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Adapting to alcohol: Dwarf hamster (*Phodopus campbelli*) ethanol consumption, sensitivity, and hoard fermentation☆

Gwen Lupfer[a,*], Eric S. Murphy[a], Zoe Merculieff[c], Kori Radcliffe[a], Khrystyne N. Duddleston[b]

[a] Department of Psychology, University of Alaska Anchorage, 3211 Providence Drive, Anchorage, AK 99508, USA
[b] Department of Biological Sciences, University of Alaska Anchorage, 3211 Providence Drive, Anchorage, AK 99508, USA
[c] Department of Psychology, Stanford University, 450 Serra Mall, Stanford, CA 94305, USA

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ABSTRACT

Ethanol consumption and sensitivity in many species are influenced by the frequency with which ethanol is encountered in their niches. In Experiment 1, dwarf hamsters (*Phodopus campbelli*) with *ad libitum* access to food and water consumed high amounts of unsweetened alcohol solutions. Their consumption of 15%, but not 30%, ethanol was reduced when they were fed a high-fat diet; a high carbohydrate diet did not affect ethanol consumption. In Experiment 2, intraperitoneal injections of ethanol caused significant dose-related motor impairment. Much larger doses administered orally, however, had no effect. In Experiment 3, ryegrass seeds, a common food source for wild dwarf hamsters, supported ethanol fermentation. Results of these experiments suggest that dwarf hamsters may have adapted to consume foods in which ethanol production naturally occurs.

1. Introduction

Naturally-occurring fermentation produces ethanol in the food sources of many species. For example, Wiens et al. (2008) reported that pentailed treeshrews (*Ptilocercus lowii*), slow lorises (*Nycticebus coucang*), plantain squirrels (*Callosciurus notatus*), and several varieties of rats feed on Malaysian bertam palm tree (*Eugeissona tristis*) nectar, which can contain up to 3.8% alcohol. The small mammals pollinate the palm trees while consuming nectar in a mutually beneficial symbiotic relationship which may have persisted more than 55 million years. Analyses of ethyl glucuronide, a biomarker of ethanol metabolism, in hair samples indicated extremely high ethanol consumption in the treeshrews and plantain squirrels, whereas behavioral observations of these animals suggested only moderate drinking and impairment. Wiens et al. (2008), therefore, suggested that the treeshrews and squirrels may have adapted to regular ethanol consumption with the ability to metabolize it rapidly.

Ripe fruit, serving as food to many insects, birds, and mammals can ferment and reach ethanol levels of 5%. Ethanol itself is calorically rich (Dudley, 2002), but not all frugivorous animals readily utilize alcohol-containing fruits as sources of energy. For example, the yellow-vented bulbul bird of Israel (*Pyenonotus xanthopygos*) ate less of a banana-based diet when it contained 3% ethanol than when it contained none (Mazeh et al., 2008). Egyptian fruit bats (*Rousettus aegyptiacus*) similarly avoided ethanol-containing solutions unless food-deprived (Sánchez et al., 2008). Minimizing alcohol ingestion may serve to protect the bats from intoxication; consumption of a 1% ethanol solution slowed their flight and affected their echolocation in experimental trials (Sánchez et al., 2010). Sánchez et al. (2010) reasoned that ethanol intoxication may be especially dangerous to flying animals that move at higher speeds than terrestrial organisms, and proposed that flying animals may have evolved to avoid alcohol rather than metabolize it efficiently.

Syrian hamsters (*Mesocricetus auratus*), unlike Egyptian bats and yellow-vented bulbul birds, are well known for readily consuming ethanol (*e.g.*, DiBattista and Joachim, 1999; Gulick and Green, 2010). Their ethanol intake has been described as very high. On average

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* Corresponding author. Tel.: +1 907 786 1722.
E-mail address: gjlupfer@uaa.alaska.edu (G. Lupfer).

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7.6 and 17.9 g/kg of ethanol was consumed per day as reported by DiBattista and Joachim (1999) and Piercy and Myers (1995), respectively. Subjects reduced their intake considerably when fed a high fat diet (DiBattista and Joachim, 1999) or when sweetened chocolate or juice beverages were made available (Piercy and Myers, 1995). Despite high consumption, however, Syrian hamsters have been reported to show no signs of ethanol withdrawal (Harris et al., 1979).

Syrian hamsters' innate insensitivity to ethanol is of interest to both basic and applied scientists. Their regular consumption of ethanol and the effects of ethanol on some metabolic processes resemble effects observed in human alcoholics with schizophrenia (Green et al., 2004). Also, studies in which antipsychotic drugs reduced ethanol consumption in hamsters (*e.g.*, Green et al., 2004; Gulick et al., 2014) may provide insights into treating co-occurring schizophrenia and alcoholism in humans.

In addition to the potential applied benefits of using hamsters as a model of human alcoholism, hamsters' innate insensitivity to ethanol is of interest to evolutionary biologists and comparative psychologists. Compared to rats, Syrian hamsters exhibit high hepatic alcohol dehydrogenase activity (Kulkosky and Cornell, 1979). They also hoard wheat grains in their burrows (Murphy, 1971), and thus, may have evolved to consume and metabolize ethanol due to ethanol-producing fermentation of seeds in their hoards (Newcomer et al., 1987). Therefore, in pentailed treeshrews, plantain squirrels, and Syrian hamsters, a pattern emerges in which a species' natural history influences its innate sensitivity (or insensitivity) to ethanol.

