IS4250 Healthcare Analytics

Prenatal Alcohol Exposure Increases Postnatal Acceptability of Nicotine Odor and Taste in Adolescent Rats

Group 14

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1. Introduction of Paper

Human studies have proven to us two statements that are well accepted today. Firstly, there is a strong relationship between prenatal alcohol exposure and the increased chance for alcohol abuse during adolescence. Secondly, if exposed to the drug during earlier stage, there is a higher probability that the subject will continue the abuse. However, there is also a growing body of evidence showing that prenatal alcohol exposure does not only increase the probability for later alcohol abuse, but also increases acceptance of nicotine. In most cases, the use of tobacco products and alcohol consumption are highly correlated behaviors. Study has shown that children who became alcoholics had a higher chance to have tobacco products abuse as well (DiFranza JR, Guerrera MP, 1990).

This co-morbid relationship between the alcohol consumption and nicotine dependence can be caused by several factors such as socio-cultural factors, shared genetic influences and pharmacologic cross-tolerance. Even though there are studies investigating the possible basis for the interactions between alcohol and tobacco in both humans and rodents, the effect of prenatal alcohol exposure on postnatal alcohol and nicotine consumption still remains largely unknown. In terms of neurobiological synergies of the two drugs, there is evidence showing that prenatal alcohol exposure affects many of the same cellular and molecular targets influenced by nicotine.

The flavor attributes, including smell, taste and oral irritation, of both drugs determine one's likely level of acceptance towards them. With specific reference to alcohol, there are strong evidences showing epigenetic chemosensory[1] mechanisms through which fetal alcohol exposure increased later alcohol acceptability by decreasing the aversive flavor attributes of alcohol's bitter taste, oral burning sensations and irritating smell(Glendinning JI, Simons YM, 2012) (Youngentob SL, Glendinning JI, 2009). This was achieved by decreasing the expression of bitter and oral irritation receptor genes in the subject's body. Nicotine, sharing the common component chemosensory qualities of an aversive odor, bitter taste and oral irritation with alcohol, is studied in the paper to investigate whether fetal exposure to alcohol increases the acceptability of nicotine's odor and taste in the age of adolescence.

The researcher carried out two experiments to test the odorant-induced innate behavioral responses to nicotine odor and the orosensory[2]-mediated responses to nicotine solutions

[1] Chemosensory: pertaining to the sensing of chemicals, as by olfaction (the sense of smell).
[2] Orosensory: relating to oral senses.

respectively. The experiment results have demonstrated that: 1) prenatal alcohol exposure increases the nicotine acceptability by decreasing the aversion of both its taste and smell; and 2) the potential chemosensory-based mechanisms by which fetal alcohol exposure increases the later initial risk for the use of nicotine, which again contributing to the co-morbid relationship with increased alcohol avidity.

2. Experiments

2.1 Experiment subjects

On fetal development day 5, pregnant female rats were divided into groups containing two weight-matched dams and randomly assigned to one of the two maternal groups. Alcohol exposed dams (ET) received an ad-lib liquid diet which provided 35% of the daily calories via alcohol. The alcohol diet was offered during a period of rapid development of olfactory, gustatory and oro-somatosensory systems. The control group, aka free-choice liquid (FCL) group was provided with ad lib access to an iso-nutritive and iso-caloric liquid diet of maltose dextrin.

For experiment 1, 20 ET (10 male, 10female) and 20 FCL (10 male, 10 female) rats were randomly selected. For experiment 2, 22 ET (11 male, 11 female) and 22 FCL (11 male, 11 female) were randomly selected.

2.2 Experiment procedures

Experiment 1 uses whole-body plethysmography[3] to monitor the sniffing responses after the delivery of air or odorant nicotine of different concentration. Odorant stimuli, together with a continuous airflow, were delivered into test chambers. For any given animal, a habituation period of 40 air only trials was conducted, followed by the delivery of 5 different concentration of nicotine odor in an ascending series ($3.123x10^{-3}$, $6.25x10^{-3}$, $1.25x10^{-2}$, $2.5x10^{-2}$, and $5x10^{-2}$). For each concentration of the odorant stimuli, an animal received randomized 10 trials of air and 10 trials of odorant stimuli. The computer then monitored the sniffing responses regarding 14 variables: sniffing frequency, the number of inspiratory and expiratory sniffs, the duration, volume, average flow rate, and peak flow rate of an inspiratory and expiratory sniff the total inspiratory and expiratory volume; and the total apneic duration.

