Amygdalo-Striatal Interaction in the Enhancement of Stimulus Salience in Associative Learning

Guillem R. Esber
National Institute on Drug Abuse, Bethesda, Maryland

Karina Torres-Tristani
University of Puerto Rico, Rio Piedras

Peter C. Holland
Johns Hopkins University

Function of the central nucleus of the amygdala (CeA) is critical to 2 aspects of attention in associative learning: the conditioning of orienting responses (ORs) to cues paired with food, and the enhancement of cue salience by the surprising omission of expected events. Such salience enhancements have been found to depend on interactions within a circuit that includes CeA, the substantia nigra pars compacta (SNc), the substantia innominata (SI), and the posterior parietal cortex (PPC). The acquisition and expression of conditioned ORs requires interactions among CeA, SNc, and the dorsal lateral striatum (DLS), but not SI or PPC. Here, we considered whether CeA-DLS interactions are also important in surprise-induced salience enhancements in a serial prediction task. Rats received unilateral lesions of CeA and DLS, either contralaterally, which disrupted interactions between those structures, or ipsilaterally, which produced comparable damage to each structure but permitted interactions between them in 1 hemisphere. Rats with ipsilateral lesions of CeA and DLS showed the salience enhancements normally observed in this task, but rats with contralateral lesions of those structures did not. Thus, convergence of information processing by CeA and DLS is essential for surprise-induced salience enhancements, as well as for conditioned ORs.

Keywords: amygdala central nucleus, associative learning, attention, dorsal striatum, salience
general deficits in learning, motivation, motor control, or orienting in general.

Surprise-induced enhancements in cue salience are also impaired after disruptions in function of brain regions not critical to conditioned ORs. Although, as with conditioned ORs, rats with compromised CeA function (Holland & Gallagher, 1993a,b; 2006) or functional disconnections of CeA and SNc (Lee et al., 2006, 2008) showed deficits in cue salience enhancements, disruptions in function of substantia innominata (SI; Chiba et al., 1995; Holland & Gallagher, 2006), posterior parietal cortex (PPC; Schiffino, Zhou, & Holland, 2014), certain subregions of medial prefrontal cortex (Maddux, 2008), or functional disconnections between CeA and SI (Han, Holland, & Gallagher, 1999) or SI and PPC (Bucci & Chess, 2005; Bucci, Holland, & Gallagher, 1998) produced deficits in enhanced cue salience but did not affect conditioned ORs. Holland and Gallagher (2006) suggested that SNc-SI projections might mediate amygdalar-cortical communication in cue salience enhancements, but it remains possible that the same CeA-SNc-DLS circuit critical to conditioned ORs is also important for salience increases, via an assortment of striatal-cortical loops.

Recently, we found that bilateral lesions of the DLS eliminated surprise-induced cue salience enhancements in a serial prediction task (Asem et al., 2014) that was previously shown to require intact CeA function (Holland & Gallagher, 1993a, 2006). Although the observation that bilateral disruption of CeA or DLS prevents salience enhancements could suggest that those regions are part of the same, serial circuit, it is possible that striatal influences act independently of the CeA circuit previously studied. Here, we examined the importance of convergence of processing by CeA and DLS in surprise-induced salience enhancements, using that same serial prediction task (Figure 1A; Wilson et al., 1992), and an asymmetrical “disconnection lesion” procedure. Because projections of CeA are almost exclusively ipsilateral, a unilateral lesion of CeA in one hemisphere and a contralateral lesion of DLS would substantially disrupt the opportunity for convergence of information processing by CeA and DLS. Control rats received...
unilateral lesions of CeA and DLS made ipsilaterally, which would leave such convergence intact in one hemisphere, despite damage to both CeA and DLS comparable with that sustained by rats with contralateral lesions. Thus, if the enhancement of cue salience by surprise required converging CeA and DLS processing, rats with contralateral lesions would display greater deficits than rats with ipsilateral lesions. By contrast, if DLS acted independently of CeA processing, then this action would be equally disrupted (or preserved) in contralateral and ipsilateral lesion conditions. Such independence would not be surprising in view of recent findings that although lesions of the lateral hypothalamus (LH) eliminate cue salience enhancements in the same serial prediction task, those deficits occur regardless of whether unilateral LH and CeA lesions are made contralaterally or ipsilaterally (Wheeler et al., 2014).

