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Recent encounters with rattlesnakes enhance ground squirrel responsiveness to predator cues

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Abstract

A diverse range of prey taxa exhibit stereotyped antipredator behaviors when confronting live predators. Predator cues also elicit antipredator responses, and previous research indicates that prey possess mechanisms to discriminate between the relative risk posed by particular predator cues, which mediates their investment in antipredator behaviors (i.e., "threat-sensitive" behavior). However, the salience of such predator cues may change according to perceived predation risk, which can vary temporally or spatially. We hypothesized that prey modify responses to predatory cues according to their recent experience with predators, which would prime prey to attend to predator-related cues. In the present study, we found that recent encounters with live tethered rattlesnakes increased perceived predation risk in free-ranging California ground squirrels (*Otospermophilus beecheyi*), leading to concomitant increases in the intensity of antipredator behaviors displayed toward a predator model. The increase in antipredator behavior following snake encounters was higher toward the predator model than a novel object, suggesting that squirrels exhibit threat-sensitive responses toward objects resembling predators. However, squirrels did not treat either of these objects with caution, nor did they differ in antipredator behavior, when presented with the model or novel object when no live snake was encountered. This suggests that increased vigilance following predator encounters modulates responses to predator cues. Such increases in antipredator behavior could assist in detecting nearby predators quickly but could also have indirect effects on prey.

Significance statement

Underresponding to predators could lead to death, while overresponding could reduce time spent on other fitness-related activities. Prey balance these costs by using environmental cues to modify antipredator responses. Detecting a live predator in the environment could indicate that the chance of predation is high, which may enhance preys' antipredator responses. We show that ground squirrels significantly enhance antipredator behaviors toward snake models and novel objects after encountering a live tethered rattlesnake predator. However, the predator model elicited greater antipredator behavior than the novel object following snake encounters, suggesting that squirrels are primed to attend to predator-related cues in this context. Both objects were treated similarly, and with less caution, when no snake was detected. Vigilance following predator interactions therefore mediates threat-sensitive responses to subsequent predator cues.

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Introduction

Predators exert strong selective pressures on animals, through both direct mortality and indirect effects of perceived predation risk (Abrams 2000; Preisser et al. 2005; Travers et al. 2010). Animals failing to respond to stimuli indicating predation risk may be less likely to survive, but overresponding can also incur costs. For example, when prey display antipredator behavior toward inappropriate stimuli, they inevitably expend time and energy that could be used for other fitness-related activities (Lima and Bednekoff 1999a). Previous research has shown that prey are sensitive to varying degrees of predation risk (Lima and Dill 1990; Sih 1992), and risk allocation theory predicts that prey will respond to differences in risk by trading off their relative investment in antipredator behavior against other activities, such as foraging and reproduction (Lima and Bednekoff 1999b; Ferrari et al. 2009).

An increase in antipredator behavior, when appropriate, will likely enhance the probability of survival. For instance, lizards that respond strongly to snake predator scent are more likely to survive subsequent snake encounters (because of decreased activity and mobility) than less-responsive lizards (Downes 2002). However, overresponding in this fashion may limit foraging or reproductive opportunities. The "threat-sensitive hypothesis" therefore states that the intensity of a prey's antipredator responses will be commensurate with the degree of risk posed by a particular set of predatory cues (Helfman 1989; Helfman and Winkelman 1997). As such, prey responding with threat-sensitive behavior toward predator cues should display antipredator responses that vary with the magnitude of perceived predation risk.

The salience of predator cues used to modify antipredator responses may not be constant, as predation risk varies contextually and across space and time (Lima and Dill 1990; Lima and Bednekoff 1999b). Time of day/year (Bosiger et al. 2012), ambient temperature (Riessen 2015; Pink and Abrahams 2016), and presence of conspecifics (Elgar 1989; Roberts 1996) or heterospecifics (Hughes et al. 2010; Hamer et al. 2011) are known to modify perceived predation risk and subsequent investment in antipredator behavior. All else being equal, when perceived risk is high, prey might be more likely to respond to predator cues, even if their initial responses to such cues were weak. For instance, tadpoles do not respond to predatory visual cues alone but require additional chemical cues of consumed conspecifics to elicit predator avoidance behavior (Mogali et al. 2012). Similarly, these chemical alarm cues at low concentrations do not elicit strong antipredator responses in fish, but when coupled with the sight of a visually alarmed conspecific, they significantly increase the intensity of antipredator behavior (Brown et al. 2004). In addition, non-lethal interactions with predators might prime prey to respond in ways that increase the chances of avoiding predators during successive encounters. Thus, prey may use recent encounters with predators to update their vigilance and sensitivity (i.e., perceived risk) toward successive predator cues to manage potentially costly antipredator behaviors. Such a mechanism is comparable to perceptual priming described in the human psychology literature (reviewed in Wasserman and Zentall 2009), whereby an object that is seen and recognized primes the subject to more easily recognize it (or a reduced set of its perceptual cues) again.

