THE EFFECTS OF SUCROSE ON ETHANOL CONSUMPTION IN ETHANOL

NAÏVE AND NON-NAÏVE RATS

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Dove, Rachel Jolene. *The Effects of Sucrose on Ethanol Consumption in Ethanol Naïve and Non-Naïve Rats*. Master of Science (Behavior Analysis), May 2014, 30 pp., 2 tables, 12 figures, references, 52 titles.

Sucrose fading and intermittent access are two common procedures that induce alcohol consumption in rodents. Sucrose fading procedures involve exposing ethanol naïve rats to a mixture of ethanol and sucrose and gradually reducing the concentration of sugar. Intermittent access procedures involve providing rats with access to ethanol on alternating days. Given that rats will consume ethanol without sucrose, the role of sugar in the sucrose fading procedure is unclear. Rats must be ethanol naïve when they are exposed to treatment with sucrose fading, so there is no point of comparison to show that exposure to sugar in sucrose fading produces higher levels of drinking. There has yet to be any work that isolates the effects of sugar on the consumption of alcohol. The purpose of the present experiment was to examine the effects of sucrose on ethanol consumption in rats with different alcohol histories. Two groups of six rats were exposed to two successive sucrose fading procedures, 30 days apart and their drinking was measured 30 days after each one. One group was exposed to an intermittent access procedure to establish drinking prior to treatment with sucrose fading, the other was ethanol naïve. Following sucrose fading, all rats drank pharmacologically active doses of ethanol. For both groups consumption correlated with the concentration of sucrose and decreased in a step-wise manner as it was faded. For the ethanol experienced rats, consumption dropped below baseline levels as sucrose was faded and decreased further with the second exposure. In contrast, the ethanol-naïve rats did not decrease consumption from the first sucrose fading procedure to the second. Slight differences in peak force of responses were also observed.

THE EFFECTS OF SUCROSE ON ETHANOL CONSUMPTION IN

ETHANOL NAÏVE AND NON-NAÏVE RATS

Many factors contribute to alcohol consumption. One important set of variables

determining consumption is training history, particularly the variables involved when a person

learns to drink (Samson and Grant, 1990, Sharpe and Samson, 2003). Controlling drinking

history in humans is difficult, if not impossible, so animal research has been useful because it

affords experimenters to control many aspects of the subjects' first encounter alcohol. Rats are

often used as a model to study the effects of environmental manipulations on alcohol

consumption. Richter and Campbell (1940) reported an aversion to alcohol at concentrations

greater than 4.375 % (w/v) in rats, leading to a major effort among ethanol researchers to

develop methods of alcohol exposure that would overcome its aversive effects. Early attempts

involved involuntary methods of exposure. Forced-delivery methods such as intubation and

passive exposure to ethanol vapors did not lead to reliable self-administration (Mello, 1973).

Later procedures incorporated opportunities for animals to consume alcohol by

manipulating the motivation operations for food and/or water. Typically, the procedures

involved deprivation of food or water and then providing access to food or water that

contained ethanol. Falk (1969) developed a paradigm of schedule induction in which rats that

were fed dry food consumed surprisingly large amounts of alcohol when it was the only liquid

available. Freund (1969) demonstrated that food-deprived mice would develop signs of physical

dependence in 4 days when maintained on a liquid diet in which ethanol supplied 35% of its

caloric value. Many of these procedures resulted in physical dependence; arguably,

demonstrating the potential negative reinforcing effects of alcohol withdrawal, but do not

show positive reinforcing effects of alcohol (Mello, 1973). In other words, by manipulating motivating operations for other stimuli, the drinking of ethanol was maintained by access to some other reinforcer, not by the ethanol itself.

Realizing the need for a procedure that produces consumption maintained by the alcohol, and the limitations inherent in methods that relied on food or water deprivation to establish naturalistic oral self-administration, researchers began to investigate the effects of environmental variables such as sucrose pairing and substitution on drinking in rats (Tolliver et al., 1987; Samson, 1986). The goal was to overcome the aversive taste of alcohol at high concentrations such that rats would consume sufficient amounts quickly enough to experience its physiological effects (Samson et al., 1988).

Sucrose fading involves introducing naïve rats to ethanol mixed with sucrose and reducing the sugar concentration over the course of several sessions. By the end of most sucrose fading schedules the sugar is entirely withdrawn. The idea behind sucrose fading is that the sugar in the ethanol solution overcomes the aversive stimulation of the alcohol and induces the animal to drink high concentrations in a short period of time, allowing the animal to experience pharmacological effects. Sucrose fading has been a standard procedure in ethanol research labs for its speed and demonstrated effectiveness in inducing self-administration (Samson et al., 1998; Simms 2010). This approach also has face validity in that it mirrors the way that many people first experience alcohol; most humans learn to drink alcohol delivered with sugars and carbohydrates (Simms, 2010).

Though sucrose fading was the most common strategy used to produce drinking in rodents in the 1980s and 1990s (Simms, 2010), there are drawbacks associated with the

procedure. Though it produces drinking, many labs have reported that sucrose fading doesn't result in long–lasting, high levels of consumption (Carillo et al, 2008; Koob and Weiss, 1990; Samson, 1986; Samson et al, 1999).

There is some concern that using sugar to increase ethanol consumption might complicate the interpretation of the drinking that results from sucrose fading procedures. Sucrose exposure has been shown to cause sucrose addiction in rats, which includes such behavioral effects as bingeing, withdrawal, craving, and sensitization (Avena et al., 2008; Colanuoni et al., 2002). Sweetened solutions can be highly reinforcing to rodents and have been demonstrated to be more addictive to rodents than human drugs of abuse such as cocaine (Lenior et al., 2007) and opiates (Spangler et al., 2004). If sucrose is addictive, rats that have learned to drink when it is present and continue to drink after it is removed could be responding to a state of sugar deprivation, ethanol deprivation, or some combination thereof.

Intermittent access procedures offer an alternative approach to induce alcohol self-administration. Like the sucrose fading procedures, intermittent access procedures do not require the deprivation of food or water, but unlike sucrose fading procedures, intermittent access does not require the addition of sugar (Simms, 2008; Simms et al, 2010). Intermittent access procedures built upon early studies known as 2-bottle choice procedures. In these procedures both water and ethanol were freely available in home cages and rats could freely drink for either bottle at any time. Rats exposed to these procedures were shown to consume ethanol (Nielsen et al., 2008; Simms et al 2008; Steensland et al, 2007) however, procedures that provided continuous access to ethanol did not produce the desired high levels of drinking required for an animal model of alcohol use (Simms, 2010).