In an initial “expectancy” phase, rats received consistent serial light—tone pairings to establish the light as a reliable predictor of the tone. Next, in a “surprise” phase, for some rats the tone was omitted on half of the trials, while other rats received additional consistent light—tone pairings. Finally, the salience of the light was assessed in a test phase in which the light was paired with food. Within the Pearce-Hall model (Pearce & Hall, 1980), as the light comes to predict the tone in the expectancy phase, its salience decreases, whereas violation of that prediction in the surprise phase restores or enhances that salience. Intact rats for which the tone was unexpectedly omitted in the surprise phase routinely show substantially more rapid learning of the new light-food relation in the subsequent test phase than rats that previously received consistent light—tone pairings (reviewed in Holland & Maddux, 2010).

Method and Materials

Subjects

The subjects were 40 male Long-Evans rats (Charles River Laboratories, Raleigh, NC), which weighed 300 g–325 g on arrival to the laboratory vivarium. Rats were individually housed in a colony room with a 12:12 hr light–dark cycle. They received a week of free access to food and water prior to lesion surgery. Five days before the beginning of behavioral training, their access to food was restricted, so that their weights reached and were then maintained at 85% of their free feeding weights. Behavioral training sessions were conducted during the light portion of the light–dark cycle. The care and experimental treatment of rats was conducted according to the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals, and protocols were approved by the Johns Hopkins University Animal Care and Use Committee.

Apparatus

The behavioral training apparatus consisted of eight individual chambers (20.5 cm × 22.0 cm × 22.5 cm) with stainless steel front and back walls, clear acrylic sides, and a floor made of 0.48-cm stainless steel rods spaced 1.9 cm apart. An illuminated clear acrylic food cup was recessed in a 5.0 × 5.0 cm opening in the front wall, and photocells at the front of the food cup recorded entries and time spent in the cup. Sucrose pellets (45 mg, Formula 5TUT, Test Diets, Richmond, IN) were delivered to the food cups by pellet feeders (Coulbourn H14-22; Allentown, PA). A 1-w lamp was mounted behind a 2.5-cm diameter perforated steel hemisphere on the front wall, 10 cm above the food cup; illumination of this lamp served as the “light” stimulus. An infrared activity monitor (Coulbourn H24-61) and a bank of infrared LEDs to provide illumination for video recordings were mounted on the top of each chamber. Each chamber was enclosed inside a sound attenuating shell. A piezoelectric device for presenting an intermittent (3 Hz) 79-dB, 1900-Hz tone was mounted on the side wall of the shell. A video camera mounted near that device allowed for TV viewing and behavioral scoring. Constant dim illumination visible to the rats was provided by a 1-W lamp mounted behind a red lens mounted near the piezoelectric device, and ventilation fans provided masking noise (66 dB).

Surgery

Each of the rats received a unilateral lesion of DLS and a unilateral lesion of CeA. Half of the rats received the DLS and CeA lesions in opposite hemispheres (contra lesions) and half received them in the same hemisphere (ipsi lesions). Both ipsi and contra lesions were counterbalanced across hemispheres. Stereotoxic (Kopf Model 902, Tujunga, CA) surgery was conducted under aseptic conditions. Rats were maintained under anesthesia with 2%–3% isoflurane mixed with oxygen. Unilateral DLS lesions were made using 0.2 μl of 15 mg/ml quinolinic acid (Sigma, St. Louis, MO) in PBS solution infused into each of two sites in one hemisphere with a Hamilton 2.0-μl syringe over a 4-min period. The injectors remained in place for 3 min after infusions before they were removed. The coordinates used were 0.2 mm anterior to bregma and 3.8 mm right or left of the midline, with infusions at a depth of 5.0 mm from the skull surface for one site, and 1.2 mm, 3.0 mm, and 5.5 mm, respectively for the other site. Unilateral CeA lesions were made in a similar manner, but using 0.10 μl of 10 mg/ml ibotenic acid (Sigma) in PBS, at coordinates 2.2 mm posterior to bregma, 4.3 mm from the midline, and 8.1 mm ventral from the skull surface at the injection site. The incisions were closed with surgical staples and topical antibiotic ointment was applied to the wound edges. After removal of the stereotoxic apparatus, each rat received a single 0.3-ml subcutaneous injection of 0.02 mg/ml buprenorphine hydrochloride (Sigma) for amelioration of pain, and was allowed to recover from surgery for 7–10 days before beginning behavioral training. Surgery was followed by 10–14 days of recovery before behavioral training. The rats were handled daily during the recovery period.

