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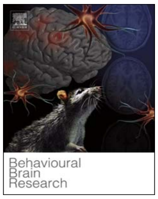

Research report

Observational fear learning in degus is correlated with temporal vocalization patterns

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ABSTRACT

Some animals learn to fear a situation after observing another individual come to harm, and this learning is influenced by the animals' social relationship and history. An important but sometimes overlooked factor in studies of observational fear learning is that social context not only affects observers, but may also influence the behavior and communications expressed by those being observed. Here we sought to investigate whether observational fear learning in the degu (*Octodon degus*) is affected by social familiarity, and the degree to which vocal expressions of alarm or distress contribute. 'Demonstrator' degus underwent contextual fear conditioning in the presence of a cagemate or stranger observer. Among the 15 male pairs, observers of familiar demonstrators exhibited higher freezing rates than observers of strangers when returned to the conditioning environment one day later. Observer freezing during testing was, however, also related to the proportion of short- versus long-inter-call-intervals (ICIs) in vocalizations recorded during prior conditioning. In a regression model that included both social relationship and ICI patterns, only the latter was significant. Further investigation of vocalizations, including use of a novel, directed k-means clustering approach, suggested that temporal structure rather than tonal variations may have been responsible for communicating danger. These data offer insight into how different expressions of distress or fear may impact an observer, adding to the complexity of social context effects in studies of empathy and social cognition. The experiments also offer new data on degu alarm calls and a potentially novel methodological approach to complex vocalizations.

1. Introduction

Many animals rely on the experiences of others to learn about their environment and danger ranks among the most important types of knowledge. Studies of mice have found that observers of familiar conspecifics in distress will exhibit greater fear learning [1,2] also deer mice: [3] see also [4,5] for data in rats), and emotional contagion [6–8] compared with observers of strangers in distress. These studies are consistent with an extensive literature demonstrating a more general effect of social familiarity on vicarious learning across phyla (e.g., [9–12,44,45]. In cases where social relationship has been found to affect observational fear learning and emotional contagion, the effect has also been cited as evidence that the brain is using higher-level representations of other's emotional states [13–15]. Another possibility, raised by work by Martin et al. [7], is that differences between familiar and stranger dyads may arise from an interference of perceptual or emotional processes brought-on by the stress of encountering a stranger conspecific (see also review by Sivaselvachandran et al. [43]).

In general, this research has emphasized the importance of neural processes that are engaged in an observing animal, rather than differences in how an observed familiar versus stranger animal may express its distress. Expressions of both alarm and distress have been found to vary with social context; for example, squirrels exhibit different alarm calls in the presence of related conspecifics [16] and mice will writhe in response to a painful stimulus to differing degrees in the presence of familiar versus stranger animals [6]. It is possible that in many of these cases, social context effects are due—at least in part—to differences in the expressions by the harmed, "demonstrator" individual.

Vocalizations, such as alarm calls, may be particularly important in mediating social fear learning and offer a valuable tool for examining context effects on emotional expression. Rats emit a 22 Hz ultrasonic vocalization during shock conditioning that appears to vary with levels of distress and freezing [17–22]. Although vocalizations may not always correlate with the behavior of observing animals [22], observers

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who are unable to process auditory information, due to inactivation or lesions to the medial geniculate nucleus, also fail to show social fear responses [23]. There are likely limits to what ultrasonic vocalizations in rats and mice can provide to studying social transmission of emotion. For example, many other species use different vocalization types, or type combinations, under varying types or degrees of danger (e.g., squirrels: [24,42,46] prairie dogs: [25] chickadees: [26]. Many species also show audience effects, in which an animal will only exhibit alarm calls when in the presence of a member of its own species (e.g., in birds: [27,28]. To best capture how social context influences communication of emotion, it will be useful to examine behavior in animals that make use of a wide spectrum of communication signals.

The degu offers a valuable experimental subject for studying social behavior and social communication. The species uses an extensive repertoire of vocalization types in distinct behavioral and social contexts [29]; see also [30]. Alarm signals have also been found to vary between individuals, with differential influence on observers [31]. The goal of the present experiment was to investigate social context effects on vocalizations and observational fear learning (alternatively, "vicarious fear learning"; although "observational" and "vicarious" may imply different signaling processes, for present purposes they are used interchangeably). This was addressed by first establishing whether degus, like other rodents, show social relationship effects on observational learning, and subsequently examining relationships between vocal behavior and observer learning.

