Improvement of auditory discrimination learning by Ginkgo biloba extract EGb 761®

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A large number of clinical and pre-clinical studies have investigated the therapeutic value of Ginkgo biloba extract EGb 761®, and beneficial effects on cognitive functions have been reported for both human [1,5] and animal subjects [6]. So far, most of these effects have been tentatively explained by the stabilizing or protective effects of EGb 761® on mitochondrial functions. Recently, Kehr et al. have reported that EGb 761® increases the extracellular levels of dopamine in the prefrontal cortex of rats [3] which points to a more specific potential of increasing cognitive functions. Since the relevance of prefrontal dopamine release for auditory discrimination learning of gerbils in a shuttle-box paradigm has been demonstrated [9], we have evaluated a possible beneficial effect of EGb 761® in this animal model of learning. For a survey of the range of action of EGb 761®, three discrimination tasks with different degrees of difficulty were employed.

Twelve groups (cf. Table 1) of 8 individuals each of 3–6 months old male Mongolian gerbils (Meriones unguiculatus) were trained in a footshock-motivated shuttle-box GO/NOGO-paradigm (unconditioned stimulus (UCS): 150–300 μA; cf. [8]) to discriminate sound stimuli. Stimuli were either 100% sinusoidal amplitude modulated (AM) tones with carrier frequencies of 2 kHz or noise stimuli. Three different stimulation paradigms were chosen based on known differences in discrimination difficulty: AM tones with modulation frequencies (fm) of 20 Hz vs. 40 Hz (easy, paradigm I), AM tones with fm of 160 Hz vs. 320 Hz (more difficult, paradigm II) or white noise stimuli (WN) vs. periodic noise stimuli (PN) with a period of 80 ms (most difficult, paradigm III, cf. [2]). The AM with the lower fm or the PN, respectively, served as the CS+ (=conditioned stimulus followed by the UCS), and the AM with the higher fm or the WN, respectively, as the CS− (not followed by the UCS).

Crossings of the hurdle during a 4 s presentation of the CS+ or CS− were considered as conditioned responses, CR+ and CR−, respectively. If the animal did not cross the hurdle within 4 s after the onset of the CS+, the UCS was turned on and the CS+ presentation continued until the gerbil crossed the hurdle, but maximally for 8 s. An additional UCS (duration 0.5 s) was applied upon false hurdle crossing in response to the CS−.

Training covered 15 days with 60 trials on each day in one block (random presentations of 30 CS+ and 30 CS−). Groups treated with EGb 761® (daily oral administration of 100 mg/kg) were compared to placebo-treated controls. EGb 761® (G) or placebo (P) treatment started either at the first day of training (1 h before training, “acutely treated groups”; a) or 2 weeks prior to the start of training (“pre-treated groups; p) and continued until end of training in all groups.

The behavior in the shuttle-box was analysed using discrimination performance defined as conditioned hurdle jumps (CR+) to CS+ stimuli minus conditioned hurdle jumps (CR−) to CS− stimuli (cf. Fig. 2). These data were tested across all groups for normal distribution using a Shapiro–Wilk test as well as a Kolmogorov–Smirnov-test. Both test revealed that the distribution of our data was highly significantly different from a
normal distribution ($P < 0.001$ for both test). We therefore used non-parametric test for further evaluations of our data: Friedman-ANOVA was used to detect improvements of discrimination performance across training sessions within an individual group. Differences between daily values of G and P groups were analyzed using the Wilcoxon-test ($P < 0.05$) and by comparison of the linear regression lines fitted through the discrimination performance values of the last 6 days of training using $F$-statistics (cf. Results). For comparisons between corresponding groups across all training sessions, a Kruskal–Wallis-test was used.

All experiments were conducted in accordance with the NIH Guidelines for Animals in Research and with the German law for the protection of experimental animals. The experimental protocol was approved by the ethics committee of the State of Sachsen-Anhalt, Germany.