Experiment 2 conducted a standard brief-access taste tests. Tastants of nicotine and sucrose of different concentrations were presented to experiment subjects on different days. The order

[3] Plethysmography: An instrument for measuring changes in volume within an organ or whole body (usually resulting from fluctuations in the amount of blood or air it contains).

of tastants testing was sucrose following by nicotine for any given animal. The two testing days were separated by one day for the experiment subjects to recover, where they were provided with ad lib access to food and water. Licking responses was monitored during 10-second trials in a 30-minute test session. The average number of licks and latency to first lick to each stimulus concentration was measured. In order to prepare the rats for the licking tests, 3-day training was conducted prior to the training.

The three-day training began on postnatal day 25. Before the training was conducted, the experiment subjects were deprived of water for 22.5 hours to encourage solution intake. First day's training was a 30-minutes training session which allowed subjects to drink de-ionized (DI) water from stationary sipper tube without restriction. After the 30-minutes session, subjects were allowed to access water and food for 1 hour. And then, the subjects were again deprived of water for 22.5 hours. On the second day of training, subjects were allowed to drink from the sipper tube during 10 seconds trial. Trials were separated by 7.5 seconds inter-trial interval. The sipper tube was controlled by a computer-activated shutter. The subjects were deprived of water for another 22.5 hours. On experiment day three, the same procedure as the second day training was repeated.

3. Results and Analysis

3.1 Experiment 1 - odorant-induced innate behavioral responses to nicotine odor

This experiment intends to find the orosensory-mediated responses of the experiment subject to nicotine solution. Experimental plot of the experiment can be found in section 5.1 Appendix A – result of experiment 1. Analysis of the experiment result shows that prenatal alcohol exposure would decrease the aversion of odor of nicotine. Besides, though in real life we do see more men smoking than women, there is no different effect of chemosensory mechanism observed for different sex.

3.2 Experiment 2 - orosensory-mediated responses to nicotine solutions

This experiment aims to find out the taste-responsiveness of the experiment subject to the taste of the nicotine solution. The author adopted two different approaches to measure the taste response of the subjects. First approach is to measure the average number of licks，whereas the second approach is to measure the latency to first lick. As a control of the experiment, both figures were measured using sucrose solution as well. Experimental plot of the experiment can be found in section 5.2 Appendix B – result of experiment 2. Experiment

result shows that prenatal alcohol exposure would decrease the aversion of taste of nicotine. Similar as in experiment 1, no different effect is observed for different sex.

4. Discussions

4.1 Challenges

4.1.1 Valence of the mechanism remains unknown

The author presents a statistically convincing model proving the relationship between prenatal alcohol exposure and postnatal acceptance of odor and taste of nicotine, which is likely to result in increased likelihood for postnatal nicotine use.

However, the valence of the mechanism is largely unknown. How much the chemosensory mechanism contributes to the postnatal nicotine and alcohol use remains an open question. Importantly, the relationship between the prenatal alcohol exposure and co-morbid expression of alcohol and nicotine addiction should not be overstated. There are many other factors which may contribute significantly to the postnatal alcohol and nicotine use.

Often, alcohol and nicotine are called “gateway drugs”, meaning that they are often the initial addictive substances that people would start with. Proven by previous research, the start and maintenance of nicotine drugs is positively correlated with alcohol consumption (Jackson KM, Sher KL, Cooper ML, Wood PK, 2002).

Without the effort to find out the valence of the chemosensory mechanism, the contribution of this paper in understanding the factors causing postnatal nicotine use is difficult to be determined.

4.1.2 Other mechanisms may be involved

Even if the relationship of prenatal alcohol exposure and postnatal acceptance of nicotine is substantial, there may be other mechanisms involved in this relationship other than the chemosensory mechanisms discussed in this paper.

Despite the understanding of the issue achieved which tied up the link between prenatal alcohol exposure and postnatal nicotine use, whether other mechanisms are involved in this process remains unveiled. For example, genetic and environmental factors may have been contributing to the relationship between prenatal alcohol exposure and postnatal nicotine use. This may encompass many mechanisms such as psychological influence of prenatal alcohol exposure and genetic expression which regulate the specific brain neurotransmitters.

Without addressing the impact of other mechanisms involved, the finding of relationship between prenatal alcohol exposure and postnatal nicotine use may be challenged by the presence of other mechanisms.

4.1.3 Failure in addressing the amount of alcohol that would cause the effect

The proposed hypothesis of this paper is that there would be reduced aversion towards odor and taste of nicotine with prenatal alcohol exposure. Though the hypothesis has been proven, this is simply a yes or no question without answering the question that how much consumption of alcohol during gestation period is likely to trigger the effect.

