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The effects of ethanol and silymarin treatment during gestation on spatial working memory.

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Abstract

Background: Using a rat model we have found that the bioflavonoid silymarin (SY) ameliorates some of the negative consequences of in utero exposure to ethanol (EtOH). In the current study our aim was to determine if spatial working memory (SWM) was impaired in offspring whose mothers were maintained on a liquid diet containing EtOH during different gestational weeks. We also determined if SWM was altered with a concomitant administration of SY with EtOH during specific gestational weeks.

Methods: We provided pregnant Fischer/344 rats with liquid diets containing 35% EtOH derived calories (EDC) during specific weeks of the gestational period. A silymarin/phospholipid compound containing 29.8% silybin co-administered with EtOH was also administered during specific weeks of the gestational period. We tested SWM of the offspring with a radial arm maze on postnatal day (PND) 60. After testing the rats were sacrificed and their brains perfused for later analysis.

Results: We observed SWM deficits, as well as a significantly lower brain weight in female offspring born of mothers treated with EtOH during the third week of gestation in comparison to mothers treated during either the first or second weeks of gestation. Rats from any group receiving EtOH in co-administration with SY showed no significant deficits in SWM.

Conclusion: EtOH treatment during the last week of gestation had the greatest impact on SWM. The addition of SY to the EtOH liquid diet appeared to ameliorate the EtOH-induced learning deficits.

Background

Fetal alcohol syndrome (FAS) produces a variety of physical and neuropsychological disorders in humans and animals [1]. In humans, FAS children tend to have a lower average IQ and also display a propensity for hyperactivity [2]. In rats, exposure to ethanol (EtOH) during gestation produces a cascade of neuroanatomical abnormalities. In particular, EtOH treatment during the later part of gestation produces increased apoptosis in the hippocampus of

rats [3], which leads to subsequent neurobehavioral deficits.

Olton and Papas [4], through testing on the radial arm maze (RAM), found the hippocampus to be the primary brain region involved in spatial working memory (SWM). Prior to testing, the fimbria-fornix (the major input-output source of the hippocampus) of rats was lesioned. Reference memory (memory for a response set that is the

same on every trial) and working memory (memory that included proactive interference and involves procedures that require memory for events consistent in all trials) were tested. Large lesions to the fimbria-fornix impaired postoperative performance on the working memory task, but no reference memory deficits were seen. Seizures produced in the CA1 area of the hippocampus are also shown to produce deficits in working memory, but not in reference memory [5].

Exposure to EtOH during gestation has toxic effects on the developing hippocampus. EtOH has been shown to disrupt the development hippocampal mossy fibers when administered during gestation [6]. Pyramidal cell loss also occurs, particularly in the CA1 area of the hippocampus when exposed during gestational days (GD) 10–21. Long-term potentiation (LTP) is also disrupted in rats prenatally exposed to EtOH [7]. Hippocampal slices from adult rats exposed to EtOH during gestation show a decreased propensity toward LTP in area CA1 of the hippocampus in comparison to pair-fed controls. Prenatal exposure to EtOH in rats also significantly reduces the sensitivity of area CA1 of the hippocampus to NMDA [8]. Rats exposed to EtOH during gestation show an enhancement of the Mg²⁺ block of the NMDA channel, further diminishing the capacity for hippocampal LTP.

Linked to the physical evidence of EtOH-induced neurodevelopmental abnormalities are the behavioral deficits that reflect the damage to the brain areas critical to the behavioral performance. Tests using the Morris Water Maze (MWM; a test of spatial memory) have shown an EtOH-induced impairment in spatial memory [9–12]. Rats treated with EtOH during gestation take significantly more time to reach the escape platform than do rats without EtOH exposure. T-maze testing also shows an EtOH-induced impairment to achieve the goal-arm in prenatally exposed rats [13].

