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The levo enantiomer of amphetamine increases memory consolidation and gene expression in the hippocampus without producing locomotor stimulation

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ABSTRACT

Dextro-amphetamine enhances memory and other cognitive functions in animals and humans. The use of *d*-amphetamine as a memory enhancer, however, is limited by a robust stimulatory side-effect profile caused by release of dopamine. The levo enantiomer of amphetamine has been shown to be considerably less effective as a dopamine releaser and less potent in producing the stimulatory effects characteristic of *d*-amphetamine. In order to determine whether *l*-amphetamine and the structurally related compound, *l*-methamphetamine, retain cognitive-enhancing effects despite their lack of stimulatory activity, we administered the compounds to rats prior to activity monitoring experiments, and in different animals, immediately after training on inhibitory avoidance and object recognition tasks. Results demonstrated that *l*-amphetamine and *l*-methamphetamine did not increase locomotion and stereotypies beyond control levels, but did produce significant memory enhancement. In addition, *l*-amphetamine and *l*-methamphetamine alleviated scopolamine-induced amnesia in the inhibitory avoidance task. In all cases, these compounds produced an effect comparable to that of *d*-amphetamine, but required only one quarter of the *d*-amphetamine dose to produce the same effect size. We also found that *l*-amphetamine modulates learning-induced changes in hippocampal Arc/Arg3.1 protein synthesis that correlate with memory consolidation. These results suggest that *l*-amphetamine and *l*-methamphetamine are potent memory enhancers in rats and may ultimately be useful for treating memory disorders in humans.

1. Introduction

In recent years, a considerable amount of research has focused on discovering compounds that improve memory in animals and in humans. One such compound is amphetamine. Two stereoisomeric forms of amphetamine exist: *d*-amphetamine (dextroamphetamine, trade name Dexedrine), and *l*-amphetamine (levoamphetamine). *d*-amphetamine is more potent than *l*-amphetamine in producing many behavioral effects, and has long been considered the "active" isomer (Schechter, 1978; Segal, 1975). *d*-amphetamine has been shown to improve memory in normal animals (Brown, Bardo, Mace, Phillips, & Kraemer, 2000; Krivanek & McGaugh, 1969; Lee & Ma, 1995; Martinez et al., 1980; Packard, 1989; Sara & Deweer, 1982; Strupp & Bunsey, 1991; White, 1988), as well as in animals with permanent brain damage induced by lesions (M'Harzi, 1988), X-irradiation (Highfield, Hu, & Amsel, 1998) or global forebrain ischemia (Wishart, Ijaz, & Shuaib, 1994). In addition, *d*-amphetamine has been shown to enhance memory in humans (Brown et al., 2000; Lee & Ma, 1995; M'Harzi, 1988; Quartermain, Judge, & Jung, 1988; Sara & Deweer, 1982; Soetens, Casaer, d'Hooge, & Hueting, 1995; Soetens, Hooge, & Hueting, 1993; Strupp & Bunsey, 1991; Wishart et al., 1994). Memory enhancement has been observed when *d*-amphetamine is administered after the training session, suggesting that it affects consolidation processes.

It is generally believed that *d*-amphetamine improves memory by activating the central and perhaps peripheral catecholamine systems (Martinez et al., 1980; White, 1988). The major mechanism of action of *d*-amphetamine is to stimulate dopamine release in the brain and to prolong the synaptic action of dopamine by blocking its reuptake (Creese & Iversen, 1975). Dopamine has previously been implicated in memory processing in animals (Castellano, Cestari, Cabib, & Puglisi-Allegra, 1991; Castner & Goldman-Rakic, 2004; Packard, 1989). For example, direct infusions of dopamine into the amygdala enhance performance on the inhibitory avoidance task (LaLumiere & McGaugh, 2005; LaLumiere, Nguyen, & McGaugh, 2004). Conversely, intrahippocampal (O'Carroll et al., 2006) or cortical infusions (Rinaldi, Mandillo, Oliverio, & Mele, 2007) of dopaminergic antagonists impair memory for spatial learning tasks. While increased levels of dopamine have been associated with improved memory, elevated levels have also been correlated with adverse effects such as hyperactivity, stereotypy,

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addiction and psychosis (Mason, 1983; Szechtman, Ornstein, Teitelbaum, & Golani, 1985; Wise & Rompre, 1989).

A second known mechanism of action of *d*-amphetamine is to increase levels of norepinepherine in the cortex (Creese & Iversen, 1975). Norepinephrine has also been associated with memory processes; infusion of noradrenergic agonists, including norepinephrine, into the amygdala or hippocampus after training on inhibitory or spatial memory tasks enhances long-term memory for the training events (Ferry & McGaugh, 1999; Hatfield & McGaugh, 1999; LaLumiere, Buen, & McGaugh, 2003; Lee & Ma, 1995). In addition, inhibition of norepinephrine synthesis at the dopamine-β-hydroxylase (DBH) stage by diethyldithiocarbamate results in very severe memory impairment for aversively mediated tasks (Randt, Quartermain, Goldstein, & Anagnoste, 1971; Stein, Belluzzi, & Wise, 1975), and this impairment can be reversed by the administration of norepinephrine (Stein et al., 1975). More recently it has been demonstrated that mutant mice conditionally lacking norepinephrine and epinephrine are severely impaired in the retrieval of contextual memories and that this impairment can be alleviated by restoration of NE levels in the brain (Murchison et al., 2004).

