Published in final edited form as:

Physiol Behav. 2014 January 17; 123: 20–32. doi:10.1016/j.physbeh.2013.09.015.

Early Ethanol and Water Consumption: Accumulating Experience Differentially Regulates Drinking Pattern and Bout Parameters in Male Alcohol Preferring (P) vs. Wistar and Sprague Dawley Rats

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Abstract

Alcohol-preferring (P) rats develop high ethanol intake over several weeks of water/10% ethanol (10E) choice drinking. However, it is not yet clear precisely what components of drinking behavior undergo modification to achieve higher intake. Our concurrent report compared precisely measured daily intake in P vs. non-selected Wistar and Sprague Dawley (SD) rats. Here we analyze their drinking patterns and bouts to clarify microbehavioral components that are common to rats of different origin, vs. features that are unique to each. Under sole-fluid conditions P, Wistar and SD rats all consumed water at a high initial rate followed by a slow maintenance phase, but 10E - in a distinctly different step-like pattern of evenly distributed bouts. During choice period, 10E vs. water patterns for P rat appeared as an overlap of sole-fluid patterns. The SD rat choice patterns resembled sole-fluid patterns but were less regular. Choice patterns in Wistar differed from both P and SD rats, by consisting of intermixed small frequent episodes of drinking both 10E and water. Wistar and SD rats increased choice ethanol intake by elevating the number of bouts. A key finding was that P rat increased choice ethanol intake through a gradual increase of the bout size and duration, but kept bout number constant. This supports the hypothesis that genetic selection modifies microbehavioral machinery controlling drinking bout initiation, duration, and other pattern features. Precision analysis of drinking patterns and bouts allows differentiation between genetic lines, and provides a venue for study of localized circuit and transmitter influences mediating mesolimbic control over ethanol consumption.

1. Introduction

An important issue in alcohol research is to determine the variable components of drinking and the specific changes in behavior responsible for elevated ethanol intake. Selective breeding of high alcohol consuming rats has yielded at least seven different lines [1]. The

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Indiana University alcohol preferring (P) rats, derived from the non-selected ('common-stock') Wistar line, has received most attention [1,2] and meets all criteria [3-5] for an animal model of alcoholism. P rats develop a characteristic drinking of 10% v/v ethanol (10E) over three weeks, reaching selection criteria of at least 5 g/kg/day intake and 2:1 preference [6,7]. By utilizing traditional daily measures of ethanol consumption, the existing studies do not allow for a precision analysis of moment-to-moment behavior. There is a need to identify temporal patterns and precise component modifications during initial development of ethanol drinking. Our aim is to provide a comprehensive strategy to deal with the entanglement of motivations and regulating CNS influences over time that drive decision-making under parallel consumption of ethanol and water solutions.

Consumption of food constitutes the major source of confounding influence for drinking behavior in normal conditions [8-10]. Parameters of feeding and associated drinking vary between rats, carrying both individual and line related differences [11,12], which makes it impossible to clarify unbiased, fluid-specific drinking patterns [13]. To eliminate feeding-imposed modulation of level and timing of 10E and water intake, we introduced a novel protocol design separating the food from fluid access time within each 24-hour cycle across days. This allowed determining specific differences in drinking behavior between P and non-selected Wistar and Sprague Dawley (SD) rats under conditions that follow the timeline of a drinking test used in the P-rat selective breeding program. First water, then 10E was available for 5 days each as the sole fluid, and finally both together for 12 days under choice conditions. Daily access to fluid lasted for 15 hours, overlapping the normal dark phase. Daily access to food with no fluid, lasting for eight hours within the light phase, allowed normal weight gain. We have found already in this study (see our concurrent report) that P relative to Wistar and SD rats show an initial elevated consumption of water, then 10E as sole fluids and next of 10E during the ethanol / water choice period. All three groups exhibit a gradual elevation of ethanol intake and preference during the choice period, yet at different rates. The variable on-going selection of ethanol vs. water appears integrated over time, so that both P and non-selected rats maintain similar stable levels of total fluid intake during choice access.

A motivation for the present work was to evaluate ethanol consumption at a very high level of temporal resolution. This information is necessary to analyse microbehavioral sequences involved in regulation of elevated ethanol intake and to reveal, at a later stage, spatial-temporal patterns of single-neuron activity within specific CNS circuits that are associated with ethanol selection in choice drinking. In this respect, a number of questions arise regarding detailed volumetric and temporal parameters of fluid consumption. Necessary water is available both in the form of free water and as part of an ethanol solution. (a) - Are temporal structures of water and ethanol consumption pattern during daily access similar or distinctly different from each other and relative to rat line? (b) - During daily access, does the preference for ethanol change? Rats often take fluid in coordinated episodes of active drinking – 'bouts', where they approach and remain close to a spout while a sizable volume is consumed. (c) - Is an increase of daily ethanol intake during choice conditions due to specific change in drinking bouts or due to elevation of scattered drinking outside of coordinated bouts? (d) - What micro components of drinking behavior are characteristic of P rat vs. outbred albino rats of Wistar – which was the source for selective breeding of P rat, and SD – which is independent from the first two - lines? (e) - Do the differences between lines represent evidence for differential self-exposure to the sought-after pharmacological effects of ethanol? To render such issues accessible for analysis, a new level of precision in measuring fluid consumption is required. We introduced in this study, as described both in the present and concurrent report, new instrumentation that uses syringe pumps to deliver unit volumes of fluid into spouts and real-time lick counts to coordinate precisely the timing

of fluid deliveries. The software for instrumentation control and data collection maintains one-msec accuracy of all licks and fluid deliveries over many days.

An emerging hypothesis is that consumption of ethanol and water is based on common microbehavioral subsystem actualized by a core timing process for initiating drinking behavior. At these 'drink-ON' points, presumed duration control for the 'ON' state often results in a coordinated drinking bout, while parallel choice mechanism defines a decision to select ethanol or water. Therefore, selective breeding may alter this neurobiological control machinery to modify ethanol bout parameters and other features of drinking patterns. A collateral output from this machinery may contribute to a universal regulating system that maintains consistent level of total fluid intake regardless of line related specifics, as shown in our concurrent report.

2. Material and methods

2.1 Animals

Adult male P-rats (59th-60th generation, n=10, Indiana U., IN) weighed 200 g, and Wistar (n=8) and Sprague Dawley (SD, n=14) rats (both from Harlan, IN) weighed 325-350 g upon arrival. P-rats were double-housed until growing to 325-350 g, and from this weight all animals were single-housed, in filter-ventilated cages (Thoren Caging System, Inc., Hazleton, PA) at $22 \pm 1.5^\circ \text{C}$ and $42 \pm 8\%$ humidity under normal lighting cycle ("OFF"=18:00, "ON"=06:00) with food and water ad lib. Body weights were measured once a week until the start of experiment. All animals entered the study upon reaching the weight of 400 g. Experimental procedures were approved by the Institutional ACUC and are in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2 Apparatus

Plexiglas cages measured $40 \times 35 \times 35 \text{ cm}$ (L $\times$ W $\times$ H) and were located in separate light-insulated, sound-attenuated and individually ventilated cubicles. Each cage contained a house light and a fan on top and two spouts, located symmetrically on the sidewalls, 6 cm from the back corners, and 4 cm above the floor. Spouts, custom made in the laboratory, required insertion of the tongue down into a spout well to obtain fluid. Lick movements were monitored by an infrared beam-photosensor setup. Fluids were delivered into the spouts at a rate of $90.25 \mu\text{l/sec}$ with model R-E syringe pumps (Razel Scientific Instruments, St. Albans, VT). The data collection and instrumentation control software (Neuroplex, Inc., Winston-Salem, NC) provided monitoring, execution and recording of each lick and fluid delivery at one millisecond resolution.

