Cognitive Functions and Drug Sensitivity in Adult Male Rats Prenatally Exposed to Methamphetamine

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Summary

The aim of the present study was to investigate the impact of prenatal methamphetamine (MA) exposure and application of the same drug in adulthood on cognitive functions of adult male rats tested in Morris water maze (MWM). Adult male rats prenatally exposed to MA (5 mg/kg), saline or no injection were examined. Half of the animals were injected daily with MA (1 mg/kg) after finishing the testing. Three types of tests were used: (1) "Place navigation test" (Learning), (2) "Probe test" and (3) "Retention memory test" (Memory). Our results showed that prenatal MA exposure did not affect the test of learning and the Probe test. In the test of memory prenatally MA-exposed rats showed smaller search errors and used spatial strategies more than both control groups. Further, MA application in adulthood prolonged trajectories, increased the incidence of random search and decreased the incidence of direct swim in the Place navigation test. In addition, MA administration in adulthood increased the speed of swimming regardless of prenatal exposure. The present study thus demonstrates that: (1) Prenatal MA exposure does not affect learning in the MWM. (2) Prenatal MA exposure improves performance in the Retention memory test in the MWM. (3) MA application in adulthood impairs learning in the MWM.

Key words:

methamphetamine, Morris water maze, spatial learning and memory, sensitization

Introduction

In recent years, methamphetamine (MA) is becoming a more "popular" street drug in many countries of the world because of its relatively uncomplicated production and low price compared to cocaine or heroin (Marwick 2000). Approximately half of MA users are women, mostly of reproductive age, and consequently some percentage of them become pregnant while using the drug (Williams *et al.* 2003). Since MA readily crosses the placenta, such cases will result in intrauterine exposure. Although the long-term consequences of these exposures are relatively unknown, there are studies suggesting that MA exposition during pregnancy can impair the development of neonatal central nervous system (Šlamberová *et al.* 2006, Williams *et al.* 2003). Increased creatine metabolism in striatum (Little *et al.* 1988) and deficiencies in visual recognition task, which are thought to rely upon hippocampal function (Clark *et al.* 2000), have been demonstrated after prenatal MA exposure in humans (Struthers and Hansen 1992). Both hippocampus and striatum are regions important in spatial learning and memory in humans and rodents (Barnes 1988, Iaria *et al.* 2003, McDonald and White 1994). However, studies existing on the impact of MA on cognition are rather inconsistent. This inconsistency seems to be caused by different dose of the drug and duration of the drug exposure used in the studies. Acute MA was shown to produce improvements in cognitive processing when given to drug-naive subjects (Kornetsky *et al.* 1959). In contrast, more recent studies in humans have shown, that long-term MA use is associated with impaired performance on a number of cognitive tasks (Rogers *et al.* 1999, Salo *et al.* 2002, Simon *et al.* 2000).

In rats, Acuff-Smith et al. (1996) investigated the effect of MA administered at different times during gestation on cognitive functions of the progeny. The same authors found that high doses (15 and 20 mg/kg) administered in early days of gestation impair spatial memory in the Morris water maze (MWM), while lower doses (5 and 10 mg/kg) did not have any effect on cognition in adult offspring. There are also studies showing sensitization after

repeated administration of psychostimulants (amphetamine, cocaine), such as a progressive increase in their psychomotor activating effects (Robinson and Becker 1986, Segal *et al.* 1981, Stewart and Badiani 1993). Furthermore, it has been reported that in mice exposed to MA prenatally, there is an intensification of adult MA-induced monoamine neurotoxicity when examined in vitro (Heller *et al.* 2001), suggesting an increased susceptibility to the drug from prior exposure. Vorhees et al. (1994) also showed altered responsivity to later pharmacological challenge in rats exposed neonatally to MA. Interestingly, Williams et al. (2003) showed increased hypoactivity in animals neonatally treated with MA after re-exposure to the drug in adulthood. There are, however, no behavioral studies focused on cognition and investigating the effect of prenatal MA exposure on the sensitivity to the same drug in adulthood.

The present study was therefore designed to investigate 3 objectives: (1) to find the effect of prenatal MA exposure on cognitive functions of adult male rats, (2) to find the effect of MA administered in adulthood on cognitive functions of adult male rats, (3) to determine whether prenatal MA exposure changes sensitivity to MA injection in adulthood.

Methods

Animals and Drug administration

Adult female Wistar rats (250-300g) from Anlab farms (Prague, Czech Republic) were randomly assigned to MA-treated, saline-treated or control group. They were smeared by vaginal lavage to determine the phase of estrous cycle. At the onset of the estrus phase of the estrous cycle females were housed overnight with sexually mature stimulus males. There was always one female and one male in each cage. The next morning the females were smeared again for the presence of sperm and returned to their previous home cages. The day after impregnation was counted as day 1 of gestation. MA-treated females were injected subcutaneously (s.c.) with D-methamphetamine HCl in a dose of 5 mg/kg through the entire

gestation (i.e. from the first to the last days of gestation) (Šlamberová *et al.* 2005). Saline-treated dams were administered saline s.c. at the same time and in the same volume as MA. Control females were not exposed to any injection. Two control groups (Peters 1982) (i.e. saline and control) were used to differentiate the possible effect of injection-induced stress. The day of birth was counted as postnatal day (PD) 0. On PD 1, MA-exposed pups were injected intradermally with black India ink in the left foot pad and saline-exposed pups in the right foot pad for identification. Control pups were not tattooed. A total of 24 litters were used in the experiment. The number of pups in each litter was adjusted to 12. Whenever possible, the same number of male and female pups was kept in each litter. To avoid litter bias pups were cross-fostered on PD 1, so that one mother usually raised 4 control, 4 saline and 4 MA pups. Three animals from each litter were used – 1 for each of the prenatally treated group (control, saline, MA) (Holson and Pearce 1992). On PD 21, animals were weaned and housed in groups, separated by sex. Animals were left undisturbed until adulthood.