Drinking during pregnancy has always been a controversial issue. Studies have been conducted to investigate the detriments of alcohol consumption during pregnancy. However, some researchers have arrived at a conclusion that, if "moderate" amount of alcohol is consumed on a daily basis may not have detrimental effect on the fetus (ALSPAC Study Team, 2001). Although only one aspect of neurodevelopment was addressed in this paper, it is a useful research which has shed light into the field of study by suggesting that whether drinking during pregnancy is harmful for the fetus would actually have a positive relationship on the amount of alcohol consumed by to-be mothers.

Undeniably, the paper we are studying has proven the negative effect of prenatal alcohol exposure. However, it is not in the scope of this paper to study how much alcohol intake in a daily diet would cause the consumption during pregnancy brings about negative impacts on the fetus. In the designed experiments, 35% of the daily calorie intake comes from alcohol for the alcohol-exposed dam. If alcohol consumption takes up smaller proportion of daily diet, there is a likelihood that the study may arrive at a different conclusion.

We agree that this paper has shown important findings in proving the link between prenatal alcohol exposure and likely decrease in aversion towards flavor of nicotine of the fetus, the experiment result might suggest otherwise if the amount of alcohol consumption during gestation is altered. Hence, in conclusion, the statement that alcohol exposure during pregnancy will lead to higher level of nicotine acceptance may not be sufficiently accurate without addressing whether the mechanism is at play because a significantly large proportion of the mother's daily diet comes from alcohol.

4.2 Limitations

Though this paper presents thorough and convincing analysis on the relationship between prenatal alcohol exposure and increased postnatal nicotine acceptance, according to our analysis, there are limitations inherent to the design of the experiment which could negatively impact the accuracy of experiment results.

4.2.1 Lack of justification for the chosen sample size

For the olfactory behavioral testing (experiment 1), the experiment result was collected from 20 participating alcohol-exposed subjects and 20 participating free-choice liquid subjects. For the orosensory-mediated behavioral testing (experiment 2), the experiment result was collected from 22 participating alcohol-exposed subjects and 22 participating free-choice liquid subject.

Although the author implemented control to ensure that experiment subjects selected demonstrate similar characteristics to eliminate possible bias of results, having limited sample size would be likely to impose negative impact on the accuracy of the experiment results.

Notably, it is not always desirable to have large sample size. More often than not, small sample size is appropriate to detect huge effect whereas large sample size is appropriate to detect tiny differences. An experiment with too few, too many or inappropriate number of experiment subjects as the sample population may compromise its accuracy and quality of result (J. Bartlett, J. Kotrlik, C. Higgins, 2001). In the case of this paper, it is advisable for the author to supplement the choice of sample size with rigorous justification for the paper to be more convincing.

With the information presented in the report, there is insufficient evidence to suggest whether the sample size is chosen at random or it is derived from sophisticated statistical model. Assuming that the absence of explanation on choice of sample size is due to the lack of statistical model used, we identify that the lack of justification for chosen sample size is a potential limitation of the experiment.

For an experiment to have appropriate sample size, several factors need to be analyzed. This include, but is not limited to, nature of hypothesis, effect size, population standard deviation, desired confidence of experiment result to determine the correctness of the hypothesis and the significance level (National Academic Press, 2003).

In conclusion, we advise that, to improve the accuracy of the result and robustness of analysis, the author should include justification on determination of appropriate sample size base on statistical models.

4.2.2 Using animals as experiment subjects

For this experiment, the author collected sample of rats as subjects to test the validity of the proposed hypothesis. However, despite the previous studies investigating the possible basis for alcohol and nicotine interactions in both rodents and humans, the author did not support the judgment of how applicable the experiment results would be for human.

Indeed, using human to conduct this experiment may not be ethically correct, especially so because the hypothesis is to be tested on pregnant women and their children. Moreover, it would be too difficult for result collation because the experiment may span for decades if the hypothesis is to be tested on human. Even if the methodology is collecting of results instead of conducting experiments, it would still be more difficult than resorting to animal research due to reasons such as difficulty in identifying the pregnant women who would consume alcohol during their pregnancy. Study has shown that only 10% of pregnant woman would actually drink alcohol in the United States, further limiting the scope of experiment subjects (Centers for Disease Control and Prevention, 2004).

However, this does not justify using animal as subjects is appropriate, mainly due to the fact that it may give rise to inaccurate result. The idea that animal research might be a poor indicator of human reaction is not new. In fact, in many areas, animal research has been proven to be ineffective in testing human experience. Recent study of monoclonal antibody TGN 1412 which threatened life of six healthy volunteers shows the inadequate prediction of animal research (Bracken MB, 2008).

The reasoning behind why animal research may not be entirely applicable to humans can be complicated. Very often, the difficulty in extrapolating animal research result to human is the poor design or conduct of experiment. Further, other reasons which lead to possible inaccuracy or even failure of animal research include different models as compared to human condition, variable metabolic pathways, nuances in laboratory settings as well as selection of measures (Pound P, Ebrahim S, Sandercock P, Bracken MB, Roberts I, 2004).