Interestingly, the *d* and *l* isomers of amphetamine differ markedly in terms of their neurochemical profiles. Microdialysis studies have shown that *d*-amphetamine is significantly more potent than *l*-amphetamine in increasing dopamine in striatum. However, perfusion of *d* or *l*-amphetamine produce equivalent elevations of norepinephrine in cortex (Heikkila, Orlansky, Mytilineo, & Cohen, 1975). Because norepinephrine is known to play a role in memory, we wondered if *l*-amphetamine might retain the memory enhancing activity of *d*-amphetamine without causing the side effects related to dopamine release. To examine this hypothesis, we compared the effects of the enantiomers of amphetamine and methamphetamine on locomotor activity, stereotypy and memory in rats. Our results show that the *l* enantiomers of amphetamine and methamphetamine are not only as effective as *d*-amphetamine in terms of memory enhancement, but they are also considerably more potent.

2. Materials and methods

2.1. Subjects

Male, Long-Evans rats weighing between 250 and 300 g at the time of testing served as subjects in these experiments. Naïve rats were used for each experiment. Animals were housed two per cage in plastic cages with corn-cob bedding. Rats were allowed free access to food and water and were maintained on a 12-hour on, 12-hour off light-dark cycle. Housing, care and handling of animals were in accordance with standards recommended by the Guide for the Care and Use of Laboratory Animals, AALAC and the IACUC committee. All behavioral testing was conducted during the light phase of the cycle, during the hours of 8:00 AM and 1:00 PM.

2.2. Drugs

Scopolamine hydrobromide, *d*-amphetamine, *d*-methamphetamine (Sigma, St Louis, MO), *l*-amphetamine and *l*-methamphetamine (Sention, Providence, RI) were dissolved in 0.9% sterile saline and were administered via i.p. injection in a volume of 1 ml/kg. All solutions were made on the day of testing.

2.3. Experiment 1: Locomotor activity

2.3.1. Apparatus

Activity monitoring was conducted in automated activity chambers measuring 42 × 42 × 30 cm (Accuscan Instruments, Columbus, OH). The animals' locomotor activity was detected by a grid of 48 infrared light beams which traversed the chamber in both horizontal and vertical planes. Data was collected and analyzed using VersaMax software (Version 1.83, Accuscan Instruments, Columbus, OH).

2.3.2. Procedure

For activity testing, each rat ($n = 8$ per group) was placed in an automated activity monitoring chamber and activity levels were recorded for a 40 min baseline period. The animals were then removed from the chamber and injected with saline, *d*-amphetamine, *d*-methamphetamine, *l*-amphetamine or *l*-methamphetamine (0.25, 0.5, 1.0, 2.0 or 4.0 mg/kg, i.p.) and their activity levels were monitored for three consecutive hours. The analyzed behaviors included total distance moved and number of stereotyped movements. Activity data was analyzed using repeated measures ANOVA with dose group as a between-subjects variable and time as a within-subjects variable. Planned comparisons of significant main effects and interactions were assessed with appropriate contrasts.

2.4. Experiment 2: Inhibitory avoidance in normal rats

2.4.1. Apparatus

The Inhibitory Avoidance apparatus (Coulbourn Instruments, Allentown, PA) consisted of a light chamber and a dark chamber (both measuring 50.8 × 25.4 × 30.5 cm), which were joined by means of an automatically operated guillotine door. The light chamber was illuminated by means of a small ceiling light. The dark chamber was not illuminated and had a floor made of 2.4 mm diameter steel rods, through which a foot-shock could be administered via a constant current 18-pole shock scrambler. The entire apparatus was enclosed in a ventilated, sound-attenuating cabinet. Graphic State Notation Software (Version 1.013, Coulbourn Instruments, Columbus, OH) controlled hardware, experimental parameters and data collection.

2.4.2. Procedure

Training was initiated by placing the rat into the illuminated chamber of the apparatus. After 10 s, the sliding door was opened, allowing the rat access to the adjacent dark chamber. Two seconds after entering the dark chamber, a continuous 0.4 mA (normal conditions) or 0.6 mA (scopolamine experiments) foot-shock was delivered through the floor grid for 2 s. The animal was then removed from the apparatus, injected with saline or different doses of *d*-amphetamine (0.1, 0.25, 0.5, 1.0, 2.0, 4.0 mg/kg, i.p.), *l*-amphetamine (0.1, 0.25, 0.5, 1.0, 2.0 mg/kg, i.p) or *l*-methamphetamine (0.1, 0.25, 0.5, 0.75, 1.0 mg/kg, i.p.), and returned to their home cage. Retention testing was conducted 24 h after training. The retention test was identical to training except that no foot-shock or drug was delivered. Latency to re-enter the dark chamber was recorded, and the animals were then returned to their home cages. A total of 284 rats were used in these experiments.