It remains unclear whether recent encounters with live predators mediate the expression of antipredator behaviors toward subsequent predator cues, particularly in natural settings (i.e., in completely free-ranging individuals). Since it is usually logistically difficult to control the behavior of predators in the wild, most studies expose prey to indirect predator cues, such as predator vocalizations (e.g., MacLean and Bonter 2013; Hettena et al. 2014), predator scent or odors (Apfelbach et al. 2005), or alarm cues composed of conspecific body fluids and/or predator odors in aquatic prey (e.g., Atherton and McCormick 2015; Preston and Forstner 2015; Stephenson 2016). However, due to the multimodal nature of predator recognition, studies implementing naturalistic interactions with live predators are also critical for answering questions regarding threat-sensitive behaviors in prey (Lima 2002). In the present study, we determined whether recent interactions with live rattlesnake predators (*Crotalus oreganus*) increase free-ranging California ground squirrels' (*Otospermophilus beecheyi*) assessment of predation risk and their subsequent sensitivity to predator cues.

Ground squirrels are ideal for investigating threat-sensitive behavior in vertebrates because their relatively small home ranges (Boellstorff and Owings 1995) facilitate both experimental approaches and longitudinal observations on free-ranging individuals. Rattlesnakes are sit-and-wait ambush predators that hide and hunt in cover, waiting for prey to come within strike range (Clark et al. 2012). They are cryptic in both coloration and habits (Fitch 1949) and use rapid strikes to envenomate prey (Hennessy and Owings 1988; Goldthwaite et al. 1990). Rattlesnakes often occupy ground squirrel burrows which leads to frequent natural interactions with squirrels (Barbour and Clark 2012; Putman et al. 2016), making them responsible for high predation rates in many California ground squirrel populations (Fitch 1949). Consequently, squirrels must face the constant threat of predation from a cryptic predator that can be found throughout most of their environment.

Because of this strong predation pressure, California ground squirrels have evolved an arsenal of "antisnake" behaviors including tail flagging (repeatedly waving the tail from side to side), tail piloerection (conspicuous bristling of tail fur), substrate throwing, and close-range investigation of snakes (Owings and Coss 1977; Hennessy et al. 1981; Rowe and Owings 1990), all of which deter snakes from striking and lead them to move to new hunting areas (Barbour and Clark

2012; Putman et al. 2015). Ground squirrels display these behaviors when confronting snakes (Owings and Coss 1977) and conspicuously increase their vigilance for considerable amounts of time following snake encounters (at least up to 1 h; Putman and Clark 2015). The continued expression of antisnake behaviors following interactions with snakes allowed us to ask whether recent rattlesnake encounters enhance the responses of squirrels toward predator cues in a threat-sensitive manner. We directly tested this hypothesis in a two-part experiment in which we compared the responses of free-ranging California ground squirrels to predator models and novel objects after they had interacted with a live rattlesnake. If recent encounters with rattlesnakes increase perceived risk and sensitivity to predator cues, we predicted that (1) squirrels that had recently encountered a rattlesnake would display greater antisnake behaviors than squirrels without a recent rattlesnake interaction; and (2) heightened antisnake behaviors would be greater in response to predator cues than to novel stimuli (i.e., threat-sensitive).

Methods

Study site

The study was conducted from May to July 2011–2013 at the Blue Oak Ranch Reserve (BORR), a University of California ecological reserve perched at ca. 760 m on the west slopes of the Diablo Mountain Range in northern Santa Clara County, California. The habitat is characterized by steep to moderate hills covered by a mixed oak woodland landscape. Initial surveys of the area confirmed dense populations of northern Pacific rattlesnakes, with our estimates indicating that rattlesnake densities could exceed 30 rattlesnakes per hectare within ground squirrel colonies. Thus, squirrels could potentially encounter snakes daily at this site.

Trapping and identification

Trapping and marking methods followed those of Owings et al. (2001). We trapped free-living California ground squirrels throughout the study site with Tomahawk live traps baited with black oil sunflower seeds. For long-range identification of individuals during each field season, squirrels were uniquely marked with temporary Nyanzol pelage dye while anesthetized with ketamine HCl (40 mg/kg injected intramuscularly). While anesthetized, squirrels were also measured for various morphological traits, weighed, sexed, and marked with metal ear tags for permanent identification. We released all squirrels where they were captured following recovery from anesthesia within the same day of capture.