Morris water maze

The male offspring (n = 72; 12 per each treatment group) were tested in adulthood (PD 60-90) for learning and memory in the MWM (blue circular tank, 2 m in diameter) filled with opaque water. On the rim of the pool, four starting positions were marked north (N), south (S), east (E), west (W), thus dividing the pool into four quadrants. A transparent circle platform (13 cm in diameter) 1 cm below the water surface was used for learning and memory tasks. Various pictures hanging on the walls were available to the rats as extra-maze cues. Rats' performance was tracked automatically using a video tracking system EthoVision 3.1 (Noldus Information Technology, Netherlands). Rats were tested over a 12 day period. To determine the effect of MA in adulthood half of the animals from each prenatally exposed group (i.e. MA, saline, control; n = 24) received a low dose of MA (1 mg/kg) s.c. after finishing testing each day, while the other half were not exposed to any injections in

adulthood. The dose 1 mg/kg was used because it does not cause stereotypes, unlike the dose of 5 mg/kg used in gestation. On the days 7 – 11 when no tests were performed, MA was administered at the same time as in the days of testing. Three types of tests were used in the present study: "Place navigation test" (Learning), "Probe test" and "Retention memory test" (Memory).

In the learning test, which was performed on the first 5 consecutive days, an animal was supposed to find the platform within the limit of 60 seconds. The animal unable to find the platform within the limit was guided to the platform manually. Each rat was exposed to 8 trials daily starting from 4 different positions with intertrial interval (ITI) of 30 seconds. The position of the platform was the same in all trials. In the probe test, which was administered on the 6^{th} day, the platform was removed and the animal was left to swim in the pool for 60 seconds. The memory test was performed on the 12^{th} day and the rat was supposed to find the platform located in the same position as in the learning test within 60 seconds. Each rat was exposed to 8 trials starting from 4 different positions with ITI of 30 seconds.

In the tests of learning and memory latency to reach the hidden platform, length of the trajectory, search error (a measure of proximity to the escape platform) and speed of swimming were recorded. Swim paths for each rat were manually analyzed, so that predominating strategy in each trail was always identified and frequency of the following search strategies per day was recorded: (1) Thigmotaxis (wall-hugging) – a persistent swim along the wall of the pool that could include sporadic swims towards the centre of the pool, (2) Random search – swimming over the entire area of the pool in straight swims or in wide circular swims, (3) Scanning – swimming over the central area of the pool, (4) Chaining – circular swimming at a fixed distance from the wall, in which the platform was located, (5) Focal search in an incorrect quadrant – direct swim to an incorrect quadrant of the pool followed by loops and turns there, (6) Focal search in the target quadrant - direct swim to the

correct quadrant of the pool followed by loops and turns there, (7) Spatial search – a direct swim path to the platform. This method was previously used for analyzing search strategies in mice (Janus 2004). In the "Probe test" the following measures were recorded: speed of swimming; frequency and duration of presence in the quadrant where the platform was located in the learning and memory tests.

Statistical methods

As there were no differences in the animals of the same prenatal exposure that were raised by mothers of different drug treatment, the raising mother (biological vs. foster) was not taken as a factor for statistical analyses.

Two-way ANOVA (Prenatal exposure x Treatment in adulthood) with multilevel repeated measure (days x trials/day) was used to analyze the data from the Place navigation test. Two-way ANOVA (Prenatal exposure x Treatment in adulthood) repeated measure (trials) was used to analyze the data from the Retention memory test. Chi^2 test was used to analyze the frequency of the search strategies. Two-way ANOVA (Prenatal exposure x Treatment in adulthood) was used to analyze the data from the "Probe test". LSD test was used for post-hoc comparisons. Differences were considered significant if $p < 0.05$.

Results

One rat from control group with no MA in adulthood was excluded from the study because it became evident that it was unable to swim properly and to keep its head above water during the last trials.

Place navigation test

For the latency (Fig. 1A) to reach the hidden platform and the search error, no effects of Prenatal exposure and Treatment in adulthood were found. For the length of the trajectory the main effect of Treatment in adulthood was demonstrated [$F(1,65) = 5.37$, $p<0.05$]; the animals with MA application in adulthood had longer trajectories than the animals without it,

regardless of prenatal exposure as shown in Fig.1B. All animals, regardless of treatment, demonstrated learning ability over the 5-day test period as represented by a decrease in latency [$F(4,260) = 202.80$, $p<0.0001$], trajectory length [$F(4,260) = 162.18$, $p<0.0001$] and search error [$F(4,260) = 155.61$, $p<0.0001$]. Main effect of Treatment in adulthood for swimming speed was found [$F(1,65) = 10.58$, $p<0.01$], such that MA-treated animals were faster than the animals without MA administration regardless of prenatal exposure (Fig. 1C).

Analysis of the search strategies showed significant effect of Treatment in adulthood for random search and spatial search [$\chi2 = 105.24$; $p<0.0001$]. Thus, rats treated with MA in adulthood displayed more random searching and less spatial searching in the test of learning than rats without MA administration, regardless of prenatal drug exposure (Fig.2).

Further, we found increased incidence of both random and spatial search strategies in rats prenatally exposed to MA and saline relative to rats without any prenatal exposure [$\chi2 = 102.25$; $p<0.0001$; $\chi2 = 33.53$; $p<0.0001$, respectively], regardless of treatment in adulthood. Table 1A displays an overview of incidence of the search strategies used by the rats in the five days in the test of learning.

Probe test

For the frequency and duration of presence in the quadrant with the hidden platform no effects were demonstrated. For the speed of swimming, there was main effect of Treatment in adulthood [$F(1,65) = 8.47$, $p < 0.01$]; animals with MA treatment swam faster than animals without MA injections.