2.5. Experiment 3: Inhibitory avoidance in scopolamine-treated rats

To induce amnesia for the inhibitory avoidance task, animals were injected with scopolamine hydrobromide (0.75 mg/kg, i.p.) 30 min prior to training. Animals were trained as described above, and then injected with varying doses of *d*-amphetamine (0.5, 1.0, 2.0 and 4.0 mg/kg, i.p.), *l*-amphetamine (0.1, 0.25, 0.5, and 1.0 mg/kg, i.p.) or *l*-methamphetamine (0.1, 0.25, 0.5 and 1.0 mg/kg, i.p.) immediately after training. Retention for the task was tested 24 h later. No drug was administered prior to the retention test. Groups injected with saline followed by a second saline injection, or with scopolamine followed by saline were included as controls. A total of 214 rats were used in these experiments.

As the inhibitory avoidance data was not normally distributed, it was analyzed using non-parametric analysis of variance (Kruskall Wallace) with dose group as the between-subjects variable. Post-hoc comparisons of individual dose groups were performed using Mann Whitney U tests. For graphical representation of inhibitory avoidance performance in normal rats (Fig. 2A–C), data from drug-treated animals were normalized to data from control animals and means and SEM's were generated and plotted.

2.6. Experiment 4: Object recognition

2.6.1. Apparatus

The apparatus used for object recognition testing consisted of a Plexiglas open-field activity chamber, measuring 42 × 42 × 30 cm. A video camera was mounted on the wall above the apparatus to record all trials. Objects of varying shapes were used as stimuli for the experiment. The objects were approximately 8 cm × 8 cm in size, and were secured to the bottom of the cage with Velcro adhesive tape. All objects were made out of plastic, and triplicate copies of each object were obtained in order to eliminate odor cues. Objects and the test arena were cleaned thoroughly between trials.

2.6.2. Procedure

Rats were individually habituated to the open-field box for three consecutive days. Habituation sessions were 6 min in duration. Twenty-four hours after the last day of habituation, a training session was conducted, in which two identical objects were placed in the open-field box, 10 cm from the back wall. The animal was placed into the box and was allowed to explore freely for a period of 4 min. The amount of time spent exploring the objects was recorded. Exploration was defined as directing the nose at a distance of 2 cm or less to the object, and/or touching the object with the nose. Walking over or sitting on the object was not considered exploration. Immediately following the training session, rats were injected with saline ($n = 6$, 20 or 13 for the *d*-amphetamine, *l*-amphetamine or *l*-methamphetamine experiments respectively) or the previously determined optimal dose of *d*-amphetamine (2.0 mg/kg, i.p., $n = 6$), *l*-amphetamine (0.5 mg/kg, i.p., $n = 19$) or *l*-methamphetamine (0.5 mg/kg, i.p., $n = 13$). Twenty-four hours later, retention testing was conducted. During retention testing, the rat was placed back into the same activity box with one of the familiar objects used during the training session and a novel object that the rat had not seen before. The animal was allowed to explore the box and objects for a period of 4 min, and the amount of time spent exploring each of the objects was recorded. Testing was conducted at the same time each day and was videotaped for off-line analysis. Discrimination between the familiar and novel objects was measured by calculating (time spent exploring the novel object – time spent exploring the familiar object) divided by total exploration time. Object recognition data was analyzed using independent *t*-tests.

2.7. Experiment 5: Biochemical analyses

This experiment was conducted in order to determine whether there were any changes in gene expression following conjoint behavioral testing and drug administration. *l*-Methamphetamine was used in these experiments as the behavioral experiments had demonstrated that it was the most potent compound for producing memory enhancement. Male, Sprague Dawley rats were trained on the inhibitory avoidance task. Animals in the "walk-through" condition were placed in the light chamber and allowed to enter the dark chamber without receiving a foot-shock. Animals in the "IA-trained" condition were placed in the light chamber and received a foot-shock following entry into the dark chamber. All animals were injected with either saline or *l*-methamphetamine (0.5 mg/kg) immediately after training. 1 h following behavioral conditioning, walk-through + saline-injected animals ($n = 12$), walk-through + *l*-methamphetamine ($n = 11$), IA-trained + saline ($n = 11$), and IA-trained + *l*-methamphetamine-treated animals ($n = 12$) were deeply anaesthetized with Nembutal (i.p., 65 mg/kg), decapitated, and the dorsal 1/3 of both hippocampi were dissected out rapidly in ice-cold dissection buffer (212.7 mM sucrose/2.6 mM KCl/1.23 mM NaH_2PO_4/26 mM $NaHCO_3$/10 mM dextrose/5.0 mM $MgCl_2$/0.5 mM $CaCl_2$/0.02 mM CNQX/0.1 mM D,L-APV/and saturated with 95% O_2/5% CO_2). Upon dissection, tissue samples were homogenized in ice-cold homogenization buffer, pH 7.4 (10 mM Hepes/2.0 mM EDTA/2.0 mM EGTA/0.1 mM PMSF/1:100 dilution of Phosphatase Inhibitor Cocktails I and II (Calbiochem)/1:100 dilution of Protease Inhibitor Cocktail III (Calbiochem)). Tissue was homogenized using 20 even strokes in a glass/glass tissue homogenizer; unprocessed homogenates were boiled in 1/9 volume of 10% SDS (1% SDS, final concentration) for 10 min, and stored at −80 °C. Protein concentrations were assayed using the DC Protein Assay Kit (BioRad), a Bradford-based method. Samples were subsequently diluted with 4× SDS–PAGE sample buffer (4× = 20% glycerol, 248 mM Tris–HCl pH 6.8, 12% SDS, 8% β-mercaptoethanol, bromophenol blue) to 1× final concentration and stored at −20 °C until SDS–PAGE analysis.