Regardless of whether the reason is poor design, conduct or analysis of the experiment, or it is the disparate reaction of animal and human, there is likelihood that animal research may

not be a quality indicator for this proposed hypothesis. To produce sound and robust experiment result, the author needs to justify how applicable the result of rats would be to human experience and state reasons why animal research is more desirable than human experiment in the first place (Andrew N. Rowan, 1984).

4.2.3 Prior training could affect experiment result

As mentioned before, to facilitate the experiment process of experiment 2, the author proposed a prior training plan to get experiments subject familiar with the experiment process. Indeed, it is undeniable that it is especially important for the experiment subject to get familiar with the experiment because the experiment subjects are rats and it is impossible to explain the experiment process to them. However, prior training, though effective in ensuring the experiment is successfully carried out, can also impose negative effect on the accuracy of results.

Due to the fact that experiment subjects are rats, making it difficult to control the behavior of subjects, how much their behavior would be affected by habits and trained reflex remains to be an open question. The average number of licks and latency to first lick may be largely affected by familiarity with the experiment process.

Further, the subjects were deprived of water to encourage liquid consumption. However, this may induce the subjects to make higher solution intake due to thirst. Amount of liquid consumption may depend on various factors including objective and subjective factors. This design of the training may impede the actual experiment from producing accurate result because how much the liquid consumption was induced by thirst may differ for different subjects and was relatively difficult to measure.

Hence, the experiment results may be largely affected by both factors instead of being only correlated with the subjects' acceptability of taste of nicotine, hindering the author from yielding accurate results for experiment 2.

4.2.4 Latency to first lick may not be an accurate measure

Experiment 2 consists of two approaches to measure the subjects' acceptability of taste of nicotine. The first approach is to measure acceptability of taste of nicotine by measuring the subjects' average number of licks of nicotine solution. The second approach is to measure the latency to first lick of the nicotine solution. Based on our analysis, the second approach may not be an accurate measure of the subjects' acceptability of taste of nicotine.

Latency to first lick was intended to be used to measure the speed the experiment subject makes the first lick to the nicotine solution. According to the author's assumption, the faster the subject licks the solution, the higher acceptability they have over the taste of nicotine. However, this approach has innate problems simply because that the experiment subjects are not aware of the taste of nicotine solution before they make their first lick. In this case, latency to first lick would depend on factors other than level of acceptance of taste of nicotine such as level of acceptance of odor of nicotine solution (which is intended to be determined in experiment 1). It can be argued that, experiment subjects may be able to deduce whether they can accommodate the bitter and irritating taste of nicotine based on the odor of the nicotine solution and would only make a faster lick when there is established initial acceptance of flavor of nicotine. Nonetheless, even if the above argument is valid, latency to first lick would still depend on other factors beside acceptance of nicotine flavor, such as distinctiveness of the odor.

Hence, based on our analysis, latency to first lick is not an accurate measure to determine acceptance of taste of nicotine.

4.2.5 Sucrose may not be an appropriate choice for control experiment

In experiment 2, the author proposed a control experiment by measuring the average number of licks and latency to first lick to sucrose solution to compare the result gathered for nicotine solution.

However, in our opinion, this experiment may not the most appropriate design as a control experiment to show the orosensory-mediated response of the group towards solution with no aversive taste. Although sucrose solution is liquid in nature, sucrose is considered to be food for the experiment subjects. The prior training of this control experiment is to deprive the subjects of food instead of water as compared to the training for subjects before the experiment on nicotine solution.

The author does not support the judgment of using sucrose as the control experiment instead of using other solution such as de-ionized water is also a limitation of the experiment.

4.3 Contributions

There are various previous studies on both the effect of prenatal alcohol exposure as well as the factors contributing to co-morbid use of alcohol and nicotine. However, none provides

rigorous study on the effect of prenatal alcohol exposure on the likelihood of postnatal nicotine use. With its effort in linking the tie between prenatal alcohol exposure and postnatal nicotine use, this paper has made significant contribution in the field of healthcare.

Most important contribution of this paper is that it provides statistical proof that if pregnant woman is exposed to alcohol during gestation period, her baby will be more likely to have higher tolerance towards the irritated and bitter flavor of nicotine. Given the common chemosensory characteristics of alcohol and nicotine, the chemosensory mechanism would, at least, decrease the aversion towards the odor and taste of alcohol and nicotine. Previous studies have shown that the flavor attributes are key components which contribute to the initial acceptance of nicotine and alcohol. Further, decreasing the aversion towards the flavor of these two would contribute to the initial adoption of them (Youngentob SL, Glendinning JI, 2009). Hence, by proving the validity of chemosensory mechanism, this paper provides yet another reason why pregnant mothers should not consume alcohol during pregnancy due to the fact that it would lead to higher likelihood for their children to start the consumption of alcohol and nicotine during adolescence age.

