontal lesions clearly cannot be accounted for by invoking an attentional function. For example, orbitofrontal cortex is critical in settings such as devaluation, where there is no requirement for this function (at least in the OFC-dependent parts) [11-14]. This is illustrated in considering that damage to central nucleus of the amygdala, an area clearly implicated in incrementing attention [9], does not impair devaluation [15]. Indeed, to the best of our knowledge, there is no evidence that orbitofrontal cortex is required for performance in tasks designed to isolate increases in attention, and there is evidence that it is not required for normal performance on more general attentional tasks such as attentional set-shifting [16, 17].

Second, our behavioral data suggest it is unlikely that the associability of BY increases in compound training inasmuch as controls do not show the pattern of selectively increased responding to BY that one would expect if these animals were selectively increasing attention to this specific compound cue.

Finally, it is only the outcome-specific properties of the outcome that have changed. In other words, nothing has really been omitted, at least not in the sense that Pearce and Hall describe [7]. Rather, we have substituted one outcome with another that is identical in terms of motivational value or affect. Thus, to the extent associability has increased, it is these novel outcome-specific properties that will be preferentially

encoded. This is supported here by the observation that responding to Y is highly sensitive to devaluation, and in the literature by reports that responding to a cue conditioned this way supports behaviors that require outcome-specific but not general affective representations [4, 5].

Supplemental Figure Legends

Figure S1: Experimental timeline depicting the order and timing of procedures. [*A, B, X, Y*] are training cues, [*R1, R2*] are instrumental responses, and [*O1, O2*] are different flavored sucrose pellet reinforcers.

Figure S2: Extent of neuronal loss in orbitofrontal cortex for a maximum, a minimum and a representative lesion.

Figure S3: Banana and grape flavored sucrose pellets are equally preferred but discriminable in naïve rats. The left section of the figure shows the average number of grape and banana flavored sucrose pellets consumed by food-deprived but otherwise untrained, naive rats over three preference tests. Rats showed no difference in consumption between them. The middle and right sections of the figure show the average number of grape and banana flavored sucrose pellets consumed by food-deprived rats after devaluation of the grape or banana pellets by selective satiation, in which the rats were given 20 minutes free access to one or the other flavor prior to testing. Rats consumed more of the non-devalued pellet in each case. (*, $p < 0.05$)

Figure S4: Banana and grape flavored sucrose pellets are equally preferred but discriminable after conditioning in control (A) and OFC-lesioned (B) rats. Data are from the devaluation testing shown in Figure 1C of the main text, except that here we show the first day of devaluation, to illustrate consumption before ("Before Devaluation") and after ("After Devaluation") pairing with illness, to illustrate the discriminability of the pellets. In addition, the data are shown separately according to which flavor served as the devalued O1 pellet (recall that the assignments of the flavors was counterbalanced, so for some rats O1 was banana and for some O1 was grape). Both control and OFC-lesioned rats consumed similar amounts of the two pellets before devaluation and also selectively decreased their consumption of the pellet paired with illness after devaluation. There were no differences in the effect of devaluation either between flavors within a group or within a flavor across the groups. (*, $p < 0.05$)

Figure S5: Effects of orbitofrontal lesions on conditioned reinforcement for a fully conditioned cue (B). Rats tested in this paper did not experience B in a conditioned reinforcement setting, thus we have provided data from an additional group of rats to show this responding. This figure shows average lever responses for a fully conditioned cue, B, versus the blocked cue, X, on a FR2 schedule over two days for a sham rats (n=14) and OFC lesioned rats (n = 14). OFC-lesioned rats, like shams, showed increased responding for B compared to X. (*; $p<0.05$)