Rattlesnake collection and tethering procedures

Live tethered rattlesnakes were used to elicit ground squirrel antisnake behaviors. We used a total of six snakes in this study: three were long-term captive rattlesnakes and the remaining three snakes were wild-caught within the study site at BORR. All snakes were large adult males (> 500 g). Our tethering procedure was a modification of that used by Randall and Matocq (1997). Briefly, snakes were equipped with three removable bands on their backs. Placed medially on the body and equidistant from one another, each band was made with athletic tape containing a fixed loop of string that could provide an attachment point for a tether line. Three tent stakes were then outfitted with short monofilament tether lines from which the loop of string on the bands could be fastened. After driving the stakes into the ground in a triangle shape roughly 15 cm equidistantly apart, we wrapped the snake around the three stakes in a coiled configuration. This tethering procedure allowed us to experimentally place live rattlesnakes in semicoiled postures at specific locations near the home burrows of marked squirrels. Although tethered snakes may not be viewed by squirrels as dangerous as hunting free-ranging snakes, which utilize a tightly coiled body posture, they still elicited strong antipredator responses from squirrels. Snakes did not typically exhibit defensive behaviors (rattling or striking) while tethered, similar to the free-ranging snakes we have recorded being harassed by free-ranging squirrels (Barbour and Clark 2012).

All snakes were kept at the BORR field station during the experimental period. They were housed in glass terraria with either paper towel or newspaper substratum, and were given a hide box and water ad libitum. Snakes were not fed during the study because food items in their stomachs interfere with the tethering process. It is normal for rattlesnakes to fast for several weeks to sometimes months (McCue 2007). At the end of the study period, long-term captive snakes remained in captivity, while snakes captured from BORR were released at their place of capture.

Baseline observations

We established feeding areas (hereafter called bait stations) near the home burrows of marked squirrels and habituated individual squirrels to feed at these locations by placing a small handful of black oil sunflower seeds within a metal ring. Once a squirrel was seen feeding at a bait station, we made baseline observations of its behavior from behind a camouflaged hunting blind (at least 10 m away). We began observations as soon as the squirrel reached the bait station, and squirrels were allowed to feed undisturbed until they left or for up to 10 min maximum. Baseline observations were used to ensure that the squirrel was not exhibiting behaviors indicative of a recent unaccounted rattlesnake interaction (as

in Putman and Clark 2015). We monitored multiple bait stations throughout the study site and started observations at approximately 0700 hours, before snakes emerge from their nighttime refuges (Putman et al. 2016), so we are confident we were able to determine whether a squirrel had encountered a snake the day of its trial. Any squirrels that exhibited tail flagging or head bobbing (see below) during baseline observations were excluded from further testing that day. We removed one squirrel based on its behavior during the baseline. Following baseline observations, we presented experimental stimuli. We conducted two experiments on free-ranging squirrels, which will be referred to as "nonprimed" and "snake-primed" experiments henceforth.

Experiment 1: nonprimed squirrels

In this experiment, squirrels that had not been primed by interacting with a snake (i.e., squirrels without recent rattlesnake encounters) were tested to quantify threat-sensitive responses to various stimuli. We presented one of three stimuli to focal squirrels returning to a bait station after the baseline observation: (1) a live tethered rattlesnake, (2) a rattlesnake model, or (3) a novel object consisting of a green 1-L sports canteen. Using a handheld video camera (Sony® Handycam, model DCR-SR 85), we recorded the reaction of squirrels to these stimuli until they left the area or for up to 10 min maximum. The rattlesnake model was used to represent a reduced set of predator cues. Four models were used in total, and all were made of plaster and painted to resemble a northern Pacific rattlesnake. The models were stored each night in terraria housing live captive rattlesnakes to mimic odors of live snakes. Models thus had snake scale pattern and coloration, a tightly coiled body configuration, snake odor, but lacked behaviors such as tongue flicking and body movements. The sports canteen allowed us to determine whether antisnake behaviors are also elicited by a novel object that does not contain snake-like cues. California ground squirrels respond to inanimate objects that resemble snakes or their patterns, including stones, sticks, and rulers (Coss and Owings 1985; Coss 1991), but no studies have determined whether they exhibit antisnake behaviors toward nonsnake-like objects. For these nonprimed experiments, we predicted that live rattlesnakes would evoke the greatest antisnake behaviors in ground squirrels, followed by the snake model and then the novel object.

Experiment 2: snake-primed squirrels

In this experiment, we planned two additional treatments to determine how the responses of squirrels to the rattlesnake model and novel object would differ after they had been "primed" by interacting with a live rattlesnake. Squirrels will continue to display antisnake responses following snake encounters regardless of whether a snake or other object is present, and a past study has shown that tail flagging following snake encounters indicates squirrel vigilance and readiness to evade a surprise attack (Putman and Clark 2015). Here, we tested whether rattlesnake interactions enhance responses toward an object that resembles a predator, as compared to one that does not. During these experiments, baseline observations were always followed by a live tethered rattlesnake presentation. Squirrels were allowed to interact with the rattlesnake until they left the area or for 10 min at most, after which the snake was removed and replaced by either a rattlesnake model or novel object. These objects were placed in a different location than the live snake, but within 1 m of where the live snake was tethered. Upon returning to the bait station, squirrels directed antisnake behaviors toward both the previous location of the snake and the experimental object. We only counted behaviors displayed toward the experimental objects in an attempt to distinguish responses toward objects from responses toward the previous location of the snake. We recorded squirrels' interaction with the experimental object for 10 min.