Simms' (2010) procedure for intermittent access employs the use of a higher

concentration of ethanol compared to the 2-bottle procedures, 16% w/v compared to 8% w/v,

and restricts the periods of access to ethanol in the home cage to 24-hour periods on

alternating days 3 times a week. During the scheduled periods of access, ethanol is available in

a separate bottle in addition to the regular water bottle and the animal is allowed to drink

freely from either or both. Intermittent access promotes steady increases in consumption and

is believed to work because mild alcohol deprivation appears to increase intake as the animal

experiences a cycle of access and deprivation and consumed higher amounts when alcohol is

reintroduced (Bell et al., 2008; Meisch, 1983). At the end of intermittent access the ethanol

consumed by the animal is assumed to be for the pharmacological effects alone.

Both sucrose fading and intermittent access have been shown to produce voluntary

consumption that increases over time. Since there have not yet been any studies that compare

these procedures directly, researchers lack the necessary information to make data-based

decisions when selecting a procedure to induce drinking. On the one hand, intermittent access

procedures avoid the potential complication of adding another variable, sugar to the

experimental equation. On the other hand, a course of sucrose fading takes about 2 weeks,

compared to the 16 weeks needed for intermittent exposure. Deciding between induction

procedures would be easier if it were clear that adding sugar to ethanol produces higher levels

of drinking more quickly, but without a baseline, it is unclear whether sucrose actually increases

drinking over what animals would do with simple exposure (Samson, 2000).

Simms (2010) offers a comparison of the procedures, but because the concentration of

ethanol in the challenge doses given to the animals to assess their drinking matched the

different concentrations of the two procedures, 8% w/v in the sucrose fading procedure, and 16% in the intermittent access procedure. Thus, the results provide information about the consumption of each group of rats as the result of each procedure but do not provide data that can be used for a direct comparison of the procedures; in order to compare these procedures, the effect of sugar must be isolated.

Sucrose fading requires ethanol-naïve rats whereas intermittent access requires rats be given access to high concentrations of ethanol in their home cages. Since we cannot expose the same rats to both procedures without violating one of these requirements, we used a different approach. Using 2 groups of rats, one group with previous experience with ethanol and the other group ethanol naïve, we exposed both groups to two sucrose fading treatments. We compared the drinking of the ethanol experienced rats at baseline to their drinking after each exposure. For the ethanol naïve rats, we compared the drinking following the first and second exposures to the sucrose fading procedure. This experimental design allows us to investigate the effects of sucrose on alcohol consumption in naïve and non-naïve rats in a between-groups and within-subjects fashion.

This experiment offers a novel extension of Simms (2010). In addition to consumption, we measured other dimensions of drinking that have been sensitive to drug effects. Notterman and Mintz (1965) introduced an innovative measurement strategy that allows the recording of force of a response over time. Because their measurement approach is continuous, many dimensions of the response such as duration, peak force, and time integral of force can be calculated from the force readings at specific time points. (See Figure 1 for an example of the dimensions measured in this study).

This study employed a Notterman and Mintz-style analog approach to measurement using a force transducer attached to a lick disk to read the force on the on the disk throughout each session. Ethanol was pumped up through the disk as rats licked and the force of their licking as they consumed the liquid was recorded.

In humans, the drinking of alcoholics differs from that of non-alcoholics on a drink by drink basis. Alcoholics have been shown to take larger sips, drink more quickly, and take longer time between sips than non-alcoholics (Sobel, et al., 1972; Williams and Brown, 1974). Since our measurement strategy allowed us to view the structure of each lick from onset to offset, we took an exploratory approach; we took measurements of the duration, peak force, and time integral of force of responding for both groups to determine whether the exposure to sucrose fading procedures differentially affected any of these dimensions between the two groups.
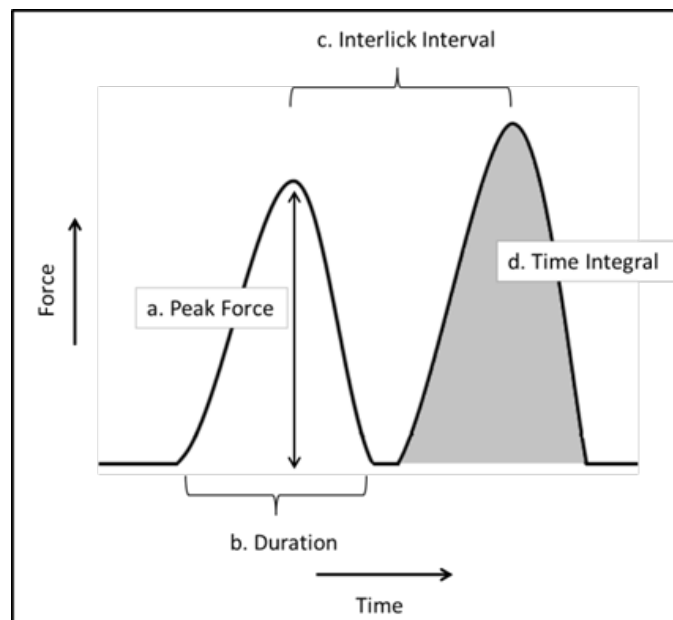

Figure 1. A sample waveform depicting force as a function of time and some dimensions of the response measured in the present study. Peak force (a.) is the highest force recorded per response. Duration (b.) is the time elapsed from the moment when the force meets the response criterion. The time integral of force (c.) represents the summation of force at every time point for the duration of the response. When shown as a waveform, the time integral of force is the area under the curve.

Methods

Subjects

Twelve male Long Evans rats (Harlan Labs, Indianapolis, IN) were used. Rats were

housed individually and maintained on a 12:12 reverse light/dark cycle (lights on at 2100 h).

Alcohol consumption was measured during the dark phase because rodents are nocturnal and

more active in the dark (Freund 1970; Goldstein and Kakihana 1977; Kurokawa et al., 2000).

Rats were housed individually and had ad libitum access to food and water in their home cages.