Retention memory test

Analysis of the data showed significant main effect of Prenatal exposure for latency [$F(2,65) = 3.76$, $p<0.05$] and search error [$F(2,65) = 4.09$, $p<0.05$]. Post-hoc test showed that animals with prenatal exposure to MA had shorter latencies (Fig. 3A) than animals prenatally exposed to saline, while the search errors (Fig. 3B) of prenatally MA-treated animals were

smaller relative to both control groups. Further, significant main effect of Treatment in adulthood for the speed of swimming (Fig. 3C) was demonstrated [$F(1,65) = 21.60$, $p<0.001$], such that all animals treated with MA, regardless of prenatal exposure, swam faster than the animals without MA application.

In addition, significant effect of Prenatal exposure for the frequency of used search strategies was found. Rats prenatally exposed to MA used more spatial search and correct target quadrant search than rats without any prenatal exposure [$\chi 2 = 18.62$; $p<0.01$], regardless of treatment in adulthood. Also, prenatally MA exposed rats swam less in the incorrect quadrants and searched the pool by using spatial search more than prenatally saline exposed rats [$\chi 2 = 25.13$; $p<0.001$], regardless of treatment in adulthood. The incidence of the search strategies in the test of memory is showed in Table 1 B.

Discussion

Our findings from this experiment are as follows. First, we found that although prenatal MA exposure at a dose of 5 mg/kg did not have an effect on the latency, search error and length of the trajectory in Place navigation task, rats prenatally exposed to MA and saline used different search strategies than rats without any prenatal exposure. Both prenatally exposed groups used more random and spatial search than the control group, regardless of treatment in adulthood. While random search is a non-spatial strategy and is used mainly in the beginning of the learning period, spatial search is a direct swim to the platform and is used if an animal remembers the exact location of the platform (Janus 2004). Therefore, it cannot be that prenatally MA and saline exposed rats demonstrated stronger spatial bias than the rats without any prenatal exposure. Instead, their behavior in the MWM was different. This might have been the effect of different coping with stress in the MWM in saline and MA exposed rats caused by the injection application rather than by MA itself, since it was shown (Peters 1982, Šlamberová *et al.* 2002) that placebo injection of saline administered to control

pregnant mothers may induce mild stress for the rat and that way indirectly affect the development of her pups. Nevertheless, more studies concerning the functioning of the hypothalamic-pituitary-adrenal (HPA) system during the tests in the MWM after prenatal exposure to MA and saline are necessary to confirm this hypothesis.

Thus, prenatal MA exposure did not influence learning in the MWM and this finding is in agreement with the work of Acuff-Smith et al. (1996), who also showed that low dose of MA (5 or 10 mg/kg) administered prenatally did not have effect on spatial memory in MWM. However, they (Acuff-Smith *et al.* 1996) further demonstrated that prenatal exposure to higher doses of MA (15, 20 mg/kg) did induce impairments of spatial memory in MWM tested in adulthood. We use the low dose of MA (5 mg/kg) in our studies, since application of MA at 5 mg/kg to pregnant female rats induces changes that are comparable with those found in fetuses of drug-abusing women (Acuff-Smith *et al.* 1996). There are other studies showing that neonatal administration of MA at doses of 5, 10 or 15 mg/kg administered four times daily from PD 11 – 20 produced lasting spatial learning and memory deficits (Williams *et al.* 2003). As the hippocampus in rats is still developing during the PD 11 – 20 and this development is analogous to human hippocampal development during the third trimester of pregnancy (Bayer *et al.* 1993), the neonatal period may be more critical for the effects of MA on cognitive functions in rats than the prenatal period.

Second, the results of search strategy analysis in Place navigation test demonstrated that the incidence of random search was significantly greater and the incidence of direct swim was significantly lower in MA-treated rats than in rats with no application in adulthood, regardless of prenatal MA exposure. Thus, rats with MA application in adulthood demonstrated weaker spatial bias than rats without adult MA. Moreover, in Place navigation test rats with MA application in adulthood had longer trajectories than rats without drug administration, regardless of prenatal exposure. The fact that MA treatment in adulthood had

no significant effect on the latency in Place navigation test was probably caused by increased swimming velocities in rats with adult MA. Furthermore, trajectory length has been shown to be a better index of spatial learning than latency (Lindner 1997). The animals with adult MA treatment found the hidden platform as fast as the animals without MA administration, because they swam faster, but their trajectories when searching for the platform were longer. This indicates that information processing but not motivation were impaired in rats with MA application in adulthood. In accordance with these findings, Friedman et al. (1998) also observed acquisition impairment in MWM induced by acute MA (12.5 mg/kg) administration, and further they demonstrated dopamine and serotonin depletions in caudate and hippocampus, respectively, 48 days after this exposure. While in the latter study the rats were tested 65 days after the neurotoxic dose of MA, in our present study the rats were given adult MA every day after testing. We showed that even the dose of MA as low as 1 mg/kg impaired learning probably by disrupting consolidation to some extent. However, by the 6th day the animals treated with MA in adulthood were able to locate the platform as successfully as the animals without MA application, as there were no differences in the performance on Probe trial between MA-treated and non-treated groups in adulthood.

As reported before, placebo injection may induce mild stress for the rat (Peters 1982). However, the rats in the present study were given adult MA each day after testing and were tested again not earlier than 24 hours after the injection. Therefore, we believe the stress caused by the injection itself was negligible when compared to stress in MWM (Akirav *et al.* 2001, Morris 1984) and did not affect the rats' performance in MWM unlike adult MA.

Third, surprisingly, in the Retention memory test rats prenatally exposed to MA displayed smaller search errors than both control groups while they had shorter latencies than prenatally saline exposed rats only. However, the analysis of search error may better reflect the accuracy of spatial learning than latency (Gallagher *et al.* 1993), since it describes the rats'

distance to the platform during the trials. Although for 2 animals the escape latencies can be almost identical, their performance can actually differ markedly. While one of the animals searches for the platform relatively close to it, the other searches in quadrants distant to the platform randomly and finds the platform accidentally. The aforementioned findings are further supported by the results of search strategy analysis, which demonstrated that rats prenatally exposed to MA searched the pool by using spatial strategies (focal search in correct target quadrant, direct swim) more, than rats with saline or no exposure in prenatal period.