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Figure S1

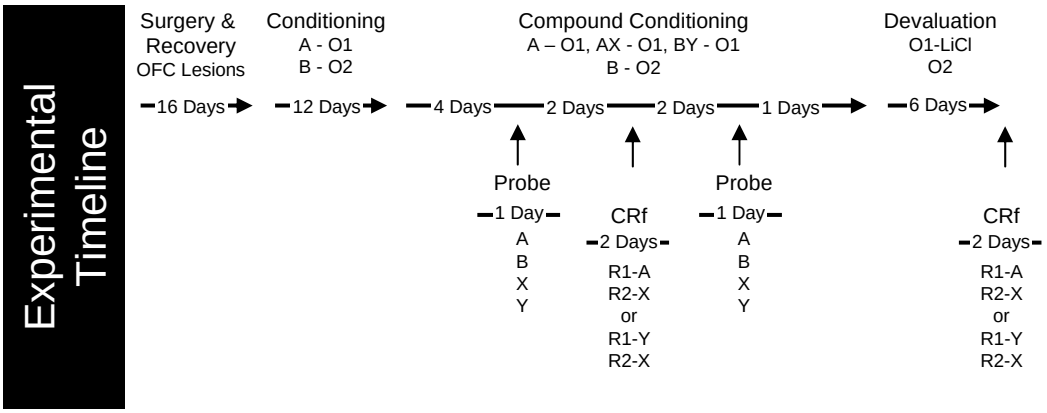

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Figure S2

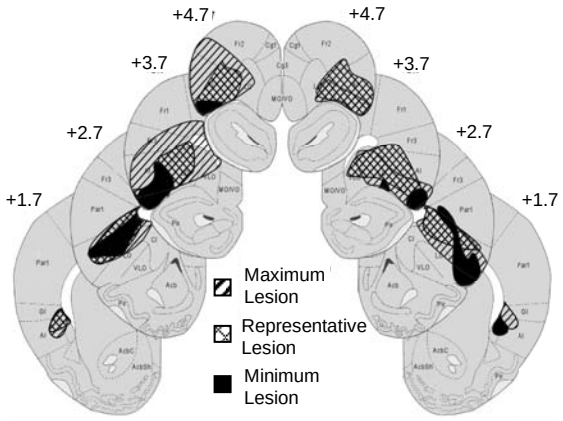

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Figure S3

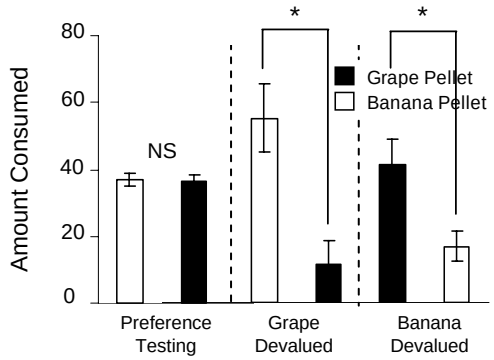

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Figure S4

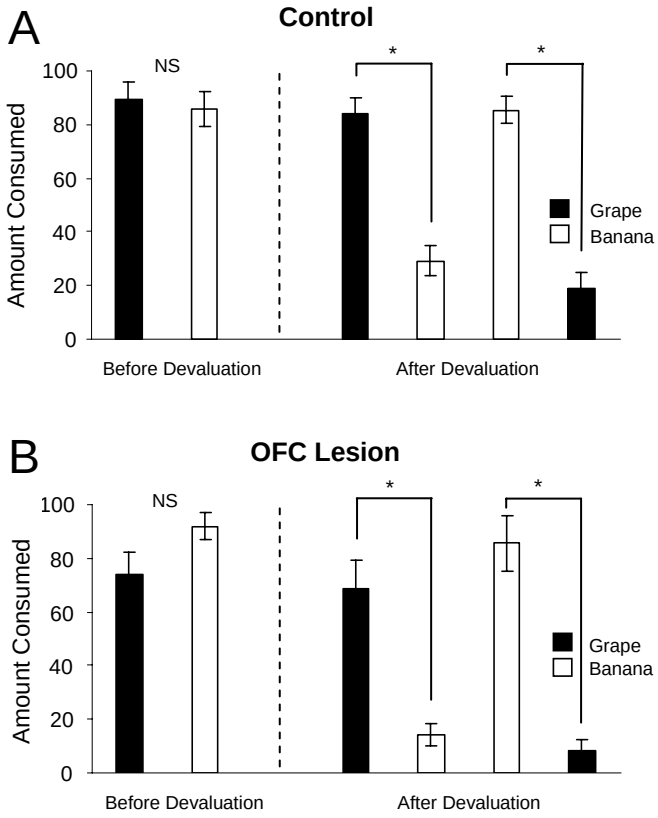

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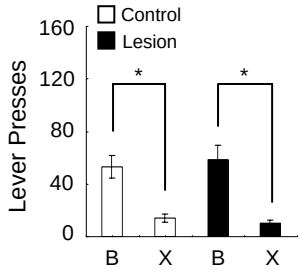
Figure S5



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