Behavioral Training Procedures

Once their weights reached 85%, rats were first given about 30 sucrose pellets (45 mg, formula 5TUT, Test Diets, St. Louis, MO) in their home cages, to familiarize them with the reinforcer. Figure 1A provides an outline of the behavioral training procedures. Each training session in each phase of the experiment included 16 trials, distributed across random intertrial intervals, which ranged from 2–6 min (M = 4 min). The rats were first trained to eat sucrose pellets from the recessed food cups, in two sessions, each including 16 unsignaled deliveries of two pellets each. Then, to establish a strong light-tone association during the expectancy phase, all rats received trials consisting of a 10-s light→10-s tone serial com-
pound. In each session of this phase, the light→tone compound was reinforced with two pellets on eight trials and nonreinforced on eight trials, randomly intermixed. After 10 sessions of expectancy training, rats received two surprise-phase sessions. During each surprise session, for rats in the shift condition, prediction error was induced by omitting the tone on the eight nonreinforced trials, while for rats in the consistent group, light→tone expectancies were confirmed by continuation of the expectancy phase protocol. Finally, in each of the five sessions in the test phase, all rats received 16 presentations of the light conditional stimulus (CS) alone followed immediately by two pellets. More rapid acquisition of food-cup conditioned responses (CRs) to the light CS was taken as evidence of enhanced salience of that CS.

**Behavioral Measures**

The primary response measure was the percentage of time spent in the food cup, as assessed by interruption of the infrared photobeam. Trial epochs were defined as a 5-s stimulus-free pre-CS period (immediately prior to the light CS), the first 5 s of the light CS, the second 5 s of the light CS, the first 5 s of the tone CS, and the last 5 s of the tone CS. Conditioned food-cup responding was assessed during the latter half of CS presentations because in that epoch, food-cup CRs are more frequent and less likely to be contaminated by conditioned ORs (e.g., Holland, 1977).

We also used the methods of Holland and Gallagher (1993a) to evaluate the acquisition of conditioned ORs in the test phase in the present experiments. Unfortunately, observation of video recordings of the 24 rats in Replication 2 of the present study yielded little evidence for the performance of ORs to the visual CS in testing in any of the treatment or lesion conditions, so we did not examine ORs further nor report those data.

**Behavioral Data Analysis**

The experiment was conducted in two identical replications, one with 16 rats and one with 24 rats. CRs during the pre-CS, light, and tone (when applicable) periods were each analyzed with separate Session (shift or consistent) × Lesion (contralateral or ipsilateral) × Sessions (when applicable) analysis of variance (ANOVA) followed by post hoc comparisons using the Tukey’s HSD procedure, adjusted for unequal ns. When the within-subject, repeated-measures sessions variable was included, the Greenhouse-Geiser procedure was used to compensate for potential violations of sphericity assumptions.

**Histological Procedures**

After completion of behavioral testing, the rats were deeply anesthetized with isoflurane and perfused intracardially with 0.9% saline followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB) solution. The brains were removed, postfixed, and cryoprotected overnight in 4% paraformaldehyde in 0.1 M PB containing 12% sucrose, frozen with powdered dry ice, and stored at −80 °C. A freezing microtome was used to take 40-μm sections from each brain. For DLS, of every six consecutive sections, the first was Nissl-stained, the second processed for NeuN immunohistochemistry to visualize cell bodies, and the third processed for tyrosine hydroxylase (TH) immunohistochemistry to assess dopamine (DA) innervation. For CeA, every sixth section was stained for Nissl, and for SNc, of each six sections the first was stained for Nissl and the second processed for TH to assess DA cells and fibers. For Nissl staining, the sections were mounted on glass slides, dehydrated in ascending concentrations of alcohol, defatted in xylene, stained with thionin, and coverslipped with Permount.

