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Selective habituation shapes acoustic predator recognition in harbour seals

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Predation is a major force in shaping the behaviour of animals[1–3], so that precise identification of predators will confer substantial selective advantages on animals that serve as food to others. Because experience with a predator can be lethal, early researchers studying birds suggested that predator recognition does not require learning[4,5]. However, a predator image that can be modified by learning and experience will be advantageous in situations where cues associated with the predator are highly variable or change over time. In this study, we investigated the response of harbour seals (*Phoca vitulina*) to the underwater calls of different populations of killer whales (*Orcinus orca*). We found that the seals responded strongly to the calls of mammal-eating killer whales and unfamiliar fish-eating killer whales but not to the familiar calls of the local fish-eating population. This demonstrates that wild harbour seals are capable of complex acoustic discrimination and that they modify their predator image by selectively habituating to the calls of harmless killer whales. Fear in these animals is therefore focused on local threats by learning and experience.

The northeastern Pacific Ocean is home to two distinct forms of killer whales. Resident killer whales live in large stable groups and feed exclusively on fish. Transient killer whales live in smaller social groups and prey only on marine mammals[6,7]. The two different forms do not interbreed and rarely interact. Resident killer whales along the west coast of North America fall into three distinct communities with adjacent home ranges (Alaskan residents, northern residents and southern residents). Transients, on the other hand, form a continuous population from northern California to southeastern Alaska[8–11].

Resident and transient killer whales show striking differences in their vocal behaviour. Residents frequently emit echolocation clicks and communicative calls whereas transients are usually silent[12,13]. Resident killer whales have a complex system of vocal dialects: different social groups have repertoires of 7–17 structurally distinct stereotyped call types (see Fig. 1b and c). The degree of sharing of call types between groups within a community varies from no shared call types to complete sharing of the repertoire[14,15], and the structure of stereotyped call types slowly changes with time[16]. In contrast, all members of the transient population share most of their call types. No call types are shared among members of different resident communities or between residents and transients.

Harbour seals are the most commonly taken prey of transient killer whales in the coastal waters of British Columbia, Canada[6], and predation from transients is likely to be a significant source of mortality. Because harbour seals have good underwater hearing at the frequencies of killer whale vocal communication[17], and because underwater calls of killer whales can be heard over long distances[18], it would be beneficial for harbour seals to respond to the calls of transients with anti-predator behaviour. When the salmon migrate through these waters, groups of resident killer whales, which pose no predatory threat to seals, will often spend several weeks in a

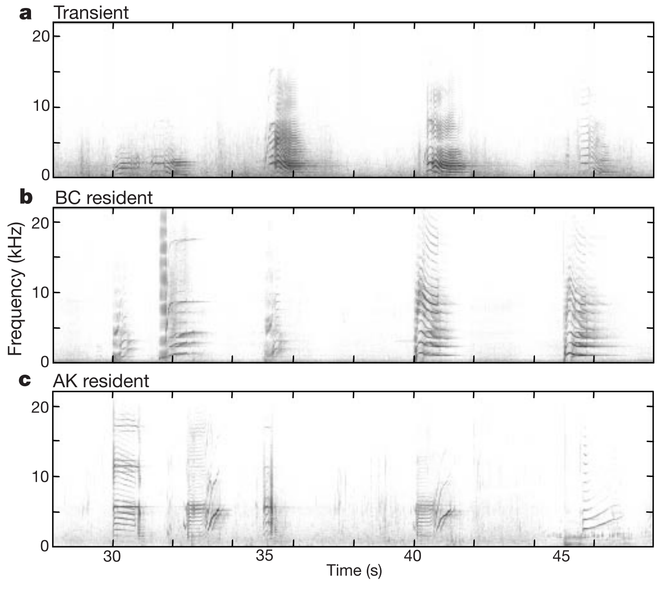

Figure 1 Playback sequences for experiment 2. Spectrograms of sections ($t = 28$ s to $t = 48$ s) of playback sequences illustrating the differences between the calls of mammal-eating killer whales (transients), familiar fish-eating killer whales (British Columbia (BC) residents) and unfamiliar fish-eating killer whales (Alaskan (AK) residents). Several (four transient, seven BC resident and four AK resident) sequences were used for each playback type.

relatively small area[19]. Seals would therefore waste time and energy if they responded to all killer whale calls indiscriminately. However, given the complexity of killer whale vocal communication, especially of the resident dialect system, consistent discrimination between calls of the two forms represents a formidable learning task.

We investigated the response of harbour seals to killer whale calls by conducting two playback experiments near reefs where seals haul out onto land off southern British Columbia, Canada. In experiment 1, we used a paired design in which test and control sequences were played in random order at the same seal haulout site on consecutive days, to test whether harbour seals show a significant response to the calls of transient killer whales. The two types of playback sequences only differed in the fact that test sequences contained killer whale calls, whereas control sequences did not. Extraneous stimuli (for example, missed echolocation clicks, or artefacts generated by either the digital editing or the playback equipment) were therefore controlled for (see Methods for details). The number of seals at the surface showed a significantly stronger decrease after the playback of calls of transient killer whales than after control sequences (paired *t*-test, $t_{1,3} = -6.77$, $P < 0.01$). The distance of the nearest seals to the playback source increased after both playback types, and the difference in this measure was not significant (paired *t*-test, $t_{1,3} = 0.83$, $P = 0.47$).