EtOH exposure during gestation also causes deficits in SWM in pups tested on the RAM [14,15]. Rats exposed to 35% ethanol-derived calories (EDC) during gestation show an impaired performance on the RAM. Those that reached criterion needed significantly more trials to complete testing and committed significantly more mistakes. Rats treated with 17% EDC during gestation showed no significant RAM deficiencies, demonstrating the dose-dependent manner in which EtOH works.

Also relevant to the consequences of in utero exposure to EtOH is the fact that particular areas of the brain are sexually dimorphic, which subserves sexually dimorphic behavior. Spatial working memory is an example of sexually dimorphic behavior. Male rats are found to consistently learn the RAM more rapidly than females [16].

Females also performed significantly worse than males on the RAM following a treatment of 1.5 g/kg/day of EtOH [17]. This impairment persisted even after treatment was completed.

We have investigated the potential fetoprotective capacity of silymarin (SY) against the toxic effects of in utero exposure to EtOH. SY is a flavanoid that has shown antioxidant properties. The mechanism through which SY protects the fetus from EtOH toxicity is not fully understood, but possible mechanisms include neutralization of free radicals by the bioflavonoids leading to decreased lipid peroxidation; increased protein synthesis, thereby promoting regenerating processes; stabilization of cell membranes; and possible binding of SY to an estradiol binding site situated on a subunit of RNA polymerase I, which would stimulate the synthesis of ribosomal RNAs, possibly restoring structural proteins and damaged enzymes in the cells nucleus [18–22]. SY has also been shown to cross the placental barrier, and appears to protect the fetus from weight loss produce by EtOH-exposure during gestation [23].

Busby, LaGrange, Edwards, and King [24] found SY to protect the fetus in utero from the toxic effects of EtOH. Rats born to mothers treated with EtOH and SY showed no significant differences in RAM performance in comparison to a pair-fed group. Rats born to mothers treated with only EtOH showed an impaired performance on the RAM in comparison to a pair-fed control group. They also found males to perform significantly better than females between groups, as females took significantly more days to reach criterion than males on the RAM.

Given that SY has been successful in preventing SWM deficits on the RAM (in the rat model) we again examined its fetoprotective properties. We further tried to determine which week of the rats' three-week gestational period was the most critical in terms of EtOH-induced SWM deficits (as measured by RAM performance).

Methods

Subjects

Subjects were 224 Fisher/344 rats (112 females, 111 males), the offspring of 58 female Fisher/344 rats that had been assigned to experimental groups. The 58 female rats received Purina lab rat chow and water *ad libitum* until they reached pregnancy weight (130 g). The females were housed with male rats until pregnancy was confirmed by vaginal smear. Once pregnancy was confirmed, the rats were placed in standard plastic laboratory maternity tubs and assigned to one of fourteen gestational groups (Table 1). Group 1 (EtOH 1–7) received a liquid Bio-Serv diet (Frenchtown, NJ) containing 35% ethanol-derived calories (EDC) during days 1–7 of the gestational period.

Table 1: Experimental Group Treatment Per Week Over the Three-Week Gestation Period.

	Group Name	Treatment GD 1-7 (Week 1)	Treatment GD 8-14 (Week 2)	Treatment GD 15-21 (Week 3)
1	EtOH 1-7	EtOH	LD	LD
2	EtOH 8-14	LD	EtOH	LD
3	EtOH 15-21	LD	LD	EtOH
4	EtOH/SY 1-7	EtOH/SY	LD	LD
5	EtOH/SY 8-14	LD	EtOH/SY	LD
6	EtOH/SY 15-21	LD	LD	EtOH/SY
7	LD/SY 1-7	LD/SY	LD	LD
8	LD/SY 8-14	LD	LD/SY	LD
9	LD/SY 15-21	LD	LD	LD/SY
10	EtOH Full	EtOH	EtOH	EtOH
11	EtOH/SY Full	EtOH/SY	EtOH/SY	EtOH/SY
12	LD/SY Full	LD/SY	LD/SY	LD/SY
13	LD Full	LD	LD	LD
14	Chow	Chow	Chow	Chow