2.3 Experimental design

The experiment consisted of three test phases (periods). (1) - Water phase: access to 100% tap water alone, as the sole fluid, with no additives (free water, water), for 5 days. (2) - Ethanol phase: access to 10% v/v solution of ethanol in tap water (10E) alone, as the sole fluid, for 5 days. (3) - Choice period, also called 'ethanol / water choice': access to concurrent 10E and free water for 12 days. In sole fluid conditions, on day 1 fluid was accessible at both sides, on day 2 at the left side only, on day 3 at the right side only, and so on. In choice conditions, on day 1 water was accessible at the left side and 10E at the right, on day 2 access sides were reversed and fluid presentation continued with daily side alternation. Phases of experiment followed each other with no interruptions, each animal passed through all 22 sessions.

2.4 Procedures

2.4.1 Adaptation—Before the experiment, animals went into experimental cages for eight-hour exposure on each of three successive days. In these pre-runs, animals stayed in experimental cages from 08:30 to 16:30, with isolating cubicles open, lights in the experimental room “ON” and ad-lib food placed on the cage floor but no fluid access and no data collection. Between and after the pre-runs animals stayed in their home-cages.

2.4.2 Immediate preparation—On the day before the start of experiment, at 17:00 animals were water deprived a single time for 24 hours. On the 1st day at 08:30, animals went into experimental cages with food ad-lib and no fluid. At 16:30, food was removed; at 17:00, the experiment began.

2.4.3 Fluid access—Daily 15-hr access to fluid lasted from 17:00 to 08:00, under normal lighting cycle (“OFF”=18:00, “ON”=06:00). 10E was prepared in tap water from 95% v/v ethanol (Sigma-Aldrich, St. Louis, MO), 24 hours in advance. Spouts were loaded with 0.1 cc unit volumes of fluids at the start of access. Fluids were freely available from spouts; licks were monitored constantly. Every time when licks into a spout reached 32 (the number of licks to consume 0.1 cc in the utilized setup), the lick counter was reset and the delivery started of the next 0.1 cc unit volume into the respective spout. Licks lasted typically 175 – 180 msec in all groups, occurring at an average frequency of ~5.6 Hz, and it would take roughly 5.7 sec to consume a 0.1 cc of fluid. Rate of evaporation from spouts in the utilized setup was evaluated. Evaporation was compensated by deliveries of 10 µl every 600 sec of no licking, which kept a volume in the spout close to what was left by a rat after the previous completed lick. Hence, every consumed 0.1 cc was accounted for, and fluid was always in the spout. Precise timing of licks and coordinated fluid deliveries allowed smooth generation of the spontaneous drinking behavior by an animal.

2.4.4 Feeding—From 08:00 to 17:00, animals remained in experimental cages with no fluid access and no data collection. Animals were weighed on Mon-Wed-Fri between 08:00–08:30 when cages were serviced. Food was available for 8 hours on the cage floor, the amount of 42-52 g/kg/day given at 08:30 and leftovers - at least 0.5 g and usually no more than 3 g - removed at 16:30. Separation of food from fluid access with two 30-min intervals removed prandial confounds and rendered pure drinking behavior for an evaluation. Feeding conditions appeared adequate based on the reported body weight gain of up to 32 g from the starting level of 400-424 g, over 3-4 weeks under 24-hr concurrent access to 10E, water and food [14-16]. The regimen in our study kept body weight close to the level at the start of experiment, preventing loss and supporting a gain of up to ~10% at the completion of the experiment. Over the experiment, the average body weight was 428 g.

2.5 Data preparation and statistical analysis

Every day a dedicated data file was collected from each animal, with total counts of, and one-msec-precise timestamps for each, of the licks and 0.1 cc fluid drops. Total drop counts yielded the daily intake and preference data that are presented in our concurrent report. For this report, total counts together with precise timestamps of all unit volumes from each datafile served to reconstruct drinking patterns for careful visual examination. The definition of a drinking bout was developed and ethanol and water bout parameters calculated, using the neurophysiological data analysis software “NeuroExplorer” (Nex Technologies, Littleton, MA). The timing of patterns and bout parameters were analyzed by t-Test or individual one way ANOVA, applied to a pair of values as necessary. To compensate for influence of side bias, alternation of an access side and day-to-day variation of fluid consumption in choice conditions, the data were averaged over two-day intervals – days

1+2, 3+4, etc., yielding a series of six values for each group over the 12 days of the choice period. The results are expressed as mean values $\pm$ SEM with significance set at $p < 0.05$.

3. Results

3.1 Patterns and general timing of fluid intake

Drinking patterns at the start of each phase varied substantially but gradually stabilized. In each group, characteristic patterns developed by day 4 in the sole fluid conditions and by day 7 in the choice period. During sole fluid conditions, drinking patterns were similar in all groups - Fig. 1. Drinking in water phase was active at the start of access, after 2-3 hours, drinking slowed and shifted to distinct episodes separated by ~ 0.5 hr. After 4-6 hours, it transformed into small and sporadic “maintenance” episodes. Drinking in ethanol phase was active within initial 0.5 hr and continued in steadily distributed episodes.

During choice period, patterns in all groups showed both similarities and differences - Fig. 2. Water intake in P and SD rats resembled that in water phase but with a shorter active period at the start and an earlier shift to “maintenance” drinking - Fig. 2: P-rat, SD. Pattern of ethanol intake in P rat was the same as in ethanol phase, and in SD – similar but less regular. In Wistar rats, water and ethanol intake were intermixed in small and frequent drinking episodes – Fig. 2: Wistar.