Presentation of experimental treatments

Experiments were conducted daily during the study period from 0800 to 1600 hours. All squirrels were assigned a particular experiment type (i.e., nonprimed or snake-primed) for the day, and experiment type and treatment type were counterbalanced to control for order effects. Squirrels assigned to the nonprimed experiment were presented with only one treatment (i.e., rattlesnake, model, or novel object presentation). Squirrels assigned to the snake-primed experiment were first shown the live tethered snake followed by a stimulus presentation of either snake model or novel object. Squirrels were retested under a different experiment/treatment only after at least 24 h had elapsed. Since ground squirrels often encounter snakes daily (Putman et al. 2016), we were confident in the ecological relevance of the time frame used for sequential treatment presentations. Rattlesnakes or snake models used in our experiments were selected randomly each day; a rattlesnake was selected for testing only if at least 48 h had elapsed since the previous time it had been used (to reduce stress on the snakes). We attempted to expose each individual squirrel to all treatments in each experiment, but because these squirrels were free-ranging, some individuals could not be relocated after participating in initial trials.

Behavioral measurements

We quantified ground squirrel behaviors from video recordings. We counted the number of tail-flagging bouts, number of head-bobbing events, minutes spent investigating the stimulus (investigation time), number of substrate throws (pushing dirt

toward the stimulus), number of alarm calls, occurrence of tail piloerection (y/n), and close proximity to the snake or object (y/n). Close proximity was defined as whether or not the squirrel came within 30 cm of the snake or stimulus, since the average strike distance of rattlesnakes is approximately 30 cm (Clark et al. 2012). We used ImageJ to estimate distance within video stills.

The various antisnake behaviors we considered followed the original descriptions of Owings and Coss (1977). However, the head-bobbing behavior exhibited during rattlesnake interactions has not been formally quantified in previous studies. Thus, we defined head bobs as the rapid, repeated lifting and lowering of the head while staring in the direction of the stimulus. Since virtually every independent head-bobbing event was of similar duration (2–5 s), head bobs were counted as events that were separated by at least 2 s of inactivity. We quantified this behavior because it was one of the most consistent behaviors that ground squirrels exhibited toward live rattlesnakes.

Our counts of tail flags and head bobs were highly correlated (Pearson's $r = 0.92$), so rather than analyze these variables independently, we combined them into a single measure. Thus, "snake-directed behavior" in our models was defined as the summation of all tail flags and head bobs directed toward the stimulus. A recent study demonstrated that tail flagging can function as a signal of vigilance in California ground squirrels (Putman and Clark 2015). Hence, the combined frequency of tail-flagging and head-bobbing behavior (i.e., snake-directed behavior) exhibited during a given experimental trial could indicate a squirrel's level of vigilance.

Statistical analyses

The mean number of trials per squirrel was 2.8. Six squirrels were tested under all five treatments in both experiments and 12 were only tested once. Within a given year, the maximum number of days between treatments for an individual was 8, with an average ± standard deviation of 2.1 ± 1.9 days between treatments. Additionally, five individuals were tested in different years, with between 360 and 395 days between treatments. Because most squirrels participated in more than one treatment in both experiments, we used generalized linear mixed models (GLMMs) fit by the Laplace approximation (lme4 package in R, version 3.0.9) to analyze the effects of our treatments on ground squirrel antipredator behaviors. We included squirrel identity as a random effect in all models, allowing individuals to have both random slopes and intercepts. Prior work has shown that females are more responsive toward snakes than males (Swaisgood et al. 2003). However, because we had considerably fewer male squirrels in our sample ($N_{males} = 7$, $N_{females} = 26$), we elected to not examine the behavior of males and females separately. We combined gender together because of low sample sizes and because this was

the more conservative statistical approach (that is, males should be less responsive).

For experiment 1, the nonprimed experiment, we evaluated the effect of treatment (type of stimulus presented) on four response variables: snake-directed behavior, investigation time, tail piloerection (y/n), and close proximity (y/n). We used separate GLMMs with a Poisson distribution and log link function for data that were not overdispersed (i.e., when mean and variance were similar). We used lognormal Poisson GLMMs for overdispersed data by incorporating an additional observation level random effect (Bolker et al. 2009). Binomial GLMMs were used to model tail piloerection and close proximity to stimulus. We compared treatment levels in models using Wald's Z tests. Because we had three treatment levels (novel object, rattlesnake model, and rattlesnake), we used Tukey post hoc tests implemented in the multcomp package in R to conduct pairwise comparisons among the three treatments.

