Research paper

Acta Neurobiol Exp 2017, 77: 86–93

ACTA NEUROBIOLOGIÆ EXPERIMENTALIS

Effect of combined administration of aripiprazole and fluoxetine on cognitive functions in female rats exposed to ethyl alcohol

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Alcoholism is a chronic and recurrent disease. The studies on ethyl alcohol show a progressive deterioration of cognitive functions (motor hyperactivity, operating memory). The aim of the study was to establish whether combined single and chronic administration of aripiprazole (ARI) and fluoxetine (FLU) affects animal locomotor activity or modifies spatial memory functions in female rats exposed to ethyl alcohol. Female Wistar rats were studied in the Morris Water Maze (MWM) and locomotor activity test. Rats undergoing the MWM and locomotor activity test were injected with saline on day 1, 7, 14 and 21 of testing. Results showed a statistically significant mobility increase in the group of ethanol-exposed females (CEt) (21 days) compared to the non-ethanol-exposed group (CNEt). Upon ARI administration to CEt, no statistically significant differences in animal mobility were found, either upon single or chronic administration. Chronic administration of FLU (21 days) as well as combined administration of ARI+FLU (14 and 21 days) caused a statistically significant reduction of the females' mobility compared to the control CEt group. Single and chronic administration of ARI (7x) both show a spatial memory improvement in CEt. No memory improvement was observed, however, after 14 and 21 days of ARI administration. FLU, likewise, improved spatial memory both upon single and chronic administration. Combined administration of ARI+FLU improved memory in CEt only upon single administration. Lack of effect upon chronic administration may be due to tolerance to memory improvement developing upon combined administration of ARI+FLU. It can be concluded that ARI (1.5 mg/kg), FLU (5 mg/kg), and combined administration of these drugs improves spatial memory in CEt.

Key words: aripiprazole, fluoxetine, cognitive functions, ethanol, female

INTRODUCTION

Excessive consumption of ethyl alcohol leads to development of an addiction and impairs emotional processes and motivational behaviors of the drinker. Ethyl alcohol addiction is a set of mental and somatic disorders with alternate periods of exacerbation (binge drinking) and remission (abstinence). Ethanol withdrawal entails numerous mental complaints (anxiety, depression) and psychomotor complaints (e.g. motor hyperactivity) (Allsop et al. 1997). Neuropsychological studies on patients addicted to ethyl alcohol show a progressive deterioration of cognitive functions, mainly operating memory and executive functions (Guerrini et al. 2005). These impairments are probably related to enhanced transmission in the dopaminergic system (DA) (Diana et al. 2003). Some atypical antipsychotics (olanzapine, quetiapine) have been shown to reduce ethyl alcohol consumption in humans; however, due to their adverse effects (body weight gain, sedation), their use is limited (Zając et al. 2006).

Aripiprazole (ARI) is a new atypical antipsychotic agent with a unique mechanism of action and few adverse effects (Zając et al. 2006, Marcus et al. 2008). Its mechanism of action is related to its agonist-antagonist effect on DA receptors (D2 and D4) and warrants the use of this drug to treat ethyl alcohol addiction (Ratajczak et al. 2013).

Fluoxetine (FLU) is an antidepressant being a selective serotonin reuptake inhibitor (SSRI) used to treat major depressive disorders (MDDs) (Holladay et al. 1998). Fluoxetine has been shown to have neuroprotective properties and to improve cognitive functions – memory in particular – in both animals (Li et al. 2009, Malinowska et al. 2016) and humans (Gudayol-Ferré et al. 2015) and, thus, may be used to treat memory disorders in alcohol addicts (Szymańska et al. 2009). Memory improvement was also observed in animals exposed to ethyl alcohol (Szymańska et al. 2009, Ratajczak et al. 2015).