Group 2 (EtOH 8-14) received a liquid Bio-Serv diet containing 35% EDC during days 8-14 of the gestational period. Group 3 (EtOH 15-21) received a liquid Bio-Serv diet containing 35% EDC during days 15-21 of the gestational period. Group 4 (EtOH/SY 1-7) received a Bio-Serv diet containing 35% EDC along with 400-mg/kg silymarin during days 1-7 of the gestational period. Group 5 (EtOH/SY 8-14) received a liquid Bio-Serv diet containing 35% EDC along with 400-mg/kg silymarin during days 8-14 of the gestational period. Group 6 (EtOH/SY 15-21) received a liquid Bio-Serv diet containing 35% EDC along with 400-mg/kg silymarin during days 15-21 of the gestational period. Group 7 (LD/SY 1-7) received a Bio-Serv diet containing an isocaloric quantity of dextrose maltose that matched the caloric amount in the ethanol diet along with 400-mg/kg silymarin during days 1-7 of the gestational period. Group 8 (LD/SY 8-14) received a Bio-Serv diet containing an isocaloric quantity of dextrose maltose that matched the caloric amount in the ethanol diet along with 400-mg/kg silymarin during days 8-14 of the gestational period. Group 9 (LD/SY 15-21) received a Bio-Serv diet containing an isocaloric quantity of dextrose maltose that matched the caloric amount in the ethanol diet along with 400-mg/kg silymarin during days 15-21 of the gestational period. The previous 9 groups received a liquid Bio-Serv diet containing an isocaloric quantity of dextrose maltose that matched the caloric amount in the ethanol diet for any period of gestation in which a treatment was not specified.

Group 10 (EtOH Full) received a liquid Bio-serv diet containing 35% EDC throughout the gestational period. Group 11 (EtOH/SY Full) received a liquid Bio-Serv diet containing 35% EDC along with 400-mg/kg silymarin throughout the gestational period. Group 12 (LD/SY Full) received a liquid Bio-Serv diet containing an isocaloric

quantity of dextrose maltose that matched the caloric amount in the ethanol diet along with 400-mg/kg silymarin throughout the gestational period. Group 13 (LD Full/Pair-Fed) received a liquid Bio-Serv diet containing an isocaloric quantity of dextrose maltose that matched the caloric amount in the ethanol diet restricted to the consumption rate per week of EtOH 1-7 for week 1, EtOH 8-14 for week 2, and EtOH 15-21 for week 3. Group 14 (Chow) received chow and water *ad libitum* throughout the gestational period. All pups were kept with birth mothers until weaning. Daily intake of liquid diet across mothers was 68 ml \pm 5 ml/day.

Once pups reached 21 days of age they were weaned and placed in an individual standard laboratory cage. On the day of weaning 8 males and 8 females from each group were randomly selected for subsequent RAM testing. A total of 16 pups were taken from each group.

Apparatus and procedures

Testing Apparatus for Spatial Working Memory

All testing took place on an 8-arm radial arm maze. The central platform measured 35.56 cm in diameter, each arm was 82.55 cm long and 10.16 cm wide, and the platform was mounted on 53.34 cm legs. The apparatus was painted flat black. All test sessions were videotaped.

Spatial Working Memory Testing Procedure

The rats began SWM testing at 12 weeks. They were food-deprived to 85% free-feeding body weight beginning two-weeks prior to RAM testing. RAM testing consisted of 3 phases: habituation, training, and testing. Habituation [postnatal day (PND) 58]: rats were placed individually in the center of the maze and allowed to explore for 25 minutes. Training (PND 59): 7 arms of the maze were blocked, allowing access to only one arm. Food rewards (a

Table 2: Female Data on RAM Performance and Mistakes for EtOH weekly treatment groups.

