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Effect of the *Ginkgo biloba* extract, EGb 761, on memory formation in day-old chicks

N.S. Rickard[a,*], N. Kowadlo[a], M.E. Gibbs[b]

[a]*Psychology Department, Monash University, Caulfield, Victoria 3145, Australia*
[b]*Departments of Psychology and Pharmacology, Monash University, Victoria 3800, Australia*

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Abstract

Previous studies indicate that the *Ginkgo biloba* extract, EGb 761, has a facilitative effect on deficient memory. The temporal parameters of this effect, however, have not been clearly defined or distinguished from the effect on normal memory. The aim in the current study was to investigate the effects of EGb 761 on memory using a well-controlled animal model. Day-old chicks were trained on either a weakly or strongly reinforced version of a passive avoidance task. Long-term memory formation of the weakly reinforced version of the task was improved significantly by EGb 761 (3 mg/ml) when administered between 10 and 30 min after training. However, the same dose of EGb 761 impaired retention when administered prior to strongly reinforced training. These data provide convincing evidence that posttraining administration of EGb 761 initiates long-term memory in chicks with only short-term memory, but that the same dose-administered pretraining can be deleterious for normal retention. This dual effect has important implications for the clinical use of *Ginkgo biloba* extracts.

Keywords: Passive avoidance learning; Day-old chick; *Ginkgo biloba*; Herbal extract; EGb 761; Long-term memory consolidation; Weakly reinforced learning; Strongly reinforced learning

1. Introduction

EGb 761 is a chemically complex extract of *Ginkgo biloba*, standardized for the active ingredients 24% ginkgo flavone glycosides and 6% terpenoids (ginkgolides and bilobalide) (Mills and Bone, 2000). A combination of these active ingredients is thought to be responsible for a range of beneficial effects (Robbers and Tyler, 1999; Stein and Hoffman, 1992), which include increased cerebral blood flow, antioxidant activity and platelet-activating factor antagonism (see review in Mills and Bone, 2000). These effects may underlie ginkgo's reported capacity for neuroprotection (Brailowsky et al., 1992; Krieglstein et al., 1995) and enhancement of sensory, attentional and cognitive functioning (Itil et al., 1998; Mills and Bone, 2000). There appear to be few side effects with the doses used (DeFeudis, 1991).

Ginkgo biloba has been reported to improve memory in normal animals. Daily oral administration of EGb 761 (100 mg/kg body weight) for either 14 or 18 weeks was found to improve memory for an appetitive operant conditioning task in mice, when compared with controls (Winter, 1989). Mice that received *Ginkgo biloba* as a dietary supplement showed an improved memory for a maze task as evidenced by a decrease in the number of errors in reaching a goal box (Gajewski and Hensch, 1999). With continuous learning and delayed nonmatching to position tasks in a radial arm maze, rats needed fewer sessions to reach criterion performance and made fewer errors when administered 50 mg/kg body weight EGb 761. However, this improvement only occurred when the agent was administered before the training sessions, with no improvement when it was given after training (Winter, 1998). Administration of 50 or 100 mg/kg EGb 761 was found to reduce the impact of stress on rodents, possibly explaining why the treatment had no facilitatory effect on learning a shock-based passive avoidance task (Porsolt et al., 1990).

Facilitatory effects of *Ginkgo biloba* extracts have also been found for humans with normal memory. A single dose of 600 mg of EGb 761 administered 1 h prior to training was found to improve performance on a memory scanning task

* Corresponding author. Tel.: +61-3-9903-2221; fax: +61-3-9903-2501.
E-mail address: n.rickard@sci.monash.edu.au (N.S. Rickard).

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(Subhan and Hindmarch, 1984) and long-term memory (Lacomblez et al., 1990) when compared with placebo. It should be noted, however, these effects occurred with a dose approximately five times that usually administered (Mills and Bone, 2000). There is less consistency in the effects of more typical doses of ginkgo extract on patients with mild or no memory loss (see review in Wong et al., 1998). However, the evidence that *Ginkgo biloba* is more likely to be beneficial for subjects with a moderate to severe memory deficit, for instance, aged subjects or dementia patients (Allard, 1992; Oswald et al., 1997; Winther et al., 1998), is more substantial.