Standard immunohistochemical protocols (e.g., Lee et al., 2005) were used to visualize TH or NeuN. The primary antibodies used were NeuN antibody (AB153; Millipore, Temecula, CA) and TH antibody (ImmunoStar, Hudson, WI), and the secondary antibody was biotinylated goat antirabbit IgG (Vector, Burlingame, CA). Sections were incubated in avidin-biotin peroxidase conjugate (Vector) and reacted with DAB-NiCl2 to visualize cells immunoreactive for NeuN or TH. Sections were mounted on slides, dehydrated in ascending concentrations of alcohol, and coverslipped with Permount.

**Lesion Evaluation**

The lesions were evaluated from photographs of the Nissl-stained sections at six coronal planes of DLS (+1.70, +1.20, +0.70, +0.20, −0.30, and −0.80 mm relative to bregma) and five planes of CeA (−1.80, −2.12, −2.30, −2.80, and −3.14 mm from bregma). Outlines of the lesion extents were drawn on digital images from Paxinos and Watson (1998) using Adobe Photoshop.
Figure 2 (opposite)
11.0.2. Calculation of the percentage damage to amygdala regions for each section was performed within Photoshop by comparing the area of the lesion within a region’s borders with the area of the region. Lesion area for DLS was the sum of the lesion areas calculated by Photoshop for each section. We did not convert these areas to percentage damage because there are no agreed-on demarcations of the borders of striatal subregions. Finally, the lesion outlines for each rat at each plane were then filled in Photoshop with an opacity of 3% (100 divided by the number of lesions represented) and stacked onto a single atlas section image, such that the darkness of an area reflected the number of lesions that included that area (Figures 2a and 2b).

The extent of TH staining was analyzed using the mean density (pixel brightness) function of NIH ImageJ. For DLS, a 1.2 × 1.6 mm (w × h) region was sampled from DLS sections +1.70, +0.70, and −0.30 in each hemisphere, in each rat. For SNc, each section (+5.30 and +5.80) was first rotated to permit extracting a 2.4 × 0.6 mm (w × h) mm sample that included most of SNc. For each region, overall mean densities for each hemisphere were then calculated for each rat by averaging across section densities. A 1 × 1 mm region was also sampled from visual cortex, to provide a comparison background level.

**Results**

**Histological Results**

Seven of the 40 rats were removed from the study because their lesions of CeA or DLS were judged as too large (n = 1) or too small (n = 6), leaving ns of nine in the shift–contra and shift–ipsi conditions, eight in the consistent–contra condition, and seven in the consistent–ipsi condition. Figures 2a and 2b show the extent of the remaining 33 DLS and CeA lesions, respectively, collapsed across treatment groups and hemispheres. Figures 2c and 2d show representative lesioned and unlesioned Nissl-stained sections of CeA, and Figures 2e–2h show NeuN-stained sections for DLS. Across the four lesion/treatment conditions, DLS lesion areas ranged from 3.06 ± 0.19 mm² to 3.43 ± 0.42 mm² per section. Lesion × Treatment ANOVA showed no significant effects or interactions, p > .376. A small number of DLS lesions extended into portions of dorsocentral (e.g., Cheatwood, Reep, & Corwin, 2003) and/or ventral striatum, but none included significant portions of the dorsomedial striatum nor any portion of the nucleus accumbens. The percentage damage to CeA ranged from 72.7 ± 7.0% to 79.9 ± 6.9% across the four groups, with comparable damage across medial and lateral/capsular subregions; Lesion × Treatment ANOVA showed no significant effects or interactions, p > .741. Some of the CeA lesions extended to basolateral amygdala (BLA); damage to that region ranged from 13.9 ± 4.6 to 17.2 ± 5.4% across the four groups, p > .409. BLA damage estimates are likely inflated because they were calculated only from sections that also included CeA, whereas BLA extends caudally considerably further, and those more caudal sections showed little or no damage. Regardless, damage to BLA was unlikely to contribute to the behavioral deficits reported here, because Holland, Hatfield, and Gallagher (2001) found no effects of much larger, targeted lesions of the BLA on performance in this same task.