All housing and experimental procedures were approved by the Institutional Animal Care and

Use Committee at the University of North Texas.

Apparatus

Alcohol consumption was measured in custom-built lickometers. Each lickometer was

placed inside a sound-attenuating experimental enclosure equipped with a fan. The main

compartment of the lickometers were made of clear acrylic and measured 20 x 21 x 24 cm. The

floor consisted of two parts; a perforated metal square of 20 x 21 cm that comprised the main

chamber and a rectangular ledge constructed of acrylic walls and a steel floor.

The ledge provided access to the measurement device. Two holes with a diameter of

about 1 cm were located in the floor, about 1 cm from the edges, in the front and rear right

corners of the chamber. For this experiment, the rear hole was covered by a steel plate

because it was not used. A metal divider was installed on the ledge to keep the rat from

standing on the platform, but permitted the rat access to the apparatus. The apparatus was a

custom built lickometer; briefly, a 1.2-aluminum disk was attached to an isometric force

transducer (Model 31, Sensotec, Columbus, OH). The disk was accessible through at 1-cm hole

in the floor of the chamber. The disk was situated beneath the hole and displace from

underneath the floor by 6 mm. In general, the size of the hole ensured that only the rat's

tongue could come into contact with the disk

Data from the transducer was sampled at 100 Hz using a commercial data acquisition

module (USB-6009, National Instruments, Austin, TX) and amplified (Model UV-10, Sensotec,

Columbus, OH) for analysis. The transducer was calibrated to an accuracy of 0.2-g units. With

no rat in the chamber, the normal fluctuations in readings varied from $\pm$ 1 g; this may be

considered the error of measurement.

Fluid could be pumped onto the disk via a peristaltic pump (Model YO-73160-05, Cole-

Parmer, Court Vernon Hills, IL). Fluid flowed at a rate of 1.2 ml/min. At the beginning of each

30-min session, the pump primed 20 µl of fluid onto the disk. From that point on, every fourth

lick would turn the pump on for 1 s. Timing was not cumulative. If the rat made 4 licks before

the pump cycle ended, the timer was reset for 1 s. In effect, rats could keep fluid continuously

flowing by licking more often than 4 licks/s, which they easily do. Visual inspection of the

chambers and lick disks showed no residual ethanol after each session, suggesting rats

consumed all the fluid delivered.

Data Analysis

Our method of data collection provides several quantitative dimensions of licking. A lick

was defined by any force that exceeded a 2.5 g threshold. Before a subsequent lick could be

registered, the force had to fall below the threshold. The duration of a lick was defined as the

time elapsed from the point forces exceeded threshold to the time when force fell below

threshold. The inter-response interval was the time elapsed from end of a lick until forces again

rose above threshold, defining the onset of a new lick. Peak force was defined as the maximum force exerted during a lick. The time-integral of force was calculated as the sum of all super-threshold forces recorded during a lick and reflects the area under the curve of the waveform produced by the lick gram-seconds. The number of licks was a count of the discrete sequences of criterion responses. Volume consumed was calculated using the cumulative duration the pump was on multiplied by the flow rate and dose (g/kg) was derived using volume consumed and the weight of each animal.

Drugs

Ethyl alcohol (ethanol) (190 Proof, Pharmaco-Aaper, Shelbyville, KY) was mixed with tap water to desired concentrations. Ethanol solutions were changed every 2-3 days throughout the study.

Procedure

This experiment employed two groups of six rats each. Prior to exposure to the sucrose fading procedures, the rats in the first group were trained to drink ethanol under an intermittent access procedure; the rats in the other group were ethanol-naive. Both groups were exposed to the same sequence of sucrose fading treatments. Any difference in consumption following exposure to the sucrose fading procedures is interpreted as the effects of sucrose on ethanol consumption in the presence or absence of previous ethanol exposure. In addition to this between-group comparison, the consumption of the animals in the intermittent access group was compared before and after sucrose fading to determine whether a history with sugar would augment drinking. Finally, sucrose exposures were replicated to

determine the reliability of those effects. (See Table 1 for the order and duration of conditions

for each group of rats.)

Table 1

Order and Length of Conditions

Condition	% sucrose	Session	Condition Criteria	Values calculated
BL*	0	1-3	Stability criteria	Average value for 3 days
SF1	10	4-6	3 days	Average value 2nd and 3rd days
	5	7-8	2 days	Average value for 2 days
	2.5	9-10	2 days	Average value for 2 days
	1.25	11-12	2 days	Average value for 2 days
	0	13-14	2 days	Average value for 2 days
RTBL1	0	44-47	30 days & stability criteria	Average value for 3 days
SF2	10	48-50	3 days	Average value 2nd and 3rd days
	5	51-52	2 days	Average value for 2 days
	2.5	53-54	2 days	Average value for 2 days
	1.25	55-56	2 days	Average value for 2 days
	0	57-58	2 days	Average value for 2 days
RTBL2	0	59-61	30 days & stability criteria	Average value for 3 days

Note: Asterisks denote sessions unique to the rats with intermittent access to ethanol in their home cages.

Intermittent Access Procedure

To establish a history of ethanol consumption without the use of sucrose, we exposed

one group of rats, to an intermittent access procedure for sixteen weeks in a to 16% w/v

ethanol in their home cages in a modified version of a procedure described by Simms et al

(2010). Bottles were hung within an hour of 0930 on Monday, Wednesday, and Friday and

removed 24 hours later. Placement was timed to occur shortly after the offset of lights. The

total amount of ethanol consumed (g) was calculated from the difference in weight of the

bottle before and after it was hung on the cage, and the rats were weighed each day to

calculate the total dose (g /kg) over 24 hours. After 16 weeks of intermittent access the rats

were consuming between 4 and 6 (g/kg) daily. The duration of access to ethanol was gradually

reduced to 4 hours in home cages over a period of 4 days in preparation for the shorter

sessions to be used in the lickometer chambers.

Adaptation Sessions

After drinking was established in the home cages, the rats in the ethanol experienced

group were exposed to several adaptation sessions. During these sessions, rats were put into

the lickometer chambers and given time to habituate to the chamber environment. For the

ethanol experienced rats, the lickometer was filled with ethanol to control for olfactory cues,

but the pump was not operative. Following the adaptation sessions, the rats were placed into

the lickometers to allow them to encounter the alcohol in the chambers and establish

consumption during sessions in the chambers. The concentration of ethanol in the chambers

was 8% (w/v) to mirror the concentration that would be used in the sucrose fading procedure

described by Samson (1986) and the concentration remained constant within the chambers for

the duration of the experiment.