In several well-controlled double-blind studies, patients with mild to moderate dementia received the more typical doses of 120 or 240 mg EGb 761, or placebo, for 12–24 weeks (Kanowski et al., 1997; Le Bars et al., 1997; Rai et al., 1991; Wesnes et al., 1987). Subjects administered the ginkgo extract demonstrated significant improvement on a number of psychometric and clinical measures in each of these studies when compared with subjects receiving placebo. However, measures of memory were not consistently improved across these studies. In a meta-analysis of over 50 studies on the effect of *Ginkgo biloba* extract on cognitive function in Alzheimer's patients, the majority of studies were found to be poorly designed (absence of double-blind, placebo controls) or poorly reported (for instance, insufficient information on diagnosis of participants) (Oken et al., 1998). Nonetheless, in the four studies that met the inclusion criteria, *Ginkgo biloba* produced a significant improvement (effect size 0.40) in cognitive functioning.

The facilitative effect of *Ginkgo biloba* extract on poor memory has been confirmed using animal models of aging. For instance, administration of 100 mg/kg EGb 761 was found to improve passive avoidance learning in aged mice, but to have no effect on memory of young or middle-aged mice (Stoll et al., 1996). In contrast, Blavet (1992) found that the performance of both adult and aged rats on a radial arm maze was improved by a 30 mg/kg dose of EGb 761 when compared with the performance of vehicle-treated rats. A higher dose (60 mg/kg) was effective only for the aged rats, which already demonstrated poorer baseline levels of retention. These data indicate that *Ginkgo biloba* may have quite different effects on normal and deficient memory. In contrast, 40 mg/kg EGb 761 was found to cause similar improvement on a discrimination task learning in adult mice with normal memory and aged mice with deficient memory (Raffalli-Sebille et al., 1992). This enhancement only occurred while the extract was being administered, indicating that the effects of *Ginkgo biloba* on memory may not be persistent. The finding that the effect of *Ginkgo biloba* on memory processes is immediate would suggest that an acute administration of the drug might be just as effective as chronic administration.

Surprisingly, previous studies have neglected to systematically investigate the temporal characteristics of EGb 761 effects on memory. Differentiating the effects of a drug on learning from those on memory processes can be problematic, because many tasks require multiple trials, sometimes over several days or weeks, making the actual time of learning difficult to identify. In contrast, the passive avoidance paradigm devised for day-old chicks (Cherkin, 1971; Mark and Watts, 1971) involves a very brief training trial (10 s) from which three stages of memory formation can be clearly distinguished (Ng and Gibbs, 1988; Rosenzweig et al., 1993). Chicks are trained to associate a colored bead with an aversive taste, after which they demonstrate retention by avoiding similarly colored dry beads while continuing to peck previously neutral beads. The time at which pharmacological agents are administered relative to learning can be tightly controlled, and the time at which any effects of the drug occur can be measured to the minute. In addition, deficient or poor retention can be modeled using a weakly reinforced form of the training, in which the aversive chemical coating the colored bead during training is diluted to 10–20%. Weakly reinforced learning results in normal memory for approximately 30 min after training, but does not initiate long-term memory formation (Crowe et al., 1989). In the current study, the passive avoidance task was used to investigate the effects of the *Ginkgo biloba* extract, EGb 761 on retention in the day-old chick.

2. Method

2.1. Subjects

Subjects were 1–2-day black Australorp–white Leghorn cross cockerels (Research Poultry Farm, Victoria). Chicks were housed in pairs to reduce isolation stress (DeVaus et al., 1980). One chick from each pair was marked with a black indelible pen for identification purposes. Crushed poultry food was made available to chicks throughout the experiment.

2.2. Apparatus

Chicks were housed in pens ($18 \times 25 \times 20$ cm) lit by 15 W white globes suspended above the pens that maintained the temperature between 26°C and 29°C. The training materials consisted of a small chrome bead (2 mm in diameter) and two small glass beads (4 mm in diameter; one colored blue and one colored red) attached to stiff wire rods (32 cm long). The chemical aversant, methylanthranilate (MeA; APS Ajax Finechem, Auburn, Australia) was used either undiluted (100%) or diluted (15–20% in ethanol) to coat the beads during training.

A hand-held recorder was used to collect data on number of pecks within a 10-s interval, and data were later downloaded onto an IBM-compatible computer for decoding (software designed by Psychology Department, Monash University). All statistical analyses were performed using SPSS for Windows statistical software.