The change in the number of seals shows that harbour seals respond to the calls of transients, either because they perceive them as dangerous or, since transients vocalize only infrequently, because they represent an unfamiliar and hence potentially dangerous cue. Moving away from the surface, where a seal would be very visible and present a good echolocation target, is probably an effective strategy to avoid detection by a visual or echolocating marine predator. This response suggests that transient killer whales pay an ecological cost for vocalizing, because calling decreases their chance of a successful attack and this cost may explain why transient killer whales vocalize less frequently than residents.

In experiment 2, we played calls of transients and British Columbia (BC) residents to see whether seals responded differently to calls of familiar fish-eating and mammal-eating killer whales. To test whether any difference in the response resulted from associative learning (that is, learning to associate the calls of transients with danger) or selective habituation[20] (habituating to the calls of residents, because these calls are never followed by an attack), we played calls of Alaskan (AK) residents. These are ecologically similar to BC residents[7], and occasionally interbreed with northern residents[10,11]. Because AK residents occur more than 600 km to the north of our study area, seals would be unfamiliar with their calls. If seals generally do not respond to killer whale calls, but have learned to associate calls of transients with danger, they would not respond to these unfamiliar calls. However, if seals initially responded to all calls, but had habituated to those of the harmless residents, they would respond to unfamiliar calls. Examples of playback sequences are shown in Fig. 1.

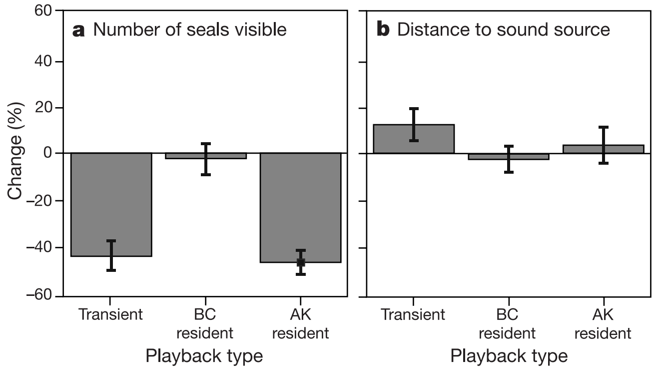

Figure 2 Response of harbour seals to the calls of different killer whale populations. Change in the number of seals visible at the surface (**a**) and the distance of the nearest seal to the playback source (**b**) after playbacks of calls of mammal-eating (Transient), familiar fish-eating (BC resident) and unfamiliar fish-eating (AK resident) killer whales (experiment 2). Ten trials were conducted at different seal haulout sites for each playback type. Error bars give mean ± 1 s.e.

The changes in the number of seals visible at the surface (Fig. 2) showed that seals responded differently to the playback of calls of different forms of killer whales (one-way analysis of variance (ANOVA), $F_{2,12} = 12.11$, $P < 0.01$). Seals responded significantly less to calls of familiar fish-eating killer whales than to mammal-eating killer whales (Tukey's test, $P < 0.01$) and unfamiliar fish-eating killer whales (Tukey's test, $P < 0.01$). The responses to mammal-eating and unfamiliar fish-eating killer whales were statistically indistinguishable (Tukey's test, $P = 0.99$). The changes in the distance to the playback source follow the same pattern, but differences are not significant (one-way ANOVA, $F_{2,12} = 0.69$, $P = 0.52$). The different response of harbour seals to the calls of transients and familiar resident killer whales suggests that seals are able to discriminate between the calls of the two populations. Owing to the complexity of killer whale vocal communication, this is not a small feat: since some groups within a resident community have no call types in common[14], the amount of variation among resident groups is as great as the difference between residents and transients. The fact that the seals responded strongly to the calls of fish-eating killer whales with which they had no experience shows that the difference in the response is due to selective habituation to the calls of harmless resident killer whales rather than to associative learning.

Imprecise predator recognition generates fitness costs. However, the currencies of cost for a predator image that is too general and one that is too specific are not the same: an over-general predator image causes an animal to respond to harmless cues and thus waste time or energy. In contrast, an over-specific predator image leads to missed detections of predators, thus posing a risk to life. Animals are limited in their sensory abilities and cannot at the same time minimize the probability of a false alarm while maximizing the probability of correct detection of predators[21,22]. Because losing a life is a far higher penalty than losing a meal or a mating opportunity, animals should maximize the probability of correct detection of predators at the expense of false alarms.