The ethanol-naive rats were given adaptation sessions similar to those described above

with one difference; water was used after a period of 23 hours of water deprivation instead of

the ethanol that was used for the ethanol experienced. Following adaptation the sessions, all

twelve rats experienced the same experimental conditions.

First Exposure to Sucrose Fading

Rats were exposed to a ten-day sucrose fading procedure. Sucrose was added to the 8%

ethanol solution at a concentration of 10% and faded entirely by the tenth day. (See Table 2 for

concentration by day.) The 10% condition was run for three days instead of two to provide at

least two days of each condition in case the novelty of the sucrose lowered drinking on the first day.

Table 2

Concentration of Sucrose during each day of sucrose fading treatment

Day of Sucrose Fading Procedure	Concentration of Sucrose (w/v) in 8% (w/v) Ethanol
1	10
2	10
3	10
4	5
5	5
6	2.5
7	2.5
8	1.25
9	1.25
10	0

Return to Baseline 0% Sucrose

Following the removal of sucrose from the ethanol solution on day 10 of the sucrose fading procedure, the rats continued M-F sessions with 8% w/v ethanol in the chambers. Rats were run in this condition for 30 days plus stability.

Second Sucrose Fading Procedure

Following consumption stability in the first return to baseline 0% sucrose condition, the rats were exposed to the sucrose fading procedure a second time. Following the second exposure to sucrose fading, a second return to baseline was conducted.

Results

Baseline

Only the ethanol experienced rats were exposed to this condition. After drinking was established in the home cages for all six rats, rats were transferred to the lickometers.

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Consumption levels stabilized within the prescribed 30 days and the baseline reflected levels consistent with consumption in home cages for 5 of 6 rats. Five of the ethanol experienced rats generalized drinking to the lick chambers. Rat 4 however, appears to have failed to generalize drinking to the chambers. During intermittent access in the home cage this rat drank each day alcohol was available. Its consumption was in line with the other rats in the group; it consumed an average dose 4.63 g/kg in 24 hours of ethanol on the last 3 sessions of intermittent access in the home cage compared to 4.66, 5.63, 5.87, 5.21, and 6.15 g/kg for rats 1-3 and 5-6, respectively.

However, once transferred into the drinking chambers, rat 4 drank only 1 of every 2-3 sessions, a schedule reminiscent of drinking during the intermittent access procedure. On the days it did drink, it drank enough to contact the sucrose-ethanol solution (greater than 35 licks), but on non-drinking days it did not lick at all. Given the periodicity of the drinking and the history of consumption in the home cage, this rat was included in the study.

Dose

For the rats with previous ethanol experience, the baseline dose ranged from 0.01 to 0.85 g/kg. Following the first sucrose fade and return to stability, the dose ranged from 0.02 to 0.65 g/kg. Five of six rats showed a decrease in total amount consumed following the first exposure to the first sucrose fading (see Figure 2). The exception was Rat 4 who showed an increase from 0.01 g/kg in baseline to 0.36 g/kg following sucrose fading. Following the second exposure to sucrose fading, the average dose ranged from 0.11 to 0.52 g/kg. Five of six rats consumed a smaller dose of alcohol after the second exposure to sucrose fading compared to baseline, with the exception of Rat 4 which increased the dose from 0.01 g/kg in baseline to

0.32 following the second sucrose pairing. The dose consumed decreased for 5 rats from SF1 to

SF2. For all animals consumption varied with sucrose concentration in a step-wise manner (see

Figure 3).

For the ethanol-naive rats, the dose (g/kg) of alcohol consumed following the first

exposure to sucrose fading ranged from 0.02 to 0.65 g/kg. Following the second exposure, the

average dose ranged from 0.25 to 0.47 g/kg. For three of the rats the dose was decreased after

the second exposure to sucrose fading compared with the first and for three of the rats the

dose was increased (Figure 2). For all animals dose varied with sucrose concentration in a step-

wise manner (Figure 3).
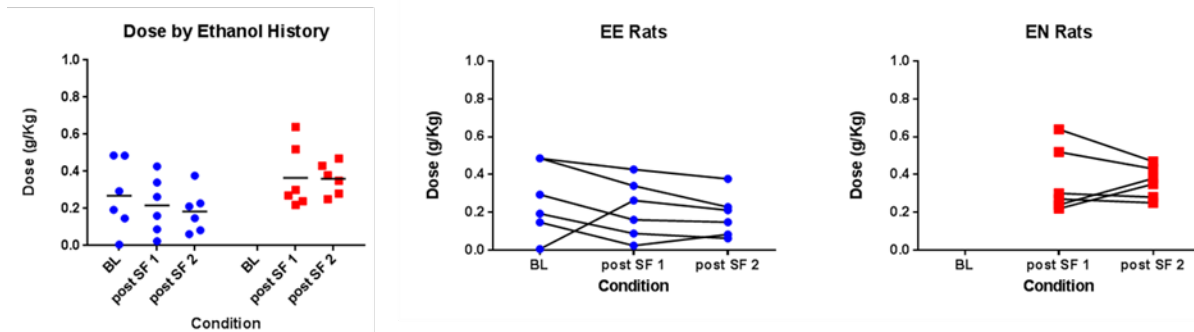
Figure 2. The dose consumed in each 0% sucrose condition is shown above. The graph on the
left shows the mean and the distribution of responses for the ethanol experienced rats (circles)
and the ethanol naïve rats (squares at each 0% sucrose condition. The middle graph tracks the
dose at each 0% sucrose condition for each of the ethanol experienced rats. The graph on the
right shows the dose at each 0% condition for the ethanol naïve rats. Data points represent 3
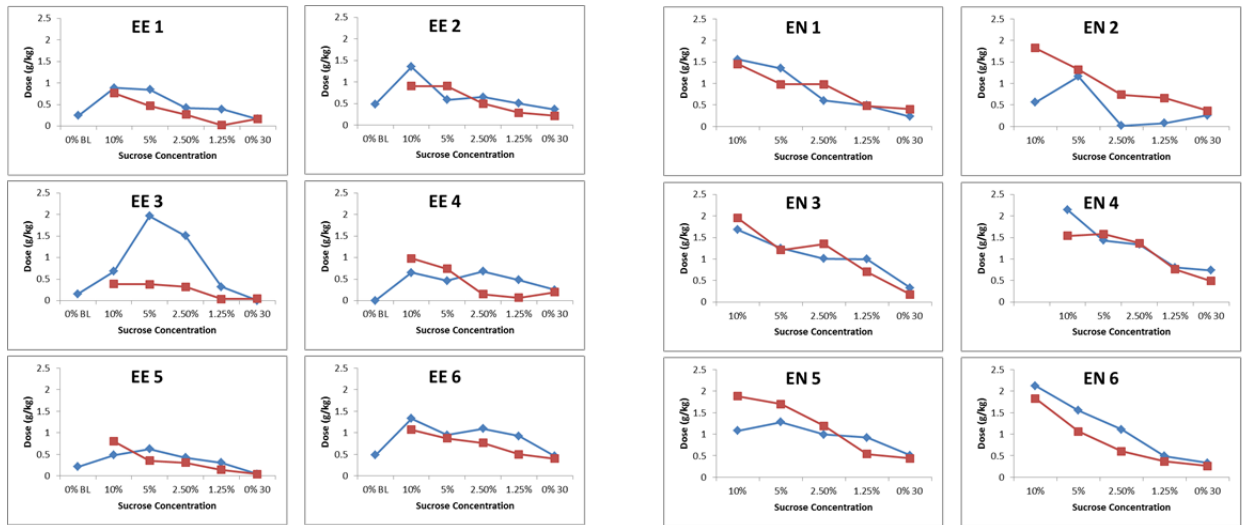day averages.