The EGb 761 tincture was an ethanolic extract of *Ginkgo biloba* obtained from Mediherb (Warwick, Queensland, Australia). The standardized extract contained 24% ginkgo glavone glycosides and 6% terpenoids (Mills and Bone, 2000), and was made up in 50% ethanol. The main constituents of EGb 761 have half-lives of between 3.2 and 10.6 h with peak plasma activity occurring between 1.5 and 3 h (Mills and Bone, 2000; Schulz et al., 1998), and there are no known interactions between EGb 761 and any other drugs (Schulz et al., 1998). The concentration of extract was 9 mg/ml, and various dilutions were made with physiological (154 mM) saline. Dilutions were performed in the morning of the experiment and brought to room temperature. The saline solution used as a control contained the concentration of ethanol that matched the ethanol content of the maximum EGb 761 dose for each experiment. A 1-ml disposable Terumo syringe fitted with a 27-gauge needle was used to administer solutions to chicks.

2.3. Procedure

Chicks were trained on a passive avoidance task, which has been described in detail elsewhere (Ng and Gibbs, 1988). Briefly, chicks were pretrained to peck freely at small chrome beads, and then red and blue glass beads, coated with water and presented in succession approximately 10 s each. The training trial consisted of a single 10-s trial during which chicks were presented a red-colored glass bead coated with the chemical aversant, MeA. Chicks pecking the training bead show typical disgust reactions, such as shaking the head and wiping the beak on the floor. In Experiments 1 and 2, the training bead was coated in a 15–20% dilution of MeA for weakly reinforced learning (Crowe et al., 1989). In Experiments 3 and 4, undiluted MeA was used for strongly reinforced training trials.

Solutions (various doses of EGb 761 or the vehicle) were administered subcutaneously under a fold of skin in each chick's abdomen. Changes in the brain in both humans' electroencephalograph profiles (Itil et al., 1998) and radioactively labelled uptake in the brain of animals (Moreau et al., 1986; Stoll et al., 1996) suggest that peripheral administration of EGb 761 results in adequate absorption and metabolism of the extract, as well as penetration of the blood–brain barrier. Each chick received a 0.1-ml volume of the extract or vehicle solution at various times before or after the training trial. Retention tests were performed at various training–test intervals and consisted of a 10-s presentation of a dry red bead, followed by a dry blue bead. (Details of specific doses, administration times and retention test times are given under the Results section for each experiment.) Number of pecks at each bead within a 10-s interval was recorded using the hand-held recorder. A different group of 20 chicks was used for each data point. Chicks that failed to peck the aversive (red) bead during the training trial or to peck the blue bead on the retention trial were excluded from subsequent data analyses. Typically, no more than 20% of chicks in any one group were excluded for these reasons, resulting in 15–20 different chicks for each data point (and occasionally 30–35 when a data point required replication) being included in final statistical analyses. Retention levels were indexed by a discrimination ratio, which is defined as the ratio of number of pecks at the blue bead to the total number of pecks at both beads, on the retention trial. This index provides a continuous measure of retention, and enables avoidance due to discrimination memory (where the red bead would be avoided, but chicks would continue to peck the blue bead) to be distinguished from avoidance due to nonspecific performance effects (where avoidance of both beads would be expected).

The experimental protocol in these studies was approved by the Monash University Animal Ethics Committee, in accordance with the standards of the Victorian Prevention of Cruelty to Animals Act and Regulations 1986 (the law), and the NHMRC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (6th edition, 1997).

3. Results

3.1. Effects of EGb 761 on poor retention

Previous literature suggests that *Ginkgo biloba* extracts are most likely to improve memory when retention is already deficient. A weakly reinforced version of the passive avoidance task (20% dilution of MeA) was therefore used in Experiments 1 and 2 as a model of deficient memory. Retention following weakly reinforced training in this task typically lasts for a short period (approximately 30 min), following which there is no long-term memory formation.