Equal amounts of homogenate (15 μg) were resolved on 10% polyacrylamide gels and transferred to PVDF membranes. Membranes were blocked with 5% BSA in TBS–Tween 20 (0.1%) for 1 h and incubated in primary antibody overnight at 4 °C (Arc 1:750 (Synaptic Systems). Blots were then washed 3 × 10 min in TBS–Tween 20 and placed in HRP-conjugated anti-rabbit secondary antibody (1:5000, (Amersham)) for 45 min at room temperature. Blots were washed 3 × 10 min in TBS–Tween 20 and reacted with enhanced chemiluminescence reagents (Amersham, ECL-plus). Signal was visualized using the Versadoc imaging system (BioRad). Optical densities of detected bands were quantified via volumetric analysis with Quantity One Software (BioRad), with the local median value surrounding each band serving as background values. To determine actin protein signal, the same blots were stripped in 0.7% β-mercaptoethanol stripping buffer (62.5 mM Tris–HCL, pH 6.7/2% SDS/700 μl BME per 100 ml buffer), re-probed for f-Actin (1:20,000 (Chemicon)), and quantified again as described above. Absorbances for Arc were normalized to within-lane actin to generate an Arc/Actin ratio which was used for statistical comparisons between groups. For display purposes, Arc/Actin ratios from the experimental groups (e.g. IA train + saline; walk + *l*-methamphetamine; IA train + *l*-methamphetamine) were expressed as a percentage of the walk + saline control group.

Statistical significance for biochemical assays was assessed with a 1-way ANOVA (significance set at $P = 0.05$), followed by Fisher's probable least-squares difference (PLSD) post-hoc test.

3. Results

3.1. Experiment 1: Locomotor activity

The results of Experiment 1 (Fig. 1) demonstrated that there were marked differences between the effects of the *d* and *l* enantiomers of amphetamine and methamphetamine in terms of locomotor and stereotyped activity. Repeated measures ANOVA showed a significant effect of dose group for *d*-amphetamine ($F_{5,42} = 10.42$, $p < 0.0001$) and *d*-methamphetamine ($F_{5,42} = 12.39$, $p < 0.0001$). Planned comparisons demonstrated that animals injected with either 2.0 or 4.0 mg/kg of *d*-amphetamine or *d*-methamphetamine showed significantly elevated levels of locomotor activity compared to saline injected controls ($p < 0.01$) (see

