Comment 2: Although the authors highlight several important advances of STAR, there seems to be one important limitation that may reduce the implementation of this procedure by much of the alcohol research community. The authors note that it was not necessary to employ an experimental manipulation that elevates drinking behavior during the acquisition phase, such as sweeting the initial alcohol solution. However, the authors used "light" food restriction throughout these experiments. However minimal this restriction was, if it was necessary for acquisition of the operant procedure, it was clearly sufficient to enhance alcohol drinking-related behaviors and thus this manipulation suffers from a similar limitation as the sweeteners used in the initial training of most rodent operant alcohol drinking studies. Moreover, food restriction will likely add an element of motivation that is unrelated to AUD and not likely to be present in human subjects: the significant caloric value of alcohol.

Response 2: This is an important point, which we have informally discussed at length with several other labs in the field and continue to debate internally as well. Before addressing the central question of integration with the field at large, we would like to clarify the feeding conditions employed in the current study. Our animals are not given *ad lib* food access, but they are also not calorically restricted – they are provided with daily food slightly above the metabolic requirement to maintain a healthy adult weight. Though metabolic rates differ due to numerous factors (ambient temperature, number of cage mates, vitamin content of the chow, sex, etc.), we fed animals on a consistent schedule (males: 2.9-3g/animal/day, females: 2.6-2.7g/animal/day) and monitored body weight daily to ensure that subjects were stable and at a healthy weight throughout the course of experiments. This resulted in a metabolizable energy content of ~8.7 kcal per day for male animals and ~7.5 kcal per day for female animals, which is in the middle of values given to maintain caloric stability (Keenan et al., 1996; National Research Council (US) Subcommittee on Laboratory Animal Nutrition, 1995; Škop et al., 2021). We used the phrase 'light food restriction' for lack of a field standard term, but in hindsight our terminology was vague, and we have removed this phrase from the manuscript in favor of a quantitative description.

Though *ad lib* food access is often the default condition for mouse studies, to our knowledge a formal justification for defaulting to this condition has not been presented in the literature, though there are several literatures that have warned against long-term *ad lib* food access, including the Guide (2011). Under *ad lib* conditions, most rodents become severely overweight within a few months and many studies have detailed a host of resulting health issues including insulin resistance, heightened rates of spontaneous cancers and neurodegenerative diseases, and dramatically shortened lifespan compared to animals given enough food to meet caloric needs (Surwit et al. 1988; Keenan et al. 1996, 1994; Duffy et al. 2001; Blackwell et al. 1995). Because most of our experiments require long viral incubation periods and longitudinal behavioral tests, the lab default is to maintain subjects at a healthy adult weight throughout all experimental procedures – these conditions were implemented in the current study as well but are not an explicit component of STAR. Critically for interpretation of our findings, animals' weights did not vary over the course of behavioral testing and did not vary by phenotype, demonstrating that individual differences in drinking are not driven by weight differences for male **(Figure S5)** or female **(Figure S17)** subjects.

It is also worth noting that the initial issues raised in the field regarding caveats of caloric drive in animal models of alcohol use were related to severe deprivation / forced intake models used at the time, many of which replaced >50% of daily caloric requirements with calories from ethanol (Mello, 1973; Woods and Winger, 1974). In contrast, the caloric content of the ethanol consumed during the 1-hour STAR sessions is minimal. For example, consider 2.5 g/kg intake (near the top of the range achieved during STAR sessions) in a 23 g mouse (roughly average male weight in this study), which results in BACs nearly double the 80 mg/dl binge intoxication line **(Figure S3B)**. Total raw energy of the alcohol consumed in this case is 0.41 kcal [0.058 g, 7 kcal/g, raw energy], equivalent to 0.101 g of chow used in our facility, of which they receive 3 g per day. Given that in many cases our animals do not finish all of the food provided, as evidenced by small bits of chow remaining in the hopper from the previous day when food is given daily, it is unlikely that caloric content per se is a significant factor driving motivated behavior for alcohol under these conditions.

Finally, to directly answer the reviewers' question regarding the effect of feeding schedule on the STAR paradigm and its applicability to the field, food access conditions are not an essential element of the STAR framework and should be altered as needed for specific questions – provided that all animals included in the

phenotyping analysis are treated identically. We have clarified these points in the methods section, and welcome additional feedback on this issue.

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