3.1.1. Experiment 1: dose response study for weakly trained chicks

Previous studies indicate that quite large acute doses of *Ginkgo biloba* are required to improve memory. For instance, single doses of 600 mg (which assuming an average body weight between 50 and 100 kg yields an approximate dose of between 6 and 12 mg/kg) are effective in humans (Lacomblez et al., 1990; Subhan and Hindmarch, 1984), while doses ranging from 30 to 60 mg/kg have been effective in smaller animals (Blavet, 1992; Raffalli-Sebille et al., 1992). Since this agent has not previously been used in the chick, a wide range of doses was tested. A pilot study revealed that undiluted extract containing up to 50% ethanol caused behavioural disturbances in the chick (circling, unsteadiness, poor righting reflex), whereas no behavioural disturbance was observed once the pure extract was diluted in 50% saline. There appeared to be slight irritation at the site of injection in both Egb 761 and control chicks (probably due to the ethanol content of the injections), but this was short-lived and did not affect behaviour in any way. The strongest dose used, therefore, was a 1:1 dilution of the

tincture (4.5 mg/ml), which assuming the average body weight of a day-old chick is 40 g translates to approximately 11 mg/kg (via a 0.1 ml volume). A range of dilutions was performed, yielding doses between 0.56 (1:15 dilution) and 4.5 mg/ml (1:1 dilution). A control group was administered the vehicle for the strongest dose, which contained 25% ethanol. A 'positive control group' was trained on the strongly reinforced version of the task, and was administered the vehicle immediately after training to indicate optimum retention of chicks performing this task. No behavioural disturbances were observed in any of the experimental or control chicks, including those administered the highest quantity of ethanol (0.025 ml), which also suggests that no interaction between EGb 761 and ethanol on behavioural performance.

A posttraining administration time (immediately after training) was selected for this experiment to differentiate effects on memory formation from potential nonspecific effects that pretraining administration of the extract could have on chicks while learning the task. Since the appropriate time of administration relative to learning is not clear from previous studies, a second dose response study (with doses ranging from 0.75 (1:11 dilution) to 3 mg/ml (1:2 dilution)) was performed with the agent administered 10 min after training. Control chicks were administered the vehicle for the strongest dose, which contained 16.7% ethanol. A more extensive exploration of administration times is described in Experiment 2 following determination of a suitable dose. All chicks were tested 2 h after training.

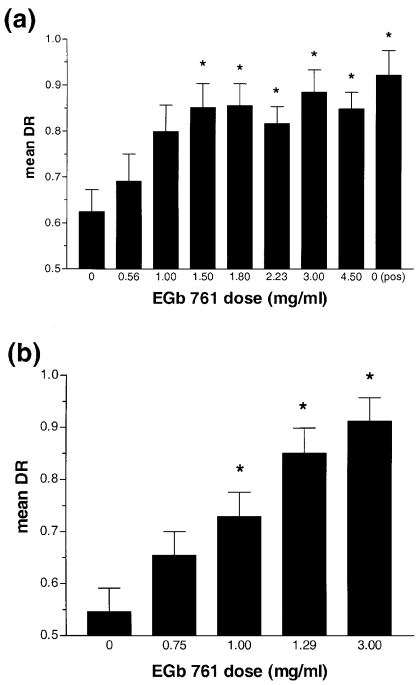

Fig. 1. Dose response functions for EGb 761 (mg/ml) administered (a) immediately after weakly reinforced training (20% MeA) and (b) 10 min after training. All chicks were tested 2 h posttraining. * Significantly different from vehicle control, $P < .05$.

It can be seen that a range of EGb 761 concentrations is capable of improving retention when tested 2 h after the weakly reinforced version of the passive avoidance task, whether the agent is administered immediately after training or 10 min after training (see Fig. 1). A one-way ANOVA revealed a significant dose effect when the extract was administered immediately after training [$F(8,178) = 3.51$, $P < .005$]. Post-hoc Dunnett's tests (against the negative control) indicated that retention levels of chicks administered doses between 1.5 and 4.5 mg/ml EGb 761 and retention of the positive control chicks (administered vehicle and strongly trained) were significantly higher than retention levels of weakly trained chicks administered the vehicle (see Fig. 1a). Given the average weight of chicks in this study was 40 g, this dose range can be translated to between 3.75 and 11.25 mg/kg. A similar dose response function was observed when EGb 761 was administered 10 min after training [$F(4,87) = 9.54$, $P < .001$]. Post-hoc Dunnett's tests indicated that retention levels for chicks administered 1, 1.5 or 3 mg/ml EGb 761 were significantly higher ($P < .05$) than retention levels for chicks administered the vehicle (see Fig. 1b). Given the average weight of chicks in this study was 40 g, this dose range can be translated to between 2.5 and 7.5 mg/kg.