The pixel density of TH staining in DLS did not differ between lesioned (44.58 ± 2.39; Figure 2i) and unlesioned (44.47 ± 2.11; Figure 2j) hemispheres, nor across lesion or treatment conditions, ranging from 43.650 ± 4.72 to 45.320 ± 5.90. Thus, although the DLS lesion procedure produced substantial neuron loss, DA fibers, presumably from SNc, were intact. Similarly, the density of TH staining in SNc itself did not differ between lesioned (84.350 ± 3.64; Figure 2k) and unlesioned (83.15 ± 3.68; Figure 2l) hemispheres, nor across lesion or treatment conditions, ranging from 81.030 ± 6.90 to 85.15 ± 6.61. By comparison, TH staining levels in visual cortex (where much lighter TH staining would be expected) ranged from 155.55 ± 11.95 to 161.42 ± 11.64. Separate Replication × Lesion × Treatment × Hemisphere ANOVAs of DLS and SNc densities showed no significant main effects or interactions, ps > .100, with the hemisphere effect and Lesion × Hemisphere interaction Fs(1, 25) < 0.167, ps > .686.

**Behavioral Results**

**Expectancy phase.** In the expectancy phase, in which all rats were treated identically, all rats acquired considerable conditioned food-cup responding to the tone, and showed little food-cup responding to the light (left portion of Figure 1B). As training progressed, responding in the pre-CS intervals decreased, responding to the tone increased, and responding during the light, although varying from session to session, showed no systematic pattern. For each of the three measurement epochs (pre-CS, light, and tone), Replication × Treatment × Lesion × Sessions ANOVAs showed significant main effects of replication (responding was higher in the first replication), Fs(1, 25) > 15.50, ps < .001, and sessions, Fs(9, 225) > 4.59, ps < .001, and a significant Replication × Sessions interaction; the replication differences emerged over training, Fs(9, 225) > 3.45, ps < .003. Most important however, there were no significant main effects of treatment or lesion, or any of their interactions, including those with replication or sessions, for any of the measurement epochs, ps > .171. Similarly, Replication × Treatment × Lesion ANOVAs of performance over the final two training sessions alone showed only significant main effects of replication for all three epochs, Fs(1, 25) > 8.64, ps < .007; other ps > .091. Thus, conditioning proceeded similarly in all treatment/lesion groups, and all groups entered the surprise phase with similar levels of responding.

**Surprise phase.** Responding during the surprise phase was maintained as in the expectancy phase. The center portion of Figure 1B shows the percentages of time spent in the food cup during the pre-CS, light, and tone measurement epochs in the two surprise phase sessions. For each of the three measurement epochs, Replication × Treatment × Lesion ANOVAs showed only the main effect of replication to be significant, Fs(1, 25) > 10.02, ps < .005; other ps > .117. Thus, all treatment/lesion groups entered the test phase with similar responding.

**Test phase.** The right portion of Figure 1B shows the primary data of this study, the acquisition of food-cup responding to the light during the test phase. Among rats with ipsilateral lesions of CeA and DLS, those in the shift condition showed faster learning to the light than rats in the consistent condition, as is typically observed in intact rats. No such shift advantage was seen in rats with contralateral lesions. These assertions are supported by the results of a Replication × Treatment × Lesion × Session
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ANOVA, which showed significant main effects of replication, $F(1, 25) = 23.11, p < .001$, and session, $F(4, 100) = 60.65, p < .001$, but more importantly, a significant Treatment $\times$ Lesion interaction, $F(1, 25) = 7.10, p = .013$. Post hoc Tukey’s HSD tests exploring this interaction showed that responding in the shift-ipsilateral rats was significantly greater than responding in each of the other three groups, $ps < .047$, which did not differ from each other, $ps > .781$. The Replication $\times$ Treatment $\times$ Sessions interaction was also significant, $F(4, 100) = 7.24, p < .001$. A comparable ANOVA of pre-CS responding showed only significant main effects of replication, $F(1, 25) = 32.04, p < .001$, and sessions, $F(4, 400) = 3.41, p = .012$, and a significant Replication $\times$ Sessions interaction, $F(4, 100) = 3.99, p = .005$.