	RAM (days)		Mistakes	
	M	SD	M	SD
EtOH 1-7	3.88	2.23	15.13	10.93
EtOH 8-14	4.13	2.59	17.25	8.41
EtOH 15-21	6.75*	2.43	13.75	7.17

* $p < 0.05$

small piece of red Froot Loop) were placed at short intervals down the arm in order to entice the rat to the end of the arm. Once the rat reached the end of the arm, the rat was returned to the center platform and the arm was re-baited in order to continue the training procedure. Near the end of the 15-min phase, food rewards were only placed at the end of the arm to train the rat to run to the end of the arm to receive the reward. Testing (PND 60): rats had 5 min to reach the end of all 8 arms (criterion). The end of each arm was baited with a food reward. Reaching criterion was not contingent upon consumption of the bait. Each rat was allowed 8, 5-min sessions to reach criterion. Mistakes were also recorded and defined as full exit of an arm to the central platform after the food reward was consumed followed by subsequent re-entry to the complete end of the arm in search of the previously consumed reward anytime during the five minute testing period. The RAM was washed with a 70% ethanol solution between each trial.

Perfusion

Two pups from each group were sacrificed on the day they were born and their brains extracted for use in later protein analysis (data not presented here). On PND 90, all rats were sacrificed and their brains extracted. Rats were injected with 1 cc of euthasol and placed back into their home cage. When the rats did not respond to tactile stimulation, they were taken to the perfusion tray. A cut was made from the sternum to the neck and the ribs were pulled away exposing the still-beating heart. A perfusion needle containing 30 ml Ringers solution was administered intracardially, followed by 30 ml of fixing solution. The brains were then extracted, weighed, and preserved in formalin for later analysis (data not presented here).

Statistics

A priori contrasts were conducted to determine if there were significant differences between groups on all dependent measures (RAM, mistakes, birth weight, weaning weight, RAM testing weight, perfusion weight, and total brain weight). SPSS Reliability Analysis was used for inter-rater reliability.

Results

An independent groups t-test was conducted in order to assess gender differences in performance on the RAM. This test revealed a significant difference between males ($M = 3.84$) and females ($M = 4.62$), $t(221) = 2.38$, $p = .01$. These results provided the rationale for analyzing male and female data separately.

A priori contrasts were conducted to assess group differences on the RAM for females. The EtOH 15-21 group ($M = 6.75$) took longer to complete RAM testing than the EtOH 1-7 group ($M = 3.88$), $t(98) = 2.26$, $p = .02$ as well as the EtOH 8-14 group ($M = 4.13$), $t(98) = 2.07$, $p = .04$ (Table 2). The contrasts also revealed the EtOH/SY full group ($M = 2.50$) took less time to complete RAM testing than the EtOH full group ($M = 6.13$), $t(98) = 2.85$, $p = .005$ as well as the LD full group ($M = 5.13$), $t(98) = 2.08$, $p = .04$ (Table 3). A priori contrasts were also conducted to assess group differences on mistakes made by the females. The EtOH full group ($M = 19.25$) committed more mistakes than the LD full group ($M = 9.38$), $t(98) = 2.08$, $p = .04$ as well as the EtOH/SY full group ($M = 10.25$), $t(98) = 2.08$, $p = .04$ (Table 3). The results of a reliability analysis indicated a 98% agreement among the raters who recorded the mistakes.

No significant differences were found in RAM testing for the males. A priori contrasts were conducted in order to assess group differences on mistakes made by the males. The EtOH 1-7 group ($M = 7.88$) committed less mistakes than the EtOH 15-21 group ($M = 18.75$), $t(94) = 2.57$, $p = .01$. No other significant differences were found. The results of a reliability analysis indicated a 98% agreement among the raters who recorded the mistakes.

A priori contrasts were also conducted to assess female group differences in weight as recorded at birth, weaning, on the first day of RAM testing, and at the time of brain perfusion. The contrasts revealed the EtOH 15-21 group ($M = 5.14$) weighed more at birth than the EtOH 1-7 group ($M = 4.65$), $t(98) = 2.81$, $p = .006$ as well as EtOH 8-14 group ($M = 4.76$), $t(98) = 2.18$, $p = .03$ (Table 4). At

Table 3: Female Data for RAM Performance and Mistakes for selected full treatment groups.