3.1.2. Experiment 2: effective time of administration for weakly trained chicks

Previous studies have typically administered ginkgo daily or continuously over a period of time (Gajewski and Hensch, 1999; Kanowski et al., 1997; Le Bars et al., 1997; Rai et al., 1991; Wesnes et al., 1987; Winter, 1989), so it is difficult to know exactly which aspect of learning or memory ginkgo is facilitating. However, the 10-s training trial in the passive avoidance task enables greater temporal precision when defining the efficacy of various treatments since the exact time of learning (within 10 s) is known. The aim of Experiment 2 was to determine the period during which EGb 761 could be administered to promote long-term memory formation. The dose found to be most effective in enhancing retention in Experiment 1 (3 mg/ml) was administered subcutaneously to chicks at various times relative to the weakly reinforced training trial, ranging from 30 min prior to training to 50 min after training. A second experiment was performed using a second effective dose (2.23 mg/ml) to assess whether long-term memory was sensitive to enhancement from another dose over a similar period of administration times relative to training. Control chicks were administered the vehicle with ethanol concentration corresponding to the relevant EGb 761 solution used.

It appears from Fig. 2 that EGb 761 enhances retention if administered at any time from soon after training up to 30 min after training. A two-way ANOVA showed a significant drug effect [$F(1,468) = 20.72$, $P < .001$], a significant time of administration effect [$F(1,468) = 2.09$, $P < .05$] and a significant interaction effect [$F(11,468) = 5.21$, $P < .001$]. Simple main effects analysis confirmed that retention levels

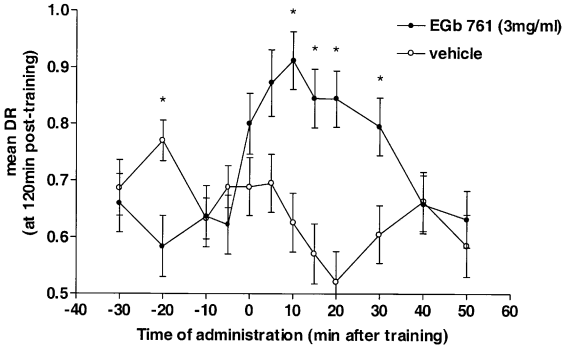

Fig. 2. Time of administration functions for EGb 761 (3 mg/ml) or the vehicle. All chicks were trained on the weakly reinforced version of the task (20% MeA), and tested at 2 h posttraining. * $P<.05$.

of chicks administered the extract at times from 10 to 30 min posttraining were significantly higher ($P<.01$) than that of controls. In addition, retention levels of chicks administered the vehicle 20 min prior to training were significantly higher than those of chicks administered EGb 761 at this time-point. Nonetheless, the time of administration effect was significant for chicks administered EGb 761 [$F(11,469)=4.72$, $P<.001$], but not for chicks administered the vehicle.

Retention at 2 h posttraining (data not shown) was also similarly enhanced by administration of a lower effective dose (2.23 mg/ml) at times between training and 30 min posttraining [$F(7,102)=3.95$, $P<.005$]. Post-hoc Dunnett's tests revealed that retention levels were significantly higher ($P<.05$) than controls when the extract was administered at either 10 or 15 min posttraining.

3.2. The effect of ginkgo extract on good retention

EGb 761 appears to be an effective cognitive enhancing agent when administered within 30 min of training to chicks trained on the weakly reinforced version of the passive avoidance task. This is consistent with the findings in rodents (Stoll et al., 1996) and humans (Allard, 1992), which indicate that ginkgo extracts are particularly beneficial for subjects in whom a memory deficit already exists. There is also evidence that ginkgo can facilitate retention when memory is already good (Lacomblez et al., 1990; Subhan and Hindmarch, 1984; Winter, 1989), although this is not as consistent (see Wong et al., 1998). Either way, previous findings suggest that the effects of *Ginkgo biloba* on memory may be different for subjects with normal memory than for those with deficient memory. In addition, while pretraining administration of the extract did not appear to have any effect on retention in Experiment 2, any deleterious effects would be masked by retention levels already being at their minimum due to weakly reinforced learning. The aim of the following experiments, therefore, was to examine the effects of EGb 761 on the retention of chicks with good memory, as produced by the strongly reinforced version of the passive avoidance task. Long-term memory for this version of the task forms by about 60 min posttraining, and is maintained for at least 24 h (Ng and Gibbs, 1988; O'Dowd et al., 1994).