Figure 3. Dose consumed in each condition by each rat is shown for the first (diamonds) and second (squares) exposures to sucrose fading. The points during the 0% conditions (0% BL and 0% 30) are 3 day averages. The points during sucrose present sessions represent two day averages.

Peak Force

For the ethanol experienced rats, peak force ranged from 4.0 to 9.84 g in baseline. Following the first exposure to sucrose fading, peak force ranged from 3.0-7.6 g. Four of six rats showed a decrease in peak force following the first sucrose fading procedure, two of six showed an increase. Following the second exposure to sucrose fading peak force ranged from 3.0-5.8 g. Compared to baseline, six of six rats showed a reduction in peak force following the second exposure to sucrose fading (see Figure 4). Peak force did not vary with sucrose concentration (see Figure 5).

Peak force of responses emitted by the ethanol-naïve rats ranged from 4.1-7.4 g after exposure to the first sucrose fading procedure. Following the second exposure to sucrose fading peak force ranged from 3.9-6.8 g. Across exposures to sucrose fading, three of the six

rats showed a decrease in peak force and 3 showed an increase (Figure 4). Peak force varied as

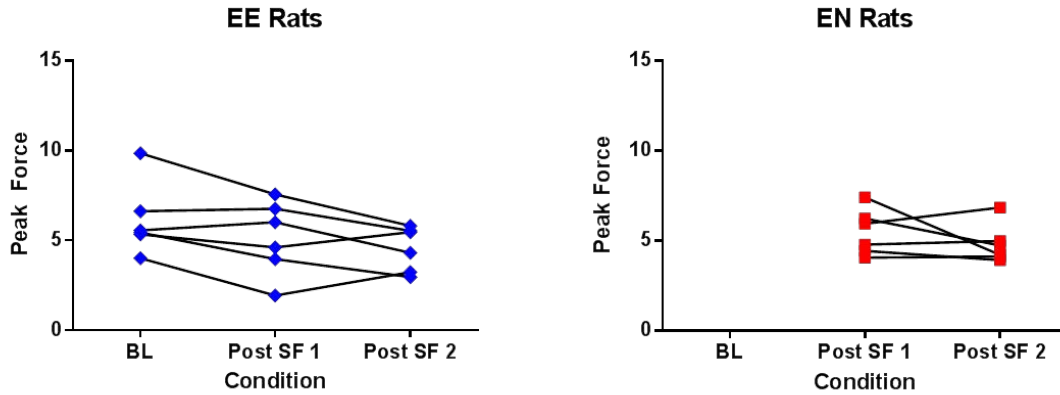a function of sucrose concentration, in both exposures (Figure 5).

Figure 4. The peak force of responses in each 0% sucrose condition is shown for each of the ethanol experienced rats in the graph on the left and for the ethanol naïve rats in the graph on the right. Data points represent 3 day averages for each 0% sucrose solution.
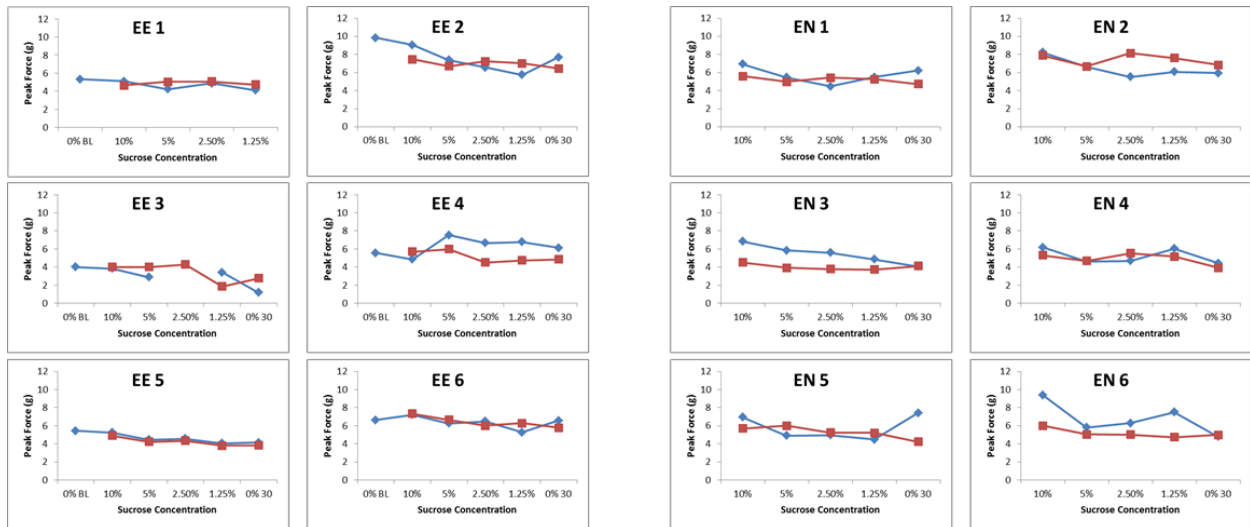