General Discussion

Rats with unilateral damage to CeA and DLS in the same hemisphere, but with both structures intact in the other hemisphere, showed surprise-induced enhancement of the salience of the visual CS. In the test phase, rats in the shift–ipsi condition acquired greater conditioned food-cup responding to that cue than rats in the consistent–ipsi condition. By contrast, rats with contralateral “disconnection” lesions of those two regions failed to show those enhancements: Rats in the shift–contra group acquired lower levels of CRs, comparable with those found in the consistent–contra and consistent–ipsi rats. This result suggests that convergence of information processing by CeA and DLS is needed for surprise-induced enhancements in cue salience. Notably, our results are not easily explainable in terms of the sensory neglect sometimes observed with unilateral lesions of dorsal striatum (e.g., Carli, Jones, & Robbins, 1989; Van Vleet, Burcham, Corwin, & Reep, 2000), because all of our rats received equivalent unilateral DLS lesions, but only those in the shift condition showed learning deficits.

Perhaps the simplest conjecture for the route by which information processing by CeA and DLS converges is that information is processed in CeA and passed to DLS via CeA-SNc and SNc-DLS projections. Notably, Lee et al. (2006) showed that rats with contralateral lesions of CeA and SNc also failed to show salience enhancements in this task. Furthermore, the lack of any noticeable loss in SNc’s dopaminergic innervation of DLS in the present study suggests the processing deficit observed here was produced by damage to DLS itself, rather than to indirect damage to SNc due to removal of some of its major inputs and/or outputs in CeA and DLS.

Data from experiments that transiently perturbed neural function in CeA or DLS pharmacologically suggest that the distribution of processing functions of these regions is comparable for conditioned ORs and surprise-induced salience enhancements. Integrity of CeA function was required only when conditioned ORs were initially acquired or at the time of surprise in the serial prediction task, and not when previously established ORs were performed or when increased cue salience was expressed in test (Holland & Gallagher, 2006; McDannald et al., 2004). By contrast, DLS function was critical only for the expression of conditioned ORs or of increased cue salience, and was unnecessary when the cue–reinforcer associations that underlie conditioned ORs were originally established or at the time of surprise in the serial prediction task or (Asem et al., 2015; Han et al., 1997). Thus, CeA and DLS play important and complementary roles in both of these aspects of attentional change in associative learning, which differ in several other ways (Holland & Maddux, 2010). It is tempting to suggest that DLS may serve as a common path for expression of many examples of enhanced processing of sensory cues. Our data (together with those of Asem et al., 2014) suggest that expression of associability changes may require convergence of enhanced associability produced and encoded elsewhere (e.g., Schiffino et al., 2014) with enhanced sensory drive provided by DLS processing.

DLS is frequently assigned a major role in orienting and sensory-motor integration in general, but typically the motor end of this continuum is emphasized. For example, recording studies suggest DLS mediation of head, neck, and other motor movements but not of the detection of visual stimuli (e.g., Pawlak et al., 2010; Reig & Silberberg, 2014; Root et al., 2010). Observations that DLS function is critical when attentional modifications are expressed and not when they are acquired are consistent with that general view. However, it is not obvious how the expression of enhanced cue salience in more rapid learning of food-cup responding can be easily construed as a simple motor-related function of DLS. It is easy to see how the expression of cue salience would be enhanced through the test phase in the serial prediction task, the previously inactivated rats in the shift condition continued to lag control rats in that condition. This finding implies that DLS inactivation in test did not merely block the expression of higher levels of conditioned responding to the test cue, but instead impaired learning about that cue. Thus, DLS was critical to the expression of the altered cue salience itself at the time of new learning, not simply to the expression of motor aspects of conditioned responding.

The relation of the present findings, which link DLS and CeA functions in surprise- and associability enhancements, to previous findings that show the expression of these enhancements to depend on the functional integrity of SI (Holland & Gallagher, 2006) and PPC (Schiffino et al., 2014), remains a subject of speculation. For example, cholinergic projections from SI to PPC are known to influence performance in the serial prediction task (Bucci et al., 1998), and PPC projects to the dorsocentral striatum (Reep & Corwin, 2009), which in turn innervates the substantia nigra pars reticulata (SNr), another key output structure of the basal ganglia (Antal et al., 2014; Tulloch et al., 1978). Likewise, SNr innervates thalamic nuclei that in turn project back to PPC (Deniau & Chevalier, 1992; Sakai & Bruce, 2004; Sakai et al., 1998). A network involved in the expression of enhanced cue salience in faster learning may be quite extensive, encompassing amygdalo-nigro-striatal and thalamo-striato-cortical loops.

References


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