Women have been proved to be more prone to organ damage due to alcohol abuse (Tuyns and Pequignot 1984, Gavaler and Arria 1995). Alcohol in women is metabolized

Received 29 January 2016, accepted 16 March 2017

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differently than in men; for instance, with the same amount of alcohol consumed women will have a higher blood alcohol concentration than the opposite sex (Frezza et al. 1990, Taylor et al. 1996). Alcohol is metabolized mainly in the liver by P-450 cytochrome (CYP2E1) (Lieber 1999, 2004). Alcohol damages liver in women more frequently due to the greater volume of liver tissue per dry matter weight unit (Li et al. 1998, Kwo et al. 1998). Alcohol absorption in women is enhanced by estrogens – this is why women get drunk more easily in the premenstrual phase. Higher blood alcohol concentration in women may be due to lower activity of alcohol dehydrogenase (ADH) in the stomach and the liver (National Institute on Alcohol Abuse and Alcoholism – NIAAA 1990). Women have also been found to experience alcohol-related brain damage (Hommer et al. 1996) corresponding to memory impairment.

Therapeutic effect of the drugs used depends on pharmacokinetic parameters (LADME) which vary between ages, sexes, or drug doses used (Beirle et al. 1999, Koren 2012). In the absorption phase, differences were observed in release of the medicinal substance due to smaller secretion of gastric acid in women caused by the predominantly alkaline environment (Beirle et al. 1999, Robinson 2002). This may result in slower absorption and reduced Cmax (Robinson 2002), in particular in women (regardless of the menstrual cycle phase) (Wilson 1984). Women also show a weaker first pass effect caused by increased CYP2D6 isoenzyme expression (Luzier et al. 1999).

Our previous studies on male rats have shown no effect of ARI in higher doses (6 mg/kg) on memory of alcohol-exposed rats and memory impairment upon combined administration of ARI (6 mg/kg) and FLU (5 mg/kg) (Burda-Malarz et al. 2014a, 2014b).

Considering the fact that the available references lack any data on combined administration of ARI and FLU on cognitive functions in ethyl alcohol abusing women, our study objective was to determine whether combined single and chronic administration of aripiprazole (ARI) and fluoxetine (FLU) affected animal locomotor activity or modified spatial memory functions in female rats exposed to ethyl alcohol.

MATERIALS AND METHODS

Animals

Timed female Wistar rats (100) were purchased from Poznan University of Medical Sciences, Poland (licensed by Ministry of Agriculture in Warszawa, Poland). The animals were housed individually in cages (size 42×26 cm) in a light-controlled (lights on 7 a.m.–7 p.m.), temperature-controlled (18–20°C), and humidity-controlled (50–60%) animal facility. The animals had free access to rat chow (Labofeed B) and water. All females used in our experiment were from litters dropped over 2 days' time (hormonally homogenous group, with an average reproductive cycle duration of 4–6 days).

All procedures related to the use of rats in these experiments were conducted with due respect to ethical principles regarding experiments on animals (directive 2010/63/EU). The study protocol was approved by the Local Ethics Committee for Research on Animals.

Drugs

Aripiprazole ARI – Otsuka Pharmaceutical Europe, Bristol-Myers Squibb Poland.

Fluoxetine FLU – Polpharma SA, Poland.

Saline – Sodium chloride (0.9%) solution was acquired from Baxter Poland Company (Warsaw, Poland).

The female rats were administered ARI (1.5 mg/kg) *ip* 30 min before the test and FLU (5 mg/kg) *po* 60 min before the test and for 7, 14, and 21 days. ARI and FLU were prepared in saline. Between the tests with different assays, there was a 24 h washout period to wash out the drug residues or their active metabolites. The controls were given saline only (2 ml, *ip* saline) according to the same schedule. Separate groups of animals were used for different tests.