Figure 5. Peak force of responses emitted in each condition for each ethanol experienced rat (left) and for each ethanol naïve rat (right.) The values for the first exposure to sucrose fading are represented by diamonds and for the second exposure values are represented by squares. The points during the 0% conditions (0% BL and 0% 30 are 3 day averages. The points during the sucrose present conditions represent two-day averages. The data for the 2.50% sucrose condition for during the first exposure is missing for EE 3 due to experimenter error.

Both sets of rats show a peak force that varies with sucrose, but the relation of peak force to sucrose concentration differs across groups as shown in Figure 6. The peak forces emitted by the ethanol naïve rats track more closely to sucrose concentration and show a tighter distribution than the forces emitted by the ethanol experienced rats. Though small, the difference is orderly and consistent within and across rats in each group.
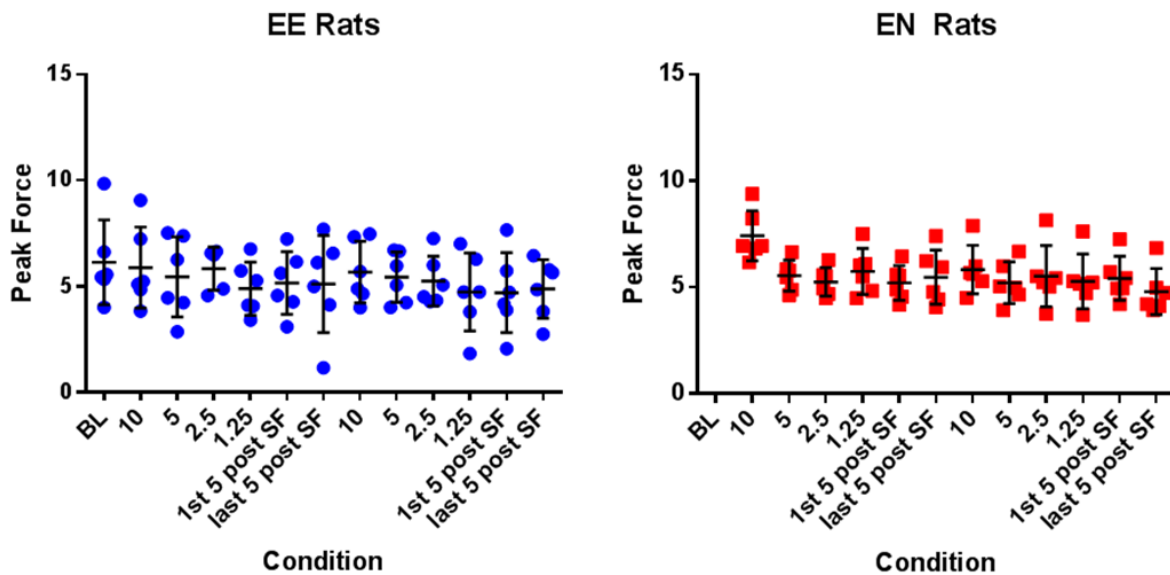

Figure 6. The peak force emitted by each rat in each condition is shown for the first (diamonds) and second (squares) exposures to sucrose fading. The points during the 0% conditions (0% BL and 0% 30) are 3 day averages. The points during sucrose present sessions represent two day averages.

Duration

For the ethanol experienced rats, duration ranged from 0.03-0.05 s in baseline. Following the first sucrose fading treatment six of six rats showed a decrease in duration, this ranged from 0.02-0.04 s. Following the second exposure to sucrose fading duration ranged from 0.02-0.03 s. Compared to baseline, all six rats showed a reduction in duration following each exposure to sucrose fading (see Figure 7). Duration did not vary with sucrose concentration (see Figure 8).

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For the ethanol-naïve rats, duration ranged from 0.03-0.04 s following the first sucrose fading procedure. Following the second exposure, duration ranged from 0.02-0.03 s. Four of six rats showed a decrease in duration compared to the first exposure, and two showed no change (Figure 7). Duration varied as a function of sucrose concentration (Figure 8).
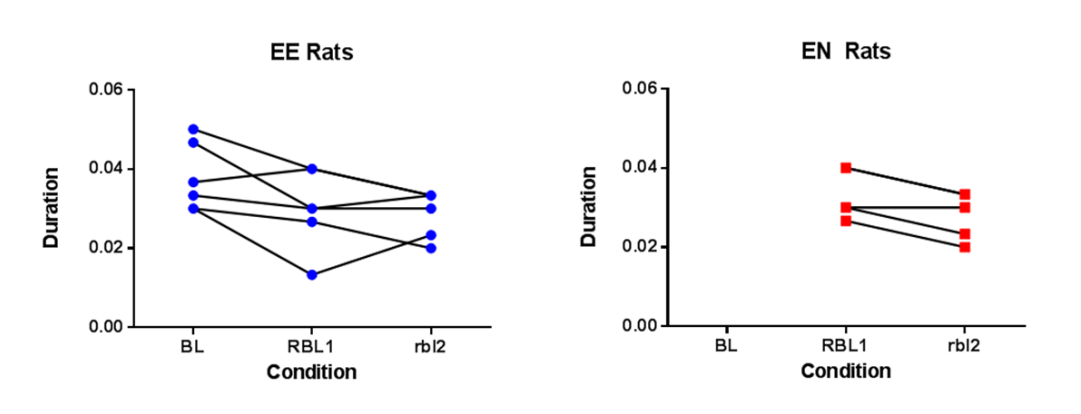