Water vs. ethanol was consumed at different times during a daily access period. Start time of intake was similar between groups during both sole-fluid conditions - Table 1A. Time to consume the 1st 50% of daily intake (T'_{50}) in sole-fluid conditions for ethanol was roughly twice the duration for water in P [$F(1,34) = 20.77$, $p < 0.0001$], Wistar [$F(1,26) = 11.49$, $p < 0.01$] and SD rats [$F(1,46) = 142.13$, $p < 0.000001$]. Start time of water intake during choice period was longer vs. the water phase in P [$F(1,62) = 6.31$, $p < 0.05$], Wistar [$F(1,58) = 8.87$, $p < 0.01$] and SD rats [$F(1,96) = 4.51$, $p < 0.05$]. Start time of ethanol intake during choice period was longer vs. the ethanol phase in P [$df(69)$, $t = 1.74$, $p < 0.05$ one-tail], Wistar [$F(1,54) = 5.67$, $p < 0.05$], and SD rats [$F(1,95) = 5.95$, $p < 0.05$] - Table 1B vs. Table 1A. Start time of ethanol vs. water intake during choice period was delayed in P [$F(1,104) = 4.58$, $p < 0.05$] and SD [$F(1,143) = 11.69$, $p < 0.001$], but very similar in Wistar rats - Table 1B. T'_{50} for water shortened during choice period to less than a half vs. water phase in P [$F(1,72) = 21.82$, $p < 0.0001$] and SD [$F(1,95) = 50.92$, $p < 0.00001$], but not in Wistar rats - Table 1B vs. Table 1A. The Wistar's T'_{50} for water during choice period was longer vs. P [$F(1,94) = 5.37$, $p < 0.05$] and SD rats [$F(1,112) = 24.28$, $p < 0.00001$] - Table 1B. Overall, patterns for water vs. ethanol were quite different with water intake occurring mostly around the start of dark phase and ethanol intake continuing steadily throughout the entire dark phase.

3.2 Structure of a drinking episode and bout parameters

To define a bout, drinking patterns were examined at high temporal resolution (Fig. 3). Ethanol and water intake may alternate in drinking episodes at close temporal proximity to each other over entire daily access. A drinking episode consists of yet smaller licking bursts containing from few to several tens of licks, separated by intervals that correspond to an omission of a variable number of consecutive lick cycles. Such microbursts may have from a sub-second to an occasional multi-second duration. Multiple sets of candidate bout criteria were tested by matching bouts identified with each set vs. raw drinking patterns for 36 sessions (8+8+20 in sole-fluid and choice periods, respectively) from each group. Both 10E and water bouts were defined as consumption of at least 0.4 cc with an interval between sequential 0.1 cc unit volumes of no longer than 50 sec in single fluid and 25 sec - in choice

conditions. For an average-weighting (428g) rat, these criteria yielded a hypothesized minimal intake of 0.93 cc/kg fluid and 0.074 g/kg ethanol per bout.

The following bout parameters were calculated. (A): percentage of intake in bouts – vs. daily intake. (B): in-bout time to accumulate a 0.1 cc ($T'0.1$) – time between consecutive 0.1 cc drops in a bout. (C): number of bouts – generated per day. (D): bout size – volume of fluid consumed in a bout. (E): inter-bout interval (iBi) – a time between consumption of the last 0.1 cc drop of a previous bout and the first one of the next. (F): bout duration – a time between consumption of the first and the last 0.1 cc drop in a bout. Table 2 shows exact values at the start and the end of each phase, grouped by parameters A through F.

3.3 Drinking bouts in sole fluid conditions

During water phase of the experiment, bout parameters differed the least between groups (exact values shown in Table 2: “water $\times$ sole-fluid”), in agreement with the uniformity of drinking patterns. The following bout features were the most notable. (a): The percentage of intake in bouts was close to 95% of daily intake in all groups with quantitatively minor changes and differences within and between groups – Fig. 4A. (b): The $T'0.1$ was similar in all groups and decreased in P [df(9), $t = 3.14$, $p < 0.05$], Wistar [df(7), $t = 2.29$, $p < 0.05$ one-tail] and SD rats [F(1,26) = 25.78, $p < 0.0001$] – Fig. 4B: day 5 vs. day 1. (c): Generally greater number of water bouts in P rat vs. other groups (Fig. 4C), with the iBi becoming shorter in P vs. Wistar [df(15), $t = 2.08$, $p < 0.05$ one-tail] and SD [df(19), $t = 2.25$, $p < 0.05$] rats (Fig. 4E: day 5), correspond to greater daily water intake in P vs. other rats (see our concurrent report). By the end of water phase, a size of water bout for an average rat was in P rat 3.5 cc/kg, in Wistar 3.7 cc/kg, and in SD group 4.9 cc/kg.

At the transition between the sole-fluid phases, on the day 1 of ethanol phase vs. the day 5 of water phase, the following bout changes were common for all groups. (a): The percentage of intake in bouts decreased in P [df(9), $t = 1.84$, $p < 0.05$ one-tail], Wistar [F(1,14) = 9.47, $p < 0.01$] and SD rats [F(1,25) = 32.65, $p < 0.00001$] – Fig. 5A: day 1 vs. Fig. 4A: day 5, Table 2A. (b): The $T'0.1$ extended in P [F(1,18) = 7.34, $p < 0.05$], Wistar [F(1,14) = 8.72, $p < 0.05$] and SD rats [F(1,25) = 22.24, $p < 0.0001$] – Fig. 5B: day 1 vs. Fig. 4B: day 5, Table 2B.

During ethanol phase (exact values shown in Table 2: “10E $\times$ sole-fluid”), common for all groups were the following bout changes. (a): The percentage of intake in bouts elevated in P [F(1,18) = 17.76, $p < 0.001$], Wistar [F(1,14) = 13.05, $p < 0.01$] and SD rats [F(1,25) = 9.00, $p < 0.01$] – Fig. 5A: day 5 vs. day 1. (b): The $T'0.1$ shortened in P [F(1,18) = 13.45, $p < 0.01$], Wistar [F(1,14) = 4.80, $p < 0.05$] and SD rats [F(1,25) = 11.80, $p < 0.01$] – Fig. 5B: day 5 vs. day 1. (c): The bout size increased in P [F(1,18) = 20.03, $p < 0.001$], Wistar [F(1,14) = 25.14, $p < 0.001$] and SD rats [F(1,25) = 38.03, $p < 0.00001$] – Fig. 5D: day 5 vs. day 1. (d): The bout duration extended in P [F(1,18) = 6.85, $p < 0.05$], Wistar [df(7), $t = 2.01$, $p < 0.05$ one-tail] and SD rats [F(1,25) = 13.24, $p < 0.01$] – Fig. 5F: day 5 vs. day 1.

In all groups during ethanol phase, at similar levels were percentage of intake in bouts (Fig. 5A), number of bouts (Fig. 5C), iBi (Fig. 5E), and bout duration (Fig. 5F). Between groups the following bout differences appeared by the end of ethanol phase. (a): The $T'0.1$ was longer in Wistar vs. P [F(1,16) = 5.41, $p < 0.05$] and SD rats [F(1,20) = 5.48, $p < 0.05$] – Fig. 5B, day 5. (b): The bout size was smaller in Wistar vs. P [F(1,16) = 5.09, $p < 0.05$] and SD rats [df(20), $t = 1.82$, $p < 0.05$ one-tail] – Fig. 5D, day 5. Autocorrelation and interval histogram plots of a sample session from the ethanol phase (Fig. 6) show that ethanol bouts occur regularly at intervals near the average iBi (~1.5 hr, as per Fig. 5E). The absence of bouts in the autocorrelogram at ~0.6 hr and ~2.0 hrs indicates that bouts are not random and result from a systematic sequencing process. A small peak in the histogram at short intervals (at ~0.25 hr) indicates that a burst of two bouts, instead of a single one, may appear at

regular main intervals of ~1.3 hr as per sample (~1.5 hr as per Fig. 5E). By the end of ethanol phase, a size of ethanol bout for an average rat was in P 6.3 cc/kg 10E = 0.49 g/kg ethanol, in Wistar 4.4 cc/kg 10E = 0.35 g/kg ethanol, and in SD group 5.1 cc/kg 10E = 0.40 g/kg ethanol.