These results suggest that prenatally MA-exposed animals memorized the location of the platform most accurately. Unfortunately, there is only one study investigating the effect of prenatal MA exposure on memory in adult male rats. Although, this study (Acuff-Smith *et al.* 1996) showed that MA 5 mg/kg did not influence memory in MWM, the test of memory was performed on the day following the learning trials, while in the present study, we tested memory 1 week after finishing the learning trials. To be able to explain our findings, other studies investigating the effect of prenatal MA exposure on glutamate N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors in hippocampus are necessary, since these receptors are known to be critical for hippocampal long term potentiation, the synaptic phenomenon thought to be behind memory formation (Davis *et al.* 1992, Jia *et al.* 2001). Our findings showing that prenatal MA exposure affects seizures induced by NMDA supports this hypothesis (Šlamberová and Rokyta 2005). Moreover, NMDA and AMPA receptor subunit levels were shown to be altered by MA treatment in rats (Simoes *et al.* 2007).

Fourth, we found that MA application in adulthood increased the speed of swimming in all animals regardless of prenatal exposure and the type of test, even though the animals were tested daily not earlier than 24 hours after acute MA application. Swimming velocity can also be used to measure motivation to find the hidden platform (Lubbers *et al.* 2007).

Therefore, the increased swimming velocity suggests that MA administration in adulthood increased motivation. Motivation is assumed to be mediated by meso-accumbens dopamine system (Ikemoto and Panksepp 1999, Robinson and Berridge 2001, Salamone and Correa 2002) and this system might have been altered by MA application in adulthood. Interestingly, there was no difference in the increase of the speed after adult MA application between the prenatally exposed groups. Thus, we did not prove the sensitizing effect of prenatal MA exposure to the same drug administered in adulthood. The studies showing the sensitizing effects of psychostimulants on central nervous system tested these effects after repeated intermittent administration of the drugs to adult animals with mature brain structures (Nordquist *et al.* 2007, Schoffelmeer *et al.* 2002, Suzuki *et al.* 2004, Vanderschuren *et al.* 2002, Wyvell and Berridge 2001). On the other hand, a study performed in rabbits demonstrated that offspring, having received cocaine during the prenatal period, showed profound behavioral tolerance to the amphetamine challenge while their mothers, who received cocaine at the same dose and duration and experienced the same period of withdrawal, exhibited robust behavioral sensitization (Stanwood and Levitt 2003). In addition, Williams et al. (2003) showed increased hypoactivity in animals neonatally treated with MA after re-exposure to the drug in adulthood. These findings suggest that specific adaptive changes in neural signaling that occur during sensitization may be influenced by the maturational state of the brain during which the exposure occurs.

Taken together, the present study demonstrates that prenatal exposure to MA at dose as low as 5 mg/kg does not impair learning in the MWM, while improves performance in the Retention memory test. In contrast, we found that MA (1 mg/kg) application in adulthood impairs learning in the MWM. Finally, our study shows that prenatal MA exposure does not increase sensitivity to the same drug in adulthood when tested in MWM. As there are no studies investigating the impact of prenatal MA exposure on sensitivity to the same drug in

adulthood, our present study will contribute to further knowledge of this problem. However, other studies, especially on functioning of dopaminergic, serotonergic and HPA systems, as well as levels of NMDA and AMPA receptor subunits in hippocampus after prenatal MA exposure must be done to fully understand its long-term effects on cognitive and behavioral processes.

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References

ACUFF-SMITH KD, SCHILLING MA, FISHER JE, VORHEES CV: Stage-specific effects of prenatal d-methamphetamine exposure on behavioral and eye development in rats. *Neurotoxicol Teratol* **18**: 199-215, 1996.

AKIRAV I, SANDI C, RICHTER-LEVIN G: Differential activation of hippocampus and amygdala following spatial learning under stress. *Eur J Neurosci* **14**: 719-725, 2001.

BARNES CA: Spatial learning and memory processes: the search for their neurobiological mechanisms in the rat. *Trends Neurosci* **11**: 163-169, 1988.

BAYER SA, ALTMAN J, RUSSO RJ, ZHANG X: Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology* **14**: 83-144, 1993.

CLARK RE, ZOLA SM, SQUIRE LR: Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci* **20**: 8853-8860, 2000.

DAVIS S, BUTCHER SP, MORRIS RG: The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro. *J Neurosci* **12**: 21-34, 1992.

FRIEDMAN SD, CASTANEDA E, HODGE GK: Long-term monoamine depletion, differential recovery, and subtle behavioral impairment following methamphetamine-induced neurotoxicity. *Pharmacol Biochem Behav* **61**: 35-44, 1998.

GALLAGHER M, BURWELL R, BURCHINAL M: Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. *Behav Neurosci* **107**: 618-626, 1993.