3.2.1. Experiment 3: effective time of administration for strongly trained chicks

A 3 mg/ml dose of EGb 761 was administered subcutaneously to each chick at various times before (30, 20, 10 or 5 min) and after (immediately, 10, 20 or 40 min) the strongly reinforced (100% MeA) training trial. Control animals were administered the vehicle with the same ethanol concentration as the EGb solution. Retention of all chicks was tested 120 min posttraining.

Posttraining administration of the ginkgo extract clearly had no effect on retention resulting from strongly reinforced learning. However, when administered prior to training, EGb 761 appears to impair retention in this task (see Fig. 3). A two-way ANOVA revealed a significant drug effect [$F(1,429)=10.53$, $P<.005$], a significant time of administration effect [$F(7,429)=4.18$, $P<.001$] and a significant interaction effect [$F(7,429)=4.31$, $P<.001$]. Simple main effects analyses revealed that there was a significant time of administration effect within the EGb 761-treated chicks only [$F(7,430)=8.92$, $P<.001$] and significant drug effects at each pretraining administration time ($P<.05$) except 20 min ($P=.203$).

3.2.2. Experiment 4: retention function for strongly trained chicks

The finding that long-term memory formation of the passive avoidance task is impaired by administration of the *Ginkgo biloba* extract prior to training is of considerable importance. If this agent is to be used as a cognitive enhancing agent, any deleterious effects must be clearly identified. In this context, it is important to determine which aspect of memory is impaired by this treatment. The retention function resulting from strong training after pretraining administration of EGb 761 was therefore investigated. Chicks were administered EGb 761 5 min before

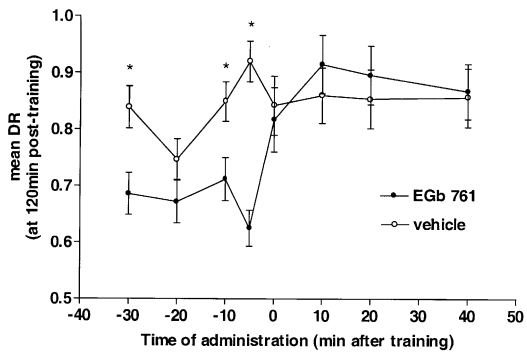

Fig. 3. Time of administration functions for 3 mg/ml EGb 761 or the vehicle. All chicks were trained on the strongly reinforced version of the task (100% MeA), and tested at 2 h posttraining. * $P<.05$.

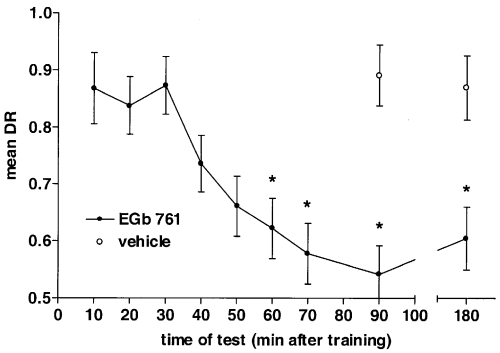

Fig. 4. Retention function for chicks trained on the strongly reinforced version of the task (100% MeA), and administered either 3 mg/ml EGb 761 or the vehicle 5 min prior to training.

training since the largest deficit in Experiment 3 resulted when the extract was administered at this time. Different groups of chicks were tested at 10, 20, 30, 40, 50, 60, 70, 90 and 180 min after training. Control chicks were administered the vehicle and tested at 90 and 180 min after training.

It can be seen in Fig. 4 that administration of EGb 761 5 min pretraining induces a persistent memory loss from about 30 min after training. A one-way ANOVA revealed a significant time of test effect [$F(10,187)=6.61$, $P<.001$], with post-hoc Dunnett's tests indicating that retention levels of chicks tested at all times 60 min posttraining and later were significantly lower ($P<.05$) than those of control chicks tested at 180 min posttraining.