Fig. 1 shows the effect of acute oral administration of EGb 761® on discrimination learning performance in animals trained to discriminate AM tones with 20 Hz fm vs. 40 Hz fm (groups PaI in blue vs. GaI in red, for group nomenclature refer to Table 1). The top panel shows the raw learning curves with the CR plotted as a function of training session. The administration of EGb 761® leads to slight improvements in learning performance: The red CR+ curve rises faster than the blue one (Fig. 1, top). This becomes clear by plotting the discrimination performance (difference between the CR+ and the CR− curves; Fig. 1, bottom; given are medians (symbols), quartiles (boxes) and non-outlier ranges (bars)). Friedman-ANOVA across all training days demonstrated a significant improvement of discrimination performance for both groups (control: $P = 0.00001$; Ginkgo: $P = 0.0002$). In addition we found larger values for the EGb 761®-treated group (red) compared to the placebo-treated group (blue). This improvement is significant (Wilcoxon-test: $P < 0.01$) on several days during the course of training, but as the overall effect of EGb 761® is small, significance between groups is not reached on every day. This improvement of discrimination performance as tested using day-by-day Wilcoxon tests only reached significance for several individual training sessions for the comparison between groups Pa and Ga, that is, for the acutely treated groups with the easiest discrimination paradigm. Using a Kruskal–Wallis-test to further evaluate differences between groups across all training sessions also revealed a significant improvement with EGb 761® between groups Pa and Ga ($P = 0.0001$), and in addition also between groups PaII and GaII ($P = 0.03$).

Table 1
Stimulation paradigms and animal groups. Code for animal groups is: P or G (Placebo or Ginkgo), a or p (acutely treated or pre-treated) and degree of difficulty (I–III).

<table>
<thead>
<tr>
<th>Group</th>
<th>CS+</th>
<th>CS−</th>
<th>Degree of difficulty</th>
<th>Ginkgo-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pal</td>
<td>AM 20 Hz fm</td>
<td>AM 40 Hz fm</td>
<td>I</td>
<td>Placebo acute</td>
</tr>
<tr>
<td>PalII</td>
<td>AM 160 Hz fn</td>
<td>AM 320 Hz fm</td>
<td>II</td>
<td>Placebo acute</td>
</tr>
<tr>
<td>PalIII</td>
<td>PN</td>
<td>WN</td>
<td>III</td>
<td>Placebo acute</td>
</tr>
<tr>
<td>Ga</td>
<td>AM 20 Hz fm</td>
<td>AM 40 Hz fm</td>
<td>I</td>
<td>EGb 761® acute</td>
</tr>
<tr>
<td>GaII</td>
<td>AM 160 Hz fn</td>
<td>AM 320 Hz fm</td>
<td>II</td>
<td>EGb 761® acute</td>
</tr>
<tr>
<td>GaIII</td>
<td>PN</td>
<td>WN</td>
<td>III</td>
<td>EGb 761® acute</td>
</tr>
<tr>
<td>PpI</td>
<td>AM 20 Hz fm</td>
<td>AM 40 Hz fm</td>
<td>I</td>
<td>Placebo pre-treated</td>
</tr>
<tr>
<td>PpII</td>
<td>AM 160 Hz fn</td>
<td>AM 320 Hz fm</td>
<td>II</td>
<td>Placebo pre-treated</td>
</tr>
<tr>
<td>PpIII</td>
<td>PN</td>
<td>WN</td>
<td>III</td>
<td>Placebo pre-treated</td>
</tr>
<tr>
<td>GpI</td>
<td>AM 20 Hz fm</td>
<td>AM 40 Hz fm</td>
<td>I</td>
<td>EGb 761® pre-treated</td>
</tr>
<tr>
<td>GpII</td>
<td>AM 160 Hz fn</td>
<td>AM 320 Hz fm</td>
<td>II</td>
<td>EGb 761® pre-treated</td>
</tr>
<tr>
<td>GpIII</td>
<td>PN</td>
<td>WN</td>
<td>III</td>
<td>EGb 761® pre-treated</td>
</tr>
</tbody>
</table>