Behavioral analyses

Ethanol administration (EA)

Animals (n=80) were forced to drink only ethyl alcohol solution (12% solution made of 95% stock ethanol; Polmos, Poland) for 2 months (~9 g/kg/day). During the next 4 weeks, the animals were presented with a free choice paradigm between tap water and ethyl alcohol. This procedure led to preparation of rats chronically exposed to alcohol. Additionally, for comparative purposes, throughout the duration of chronic ethyl alcohol treatment (rats chronically exposed to alcohol), an ethyl alcohol-naive control group of animals would receive only tap water (Okulicz-Kozaryn et al. 2004). Ethyl alcohol was the treatment continued throughout the testing period.

Measurement of locomotor activity (LA)

LA was measured in rats (Control Non-Ethanol – CNEt and Control Ethanol – CEt – groups) using eight 20.5×28×21 cm wire grid cages, each with two horizontal infrared photocell beams along the long axis, 3 cm above the floor. Photocell interruptions were recorded by electromechanical counters in an adjacent room. Before the

test, all groups of animals were habituated to a novel cage for 30 min. Rats were also treated with 1.5 mg/kg ARI (*ip*), 5 mg/kg FLU (*po*), or saline in CNEt and CEt study groups. Then, photocell activity would be recorded at 5-minute intervals. This test provided an index of basal locomotor activity of animals in a familiar environment, necessary to indicate the presence of a central stimulant or sedating effects of the drug used in the test.

Morris water maze test (MWM)

Morris water maze test (Morris et al. 1988). The water maze apparatus was a circular basin (diameter=180 cm, height=50 cm) filled with water (approximately 22–24°C) to a depth of 24c m, and pieces of Styrofoam were hiding an escape platform (diameter=8 cm) placed 1 cm below the water surface (learning place, invisible condition). Many extra-maze visual cues surrounding the maze were available, and the observer remained in the same location for each trial. The rats from the CONTROL CNEt and CEt, ARI, FLU, and ARI+FLU groups were placed in the water facing the midpoint section of the wall at one of 4 equally spaced locations: North (N), East (E), South (S), and West (W). The pool was divided into 4 quadrants: NW, NE, SE and SW. The rats were allowed to swim freely until they found and climbed onto the platform. If a rat failed to locate the platform within 60 s, it would be placed on the platform for 5 s. Each rat was submitted to 6 trials per day, and the starting position was changed at each trial (starting on the N side, followed by E, S, W sides, in that order). The interval was 5 min between trials 1–3 and 4–6, and 10 min between trials 3 and 4. For the first 3 days of maze testing, the submerged platform was placed in the NW quadrant. The platform was subsequently placed in the SE quadrant for the following 3 days. On day 7, the platform was lifted above the water level and placed in the SW quadrant, and rats were injected saline 30 min before the test (day 1, 7, 14, and 21 of the experiment). Each rat was subjected to a one probe trial consisting of 6 individual trials. The total number of times each rat crossed the probe target area and the time of the probe trial swim were recorded by the observer. The time of each of the 6 trials was noted, and a mean value for each rat was calculated (number of escape latencies). Moreover, the total number of times each rat crossed the area of quadrant – NW, NE, SE, and SW – (crossed quadrants) was recorded by the observer and a mean value for each rat was calculated (crossed quadrants). The same procedures were followed until day 21 of the experiment.

Statistical analysis

The data are shown as mean values ±SEM. The data distribution pattern was not normal (unlike Gaussian function). Statistical analyses for spatial memory test and LA test were carried out using the non-parametric Kruskal-Wallis test for unpaired data and ANOVA

Table I. Effects of single and chronic treatment with ARI and FLU and combined treatment with both drugs on locomotor activity in alcohol-exposed female rats