Further, the chemosensory mechanism proved in the paper can be applied to a larger perspective. The mechanism does not only work for alcohol and nicotine, but is applicable for many chemical substances with distinct chemosensory attributes. This essentially means that, if there is exposure to a certain chemical substance, for example, a specific drug which has certain chemosensory attributes during the gestation period, there will be increased likelihood that the fetus would be less aversive to other drugs which possess similar chemosensory attributes with likely common sensory transduction pathways. Since there are many licit and illicit drugs with prominent chemosensory drugs, this mechanism is of value to a more general level.

5. Appendix

5.1 Appendix A - Result of experiment 1 (from paper)

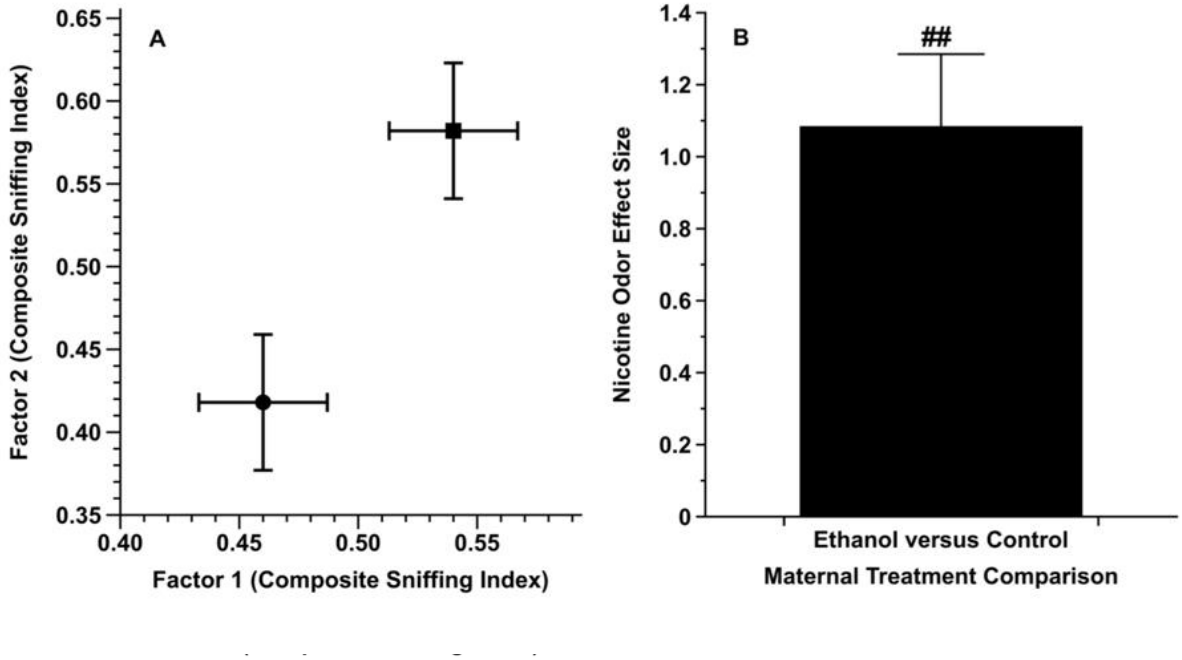


Figure 1. The consequence of prenatal alcohol exposure on the innate odor-mediated behavioral response to nicotine. Panel A illustrates the comparative location of the prenatal alcohol- versus control-exposed groups in a nicotine odor-mediated behavioral response space. The data points are the adjusted least square mean sniffing indexes (± two-dimensional se) as a function of the two prenatal treatments (Solid circles = alcohol exposed; Solid squares = control exposed). Panel B shows the nicotine response-mediated weighted effect size (mean ± se) calculated from the data illustrated in Panel A. ## = $P<0.03$; see text for details.
doi:10.1371/journal.pone.0102255.g001