Figure 7. The duration of responses in each 0% sucrose condition is shown for each of the ethanol experienced rats in the graph on the left and for the ethanol naïve rats in the graph on the right. Data points represent 3 day averages for each 0% sucrose solution.
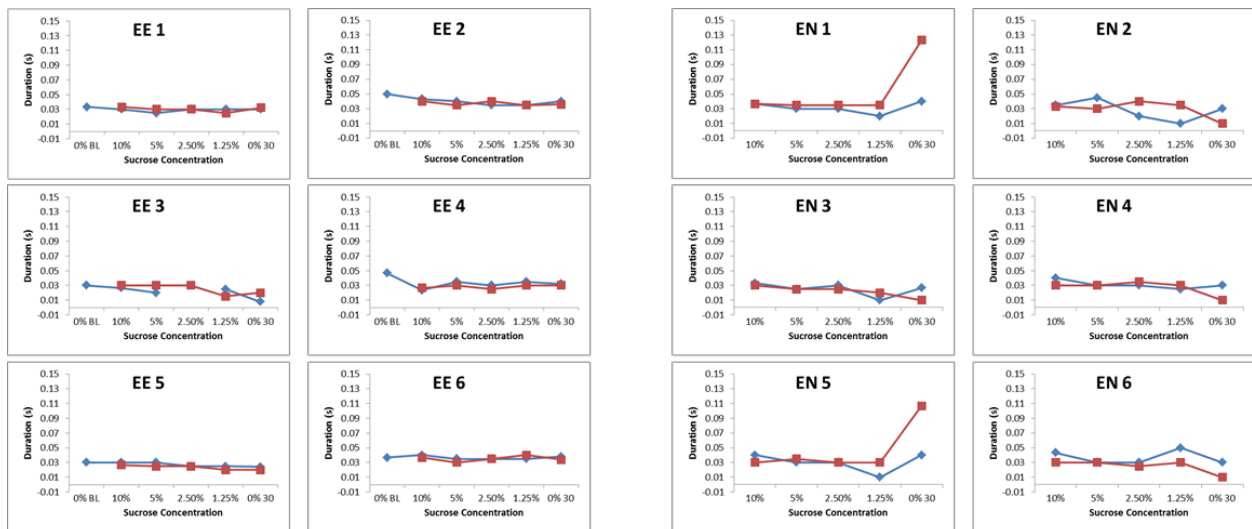

Figure 8.Duration of responses emitted in each condition for each ethanol experienced rat (left) and for each ethanol naïve rat (right.) The values for the first exposure to sucrose fading are represented by diamonds and for the second exposure values are represented by squares. The points during the 0% conditions (0% BL and 0% 30 are 3 day averages. The points during the sucrose present conditions represent two-day averages. The data for the 2.50% sucrose condition for during the first exposure is missing for EE 3 due to experimenter error.

Time Integral of Force

For the ethanol experienced rats, the time integral of force (TOF) in baseline ranged from 9.4-32 g-s. Following the first exposure to sucrose fading TOF ranged from 5.8-22 g-s with five of six rats showing a decrease in TOF compared to baseline. Following the second exposure TOF ranged from 9.2-16.8 g-s with five rats showing a decrease in TOF compared to baseline. Four of six rats showed a reduction from baseline following the first exposure to sucrose fading and an additional reduction in TOF following the second exposure (Figure 8). Time integral of force did not vary with sucrose concentration (Figure 9).

For the ethanol naive rats, following the first exposure to sucrose fading, TOF ranged from 9.2-23 g/s. Following the second exposure TOF ranged from 8.6-21; three of six rats showed a decrease in TOF and three showed an increase (Figure 8). TOF varied as a function of sucrose concentration (Figure 9).
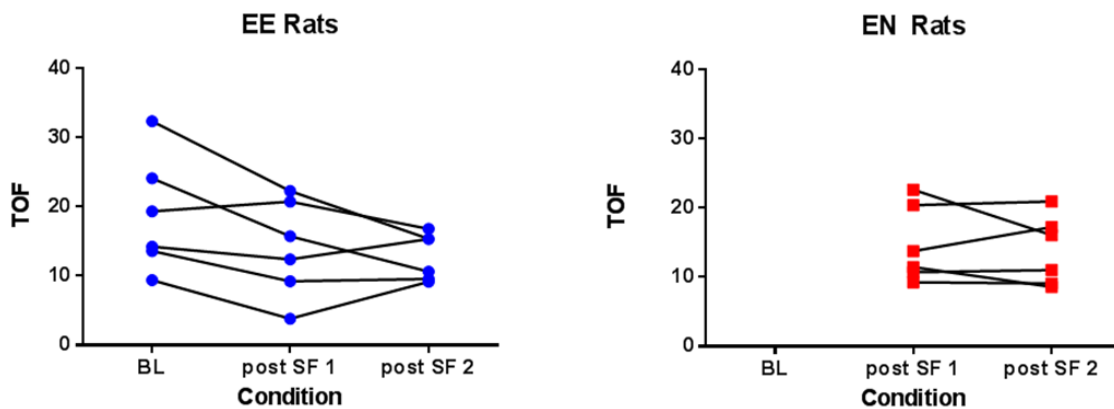