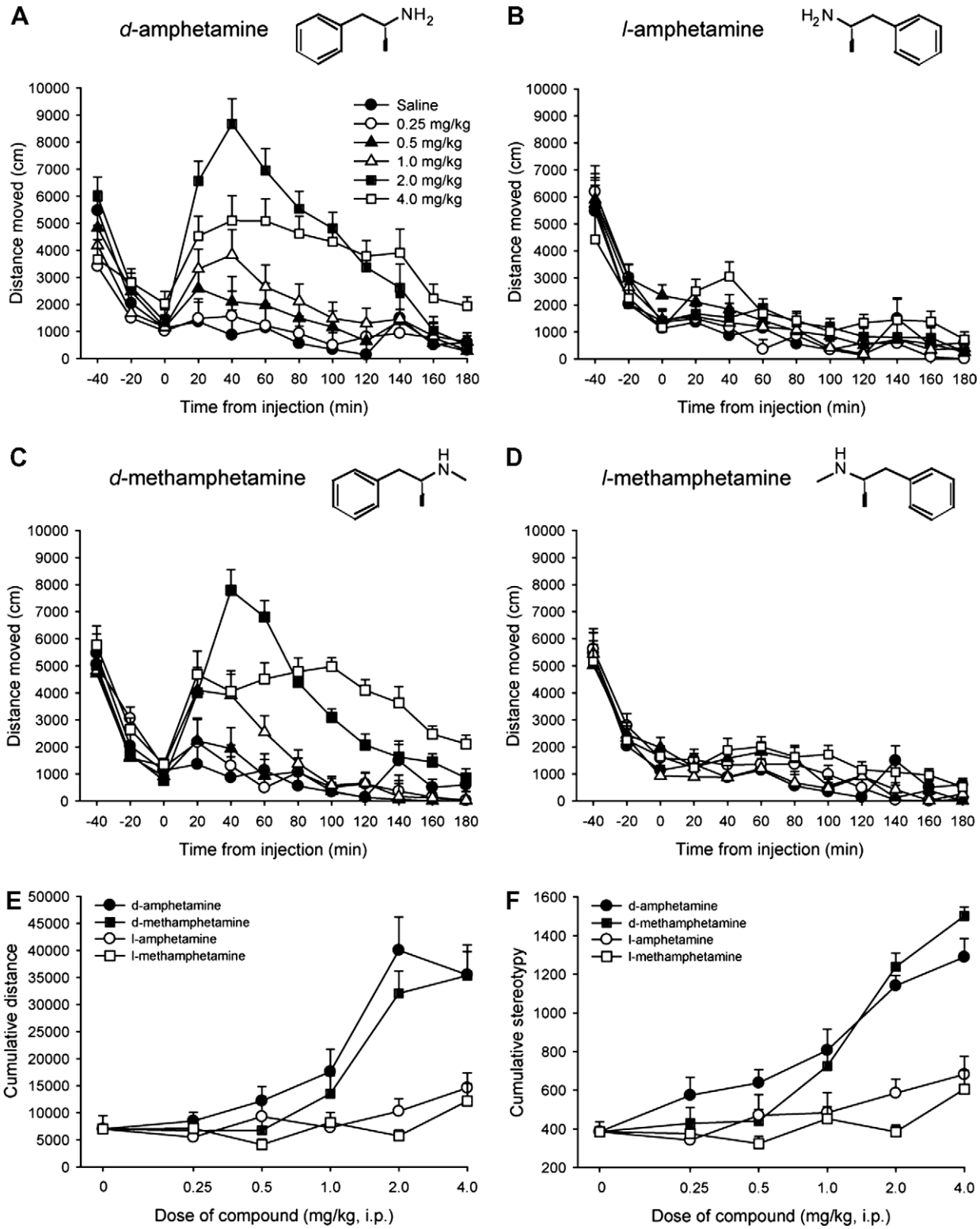

Fig. 1. Locomotor and stereotyped behavior in rats following administration of *d*-amphetamine, *l*-amphetamine, *d*-methamphetamine and *l*-methamphetamine. (A) Total distance moved following *d*-amphetamine administration. Means and SEM are plotted. (B) Total distance moved after *l*-amphetamine administration. (C) Total distance moved after *d*-methamphetamine administration. (D) Total distance moved after *l*-methamphetamine administration. (E) Cumulative distance (mean and SEM) for *d* and *l*-amphetamine and *d* and *l*-methamphetamine. (F) Cumulative stereotyped behavior for *d* and *l*-amphetamine and *d* and *l*-methamphetamine.

Fig. 1A and C). The increased locomotor activity persisted for 120 min following injection of 2.0 mg/kg *d*-amphetamine or *d*-methamphetamine and for 180 min following injection of 4.0 mg/kg of either compound (all p's < 0.01). In general, a dose dependent relationship was observed, whereby increased doses of *d*-amphetamine or *d*-methamphetamine resulted in higher total locomotor counts (see Fig. 1E). The 4.0 mg/kg dose of each compound, however, was accompanied by slightly lower locomotor

activity counts than the 2.0 mg/kg dose. This can be attributed to the observation that animals in the 4.0 mg/kg groups showed correspondingly higher levels of stereotyped behaviors such as repetitive circling, jaw movements and head bobbing (see Fig. 1F).

In contrast to the elevated activity and stereotypy levels observed after administration of the *d* enantiomers, animals injected with *l*-amphetamine or *l*-methamphetamine did not show increased levels of locomotor activity (p's > 0.05) (see Fig. 1B and D). Levels of locomotor and stereotyped behaviors were comparable to controls at all doses and experimental time points. These results clearly demonstrate that the *d* isomers of amphetamine and methamphetamine are significantly more potent than the *l* isomers in terms of producing stimulatory locomotor effects.

3.2. Experiment 2: Inhibitory avoidance

The aim of Experiment 2 was to determine whether *d*-amphetamine, *l*-amphetamine and *l*-methamphetamine have memory enhancing effects in normal rats as measured by the inhibitory avoidance task. *d*-Methamphetamine was not tested as previous research has demonstrated that its use results in behavioral and neural toxicity (Bisagno, Ferguson, & Luine, 2002; Schroder, O'Dell, & Marshall, 2003). Results, presented in Fig. 2, indicated that all three compounds were effective memory enhancing agents. Post-training administration of *d*-amphetamine resulted in significantly enhanced performance for the inhibitory avoidance task (Kruskal Wallace chi square = 15.94, $p < 0.02$). In particular, doses of 2.0 and 4.0 mg/kg (p's < 0.03) produced a significant enhancement of performance as compared to saline injected controls (see Fig. 2A). Interestingly, post-training administration of *l*-amphetamine and *l*-methamphetamine also significantly enhanced retention for the inhibitory avoidance task, and did so at lower doses than *d*-amphetamine (Kruskall Wallace chi square = 15.88, $p < 0.09$, Kruskal Wallace chi square = 14.46, $p < 0.01$, respectively). A dose of 0.5 mg/kg of *l*-amphetamine ($p < 0.01$), and doses of 0.25 and 0.5 mg/kg *l*-methamphetamine produced a statistically significant enhancement of retention (p's < 0.006 and 0.001, respectively) which was similar in magnitude to that observed after administration of 2.0 and 4.0 mg/kg doses of *d*-amphetamine (see Fig. 2B and C). It is interesting to note that an inverse U-shaped dose response curve was obtained for *l*-amphetamine and *l*-methamphetamine. This is a common phenomenon in behavioral experiments and in particular, is often seen after treatment with memory enhancing compounds. In summary, these results clearly demonstrate that *l*-amphetamine and *l*-methamphetamine are more potent in terms of facilitating memory in normal animals as compared to *d*-amphetamine.