2. Methods

2.1. Subjects

Subjects were 37 male and 15 female degus (26 pairs) taken from litters across 14 breeding pairs (12 for male-only pairs) between the ages of 5 and 18 months. These numbers were expected to be sufficient to detect observational fear learning based on calculation of Cohen's d from observational fear learning data collected in mice in the same paradigm [32]; $F_{1,\ 45} = 11.48$, $P = 0.0015$, Cohen's d = 1.02; suggesting the need for around 15 pairs per group, or 30 pairs total). Degus were kept in a temperature controlled room and maintained on a 12 h/12 h light-dark cycle with *ad libitum* access to food and water. As degus are diurnal, experiments were performed at a consistent time around the middle of animals' light cycle. All degus were socially housed with siblings (at least 2 and no more than 6 to a 15″ × 19″ × 8″ or 14″ × 11″ × 9.5″ cage) from weaning (3 mo) until experiments. Following procedures, degus were returned to their original social living conditions and exhibited normal, affiliative interactions with cagemates; inspection by experimenters and veterinary staff revealed no unusual, stress-related behaviors. All animal protocols were approved by the University Animal Care Committee (UACC) at the University of Toronto.

2.2. Observational fear learning procedure

The observational fear learning protocol was modified from that described by Jeon and Shin, [32], illustrated in Fig. 1A. On the conditioning day, pairs of degus were brought for the first time to the experiment room, which contained two fear conditioning chambers (23 × 28.5 cm; Coulbourn Instruments, Whitehall, PA). The chambers were placed side-by-side so that degus could see, hear, and (presumably) smell one another. Conditioning began when two degus—either from the same cage (cagemates) or taken from two different cages and breeding lines (strangers)—were simultaneously placed in the facing chambers. Group assignment was randomized such that both stranger and cagemate dyads were often drawn from the same cage group. Following a five minute period during which baseline behavior was recorded ("baseline"), a series of 21 footshocks (1 mA for 2 s, each separated by 10 s) was presented to one of the two degus ("demonstrator") but not the other ("observer"). Stimulations were presented by controlling the Coulbourn stimulation box with an Arduino (www.arduino.cc) running custom written Arduino software, controlled by a custom-written GUI interface built in MATLAB (MathWorks; Natick, MA). After conditioning, degus were singly-housed into new cages. On the following day, fear retention was assessed in both observers and demonstrators. During fear retention, each animal was individually (i.e., in isolation) placed in the shock chamber for five minutes and behavior was monitored. Animals from both stranger and cagemate groups were tested on the same days. Initial analysis revealed significantly higher levels of baseline freezing rates in female degus (median time freezing in males: 37 s, females: 77 s, Wilcoxon rank-sum test, p = 0.035; Fig. 1B). The proportion of male versus female observers in mixed-sex pairs was also not counterbalanced (e.g., only three mixed-sex pairs were included, and these were all stranger pairs); therefore, to reduce variance and maintain consistency with previous studies, subsequent analyses focused on only the 15 male-only pairs (learning results from pairs with females pairs are described in Results and Supplementary Materials). Across these 15 pairs, there were no consistent age differences between the observer/demonstrator and cagemate/stranger groupings (median ages ± std: observer cagemate = 6 mo, 20 d ± 5 mo, 16 d; observer stranger = 6 mo, 3 d ± 1 mo, 27 d; demonstrator cagemate = 6 mo, 20 d ± 5 mo, 16 d; demonstrator stranger = 7 mo, 3 d ± 2 mo, 29 d).

2.3. Movement & audio recording

Video data were collected with two USB2 cameras (Microsoft LifeCam) using ANY-maze Behavioral Tracking software (Stoeling; Wood Dale, IL). Audio data were sampled at 150 Hz with a Knowles SPM Series ultrasound microphone placed at the intersection of the two cages, and a laptop computer running Avisoft SASLab Pro (Avisoft Bioacoustics; Glienicke, Germany). Synchronization of video and audio data with shock presentations was archived through the custom-written experimental control software, which included timestamped presentations of a blinking LED light (1 s on alternating with 9 s off) to a third webcam and a 1s, 2 kHz tone presented at the beginning (one pulse) and end (5 pulses spaced at 10 s intervals), of the recording session.

2.4. Analysis approach

The goal of the present study was to test the following predictions: 1) degus exhibit social fear learning, 2) degu social fear learning is higher between familiar (cagemate) animals compared with unfamiliar (stranger) animals, 3) that specific communication signals—particularly those in the vocal domain—contribute to social fear learning, and vary with social relationship. The first two predictions are taken-up together, complimented with analyses of variables that might confound the effect (e.g., age). The next prediction was subsequently tested by examining, in order, the amount of vocalizing, the temporal pattern of vocalizations, and the types of vocalizations. When two variables were found to be associated with retention freezing in observers (social relationship of the dyad and vocalization pattern), a multiple regression model was created to examine the relative variance explained by each variable. Examination of whether vocalization types (calls or syllables) predicted observer retention freezing demanded a new analysis method, described below (*Vocalizations: classification*).

2.5. Freezing

Freezing was manually scored by two experimenters blind to condition. Freezing was defined as total time the degu spent visibly tense and immobile except for respiration. ANY-maze tracking was used to automatically score freezing, however for some videos the software failed to produce freezing scores. In all other videos, manual freezing scores and ANY-maze scores were highly correlated (r = 0.71,