Group	Activity counts / mean				Friedman H[3.39]
	Single administration (x±SEM)	Chronic treatment			
		7 days (x±SEM)	14 days (x±SEM)	21 days (x±SEM)	
Saline (0.5 ml/rat) CONTROL NON-ETHANOL (CNEt)	71.17±6.04	68.17±6.24	84.33±4.63	74.17±8.75	2.4
Saline (0.5 ml/rat) CONTROL ETHANOL (CEt)	82.83±4.25 *NS vs. CNEt*	85.83±6.30 *NS vs. CNEt*	92.00±3.42 NS *NS vs. CNEt*	104.50±9.35# *p=0.0293*	2.9
ARI 1.5 mg/kg *ip* 30 min before the test (CEt)	89.33±6.30 *NS*	74.50±8.89 *NS*	102.00±7.17 *NS*	98.67±8.27 *NS*	3.2
FLU 5 mg/kg *po* 60 min before the test (CEt)	90.33±5.40 *NS*	80.50±7.68 *NS*	100.33±12.13 *NS*	77.17±8.07* *p=0.0401*	3.7
ARI 1.5 mg/kg+FLU 5 mg/kg (CEt)	86.90±4.09 *NS*	95.10±5.99 *NS*	71.90±5.41* *p=0.0057*	67.50±5.67* *p=0.0033*	5.9
Kruskal-Wallis H [4.49]	1.9	2.1	8.5	10.4	

Number of housed animals=10
* Statistically significant difference p<0.05 *vs.* CEt group
# Statistically significant difference p<0.05 *vs.* CNEt group

Friedman two-way variance analysis for paired data. Statistical significance was tested using Dunn's and Dunnet's *post-hoc* test.

RESULTS

Effects of single and chronic treatment with ARI and FLU and combined treatment with both drugs on locomotor activity in alcohol-exposed female rats

There was no statistically significant difference in the activity counts between the CEt and CNEt group of rats (Table I) either in single or in chronic treatment (7 and 14 days). Only after 21 days of treatment, a statistically significant increase of locomotor activity between CEt and CNEt group of rats would be observed ($p<0.05$ *vs.* CNEt) (Table I).

ARI at the dose of 1.5 mg/kg in single and chronic treatment did not lead to locomotor activity change compared to CEt and CNEt control groups of rats (Table I).

FLU at the dose of 5 mg/kg would show a statistically significant decrease in the locomotor activity compared to the CEt control group of rats ($p<0.05$ *vs.* CEt) (Table I) only after 21 days of treatment. There was no statistically significant difference compared to CNEt control group.

After chronic treatment (14 and 21 days) with both drugs (ARI 1.5 mg/kg and FLU 5 mg/kg), a statistically significant decrease in the locomotor activity compared to CEt control group ($p<0.05$ *vs.* CEt) (Table I) was observed. There was no statistically significant difference compared to CNEt control group.

Effects of single and chronic treatment with ARI andFLU and combined treatment with both drugs on memory measured in the MWM test (escape latency) in alcohol-exposed female rats

There was no statistically significant difference in the number of escape latencies between CEt and CNEt group of rats (Table II).

Single and chronic treatment (7 days) with ARI (1.5 mg/kg) administered to alcohol-exposed animals showed a statistically significant improvement of spatial memory (decrease in the number of escape latencies) compared to CEt control group of rats ($p<0.05$ *vs.* CEt) (Table II). No statistically significant change in the number of escape latencies after ARI administration was observed in comparison to CNEt and CEt control group of rats (Table II).

Single and chronic administration of FLU (5 mg/kg) (7- and 14-days treatment) in alcohol-exposed female rats showed a statistically significant decrease in the number of escape latencies compared to CEt control group of rats

Table II. Effects of single and chronic treatment with ARI andFLU and combined treatment with both drugs on memory measured in the MWM test (escape latency) in alcohol-exposed female rats