	RAM (days)		Mistakes	
	M	SD	M	SD
EtOH/SY full	2.50	1.77	10.25	6.48
EtOH full	6.13*	3.40	19.25*	12.35
LD full	5.13	2.48	9.38	7.03

* $p < 0.05$ **Table 4: Female Weight Data for EtOH weekly treatment groups.**

	Birth (g)		Weaning (g)		RAM (g)		Perfusion (g)		TBW (g)	
	M	SD	M	SD	M	SD	M	SD	M	SD
EtOH 1-7	4.65	0.14	29.88	2.80	88.86	10.72	154.22	13.94	1.63	0.05
EtOH 8-14	4.76	0.44	30.84	3.80	92.38	8.27	157.87	8.65	1.64	0.03
EtOH 15-21	5.14*	0.32	25.74*	4.78	86.49*	14.49	139.79*	19.89	1.57*	0.10

* $p < 0.05$ **Table 5: Female Weight Data for selected EtOH/SY and LD/SY weekly treatment groups.**

	Birth (g)		Weaning (g)		RAM (g)		Perfusion (g)	
	M	SD	M	SD	M	SD	M	SD
EtOH/SY 1-7	4.11	0.57	26.06	3.36	85.65	7.59	149.18	6.56
LD/SY 1-7	4.49*	0.40	25.63	3.76	92.54	9.28	140.30	12.47
EtOH/SY 8-14	4.75	0.38	27.25	6.74	82.19	10.61	155.34	7.35
LD/SY 8-14	5.12*	0.14	31.44*	5.78	94.36*	4.96	151.28	9.00

* $p < 0.05$

weaning, this trend had reversed, revealing the EtOH 15-21 group ($M = 25.74$) to weigh less than the EtOH 1-7 group ($M = 29.88$), $t(98) = 2.00, p = .04$ as well as the EtOH 8-14 group ($M = 30.84$), $t(98) = 2.45, p = .02$. This trend continued at perfusion, with the EtOH 15-21 group ($M = 139.79$) weighing less than the EtOH 1-7 group ($M = 154.21$), $t(98) = 2.42, p = .02$ as well as the EtOH 8-14 group ($M = 157.87$), $t(98) = 3.03, p = .003$. There was also a significant difference in total brain weight (TBW) as the EtOH 15-21 group ($M = 1.57$) weighed less than the EtOH 1-7 group ($M = 1.63$), $t(96) = 2.42, p = .02$ as well as the EtOH 8-14 group ($M = 1.64$), $t(96) = 3.06, p = .003$.

Additional contrasts on the female weight data revealed significant differences in birth weight as the EtOH full group ($M = 5.13$) weighed more than the LD full group ($M = 4.54$), $t(98) = 3.38, p = .03$; RAM testing weight as the

EtOH full group ($M = 100.14$) weighed more than the EtOH/SY full group ($M = 90.56$), $t(98) = 2.15, p = .03$; and perfusion weight as the EtOH full group ($M = 168.24$) weighed more than the EtOH/SY full group ($M = 148.75$), $t(98) = 3.27, p = .002$. Further weight differences are shown in Table 5.

A priori contrasts were also conducted to assess group differences in the weight data obtained from males at birth, weaning, on the first day of RAM testing, and at the time of brain perfusion. The contrasts revealed significant differences in birth weights as the EtOH 1-7 group ($M = 4.65$) weighed less than the EtOH 15-21 group ($M = 5.29$), $t(94) = 2.71, p = .003$ (Table 6); the EtOH/SY 1-7 group ($M = 3.97$) weighing less than the EtOH/SY 15-21 group ($M = 4.80$), $t(94) = 3.53, p = .001$; the LD full group ($M = 4.51$) weighing less than the EtOH full group ($M =$

Table 6: Male Weight Data for EtOH weekly treatment groups.