For experiment 2, the snake-primed experiment, we used planned comparisons to determine the responses of squirrels to models and novel objects after they had been primed with a live rattlesnake, and to evaluate whether these responses differed from those in the nonprimed experiment. We planned three comparisons: (1) responses to snake models of primed squirrels (experiment 2) versus the responses to snake models of nonprimed squirrels (experiment 1), (2) responses to novel objects of primed squirrels (experiment 2) versus responses to novel objects of nonprimed squirrels (experiment 1), and (3) responses of primed squirrels to snake models versus responses of primed squirrels to novel objects (both experiment 2). Because each comparison involved two treatments, we conducted a single GLMM per comparison per response variable (snake-directed behavior, investigation time, tail piloerection) as above to evaluate the effect of treatment. We did not include close proximity as a response variable in these comparisons because we found no differences in this response in the nonprimed experiments (i.e., nearly all squirrels approached within 30 cm of the stimulus).

Although alarm call vocalizations and substrate throwing are behaviors elicited by snakes (Owings and Coss 1977; Owings et al. 1977), our counts for these variables were extremely low. Across all trials, only 13.8% of all squirrels alarm called and 10.8% threw substrate. Consequently, we did not analyze these behaviors statistically.

Results

Nonprimed experiment

We conducted 19 live rattlesnake ($N_{male} = 4$, $N_{female} = 15$), 21 rattlesnake model ($N_{male} = 7$, $N_{female} = 14$), and 19 novel object ($N_{male} = 5$, $N_{female} = 14$) presentations. Following
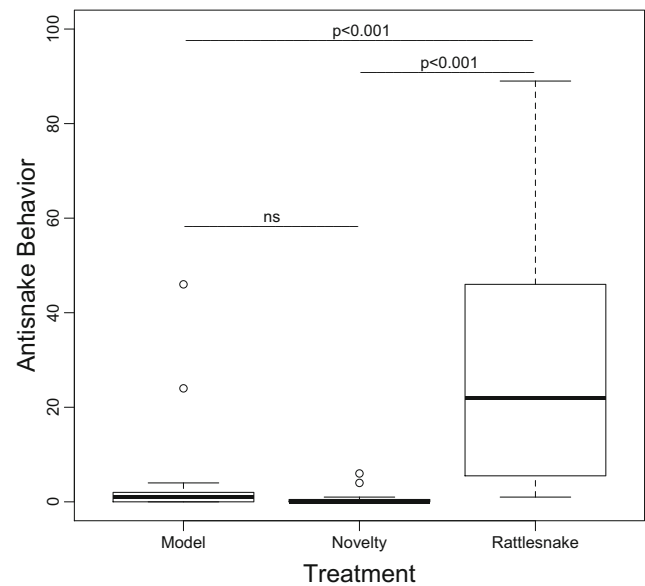
baseline observations, squirrels generally returned and encountered the stimulus within 25 min and no more than 1 h (mean ± SD, 26.6 ± 12.9 min). Squirrels displayed significantly greater snake-directed behaviors toward live rattlesnakes than snake models or novel objects (both $P < 0.001$, Table 1), yet there was no difference in snake-directed behaviors between snake model and novel object presentations ($P = 0.331$, Fig. 1, Table 1). Additionally, squirrels spent significantly more time investigating rattlesnakes than snake models or novel objects (both $P < 0.001$, Table 1) but showed no difference in investigation time between models and novel objects ($P = 0.346$, Fig. 2, Table 1). Live rattlesnakes also elicited significantly greater tail piloerection than snake models ($P = 0.018$) and novel objects ($P < 0.001$), but squirrels displayed only a marginal difference in tail piloerection when comparing snake model and novel object presentations ($P = 0.095$, Table 1). Finally, squirrels showed no differences in their tendency to closely approach live rattlesnakes, snake models, and novel objects (Table 1).

Snake-primed experiment

We conducted 19 rattlesnake model ($N_{male} = 5$, $N_{female} = 14$) and 19 novel object ($N_{male} = 3$, $N_{female} = 16$) presentations on squirrels that had recently interacted with a live tethered

Fig. 1 Comparison of snake-directed behaviors toward different stimuli for the nonprimed experiments. Snake-directed behaviors were defined as the sum of all tail-flagging and head-bobbing events directed toward the stimulus. Boxplots indicate the median value, the interquartile ranges, and the lowest and highest data points within 1.5 of the interquartile range of the lower and upper quartile, respectively (the whiskers). Outliers are shown as open circles (two outlying data points with values of 121 and 148 in the rattlesnake treatment are not shown in order to limit the y-axis to a range of 90)

Table 1 Results of GLMM models of squirrel behaviors in the nonprimed experiment, with Tukey contrasts for comparing all treatments to each other using the *multcomp* package