Group	Escape latency [s]				Friedman H[3.39]
	Single administration (x±SEM)	Chronic treatment			
		7 days (x±SEM)	14 days (x±SEM)	21 days (x±SEM)	
Saline (0.5 ml/rat) CONTROL NON-ETHANOL (CNEt)	16.32±4.67	11.88±1.52	11.54±2.16	9.58±1.63	7.2
Saline (0.5 ml/rat) CONTROL ETHANOL (CEt)	21.38±2.32 *NS vs. CNEt*	17.92±2.21 *NS vs. CNEt*	12.60±1.38 *NS vs. CNEt*	11.71±1.39 *NS vs. CNEt*	8.4
ARI 1.5 mg/kg *ip* 30 min before the test (CEt)	11.10±1.64* *p=0.0020*	11.82±1.63* *p=0.0394*	12.30±2.11 *NS*	9.24±1.09 *NS*	2.5
FLU 5 mg/kg *po* 60 min before the test (CEt)	14.65±2.16* *p=0.0479*	11.26±1.27* *p=0.0176*	8.82±1.12* *p=0.0475*	11.79±1.80 *NS*	4.2
ARI 1.5 mg/kg+FLU 5 mg/kg (CEt)	13.50±1.85* *p=0.0161*	14.24±1.75 *NS*	14.30±2.29^{x} *p=0.0454*	13.87±1.80^{+} *p=0.0411*	2.1
Kruskal-Wallis H [4.49]	10.5	12.4	9.8	7.7	

Number of housed animals=10
* Statistically significant difference $p<0.05$ *vs.* CEt group
^{x} Statistically significant difference $p<0.05$ *vs.* FLU group
$^{+}$ Statistically significant difference $p<0.05$ *vs.* ARI group

(p<0.05 *vs.* CEt) which proves spatial memory improvement. The memory-improving effect was not observed after 21 days of FLU administration compared to CEt control group of rats (Table II). There was no statistically significant difference compared to CNEt control group (Table II).

Single administration of ARI+FLU to alcohol-exposed female group of rats was sufficient to cause a statistically significant decrease in the number of escape latencies compared to CEt group of rats (p<0.05 *vs.* CEt group) which indicates a spatial memory improvement in these groups of rats (Table II). There was no statistically significant difference compared to CNEt control group (Table II). In addition to this, only after chronic treatment with both drugs (ARI+FLU) there was a statistically significant deterioration in spatial memory compared to 14 days of FLU treatment (p<0.05 *vs.* FLU) and 21 days of ARI treatment (p<0.05 *vs.* ARI) (Table II). There was no statistically significant difference following combined treatment with both drugs (ARI+FLU) compared to CNEt control group of rats (Table II).

Effects of single and chronic treatment with ARI andFLU and combined treatment with both drugs on memory measured in the MWM test (crossed quadrants) in alcohol-exposed female rats

There was no statistically significant difference in the number of crossed quadrants between the CEt and CNEt groups of rats (Table III).

Single and chronic treatment (7 days) with ARI (1.5 mg/kg) administered to alcohol-exposed animals showed a statistically significant improvement of spatial memory – decrease in the crossed quadrants compared to CEt control group of rats (p<0.05 *vs.* CEt) (Table III).

Only after chronic treatment with FLU (5 mg/kg) (7 days) a statistically significant decrease in the crossed quadrants (memory improving) compared to the CEt control group of rats (p<0.05 *vs.* CEt) (Table III) was observed. There was no statistically significant difference compared to CNEt control group (Table III).

After single and chronic administration of ARI+FLU to the alcohol-exposed group of female rats, no statistically significant difference was observed in the number of crossed quadrants compared to CEt and CNEt groups of rats (Table III).

DISCUSSION

Studies on the effect of ethanol on human and animal locomotor activity yield ambiguous results (Eckardt et al. 1998); some authors claim that, depending on the dose, alcohol has a sedative effect (Risinger et al. 1994), some that it has a stimulating effect (Wilson et al. 1998, Loftis et al. 2006). Because of the differences in ethyl alcohol metabolism between women and men (Frezza et al. 1990, Taylor et al. 1996) and higher concentration of ethyl alcohol in women's blood (at the same dose), it was reasonable

Table III. Effects of single and chronic treatment with ARI and FLU and combined treatment with both drugs on memory measured in the MWM test (crossed quadrants) in alcohol-exposed female rats