	Birth (g)		Weaning (g)		RAM (g)		Perfusion (g)	
	M	SD	M	SD	M	SD	M	SD
EtOH 1-7	4.65	0.14	32.15	1.26	117.51	10.05	221.46	15.00
EtOH 8-14	5.08	0.40	35.32*	6.73	109.47	32.03	234.51	20.12
EtOH 15-21	5.29*	0.32	30.83	2.85	116.74	16.44	224.72	12.91

**p* < 0.05

5.17), *t*(94) = 2.71, *p* = .008; and the LD full group weighing less than the EtOH/SY full group (*M* = 5.43), *t*(94) = 3.76, *p* = .000 (Table 6). Contrasts further revealed a significant difference in weaning weight as the EtOH 15-21 group (*M* = 30.83) weighed less than the EtOH 8-14 group (*M* = 35.32), *t*(94) = 2.00, *p* = .04.

Discussion

The results of this study demonstrate that EtOH exposure during gestation affects subsequent performance on the RAM in EtOH-exposed adult rats. Further, SWM deficits were found to be more severe in female EtOH treated rats than in male EtOH treated rats, as reported earlier [16,17]. The fetoprotective effects of SY were also replicated.

Female pups born to the EtOH 15-21 group showed significant RAM deficits in comparison to the EtOH 1-7 and EtOH 8-14 groups, indicating that, in females at least, the hippocampus appears to be more sensitive to EtOH exposure during the last week of gestation. Of interest is that males did not show this difference, although the EtOH 15-21 group did commit significantly more mistakes than did the EtOH 1-7 group. This may indicate that EtOH exposure later in the gestational period may have caused more hippocampal disruption in males as well as females; although this difference may be due EtOH-induced hyperactivity. Gender differences in response to in utero exposure to EtOH were evident in SWM capacity. Male rats consistently learned the RAM task quicker than female rats [16]. Females also seem to be more sensitive to the detrimental effects of EtOH as measured by RAM performance [17].

Consistent with the Busby, LaGrange, Edwards, and King [24] investigation, those rats treated with EtOH in combination with SY did not differ significantly in RAM testing or mistakes made when compared to the pair-fed group. The female rats born to the EtOH full group took significantly longer to complete RAM testing than did the EtOH/SY full group, and also committed more mistakes than did the EtOH/SY full group. Interestingly, the LD full group took significantly more days to complete RAM testing

than did the EtOH/SY full group. This may have been the result of residual hyperactivity, as in utero exposure to EtOH can cause subsequent hyperactivity in the juvenile rat [14].

Birth weight analysis (group weight mean) for females showed that initially, the EtOH 15-21 group weighed significantly more than did either the EtOH 1-7 or the EtOH 8-14 group, but at weaning this trend had reversed. Both the EtOH 1-7 group and the EtOH 8-14 group weighed significantly more than did the EtOH 15-21 group. Although no significant differences were found at RAM testing weight, the EtOH 1-7 group and the EtOH 8-14 group weighed significantly more than the EtOH 15-21 group at the time of brain perfusion. The same two groups also had larger total brain weights than did the EtOH 15-21 group. In relation to the differences found on RAM testing, these lower total brain weights could be related to the cellular/structural loss seen from the exposure of EtOH to a fetus in utero [25,26] and may provide the physiological underlying causes of the deficiencies found in RAM testing.

Female pups exposed to EtOH in co-administration with SY did not display SWM deficits on the RAM. Further, no deficits on RAM performance were found in any of the groups treated with SY. The mechanism through which SY protects the fetus from EtOH toxicity is not fully understood. SY may work by neutralizing free radicals and decreasing lipid peroxidation [19], increasing protein synthesis [20,27,28], thereby promoting regenerative processes, as well as possible competitive binding to an estradiol binding site situated on a subunit of RNA polymerase I [22,23,18], which would stimulate the synthesis of ribosomal RNAs, possibly restoring structural proteins and damaged enzymes in the cells nucleus. If SY binds to the estradiol site during gestation, the ameliorative effects of SY might be due to the regenerative properties of this binding site.