	Estimate	SE	Z	P
Snake-directed behavior				
Intercept	2.75	0.35	7.97	< 0.001
Model/snake	3.43	0.69	4.97	< 0.001
Novelty/snake	4.55	0.78	5.87	< 0.001
Novelty/model	1.12	0.79	1.42	0.330
Time spent investigating				
Intercept	4.72	0.22	21.52	< 0.001
Model/snake	3.16	0.62	5.87	< 0.001
Novelty/snake	4.37	0.82	1.42	< 0.001
Novelty/model	1.21	0.88	1.38	0.346
Tail piloerection				
Intercept	2.51	0.87	2.88	0.004
Model/snake	2.63	0.98	2.69	0.018
Novelty/snake	4.38	1.10	3.97	< 0.001
Novelty/model	1.75	0.85	2.07	0.095
Proximity				
Intercept	0.11	0.46	0.23	0.819
Model/snake	0.50	0.68	0.73	0.745
Novelty/snake	0.44	0.66	0.65	0.789
Novelty/model	0.07	0.69	0.09	0.995

rattlesnake (i.e., snake-primed). Squirrels' return rates to the bait station following snake encounters were similar to those in the nonprimed experiment, typically within 25 min and no more than 1 h (mean ± SD, 24.5 ± 14.8 min). To determine whether recent rattlesnake encounters enhanced antipredator behaviors to snake-like cues, we compared the responses of snake-primed and nonprimed squirrels to rattlesnake models. Compared to nonprimed individuals, snake-primed squirrels significantly increased snake-directed behaviors toward rattlesnake models ($\beta \pm SE = 1.65 \pm 0.65$, $Z = 2.54$, $P = 0.011$, Fig. 3). Snake-primed squirrels did not increase their time spent investigating rattlesnake models relative to nonprimed squirrels ($\beta \pm SE = 1.07 \pm 0.68$, $Z = 1.58$, $P = 0.114$). Similarly, tail piloerection toward snake models did not differ between snake-primed and nonprimed squirrels ($\beta \pm SE = 1.01 \pm 0.71$, $Z = 1.42$, $P = 0.156$).

To determine whether recent rattlesnake encounters enhanced antipredator behaviors to nonpredator cues, we compared the responses of snake-primed and nonprimed squirrels to a novel object. Snake-primed squirrels displayed significantly greater snake-directed behaviors toward novel objects than nonprimed squirrels ($\beta \pm SE = 2.08 \pm 0.88$, $Z = 2.36$, $P = 0.018$, Fig. 3). However, snake-primed squirrels did not spend significantly more time investigating novel objects than nonprimed squirrels ($\beta \pm SE = 1.26 \pm 0.77$, $Z = 1.63$, $P = 0.103$), but did display significantly greater tail
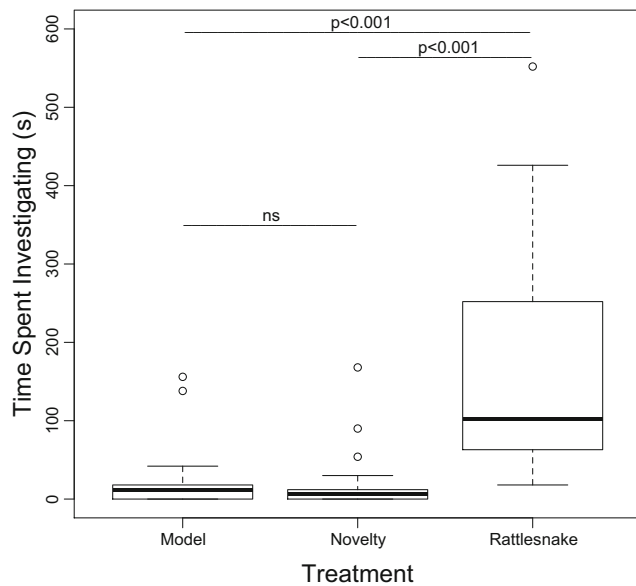
Fig. 2 Comparison of investigation times toward different stimuli for the nonprimed experiments. Boxplots indicate the median value, the interquartile ranges, and the lowest and highest data points within 1.5 of the interquartile range of the lower and upper quartile, respectively (the whiskers). Outliers are shown as open circles

piloerection than nonprimed individuals ($\beta \pm SE = 1.99 \pm 0.78$, $Z = 2.55$, $P = 0.011$).

Finally, we compared the responses of snake-primed squirrels to rattlesnake models and novel objects to determine whether squirrels exhibited threat sensitivity in responding to predator cues. Indeed, snake-primed squirrels significantly increased snake-directed behavior toward snake models than novel objects ($\beta \pm SE = 1.41 \pm 0.45$, $Z = 3.14$, $P = 0.002$, Fig. 3). However, snake-primed squirrels showed no differences in their time spent investigating snake models or novel objects ($\beta \pm SE = 0.36 \pm 0.40$, $Z = 0.889$, $P = 0.374$), or in their tendency to exhibit tail piloerection toward either stimulus ($\beta \pm SE = 0.59 \pm 0.71$, $Z = 0.828$, $P = 0.407$).