Group	Crossed quadrants				Friedman H[3.39]
	Single administration (x±SEM)	Chronic treatment			
		7 days (x±SEM)	14 days (x±EM)	21 days (x±SEM)	
Saline (0.5 ml/rat) CONTROL NON-ETHANOL (CNEt)	3.95±0.82	2.58±0.29	2.65±0.55	2.04±0.32	2.3
Saline (0.5 ml/rat) CONTROL ETHANOL (CEt)	4.41±0.51 *NS vs. CNEt*	3.88±0.49 *NS vs. CNEt*	3.53±0.43 *NS vs. CNEt*	3.08±0.39 *NS vs. CNEt*	2.7
ARI 1.5 mg/kg *ip* 30 min before the test (CEt)	2.84±0.47* *p=0.0362*	2.69±0.26* *p=0.0458*	3.03±0.52 *NS*	2.45±0.31 *NS*	3.0
FLU 5 mg/kg *po* 60 min before the test (CEt)	4.00±0.68 *NS*	2.64±0.24* *p=0.0355*	2.65±0.47 *NS*	2.79±0.47 *NS*	2.8
ARI 1.5 mg/kg+FLU 5 mg/kg (CEt)	3.26±0.50 *NS*	3.03±0.33 *NS*	2.70±0.47 *NS*	3.10±0.63 *NS*	1.9
Kruskal-Wallis H [4.49]	9.6	10.1	2.3	2.0	

Number of housed animals=10
* Statistically significant difference p<0.05 *vs.* CEt group

to study the effect of aripiprazole and fluoxetine on ethyl alcohol's effects, such as its effect on locomotor activity, in female animals. Currently, there are only a few references on ARI's and FLU's effect on locomotor activity (Richtand et al. 2012) and no research papers on combined administration of these two drugs to female rats exposed to ethyl alcohol.

Our results have shown a statistically significant mobility increase on day 21 in the group of ethanol-exposed females (CEt) compared to the non-ethanol-exposed group (CNEt). Similar results in females were obtained by Quintanilla (1999), indicating a statistically significant increase of female rats' mobility after ethanol administration. Waller (1986) reached similar conclusions suggesting that the effect of locomotor stimulation in animals may depend on the alcohol dose used. Increased locomotor activity is typical for rats receiving ethanol in low doses (0.12–0.25 g/kg) (Waller et al. 1986, June and Lewis 1994). Other authors, have also shown a stimulative effect – increased locomotor activity for small doses of ethanol (2 g/kg) would explain its anxiolytic effect (Boerngen-Lacerda and Souza-Formigoni 2000).

Upon ARI administration to ethanol-exposed females, no statistically significant differences in animal mobility were found, either upon single or chronic administration. Similar results were obtained by Ingman and others (2006) who have shown that low doses of ARI (up to 3 mg) failed to affect locomotor activity. Burda and colleagues (2011), on the other hand, observed sedation upon chronic administration of ARI (for 14 days) at the dose of 6 mg/kg to male rats (Burda et al. 2011) which may be explained with minimal affinity of the drug to histaminic and α1-adrenergic receptors (Muzina 2009). Clinical trials conducted in the United States by Owen and others (Owen et al. 2009) found only rare cases of sedation in patients receiving aripiprazole. These results corroborate with other clinical trials, e.g. by Kohen and colleagues (2010) and Muzina (2009). Therefore, it may be believed that no sedative effect is observed in ethanol-exposed female rats. ARI administered at the dose of 1.5 mg/kg 30 min prior to the experiment to female rats failed to modify locomotor activity of alcohol-exposed females.