During brain development, certain types of cells are programmed to move to precise locations in the brain (i.e.,

the hippocampus) to serve a specific purpose. The presence of EtOH can cause interference with the molecules promoting appropriate cell movement, disrupting the correct paths of these migrating cells, leading to incorrect cell migration and adhesion. Silymarin, through its scavenging of the free radicals created by the oxidation of EtOH [28] may prevent the disruption of these signals and allow for correct migration. If this movement is altered by the presence of EtOH, the incorrect migration of developing cells can occur, reaching incorrect targets and causing behavioral deficits [29].

Developing brain cells exposed to EtOH during gestation show signs of hypoxia, having a reduced oxygen supply that may lead to developmental delays and malformations. Hypoxia also causes the formation of free radicals, the toxic by-products of oxygen metabolism. Free radicals can damage the cell surface, causing swelling, cellular damage, and possibly death to these cells. Silymarin acts as an antioxidant by scavenging the free radicals that induce lipid peroxidation, and also stimulates regeneration through increased protein synthesis [18]. Rats exposed to EtOH during gestation develop fewer neurons and glial cells than control rats, including the cells located in the hippocampus [30]. When fetuses are exposed to ethanol during GD 10–21, pyramidal cell loss also occurs, particularly in CA1 of the hippocampus [6]. Dendritic spines, when exposed to ethanol prenatally, show increased variability in relation to controls [31]. Given that dendritic spines modulate the entry of information through modifications in their density and shape, the variation in spine development due to ethanol exposure might contribute to the cognitive deficits associated with FAS. Rats exposed to EtOH prenatally also develop fewer purkinje cells than controls [32]. Purkinje cells receive many excitatory inputs. The purkinje cells inhibit many of these inputs, as purkinje cells are inhibitory by nature, to allow for a clear communication of incoming information from these cells with the rest of the brain. A loss in purkinje cells can also result in behavioral deficits, as well as lowered total brain volume and weight.

When EtOH is administered gestationally, an enhancement in the Mg^{2+} block of the NMDA-receptors is observed, which suggests that prenatal EtOH exposure facilitates a more complex block of the NMDA channel by Mg^{2+} [8]. NMDA-receptor activation is critical in the induction of LTP [33]. LTP is one of the primary manifestations of plasticity in the hippocampus and consists of excitatory postsynaptic potentials that are sustained for at least 30-minutes after tetanic stimulation [7,34]. Pups prenatally exposed to EtOH would then have a diminished capacity for hippocampal LTP [7,34,33]. Lowering the capacity for LTP would affect the pups' ability to create and keep spatial maps in the hippocampus during RAM

testing. This would translate into the observed behavioral deficits on the RAM. It might also account for more severe deficits observed in the female rats receiving EtOH during the last week of gestation (the critical developmental period for the hippocampus) and female rats receiving EtOH treatment throughout gestation.

Conclusion

Because female pups born to mothers treated with EtOH during the last week of gestation showed the greatest SWM deficits, it appears that hippocampal development is most vulnerable to EtOH-induced toxicity during this gestational period. Further, SY was shown to protect the fetus in utero from the toxic effects of EtOH. The treatment groups that did not receive SY in co-administration with EtOH were far less proficient on the RAM than were EtOH/SY treatment groups. The fetoprotective properties of SY are not yet fully understood, but due to our consistent observations of protectivity, the properties of SY in co-administration with EtOH merit further study.

Competing Interests

None declared.

Authors' contributions

SN performed the behavioral assessments in the study and participated in the design of the study. LL participated in the design of the study, the statistical analysis, and drafted the manuscript. ET maintained the experimental protocol throughout the experiment and performed a portion of the behavioral assessments. DR maintained the experimental protocol throughout the experiment and performed a portion of the behavioral assessments.

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