HELLER A, BUBULA N, LEW R, HELLER B, WON L: Gender-dependent enhanced adult neurotoxic response to methamphetamine following fetal exposure to the drug. *J Pharmacol Exp Ther* **298**: 769-779, 2001.
HOLSON RR, PEARCE B: Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. *Neurotoxicol Teratol* **14**: 221-228, 1992.
IARIA G, PETRIDES M, DAGHER A, PIKE B, BOHBOT VD: Cognitive strategies dependent on the hippocampus and caudate nucleus in human navigation: variability and change with practice. *J Neurosci* **23**: 5945-5952, 2003.
IKEMOTO S, PANKSEPP J: The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. *Brain Res Brain Res Rev* **31**: 6-41, 1999.
JANUS C: Search strategies used by APP transgenic mice during navigation in the Morris water maze. *Learn Mem* **11**: 337-346, 2004.
JIA Z, LU YM, AGOPYAN N, RODER J: Gene targeting reveals a role for the glutamate receptors mGluR5 and GluR2 in learning and memory. *Physiol Behav* **73**: 793-802, 2001.
KORNETSKY C, MIRSKY AF, KESSLER EK, DORFF JE: The effects of dextro-amphetamine on behavioral deficits produced by sleep loss in humans. *J Pharmacol Exp Ther* **127**: 46-50, 1959.
LINDNER MD: Reliability, distribution, and validity of age-related cognitive deficits in the Morris water maze. *Neurobiol Learn Mem* **68**: 203-220, 1997.
LITTLE BB, SNELL LM, GILSTRAP LC, 3RD: Methamphetamine abuse during pregnancy: outcome and fetal effects. *Obstet Gynecol* **72**: 541-544, 1988.
LUBBERS ME, VAN DEN BOS R, SPRUIJT BM: Mu opioid receptor knockout mice in the Morris Water Maze: a learning or motivation deficit? *Behav Brain Res* **180**: 107-111, 2007.
MARWICK C: NIDA seeking data on effect of fetal exposure to methamphetamine. *Jama* **283**: 2225-2226, 2000.
MCDONALD RJ, WHITE NM: Parallel information processing in the water maze: evidence for independent memory systems involving dorsal striatum and hippocampus. *Behav Neural Biol* **61**: 260-270, 1994.
MORRIS R: Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* **11**: 47-60, 1984.
NORDQUIST RE, VOORN P, DE MOOIJ-VAN MALSEN JG, JOOSTEN RN, PENNARTZ CM, VANDERSCHUREN LJ: Augmented reinforcer value and accelerated habit formation after repeated amphetamine treatment. *Eur Neuropsychopharmacol* **17**: 532-540, 2007.
PETERS DA: Prenatal stress: effects on brain biogenic amine and plasma corticosterone levels. *Pharmacol Biochem Behav* **17**: 721-725, 1982.
ROBINSON TE, BECKER JB: Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Res* **396**: 157-198, 1986.
ROBINSON TE, BERRIDGE KC: Incentive-sensitization and addiction. *Addiction* **96**: 103-114, 2001.
ROGERS RD, EVERITT BJ, BALDACCHINO A, BLACKSHAW AJ, SWAINSON R, WYNNE K, BAKER NB, HUNTER J, CARTHY T, BOOKER E, LONDON M, DEAKIN JF, SAHAKIAN BJ, ROBBINS TW: Dissociable deficits in the decision-making cognition of chronic amphetamine abusers, opiate abusers, patients with focal damage to prefrontal cortex, and tryptophan-depleted normal volunteers: evidence for monoaminergic mechanisms. *Neuropsychopharmacology* **20**: 322-339, 1999.
SALAMONE JD, CORREA M: Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behav Brain Res* **137**: 3-25, 2002.

SALO R, NORDAHL TE, POSSIN K, LEAMON M, GIBSON DR, GALLOWAY GP, FLYNN NM, HENIK A, PFEFFERBAUM A, SULLIVAN EV: Preliminary evidence of reduced cognitive inhibition in methamphetamine-dependent individuals. *Psychiatry Res* **111**: 65-74, 2002.
SEGAL DS, GEYER MA, SCHUCKIT MA: Stimulant-induced psychosis: an evaluation of animal methods. *Essays Neurochem Neuropharmacol* **5**: 95-129, 1981.
SCHOFFELMEER AN, DE VRIES TJ, WARDEH G, VAN DE VEN HW, VANDERSCHUREN LJ: Psychostimulant-induced behavioral sensitization depends on nicotinic receptor activation. *J Neurosci* **22**: 3269-3276, 2002.
SIMOES PF, SILVA AP, PEREIRA FC, MARQUES E, GRADE S, MILHAZES N, BORGES F, RIBEIRO CF, MACEDO TR: Methamphetamine induces alterations on hippocampal NMDA and AMPA receptor subunit levels and impairs spatial working memory. *Neuroscience* **150**: 433-441, 2007.
SIMON SL, DOMIER C, CARNELL J, BRETHEN P, RAWSON R, LING W: Cognitive impairment in individuals currently using methamphetamine. *Am J Addict* **9**: 222-231, 2000.
STANWOOD GD, LEVITT P: Repeated i.v. cocaine exposure produces long-lasting behavioral sensitization in pregnant adults, but behavioral tolerance in their offspring. *Neuroscience* **122**: 579-583, 2003.
STEWART J, BADIANI A: Tolerance and sensitization to the behavioral effects of drugs. *Behav Pharmacol* **4**: 289-312, 1993.
STRUTHERS JM, HANSEN RL: Visual recognition memory in drug-exposed infants. *J Dev Behav Pediatr* **13**: 108-111, 1992.
SUZUKI T, FUKUOKA Y, MORI T, MIYATAKE M, NARITA M: Behavioral sensitization to the discriminative stimulus effects of methamphetamine in rats. *Eur J Pharmacol* **498**: 157-161, 2004.
ŠLAMBEROVÁ R, CHAROUSOVÁ P, POMETLOVÁ M: Methamphetamine administration during gestation impairs maternal behavior. *Dev Psychobiol* **46**: 57-65, 2005.
ŠLAMBEROVÁ R, POMETLOVÁ M, CHAROUSOVÁ P: Postnatal development of rat pups is altered by prenatal methamphetamine exposure. *Prog Neuropsychopharmacol Biol Psychiatry* **30**: 82-88, 2006.
ŠLAMBEROVÁ R, ROKYTA R: Occurrence of bicuculline-, NMDA- and kainic acid-induced seizures in prenatally methamphetamine-exposed adult male rats. *Naunyn Schmiedebergs Arch Pharmacol* **372**: 236-241, 2005.
ŠLAMBEROVÁ R, SCHINDLER CJ, VATHY I: Impact of maternal morphine and saline injections on behavioral responses to a cold water stressor in adult male and female progeny. *Physiol Behav* **75**: 723-732, 2002.
VANDERSCHUREN LJ, SCHOFFELMEER AN, VAN LEEUWEN SD, HOF L, JONKER AJ, VOORN P: Compartment-specific changes in striatal neuronal activity during expression of amphetamine sensitization are the result of drug hypersensitivity. *Eur J Neurosci* **16**: 2462-2468, 2002.
VORHEES CV, AHRENS KG, ACUFF-SMITH KD, SCHILLING MA, FISHER JE: Methamphetamine exposure during early postnatal development in rats: I. Acoustic startle augmentation and spatial learning deficits. *Psychopharmacology (Berl)* **114**: 392-401, 1994.
WILLIAMS MT, BLANKENMEYER TL, SCHAEFER TL, BROWN CA, GUDELSKY GA, VORHEES CV: Long-term effects of neonatal methamphetamine exposure in rats on spatial learning in the Barnes maze and on cliff avoidance, corticosterone release, and neurotoxicity in adulthood. *Brain Res Dev Brain Res* **147**: 163-175, 2003.
WILLIAMS MT, MORFORD LL, WOOD SL, WALLACE TL, FUKUMURA M, BROENING HW, VORHEES CV: Developmental D-methamphetamine treatment selectively