3.4 Ethanol drinking bouts in choice conditions

At the transition to ethanol / water choice, on the days 1-2 of choice period vs. the day 5 of ethanol phase, the following features of ethanol bouts were common and most notable for all groups. (a): The percentage of intake in bouts remained at the same level in each group - Fig. 7A: days 1-2 vs. Fig. 5A: day 5, Table 2A. (b): The T'0.1 remained at the same level in each group - Fig. 7B: days 1-2 vs. Fig. 5B: day 5, Table 2B. (c): The bout size decreased in P [F(1,18) = 7.13, $p < 0.05$], Wistar [F(1,14) = 26.72, $p < 0.001$] and SD rats [F(1,26) = 15.19, $p < 0.001$] - Fig. 7D: days 1-2 vs. Fig. 5D: day 5, Table 2D. (d): The bout duration shortened in P [df(9), $t = 3.41$, $p < 0.01$], Wistar [F(1,14) = 13.65, $p < 0.01$] and SD rats [F(1,26) = 17.64, $p < 0.001$] - Fig. 7F: days 1-2 vs. Fig. 5F: day 5, Table 2F.

During choice period (exact values shown in Table 2: "10E $\times$ choice"), the T'0.1 shortened in P [F(1,18) = 5.90, $p < 0.02$], Wistar [F(1,14) = 5.61, $p < 0.05$] and SD rats [df(14), $t = 2.86$, $p < 0.05$] - Fig. 7B: days 11-12 vs. 1-2. Differences of ethanol bouts between groups, which had appeared during the ethanol phase, progressed further during the choice period. By the end of choice period, these differences were as follows. (a): The T'0.1 was longer in Wistar vs. P [F(1,16) = 6.28, $p < 0.05$] and SD rats [F(1,20) = 7.96, $p < 0.05$] - Fig. 7B: days 11-12. To illustrate this further, interval histograms in Fig. 8 show that Wistar vs. P and SD rats consistently take 1 - 2 sec more of in-bout time to consume a 0.1 cc of 10E. (b): The number of ethanol bouts was the greatest in Wistar vs. P [F(1,16) = 4.84, $p < 0.05$] and SD rats [F(1,20) = 5.62, $p < 0.05$] - Fig. 7C: days 11-12. (c): The ethanol bout size was smaller in Wistar vs. P [F(1,16) = 21.88, $p < 0.001$] and SD rats [df(20), $t = 2.06$, $p < 0.05$ one-tail] - Fig. 7D: days 11-12.

P rats developed by the end of choice period the following unique features of ethanol bouts. (a): The ethanol bout size became the largest in P vs. Wistar [F(1,16) = 21.88, $p < 0.001$] and SD rats [F(1,22) = 10.91, $p < 0.01$] - Fig. 7D: days 11-12. (b): The duration of ethanol bout was the longest in P vs. Wistar [F(1,16) = 16.20, $p < 0.001$] and SD rats [F(1,22) = 8.59, $p < 0.01$] - Fig. 7F: days 11-12. By the end of choice period, a size of ethanol bout for an average rat was in P 5.8 cc/kg 10E = 0.46 g/kg ethanol, in Wistar 3.0 cc/kg 10E = 0.24 g/kg ethanol, and in SD group 3.9 cc/kg 10E = 0.31 g/kg ethanol.

3.5 Water drinking bouts in choice conditions

At the transition to ethanol / water choice, on the days 1-2 of choice period vs. the day 5 of water phase, the following features of water bouts were the most notable and common for all groups. (a): The percentage of intake in bouts remained similar - Fig. 9A: days 1-2 vs. Fig. 4A: day 5, Table 2A. (b): The T'0.1 remained similar - Fig. 9B: days 1-2 vs. Fig. 4B: day 5, Table 2B. (c): The number of bouts decreased in P [F(1,18) = 16.44, $p < 0.001$], Wistar [df(7), $t = 3.72$, $p < 0.01$] and SD rats [df(13), $t = 2.09$, $p < 0.05$ one-tail] - Fig. 9C: days 1-2 vs. Fig. 4C: day 5, Table 2C. (d): The bout size was preserved - Fig. 9D: days 1-2 vs. Fig. 4D: day 5, Table 2D. (e): The bout duration was preserved - Fig. 9F: days 1-2 vs. Fig. 4F: day 5, Table 2F.

Differences of water bouts between groups, which developed during choice period (exact values shown in Table 2: "water $\times$ choice"), corresponded to the differences in preference (see our concurrent report) and drinking patterns for water vs. ethanol between groups. By the end of choice period (days 11-12), these bout differences were as follows. (a): The iBi

was shorter in P vs. Wistar [$F(1,16) = 8.70, p < 0.01$] and SD rats [$F(1,22) = 4.88, p < 0.05$] - Fig. 9E: days 11-12. (b): Within P rats, drinking of water vs. ethanol showed lower percentage of intake in bouts [$F(1,18) = 32.32, p < 0.0001$] - Table 2A, smaller bout size [$F(1,18) = 28.74, p < 0.0001$] - Table 2D, and shorter bout duration [$F(1,18) = 24.16, p < 0.001$] - Table 2F. (c): Within Wistar rats, drinking of water vs. ethanol showed lower percentage of intake in bouts [$F(1,14) = 7.29, p < 0.05$] - Table 2A, smaller number of bouts [$F(1,14) = 12.73, p < 0.01$] - Table 2C, and longer iBi [df(7), $t = 2.03, p < 0.05$ one-tail] - Table 2E. (d): The percentage of water intake in bouts was higher in SD vs. P [$F(1,22) = 21.31, p < 0.001$] and Wistar rats [$F(1,20) = 4.66, p < 0.05$] - Fig. 9A: days 11-12. (e): The size of water bout was larger in SD vs. P [$F(1,22) = 12.23, p < 0.01$] and Wistar rats [df(20), $t = 1.77, p < 0.05$ one-tail] - Fig. 9D: days 11-12. However, each group by the end of choice period showed very similar values of T'0.1 in water and ethanol bouts - Table 2B: days 11-12. A size of water bout for an average rat by the end of choice period was in P 2.6 cc/kg, in Wistar 3.5 cc/kg, and in SD group 4.9 cc/kg.

Timing of formally identified ethanol and water bouts in Wistar rats complemented the findings of a similar intake patterns and intermixed drinking of both fluids during choice period. Plots of a Wistar rat sample session from the choice period (Fig. 10) represent a coordinated structure of bout sequences. Autocorrelogram plots show that ethanol bouts occur alone or as a group of two ~10 min apart and repeat every ~40 min, while water bouts seem more random. Crosscorrelogram plots show that relative to ethanol, water bouts are clearly consistent and appear within 20 min before repeated ethanol bouts.