3.3. Experiment 3: Inhibitory avoidance in scopolamine-treated rats

Previous research has demonstrated that *d*-amphetamine can alleviate scopolamine-induced amnesia for the inhibitory avoidance task (Quartermain & Jung, 1989). The aim of Experiment 3 therefore, was to determine whether the memory impairment induced by muscarinic blockade could be alleviated by administration of *l*-amphetamine or *l*-methamphetamine. Results are presented in Fig. 2, and demonstrate that in each experiment, pre-training administration of scopolamine produced a robust and statistically significant amnesia (p's all < 0.01). As expected, this amnesia could be alleviated by post-training injection of 4.0 mg/kg *d*-amphetamine ($p < 0.001$) (see Fig. 2D). Interestingly, *l*-amphetamine and *l*-methamphetamine also had a beneficial effect on the scopolamine-induced amnesia (see Fig. 2E and F). Animals injected with scopolamine followed by 1.0 mg/kg of *l*-amphetamine ($p < 0.03$) or 0.1 and 0.5 mg/kg of *l*-methamphetamine (p's < 0.01) performed significantly better on the 24 h retention test than animals injected with scopolamine followed by saline. Interestingly, *l*-amphetamine resulted in a moderate blockade of the scopolamine-induced amnesia, while *l*-methamphetamine was able to completely block the impairing effects of scopolamine. Further experiments will be required in order fully understand this effect. In conclusion, these results demonstrate that *l*-amphetamine and *l*-methamphetamine are able to significantly improve mnemonic performance in pharmacologically impaired rats, and are able to do so at doses lower than the effective dose for *d*-amphetamine.

3.4. Experiment 4: Object recognition

The aim of Experiment 4 was to evaluate the effects of the three compounds in normal rats using a non-aversive, object recognition task. Results, illustrated in Fig. 2G–I, demonstrate that all three compounds improved performance on this task, as the amount of time spent exploring the novel as compared to the familiar object was increased in animals that received drug. Moreover, in agreement with the inhibitory avoidance experiments, *l*-amphetamine and *l*-methamphetamine afforded similar levels of enhancement as *d*-amphetamine, but at lower doses. Administration of 2.0 mg/kg *d*-amphetamine produced a significant enhancement ($t_{10} = 5.23$, $p < 0.0001$), while administration of 0.5 mg/kg *l*-amphetamine ($t_{37} = 3.15$, $p < 0.003$) or *l*-methamphetamine ($t_{27} = 2.44$, $p < 0.021$) significantly improved discrimination between familiar and novel objects.

3.5. Experiment 5: Analysis of Arc/Arg3.1 protein expression

Memory consolidation is associated with the rapid induction of immediate early gene (IEG) transcription followed by new protein synthesis. We used the expression profile of the IEG Arc/Arg3.1 to track changes in hippocampal gene expression that might contribute to the pharmacological enhancement of memory consolidation.

Four groups of rats were either IA-trained or given the walk-through condition, and were subsequently injected with either *l*-methamphetamine (0.5 mg/kg) or saline immediately following conditioning. Animals were sacrificed 1 h following behavioral experience, at which point we dissected out the dorsal hippocampus for quantitative immunoblotting assays. We found a non-significant trend for increased Arc protein levels in IA-trained animals injected with saline (180.7% of walk + saline controls), and that this effect was significantly enhanced in trained animals treated with *l*-methamphetamine [303.5% of walk + saline controls, one way ANOVA, $F(3,42) = 5.275$, $p < 0.005$, effect of "group"; Fisher's PLSD for train + saline *vs.* train + drug, $p < 0.05$]. Furthermore, the drug-related enhancement in Arc/Arg3.1 protein was specific to IA training, as walk-through animals treated with *l*-methamphetamine did not differ from those injected with saline (Fisher's PLSD, $p > 0.8$) (see Fig. 3).

4. Discussion

To summarize the major findings of this study, we find that while the levo enantiomers of amphetamine and methamphetamine lack the stimulant effects of the dextro enantiomers, as measured by activity monitoring, they are potent memory enhancing agents. The enhancement of memory was as large as that observed with *d*-amphetamine, but it occurred at much lower doses. The memory enhancing action of amphetamine stereoisomers are not related to increased vigilance or alertness at the time of training, since the drugs were injected after training was completed. *D* and *l*-amphetamine are both rapidly eliminated from the body and have been shown to have brain half-lives of <2.5 h