The present experiments were conducted in order to explore ethanol consumption in another hamster species, the dwarf hamster (*Phodopus campbelli*). The *Phodopus* genus is believed to be the first to have diverged from other hamsters (Romanenko et al., 2007), and researchers estimate that this split occurred 13–14 million years ago (Steppan et al., 2004). *P. campbelli* hamsters are approximately mouse-sized and inhabit cold, arid regions of central Asia, including Siberia, Mongolia and northeastern China (Ross, 1995). They are crepuscular and must travel long distances – up to several km in a single night – in order to acquire sufficient food and water resources (Wynne-Edwards et al., 1999). Major food and water sources include wild onions, beetles, and ryegrass seeds (Ross, 1995). Like Syrian hamsters, dwarf hamsters have large cheek pouches in which they transport these items from their evening foraging trips to their burrows (Ross, 1995; Wynne-Edwards, 2003). Studies conducted using closely related *Phodopus sungorus* dwarf hamsters revealed that a short photoperiod increased the proportion of total food consumed that came from subjects' hoards (as opposed to from a food supply outside subjects' burrows; Wood and Bartness, 1996), a finding consistent with the idea that dwarf hamsters may rely on food hoarded in summer during harsh winter conditions. In laboratory experiments, *P. campbelli* dwarf hamsters used their pouches to carry approximately 80% of reinforcers earned in operant conditioning chambers back to their home cages (Lupfer-Johnson et al., 2010).

We quantified ethanol consumption in *P. campbelli* hamsters fed standard, high carbohydrate, and high fat diets in Experiment 1. Due to similar grain-hoarding behaviors in the two hamster species, we hypothesized that dwarf hamsters would resemble Syrian hamsters both in terms of readily consuming ethanol solutions without food or water deprivation and also in terms of reducing consumption when high-fat food was available.

Experiment 2 documented the motor impairing effects of ethanol administered both orally and i.p. in dwarf hamsters using the Wobbling Scale, an instrument designed to assess ethanol sensitivity in mice (Metten et al., 2004). When ethanol is consumed orally, gastric and hepatic alcohol dehydrogenase (ADH) metabolize much of it before it reaches the blood and nervous system, and ADH levels are often positively related to a species' evolutionary exposure to ethanol (Mazeh et al., 2008). Because i.p. injections circumvent the normal digestive process, we hypothesized that subjects would exhibit motor impairment from i.p. injections but not from orally administered ethanol.

In Experiment 3, we assessed the microbial ethanol fermentation potential of ryegrass seeds (a major food source for dwarf hamsters). To maximize seed shelf life, seeds are stored in dry conditions and low temperature to inhibit aging and production of volatile compounds (which degrade seed quality). However, the metabolic reactions producing volatiles still occur within the seeds, albeit slowly (Zhang et al., 1993). Ethanol is the most common volatile compound produced during seed storage and has been documented in many species including sunflower seeds and soybeans (Zhang et al., 1993). Seeds of peas (*Pisum sativum* and *Lathyrus pratensis*), broom plants (*Cytisus scoparius*), and oak trees (*Quercus robur*) produced ethanol and lost viability during artificial aging or desiccation; in the case of *P. sativum* seeds, volatile production was observed after only 8 days (Colville et al., 2012). Although not tested, ryegrass seeds are also likely to generate volatiles, including ethanol, when stored over long periods; thus, hamster hoards may generate ethanol. In addition to ethanol production *via* plant metabolism, fermentation of carbon substrates in the seeds by microbes (yeast) holds the potential to produce ethanol in seed hoards at a rate faster than has been observed in seed storage experiments. We hypothesized that ryegrass seeds hoarded by dwarf hamsters would also ferment and produce ethanol.

2. Methods

2.1. Animals and materials

All procedures were approved by the University of Alaska Anchorage Institutional Animal Care and Use Committee. Adult dwarf hamsters (*P. campbelli*) were bred from hamsters acquired from Queen's University (Kingston, Ontario). The Queen's University breeding colony originated from wild *P. campbelli* hamsters captured in Siberia (Wynne-Edwards and Lisk, 1984). Subjects were generally housed in same-sex groups of 2–4 except aggressive males which were housed singly. Cages measured 26.67 × 48.26 × 20.32 cm. Subjects had *ad libitum* access to Mazuri Rodent Pellets #5663 (Brentwood, MO; exceptions to this described below) and tap water in their home cages. The Mazuri pellets contained 23% protein, 6.5% fat, and 4.5% fiber. Environmental enrichment (*i.e.*, small cardboard boxes or paper bags) was added to each cage 1–2 times per week. The subjects were exposed to a 14:10 h light/dark cycle (lights on from 7:00 a.m. to 9:00 p.m.), and the animal colony room was maintained at a temperature of 21 ± 1 °C.

All subjects were housed singly during Experiment 1 in order to monitor daily ethanol consumption. Ethanol solutions (15% and 30% v/v) were presented to subjects in graduated 50 mL Nalgene conical centrifuge tubes. The tubes were placed inside dispensers created for the current experiment by drilling 4 holes into the bottom of 16-ounce cans, with sipper tubes protruding from the openings. The cans were attached to subjects' cages *via* magnets. Consumption measurements were taken at 24-h intervals. For each measurement, two experiments read the bottom of the meniscus of each graduated tube independently to the nearest .5 mL. Discrepancies were discussed, and a single value was recorded.

A high fat and a high carbohydrate diet, used in Experiment 1, were obtained from Custom Animal Diets, LLC (Bangor, PA). These diets were based on previous research with Syrian hamsters by DiBattista and Joachim (1999). The high fat diet was 76.2% fat, 23.3% protein, and 1.3% carbohydrates. The high carbohydrate diet was