4. Discussion

The purpose of this study was a precise assessment of the components of spontaneous drinking that are universal across the three rat lines vs. those unique to each one, to identify specific changes that over time mediate increase of ethanol intake.

4.1 Elimination of prandial interference revealed similarities and unique differences of drinking pattern in P, Wistar and SD rats

A key aspect of this study was elimination of prandial influence on drinking through separation of food from fluid access. In most previous reports, access to food was concurrent with fluid and 70% to 90% of all drinking occurred in the dark phase, coinciding with periods of active feeding either in anticipation of or in response to ingestion of food, uniformly across experimental subjects [8-10,17]. This nocturnal drinking in rats showed a general bi- or tri-peaked pattern [6,18-21] with an even peak distribution through a dark phase.

With elimination of prandial confounds, in present study there were no such peaks of drinking as reported with concurrent food, but a new set of differences emerged even when water was the sole fluid. Intake level during water phase in P was greater vs. the nearly identical levels in non-selected rats (see our concurrent report), but intake pattern in all groups was the same - Fig. 1: water alone. However, time to consume the 1st 50% of daily water appears linked to a shared genetic origin as in P and Wistar vs. SD rats, even though selective breeding targeted P but neither Wistar nor SD rats - Table 1A: water.

For all groups drinking during ethanol phase differed from water phase by much longer time to consume the first half of daily intake (Table 1A), while the only source of water in ethanol phase is 10E - see our concurrent report. However, ethanol has aversive properties for rats [22]. A compromise between the two limitations appears to be a reason why the animals opt for a steady distributed intake in ethanol phase - Fig. 1: ethanol alone.

4.2 Choice drinking pattern: ethanol preference changes during a day, while intake of water and ethanol appears time-linked

Quantitative data on general timing of fluid intake during choice period (Table 1B), where a single rat line differs from another two with similarities, in effect serve as internal controls indicating that the differences are systematic, not random. In the P rat, the parameter of alcohol preference, as based on daily intake (see our concurrent report), was 70%, which obscures the change over the entire duration of daily access. In fact, P rats consumed nearly 100% of all daily water early so that for entire remainder of daily fluid access the majority of intake and time varying preference of nearly 100% was for 10E – Fig. 2: P-rat. Without prandial interference, alcohol preference clearly changes during the daily access time.

In the Wistar rat, closely intermixed episodes of 10E and water consumption occurred as though driven by a common nonspecific process that frequently activated fluid ingestion and directed by a parallel mechanism for ongoing choice decisions. Wistar's un- or weakly determined ongoing choice appeared even at the level of formally identified bouts, as there were repeated switches from one fluid to the other (Fig. 10) during periods of active drinking. Resulting from the labile choice, Wistar rat's time varying preference for 10E over the entire duration of daily access remained near 65% (Fig. 2: Wistar), close to the alcohol preference as based on daily intake – see our concurrent report. In the SD rat, alcohol drinking patterns and time varying preference for 10E were less regular vs. the other groups (Fig. 2: SD), likely due to the lowest daily ethanol intake and intermediate (~50%) level of alcohol preference as based on daily intake – see our concurrent report.

4.3 The phenomenon of a 'drinking bout' is present already in sole-fluid conditions

The term 'bout' is a name we apply to closely spaced groups of consumed 0.1 cc unit volumes of fluid. Most drinking in the present study takes place within bouts (Table 2A) as rats remain at spouts continually licking and 0.1cc unit volumes are dispensed every 7 to 10 sec (Table 2B). Available reports on spontaneous drinking bouts in alcohol research target only ethanol / water choice conditions and lack the level of precision achieved in this study where each lick and 0.1 cc unit volume were timed at one-msec resolution over the 22 days. Such precision enabled us to calculate an extended set of bout parameters, beyond their number and size.

The similar percentages of water intake in bouts together with a uniform decrease of time to accumulate 0.1 cc water in a bout in all groups by the day 5 of water phase, suggest a universal for all lines process of entrainment to drink water as the sole fluid in a similar manner in the specific setup of the present experiment – Fig. 4A,B. Greater number of water bouts (Fig. 4C) with gradual development of the shortest interbout interval (Fig. 4E: day 5) allowed P rat to fit the highest vs. other groups daily level of water intake (see our concurrent report) into a visually similar drinking pattern – Fig. 1: water alone. During ethanol phase, the majority of bout parameters showed similar and significant, unidirectional changes in all groups – Fig. 5A,B,D,F. Such changes suggest development of uniform micro-behavioral adaptations to consumption of ethanol (10E) as the sole fluid by rats of different origin.

4.4 Bout size and projected levels of ethanol

The per-bout doses of ethanol in this study appear within the range of known mildly stimulating doses for rats [23-25] and are commonly considered "low alcohol doses". The ethanol doses per bout were by the end of ethanol phase in P 0.49 g/kg, in Wistar 0.35 g/kg and in SD rats 0.40 g/kg, and by the end of choice period in P 0.46 g/kg, in Wistar 0.24 g/kg and in SD rats 0.31 g/kg. P rats in early drinking are likely to generate a greater self-exposure to post-absorptive effects of ethanol vs. non-selected rats. Currently no methods

are available for precise monitoring that would provide real time data on blood or brain levels of ethanol during undisturbed bout drinking in freely behaving animals. According to ethanol pharmacokinetics studies [26-28], the aforementioned bout doses should produce projected average peak levels of ethanol in blood and brain of about 30 mg/dL (~7 mM) in Wistar and no more than 50 mg/dL (~11 mM) in P rat during ethanol phase. The respective numbers during choice period should have been about 20 mg/dL (~5 mM) in Wistar and no more than 45 mg/dL (~10 mM) in P rat. For the P rat, the projected per-bout levels are always higher vs. other groups. Ethanol in this study is most likely to be metabolized completely between bouts. Ethanol metabolizing capacity in rat is at the level of 0.33 – 0.35 g/kg/hr [29-31]. At such rate, the bout doses mentioned above should be completely metabolized within a duration of an interbout interval in each group (~ 1.4 hr, Table 2-E × “ethanol”), before the next bout during both ethanol phase and choice period.

4.5 Bout adjustments at transition to choice period

When access conditions changed to ethanol / water choice, the daily intakes for both water and ethanol decreased, becoming smaller than in their respective sole-fluid phases - see our concurrent report. The bout parameters changed immediately as well, however in a manner specific to the fluid. At the transition, water bout sizes remained the same but numbers decreased in each group – Tables 2D,C × “water”, days 1-2 vs. day 5. In contrast, ethanol bout size decreased by ~30% in each group and numbers remained mostly similar - Tables 2D,C × “ethanol”, days 1-2 vs. day 5. This dichotomy represents two fundamentally different ways of microbehavioral adjustment to a change of daily intake. Accordingly, we term the adjustment of a number of bouts with preservation of a bout size the “water strategy”, and the adjustment of a bout size with preservation of a number of bouts - the “alcohol strategy”.