Chronic administration of FLU (21 days) caused a statistically significant reduction of the females' mobility compared to the control group receiving ethanol (CEt). Our results corroborate with results of the study by Uzbay and others (2004) which also found a statistically significant reduction of mobility in females exposed to alcohol following FLU administration. Studies by Gobert and colleagues (1997) indicate that FLU administration causes DA level increase in the rats' frontal cortex which may be the reason for the animals' increased mobility (Gobert et al. 1997, Noorafshan et al. 2014). This is also confirmed in the study by Dyr (2001) who found this to be due to ethyl alcohol's effect on striatal DA levels.

Combined administration of ARI+FLU after 14 and 21 days would cause a statistically significant reduction of the animals' mobility compared to the control group receiving ethanol (CEt). It is possible that combined administration of Ari and FLU enhances the effect of these drugs on the serotoninergic and dopaminergic systems and blocks relevant receptors in the limbic system (CB1) and brain striatum (5-HT2A/2C) (Dyr 2001, Uzbay et al. 2004, Chun-Fu et al. 1998, Pietrzak et al. 2011), hence the observed effect.

Studies by some authors suggest that chronic consumption of alcohol may impair cognitive functions, mainly operating memory and executive processes (NIAAA 2001).

Our studies on spatial memory in females show no statistically significant differences compared to the non-ethanol-exposed group (CNEt). This corroborates with reports of some authors who also failed to observe any statistically significant differences in this respect (Cacace et al. 2012).

Our results show a spatial memory improvement in ethanol-exposed rats both upon single and chronic administration of ARI (7×). No memory improvement was observed, however, after 14 or 21 days of ARI administration. Improvement of spatial memory in male rats upon single and chronic administration of ARI was also reported by Burda and others (2011). In rats receiving ethanol on a long-term basis, Burda-Malarz and colleagues (2014a) noticed a spatial memory improvement also upon single administration of ARI at the dose of 6 mg/kg. In ethyl alcohol-preferring rats, Burda-Malarz and others (2014b) observed that aripiprazole had no effect on memory at the dose of 6 mg/kg (no effect upon single or chronic administration) which may be due to changes in the dopaminergic and serotoninergic system induced by ethanol. Neither did Ratajczak and colleagues (Ratajczak et al. 2012) find any memory improvement upon administration of 1.5 mg/kg of ARI to alcohol-exposed rats compared to the control group.

FLU, likewise, improved spatial memory both upon single and chronic administration (7 and 14 days, but not 21 days) which corroborates with our previous studies on male rats (Burda-Malarz et al. 2014a, 2014b). This could be explained with FLU's effect on neurogenetic processes in the hippocampus which is important in particular considering frequent damages of this brain structure in chronic alcohol consumers (Klomp et al. 2015).

Combined administration of ARI+FLU improved memory in alcohol-exposed females only upon single administration. Lack of effect upon chronic administration may be due to the tolerance to memory improvement developing upon combined administration of ARI+FLU. Semba and others (Semba et al. 1995) obtained similar results to support this hypothesis and found that high doses of FLU have an agonistic effect on D2 and D3

receptors located in the striatum, which may lead to spatial memory impairment. Preskorn (2003) also claimed that FLU limited 5-HT reuptake, thus modifying ARI's activity. In addition to this, chronic administration of FLU frequently led to saturation of DR receptors (located in the rats' pituitary glands) (Inoue et al. 1998) and subcortical structures (Sesack and Carr 2002), agonistic effect on 5-HT1A (in the new cortex) (Newman-Tancredi et al. 1996), and antagonistic effect on 5-HT2A receptors in the mesolimbic system (McGavin and Goa 2002), leading to the effect observed upon repeated combined administration of these drugs.

CONCLUSION

It can be concluded that ARI at the dose of 1.5 mg/kg, FLU at the dose of 5 mg/kg, and combined administration of these drugs improves spatial memory in female rats exposed to ethanol (which effect generally subsides upon chronic administration of these drugs). This may also be related to the alcohol's effect on DA and 5-HT systems in the brain. Due to the limited number of reports on the drugs' modifying effect on memory in alcohol-exposed female rats, further studies on this subject are necessary.

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