Fig. 2 shows a summary of the discrimination performance for all experimental groups (placebo treatment in blue, EGb 761® treatment in red). The different degrees of difficulty are seen from the declining performance from left to right panels in both placebo and EGb 761® treated groups, in line with earlier reports [8,2]. Data from acutely treated groups are given in the top row, those of pre-treated animals in the bottom row. When the Ginkgo groups were compared to the placebo groups (red vs. blue graphs), Ginkgo does seem to have a positive effect on discrimination performance for the tasks with a degree of difficulty I and II: Here the red curves always showed larger values demonstrating better discrimination performance after the first half of the training period.

To test for significant differences in learning performance, we compared linear regression functions (dotted lines) across the last six training sessions for each group using $F$-statistics. In this way significant differences in these regression functions for both groups with degree of difficulty I and for the pre-treated group with degree of difficulty II were obtained. For the first two groups we found a significant difference in ordinate intercept (top left panel: $P = 0.00006$; bottom left panel: $P = 0.0004$), for the latter we found a significant difference in the slope of the linear regression function (bottom middle panel: $P = 0.01$). For the groups with the most difficult paradigm (III) (right panels), no clear differences between learning
We have tested the effect of oral administration of Ginkgo biloba extract EGb 761® on auditory discrimination learning with three different degrees of difficulty in Mongolian gerbils. In addition, we tested if pre-treatment with Ginkgo extract starting 2 weeks prior to training has any effects different from those obtained with acute Ginkgo treatment starting at the beginning of training. Discrimination training of complex auditory patterns in the shuttle-box as used here is a type of aversive reinforcement learning that involves dopamine release in medial prefrontal cortex and auditory cortex [9] and requires dopamine-dependent protein synthesis for long-term memory formation in auditory cortex [7]. As expected for this learning paradigm from previous pharmacological manipulation of dopamine receptors, EGb 761® administration only led to mild improvements of learning performance. Poor discrimination performance as in control groups in paradigm III could not be turned into a performance comparable to those seen in easier paradigms (I or II). Nevertheless, significant beneficial effects could be demonstrated: Ginkgo extract significantly improved discrimination performance in groups that were acutely treated as well as in those groups that were pre-treated and had to learn discrimination tasks with degree of difficulty I and II. Furthermore, EGb 761® led to slightly faster discrimination in both pre- and acutely treated groups with the easy discrimination paradigm I (1 day saving). Strong improvement of learning speed based on our criterion (cf. [8,2]) was seen in comparison to placebo-treated groups in the most demanding discrimination paradigm III (6 days saving in both acutely and pre-treated animals), although overall performance remained moderate in these groups.

These results are in line with earlier reports on Ginkgo effects in both human and animal studies [1,5,6]. There the Ginkgo effect
Effects of oral EGb 761® administration on learning speed in acutely treated (top) and pre-treated (bottom) groups. Plotted is the 1st day with significant (Wilcoxon-test, \( P \leq 0.05 \)) discrimination that was followed by another day with significant discrimination for all groups.

was mainly reported for aged subjects. As we tested young adults in our study a stronger effect on aged gerbils cannot be excluded.

The observation that pre-treated groups did not exhibit markedly better learning performance compared to acutely treated groups, independent of Ginkgo administration, may be due to the fact that learning performance depends on the release of dopamine during the execution of the task in trials [9]. Thus, an unspecific increase of dopamine during pre-treatment without task execution is not likely to influence later learning considerably.

In summary, we were able to demonstrate slight but statistically significant improvements in learning performance in an auditory discrimination task in young adult Mongolian gerbils treated with Ginkgo extract EGb 761® both in easy as well as in more demanding versions of the task. As EGb 761® has been reported to increase extracellular concentration of dopamine in the prefrontal cortex of rats [3] which in turn has been shown to play a major role in this type of learning paradigm [9], we suppose that EGb 761® improves discrimination learning through its effect on the dopaminergic system.

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References