4.6 Modification of bouts during choice period

The in-bout consumption of ethanol in present study comprised nearly 100% of daily intake during choice period – Fig. 7A. Daily ethanol intake and preference increased during choice period in all groups - see our concurrent report. During this increase P rat maintained the number of bouts and – P rat’s unique feature - increased their size, developing the largest ethanol bout (Figs. 7C; 7D: days 11-12) equal to 0.46 g/kg ethanol, vs. 0.24 g/kg in Wistar and 0.31 g/kg in SD rats. Wistar rat, in contrast, increased nearly twice the number of ethanol bouts while their size remained stable and the smallest - Figs. 7C: days 11-12; 7D. In SD vs. other groups, ethanol intake was the lowest and bout modifications were minor. P rats are reported to acquire increased tolerance [14] and taste acceptance [32] of ethanol through chronic drinking in free choice conditions. Our findings may indicate initial development of both features by P rat already early in choice conditions, along with progressively increasing per-bout doses of ethanol and contracting time to consume 0.1 cc of 10E in a bout – Fig. 7B.

Our view is that accumulating experience with ethanol regulates both initiation and size of bouts in subsequent drinking. The role of pre- and post absorptive effects of ethanol in regulation of intake has been studied using i.v. and i.g. catheters and gastric fistulas [33-36]. These studies and analysis of discriminative stimulus properties of self-administered oral ethanol [37] suggest that rats quickly acquire the value of the pre-absorptive ethanol effects based on exposure to both pre- and post-absorptive effects from the occurred drinking episodes. Such experience appears to be used to gauge the amount of ongoing intake during subsequent consumption of ethanol.

Studies on spontaneous ethanol drinking bouts in common-stock lines focused on Long Evans rats [17,21], and most reported bout sizes were close to our data in SD line. The few

reports available on P rat found ethanol intake per drinking ‘episode’ / bout of 0.52 g/kg during the 2nd week of ethanol / water choice, with a further increase to 0.74 g/kg [6] or even ~1 g/kg [20,38] after several more weeks. Such studies utilized different conditions of access to ethanol / water choice concurrent with food, instrumentation with a lower time resolution, and broader criteria for a drinking ‘episode’ / bout. They did not account for prandial effects that cause ethanol bouts to differ in size by as much as 25-45% [10,17] and there were no data on water bouts. A substantial number of studies yielding data on ethanol bouts focus on operant access to ethanol [39], where bout parameters “...clearly reflect the constraints on ethanol intake imposed by the operant response schedule.” [10, p. 380]. Such and other inherent specifics of those studies impede an extended comparison to our present findings. From our data, early development of increasingly large drinking bouts emerges as the initial sign of future heightened ethanol consumption by P rat. In non-human primates, early formation of large ethanol drinking episodes [40] also appeared indicative of future heavy drinking.

What is the possibility that rats are ‘rewarded’ by ethanol per-bout doses as intake elevates over successive days during choice period? A number of studies revealed the differences between P and lower-drinking rats in parameters of mesolimbic DA system in ethanol-naïve [41,42] and -exposed [43,44] conditions. Posterior VTA was reported to mediate the reinforcing effects of ethanol in both P and Wistar rats [45], and VTA’s sensitivity to these effects was found to increase after chronic drinking [46]. To the extent that such DA-facilitated learning takes place early during choice access to ethanol as in the present study it appears to become translated in select-bred P rat into increased drinking through an enlargement of bout size, and not to an increase in seeking-like processes with an elevating number of small bouts as found in Wistar rat.

While ethanol intake increased, water intake decreased over successive days during choice period in all groups - see our concurrent report. P rats, as they did with ethanol, preserved the number of water bouts and duration of the interbout interval, and adjusted the intake by reducing the size of water bout. In contrast, both Wistar and SD rats again preserved bout sizes, but reduced numbers of water bouts with elongation of interbout intervals – Fig. 9C,D,E.

4.7 Bout timing vs. number: implications for regulation of ethanol intake

Ethanol drinking when released from prandial interference reveals its own unique ultradian rhythm independent of feeding pattern. The duration of ethanol interbout intervals in present study (~1.4 hr, Table 2-E) differs from inter-meal intervals under spontaneous unlimited feeding in adult male Wistar and SD rats, of respectively 65 and 52 min [13,47]. The in-bout timing of accumulation for 0.1 cc fluid (Table 2-B) appeared to be the most tightly controlled and uniformly changed micro-behavioral component in all groups, contracting progressively through sole-fluid and then choice conditions. The degree of this contraction becomes very small in choice conditions, so that by the end of choice period the rats likely could not consume fluids much faster - Figs. 7B, 9B. P and SD rats during a bout typically licked rapidly with only little pausing, while in Wistar occasional pauses (Fig. 3) were greater and times to accumulate 0.1 cc fluid in bouts – longer, Fig. 8. Nevertheless, within each group final values of in-bout timing were the same for water and 10E – Table 2B: days 11-12. Precision in-bout timing thus serves as a means of nearly linear microbehavioral conversion of bout duration into bout size in the present study, but is not reported elsewhere. However, identical volumes per lick for both water and ethanol in normal conditions were noted [17,48].

Bout drinking of both water and ethanol appears based on persistent activity of a brain-stem licking rhythm generator [49,50]. The output from this subsystem into consummatory

behavior must be subject to change in ‘state’ of neuronal circuits at other levels of CNS comprising yet unidentified brain machinery that controls initiation and duration of drinking which may result in a coordinated bout or scattered drinking, directed at ethanol or water according to parallel ongoing choice decisions. Integration of these drinking “pulses” over daily cycle may serve as a basis for a universal system that maintains consistent level of total fluid regardless of line specifics, as found in our concurrent report. Differences between P and Wistar rats in spontaneous firing of VTA DA neurons have been found [51] in ethanol-naïve awake immobilized state. However, no analysis of neural activity causal to choice decisions in a rat freely drinking ethanol yet has been performed [52].

How do present findings relate to concepts of ‘craving’ and ‘loss of control’? Both concepts have been cautiously deemed relevant to changes in ethanol bout drinking parameters [39,53]. An initial possible interpretation of our findings may be that ‘craving’ and ‘loss of control’ correspond to persistent internal brain states that become actualized in P rat as enlarged and elongated bouts, and in Wistar as increased numbers of bouts, to achieve higher ethanol intake. The hypothesized brain states could result from an ‘obsessive’ persistence of activity in yet unidentified circuits.

4.8 Conclusions

Patterns of drinking, when released from prandial modulation, show that P vs. Wistar and SD rats develop early during ethanol / water choice conditions a systematic shift to consume nearly 100% ethanol (10E) for a greater part of a daily access cycle. The entire mosaic of bout modifications in ethanol / water choice drinking points to a principal difference of P vs. non-selected rats in microbehavioral strategies to adjust choice intake of both ethanol and water. P rats primarily change the size of drinking bout, following over time the universal ‘alcohol’ strategy as found in all lines at transition from sole-fluid to choice conditions, while Wistar and SD rats primarily change the number of drinking bouts, following the ‘water’ strategy. This allows P rats early during choice conditions to develop the largest ethanol drinking bouts with pharmacologically meaningful doses of ethanol, nearly twice exceeding their progenitor Wistar line.