induces spatial navigation impairments in reference memory in the Morris water maze while sparing working memory. *Synapse* **48**: 138-148, 2003.
WYVELL CL, BERRIDGE KC: Incentive sensitization by previous amphetamine exposure: increased cue-triggered "wanting" for sucrose reward. *J Neurosci* **21**: 7831-7840, 2001.

Figure legend

Figure 1. Performance in Place navigation task: (A) Latency to reach the platform in days 1-5. (B) The length of trajectory - main effect of MA application in adulthood; adult MA > no MA in adulthood. Results are presented as average of trials in all five days. (C) The velocity of swimming - main effect of MA application in adulthood; adult MA > no MA in adulthood. Results are presented as average of trials in all five days. Statistics: Two-way ANOVA (Prenatal exposure x Treatment in adulthood) with multilevel repeated measure (days x trials/day). Values are means ± SEM, n = 11-12. * $p < 0.05$ vs. NO MA; ** $p < 0.01$ vs. NO MA

Figure 2. Strategies used in Place navigation task in days 1 - 5: (A) Random search strategy - effect of MA administration in adulthood on the frequency of random search regardless of prenatal exposure; adult MA > no MA in adulthood in days 3, 4, 5. (B) Spatial search strategy - effect of MA in adulthood on the frequency of spatial search regardless of prenatal exposure; adult MA < no MA in adulthood in days 2, 3, 4, 5. Statistics: Chi^2 test; 1st day: [$\chi^2 = 12.14$, $p=0.06$]; 2nd day: [$\chi^2 = 56.44$, $p<0.0001$]; 3rd day: [$\chi^2 = 48.91$, $p<0.0001$]; 4th day: [$\chi^2 = 135.82$, $p<0.0001$]; 5th day: [$\chi^2 = 150.95$, $p<0.0001$]; Values are sums of frequencies in days 1 – 5, n = 11-12. * $p<0.0001$ vs. NO MA

Figure 3. Performance in Retention memory task: (A) Latency to reach the platform - main effect of Prenatal MA exposure; prenatal MA < prenatal saline. (B) Search error - main effect of Prenatal MA exposure; prenatal MA < controls and prenatal saline. (C) The velocity of swimming - main effect of MA administration in adulthood; adult MA > no MA in adulthood. Statistics: Two-way ANOVA (Prenatal exposure x Treatment in adulthood)

repeated measure (trials). Results are presented as averages of all 8 trials in day 12. Values are means ± SEM, n = 11-12. * $p < 0.05$ vs. Control; # $p < 0.05$ vs. Saline; + $p < 0.001$ vs. NO MA