4. Discussion

The current findings suggest that the *Ginkgo biloba* extract, EGb 761, is capable of facilitating memory in chicks with poor long-term retention. Relatively low doses of the extract were found to result in the formation of long-term memory in chicks, which had been trained on a weakly reinforced version of the passive avoidance task, which usually yields only shorter term memory traces. The extract was effective in promoting long-term memory formation when administered any time between 10 and 30 min after learning, but not when administered at times between 30 and 5 min prior to training. In contrast, pretraining administration of EGb 761 to chicks with good retention (produced by the strongly reinforced training), caused a significant and persistent memory loss from around 30 min after training.

Previous animal studies have typically used chronic administration of quite high doses of *Ginkgo biloba* extract to improve memory. For instance, poor retention (Blavet, 1992; Raffalli-Sebille et al., 1992) and normal retention (Winter, 1989, 1998) of rodents was improved with ginkgo doses in the range of 30–100 mg/kg. In humans, dosage is typically between 120 and 240 mg/day (Kanowski et al., 1997; Le Bars et al., 1997; Mills and Bone, 2000) or a single dose of 600 mg (Lacomblez et al., 1990; Subhan and Hindmarch, 1984). While such acute doses seem very high, given an average weight of 60 kg/participant, this dose is approximately 10 mg/kg, which is comparable or even low compared with the effective chronic dose range. The current study found that when given immediately after training, an acute dose of between 3.75 and 11.25 mg/kg EGb 761 was effective in improving retention in chicks, which is comparable to previous animal studies. For a 60-kg human, this dose range translates to a single dose of between 224 and 675 mg extract given immediately after learning. This would suggest that there is some consistency in the effective dose of EGb 761 for improving memory across species.

The finding that EGb 761 must be administered within 30 min of training to enhance long-term memory formation following weakly reinforced learning is significant. This would suggest that the facilitatory effects of the agent are on memory formation processes initiated by learning, rather than on learning per se. Since the majority of previous studies have used chronic and often continuous administration of *Ginkgo biloba*, information on when the drug must be in the system relative to learning has not been available. In two studies in which single doses were administered to healthy human females, long-term memory was improved by a 1-h pretraining treatment (Lacomblez et al., 1990; Subhan and Hindmarch, 1984). Further, learning on a radial arm maze was enhanced only when young adult rats were administered EGb 761 prior to learning, with no effect observed when the extract was administered posttraining (Winter, 1998). This is inconsistent with the findings in the current study in which pretraining administration of EGb 761 (up to 30 min before training) had no observable effect on retention following weakly reinforced learning and, moreover, impaired retention following strongly reinforced learning. It would seem, then, that in the chick, EGb 761 does not enhance learning when administered up to 30 min pretraining at this dose. However, it is important to note that earlier pretraining injections (for instance, 1 h prior to training) were not tested in the current study and would be of considerable interest for future research. It is also possible that the dose found to facilitate poor retention is not optimal for enhancing good retention. The previous finding that a 30 mg/kg dose improved learning best in young rats, while a higher dose was optimal for older rats (Blavet, 1992) suggests that the dose required to enhance deficient memory may be higher than that required to improve normal memory. Further investigation of the effect of pretraining administration of lower doses of EGb 761 on normal retention would therefore be of interest.

The finding that EGb 761 is only effective if administered at posttraining times up to 30 min would suggest that the agent is working through mechanisms activated around that time-point. Several neurochemical mechanisms are known to be critical if memory for the passive avoidance task is to persist beyond 30 min posttraining in the chick. These include release of noradrenaline (Crowe et al., 1991) and increased glycolysis and glycogenolysis (O'Dowd et al.,

1994). Interestingly, EGb 761 is known to enhance levels of the neurotransmitters acetylcholine (DeFeudis, 1991; Taylor, 1990) and noradrenaline (Auget et al., 1982; Taylor, 1990, 1992). One possible explanation, then, is that EGb 761 achieves its facilitative effect on memory in this model by stimulating noradrenaline release at 30 min after training. Consistent with the current findings, exogenous administration of 150 μg/kg noradrenaline immediately after weakly reinforced training has been shown to result in long-term memory formation (Crowe et al., 1990).