Discussion

Recent encounters with a live predator evoked heightened states of vigilance that appeared to mediate sensitivity to predator cues (i.e., snake model). Without the influence of a recent rattlesnake encounter, ground squirrels exhibited minimal antisnake behaviors toward snake models and nearly none toward novel objects (Fig. 1). In contrast, squirrels that had recently encountered a live tethered rattlesnake significantly increased antisnake behaviors toward both snake models and novel objects. Their responses toward snake models were also greater relative to novel objects (Fig. 3), albeit the magnitude of increase was small compared to encounters with the live tethered snake (Fig. 1). Coss and Owings (1985) reported that

the perception of snake-like features (e.g., rounded stones, curled leaves, and sticks) triggered investigative behavior and tail flagging in captive California ground squirrels. Previous studies have also shown that microhabitat features indicative of snake predation risk (e.g., bushy, occluded areas) can elicit antipredator behaviors in other rodent species (Hennessy et al. 1981; Kotler et al. 1993; Wasko et al. 2014). Yet, in our study, antisnake behavior toward predator cues was dependent on whether or not squirrels had recently encountered a live rattlesnake in the area. Taken together, our results suggest that threat-sensitive behavior is not only in response to the degree of risk posed by predator cues (Helfman 1989; Helfman and Winkelman 1997), but also depends on the context in which those cues are experienced (i.e., level of perceived predation risk).

Increased vigilance and focal attention after experiencing the live predator seems to mediate discrimination of predator cues from other stimuli, as evidenced by the greater snake-directed behaviors displayed toward snake models than novel objects after interactions with live rattlesnakes. Our findings underscore the importance of recent predator encounters in mediating antipredator behaviors, and that prey may possess perceptual mechanisms by which to update or manage their sensitivity to potential risk. Such perceptual priming modifies attention in pigeons and jays, and subsequently the search image used to react to a "target," which improves visual search
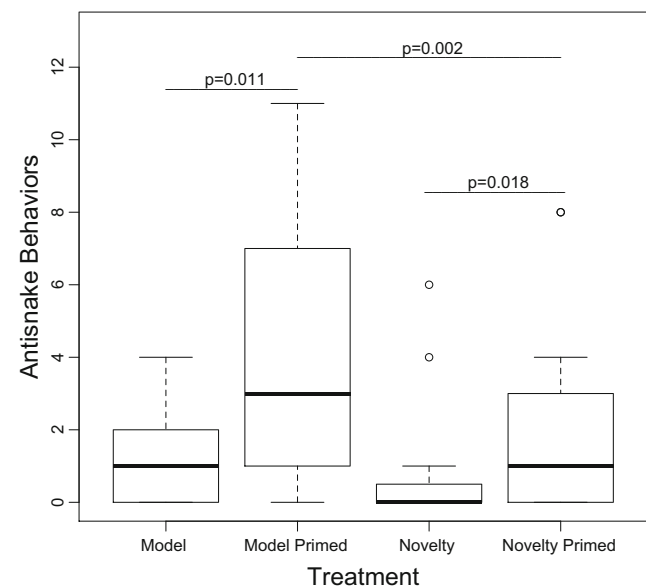
Fig. 3 Comparison of snake-directed behaviors toward models and novel objects in both primed and nonprimed experiments. Snake-directed behaviors were defined as the sum of all tail-flagging and head-bobbing events directed toward the stimulus. Boxplots indicate the median value, the interquartile ranges, and the lowest and highest data points within 1.5 of the interquartile range of the lower and upper quartile, respectively (the whiskers). Outliers are shown as open circles (two outlying data points with values of 24 and 46 in the model treatment and one outlying value of 22 in the model primed treatment are not shown in order to limit the y-axis to a range of 13)

efficacy for the "target" (Blough 1989, 1991; Goto et al. 2014). Although perceptual priming is typically studied in the context of predators searching for cryptic prey in heterogeneous environments (e.g., birds for insects on a background), such priming can in all likelihood also improve preys' search images for hidden predators. In support of this, studies have found that fish exposed to chemical alarm cues are subsequently more responsive to predatory visual cues (Brown et al. 2004; Stephenson 2016), and thus are alerted to attend to other sensory modalities. Additionally, rabbits that are alerted to olfactory cues of predatory fox increase investigative behavior, demonstrating that wariness of a potential nearby predator can cause visible shifts in antipredator behaviors or their intensity (Monclús et al. 2006).