This detailed and scalable analysis of spontaneous ethanol bouts in a course of integrated free-flowing drinking behavior, released from prandial bias, provides an important addition to recently available tools for focused study of ethanol intake and potential drug medications. The present work also allows a future search for causal patterns of neural activity in reward related brain regions during active choice drinking in freely behaving animals, to advance understanding of central neural circuit control over oral ethanol self-administration.

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Highlights

- Initial development of alcohol and water consumption in P vs. nonselected rats.
- Intake volumes and timing measured precisely, prandial confounds eliminated.
- P rats vs. non-selected develop specific drinking patterns in ethanol/water choice.
- P rats adjust choice intake through a change of bout size, nonselected – of number.
- Precise behavioral analysis allows a study of ongoing neural control over drinking.

Fig. 1. Drinking patterns in sole-fluid conditions (typical samples shown) were similar in P, Wistar and Sprague Dawley rats. **A:** water intake in all groups was active at start, slowed after 2-3 hours at the approach to water satiation and transformed after 4-6 hours into smaller and less frequent “maintenance” episodes. **B:** 10% v/v ethanol (10E) intake in all groups had a ladder-like pattern of distributed, large drinking episodes.

Fig. 2. Drinking patterns during choice period (typical samples shown) were different in P, Wistar and Sprague Dawley (SD) rats. **P rat** water intake was active at the start of a session, but after 1-1.5 hrs transformed into occasional minute drinking episodes; ethanol (10E) drinking occurred in regular distributed bouts. **Wistar rat** water and ethanol intake patterns were similar, and both fluids were consumed in small and frequent, intermixed episodes. **SD** water intake pattern was similar to P rat; ethanol intake pattern varied more and alcohol preference was lower vs. other groups.

Fig. 3. Detailed structure of drinking pattern. **A** - view of an entire session, bin = 4 sec: ethanol (10E) and water appear consumed close together in time. **B** - zoomed-in view, bin = 50 msec: adjoining episodes of ethanol and water intake stand ~168 sec apart, both 10E and water drinking consist of rapid succession of licks grouped in small bursts interspersed with short breaks. Graphs generated from the Wistar sample of choice drinking in Fig. 2.

Fig. 4.

Drinking bouts during water phase in P, Wistar (W) and Sprague Dawley (SD) rats. **A:** percentage of intake in bouts (% 'B) – constituted ~95% of intake in all groups, changes were minor. **B:** in-bout time to accumulate 0.1 cc (T '0.1) – by the day 5 in all groups decreased significantly vs. the day 1. **C:** number of bouts (# 'B) and **D:** bout size – changes were minor, P generally had more bouts than other rats. **E:** inter-bout interval (i-B 'i) – by the day 5 was the shortest in P rat. **F:** bout duration – changes were non-significant. The values represent means $\pm$ SEM. Differences significant at $p < 0.05$ or less are indicated: ^ – between the day 5 and the day 1 in a given group; + – between P and W rats; x – between P and SD rats, * – between W and SD rats.

Fig. 5.

Drinking bouts during ethanol phase in P, Wistar (W) and Sprague Dawley (SD) rats. **A:** percentage of intake in bouts (% 'B) – in each group: on the day 1 was significantly lower vs. the day 5 of the water phase (Fig. 4A: day 5), by the day 5 of ethanol phase grew significantly vs. the day 1, to ~95%. **B:** in-bout time to accumulate 0.1 cc (T'0.1) – in each group: on the day 1 was significantly longer vs. the day 5 of water phase (Fig. 4B: day 5), by the day 5 of ethanol phase shortened significantly vs. the day 1; in W rat remained the longest. **C:** number of bouts (# 'B) – in all groups was similar and by the day 5 decreased similarly vs. the day 1. **D:** bout size – in all groups by the day 5 increased significantly vs.

the day 1, in W rat remained the smallest. **E:** inter-bout interval (i-B 'i) - was similar across groups, changes were non-significant. **F:** bout duration – in all groups was similar, by the day 5 increased significantly vs. the day 1. The values represent means $\pm$ SEM. Differences significant at $p < 0.05$ or less, between the day 5 of water phase (Fig. 4: day 5) and the day 1 of ethanol phase are indicated by “ Δ ”; other symbols are as per the legend to Fig. 4.

Fig. 6. Periodicity of drinking bouts during ethanol phase, both graphs generated from the same typical session. Autocorrelogram: bout occurrence peaks at approx. ± 14 min, ± 1 hr 15 min, ± 2 hr 40 min and ± 3 hr 22 min. Histogram: inter-bout intervals (iBi-s) peak at 8 to 20 min, 1 hr 12 min with sub-peak at 1 hr 32 min, and 3 hr 22 min.

Fig. 7.

Ethanol bouts during choice period in P, Wistar (W) and Sprague Dawley (SD) rats. **A:** percentage of ethanol intake in bouts (% 'B) – in all groups was ~95% of daily intake, changes were minor. **B:** in-bout time to accumulate 0.1 cc (T'0.1) – in all groups significantly shortened by days 11-12 vs. days 1-2, in W rat remained the longest. **C:** number of bouts (# 'B) – in W by days 11-12 grew significantly vs. the days 1-2 and vs. other rats. **D:** bout size – on the days 1-2 in each group was significantly smaller vs. the day 5 of ethanol phase (Fig. 5D, day 5); by the days 11-12 in P grew significantly vs. the days 1-2 and became the largest vs. other rats, in W rat remained the smallest. **E:** inter-bout

interval (i-B 'i) – changes were non-significant. **F:** bout duration – on the days 1-2 in each group was significantly shorter vs. the day 5 of ethanol phase (Fig. 5F, day 5); by the days 11-12 in P grew significantly vs. the start and remained the longest vs. other rats. The values represent means $\pm$ SEM. Differences significant at $p < 0.05$ or less between the days 1-2 of choice period and the day 5 of the sole-fluid phase are indicated by “ ”, other symbols are as per the legend to Fig. 4.

Interval histogram of in-bout 10E drops, bin = 200 ms

Fig. 8. Interval histogram for in-bout 0.1 cc unit volumes of 10E from the sample sessions shown in Fig. 2: in Wistar vs. P and Sprague Dawley (SD) rats, the main and secondary peaks shifted to the right by 1 - 2 sec, yielding longer in-bout intervals between drops.

Fig. 9.