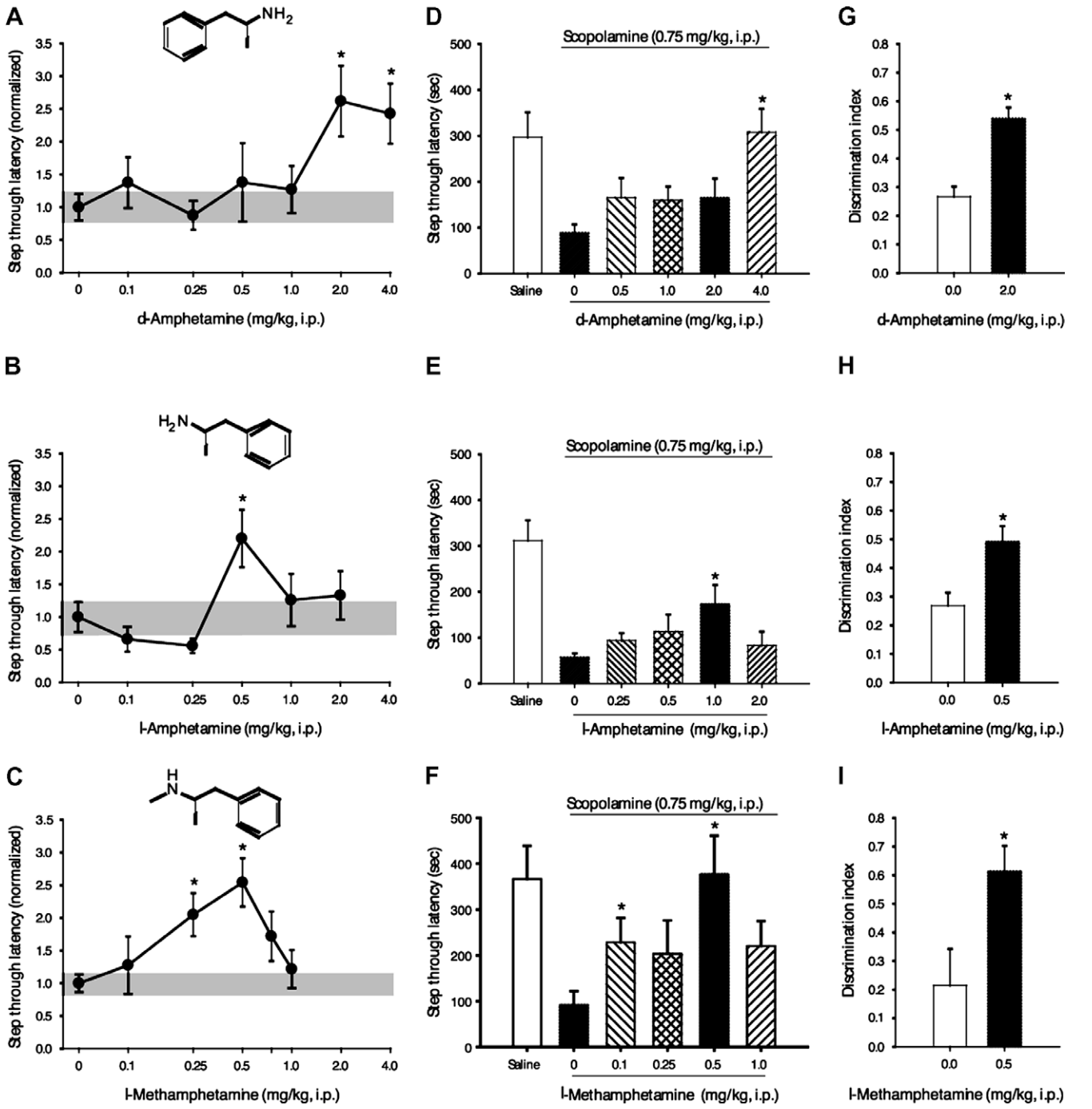

Fig. 2. Memory in rats following administration of *d*-amphetamine, *l*-amphetamine and *l*-methamphetamine. (A) Inhibitory avoidance performance in normal animals following post-training administration of *d*-amphetamine. Data were normalized to control performance and mean and SEM of the normalized data were plotted. The grey shading indicates mean and SEM of control animals. Asterisks signify significant differences from controls ($p < 0.05$). (B) Inhibitory avoidance performance in normal animals following post-training administration of *l*-amphetamine. (C) Inhibitory avoidance performance in normal animals following post-training administration of *l*-methamphetamine. (D) Inhibitory avoidance performance in animals injected with scopolamine prior to training, and with scopolamine and *d*-amphetamine after training. Means and SEM are plotted. Asterisks indicate significance from scopolamine–saline treated animals. (E) Inhibitory avoidance performance in animals injected with scopolamine prior to training, and with scopolamine and *l*-amphetamine after training. (F) Inhibitory avoidance performance in animals injected with scopolamine prior to training, and with scopolamine and *l*-methamphetamine after training. (G) Object recognition performance in normal rats following post-training injection of *d*-amphetamine. Means and SEM are plotted. Asterisks indicate significance from saline treated animals. (H) Object recognition in normal animals following post-training administration of *l*-amphetamine. (I) Object recognition in normal animals following post-training administration of *l*-methamphetamine.

following i.p. administration to rats (Lokiec, Rapin, Jacquot, & Cohen, 1978). It is unlikely therefore that the drugs directly affected performance on the recall test (e.g. by altering locomotor behavior) since 24 h elapsed from the time of drug administration before testing. Rather, the data suggest that the drugs act on the memory consolidation process that continues for several hours after training (McGaugh, 2000).

The memory enhancing effects were not confined to memory for aversive events, as they were also observed in the object recognition task. Moreover, these drugs could all reverse the memory