Having a learned component to predator recognition is advantageous in situations where the nature of the predatory threat is to some degree unpredictable[23], or where cues associated with the predator are highly variable or change with time. Animals that adjust their predator image by associative learning start out with one that is too specific and expand it by adding cues (either when attacked after sensing the cue, or by seeing conspecifics respond to it[23]) to reflect the actual predators present. This is risky, as it requires experience with the predators. In contrast, selective habituation predicts that prey animals start out with a rather general predator image from which certain harmless cues are removed by habituation. This initially generates costs from false alarms but not from missed detections. It does not require experience with the predator, since any unusual cue that falls within a certain predator class elicits a response. By selective habituation—learning what not to fear—harbour seals pursue the more conservative, and thus more advantageous, strategy. ☐

Methods

General playback procedure

Playbacks were performed to harbour seals in the water using a TCD-D8 DAT-recorder (Sony) and an LL916 underwater speaker (Lubell Labs) deployed at a depth of approximately 5 m from a small boat (6 m long aluminium vessel or 4 m long inflatable) anchored about 100 m from a seal haulout. The maximum source level of the loudest call in sequences containing killer whale calls was 148 dB (reference pressure 1 μPa at 1 m). This is 5 dB lower than the average source level of discrete calls of resident killer whales[18]. We counted the number of seals in the water and measured the distance to the nearest animal with laser rangefinders at 20 s intervals for at least 2 min preceding the playback of the calls. We played the 1-min playback sequence a single time and continued to note number of animals and distance to the nearest animal for an additional 2 min or more. Before playbacks the average number of seals at the surface was 13.6 (standard deviation, std, 7.9), and the average distance of the nearest seal to the playback source was 60.2 m (std 21.3 m). Trials where fewer than five seals were present before the playback were excluded from the analysis. The strength of the response was expressed as the percentage change in the average number of seals and average distance to the nearest seal from the 2 min before to the 2 min after the calls were played. Playback experiments were conducted off northern Vancouver Island in Johnstone and Queen Charlotte Straits and off southern Vancouver Island in Haro and Georgia straits.

Experiment 1

Test and control playbacks were conducted once each at the same haulout in random order at the same tidal height on consecutive days. Both types of playback sequences were based on identical sections of background noise from a digitized recording of transient killer whales digitally spliced into a 1-min sequence. Sections containing whistles, echolocation clicks or pulsed calls were not used for this purpose. To avoid startle responses caused by the sudden onset of unfamiliar background noise, the volume was slowly faded in over the first 30 s of the sequence and faded out during the last 10 s. For test sequences, five killer whale calls from the same recording, belonging to at least three different call types, were spliced into the sequence between the fades. For control sequences an additional five sections of background noise were spliced in instead of the calls. The volume of each sequence pair was adjusted so that the loudest call in the test sequence had a source level of 148 dB (reference pressure 1 μPa at 1 m). We generated and used four such pairs of sequences from recordings of different transient groups and played each at two different haulouts. In order to avoid pseudoreplication[24], we averaged responses obtained at haulouts where the same pair of playback sequences was played, so that the number of playback sequences, not trials, determined degrees of freedom. We used a paired t-test to test for significant differences between responses to test and control.

Experiment 2

For this experiment, we generated three types of playback sequence using the methodology explained above. Sequences for playbacks of familiar fish-eater calls contained five calls from BC resident killer whales. We used calls of northern residents for playbacks off northern Vancouver Island, and those of southern residents off southern Vancouver Island. For playbacks of unfamiliar killer whale calls we generated sequences from recordings of Alaskan residents made in Prince William Sound, Alaska. Sequences of transient calls were those used as test sequences in experiment 1. Except for the familiar fish-eating killer whales, we generated four sequences for each playback type from recordings of different social groups. For familiar fish-eating killer whales, we generated a total of seven playback sequences (three of northern residents and four of southern residents). Ten trials were conducted for each playback type and again, to avoid pseudoreplication, all responses obtained with the same playback sequence were averaged. We used a one-way ANOVA to test for statistical differences between the playback types and used Tukey's honestly significant difference test[25] to determine which playback types differed.

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Graded persistent activity in entorhinal cortex neurons

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Working memory represents the ability of the brain to hold externally or internally driven information for relatively short periods of time[1,2]. Persistent neuronal activity is the elementary process underlying working memory but its cellular basis remains unknown. The most widely accepted hypothesis is that persistent activity is based on synaptic reverberations in recurrent circuits. The entorhinal cortex in the parahippocampal region is crucially involved in the acquisition, consolidation and retrieval of long-term memory traces for which working memory operations are essential[2]. Here we show that individual neurons from layer V of the entorhinal cortex—which link the hippocampus to extensive cortical regions[3]—respond to consecutive stimuli with graded changes in firing frequency that remain stable after each stimulus presentation. In addition, the sustained levels of firing frequency can be either increased or decreased in an input-specific manner. This firing behaviour displays robustness to distractors; it is linked to cholinergic muscarinic receptor activation, and relies on activity-dependent changes of a Ca^{2+}-sensitive cationic current. Such an intrinsic neuronal ability to generate graded persistent activity constitutes an elementary mechanism for working memory.

The entorhinal cortex (EC) is a crucial component of the medial temporal-lobe memory system[4,5]. EC neurons have been shown to display persistent activity during the delay phase of delayed match or non-match to sample memory trials[6,7] and the hippo-