Table 1. Percentage of search strategies used in groups

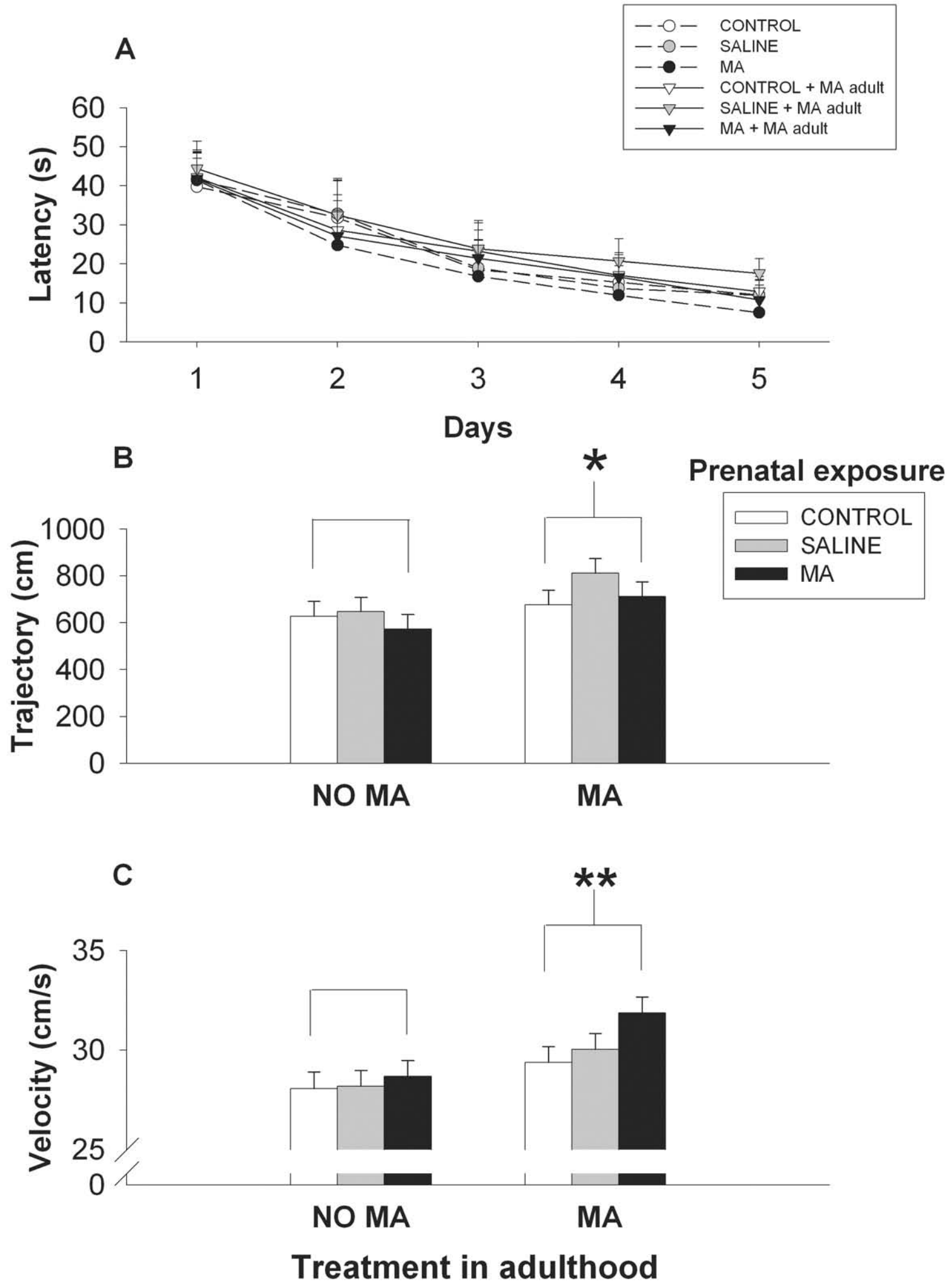
A. Place navigation task in days 1 – 5

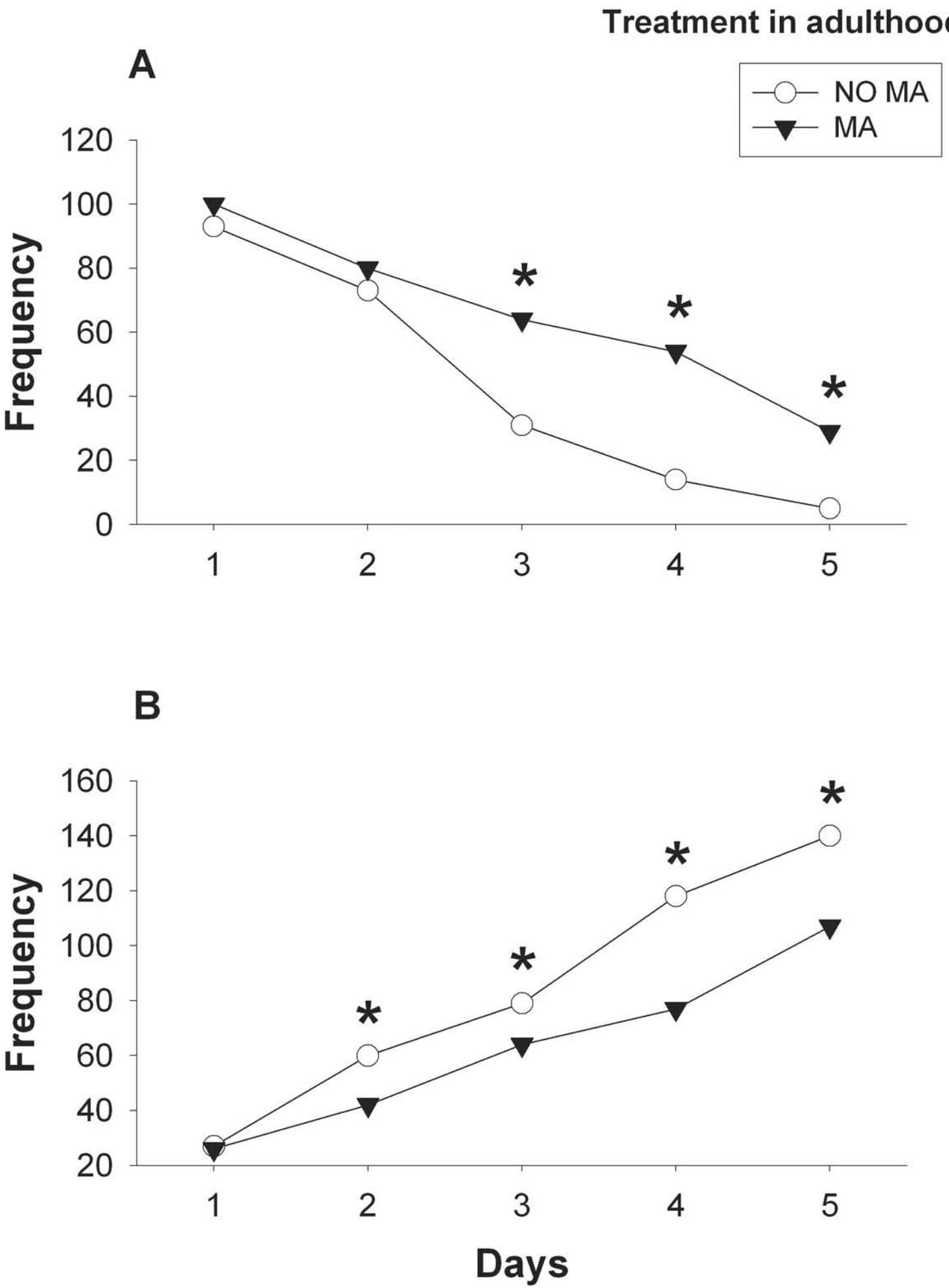
	NO MA in adulthood			**MA in adulthood**		
STRATEGY	**Control**	**Saline**	**MA**	**Control**	**Saline**	**MA**
Thigmotaxis	13	11	6**++	14	11	9**
Random	14	15	16**	16	26**	25**
Scanning	2	1	1	0	1	1
Chaining	4	4	4	5	5	3
Incorrect Q	36	37	31++	40	29**	29**
Correct TQ	5	5	4	6	6	8
Spatial search	26	27	38**++	19	22	25**