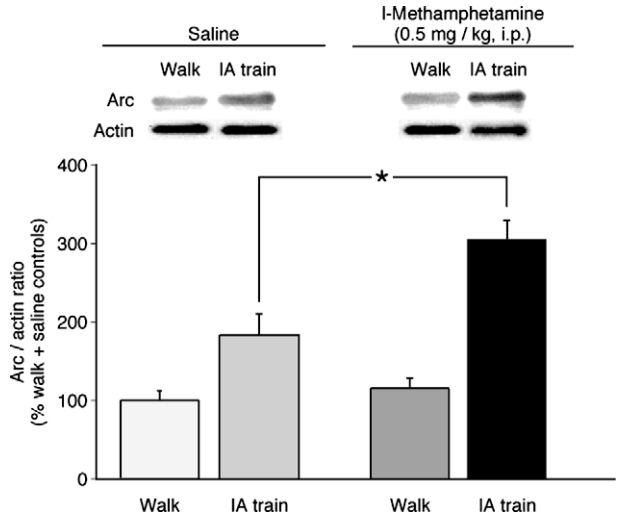

Fig. 3. *l*-Methamphetamine enhances learning-related increases in Arc protein expression in the hippocampus. Shown on the left are Arc protein levels in hippocampal homogenates from walk-through controls injected with saline ("walk + saline", hatched grey bars) or from an additional group of rats given inhibitory avoidance and injected with saline ("train + saline", hatched black bars). (Right) While treatment with *l*-methamphetamine treatment did not cause a change in walk-through animals ("walk + drug", open bar), it caused a significant ($p < 0.05$) elevation in Arc protein levels in drug-treated trained animals ("train + drug", black bar) relative to the train + saline group. For display purposes, data from all four conditions are expressed as a percentage of the mean for the walk + saline group. Representative gel bits for Arc (top) and actin (bottom) from each group are shown above. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

impairment caused by scopolamine, an effect that has previously been observed for *d*-amphetamine (Quartermain & Jung, 1989; Quartermain et al., 1988). Together, our findings suggest that *l*-amphetamine and *l*-methamphetamine may be therapeutically beneficial for certain diseases of memory consolidation in humans.

The precise site of action of *l*-amphetamine and *l*-methamphetamine remains to be determined. However, both the inhibitory avoidance and object recognition tasks depend on the hippocampus, and the inhibitory avoidance task, in particular, has been shown to produce many biochemical changes in hippocampus consistent with the possibility that information is stored, at least temporarily, in this structure (Izquierdo & Medina, 1997; Taubenfeld et al., 2001; Whitlock, Heynen, Shuler, & Bear, 2006). The memory enhancing effect of *d*-amphetamine can be blocked by local intrahippocampal administration of the norepinephrine beta receptor antagonist propranolol (Lee & Ma, 1995). Therefore, it seems reasonable to suggest that modulation of norepinephrine signaling in the hippocampus is likely to be important for the effect of the levo enantiomers of amphetamine and methamphetamine on memory consolidation. However, more work is required to determine the site(s) of action of these compounds.

Our original motivation to investigate *l*-amphetamine for memory enhancement was the early finding that both *d*- and *l*-amphetamine enantiomers have similar effects on catecholamines in the hippocampus—in contrast to the substantial difference in striatal dopamine release that is most likely responsible for changes in locomotor activity (Heikkila et al., 1975; Kanabayashi, Honda, Kodama, Mignot, & Nishino, 2000; Kuczenski, Segal, Cho, & Melega, 1995). Unlike *d*-amphetamine, *l*-amphetamine produces only small increases in dopamine, and *l*-methamphetamine has virtually no effect. On the other hand, all three compounds are effective in elevating norepinephrine. Given that increased levels of norepinephrine in the amygdala, hippocampus and cortex have been associated with enhanced memory (LaLumiere, Buen, & McGaugh, 2002; LaLumiere et al., 2003; Lee & Ma, 1995), that decreased levels have been associated with memory impairment (Luine, Bowling, & Hearns, 1990), and that noradrenergic agonists and the selective norepinephrine reuptake inhibitor atomoxetine, can improve memory in rats (LaLumiere, Nawar, & McGaugh, 2005; Tzavara et al., 2006), it is possible that the memory enhancement observed after administration of *l*-amphetamine and *l*-methamphetamine is due to effects on norepinephrine.

More work is required to identify the precise mechanism of action; however, an appealing hypothesis is that the drugs ultimately act on the hippocampal gene expression required for memory consolidation. Our biochemical data provide some evidence to support this hypothesis, as they demonstrate the enhancement of Arc/Arg3.1 protein expression in the hippocampus in response to learning. Recent studies have highlighted a critical regulatory role for Arc/Arg3.1 protein in activity-dependent glutamate receptor trafficking (Chowdhury et al., 2006; Rial Verde, Lee-Osbourne, Worley, Malinow, & Cline, 2006; Shepherd et al., 2006), long-lasting changes in synaptic strength (Plath et al., 2006), and long-term memory (Guzowski et al., 2000; Plath et al., 2006) (see Guzowski, 2002 for review). Furthermore, given that both Arc protein expression and inhibitory avoidance memory are enhanced following stimulation of amygdalar β-adrenergic receptors (McIntyre et al., 2005), it is conceivable that *l*-methamphetamine promotes endogenous learning-induced synaptic plasticity through its facilitation of noradrenergic synaptic transmission.

Regardless of the site and mechanism of action, the current data suggest the possibility that these compounds could be therapeutically effective in humans with memory disorders. *d*-Amphetamine has been shown to enhance memory consolidation in humans as well as rats, but its use is limited by adverse side effects. The present results suggest that *l*-amphetamine may be equally (or more) effective as a memory enhancer, but with fewer side effects. Indeed, inspired by these animal data, a double blind, placebo controlled clinical trial was recently conducted to investigate the use of l-amphetamine on cognitive function in patients with multiple sclerosis (Morrow et al., 2009). This study revealed a significant improvement of auditory/verbal and visual/spatial memory in treated subjects, without serious side effects, suggesting that l-amphetamine has considerable therapeutic potential in human memory disorders.

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