Water bouts during choice period in P, Wistar (W) and Sprague Dawley (SD) rats. **A:** percentage of water intake in bouts (% 'B) – by the days 11-12 in P and W rats was lower vs. ethanol (Fig. 7A). **B:** in-bout time to accumulate 0.1 cc (T'0.1) – in W was generally longer vs. other rats. **C:** number of bouts (# 'B) – on the days 1-2 in all groups was significantly smaller vs. the day 5 of water phase (Fig. 4C, day 5); by the days 11-12 in W and SD rats decreased significantly vs. the days 1-2 and in W rat was smaller vs. ethanol (Fig. 7C, days 11-12). **D:** bout size – in SD rat was the largest; by the days 11-12 in P rat was significantly smaller vs. the days 1-2 and vs. ethanol (Fig. 7D, days 11-12). **E:** inter-bout interval (i-B 'i) – on the days 1-2 in all groups was similar; by the days 11-12 in W and

SD rats increased significantly vs. the days 1-2 and in W rat - vs. ethanol (Fig. 7E, days 11-12), in P rat was the shortest. **F:** bout duration – in P rat on the days 1-2 and by the days 11-12 was significantly shorter vs. ethanol (Fig. 7F, days 1-2 and 11-12). The values represent means $\pm$ SEM. Differences significant at $p < 0.05$ or less of water vs. ethanol bouts (Fig. 7) are indicated by “e”, other symbols are as per the legend to Fig. 7.

Fig. 10.

Timing structure of bout sequences during choice period, graphs generated from the Wistar sample shown in Fig. 2. Autocorrelograms - **A**: ethanol bouts occur as a single instance or as a group of two ~10 min apart and repeat every ~40 min. **B**: water bouts occur in a loose group over ~1.5 hr, and repeat every 2.5 – 3 hours. Crosscorrelograms - **C**: ethanol and water bouts occur together in a structured sequence where water bout peaks at 18, 8 or 4 min before ethanol bout (see arrows) and then over 16th to 32nd min afterward, this time appear linked to the next instance of ethanol bout that peaks ~40 min later (A). **D**: view of the same data as (C) at a longer time scale - peaks of water bout occurrence last close to 20 min and appear ~40 min apart, just before the reference and each of the later instances of ethanol bout as in (A).

Table 1

General timing of fluid intake

condition	fluid	Start time of intake (minutes)			Time to consume the 1st 50% of daily intake (1/50, hours)		
		P-rat	Wistar	SD	P-rat	Wistar	SD
1-A: sole-fluid	water	.52 (.07)	.37(.03)	.52(.07)	2.7 (.4)	2.8 (.5)	1.4 (.1) ×*
	10E	.57 (.12)	.32(.03)	.35(.03)^	4.8 (.3) ^	4.5 (.3) ^	4.3 (.2) ^
1-B: choice	water	2.8 (.5)	3.3 (.6)	1.7 (0.3) ×*	1.0 (.2)	1.8 (.3) +	0.6 (.04) ×*
	10E	8.0 (2.3) ^	2.9 (.6) +*	8.3 (2.0) ^	4.9 (.2) ^	5.6 (2) ^ +	5.2 (.2) ^

Table 2
Parameters of drinking bouts

condition	day(s)	water			10% v/v ethanol		
		P-rat	Wistar	SD	P-rat	Wistar	SD
2-A: Percentage of intake in bouts (% 'B)							
sole-fluid	d 1	95 (1)	93 (2)	94 (1)	91 (1)	86 (3)	91 (1)
	d 5	94 (1) Δ	95(1) Δ	98 (1) ^ ×* Δ	98 (1) ^	97 (1) ^	97 (2) ^
choice	dd 1-2	90 (3) e	93 (1)	98 (1) ×*	98 (.4)	97 (1)	94 (3)
	dd 11-12	86 (2) e	91 (2) e	96 (1) ^ ×*	98 (1)	97 (1)	96 (2)
2-B: In-bout time to accumulate o.L cc (T'0.1, sec)							
sole-fluid	d 1	10.1 (.8)	9.8 (1.0)	10.7 (.5)	10.3 (.5)	12.4 (1.5) *	9.5 (.3)
	d 5	8.1 (.7) ^ Δ	8.2 (.3) ^ Δ	7.7 (.3) ^ Δ	7.6 (.6) ^	9.2 (.4) ^ +*	7.9 (.4) ^
choice	dd 1-2	7.8 (.3)	8.6 (.3) +*	7.5 (.1)	8.1 (.3)	9.2 (.4) +*	7.7 (.2)
	dd 11-12	7.4 (.4)	8.0 (.3) ^ *	7.1 (.2) ^	7.3 (.2) ^	8.1 (.3) ^+*	7.3 (.2) ^
2-C: Number of bouts (# 'B)							
sole-fluid	d 1	21 (6)	16 (3)	13 (1)	16 (2)	14 (2)	15 (1)
	d 5	22 (4) × Δ	16 (3)	12 (1)	10 (2) ^	11 (1)	10 (1) ^
choice	dd 1-2	8 (1)	10 (2)	10 (1) e	11 (1) ×	9 (2)	7 (1)
	dd 11-12	7 (1)	5 (1)^ e	7 (1) ^	8 (1)	15 (3) ^ +*	9 (1)
2-D: Bout size(cc)							
sole-fluid	d 1	1.7 (.2)	1.6 (.2)	1.9 (.2)	1.4 (.1)	1.2 (.1) *	1.5 (.05)
	d 5	1.5 (.3)	1.6 (.2) Δ	2.1 (.2) × Δ	2.7 (.3) ^	1.9 (.1) ^ +*	2.2 (.1) ^
choice	dd 1-2	1.4 (.2)	1.4 (.1)	1.9 (.2) ×*	1.8 (.1)	1.2 (.1) +*	1.6 (.1)
	dd 11-12	1.1 (.1) ^ e	1.5 (.3)	2.1 (.2) ×*	2.5 (.2) ^	1.3 (.1) +*	1.7 (.1) ×
2-E: Inter-bout interval (i-B 'i, hours)							
sole-fluid	d 1	1.3 (.2)	1.0 (.2)	1.1 (.1)	1.6 (.1)	1.5 (.2)	1.6 (.1)
	d 5	0.6 (.1) ^ +×Δ	1.2 (.3)	1.0 (.1) Δ	1.4 (.2)	1.8 (.6)	1.6 (.1)
choice	dd 1-2	1.3 (.1)	1.3 (.2)	1.3 (.2) e	1.5 (.2)	2.2 (.6)	2.2 (.4) ×
	dd 11-12	1.2 (.3) +×	2.2 (.3) ^ e	2.0 (.3) ^	1.7 (.2)	1.5 (.4)	2.0 (.3)
2-F: Bout duration (minutes)							
sole-fluid	d 1	2.5 (.3)	2.4 (.3)	3.1 (.3)	2.0 (.1)	2.2 (.2)	2.1 (.1)
	d 5	1.8 (.4)	2.1 (.4)	2.5 (.3)	3.2 (.4) ^	2.8 (.3) ^	2.7 (.2) ^
choice	dd 1-2	1.6 (.3) e	1.9 (.2)	2.3 (.2) × e	2.3 (.2) +×	1.7 (.1)	1.9 (.1)
	dd 11-12	1.2 (.2) e	1.8 (.3)	2.3 (.2) ×	2.8 (.3) ^ +×	1.6 (.1)	1.9 (.2)