5.2 Appendix B - Result of experiment 2 (from paper)

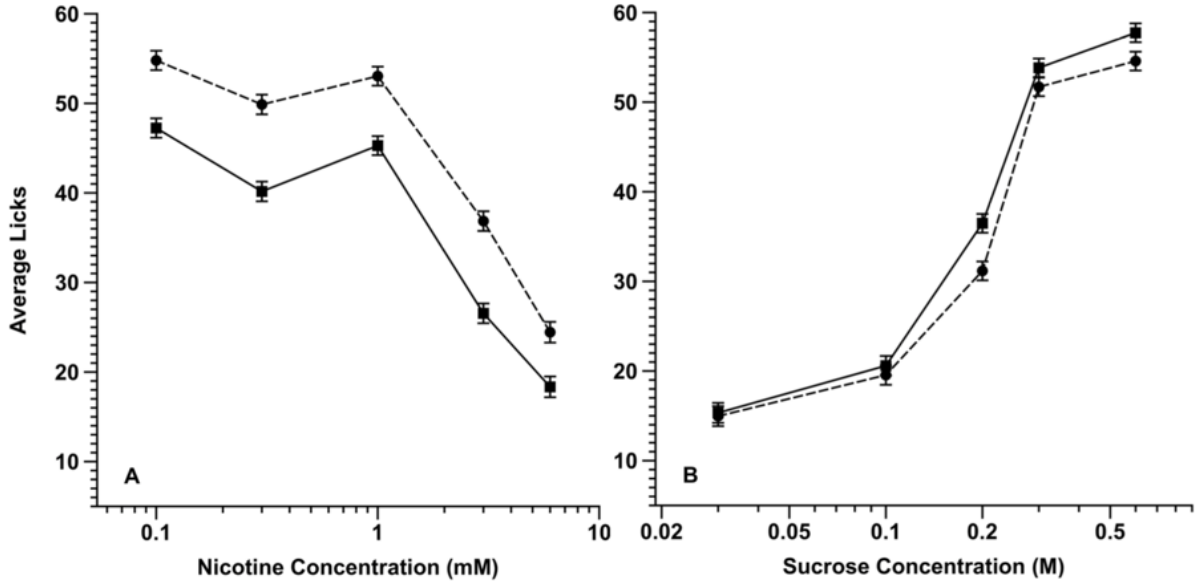


Figure 2. Oral acceptability of a concentration range of nicotine (A) and sucrose (B) solutions to prenatal alcohol- and control-exposed rats. Prenatal alcohol increased the acceptability of nicotine (A) and but not sucrose (B). The data points are expressed as the adjusted least square mean average licks (± se). Note: the scale on the x-axis of A and B differ. Solid circles = alcohol exposed; Solid squares = control exposed.
doi:10.1371/journal.pone.0102255.g002

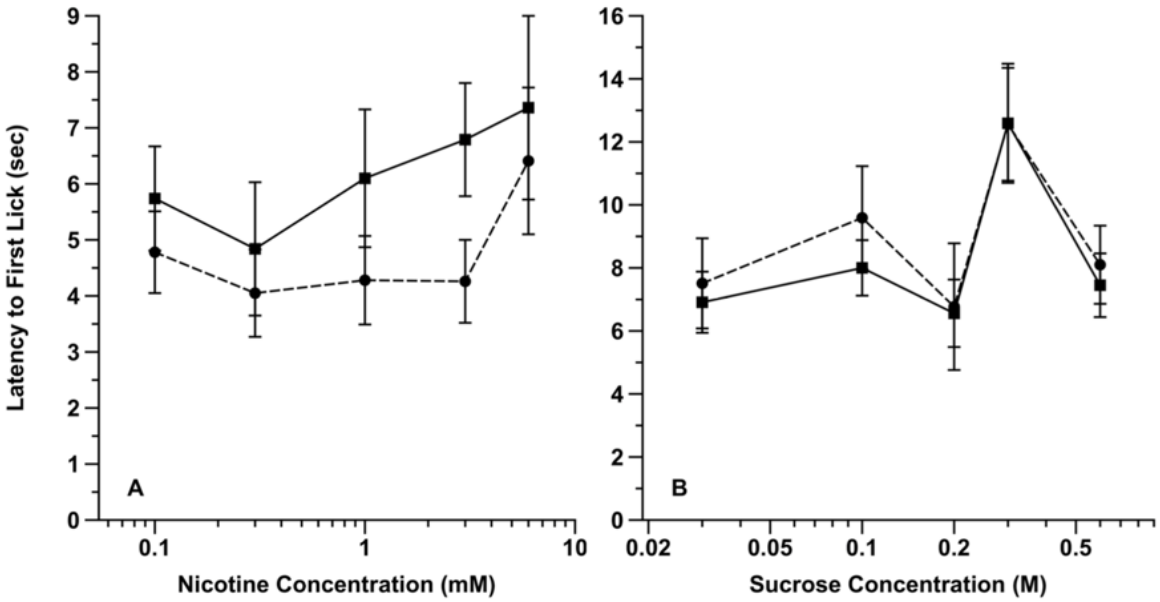
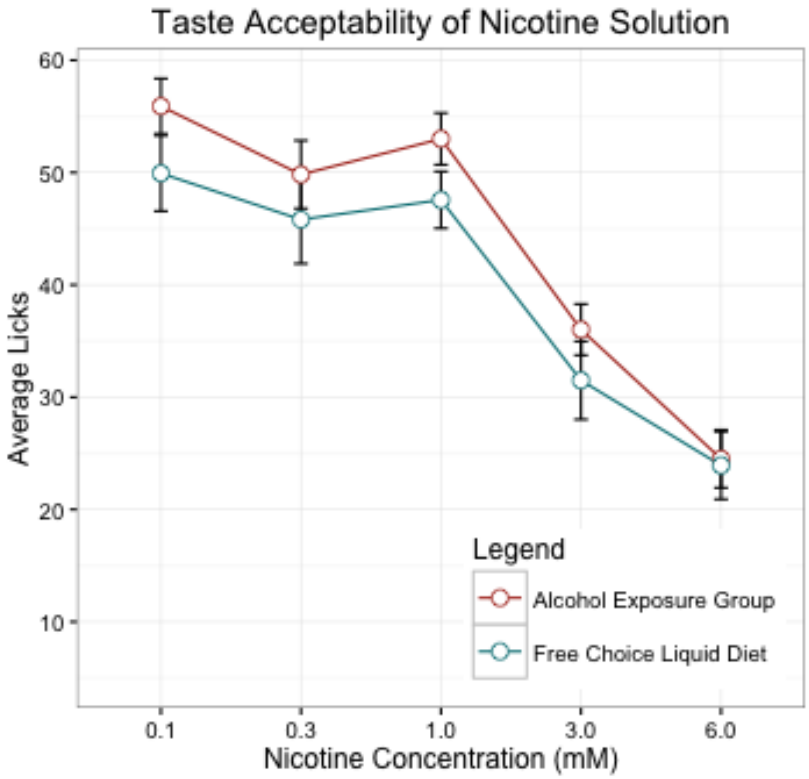