Figure 9. The time integral of force of responses emitted in each 0% sucrose condition is shown for each of the ethanol experienced rats in the graph on the left and for the ethanol naïve rats in the graph on the right. Data points represent 3 day averages for each 0% sucrose solution.
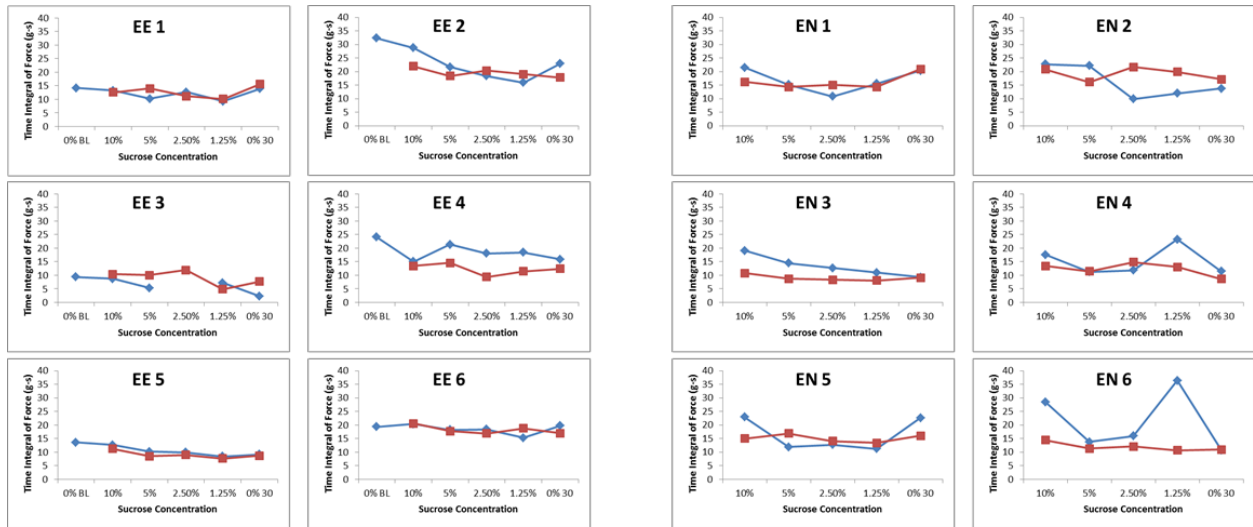
Figure 10. The time integral of force of responses emitted in each condition for each ethanol experienced rat (left) and for each ethanol naïve rat (right.) The values for the first exposure to sucrose fading are represented by diamonds and for the second exposure values are represented by squares. The points during the 0% conditions (0% BL and 0% 30 are 3 day averages. The points during the sucrose present conditions represent two-day averages. The data for the 2.50% sucrose condition for during the first exposure is missing for EE 3 due to experimenter error.

Discussion

All six rats that were exposed to the intermittent access procedure consumed pharmacologically relevant doses regularly before transfer to the chambers. These results replicate Simms' (2010) and further extend that work to include the use of an analog measurement strategy. All of the rats in both groups drank pharmacologically active doses (>0.2g/kg) of ethanol during exposure to sucrose fading. These results are in accord with studies (Sharpe and Samson, 2003; Carrillo et al., 2008) building on Samson (1986).

All of the rats in both groups consumed ethanol in a stepwise manner that varied with the concentration of sucrose during the sucrose fading procedure. Sucrose increased the dose

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for all animals while it was part of the solution, but the effects of sucrose on longer term

drinking differed according to previous ethanol history.

The ethanol experienced rats consumed more ethanol during the sucrose fading

procedure than they had in baseline. However, once sucrose was withdrawn drinking did not

return to baseline levels. The second exposure to sucrose fading, and the corresponding drop in

consumption that followed replicated the effect of the first exposure; ethanol consumption

decreased following exposure to sucrose fading. All of the rats consumed less ethanol after

sucrose fading and the amount of consumption fell considerably; after two exposures to the

sucrose fading procedure, half of the rats with previous ethanol experience failed to consume a

pharmacologically relevant dose of ethanol.

In contrast, when comparing ethanol consumption following the first sucrose procedure

to that which followed the second exposure, half of the ethanol naïve rats consumed more

ethanol and half consumed less. Unlike the rats with previous ethanol history, all of the ethanol

naïve rats consumed pharmacologically relevant amounts of alcohol following both exposures

to sucrose fading.

Without a baseline, it is impossible to say that exposure to sucrose fading procedures

caused rats to drink more than they would have otherwise consumed. Here, our results show

that exposure can *decrease* consumption when it is established previously under intermittent

access procedures.

The details of the procedure used during previous exposure could turn out to be critical

to the different effects on each group of rats. In our experiment, ethanol history was

established using Simms' (2010) procedure. The relative contributions of the length of the

intermittent access, the duration of access during each period during the procedure, and the concentration during intermittent access and at the time of sucrose fading are not clear. Additional investigation is needed to determine whether it is necessary to induce a history of consumption of pharmacologically relevant levels of ethanol to achieve the decrease in consumption following exposure to sucrose fading, or whether ethanol exposure that does not result in pharmacologically relevant dose is sufficient.

Regardless, the success of the intermittent access procedure to induce drinking of pharmacologically active levels of ethanol seems to depend on the intermittent nature of the schedule. Over the course of 16 weeks of intermittent access to alcohol the rats consumed more ethanol with increased experience. The only difference between the previous 2 bottle choice procedures that failed to produce sufficient consumption to model addiction (Simms, 2010) and the intermittent access procedures that succeeded, is the intermittent schedule. The schedule of access and deprivation seems to be the critical element for success. By allowing access only intermittently, the procedure appears to manipulate the motivating operations for ethanol consumption; supplying it when it is likely to be highly reinforcing, and limiting access when it is not as likely to be.

When exposed to the sucrose fading procedure, consumption of ethanol established under intermittent access is disrupted. Though consumption increases sharply with the addition of sucrose, it decreases as sucrose is faded. Consumption decreases after sucrose fading, and a successive exposure results in a successive decrease. If the consumption established during intermittent access was maintained by establishing operations created by the schedule of access and deprivation, the daily schedule of access during and following exposure to sucrose

fading treatments may contribute to a decrease in responding. Additionally, consumption

increases when sucrose is added to ethanol. At the first exposure to sucrose fading, the regular

pattern of intermittent consumption and deprivation of alcohol was replaced by a pattern of

increased exposure to a substance that evokes consumption. The exposure to increased

consumption driven by sucrose short circuited the process underlying the increased

consumption during intermittent access procedures. Instead, when sucrose is present in

ethanol and available daily, the establishing operation maintaining the drinking during

intermittent access isn't in force.

Aside from difference in the effect on dose consumed, exposure to sucrose fading had

different effects on peak force. Peak force for both sets of rats varies with sucrose, but the

relation of peak force to sucrose concentration differs across groups. The peak forces emitted

by the ethanol naïve rats track more closely to sucrose concentration and show a tighter

distribution than the forces emitted by the ethanol experienced rats. Because the force of the

response was not specifically required by a programmed contingency, the difference between

the two groups is interesting. If the peak force represents the value of the solution to the

animal, we might be able to use the differential forces of response to learn something about

whether the animals value ethanol and sucrose the same way regardless of history and lends

itself to a behavioral economic analysis. If the force of the consumption response reflects the

value of the solution, and the force can be manipulated via history or pairing, the next studies

should focus on the identification of the manipulations that increase or decrease the cost an

animal will pay, as well as further description of the relation between force and consumption.

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