There is also quite a substantial body of evidence that suggests that *Ginkgo biloba*'s facilitative effect on cognitive functioning may be via enhancing blood flow to the brain. *Ginkgo biloba* has been demonstrated to promote blood flow to the brain (DeFeudis, 1991; Krieglstein et al., 1995; Le Poncin-Lafitte et al., 1980), which has been associated with improvements in cognitive functioning in brain damaged subjects (DeFeudis, 1991). It has also been found to enhance cognitive functioning in aged people suffering from cerebral insufficiency, the symptoms of which have been associated with impaired cerebral blood flow and problems with the supply of oxygen and glucose (Krieglstein et al., 1995). In the chick, it appears that by 30 min after passive avoidance training, ATP supplies are reduced, and additional sources of energy (such as glycolysis or glycogenolysis) are required (O'Dowd et al., 1994). Administration of EGb 761 may, therefore, overcome memory loss at 30 min posttraining in the chick by enhancing cerebral blood flow to brain regions involved in memory processing. In addition, ginkgo extracts have been found to overcome ATP and glucose deficiencies in models of ischaemia (Le Poncin-Lafitte et al., 1980; Mills and Bone, 2000) and hypoxia (Janssens et al., 1995). Ginkgo extracts appear to reduce brain cells' requirement for glycolysis by enhancing the production of ATP production by mitochondria (Janssens et al., 1995). Therefore, administration of EGb 761 to chicks may also overcome memory loss at 30 min posttraining because ATP production is extended.

The effects of posttraining administration of EGb 761 on long-term memory formation following weakly reinforced training, then, are clearly beneficial. The mechanism by which this facilitation occurs cannot be determined from the current study, but investigations into changes in noradrenaline release or ATP production following EGb 761 administration would be worthy of further investigation. Any application of this finding, however, must take into consideration the deleterious effects of this agent on normal memory. While posttraining administration of EGb 761 improved retention of chicks trained on the weakly reinforced version of the task, this agent impaired memory formation beyond 30 min posttraining when given pretraining to chicks trained on the strongly reinforced version of the task. This time-point is during an intermediate stage of memory formation, a time at which critical processes for long-term memory formation are thought to be triggered (Ng and Gibbs, 1988).

While the reason for this effect is not clear, it is possible that the combination of strong learning and EGb 761 results in overstimulation of some critical process prior to learning. This is consistent with previous literature that indicates that moderate arousal improves memory formation (Kety, 1976), but that higher levels may be detrimental (Tsukada, 1988). Since both strong learning and EGb 761 are both known to stimulate the release of the stress-related hormone, noradrenaline, it follows that together they may be deleterious for memory formation. Similar paradoxical effects have been observed in this model previously (Zhao et al., 1994), whereby an agent that enhances poor retention can also impair good retention.

Interestingly, there appears to be a significant effect of the vehicle (containing approximately 17% ethanol) on long-term memory when it was administered to chicks 20 min prior to training. In weakly trained chicks, retention was poor for chicks administered the vehicle at all times except 20 min pretraining. On the other hand, retention was good in strongly trained chicks administered the vehicle at all time-points except 20 min pretraining. While this quantity of ethanol (0.017 ml) generally has no effect on chicks' learning or retention, this temporal consistency would suggest that learning is markedly affected when ethanol has been present in the chick's system for 20 min. This ethanol-induced state is likely to be an aversive experience to chicks, so it is possible that this strengthens the weakly reinforced training experience such that chicks form a normal retention for this usually subthreshold experience. When the reinforcement is already strong, the additional aversion caused by the ethanol may make the event too arousing, resulting in poor retention. This is again consistent with the finding that moderate levels of arousal are optimal for performance (Kety, 1976; Tsukada, 1988).

The role of *Ginkgo biloba* in treating memory loss is, on the whole, promising. EGb 761 has over the last two decades been shown to improve poor retention across several species, and there is comparability in the effective dose range. The current findings present the first evidence that *Ginkgo biloba* may enhance memory formation processes rather than acquisition, in a model of poor retention. Notably, the long-term memory loss and short-term memory preservation resulting from this version of the task is also typically observed in many forms of human amnesia. Moreover, these data suggest that for tasks on which retention is expected to be good, pretraining administration of EGb 761 may actually be detrimental to learning. This unexpected finding provides an important caveat when considering how facilitative agents can be used in practice.

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