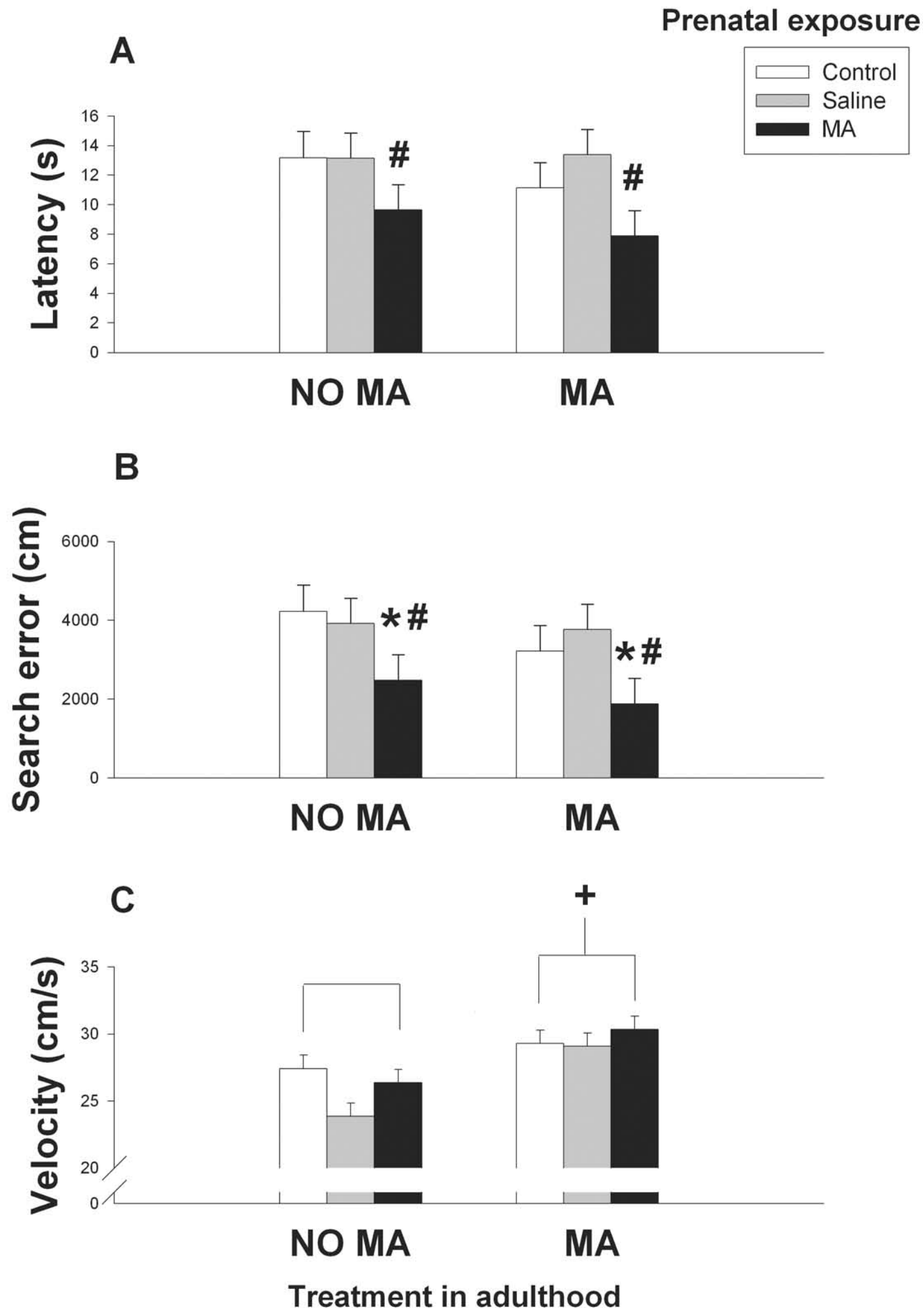
B. Retention memory task

	NO MA in adulthood			**MA in adulthood**		
STRATEGY	**Control**	**Saline**	**MA**	**Control**	**Saline**	**MA**
Thigmotaxis	2	2	0	2	0	0
Random	7	7	5	8	9	6
Scanning	0	0	0	0	0	0
Chaining	1	1	1	1	3	2
Incorrect Q	35	36	29	38	47	28
Correct TQ	8	4	10+	2	9**	6
Spatial search	47	50	55	49	32**	58*++

Statistics: Chi^2 test. Values are per cent, n = 11-12. * $p < 0.05$ vs. Control; ** $p < 0.0001$ vs. Control; + $p < 0.001$ vs. Saline; ++ $p < 0.0001$ vs. Saline. Q = Quadrant, TQ = Target Quadrant.


A
CONTROL
SALINE
MA
CONTROL + MA adult
SALINE + MA adult
MA + MA adult
Latency (s)
60
50
40
30
20
10
0
1
2
3
4
5
Days
B
*
Prenatal exposure
CONTROL
SALINE
MA
Trajectory (cm)
1000
800
600
400
200
0
NO MA
MA
C
**
Velocity (cm/s)
35
30
25
0
NO MA
MA
Treatment in adulthood


Treatment in adulthood
A
NO MA
MA
120
100
80
60
40
20
0
Frequency
1
2
3
4
5
*
*
*
B
160
140
120
100
80
60
40
20
Frequency
1
2
3
4
5
Days
*
*
*
*


Prenatal exposure
Control
Saline
MA
A
Latency (s)
16
14
12
10
8
6
4
2
0
#
#
NO MA
MA
B
Search error (cm)
6000
4000
2000
0
*#
*#
NO MA
MA
C
Velocity (cm/s)
35
30
25
20
0
+
NO MA
MA
Treatment in adulthood