It is important for ground squirrels to attend to snake-like cues after they have discovered a snake because rattlesnakes frequently move to new ambush locations within a few meters of their previous one once they have been detected by squirrels (Barbour and Clark 2012; Putman et al. 2016). Tail flagging additionally increases vigilance in nearby conspecifics (Hersek and Owinsgs 1993); therefore, sustained vigilance toward snake-like cues after a recent rattlesnake encounter may facilitate detection of relocated snakes that remain close by. Even if ground squirrels are uncertain about the presence of a snake, upregulating antisnake behaviors may still dissuade rattlesnake attacks by advertising vigilance and readiness for an attack. Vigilant squirrels that display tail flags are better at avoiding surprise attacks than nontail-flagging squirrels (Putman and Clark 2015). This is potentially why we saw an increase in antisnake behavior toward novel objects when squirrels were primed with a live snake.

Snake-primed squirrels displayed greater antisnake behavior and tail piloerection toward novel objects than squirrels in the nonprimed experiments. It is notable that the novel object elicited significant increases in tail piloerection after a rattlesnake encounter, whereas the snake model did not (primed versus nonprimed comparisons). Tail piloerection has been used as a reliable measure of sympathetic nervous system arousal in mammals (Fuchs et al. 1985; Coss and Biardi 1997; Coss 1999; Schehka and Zimmermann 2009) and is exhibited by rodents when threatened (Heynen et al. 1989). Previous studies have suggested that neophobic behavior in prey may be an adaptive mechanism by which they deal with spatial and temporal variation in predation risk (Brown et al. 2013). It is possible that when encountering novel cues after being alerted to potential predators in the area, ground squirrels exhibit caution by assessing the potential risks posed by unfamiliar contexts. However, without the comparison to a control in which primed squirrels returned to the bait station with no object present, we cannot determine whether these behaviors are the result of the novel object, per se, or just the general sustained vigilance associated with recent snake encounters (as in Putman and Clark 2015).

Understanding how prey assess predation risk is critical because it governs their investment in antipredator behaviors (Lima and Dill 1990), allowing behavioral ecologists to make predictions regarding the strength and range of responses likely to be exhibited by prey in various contexts. It would benefit prey to maintain their vigilance and attention toward predators or their cues after detecting the likelihood of predation; however, increased vigilance following predator encounters can also have implications for the indirect effects of predators on prey. Past studies show that a perceived increase in predation risk may exacerbate short-term increases in vigilance when traversing through risky environments, which could impact prey activity budgets in the long term (Mateo 2007). In our system, ground squirrels reduce foraging and increase vigilance toward potential snake predators when moving through complex environments such as rock outcrops and burrow aggregations, microhabitats where snakes may hide in ambush (Leger et al. 1983). Although vigilance helps animals detect potential threats quickly (Baldellou and Peter Henzi 1992; Cowlishaw 1997; Caro 2005), investment in vigilance also reduces time that can be allocated toward other biologically important behaviors such as feeding, mating, tending young, and forming shelter (Hennessy and Owings 1988; Lima and Bednekoff 1999b; Arenz and Leger 2000). It is therefore critical for prey to develop a balance between vigilance and engaging in other behaviors. Perceptual priming can have a long-term effect that lasts for weeks to even years (Basile and Hampton 2013), but clearly this length of investment would hinder free-ranging animals from pursuing other fitness-related activities. In an ecologically realistic study, priming in nesting shrikes only lasted for about an hour after experiencing models of nest predators (Němec et al. 2015). Future studies should address whether the duration of vigilance (or priming) following a predator encounter has possible community-level indirect effects, such as facilitating success for other predators (Kotler et al. 1992).

It is likely advantageous for prey to increase vigilance and antipredator behaviors following encounters with ambush hunting predators, like the rattlesnakes in our study, since they typically cover small distances and provide persistent cues of predation risk (Preisser et al. 2007; Miller et al. 2014; Wikenros et al. 2015). However, this effect might be lessened following encounters with active or coursing predators since they tend to range far distances in pursuit of prey. In our system, squirrels respond differently to gopher snakes (*Pituophis catenifer*), which are active predators, than rattlesnakes (Owings and Coss 1977; Rundus et al. 2007). Future work will assess how recent encounters with gopher snakes impact the perception of risk and threat-sensitive behavior exhibited by squirrels toward predator cues. Different predators may cause various priming responses, which could further impact perceived risk, the salience of predator cues, and thus the range of responses exhibited by prey. Comparisons within

our study system and other systems will allow us to make generalizations on the regulation of threat-sensitive behavior due to differential hunting strategies of various predator types.

Compliance with ethical standards

Conflict of interest   The authors declare that they have no conflict of interest.

Ethical approval   All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of San Diego State University and methods were approved by the Institutional Animal Care and Use Committee (APF 13-08-015C) prior to data collection.

Data availability statement   The datasets analyzed during the current study are available as electronic supplementary material.

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