Figure 3. Latency to first lick response for a concentration range of nicotine (A) and sucrose (B) solutions by prenatal alcohol- and control-exposed rats. Compared to control, prenatal alcohol animals responded faster to nicotine (A), but not sucrose (B). The data is expressed as the average latency (± se). Note: the scale on the x-axis of A and B differ. Solid circles = alcohol exposed; Solid squares = control exposed.
doi:10.1371/journal.pone.0102255.g003

5.3 Appendix C - Plot generated using R studio



* Our results of the number of average licks for the free choice liquid diet group are slightly different from the author's original results in the paper.

However, after crosschecking and validating the dataset, we are convinced that our results are correct when computed using the data provided by the author. One possible reason of the difference may be the author's possible manipulation of raw test data in order to make the experiment results more convincing.

Notably, our result for the average number of licks for the free choice liquid diet group is higher than the author's result. We assume that, if it is not due to the reason that author may used different dataset, the author may have manipulated the data to make the average licks lower than the alcohol group to show that the free choice liquid group make significant lower number of licks as compared to the alcohol exposed group.

5.4 Appendix D - R code to replicate the experimental plot

```
# Import library
> library("dplyr",
lib.loc="/Library/Frameworks/R.framework/Versions/3.2/Resources/library")
> library("ggplot2",
lib.loc="/Library/Frameworks/R.framework/Versions/3.2/Resources/library")
> library("plyr", lib.loc="/Library/Frameworks/R.framework/Versions/3.2/Resources/library")
```

```
> library("Rcmdr",
lib.loc="/Library/Frameworks/R.framework/Versions/3.2/Resources/library")


#read and group data
> avg_lick <- read.csv("~/Desktop/Average lick.csv")
>   View(Average.lick)
> et <- subset(avg_lick, avg_lick$PRENATAL.EXPOSURE=="alcohol")
> fcl <- subset(avg_lick, avg_lick$PRENATAL.EXPOSURE!="alcohol")


#summarySE method will calculate useful statistics for the dataset including mean, standard
#error, standard deviation and confidence interval
> summarySE <- function(data=NULL, measurevar, groupvars=NULL, na.rm=FALSE,
+                       conf.interval=.95, .drop=TRUE) {
+     library(plyr)
+
+     length2 <- function (x, na.rm=FALSE) {
+         if (na.rm) sum(!is.na(x))
+         else       length(x)
+     }
+
+     datac <- ddply(data, groupvars, .drop=.drop,
+                    .fun = function(xx, col) {
+                       c(N    = length2(xx[[col]], na.rm=na.rm),
+                         mean = mean   (xx[[col]], na.rm=na.rm),
+                         sd   = sd     (xx[[col]], na.rm=na.rm)
+                       )
+                    },
+                    measurevar
+     )
+
+     datac <- rename(datac, c("mean" = measurevar))
+
+     datac$se <- datac$sd / sqrt(datac$N)  # Calculate standard error of the mean
+
+     ciMult <- qt(conf.interval/2 + .5, datac$N-1)
+     datac$ci <- datac$se * ciMult
+
+     return(datac)
+ }
```

```
#Using summarySE function to find mean and se for average licks of alcohol exposed group
#under different concentration
> et0.1 <- summarySE(et, measurevar="AVERAGE.LICKS..0.1mM.",
groupvars=c("PRENATAL.EXPOSURE"))
> et0.3 <- summarySE(et, measurevar="AVERAGE.LICKS..0.3mM.",
groupvars=c("PRENATAL.EXPOSURE"))
> et1.0 <- summarySE(et, measurevar="AVERAGE.LICKS..1.0mM.",
groupvars=c("PRENATAL.EXPOSURE"))
> et3.0 <- summarySE(et, measurevar="AVERAGE.LICKS..3.0mM.",
groupvars=c("PRENATAL.EXPOSURE"))
> et6.0 <- summarySE(et, measurevar="AVERAGE.LICKS..6.0mM.",
groupvars=c("PRENATAL.EXPOSURE"))


#Using summarySE function to find mean and se for average licks of free choice liquid diet
#group under different concentration
> fcl0.1 <- summarySE(fcl, measurevar="AVERAGE.LICKS..0.1mM.",
groupvars=c("PRENATAL.EXPOSURE"))
> fcl0.3 <- summarySE(fcl, measurevar="AVERAGE.LICKS..0.3mM.",
groupvars=c("PRENATAL.EXPOSURE"))
> fcl1.0 <- summarySE(fcl, measurevar="AVERAGE.LICKS..1.0mM.",
groupvars=c("PRENATAL.EXPOSURE"))
> fcl3.0 <- summarySE(fcl, measurevar="AVERAGE.LICKS..3.0mM.",
groupvars=c("PRENATAL.EXPOSURE"))
> fcl6.0 <- summarySE(fcl, measurevar="AVERAGE.LICKS..6.0mM.",
groupvars=c("PRENATAL.EXPOSURE"))


#Create a dataframe with useful information
> dt <- data.frame(group=c("et","et","et","et","et","fcl","fcl","fcl","fcl","fcl"),
concentration=c("0.1","0.3","1.0","3.0","6.0","0.1","0.3","1.0","3.0","6.0"),
mean=c(et0.1$AVERAGE.LICKS..0.1mM.,et0.3$AVERAGE.LICKS..0.3mM.,et1.0$AVER
AGE.LICKS..1.0mM.,et3.0$AVERAGE.LICKS..3.0mM.,et6.0$AVERAGE.LICKS..6.0mM.
,fcl0.1$AVERAGE.LICKS..0.1mM.,fcl0.3$AVERAGE.LICKS..0.3mM.,fcl1.0$AVERAGE.
LICKS..1.0mM.,fcl3.0$AVERAGE.LICKS..3.0mM.,fcl6.0$AVERAGE.LICKS..6.0mM.),
se=c(et0.1$se,et0.3$se,et1.0$se,et3.0$se,et6.0$se,fcl0.1$se,fcl0.3$se,fcl1.0$se,fcl3.0$se,fcl6.
0$se))


#View the created dataframe
> dt
  group concentration     mean       se
1    et          0.1 55.90842 2.464086
2    et          0.3 49.82474 3.026497
```

```
3     et         1.0 53.00000 2.299911
4     et         3.0 36.01632 2.275762
5     et         6.0 24.51158 2.575578
6    fcl         0.1 49.93421 3.364068
7    fcl         0.3 45.81579 3.926318
8    fcl         1.0 47.56632 2.533158
9    fcl         3.0 31.49579 3.479205
10   fcl         6.0 23.92579 3.010563
```

```
#using ggplot function to plot the graph
#two lines indicating the mean value for different groups are plotted
> ggplot(dt, aes(x=concentration, y=mean, colour=group, group=group)) +
+     geom_errorbar(aes(ymin=mean-se, ymax=mean+se), colour="black", width=.1) +
+     geom_line() +
+     geom_point(size=3, shape=21, fill="white") +
+     xlab("Nicotine Concentration (mM)") +
+     ylab("Average Licks") +
+     scale_colour_hue(name="Legend",
+                      labels=c("Alcohol Exposure Group", "Free Choice Liquid Diet"),
+                      l=40) +
+     ggtitle("Taste Acceptability of Nicotine Solution") +
+     expand_limits(y=5) +
+     scale_y_continuous(breaks=0:10*10) +
+     theme_bw() +
+     theme(legend.justification=c(1,0),
+           legend.position=c(1,0))
```

6. References

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